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PERMAFROST MICROBIOLOGY

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METHANE AS AN INDICATOR OF PERMAFROST FORMATION CONDITIONS IN ANTARCTICA

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Research of permafrost in Antarctica revealed the presence of methane in lacustrine (Larsemann, Schirmacher and Bunger Hills oases), marine (King George Island) and temporary stream (Larsemann Hills) sediments. In moraine and deluvium deposits, we found no methane. The methanogenic activity has been proved by experiments with isotopically marked substrates. In permafrost, there are number of viable methanogenic archaea, that start producing methane as ground temperature rises. The methane flux could be increased in the future due to deglaciation, active layer deepening, activation of methanogenic microorganisms and release of entrapped methane.

Antarctica, methane, permafrost, radioactive label

INTRODUCTION

Methane, which is known to be a terminal product of the anaerobic degradation of organic matter and a side product of carbon dioxide hydrogenation, is one of the key players in ecosystems and is, traditionally, indicative of highly hydromorphic and reducing environments [*Whiticar et al., 1986; Evans et al., 2019*]. Being an active greenhouse gas, methane absorbs infrared radiation (also called longwave or terrestrial radiation) in the atmosphere [*Wuebbles, Hayhoe, 2002; Feldman et al., 2018*], thereby contributing to air temperature rise.

Methanogenesis occurring in sediments of modern lakes and beneath the Antarctic Ice Sheet has long been a subject of study by many researchers [*Ellis-Evans, 1984; Wand et al., 2006*]. However, the existence of methanogens in Antarctic permafrost had not been confirmed in published literature until the mid 1990s. Measurements of biogenic methane content as part of the study of viable microorganisms in permanently frozen ground in the Dry Valleys (Taylor Valley, Miers Valley, Mount Feather) initiated by David A. Gilichinsky back in 1995, during the first joint U.S./Russian Antarctic expedition, were continued in 1999 (Beacon Valley). A summary of the research results was reported in a paper published in the Journal of Astrobiology [*Gilichinsky et al., 2007*], which *inter alia* included results of methane detection in drill cores. Since 2007, the Soil Cryology Laboratory has been involved in the study of the Antarctic coastal oases within the Russian Antarctic Expedition (RAE), where, alongside other research goals, the sediments were sampled for methane detection. Despite the numerous publications of most of the research findings covering a wide range of biogeochemical processes [*Abramov et al., 2011; Demidov et al., 2013; Karaevskaya et al., 2014*], the patterns of methane distribution and its role as an indicator of the conditions preceding sediments cryopreservation did not receive adequate research attention; these therefore were chosen to be the object of this study.

Analysis of the Arctic permafrost samples revealed that besides methane, genes of microorganisms are also involved in methane cycling, which can serve as an indicator of the conditions favorable for permafrost formation and evolution (cryogenesis) [*Rivkina et al., 2016*].

Exogenous methane in perennially frozen sediments is formed by methanogenic microorganisms (archaea), with CO_2 and organic compounds such as acetate, methylamines and methanol being the main substrates for its production. Recent data have provided some compelling evidence for methanogenesis to occur in oxic environments as well.

It was shown that in phosphorus deficient Antarctic lakes, some aerobic bacteria are capable to utilize methylphosphonate as phosphate source, with methane being formed as a by-product of this reaction [*Li et al., 2020*], although in minute amounts, as compared to methane produced as a result of biological activity of methanogenic archaea in anoxic conditions. The effects of temperature rise in high-latitude regions in the northern hemisphere that serve as significant source of methane flux will result in permafrost degradation, thereby intensifying methanogenesis with the labile organic matter increasingly involved in the biogeochemical processes [*Schuur et al., 2013*]. Unlike the Arctic permafrost, the Antarctic perennially frozen soils are characterized by low total organic carbon (TOC), which is corroborated by direct measurements in drillcores from boreholes that estimated TOC concentrations to be from hundredths of a fraction in moraine sediments to 1.7 % in lacustrine-lagoon sediments [*Gilichinsky et al., 2007; Demidov et al., 2013*]. Because of the insufficient coverage of boreholes for ground temperature monitoring, the Antarctic permafrost temperature departures and trends appear statistically not significant, however, over the past decade permafrost warming was observed in some parts of the continent [*Biskaborn et al., 2019*]. The accelerated rate of the Antarctic Ice Sheet mass loss during the past few decades is discussed in [*Rignot et al., 2019*]. According to these estimates, the total rate of ice mass loss showed a 6-fold increase in Antarctica: from 40 Gt/yr in the period 1979–1990 to 252 Gt/yr in 2009–2017. In the past decade, the continent was loosing ice at a rate of 159 Gt/yr (West Antarctica) and 51 Gt/yr (East Antarctica).

