Potential methane production rates and its carbon isotopic composition from ornithogenic tundra soils in coastal Antarctic

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Abstract   Methane (CH_4) is one of important greenhouse gases with chemical activity. The determination of isotopic compositions for CH_4 emitted from the soils helps us to understand its production mechanisms. CH_4 isotope measurements have been conducted for different types of global terrestrial ecosystems. However, no isotopic data of CH_4 have been reported from Antarctic tundra soils. In this paper, ornithogenic soil profiles were collected from four penguin colonies, and potential CH_4 production rates and its ^{13}C ratio ($\delta^{13}C$) were investigated based upon laboratory incubation experiments. The mean CH_4 production rates are highly variable in these soil profiles, ranging from 0.7 to 20.3 µg CH_4−C $kg^{-1} \cdot h^{-1}$. These ornithogenic soils had high potential production rates of CH_4 under ambient air incubation or under N_2 incubation, indicating the importance of potential CH_4 emissions from penguin colonies. Most of the soil samples had higher $\delta^{13}C$-CH_4 under N_2 incubation (−39.28‰~−43.53‰) than under the ambient air incubation (−42.81‰~−57.19‰). Highly anaerobic conditions were conducive to the production of CH_4 enriched in ^{13}C, and acetic acid reduction under N_2 incubation might be a predominant source for soil CH_4 production. Overall the $\delta^{13}C$-CH_4 showed a significant negative correlation with CH_4 production rates in ornithogenic tundra soils under N_2 incubation (R^2=0.41, p<0.01) or under the ambient air incubation (R^2=0.50, p<0.01). Potential CH_4 production from ornithogenic soils showed a significant positive correlation with total phosphorus (TP) and NH_4^+−N contents, pH and soil moisture (Mc), but the $\delta^{13}C$-CH_4 showed a significant negative correlation with TP and NH_4^+−N contents, pH and Mc, indicating that the deposition amount of penguin guano increased potential CH_4 production rates from tundra soils, but decreased the $\delta^{13}C$-CH_4. The CH_4 emissions from the ornithogenic soils affect carbon isotopic compositions of atmospheric CH_4 in coastal Antarctica.

Keywords CH_4, Antarctica, ornithogenic soil, carbon isotope, penguin colony

1 Introduction

Methane (CH_4) is one of the important, chemically active greenhouse gases (GHGs), and its contribution to global greenhouse effect is about 15% at the one-hundred-year time scale[1-4]. The CH_4 emissions from Arctic terrestrial ecosystems, especially boreal wetlands, play a special role in the global carbon cycle due to the amplified warming of the region during the past few decades[5-6]. In maritime Antarctica, the coastal ice free areas contain some of the largest marine animal colonies on a global scale. Marine animal colonies, tundra vegetation and the interactions between them form a special terrestrial ecosystem[7]. Marine animals including penguins and other seabirds play an important role in the nutrient cycling of the ecosystems by transferring carbon and nitrogen from the marine to the terrestrial environment[7-9]. The deposition of a large amount of penguin guano strongly influences the physical and chemical properties of local soils, and produces a kind of special soil named ornithogenic

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soil[7,10-12]. Recently, field observations have indicated that penguin colonies are a significant source for atmospheric CH_4 in maritime Antarctica[13-15]. Nevertheless, CH_4 production from fertile ornithogenic tundra soils are still poorly understood in coastal Antarctica. Tundra CH_4 emission is an integrated effect of the production, oxidation and transport of CH_4 in the soils. A better knowledge of these processes affecting CH_4 emission may provide more information about the response of tundra CH_4 emission to changing climate.

The technique of stable carbon isotopes has been proved to be a useful tool in studying the complicated processes of CH_4 production and emission[16-19]. CH_4 emitted from various sources may have different isotopic characteristics because of its isotopic fractionation effects. Isotope fractionation happens in all the major processes of CH_4 emission. The ^{12}C-substrate is preferentially utilized by methanogens for CH_4 production, and once formed, $^{12}CH_4$ is consumed faster than $^{13}CH_4$ by methanotrophs, and $^{12}CH_4$ is transported faster than $^{13}CH_4$ as well[20-23]. The $\delta^{13}C$-CH_4 produced through acetic acid fermentation ranged from −60‰ to −50‰, whereas the $\delta^{13}C$-CH_4 in the reduction of CO_2/H_2 was below this range[24-25]. Generally the CH_4, emitted from the combustion of fossil fuel, had relatively heavy isotopic compositions with the $\delta^{13}C$-CH_4 range of −50‰~−30‰, whereas CH_4 from biomass combustion had especially high ^{13}C-CH_4 (−28‰~ −12‰). In addition, the relative contribution of acetate to CH_4 production (f_{ac}) and the fraction of CH_4 oxidized (f_{ox}) can be quantitatively estimated[19,26-27] using mass balance equations based on the measurements of $\delta^{13}C$ in CH_4, CO_2 and acetate, and of the isotope fractionation factors (α_{CO_2/CH_4}, $\varepsilon_{acetate/CH_4}$, α_{ox} and $\varepsilon_{transport}$). At present, studies on isotopes of soil CH_4 have been conducted in different ecosystems[25,28-31]. However, few data are available on the isotopes of CH_4 emitted from tundra soils in coastal Antarctica.

In this study, the ornithogenic tundra soil profiles were collected from four penguin colonies in coastal Antarctica during the 22nd Chinese National Antarctic Research Expedition (22nd CHINARE). Potential CH_4 production rates and its $\delta^{13}C$ were measured based upon laboratory incubation experiments. Our objectives are (1) to measure potential CH_4 production rates from ornithogenic tundra soils and the carbon isotopic compositions of CH_4 emitted from the soils; (2) to discuss the factors affecting CH_4 production, consumption and its isotopic compositions.

