Potential methane and nitrous oxide production and respiration rates from penguin and seal colony tundra soils during freezing–thawing cycles under different water contents in coastal Antarctica

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Abstract   In coastal Antarctica, frequent freezing–thawing cycles (FTCs) and changes to the hydrological conditions may affect methane (CH_4) and nitrous oxide (N_2O) production and respiration rates in tundra soils, which are difficult to observe *in situ*. Tundra soils including ornithogenic tundra soil (OAS), seal colony soil (SCS) and emperor penguin colony soil (EPS) were collected. In laboratory, we investigated the effects of FTCs and water addition on potential N_2O and CH_4 production and respiration rates in the soils. The CH_4 fluxes from OAS and SCS were much less than that from EPS. Meanwhile, the N_2O fluxes from OAS and EPS were much less than that from SCS. The N_2O production rates from all soils were extremely low during freezing, but rapidly increased following thawing. In all cases, FTC also induced considerably enhanced soil respiration, indicating that soil respiration response was sensitive to the FTCs. The highest cumulative rates of CH_4, N_2O and CO_2 were 59.5 mg CH_4-C·kg^{-1} in EPS, 6268.8 μg N_2O-N·kg^{-1} in SCS and 3522.1 mg CO_2-C·kg^{-1} in OAS. Soil water addition had no significant effects on CH_4 production and respiration rates, but it could reduce N_2O production in OAS and EPS, and it stimulated N_2O production in SCS. Overall, CH_4 and N_2O production rates showed a trade-off relationship during the three FTCs. Our results indicated that FTCs greatly stimulated soil N_2O and CO_2 production, and water increase has an important effect on soil N_2O production in coastal Antarctic tundra.

Keywords   Antarctica, CH_4, N_2O, soil respiration, freezing–thawing cycles, tundra

1   Introduction

Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are three important reactive greenhouse gases (GHGs). Soils are important sources or sinks for atmospheric CH_4 and N_2O in terrestrial ecosystems (Arnold et al., 2005; Tang et al., 2006; IPCC, 2007; Tian et al., 2012; Imer et al., 2013), whereas soil respiration is the primary path for carbon from soil to the atmosphere (Schlesinger et al., 2000). Freezing and thawing cycles (FTCs) affect the

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soil microbial activities, the composition of soil microbial communities, and the dynamics of carbon and nitrogen, especially in cold polar regions (Zhu et al., 2009; Foster et al., 2016). Significantly increased GHG emissions from the soil have been reported during the thawing process (Zhu et al., 2009; Wu et al., 2014). In addition, hydrological conditions have an important effect on the activities of methanotrophic and nitrifying bacteria, oxygen diffusion and the supply of nutrients in the soils, and correspondingly on CH_4 and N_2O production rates and soil respiration (Teepe et al., 2004; Ou et al., 2013). The effects of changing soil moisture (Mc) during FTCs on GHG fluxes have been experimentally studied, but the observed relationship remains variable (Nielsen et al., 2001; Freppaz et al., 2008; Gilliam et al., 2010; Zhang et al., 2011). For example, enhanced N_2O emissions has been observed in a narrow range of soil water content during FTCs, whereas in other experiments Mc had no significant effect on N_2O emissions (Teepe et al., 2004). Therefore, the effects of soil water regimes during FTCs on GHG emissions remain unclear.

In coastal Antarctica, the ice-free coastal zones are often colonized by a large number of sea animals such as penguins, seals and other seabirds. Sea animals play a crucial part in the nutrient cycling of ecosystems by transferring carbon and nitrogen from the marine to the terrestrial environment (Turner et al., 1997; Sun et al., 2004b). The deposition of a large amount of penguin guano strongly influences the physical and chemical properties of tundra soils via the effects of microbes, and produces a special type of soil called ornithogenic soil, which is particularly high in organic carbon, nitrogen and phosphorus (Sun et al., 2002; Zhu et al., 2006; Ball et al., 2015). In addition, the activity of microorganisms is higher in ornithogenic soils than in non-ornithogenic soils (Roser et al., 1993; Tscherko et al., 2003; Zdanowski et al., 2005). The ice and snow melt water and frequent FTCs affect microbial activity and soil properties. Significant GHG emissions have been observed *in situ* in Arctic and Antarctic tundra soils, and ground temperature as well as Mc is considered as important factors affecting GHG emissions (Teepe et al., 2001; Zhu et al., 2005, 2007, 2008b, 2009). However, effects of soil FTCs and changing hydrological conditions on CH_4 and N_2O production in sea animal colony soils remain poorly understood in coastal Antarctica. Increased understanding of these processes affecting GHG production might provide more information about the impacts of future climate change on tundra GHG emissions.

During the 22nd Chinese National Antarctic Research Expedition (CHINARE-22), we collected ornithogenic tundra soils (OAS), seal colony soils (SCS) and the soils added by fresh emperor penguin guano (EPS) in coastal Antarctica, and conducted laboratory simulation experiments to investigate the effects of soil FTCs and water addition on potential N_2O and CH_4 production and soil respiration rates in sea animal colony tundra. The objectives of this study were (1) to determine potential N_2O and CH_4 production and soil respiration rates under different water treatments during soil FTCs, (2) to investigate the effects of soil FTCs on N_2O and CH_4 production and soil respiration rates, and (3) to discuss the effects of water addition and soil properties on N_2O and CH_4 production and soil respiration rates during soil FTCs. The research may be helpful to understand the combined effects of temperature and soil moisture on GHGs production rates in Antarctic tundra.

2 Study methods

2.1 Sampling sites

Tundra soil samples were collected in sea animal colonies of coastal Antarctica from the following three sites:

The first site was situated on Ardley Island (62°13′S, 58°56′W) in western Antarctica, with 2.0 km length and 1.5 km width. This island was designated as Site of Special Scientific Interet (SSSI33) in 1991, and redesignated as Antarctic Specially Protected Area (ASPA 150) in 2002 by Antarctic Treaty Consultative Meetings and is one of the most important penguin colonies in the maritime Antarctic region. During the breeding period, about 10200 penguins colonize Ardley Island and discharge about 139 tons of guano (Sun et al., 2000, 2001). In the active colony, continuous deposition of fresh guano and penguin trampling inhibit vegetation establishment, and soils are covered by a large amount of fresh guano (Sun et al., 2004b; Zhu et al., 2008b, 2009). One ornithogenic soil core (named OAS) was collected from a poorly drained tundra area about 100 m from the active penguin colony in the southeast of the Island.

The second site was located on the Fildes Peninsula of western Antarctica (61°51′–62°15′S, 57°30′–59°00′W). This peninsula is characterized by oceanic climate because of the effects of polar cyclones and is an important location for seal colonies (Zhu et al., 2005, 2007). The seals include Weddell seal (*Leptonychotes weddellii*), elephant seal (*Mirounga leonine*), leopard seal (*Hudrurga leptonyx*), fur seal (*Arctocephalus gazella*) and crabeater (*lobodon carcinophagus*). A total of about 10000 sea animals including seals colonize on this peninsula every summer, and their excreta deposited into local soils through ice and snow melt water (Sun et al., 2004a). One soil core (named SCS) was collected from seal colony on the western coast.

