

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

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Abstract Methane (CH₄) is one of important greenhouse gases with chemical activity. The determination of isotopic compositions for CH₄ emitted from the soils helps us to understand its production mechanisms. CH₄ isotope measurements have been conducted for different types of global terrestrial ecosystems. However, no isotopic data of CH₄ have been reported from Antarctic tundra soils. In this paper, ornithogenic soil profiles were collected from four penguin colonies, and potential CH₄ production rates and its ¹³C ratio (δ¹³C) were investigated based upon laboratory incubation experiments. The mean CH₄ production rates are highly variable in these soil profiles, ranging from 0.7 to 20.3 μg CH₄-C kg⁻¹·h⁻¹. These ornithogenic soils had high potential production rates of CH₄ under ambient air incubation or under N₂ incubation, indicating the importance of potential CH₄ emissions from penguin colonies. Most of the soil samples had higher δ¹³C-CH₄ under N₂ incubation (-39.28%~-43.53%) than under the ambient air incubation (-42.81%~-57.19%). Highly anaerobic conditions were conducive to the production of CH₄ enriched in ¹³C, and acetic acid reduction under N₂ incubation might be a predominant source for soil CH₄ production. Overall the δ¹³C-CH₄ showed a significant negative correlation with CH₄ production rates in ornithogenic tundra soils under N₂ incubation ($R^2=0.41$, $p<0.01$) or under the ambient air incubation ($R^2=0.50$, $p<0.01$). Potential CH₄ production from ornithogenic soils showed a significant positive correlation with total phosphorus (TP) and NH₄⁺-N contents, pH and soil moisture (Mc), but the δ¹³C-CH₄ showed a significant negative correlation with TP and NH₄⁺-N contents, pH and Mc, indicating that the deposition amount of penguin guano increased potential CH₄ production rates from tundra soils, but decreased the δ¹³C-CH₄. The CH₄ emissions from the ornithogenic soils affect carbon isotopic compositions of atmospheric CH₄ in coastal Antarctica.

Keywords CH₄, Antarctica, ornithogenic soil, carbon isotope, penguin colony

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1 Introduction

Methane (CH₄) 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 CH₄ 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 CH₄ in maritime Antarctica^[13-15]. Nevertheless, CH₄ production from fertile ornithogenic tundra soils are still poorly understood in coastal Antarctica. Tundra CH₄ emission is an integrated effect of the production, oxidation and transport of CH₄ in the soils. A better knowledge of these processes affecting CH₄ emission may provide more information about the response of tundra CH₄ emission to changing climate.

The technique of stable carbon isotopes has been proved to be a useful tool in studying the complicated processes of CH₄ production and emission^[16-19]. CH₄ emitted from various sources may have different isotopic characteristics because of its isotopic fractionation effects. Isotope fractionation happens in all the major processes of CH₄ emission. The ¹²C-substrate is preferentially utilized by methanogens for CH₄ production, and once formed, ¹²CH₄ is consumed faster than ¹³CH₄ by methanotrophs, and ¹²CH₄ is transported faster than ¹³CH₄ as well^[20-23]. The $\delta^{13}\text{C-CH}_4$ produced through acetic acid fermentation ranged from -60‰ to -50‰ , whereas the $\delta^{13}\text{C-CH}_4$ in the reduction of CO₂/H₂ was below this range^[24-25]. Generally the CH₄, emitted from the combustion of fossil fuel, had relatively heavy isotopic compositions with the $\delta^{13}\text{C-CH}_4$ range of -50‰ ~ -30‰ , whereas CH₄ from biomass combustion had especially high ¹³C-CH₄ (-28‰ ~ -12‰). In addition, the relative contribution of acetate to CH₄ production (f_{ac}) and the fraction of CH₄ oxidized (f_{ox}) can be quantitatively estimated^[19,26-27] using mass balance equations based on the measurements of $\delta^{13}\text{C}$ in CH₄, CO₂ and acetate, and of the isotope fractionation factors ($\alpha_{\text{CO}_2/\text{CH}_4}$, $\epsilon_{\text{acetate}/\text{CH}_4}$, α_{ox} and $\epsilon_{\text{transport}}$). At present, studies on isotopes of soil CH₄ have been conducted in different ecosystems^[25,28-31]. However, few data are available on the isotopes of CH₄ 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 CH₄ production rates and its $\delta^{13}\text{C}$ were measured based upon laboratory incubation experiments. Our objectives are (1) to measure potential CH₄ production rates from ornithogenic tundra soils and the carbon isotopic compositions of CH₄ emitted from the soils; (2) to discuss the factors affecting CH₄ 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, CH₄ and N₂O 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 -10°C and transported to the laboratory in China for the incubation experiment.