2 Materials and methods

2.1 Study sites and microcosm sampling

Tundra ornithogenic soils were collected from the following four penguin colonies during the 22nd CHINARE.

The first site is located on Ardley Island, West Antarctica (62°13′S, 58°56′W), with 2.0 km length and 1.5 km width. This island was defined as an area of special scientific interest by the Scientific Committee of Antarctic Research (SCAR). It has one of the most important penguin colonies in maritime

Antarctica[8]. Approximately 10200 penguin individuals colonized this island during the breeding season including Gentoo penguins (*Pygoscelis papua*), Adélie penguins (*P. adeliae*), and Chinstrap penguins (*P. antarctica*)[8,32]. Recently, CH_4 and N_2O emissions from this penguin colony have been measured *in situ* by Zhu et al.[14-15]. In the active colony, continuous deposition of fresh guano and penguin trampling inhibits vegetation establishment, and soils are covered by layers of guano[8]. The ornithogenic soils had well expressed O (organic and A (accumulation) horizons, and were covered by thick continuous moss cover. One ornithogenic soil core (named AI) with a depth of 30 cm was collected at a poorly drained area about 100 m from the active penguin colony.

The second site is located in an emperor penguin colony at Prydz Bay, East Antarctica (69°22′S, 76°24′E). This colony is about 10 km from the Chinese Antarctic Zhongshan Station. This area has a typical cold, dry polar continental climate. It is one of the most important emperor penguin colonies in coastal Antarctica with about 10000 emperor penguins breeding here every year. One ornithogenic soil profile (named PB, about 25 cm depth) was collected from the colony. Only some algae were present on the surface of the ornithogenic soils at the sampling site[14].

The third site is located on Gardner Island (68°34′S, 77°52′E) and the fourth site is located on Magnetic Island (68°32′S, 77°54′E), both in East Antarctica. These two islands are important Adélie penguins (*P. adeliae*) colonies. Two ornithogenic soil profiles (named GI and MI) were sampled from Gardner Island and Magnetic Island, respectively. Sparse algae grew on the ornithogenic tundra soils. All the areas above are covered by accumulated snow and ice during winter and the ornithogenic soils are frozen. Every summer the snow and ice melt, and soil freezing–thawing frequency increases considerably in these ice−free areas. The sampling sites are shown in Figure 1.

Ornithogenic soil profiles (about 20 cm depth) were collected from the different penguin colonies by *in situ* sectioning of the soil layer from top to bottom using a bamboo scoop during January and February 2006. Intact soil cores (6 cm inner diameter, about 30 cm depth) were obtained from penguin colonies by hammering 30 cm long PVC tubes into the soils and carefully digging the tubes out[8,33]. These samples were kept at −10ºC and transported to the laboratory in China for the incubation experiment.

2.2 Incubation experiment and measurements of potential CH_4 production rates

In the laboratory, all ornithogenic soil profiles were sectioned into three parts, and then mixed homogeneously. About 100 g (fresh weight) of soils were put into glass vessels (500 mL) for the incubation experiments. To investigate the potential flux of CH_4 in the field, we added 1 mL water to make up for water lost during the experiment in order to maintain field moisture. Additionally, to avoid the disturbance associated with thawing, which may lead to unusually high
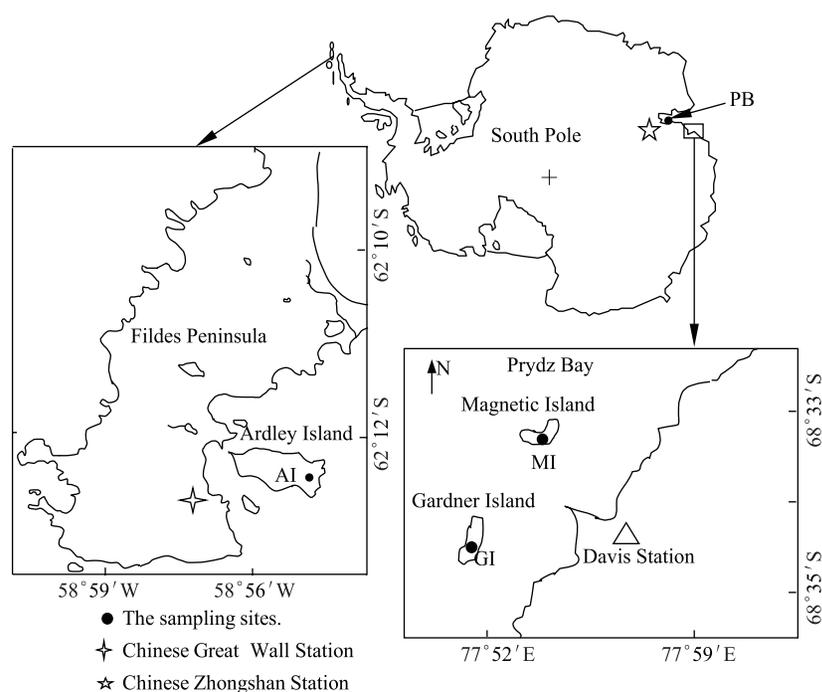
Figure 1 Sampling sites for ornithogenic tundra soil profiles in penguin colonies of coastal Antarctica. (1) AI: penguin colony on Ardley Island, West Antarctica; (2) PB: emperor penguin colony at Prydz Bay, East Antarctica; (3) GI and MI: Adélie penguin colonies on Gardner Island and Magnetic Island, respectively, East Antarctica.