The third site was located within an emperor penguin colony at Prydz Bay in eastern Antarctica (69°22′S, 76°24′E). This area has a cold and dry Antarctic climate due to the effects of circular cyclones and high pressure from the Antarctic continent (Zhu et al., 2007). It is one of the most important emperor penguin colonies in coastal Antarctica. There are about 10000 emperor penguins breeding in this area every year (Zhu et al., 2009). Emperor penguin colony soils (named EPS) were also collected. All the sampling sites are shown in Figure 1, and a more detailed description of study sites can be found in our previous publications (Sun et al., 2004a, 2004b; Zhu et al., 2009).
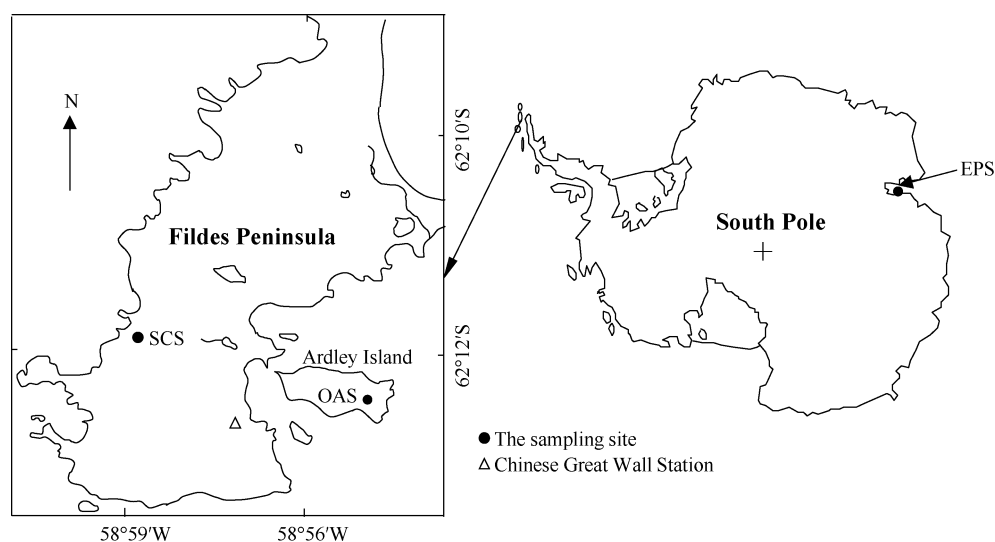
Figure 1   The sampling sites for tundra soil profiles in sea animal colonies of coastal Antarctica: (1) OAS: the ornithogenic tundra soil in penguin colony on Ardley Island, West Antarctica; (2) SCS: the soil in seal colony on Fildes Peninsula, West Antarctica; (3) EPS: the soils in emperor penguin colony at Prydz Bay, East Antarctica.

2.2   Collection of soil samples

In the summer of 2005/2006 during the 22nd Chinese National Antarctic Research Expedition (CHINARE-22), two soil cores (6 cm inner diameter, about 30 cm long) were sampled from the penguin and seal colony tundra, respectively, by hammering 30 cm PVC tubes into the soils and carefully excavating the tubes (Zhu et al., 2009). The soils added with fresh penguin guano were collected from the emperor penguin colony by sectioning the accumulative guano-soil layers *in situ* with a bamboo scoop. All the samples were completely sealed, transported to the laboratory in China, and stored at −10°C before the experiment.

2.3   Experiment design and laboratory incubation

In the laboratory, all the samples were homogeneously mixed and then divided into four equal parts. About 50 g (fresh weight) of tundra soils were put into four 250 mL glass vessels for the freezing–thawing experiments. In the austral summer, ice and snow melt water is likely to alter tundra Mc during soil FTCs. To investigate the effects of Mc on the potential production rates of soil GHGs during the FTCs, 5, 10, 15 and 20 mL pure water was added into four vessels for the simulation experiment. The different amounts of water added to the simulated soil samples approximately represented the Mc gradient of in the field. Each soil sample was submitted to three FTCs under the addition of 5, 10, 15 and 20 mL pure water, respectively. A single sample was used for the experiment simulation in each treatment. All the soil samples were incubated in ambient air for three FTC experiments (Zhu et al., 2009). In every FTC process, soil sample was frozen for 48 h at −10°C, and then thawed for 72 h at 4°C. The whole incubation period lasted 16 d. Headspace gases were

collected every 12 h and stored in 18 mL evacuated vials before the analysis (Wang et al., 2004). After each gas sampling, the headspace gas was renewed to avoid excessive accumulation of N_2O, CH_4 and CO_2 in the headspace by re-evacuating and re-flushing the glass vessel with highly pure air (Wang et al., 2004; Zhu et al., 2009).

2.4   Determination of GHG concentrations and calculation of potential production rates

The CH_4 concentrations in all air samples were determined by a gas chromatograph GC-12A (Shimadzu Co., Japan) using a flame ionization detector (FID) (Zhu et al., 2009). The variance coefficient for standard samples was within 0.1%–0.6% in 24 h. The N_2O concentrations were measured with a gas chromatograph GC-HP5890 II (Agilent Co., USA) equipped with electron capture detector (ECD) (Zhu et al., 2005). Compressed air was used as standard gas with the concentration of 303 ppbv. The variance coefficient for standard samples was within 0.2%–0.5% in 10 h. The CO_2 concentrations were measured by gas chromatograph GC-12A (Shimadzu Co., Japan) equipped with thermal conductivity detector (TCD). The potential production rates were estimated from the changing rate of GHG concentrations in the headspace of the glass vessels over time, and the cumulative rates were calculated by integrating the production rates over the incubation period of three FTCs (Zhu et al., 2009). According to our measurements, the GHG concentrations in the initial and final gas samples from the headspace of the glass vessels showed a significant variation within 12 h. Therefore we used the mean change rates of GHG concentrations in the two gas samples to estimate their potential production rates within 12 h (Wang et al., 2004; Zhu et al., 2009). The positive values indicate net GHG production, and negative values indicate net GHG uptake from the atmosphere.

2.5   Analyses of soil samples

The soil samples were mixed homogeneously for the general analyses after the incubation experiments had finished. Soil gravimetric Mc was determined by drying the soil at 105°C for 12 h. The Mc was calculated as the weight of lost water/fresh soil weight ×100%. Total organic carbon (TOC) content was analyzed from the dry samples by potassium dichromate volumetric method with an analytical error of 2.5% (Sun et al., 2004a). Total nitrogen (TN) content was determined using a Vario MACRO CHNS analyzer (Elementar Co., Germany). Soil NH_4^+-N and NO_3^--N contents were determined by ion selective electrode with an analytical error of <5.0% (Zhu et al., 2008b, 2009).

2.6   Statistical analysis

Statistical analyses were conducted with OriginPro 8 (OriginLab Co., USA), SPSS Statistics 20 (IBM Co., USA) and Microsoft Excel 2007 (Microsoft Co., USA). In all analyses where $P<0.05$, the factor tested and the relationship were considered statistically significant. The differences in the GHG production rates between the different water treatments were tested using one-way repeated analysis of variance (ANOVA) with multiple comparisons using the least significant difference test at $P<0.05$, and different letters (a, b or c) were used to indicate statistically significant differences.

3   Results

3.1   Soil physiochemical properties

As summarized in Table 1, the contents in all samples ranged from 0.3% to 5.1% for TOC, and from 0.2% to 2.0% for TN. The TOC and TN contents in EPS and OAS were clearly higher than those in SCS. The NH_4^+-N concentrations in OAS were one order of magnitude higher than those in EPS and SCS. The NO_3^--N concentrations in all the samples showed a large fluctuation with the range from 1.3 to 65 $\mu g \cdot g^{-1}$, and the levels for OAS were generally higher than those for EPS and SCS. The Mc for all three sites gradually increased under the increasing amounts of addition water. However, the Mc was much higher in EPS and OAS than in SCS due to the differences in the *in situ* Mc.