2.2 Incubation experiment and measurements of potential CH₄ 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 CH₄ 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

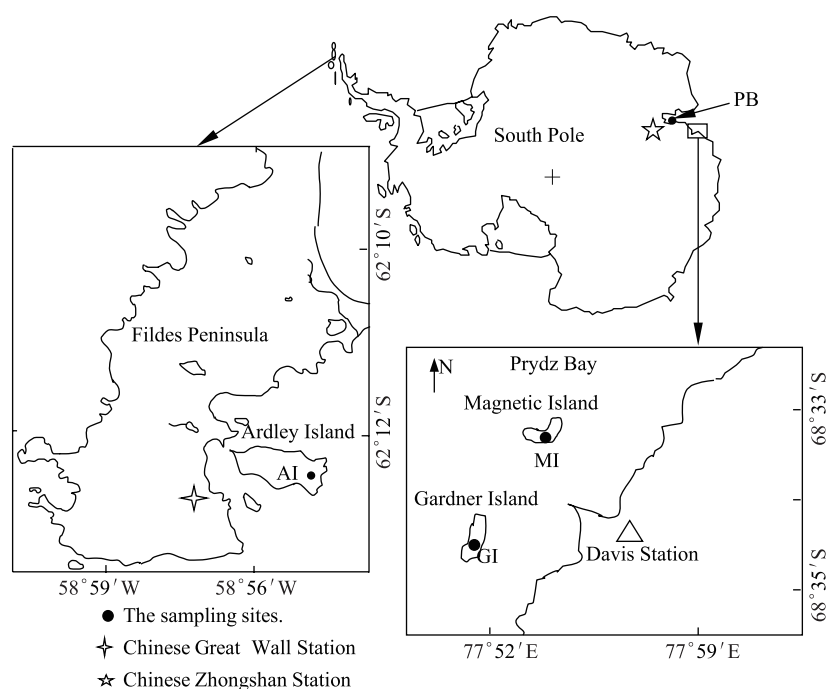


Figure 1 Sampling sites for ornithogenic tundra soil profiles in penguin colonies of coastal Antarctica. (1) AI: penguin colony on Ardley Island, West Antarctica; (2) PB: emperor penguin colony at Prydz Bay, East Antarctica; (3) GI and MI: Adélie penguin colonies on Gardner Island and Magnetic Island, respectively, East Antarctica.

trace gas fluxes, the samples were completely thawed and then incubated in the dark at 4°C, 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 ambient air conditions, to simulate the potential CH₄ fluxes from ornithogenic soils under local natural conditions. The second group was incubated under N₂ to establish anaerobic conditions, which was used to simulate the potential CH₄ 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 N₂ five times to avoid CH₄ build-up in the headspace^[34]. Two repetitions were made for each soil sample^[35].