trace gas fluxes, the samples were completely thawed and then incubated in the dark at 4ºC, which is very close to local mean air temperature in the austral summer. The glass vessels with the soil samples were divided into two groups: the first group was incubated under mbient air conditions, to simulate the potential CH_4 fluxes from ornithogenic soils under local natural conditions. The second group was incubated under N_2 to establish anaerobic conditions, which was used to simulate the potential CH_4 fluxes under waterlogged−soil conditions from the effects of snowmelt water on the ornithogenic tundra soils. During the incubation, the headspace gases were collected every two hours, and then stored in 18 mL evacuated vials before analysis. After each gas sampling, the headspace gas was renewed by flushing with ambient air repeatedly or re-evacuating and re-flushing the glass vessel with N_2 five times to avoid CH_4 build-up in the headspace[34]. Two repetitions were made for each soil sample[35].

The CH_4 concentrations were determined by gas chromatography (Shimadzu GC−12A, Japan) equipped with a flame ionization detector (FID) and a molecular sieve 5A column (2.6 mm i.d. × 2.0 m) under N_2 (40 mL· min^{-1}) as a carrier gas at 80ºC[36]. The standard gas for CH_4 was 8 ppmv. The variance coefficient for standard samples was within 0.1%–0.6% in 24 h. Potential CH_4 production rates were estimated from the changing rate of headspace CH_4 concentrations. The incubation time intervals were 24 h for the experimental samples. The cumulative flux was calculated by integrating the potential fluxes over the incubation period.

2.3 Measurement of δ^{13}C-CH$_4$

The carbon isotopic compositions of CH_4 were analyzed by using a continuous flow technique coupled to a Finnigan MAT−253 isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany)[37]. The CO_2 in gas samples was directly analyzed while CH_4 in gas samples was converted into CO_2 and separated primarily on a PreCon (pre−concentration device). Then, the gas was piped into a GC equipped with a Pora PLOT Q column (25 m length; 0.35 mm i.d.) at 25°C under 2.0×10^5 Pa for further separation. The separated gases were finally transferred into the mass spectrometer for δ^{13}C determination. The reference and carrier gases used were CO_2 (99.999% purity and –23.73‰ δ^{13}C$_{PDB}$-value) and He (99.999% purity, 20 mL·min^{-1}). The precision of the repeated analysis was ±0.2‰ when 2.02 μL·L^{-1} CH_4 was injected. The Isotope ratios are presented as δ values, which are defined as: $\delta_x=[(R_{sample}/R_{standard}) -1]\times1000‰$, where δ_x is the δ value of the heavy isotope x and R is the ratio of the heavy isotope (at%, atom percent) to the light isotope (at%).

2.4 General analyses of ornithogenic tundra soils

The ornithogenic soil samples were separated from soil profiles and mixed homogeneously for the general analyses. The pH was determined in distilled water and in a 1M KCl solution (soil: solution ratio 1:3). Total organic carbon (TOC) was analyzed from the dry soil by the potassium dichromate volumetric method with an analytic error of 2.5%[33]. Total

nitrogen (TN), NH_4^+-N and NO_3^--N were determined by an ion−selective electrode method with an analytical error of <5.0%[15]. Total phosphorus (TP) content was determined by ultraviolet visible spectrometry (UVS) with an analytical error of <2.0%. Sulphur (S) was analysed by the KI volume method after combustion in a SRJK−2 high-temperature furnace with an analytical error of <5.0%[33]. Soil gravimetric moisture content (M_C) was determined by drying the soil at 105 °C for 12 h. M_C was calculated as: M_C= the weight of the lost water/dry soil weight×100%. The precision and accuracy of our results were monitored using reference materials (GBW07) in every batch of analysis. The measured values of the reference materials were in good agreement with the reference values, and the differences were within ±5%.

2.5 Data analysis

Statistical analysis was made with OriginPro 7.5 and Microsoft Excel 2007 for Window XP. Statistically significant differences in potential fluxes of CH_4 and $\delta^{13}C$-CH_4 between incubation groups over each incubation period were assessed using a two-sample t-test. In all analyses where p<0.05, the factor tested and the relationship were considered statistically significant[35, 38].

3 Results and discussion

3.1 Physicochemical properties of ornithogenic tundra soils

As summarized in Table 1, there was a different soil moisture environment between these ornithogenic soils. Soil moisture in AI and PB was 38% and 76%, respectively. The ornithogenic soils were slightly acidic to neutral, with the pH values of 5.2−7.3. The TOC and TN contents in these samples were highly variable, ranging from 5.5%−14.7% for TOC, and from 0.49%−3.60% for TN. The TOC and TN contents in the ornithogenic soils from AI were one order of magnitude lower than those in other ornithogenic soils due to the deposition of less penguin guano. However,

the AI soil had much higher C/N ratio (11.1) than other soils. The highest NH_4^+—N concentration in the soils was that from MI, followed by PB, and the lowest was AI. The NH_4^+—N concentration in the soils AI was two magnitudes lower than those in other ornithogenic soils. There was no great difference in NO_3^-—N concentrations between the ornithogenic soils. These soils in coastal Antarctica also had particularly high P (3.74—37.18 mg·g⁻¹) and S (1.65—2.38 mg·g⁻¹) contents (Table 1).