The predicted expansion of ice-free areas in Antarctica next to the deepening seasonally thawing layer (active layer, AL) can entail enhancement of methane emissions from the Antarctic permafrost both due to activated microbial activity in areas that have become ice-free, and subglacial methane discharge [*Wadham et al., 2012*].

RESEARCH METHODS

There are numerous descriptions of core sampling for determination of methane concentrations in frozen sediments through controlled degassing of the cores [*Rivkina et al., 2007*]. During the 2007–2011 drilling operations with no flushing or purging, the permafrost cores collected for microbiological and other analyses were placed in a freezer for their transportation to the laboratory and storage at a temperature maintained to be –18 °C, prior to their analyses. Sediments were sampled using the "head space" gas equilibration method [*Alperin, Reeburgh, 1985*] which enabled collecting gas specimens using headspace degassing in 150 mL syringes. Their methane content in samples was determined using a KhPM-4 (Russia) gas chromatograph furnished with a flame ionization detector [*Rivkina et al., 2007*]. The carbon isotope composition ($\delta^{13}C$) of methane was determined on a Deltaplus XL, GC Combustion III system for gas chromatography–mass spectrometry (ThermoFinnigan, Germany) in the Center of Isotopic Research of Karpinsky Russian Geological Research Institute (VSEGEI). The total carbon abundance was determined by dry incineration using an AN-7529 express analyzer (Russia). The method of radioactively labeled substrates enabled the sensitivity enhancement of methane formation detecting in cultivated anaerobic microcosms. We used sodium acetate labeled with radioactive carbon in the methyl group ($Na^{14}CH_3CO_2$), and bicarbonate ($NaH^{14}CO_3$) with their radioactivities (*R*) equal $108{\cdot}10^5$ cpm and $702{\cdot}10^5$ cpm, respectively. After a week of incubation at room temperature (20 °C), we measured radioactivity of newly formed methane (*r*). The experimental method for detecting methanogenesis in microcosms using a radioactive label is discussed in detail in the paper authored by E.M. Rivkina and colleagues [*Rivkina et al., 2007*]. The redox potential (Eh) was estimated in samples exposed to thawing immediately prior to measuring by the "Ecotest-120" ionomer (Russia) furnished with platinum electrode EPV.1 (the indicator) and EVL.1M3.1 silver chloride electrode (the reference).

DESCRIPTION OF SAMPLING SITES

Boreholes A11-08 and A1-09 drilled on King George island (Fig. 1) in the vicinity of the meteorological observation site of Bellingshausen Station, penetrated sediments of the 1st marine terrace. The active layer (thickness: 3 m) composed of shingle is underlain to a depth of 7.5 m by sand with inclusions of gravel, which passes into loamy soil and then, at a depth of 9 m, into a bluish clay (Fig. 2). The sediments have a strong smell of hydrogen sulfide. Diatom assemblages inhabiting the terrace are typical of the Antarctic zone of the Southern ocean. Sublittoral benthic species amassed in the lowermost part of the section indicate the shallow sea and quiescent sedimentation conditions, while benthic and cold-water plankton species accumulated in the topmost parts. The water extract having salinity as low as 0.1–3.5 % suggests that marine sediments were probably diluted by mixing with fresh water (e.g. washed with glacier meltwater) prior to their freezing [*Abramov et al., 2011*].