The CH₄ 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 N₂ (40 mL·min⁻¹) as a carrier gas at 80°C^[36]. The standard gas for CH₄ was 8 ppmv. The variance coefficient for standard samples was within 0.1%–0.6% in 24 h. Potential CH₄ production rates were estimated from the changing rate of headspace CH₄ 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 δ¹³C-CH₄

The carbon isotopic compositions of CH₄ were analyzed by using a continuous flow technique coupled to a Finnigan MAT-253 isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany)^[37]. The CO₂ in gas samples was directly analyzed while CH₄ in gas samples was converted into CO₂ 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 2.0×10⁵ Pa for further separation. The separated gases were finally transferred into the mass spectrometer for δ¹³C determination. The reference and carrier gases used were CO₂ (99.999% purity and -23.73‰ δ¹³C_{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 CH₄ was injected. The Isotope ratios are presented as δ values, which are defined as: δ_x=[(R_{sample}/R_{standard})-1]×1000‰, 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), $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ 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 $105\text{ }^\circ\text{C}$ for 12 h. M_c was calculated as: $M_c = \frac{\text{weight of the lost water}}{\text{dry soil weight}} \times 100\%$. 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 $\pm 5\%$.

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 $\delta^{13}\text{C}\text{-CH}_4$ 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 $\text{NH}_4^+\text{-N}$ concentration in the soils was that from MI, followed by PB, and the lowest was AI. The $\text{NH}_4^+\text{-N}$ concentration in the soils AI was two magnitudes lower than those in other ornithogenic soils. There was no great difference in $\text{NO}_3^-\text{-N}$ concentrations between the ornithogenic soils. These soils in coastal Antarctica also had particularly high P (3.74–37.18 $\text{mg}\cdot\text{g}^{-1}$) and S (1.65–2.38 $\text{mg}\cdot\text{g}^{-1}$) 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 $19.63 \pm 3.35\ \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and under N_2 incubation (mean $20.32 \pm 5.40\ \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). 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 $3.07 \pm 0.63\ \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) under ambient air while GI produced significant amounts of CH_4 emission under N_2 incubation (mean $6.10 \pm 1.95\ \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$), 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 ($0.20\ \text{mg}\ \text{C}\cdot\text{kg}^{-1}$ and $0.02\ \text{mg}\ \text{C}\cdot\text{kg}^{-1}$, respectively) than under ambient air incubation ($0.13\ \text{mg}\ \text{C}\cdot\text{kg}^{-1}$ and $0.006\ \text{mg}\ \text{C}\cdot\text{kg}^{-1}$, 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	M_c /%	pH	TOC /%	TN /%	$\text{NH}_4^+\text{-N}$ /($\mu\text{g}\cdot\text{g}^{-1}$)	$\text{NO}_3^-\text{-N}$ / ($\mu\text{g}\cdot\text{g}^{-1}$)	C/N	P/($\text{mg}\cdot\text{g}^{-1}$)	S/($\text{mg}\cdot\text{g}^{-1}$)
MI	65.9	7.3	8.31 ± 1.08	2.47 ± 0.07	$(2.38 \pm 0.30) \times 10^3$	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	$(1.16 \pm 0.04) \times 10^3$	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	$(1.76 \pm 0.18) \times 10^3$	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.