Table 1   Physiochemical properties for all the soil samples under different incubation treatments

Type	Mc/%	TOC/%	TN/%	C/N	NH_4^+-N/($\mu g \cdot g^{-1}$)	NO_3^--N/($\mu g \cdot g^{-1}$)
Ornithogenic soils						
OAS-5	45	3.5	1.8	1.9	4852	65.0
OAS -10	50	2.7	1.5	1.8	4260	13.5
OAS -15	54	3.5	2.0	1.7	4984	26.0
OAS -20	57	2.6	1.6	1.6	6205	8.1
Seal colony soils						
SCS-5	25	1.0	0.4	2.9	380	1.3
SCS -10	31	0.6	0.3	2.0	642	2.8
SCS -15	37	0.3	0.3	1.1	456	1.5
SCS -20	41	0.5	0.2	2.2	431	8.2
Emperor penguin colony soils						
EPS-5	40	3.4	1.5	2.3	710	15.3
EPS -10	45	5.1	1.6	3.2	793	5.8
EPS -15	49	4.0	1.3	3.2	717	3.4
EPS -20	53	4.6	1.3	3.6	490	2.6

Notes: The soil samples were mixed homogeneously for the general analyses after the incubation experiments had finished, and freezing-thawing cycles could alter soil physiochemical properties during the incubation period, which might lead to the big differences in the levels of TOC, TN, NH_4^+-N and NO_3^--N for the same soil with different amount of water addition. Each sample was labelled according to the water addition amount (5, 10, 15 or 20 mL).

3.2   CH₄ production rates in tundra soils

The CH₄ production rates from OAS, SCS and EPS showed different patterns of variation during three FTCs under different water treatments (Figures 2–4). The CH₄ production rates from OAS, SCS and EPS ranged from −0.1 to 0.9 μg CH₄-C·kg^{-1}·h^{-1}, from −0.03 to 0.6 μg CH₄-C·kg^{-1}·h^{-1} and from −0.1 to 127.4 μg CH₄-C·kg^{-1}·h^{-1}, respectively. The CH₄ production rates from OAS and SCS were extremely low, even negative during the FTCs under different water treatments, and they did not show any regular variation pattern. Enhanced CH₄ production did not occur following the soil thawing. Higher CH₄ production rates from OAS occurred under the 20 mL water treatment during the second and third FTCs with the maximum of 0.9 μg CH₄-C·kg^{-1}·h^{-1}. Higher production rates from SCS occurred under the 5 mL water treatment during the third FTCs with the maximum of 0.6 μg CH₄-C·kg^{-1}·h^{-1}.
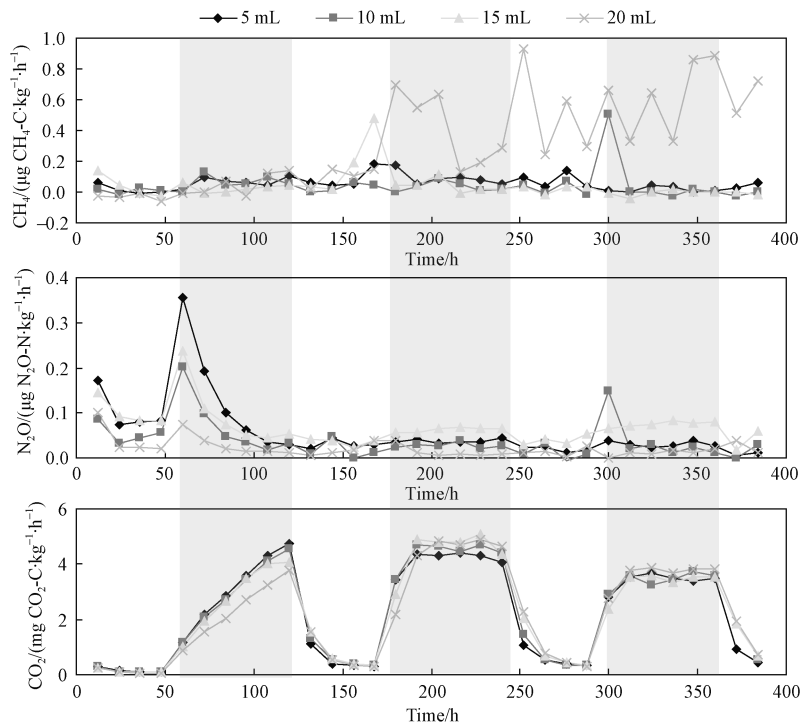
Figure 2   The CH$_4$ and N$_2$O production rates and soil respiration rates in ornithogenic tundra soil OAS during three freezing-thawing cycles (FTCs) under different water treatments. The shaded areas indicate the stage of soil thawing, whereas non-shaded areas indicate the stage of soil freezing.
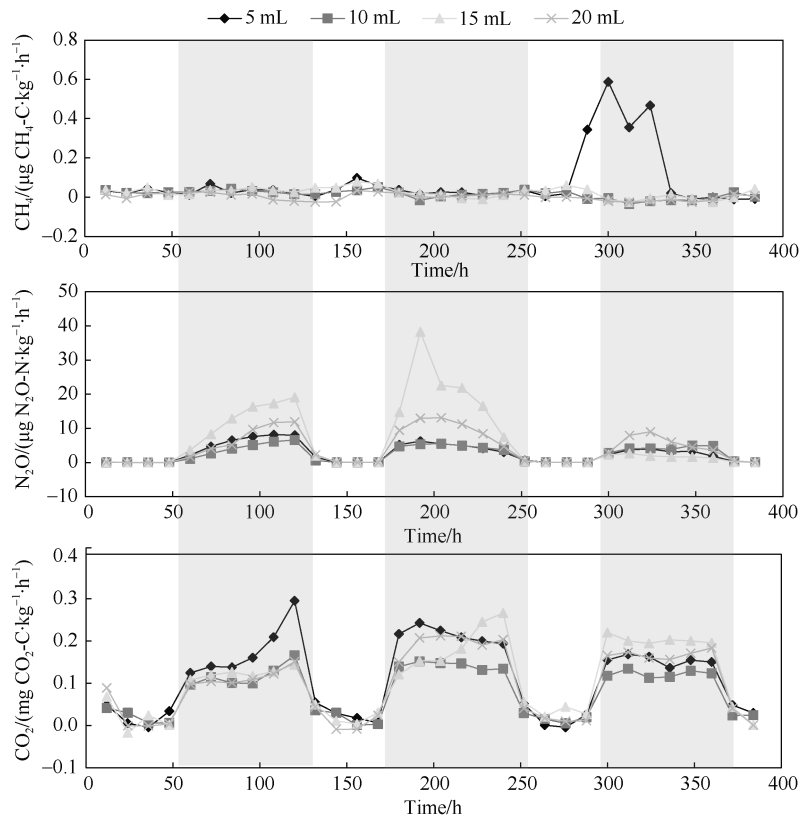

Figure 3   The CH$_4$ and N$_2$O production rates and soil respiration rates in seal colony soil SCS during three freezing-thawing cycles (FTCs) under different water treatments. The shaded areas indicate the stage of soil thawing, whereas non-shaded areas indicate the stage of soil freezing.