3.2 Potential CH_4 production rates from ornithogenic tundra soils

As shown in Figure 2, the production rates of CH_4 from the ornithogenic soil MI increased gradually with the time both under ambient air or under N_2 incubation, and then reached a plateau after 15 h. Overall, the soil MI had almost the same CH_4 production rates under ambient air (mean 19.63± 3.35 μg·kg⁻¹·h⁻¹) and under N_2 incubation (mean 20.32± 5.40 μg·kg⁻¹·h⁻¹). The CH_4 cumulative emissions in the headspace showed a linear increase during the laboratory incubation (linear regression: positive slope with R^2=0.99, p<0.01 for the MI soils). Similarly, the CH_4 production rates from the GI soil rapidly increased to the highest level within 18 h, and then decreased to a low level under ambient air or under N_2 incubation. The GI soil produced low CH_4 emissions (mean 3.07±0.63 μg·kg⁻¹·h⁻¹) under ambient air while GI produced significant amounts of CH_4 emission under N_2 incubation (mean 6.10±1.95 μg·kg⁻¹·h⁻¹), indicating that anaerobic conditions might stimulate soil CH_4 production and emission because methanogen is a obligate anaerobe[39]. In addition, the fertile ornithogenic soils generally contained considerable organic matter due to the deposition of penguin guano, and this provided rich a matrix for soil CH_4 production[24]. The cumulative CH_4 emissions in the headspace from MI and GI both showed a linear increase during the ambient air or N_2 incubation, but the mean cumulative emissions from MI and GI were higher under N_2 incubation (0.20 mg C·kg⁻¹ and 0.02 mg C·kg⁻¹, respectively) than under ambient air incubation (0.13 mg C·kg⁻¹ and 0.006 mg C·kg⁻¹, respectively), further confirming that anaerobic conditions in the soils might increase CH_4 production rates.

Table 1 Physicochemical properties of ornithogenic soils at the sampling sites

Sources	Mc /%	pH	TOC /%	TN /%	NH_4^+—N /(μg·g⁻¹)	NO_3^-—N / (μg·g⁻¹)	C/N	P/(mg·g⁻¹)	S/(mg·g⁻¹)
MI	65.9	7.3	8.31±1.08	2.47±0.07	(2.38±0.30)×10³	18.79±3.82	3.4	37.18±1.37	1.65±0.03
GI	64.1	7.1	14.65±1.36	3.60±0.46	(1.16±0.04)×10³	37.57±2.44	4.1	27.30±0.97	2.27±0.04
AI	38.0	5.2	5.45±0.59	0.49±0.09	35.96±17.65	7.19±1.41	11.1	4.41±0.16	2.07±0.15
PB	76.4	6.8	12.29±1.73	2.60±0.29	(1.76±0.18)×10³	26.29±1.83	4.7	3.74±0.39	2.38±0.46

Notes: (1) GI and MI: the sites for ornithogenic soil profiles in Adélie penguin colonies on Gardner Island and Magnetic Island, respectively, East Antarctica; (2) AI: the sites for ornithogenic soil core in penguin colony on Ardley Island, West Antarctica; (3) PB: the site for ornithogenic soil profile in emperor penguin colony at Prydz Bay, East Antarctica.
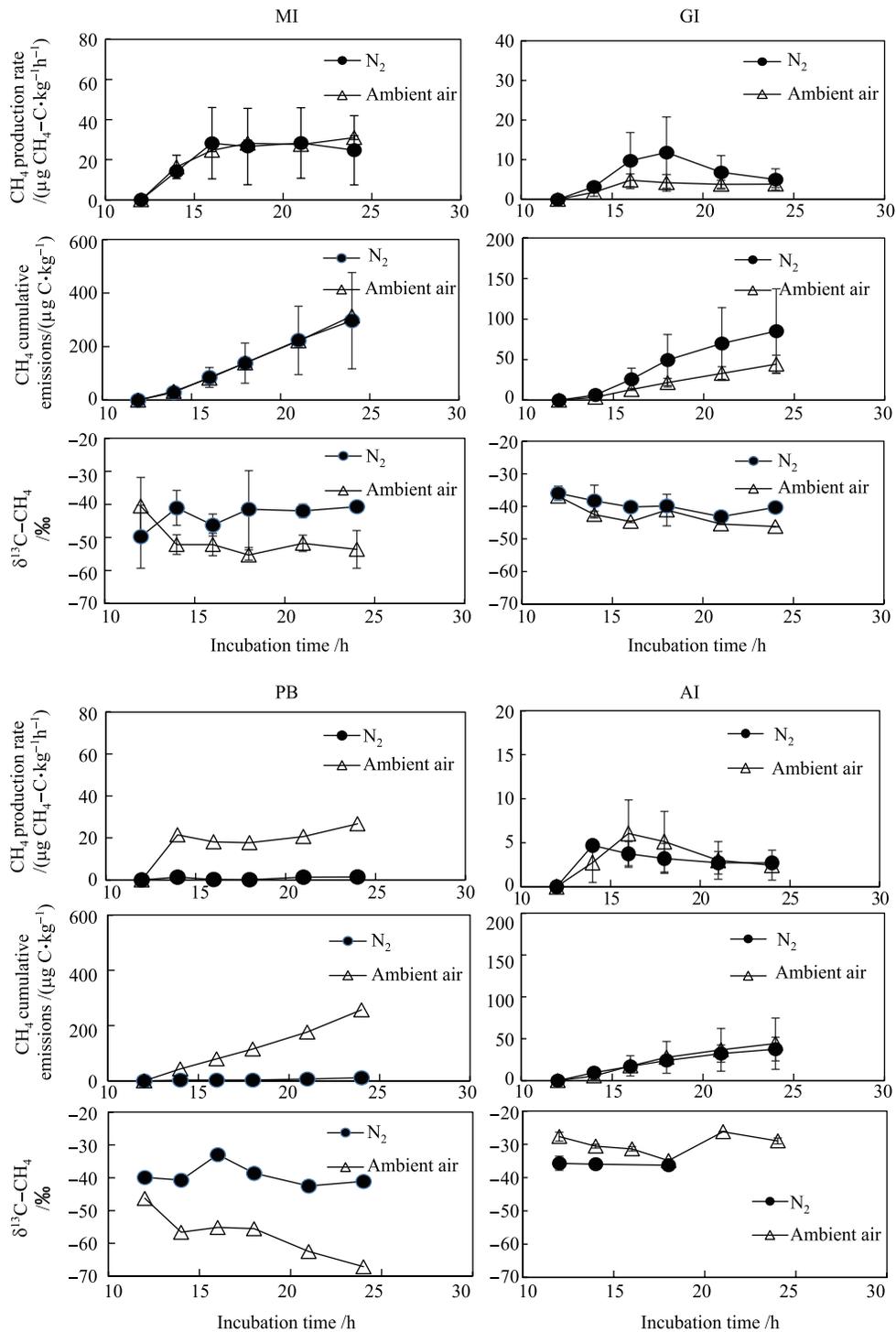
Figure 2 The CH$_4$ production rates and δ^{13}C-CH$_4$ from MI, GI, PB and AI ornithogenic tundra soils during ambient air and N$_2$ incubations. Note: these are the mean values at each soil sample, and the vertical bars indicate standard errors of CH$_4$ production rates and δ^{13}C-CH$_4$.