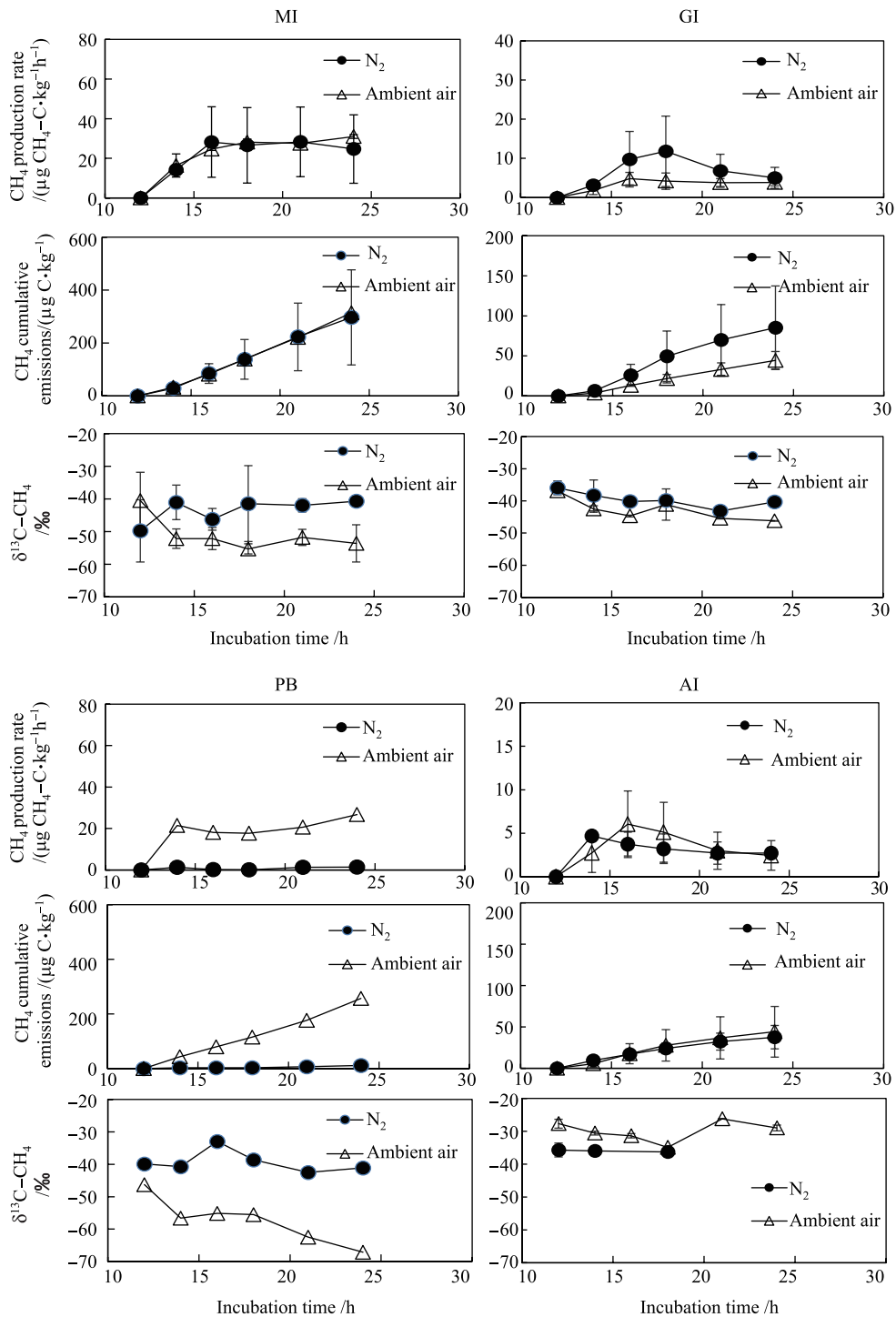


Figure 2 The CH₄ production rates and δ¹³C-CH₄ from MI, GI, PB and AI ornithogenic tundra soils during ambient air and N₂ incubations. Note: these are the mean values at each soil sample, and the vertical bars indicate standard errors of CH₄ production rates and δ¹³C-CH₄.

Similarly to GI, the CH₄ 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 N₂ incubation. The AI soil produced almost equal CH₄ emission amounts under ambient air (mean 3.23±0.97 μg·kg⁻¹·h⁻¹) and under N₂ incubation (mean 3.35±0.96 μg·kg⁻¹·h⁻¹). The cumulative CH₄

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 CH₄ emissions (17.47±3.74 μg·kg⁻¹·h⁻¹) under ambient air incubation, but

negligible CH₄ emissions (0.70±0.28 μg·kg⁻¹·h⁻¹) under N₂ incubation. The cumulative CH₄ emissions were also much higher under ambient air (mean 0.11 mgC·kg⁻¹) than under N₂ incubation (mean 0.01 mgC·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 N₂ incubation could inhibit the CH₄ production or prevent CH₄ 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 CH₄ 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 CH₄ 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 CH₄ production rates under moisture conditions present in the field.