Figure 4 The CH_4 and N_2O production rates and soil respiration rates in emperor penguin colony soil EPS during three freezing-thawing cycles (FTCs) under different water treatments. The shaded areas indicate the stage of soil thawing, whereas non-shaded areas indicate the stage of soil freezing.
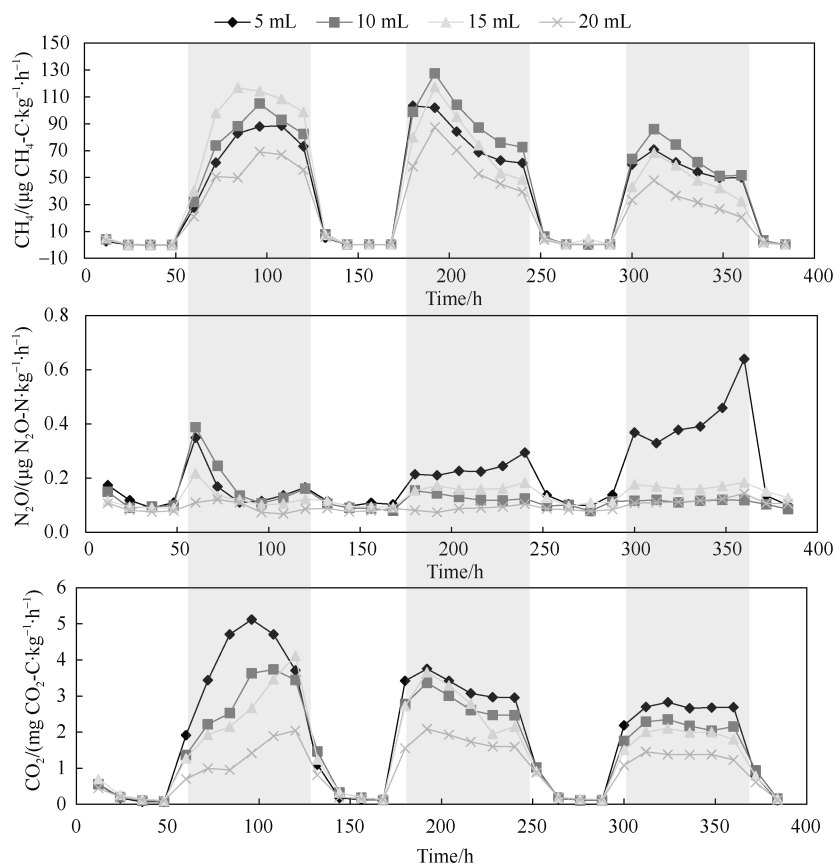
Overall, EPS emitted considerably more CH_4 than OAS and SCS (Table 2). The CH_4 production rates from EPS showed a consistent pattern of change during three FTCs under the four different water treatments (Figure 4). The production rates from EPS were close to zero during soil freezing, but rapidly increased following thawing during each FTC. They reached the highest rates during the second FTC, and decreased during the third FTC. The production rates from EPS were lowest under the 20 mL water treatment. During the three FTCs, the highest cumulative CH_4 production (59.5 mg CH_4-C·kg^{-1}) occurred in EPS under the four different water treatments (Figure 5).

During the three FTCs, no significant effects of soil water on CH_4 production rates were found in the tundra soils. Combined with all the data from each treatment, the mean CH_4 production rates showed a significant exponential correlation (r_{CH_4-TOC}=0.793, $P<0.05$) with soil TOC (Figure 6), suggesting that TOC had an important effect on the CH_4 production rates. Compared with OAS and SCS, higher CH_4 production rates from EPS might be related to the higher TOC content (Table 1).

3.3 N_2O production rates in tundra soils

The N_2O production rates from OAS and EPS fluctuated within a small range, whereas SCS produced substantially more N_2O during the three FTCs, one to two orders of magnitude higher than OAS and EPS (Figures 2–4). The N_2O production rates of OAS, SCS and EPS ranged from 0 to 0.4 μg N_2O-N·kg^{-1}·h^{-1}, from 0 to 38 μg N_2O-N·kg^{-1}·h^{-1} and 0.1 to 0.6 μg N_2O-N·kg^{-1}·h^{-1}, respectively. Under different water addition levels, the N_2O production rates from the soils were extremely low during freezing, but rapidly increased following thawing during each FTC.

Almost all the samples produced more N_2O during the first or second FTCs, and then declined during the third cycle. The highest N_2O production from OAS occurred under 5 mL water addition, and the maximum decreased following the water addition from 5 to 20 mL during the first FTC, which was not observed during the second and third FTCs (Figure 2). In SCS, the production rates under 15 mL water addition showed the highest pulses during the first and the second FTCs, and then the pulses decreased in magnitude during the third FTC. However, N_2O production from SCS showed small pulses under the 5, 10 and 20 mL water addition during the three FTCs (Figure 3). Similar production patterns were also observed from the soils EPS. However, the highest N_2O production from EPS occurred under 5 mL water addition during the third FTC (Figure 4).

Table 2 The mean N_2O and CH_4 production rates and respiration rates in tundra soils under different water treatments during three FTCs ($n=32$)

Soil types	Water treatment	N_2O/(μg N_2O-N·kg^{-1}·h^{-1})			CH_4/(μg CH_4-C·kg^{-1}·h^{-1})			Respiration rates/(mg CO_2-C·kg^{-1}·h^{-1})		
		Freezing	Thawing	FTC	Freezing	Thawing	FTC	Freezing	Thawing	FTC
OAS	5 mL	0.04±0.04ab	0.07±0.08a	0.06±0.07ab	0.06±0.05b	0.06±0.04b	0.06±0.05b	0.46±0.34a	3.56±0.89a	2.20±1.71a
	10 mL	0.03±0.03b	0.05±0.05ab	0.04±0.04bc	0.02±0.03b	0.06±0.12b	0.04±0.09b	0.49±0.43a	3.60±0.96a	2.30±1.74a
	15 mL	0.06±0.03a	0.08±0.04a	0.07±0.04a	0.07±0.13b	0.02±0.03b	0.04±0.09b	0.67±0.65a	3.54±1.09a	2.29±1.71a
	20 mL	0.03±0.02b	0.02±0.02b	0.02±0.02c	0.26±0.32a	0.36±0.31a	0.32±0.31a	0.69±0.71a	3.41±1.17a	2.25±1.69a
SCS	5 mL	0.20±0.41a	4.69±1.99b	2.72±2.71b	0.05±0.09a	0.10±0.18a	0.08±0.15a	0.02±0.02a	0.18±0.04a	0.11±0.09a
	10 mL	0.11±0.18a	4.38±1.30b	2.51±2.36b	0.02±0.01ab	0.01±0.02b	0.02±0.02b	0.02±0.01a	0.13±0.02b	0.08±0.06a
	15 mL	0.23±0.52a	11.68±10.12a	6.67±9.46a	0.04±0.02ab	0.01±0.02b	0.02±0.03b	0.03±0.02a	0.17±0.05a	0.11±0.08a
	20 mL	0.26±0.60a	7.65±3.69b	4.42±4.64a	0.01±0.02b	−0.001±0.02b	0.002±0.02b	0.02±0.03a	0.16±0.04a	0.10±0.08a
EPS	5 mL	0.12±0.02a	0.28±0.14a	0.21±0.13a	1.10±1.89a	69.35±19.45a	39.49±37.31a	0.34±0.37a	3.27±0.87a	1.99±1.63a
	10 mL	0.10±0.02bc	0.15±0.07bc	0.13±0.06bc	1.59±2.61a	79.37±22.75a	45.35±42.70a	0.40±0.44a	2.58±0.65b	1.62±1.23a
	15 mL	0.11±0.02ab	0.16±0.03b	0.14±0.03b	1.80±2.49a	74.31±29.73a	42.59±42.69a	0.39±0.37a	2.42±0.78b	1.53±1.20ab
	20 mL	0.09±0.01c	0.10±0.02c	0.09±0.02c	1.10±1.81a	47.96±18.32b	27.46±27.26a	0.30±0.27a	1.47±0.38c	0.96±0.67b