Similarly to GI, the CH$_4$ production rates from the AI soil rapidly increased to the highest level, and then decreased to a low level under both ambient air and under N$_2$ incubation. The AI soil produced almost equal CH$_4$ emission amounts under ambient air (mean 3.23±0.97 μg·kg^{-1}·h^{-1}) and under N$_2$ incubation (mean 3.35±0.96 μg·kg^{-1}·h^{-1}). The cumulative CH$_4$

emissions from AI were significantly lower than from other ornithogenic soils due to very low soil TOC and TN contents.

Contrary to the MI and GI soils from Adélie penguin colonies, the PB ornithogenic soil from an emperor penguin colony produced a significant amount of CH$_4$ emissions (17.47±3.74 μg·kg^{-1}·h^{-1}) under ambient air incubation, but

negligible CH_4 emissions (0.70 ± 0.28 $\mu g \cdot kg^{-1} \cdot h^{-1}$) under N_2 incubation. The cumulative CH_4 emissions were also much higher under ambient air (mean 0.11 $mgC \cdot kg^{-1}$) than under N_2 incubation (mean 0.01 $mgC \cdot kg^{-1}$). The PB ornithogenic soil had the highest soil moisture of all the samples, and soil water was highly saturated as shown in Table 1. Extremely anaerobic conditions under N_2 incubation could inhibit the CH_4 production or prevent CH_4 emissions escaping[40]. Another possible reason was that the ornithogenic soils might emit more bioavailable organic carbon under aerobic conditions than under anaerobic conditions, leading to much higher CH_4 production rates under ambient air incubation[24].

It should be emphasized that water content is an important factor in determining soil microbial activity in Antarctic environments[41]. Therefore soil moisture is probably an important contributing factor to the higher production rates of CH_4 associated with ornithogenic tundra soils in coastal Antarctica. In our assessments, there was no need to saturate the ornithogenic soils to produce slurries, as in the methods used by Harris and Tibbles[42] and Cocks et al.[43], as our samples were at typical moisture contents for the different penguin colonies, and thus our results could reflect potential CH_4 production rates under moisture conditions present in the field.

3.3 The δ^{13}C-CH_4 emitted from ornithogenic tundra soils

The mean δ^{13}C-CH_4 emitted from MI and GI were higher under N_2 incubation (mean -43.53 ± 2.26‰ and -39.63 ± 0.95‰, respectively) than under the ambient air incubation (mean -50.91 ± 2.04‰ and -42.81 ± 1.13‰, respectively), indicating that the anaerobic conditions were conducive to ^{13}C enrichment in the CH_4 (Figure 2 and Table 2). Similarly, the δ^{13}C-CH_4 emitted from PB had significant differences under ambient air incubation and under N_2 incubation. The δ^{13}C-CH_4 ranged from -32.9‰ to -41.1‰ under N_2 incubation, whereas it ranged from -67.2‰ to -36.28‰ under ambient air incubation, further confirming that the CH_4 emitted from the ornithogenic soils could enrich ^{13}C under highly anaerobic conditions. The δ^{13}C-CH_4 emitted from AI ranged from -35.0‰ to -29.0‰ under ambient air incubation, whereas it ranged from -37.8 ‰ to -33.6‰ under N_2 incubation. The δ^{13}C values of CH_4 emitted from AI were significantly higher than those from MI, GI and PB. Furthermore, the δ^{13}C-CH_4 from AI was significantly higher

under the ambient air incubation (mean -29.93 ± 0.87‰) than under N_2 incubation (mean -35.88 ± 0.76‰).