3.3 The δ¹³C-CH₄ emitted from ornithogenic tundra soils

The mean δ¹³C-CH₄ emitted from MI and GI were higher under N₂ 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 ¹³C enrichment in the CH₄ (Figure 2 and Table 2). Similarly, the δ¹³C-CH₄ emitted from PB had significant differences under ambient air incubation and under N₂ incubation. The δ¹³C-CH₄ ranged from -32.9‰ to -41.1‰ under N₂ incubation, whereas it ranged from -67.2‰ to -36.28‰ under ambient air incubation, further confirming that the CH₄ emitted from the ornithogenic soils could enrich ¹³C under highly anaerobic conditions. The δ¹³C-CH₄ emitted from AI ranged from -35.0‰ to -29.0‰ under ambient air incubation, whereas it ranged from -37.8‰ to -33.6‰ under N₂ incubation. The δ¹³C values of CH₄ emitted from AI were significantly higher than those from MI, GI and PB. Furthermore, the δ¹³C-CH₄ from AI was significantly higher

under the ambient air incubation (mean -29.93±0.87‰) than under N₂ incubation (mean -35.88±0.76‰).

The CH₄ emitted from different sources might have different isotopic characteristics because of isotopic fractionation effects. Biogenic CH₄ generally has depleted ¹³C (-65‰~-55‰) compared to the CH₄ emitted from the burning of fossil fuels (-50‰~-30‰) and biomass burning (-28‰~-12‰)^[24]. In the soil systems, acetate fermentation and CO₂/H₂ reduction are the two main mechanisms of CH₄ production. Generally CH₄ produced through CO₂/H₂ reduction is poor in ¹³C (δ¹³C=-60‰~-110‰), while CH₄ produced through acetic acid reduction is enriched in ¹³C (δ¹³C=-50‰~-65‰)^[44-47]. In addition, the CH₄ oxidation process can lead to ¹³C enrichment^[8,48]. In this study, the mean δ¹³C-CH₄ emitted from the MI, GI and PB soil profiles was 7.54‰, 3.19‰ and 9.21‰ higher, respectively, under N₂ incubation than under ambient air incubation. This indicated that soil CH₄ production in MI, GI and PB might be predominantly due to acetate fermentation under N₂ incubation, whereas ¹³C-depleted CH₄ emitted from MI, GI and PB might be predominantly produced through CO₂/H₂ reduction under ambient air incubation. The ¹³C-CH₄ enrichment under N₂ incubation confirmed that acetate fermentation might become an important source for atmospheric CH₄ in highly waterlogged tundra soils in coastal Antarctica.

For the AI ornithogenic soil, δ¹³C-CH₄ was 5.63‰ higher under ambient air incubation than under N₂ incubation. The ¹³C enrichment in CH₄ might be due to CH₄ 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 ¹³C, therefore the δ¹³C-CH₄ for AI was higher under ambient air incubation.

3.4 Correlations between δ¹³C-CH₄ and CH₄ production rates

Overall δ¹³C-CH₄ showed a significant negative correlation with soil CH₄ production rates under N₂ incubation ($R^2=0.41$, $p<0.01$) or under ambient air incubation ($R^2=0.50$, $p<0.01$). This indicated that δ¹³C-CH₄ had a close relationship with