Notes: Different letters indicate significant differences between the two treatments; the same letters indicate no significant differences between the two treatments.
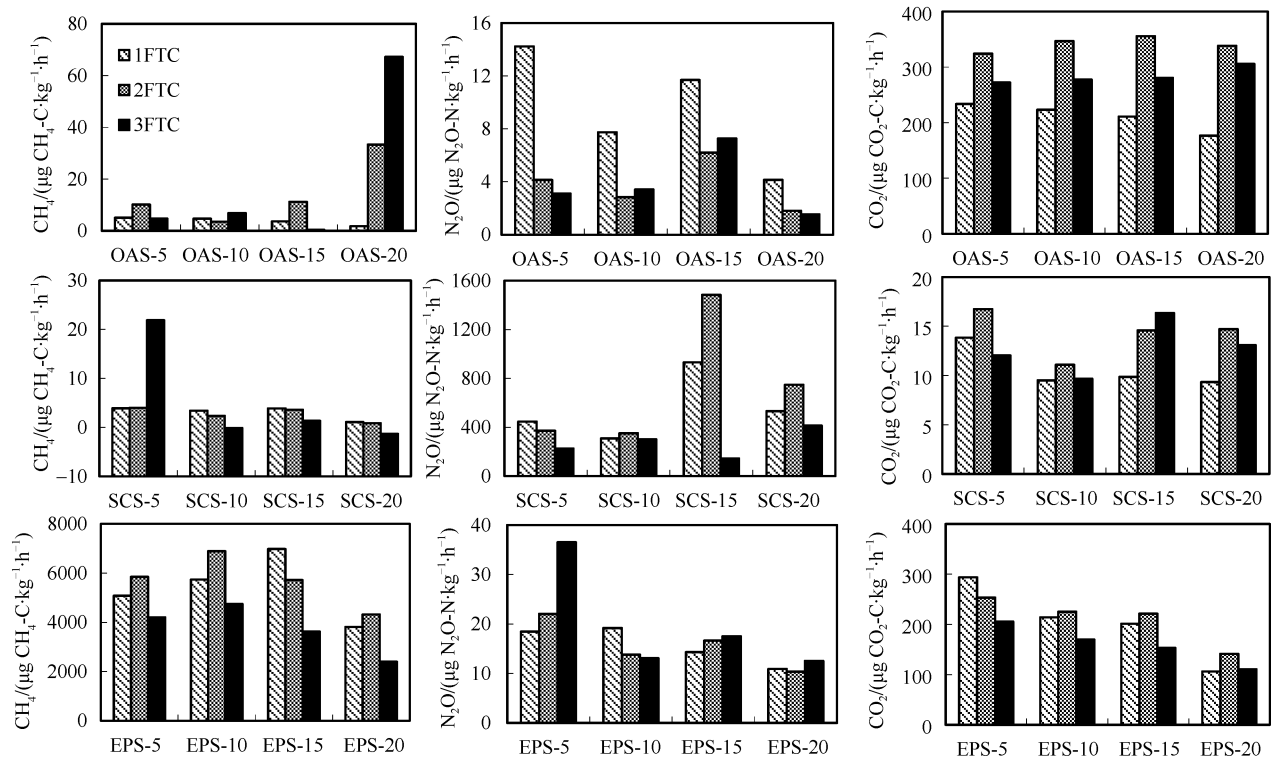

Figure 5 Cumulative production of CH_4, N_2O and CO_2 from the tundra soil OAS, SCS and EPS during the three FTCs under different water treatments. The stripe, diagonal and solid column indicate the first, second and the third FTC, respectively.

The highest cumulative N_2O production (6.3 mg N_2O-N·kg^{-1}) occurred in SCS under the four different water addition levels during the three FTCs (Figure 5). The water addition reduced N_2O production in OAS and EPS, but stimulated N_2O production in SCS (Table 2). Overall the mean N_2O production rates for all the soil samples showed an exponential decrease ($r_{N_2O-Mc}=0.860$, $P<0.05$) with

theaddition of soil water (Figure 6).

3.4 Soil respiration

The soil respiration rates showed similar variation patterns during the FTCs under the addition of 5–20 mL of water. In all cases, FTC induced enhanced soil respiration during the first FTC, and the same patterns were observed during the
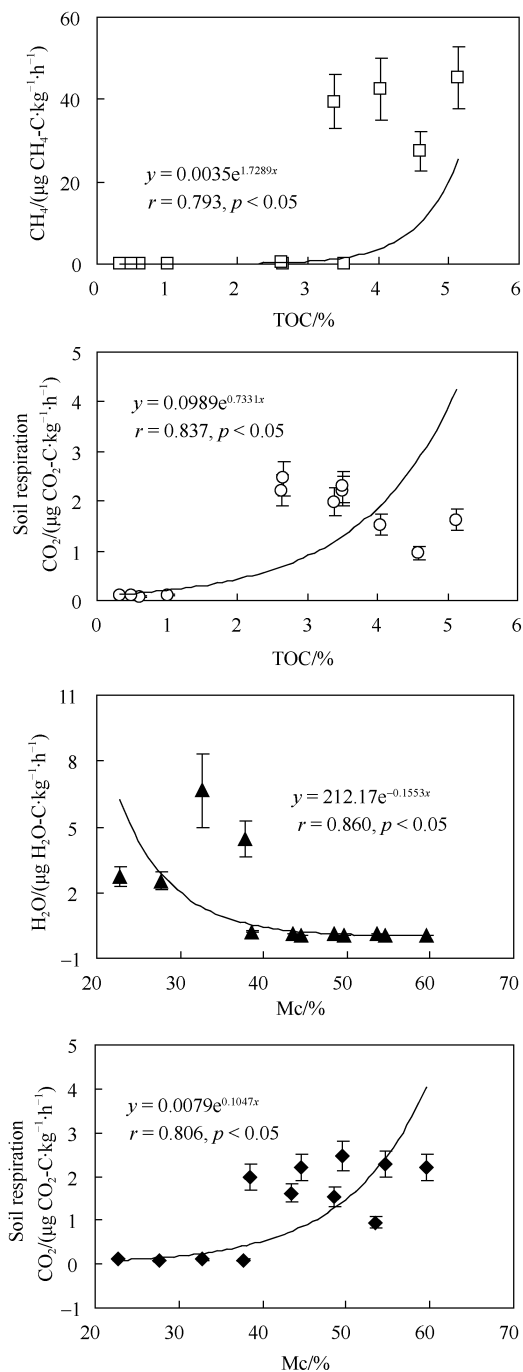
$y = 0.0035e^{1.7289x}$
$r = 0.793, p < 0.05$

$y = 0.0989e^{0.7331x}$
$r = 0.837, p < 0.05$

$y = 212.17e^{-0.1553x}$
$r = 0.860, p < 0.05$

$y = 0.0079e^{0.1047x}$
$r = 0.806, p < 0.05$

Figure 6   Correlation between mean CH_4 production, soil respiration rates and TOC, soil water content M_c in the tundra soils OAS, SCS and EPS.

second and third FTCs, indicating that the response of soil respiration was very sensitive to the change from soil freezing to thawing during the FTCs (Figures 2–4). The soil respiration rates from OAS, SCS and EPS ranged from 0.1 to 5.1 mg CO_2-C·kg^{-1}·h^{-1}, 0 to 0.3 mg CO_2-C·kg^{-1}·h^{-1} and 0.1 to 5.1 mg CO_2-C·kg^{-1}·h^{-1}, respectively. The respiration rates from OAS and EPS were substantial under different water contents, one order of magnitude higher than those from SCS (Table 2). The highest cumulative CO_2

production (3.5 g CO_2-C·kg^{-1}) occurred in the OAS during the three FTCs (Figure 5).