The CH_4 emitted from different sources might have different isotopic characteristics because of isotopic fractionation effects. Biogenic CH_4 generally has depleted ^{13}C (-65‰~-55‰) compared to the CH_4 emitted from the burning of fossil fuels (-50‰~-30‰) and biomass burning (-28‰~-12‰)[24]. In the soil systems, acetate fermentation and CO_2/H_2 reduction are the two main mechanisms of CH_4 production. Generally CH_4 produced through CO_2/H_2 reduction is poor in ^{13}C (δ^{13}C=-60‰~-110‰), while CH_4 produced through acetic acid reduction is enriched in ^{13}C (δ^{13}C=-50‰~-65‰)[44-47]. In addition, the CH_4 oxidation process can lead to ^{13}C enrichment[8,48]. In this study, the mean δ^{13}C-CH_4 emitted from the MI, GI and PB soil profiles was 7.54‰, 3.19‰ and 9.21‰ higher, respectively, under N_2 incubation than under ambient air incubation. This indicated that soil CH_4 production in MI, GI and PB might be predominantly due to acetate fermentation under N_2 incubation, whereas ^{13}C−depleted CH_4 emitted from MI, GI and PB might be predominantly produced through CO_2/H_2 reduction under ambient air incubation. The ^{13}C-CH_4 enrichment under N_2 incubation confirmed that acetate fermentation might become an important source for atmospheric CH_4 in highly waterlogged tundra soils in coastal Antarctica.

For the AI ornithogenic soil, δ^{13}C-CH_4 was 5.63‰ higher under ambient air incubation than under N_2 incubation. The ^{13}C enrichment in CH_4 might be due to CH_4 oxidation by methane—oxidizing bacteria under ambient air incubation. In addition, soil moisture was much lower in AI than in other ornithogenic soils (Table 1), and such low soil moisture might lead to the formation of highly unsaturated soil. Generally unsaturated soils could provide more organic carbon that soil microorganisms need under aerobic conditions than under anaerobic conditions[24], and more organic carbon was transformed into ^{13}C, therefore the δ^{13}C-CH_4 for AI was higher under ambient air incubation.

3.4 Correlations between δ^{13}C-CH_4 and CH_4 production rates

Overall δ^{13}C-CH_4 showed a significant negative correlation with soil CH_4 production rates under N_2 incubation (R^2=0.41, $p<0.01$) or under ambient air incubation (R^2=0.50, $p<0.01$). This indicated that δ^{13}C-CH_4 had a close relationship with

Table 2 The mean δ^{13}C-CH_4 and CH_4 production rate from Antarctic ornithogenic soils during the laboratory incubation experiments

Sources	Under N_2			Under ambient air		
	δ^{13}C-CH_4 /‰	CH_4 production rate /($\mu g \cdot kg^{-1} \cdot h^{-1}$)	n	δ^{13}C-CH_4 /‰	CH_4 production rate /($\mu g \cdot kg^{-1} \cdot h^{-1}$)	n
MI	-43.53 ± 2.26a	20.32 ± 5.40a	12	-50.91 ± 2.04b	19.63 ± 3.35a	12
GI	-39.63 ± 0.95a	6.10 ± 1.95b	12	-42.81 ± 1.13a	3.07 ± 0.63b	12
PB	-39.28 ± 1.38a	0.70 ± 0.28b	6	-57.19 ± 2.91b	17.47 ± 3.74a	6
AI	-35.88 ± 0.76ac	3.35 ± 0.96b	4	-29.93 ± 0.87c	3.23 ± 0.97b	12

Note: The measured δ^{13}C-CH_4 and CH_4 production rate at study sites with the same suffix letter (a, b or c) are not significantly different from one another (LSD, $p<0.05$).

soil CH$_4$ production rates, and decreased with increasing CH$_4$ production rates (Figure 3). For all the soil profiles, a significant negative correlation existed between δ^{13}C-CH$_4$ and CH$_4$ concentration in the headspace under N$_2$ or ambient air incubation, further confirming that CH$_4$ emissions from the ornithogenic soils had an important effect on carbon isotopes of CH$_4$ (Figure 4). Quay et al.[49] also found the negative correlation between δ^{13}C-CH$_4$ and CH$_4$ concentrations. The negative correlation might be due to CH$_4$ oxidation[8,48]. Generally, ^{12}C-CH$_4$ had a faster reaction rate during the processes of CH$_4$ oxidation, with less ^{12}C occurring in the residual CH$_4$, leading to enrichment in ^{13}C-CH$_4$. The correlation between the CH$_4$ concentration and δ^{13}C-CH$_4$ was more significant under ambient air incubation than under N$_2$ incubation, indicating that ambient air incubation might increase soil CH$_4$ oxidation compared with N$_2$ incubation.
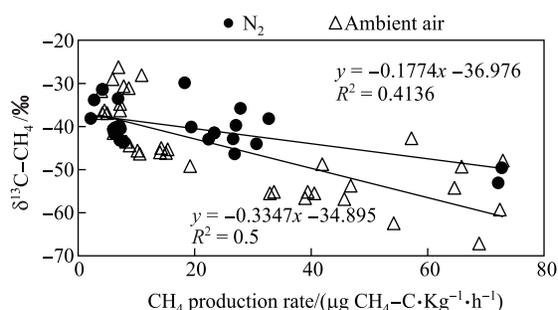
Figure 3 Correlations between δ^{13}C-CH$_4$ and CH$_4$ production rates from the ornithogenic tundra soil profiles in coastal Antarctica.

3.5 Effects of penguins on soil CH$_4$ production rates and δ^{13}C-CH$_4$

As illustrated in Figure 5, potential CH$_4$ production from ornithogenic soils showed a significant positive correlation with total phosphorus (TP) and NH$_4^+$−N contents, pH and soil moisture (Mc), but a strong negative correlation with soil total sulfur (TS) content. On the contrary, the δ^{13}C-CH$_4$ showed a significant negative correlation with TP and NH$_4^+$−N contents, pH and Mc. The deposition of penguin guano strongly effects the physical and chemical properties of tundra soils via the influence of microbes[7]. These ornithogenic tundra soils had particularly high P and S levels (Table 1), two elements which were typical in penguin guano and could be used as indicators for the amount of penguin guano deposition in soils/sediments[50]. The correlations between potential CH$_4$ production and TP, TS contents from ornithogenic soils indicated that the amount of penguin guano deposition increased potential CH$_4$ production rates from tundra soils, but decreased the δ^{13}C-CH$_4$.