Table 2 The mean δ¹³C-CH₄ and CH₄ 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 /(μg·kg ⁻¹ ·h ⁻¹)	<i>n</i>	δ ¹³ C-CH ₄ /‰	CH ₄ production rate /(μg·kg ⁻¹ ·h ⁻¹)	<i>n</i>
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 δ¹³C-CH₄ and CH₄ 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 CH₄ production rates, and decreased with increasing CH₄ production rates (Figure 3). For all the soil profiles, a significant negative correlation existed between $\delta^{13}\text{C}-\text{CH}_4$ and CH₄ concentration in the headspace under N₂ or ambient air incubation, further confirming that CH₄ emissions from the ornithogenic soils had an important effect on carbon isotopes of CH₄ (Figure 4). Quay et al.^[49] also found the negative correlation between $\delta^{13}\text{C}-\text{CH}_4$ and CH₄ concentrations. The negative correlation might be due to CH₄ oxidation^[8,48]. Generally, ¹²C-CH₄ had a faster reaction rate during the processes of CH₄ oxidation, with less ¹²C occurring in the residual CH₄, leading to enrichment in ¹³C-CH₄. The correlation between the CH₄ concentration and $\delta^{13}\text{C}-\text{CH}_4$ was more significant under ambient air incubation than under N₂ incubation, indicating that ambient air incubation might increase soil CH₄ oxidation compared with N₂ incubation.

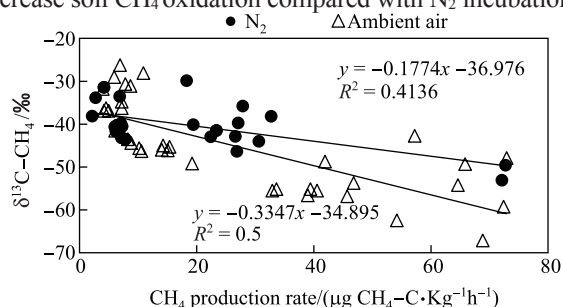


Figure 3 Correlations between $\delta^{13}\text{C}-\text{CH}_4$ and CH₄ production rates from the ornithogenic tundra soil profiles in coastal Antarctica.

3.5 Effects of penguins on soil CH₄ production rates and $\delta^{13}\text{C}-\text{CH}_4$

As illustrated in Figure 5, potential CH₄ production from ornithogenic soils showed a significant positive correlation

with total phosphorus (TP) and NH₄⁺-N contents, pH and soil moisture (Mc), but a strong negative correlation with soil total sulfur (TS) content. On the contrary, the $\delta^{13}\text{C}-\text{CH}_4$ showed a significant negative correlation with TP and NH₄⁺-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 CH₄ production and TP, TS contents from ornithogenic soils indicated that the amount of penguin guano deposition increased potential CH₄ production rates from tundra soils, but decreased the $\delta^{13}\text{C}-\text{CH}_4$.

The CH₄ production rates from ornithogenic soils are correlated with the supply of extra methanogenic substrate by the deposition of penguin guano, and high CH₄ production might be the result of dissolved CH₄, 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 NH₄⁺-N content may limit the capacity of the soil to take up CH₄, as NH₄⁺-N can inhibit the activity of methanotrophs^[54-55]. Therefore, in this study high NH₄⁺-N input from penguin guano might decrease soil CH₄ uptake, but greatly stimulate CH₄ production by methanogens in the ornithogenic soils. Favorable conditions for high CH₄ production are created by physical and chemical processes related to penguin activities: the input of penguin guano and penguin tramp^[56]. The correlations between CH₄ production rates and soil TP, TS, Mc, pH and NH₄⁺-N (Figure 5) further confirmed that soil physical and chemical processes associated with penguin activities were the predominant factors affecting CH₄ production and its $\delta^{13}\text{C}$