The Mc showed significant effects on respiration rates in the soil EPS. The respiration rates from EPS clearly decreased with the increase in Mc (Figure 4), which did not occur in OAS and SCS (Table 2). Overall the mean respiration rates of all treatments showed an exponential correlation with soil water (r_{CO_2-Mc} =0.806, $P<0.05$), and TOC contents (r_{CO_2-TOC} =0.837, $P<0.05$) (Figure 6).

3.5   Correlations between GHG production rates

The correlations between GHG production rates from the soils are shown in Figures 7–9. When CH_4 production rates were very low (<1 μg CH_4-C·kg^{-1}· h^{-1}) in OAS and SCS, they showed no obvious correlations with soil respiration rates during the freezing or thawing period (Figures 7a and 7b). In contrast, there was a significant positive linear correlation between CH_4 production and respiration rates in the soils EPS with high CH_4 production for all treatments (Figure 7c). The N_2O production rates were below 1 μg N_2O-N·kg^{-1}·h^{-1} in OAS and EPS, and they showed negative correlations with soil respiration rates during FTCs (Figures 8a and 8c). However, N_2O production rates were higher than 1 μg N_2O-N·kg^{-1}·h^{-1} in SCS, and they showed positive correlations with soil respiration rates (Figure 8b). In addition, CH_4 and N_2O production rates showed a trade-off relationship for EPS (Figure 9). When CH_4 production rates were very high, N_2O production was inhibited and was thus extremely low in the soil EPS. In contrast, when N_2O production rates were relatively high, the CH_4 production rates were inhibited in SCS.

4   Discussion

The CH_4 production rates from OAS and SCS were extremely low under different water treatments, and enhanced CH_4 production did not occur following soil thawing. Priemé and Christensen (2001) did not observe any effect of FTCs on CH_4 emission rates from farmed organic soils during a laboratory incubation, which was very similar to the findings of our study. However, the FTC induced a pulse in CH_4 production from EPS under different water addition levels (Figure 4). Recently, increased CH_4 and N_2O emission rates have been observed *in situ* from penguin or seal colonies in coastal Antarctica during FTCs (Zhu et al., 2008a, 2008b). Significantly increased CH_4 production rates were also observed in high-Arctic wet tundra during FTCs (Tagesson et al., 2012). The variability in CH_4 production rates from EPS in our study was consistent with those reported in the references above. Active methanogenic bacteria and methane oxidizing bacteria that adapted to the extreme conditions could live under the temperature of −3°C to −6°C (Dirk et al., 2007). Our results showed that methanogenic bacteria activity in EPS might greatly increase following soil thawing.
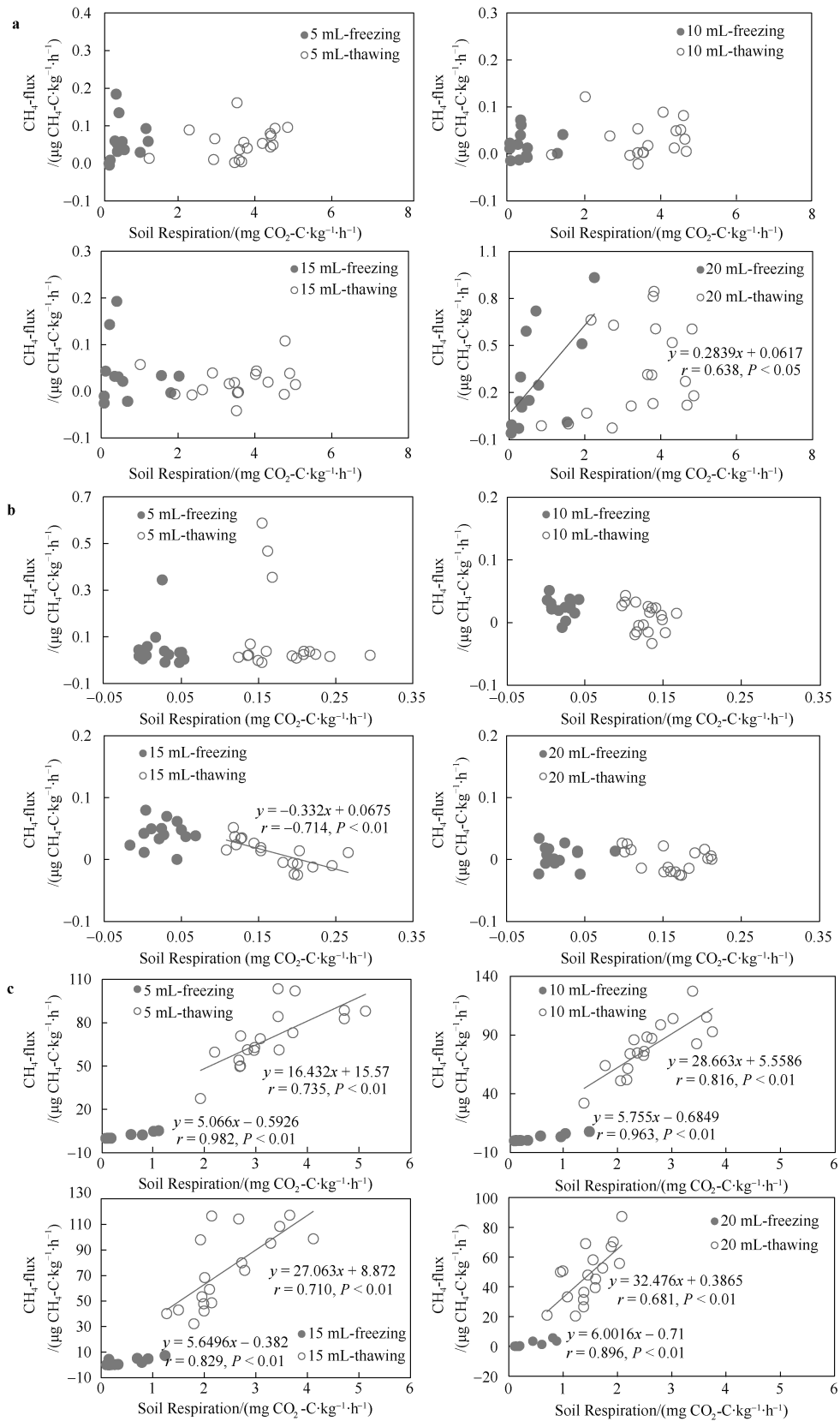
Figure 7 Relationships between CH_4 production and soil respiration rates from the tundra soils during freezing-thawing cycles (FTCs) under different water treatments. **a**, ornithogenic soils (OAS); **b**, seal colony soils (SCS); **c**, emperor penguin colony soil (EPS). If the regression is significant at $P<0.05$, regression lines and r values are shown in the figure.