The CH$_4$ production rates from ornithogenic soils are correlated with the supply of extra methanogenic substrate by the deposition of penguin guano, and high CH$_4$ production might be the result of dissolved CH$_4$, large microbial populations, rapid decomposition of guano, provision of labile organic compounds to methanogens, and anaerobic condition in the soils[51-52]. Similar results were also obtained in our previous studies[13,15,36,38,53]. Soil NH$_4^+$−N content may limit the capacity of the soil to take up CH$_4$, as NH$_4^+$−N can inhibit the activity of methanotrophs[54-55]. Therefore, in this study high NH$_4^+$−N input from penguin guano might decrease soil CH$_4$ uptake, but greatly stimulate CH$_4$ production by methanogens in the ornithogenic soils. Favorable conditions for high CH$_4$ production are created by physical and chemical processes related to penguin activities: the input of penguin guano and penguin tramp[56]. The correlations between CH$_4$ production rates and soil TP, TS, Mc, pH and NH$_4^+$−N (Figure 5) further confirmed that soil physical and chemical processes associated with penguin activities were the predominant factors affecting CH$_4$ production and its δ^{13}C
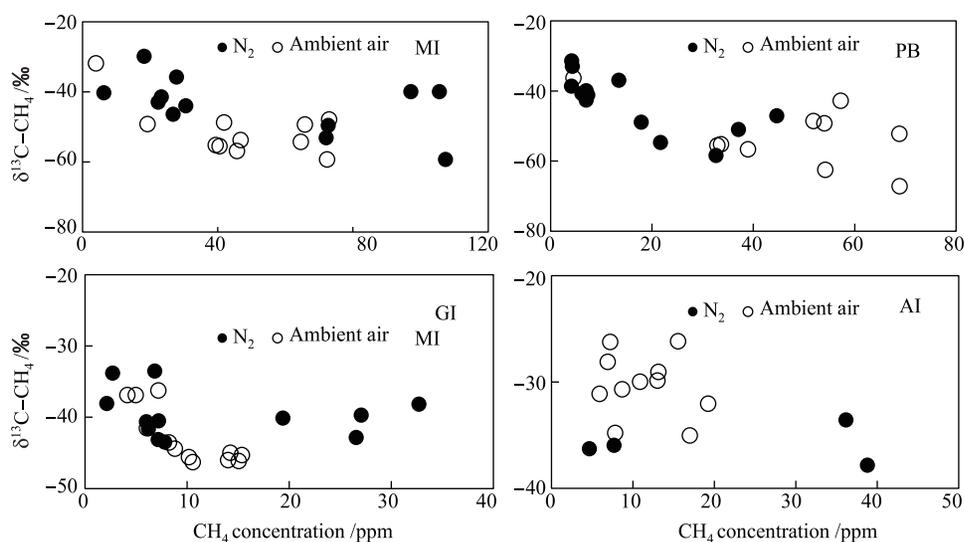
Figure 4 Relationships between the δ^{13}C values and CH$_4$ concentration in the headspace under ambient air and N$_2$ conditions during the ornithogenic soil incubations.
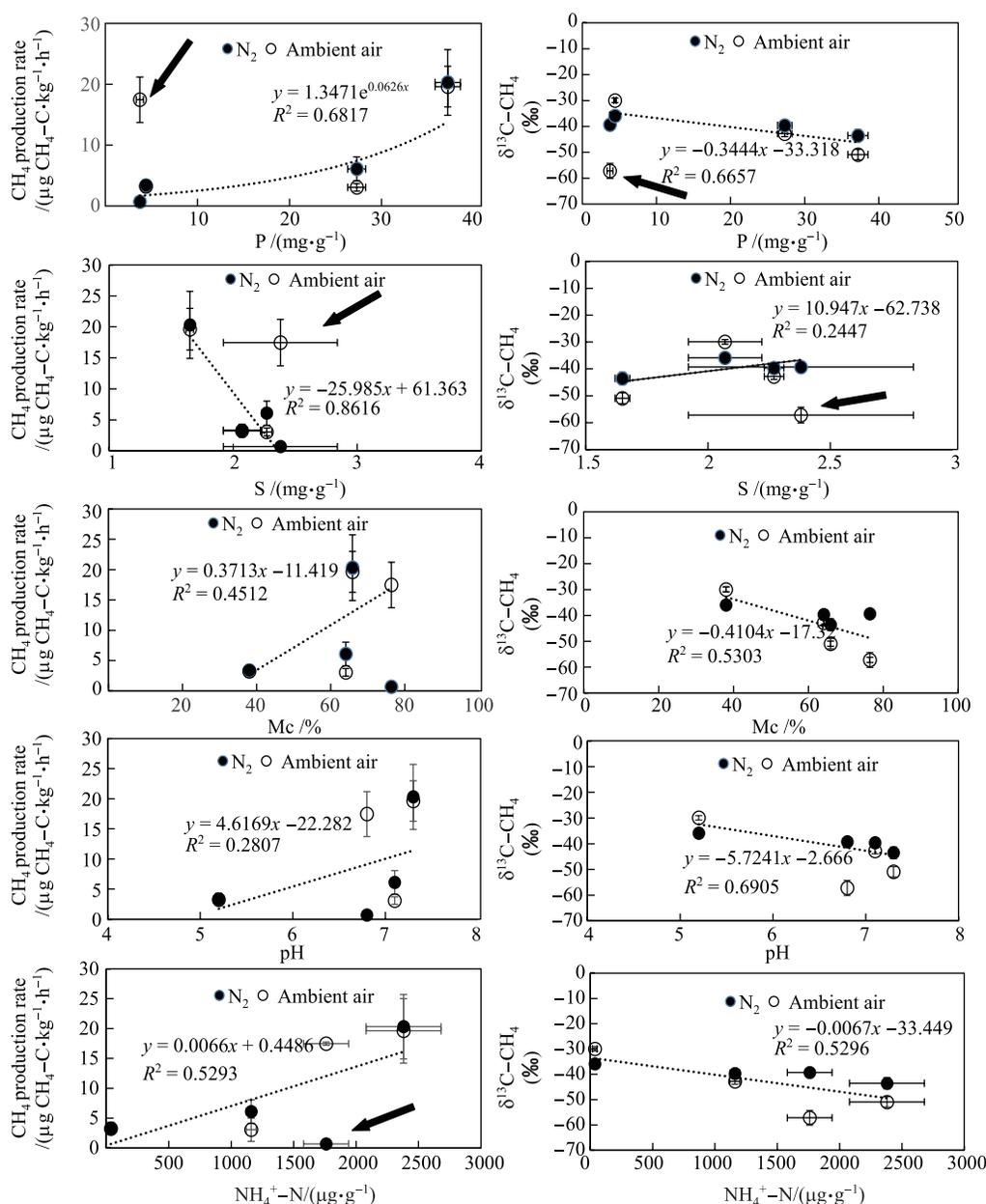
Figure 5 Correlation between CH_4 production rates, $\delta^{13}C\text{-}CH_4$ and P, S, pH, $NH_4^+\text{-}N$ and soil moisture in the ornithogenic tundra soils. Note: these are the mean values at each soil sample, and the vertical bars indicate standard errors of CH_4 production rates and $\delta^{13}C\text{-}CH_4$. If the regression is significant at $p<0.05$, regression lines and R values are shown in the figure. The arrow indicates outlier data, which are excluded from the correlation.