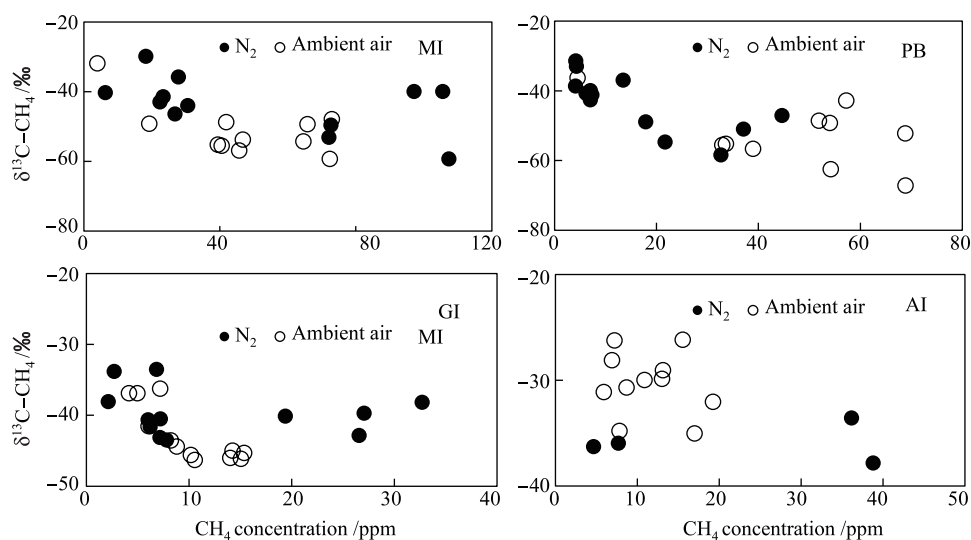


Figure 4 Relationships between the $\delta^{13}\text{C}$ values and CH₄ concentration in the headspace under ambient air and N₂ conditions during the ornithogenic soil incubations.