Figure 8 Relationships between N_2O production and soil respiration rates from the tundra soils during freezing-thawing cycles (FTCs) under different water treatments. **a**, ornithogenic soils (OAS); **b**, seal colony soils (SCS); **c**, emperor penguin colony soil (EPS). If the regression is significant at $P<0.05$, regression lines and r values are shown in the figure.
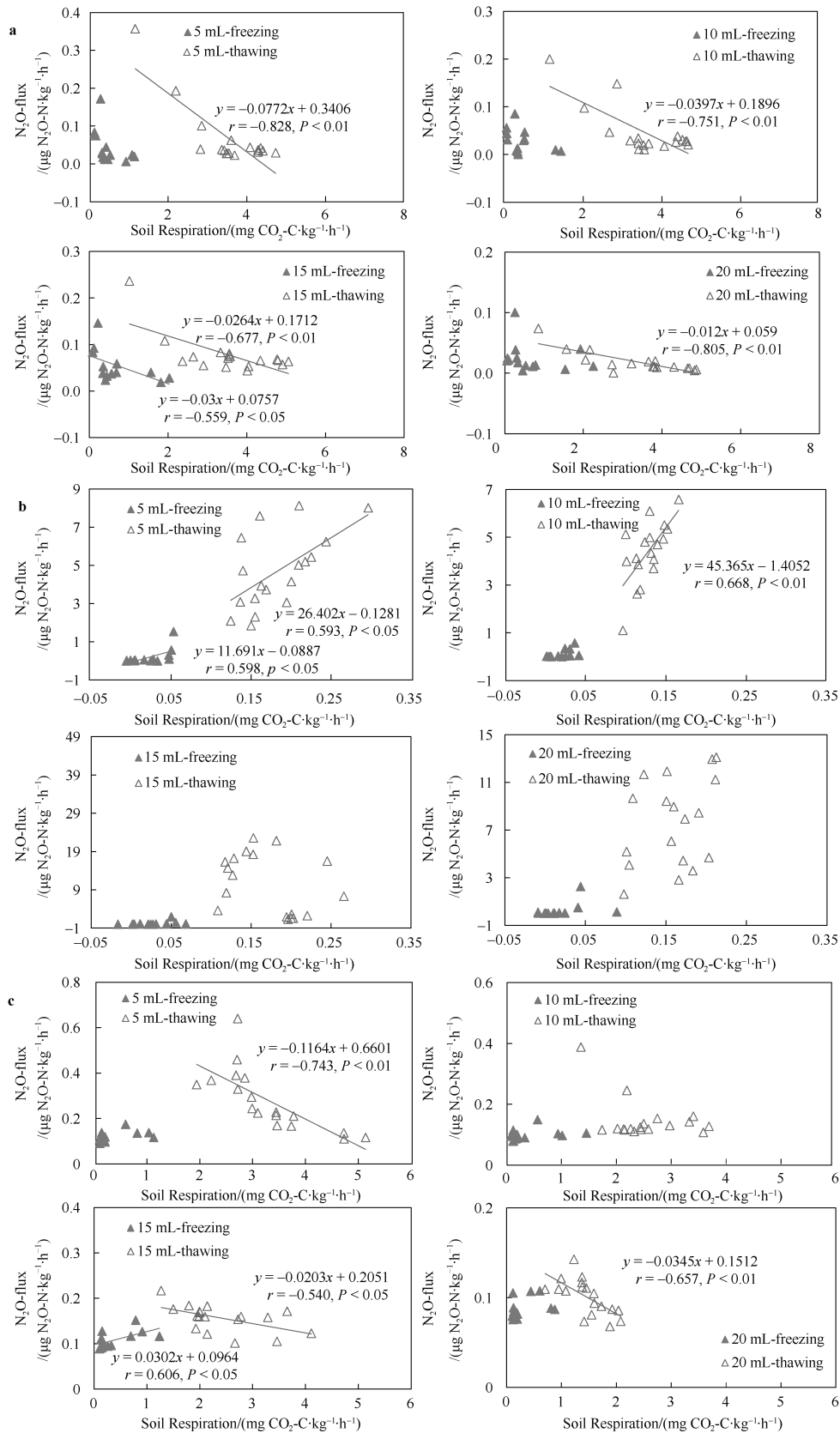
Figure 9 Relationships between CH_4 and N_2O production rates during freezing-thawing cycles (FTCs) under different water treatments. **a**, ornithogenic soils (OAS); **b**, seal colony soils (SCS); **c**, emperor penguin colony soil (EPS). If the regression is significant at $P<0.05$, regression lines and r values are shown in the figure.
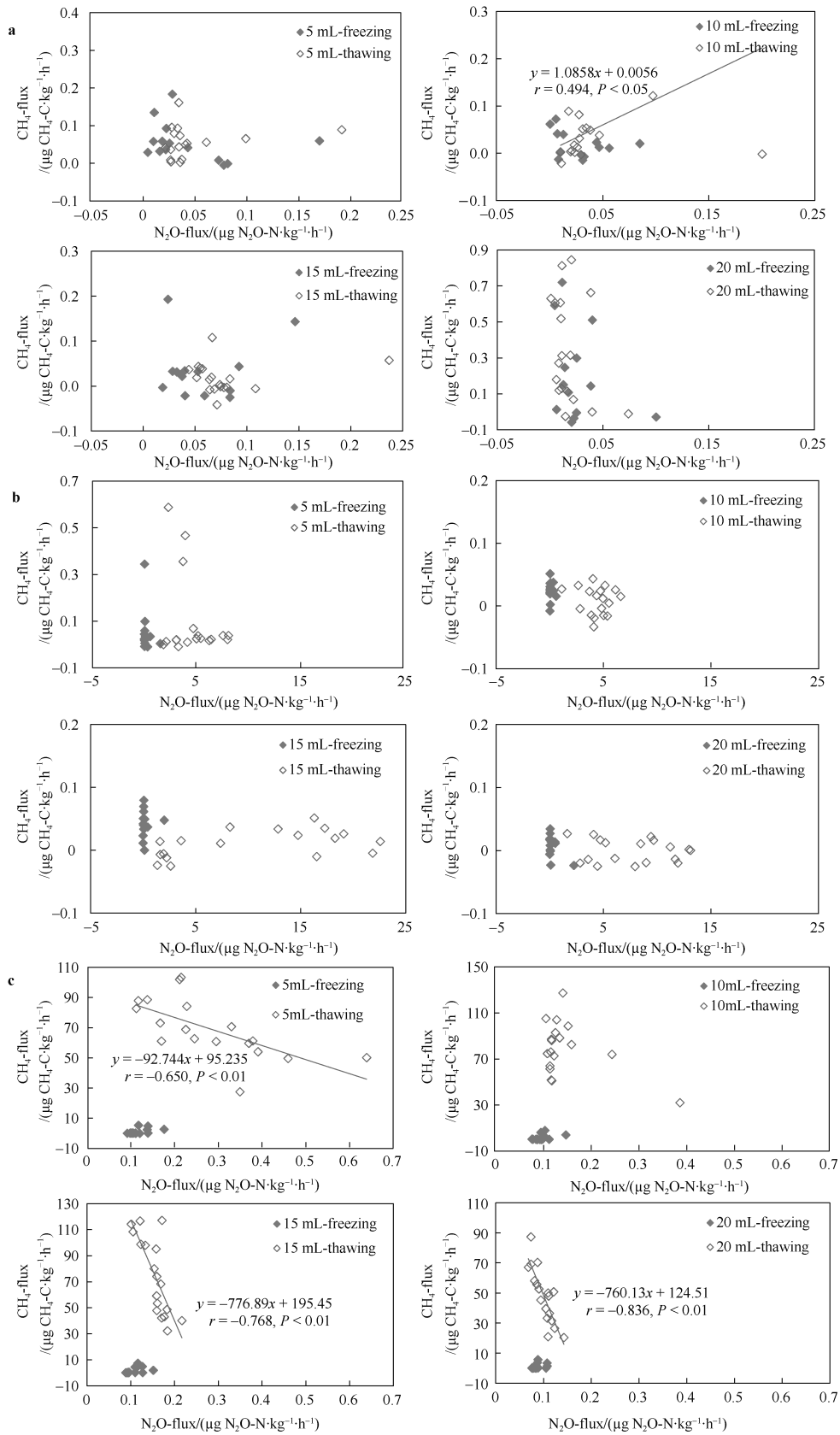
In general, CH_4 production occurred in the anaerobic environment via the effects of methanogens, which are extremely sensitive to oxygen concentration and oxygen diffusion into the soils can inhibit methanogen activity (Jones et al., 2005). The Mc could influence the O_2 diffusion and supply in the soil (Teepe et al., 2004), thus affecting soil CH_4 dynamics. In addition, EPS emitted substantially more CH_4 than OAS and SCS. Furthermore, the production rates from EPS rapidly increased following thawing during each FTC (Table 2 and Figure 4), which was highly similar to the finding for soil respiration rates. This may be caused by the release of more microbial available carbon over the incubation period. Samples of EPS had a relatively higher Mc and TOC content than other tundra soils (Table 1). The relationship between soil CH_4 production rates and TOC confirmed this (Figure 6), and the strong correlation between soil CH_4 production and respiration rates further supported this hypothesis (Figure 7c).