Borehole A5-08 was drilled from the bed of a dried-up lake in the Bunger Hill Oasis, near Oasis Station (Fig. 1). The sediments are composed of permafrost sandy, sandy-loamy and loamy varieties with inclusions of shingles, rubble material and boulders. The chemical composition of the water fraction suggests a marine or lacustrine-lagoon origin of deposits. The detected fauna remains bear no evidence of diatoms. Likewise in King George Island, lacustrine-marine sediments of the Bunger Hill Oasis are devoid of spores and pollen.

The Larsemann Oasis area (Progress station (Fig. 1)) was drilled on the Crystal lake shore (BH A1-07), in the bottom of a temporary stream valley (BH A2-07) and on the strip partitioning the lakes of Reid and Scandrett (BH Lars_bur 6/13). Here, dominantly moraine sandy sediments with inclusions of

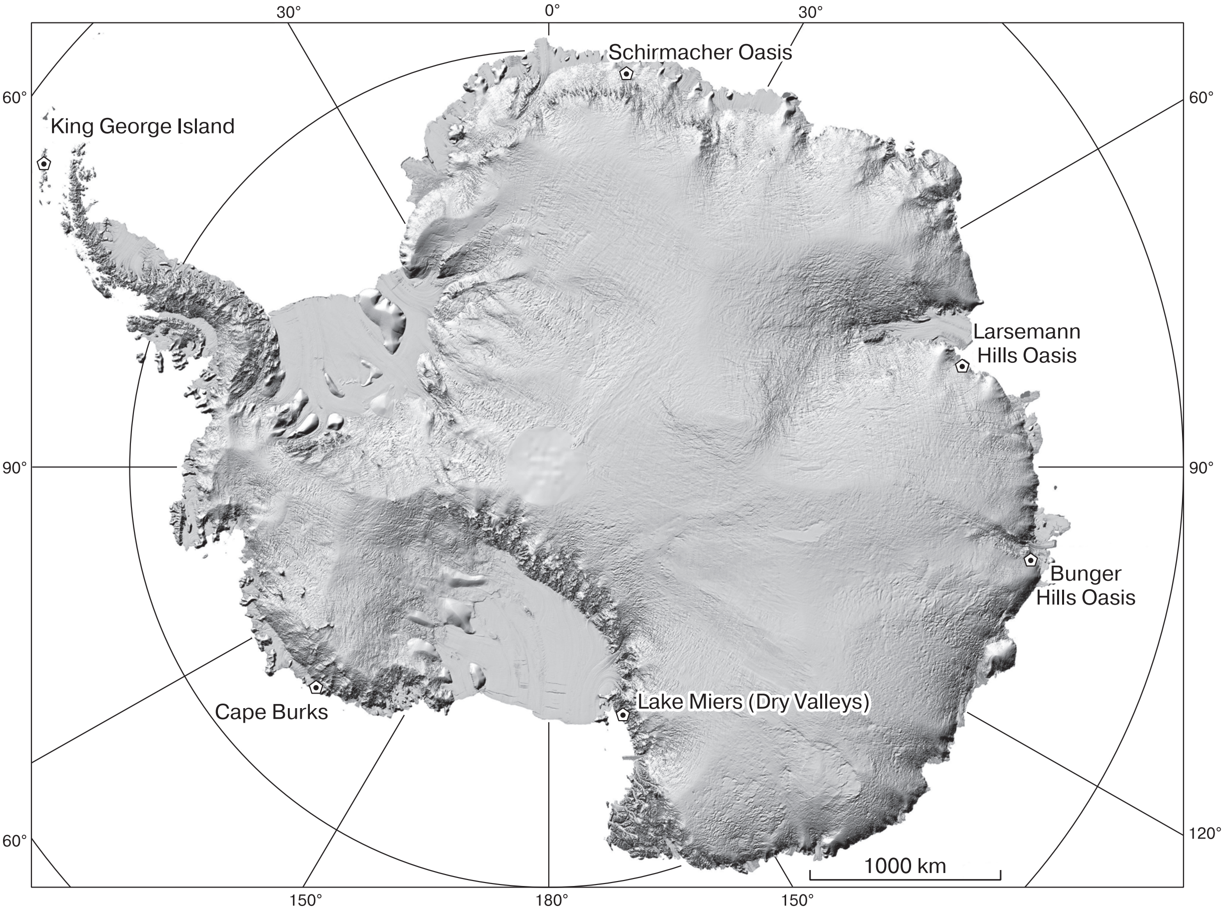

Fig. 1. Schematic of sediment sampling sites in Antarctica.