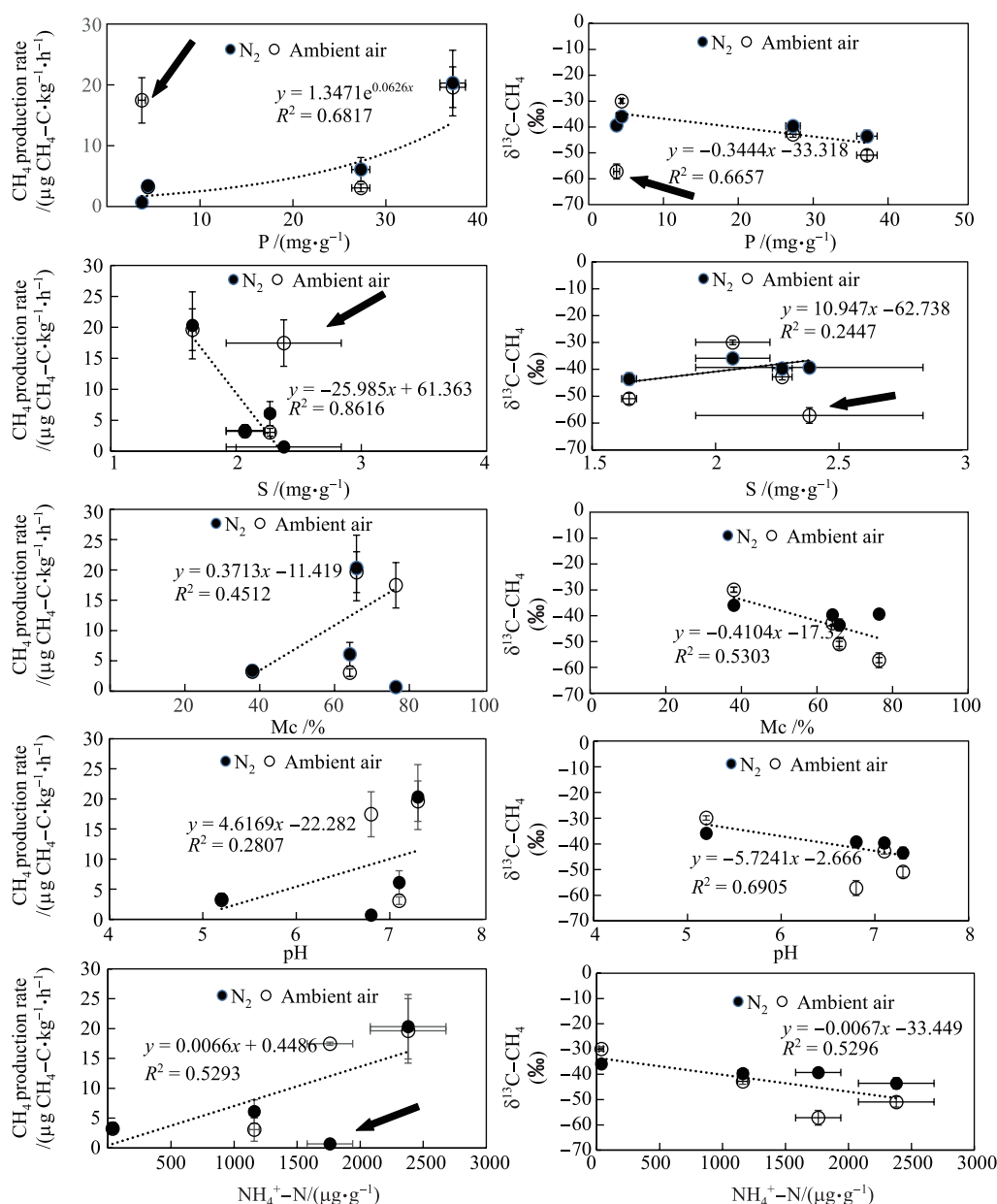


Figure 5 Correlation between CH₄ production rates, δ¹³C-CH₄ and P, S, pH, NH₄⁺-N and soil moisture in the ornithogenic tundra soils. Note: these are the mean values at each soil sample, and the vertical bars indicate standard errors of CH₄ production rates and δ¹³C-CH₄. If the regression is significant 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°C), our results showed that ornithogenic tundra soils in penguin colonies do have high potential fluxes of CH₄, indicating the importance of the CH₄ 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 CH₄ emissions from these colonies are still scarce. Much more work is needed in this region to characterize the CH₄ production and emissions from ornithogenic tundra soils in coastal Antarctica. The technique of stable carbon isotopes for CH₄ has been proved to be a useful tool in studying the processes of CH₄ production^[16-18].

The relative contribution of acetate to CH₄ production and the fraction of CH₄ oxidized can be quantitatively estimated^[19, 26-27] based on the measurements of δ¹³C in CH₄, CO₂ and acetate, and of the isotope fractionation factors. However, investigations of the isotope fractionation factors for CH₄ 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 CH₄ production rates are highly variable in the ornithogenic soil profiles, ranging from 0.7 to 20.3 μg·kg⁻¹·h⁻¹. Our results showed that ornithogenic tundra soils had high potential production rates of CH₄ based upon experimental incubation under ambient air and under N₂, indicating the importance of potential CH₄ emissions from ornithogenic tundra soils in Antarctic terrestrial ecosystems.

(2) The ornithogenic soil profiles MI, GI and PB had higher δ¹³C-CH₄ under N₂ conditions than under ambient air incubation. The average δ¹³C-CH₄ 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 CH₄ enriched in ¹³C, and acetic acid reduction under anaerobic conditions might be a predominant source for CH₄ production in ornithogenic soils.

(3) Overall, δ¹³C-CH₄ emitted from ornithogenic tundra soils showed a significant negative correlation with soil CH₄ production rates under N₂ incubation ($R^2=0.41$, $p<0.01$) or under ambient air incubation ($R^2=0.50$, $p<0.01$). The CH₄ emissions from the ornithogenic soils had an important effect on carbon isotopes of CH₄ in Antarctic tundra.

(4) Potential CH₄ production from ornithogenic soils showed a significant positive correlation with TP and NH₄⁺-N contents, pH and Mc, but a strong negative correlation with soil total sulfur (TS) contents. On the contrary, the δ¹³C-CH₄ showed a significant negative correlation with TP and NH₄⁺-N contents, pH and Mc, indicating that the amount of penguin guano deposition increased potential CH₄ production rates from tundra soils, but decreased the δ¹³C-CH₄.

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