In this study, N_2O production rates from OAS, SCS and EPS was extremely low during freezing in all cases, but rapidly increased following thawing during each FTC. Many studies have indicated that the freezing and thawing soils were important sources for N_2O (Zhu et al., 2008b; Kim et al., 2012; Wu et al., 2014). The positive relationship between N_2O fluxes and temperature have been found during soil thawing (Ludwig et al., 2006; Wagnerriddle et al., 2010; Dietzel et al., 2011; Kim et al., 2012). Strong bacterial activity and the supply of more nutrients in thawing soils might lead to high N_2O emissions during FTCs. Therefore, our results indicate that thawing-induced soil production was a potential important N_2O source in coastal Antarctica. However, the OAS and EPS produced much lower N_2O than SCS during the three FTCs, though the concentrations of TN and NH_4^+-N in the soil samples were considerable (Table 1), suggesting that TN and NH_4^+-N contents might not be the dominant factors limiting the N_2O production in sea animal colony soils. However, OAS and EPS showed a higher soil Mc than SCS and thus OAS and EPS might have poor permeability and furthermore facilitate anaerobic processes, and N_2O produced in the soil might be reduced to N_2 in the highly anaerobic environment. Therefore, excessive water could reduce N_2O production in the incubated soil. Teepe et al. (2004) found that the N_2O emissions from the silt during thawing significantly decreased under high Mc of 76% WFPS. Our previous laboratory experiments also showed higher N_2O emissions from seal colony soils and the production rates were much higher under N_2 incubation than under ambient air incubation (Zhu et al., 2008b), implying that denitrification might be the major pathway for N_2O production in SCS. Although TN and inorganic N contents in SCS were the lowest among the three soils in the current study, effective denitrification still produced a large amount of N_2O. Furthermore, Mc was the lowest in SCS, which might be favorable for the diffusion of N_2O

into the air. In addition, the N_2O production in SCS increased with the water addition from 5 to 15 mL, but decreased with water addition of 20 mL, which also indicates that a higher water content in SCS might decrease the N_2O production and diffusion.

Soil N_2O was predominantly produced through nitrification and denitrification via effects of the bacteria. Water content was a key factor controlling soil denitrification, and high water content could lead to the decrease in the oxygen diffusion rate, which is helpful for denitrification processes (Koponen and Martikainen, 2004). Thawing might further increase Mc, and excess water might lead to the formation of an anaerobic environment in penguin colony soils (EPS and OAS), which might limit the activities of nitrifying microorganisms and promote denitrification in these soils. The mean soil respiration rates and water contents were higher in penguin colony soils (OAS and EPS) than in the seal colony soil (SCS) (Table 2), further indicating strong microbial activity and highly anaerobic conditions. Strong active microbial activity might lead to high mineralization rates, considerable O_2 consumption and high denitrification (Zhu et al., 2008b). The N_2O produced through denitrification might be reduced to N_2 in a highly anaerobic environment, and a high ratio of N_2/N_2O further limited the N_2O production (Zhu et al., 2008b). Therefore, net N_2O production rates gradually decreased with the increase in soil respiration rates and Mc (Figures 8a, 8c).

In this study, the mean respiration rates for penguin or seal colony soils (0.08–2.5 mg CO_2-C·kg^{-1}·h^{-1}) were much higher than those for cold desert soil in Antarctica (0.001–0.065 mg CO_2-C·kg^{-1}·h^{-1}) (Burkins et al., 2000; Treonis et al., 2002). High soil respiration might be due to strong microbial respiration and increasing water content during the thawing processes. Studies in cold regions also indicated that soil respiration is sensitive to temperature increase (Peng et al., 2009; Ma et al., 2016). The number of culturable microorganisms was significantly positively correlated with Mc, TOC and TN content in frozen soils (Wang et al., 2011). The FTCs could destroy microbial cells, and more bio-available nutrients released from dead bacteria cells and thawing soils could be used for the surviving bacteria (Larsen et al., 2002; Teepe et al., 2004). As shown in Figure 6, TOC content had an important effect on CH_4 production and soil respiration when higher than 3%. Similarly, Mc also affected soil respiration rates when it was higher than 40%. In contrast, Mc could affect N_2O fluxes when it was lower than 40%. In summary, soil TOC and Mc within a threshold range might be important factors affecting GHG production in the tundra.

The positive relationship between CH_4 fluxes and respiration rates in thawing soil implied that methanogenic bacteria activity might be associated with soil respiration (Figure 7c). Similar results were also observed in mire soil (Song et al., 2004). The relationship between N_2O and CO_2 production indicated that nitrifiers and denitrifiers

preferentially had the capability to utilize extra substrates released during the soil thawing, and N_2O-reducing bacteria or other heterotrophic bacteria might be more active with increased soil respiration (Ludwig et al., 2006). Therefore, N_2O production rates in OAS and EPS showed negative correlations with soil respiration when high respiration rates occurred, and the opposite was shown for SCS (Figure 8). However, it should be noted that OAS and EPS showed greatly higher CH_4 production and soil respiration but lower N_2O production rates than SCS. In contrast, seal colony soils showed higher N_2O production but lower CH_4 production and low soil respiration, suggesting their production might be regulated by microbial activity during soil FTCs (Figure 9). In penguin colony soils, methanogenic bacteria may show a higher activity while the activity for nitrifying or denitrifying bacteria may be inhibited due to the competition for the substrate, and the opposite may occur in seal colony soils. Similar trade-off relationships between CH_4 and N_2O emissions have been reported from paddy fields (Zou et al., 2003; Jiang et al., 2005). Therefore, it is important for the evaluation of greenhouse gas budget to assess the trade-off relationships between N_2O and CH_4 fluxes from sea animal colonies in coastal Antarctica.

5   Conclusions

Effects of FTCs and water addition on potential N_2O and CH_4 production and respiration rates were elucidated in sea animal colony soils. The CH_4 production rates from an ornithogenic tundra soil (OAS) and a seal colony soil (SCS) were minimal during the FTCs under the 5–20 mL water addition. Overall, the emperor penguin colony soil (EPS) emitted considerably more CH_4 than OAS and SCS, and its CH_4 production rates rapidly increased following thawing during each FTC, whereas SCS produced much higher N_2O (one to two orders of magnitude higher than OAS and EPS). In all cases, FTC induced significantly enhanced soil respiration, indicating that soil respiration response to the FTCs was highly sensitive. Soil water addition had no significant effects on CH_4 production and respiration rates, but it could inhibit N_2O production in OAS and EPS, and it stimulated N_2O production in SCS. During three FTCs, soil CH_4 and N_2O production rates showed a trade-off relationship. Our results indicated that soil FTCs greatly stimulated the production of N_2O and CO_2, and furthermore water addition had an important effect on tundra soil N_2O production. In coastal Antarctica, the number of sea animals and their colonies is potentially large. The frequency of FTCs is considerable following soil water change in the austral summer because of the cold and unstable weather conditions. Therefore, FTC and soil water changes are important factors controlling the production of greenhouse gas from sea animal colonies in coastal Antarctica.

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