The Reference Elevation Model of Antarctica (REMA) served as the base.

gravel and boulders (2.0–5.5 m thick layer) occurring from the surface downward, have a massive, layered, and rarely basal cryogenic texture. Beneath the moraine sediments, borehole Lars_bur 6/13 penetrated lacustrine-lagoon sandy-loamy sediments, admixed with clayey particles and gravel, and diatom shells [*Demidov et al., 2013*].

Results of the dry combustion analysis showed total carbon content to be not more than 1.7 % and organic carbon not more than 0.3 % in the studied sediments of coastal oases. The value of Eh varies from +200 mV in the oxidized terrigenous facies with slightly acidic and neutral pH values 5.7–7.6 (Larsemann oasis) to –280 mV in strongly reduced alkaline and strongly alkaline horizons of marine sediments with pH 8.0–10.1 (King George Island and Bunger Hills Oasis).

RESULTS AND DISCUSSIONS

Some of the studied permafrost-affected soils of the ice-free oases in Antarctica revealed the presence of methane. Methane concentrations detected in the sediments were 20–330 μmol/kg (in the marine terrace of King George Island), trace amounts (moraine sediments), and <200 μmol/kg (lacustrine-lagoon sediments in the Larsemann, Schirmacher, and Bunger oases) (Fig. 2). The carbon isotope composition of methane analyzed in 15 samples of permafrost sediments from King George Island and the Bunger Hills Oasis spans the interval from –81 to –94 ‰ and therefore clearly indicate its biogenic origin [*Abramov et al., 2011*]. The MS chromatography methods showed that initially methane-rich specimens of the permafrost sediment from the coastal oases bear no signs of methanogenesis in their anaerobic microcosms, although results of the 16S rRNA clone libraries method analyzed the archaea community inhabiting permafrost sediments of the marine terrace of King George Island and lacustrine sediments of the Bunger Hills Oasis, revealed dominant phylotypes which are found to be most closely related to methane-producing archaea in the permafrost sedimentary rocks of marine and lake origin. The horizon of marine sediments was characterized by diverse phylo-