from tundra soils in coastal Antarctica.

Although the experiment was short-term and conducted at a stable temperature (4℃), our results showed that ornithogenic tundra soils in penguin colonies do have high potential fluxes of CH_4, indicating the importance of the CH_4 emissions from these colonies in Antarctic terrestrial ecosystems. In maritime Antarctica and the sub-Antarctic, the number of marine animals and their colonies is large. It has been estimated that about 5 million Adelie penguins, 3 million Chinstrap penguins and more than half a million emperor penguins are distributed around the Antarctic coast[57].

Penguins play an important role in the nutrient cycling of Antarctic terrestrial ecosystems by transferring C and N from the marine to the terrestrial environment[7,9]. Penguin guano and fertile ornithogenic soils are the most important organic C and N reservoirs in the Antarctic terrestrial ecosystems[12,53]. At present, data on CH_4 emissions from these colonies are still scarce. Much more work is needed in this region to characterize the CH_4 production and emissions from ornithogenic tundra soils in coastal Antarctica. The technique of stable carbon isotopes for CH_4 has been proved to be a useful tool in studying the processes of CH_4 production[16–18].

The relative contribution of acetate to CH_4 production and the fraction of CH_4 oxidized can be quantitatively estimated[19, 26-27] based on the measurements of $\delta^{13}C$ in CH_4, CO_2 and acetate, and of the isotope fractionation factors. However, investigations of the isotope fractionation factors for CH_4 production and emission from tundra soils have not been conducted in coastal Antarctica, and need to be further studied in the future.

4 Conclusions

The results are summarized as follows:

(1) The mean CH_4 production rates are highly variable in the ornithogenic soil profiles, ranging from 0.7 to 20.3 $\mu g \cdot kg^{-1} \cdot h^{-1}$. Our results showed that ornithogenic tundra soils had high potential production rates of CH_4 based upon experimental incubation under ambient air and under N_2, indicating the importance of potential CH_4 emissions from ornithogenic tundra soils in Antarctic terrestrial ecosystems.

(2) The ornithogenic soil profiles MI, GI and PB had higher $\delta^{13}C$-CH_4 under N_2 conditions than under ambient air incubation. The average $\delta^{13}C$-CH_4 emitted from MI, GI and PB, respectively, was 7.54‰, 3.19‰ and 9.21‰ higher under anaerobic conditions than under aerobic conditions. Our results indicated that anaerobic conditions were conducive to the emissions of CH_4 enriched in ^{13}C, and acetic acid reduction under anaerobic conditions might be a predominant source for CH_4 production in ornithogenic soils.

(3) Overall, $\delta^{13}C$-CH_4 emitted from ornithogenic tundra soils showed a significant negative correlation with soil CH_4 production rates under N_2 incubation (R^2=0.41, p<0.01) or under ambient air incubation (R^2=0.50, p<0.01). The CH_4 emissions from the ornithogenic soils had an important effect on carbon isotopes of CH_4 in Antarctic tundra.

(4) Potential CH_4 production from ornithogenic soils showed a significant positive correlation with TP and NH_4^+–N contents, pH and Mc, but a strong negative correlation with soil total sulfur (TS) contents. On the contrary, the $\delta^{13}C$-CH_4 showed a significant negative correlation with TP and NH_4^+–N contents, pH and Mc, indicating that the amount of penguin guano deposition increased potential CH_4 production rates from tundra soils, but decreased the $\delta^{13}C$-CH_4.

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