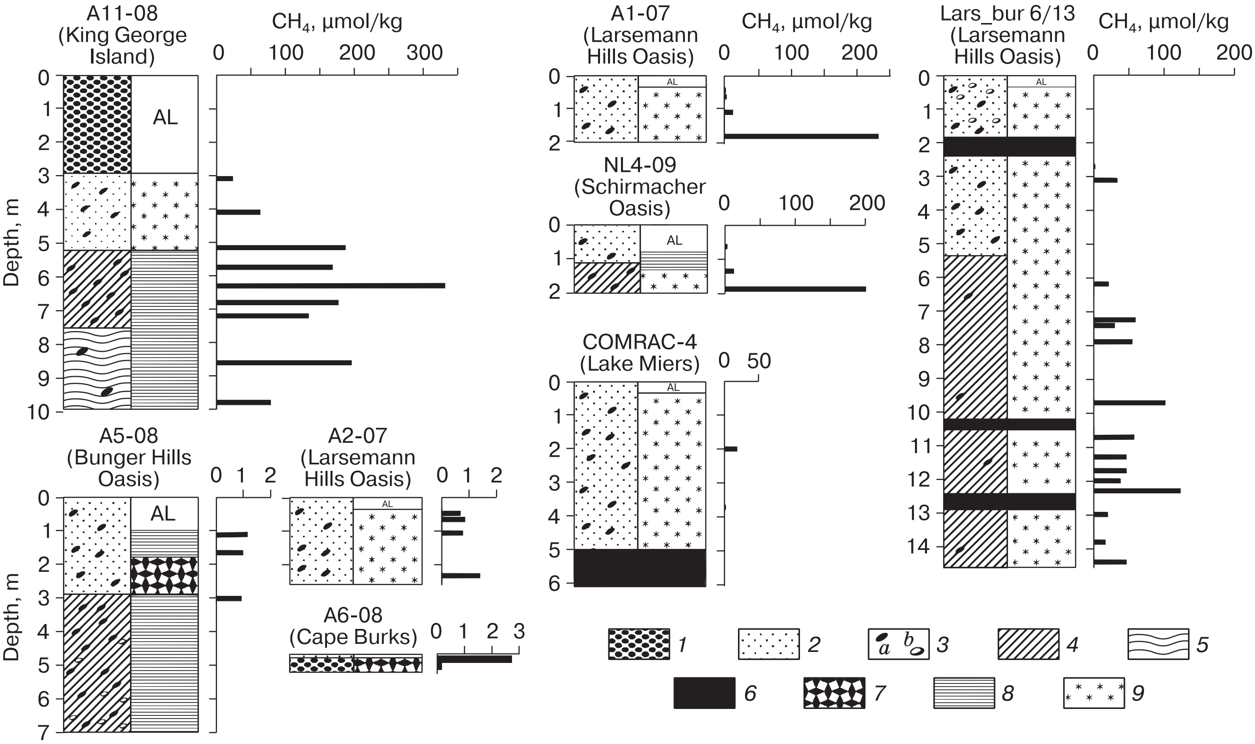

Fig. 2. Lithological composition, cryogenic texture and methane concentrations of headspace samples from boreholes.

1 – shingle; *2* – sand; *3* – inclusions of rubble material (*a*) and boulders (*b*); *4* – loamy soil; *5* – clay. Cryogenic texture: *6* – ice, *7* – basal, *8* – laminated, *9* – massive.

types of the genera Methanosarcina, Methanobrevibacter, Caldivirga, Methanogenium, Methanolobus and Methanoculleus, and by two dominant (i.e. represented by at least three clones) phylotypes of the class Methanomicrobia. The archaeal diversity in the sediments of the Bunger Hills Oasis was found to be lower and was characterized by only two representatives of the class Methanomicrobia [*Karaevskaya et al., 2014*]. Utilization of the radioactively labeled substrates allowed to identify active methanogenesis in these sediments (Table 1). As follows from Table 1, the most active methane formation occurred in specimens sampled from the sediments in the marine terrace of King George Island (BH A11-08) at depths of 4 and 9 m.

As it was highlighted in by [*Gilichinsky et al., 2007*], biogenic methane (up to 16 μmol/kg) was also discovered in epicryogenic mid-Pleistocene lake sedi-

Table 1. **Introduction of the radioactive label into methane under anaerobic cultivation using radioactive-labeled substrates**

Borehole	Depth, m	CH_4 content in the sample, μmol/kg	Methane generation from			
			$Na^{14}CH_3CO_2$		$NaH^{14}CO_3$	
			count, cpm	*r*/*R*, %	count, cpm	*r*/*R*, %
Larsemann Hills Oasis (Progress Station)						
A1-07	1.9–2.0	230	757.99	0.007	163.67	0
King George Island (Bellingshausen Station)						
A11-08	4.1–4.2	62	1318	0.012	88	0
A11-08	5.0–5.2	186	458	0.004	4132	0.005
A11-08	6.1–6.2	331	452	0.004	155	0
A11-08	8.8–9.0	194	671	0.006	159 570	0.23

Note. Blanc count did not exceed 70 cpm; *r* is radioactivity of newly generated methane; *R* is radioactivity of the introduced label.

ments of the Dry Valleys (Miers Lake, Fig. 2), while the presence of abiogenic methane, as well as its homologues (ethane, propane) and ethylene, was identified in sandstones of Mount Feather (Sirius Group) in the Dry Valleys.

Despite the fact that during anaerobic incubation of a methane-containing specimen, we observed active methane production recorded chromatographically, the isolation of methane-producing organisms into a pure culture from this microcosm failed to be successful. Methane abundance in culture headspace of the samples from permafrost sediments in the lake Miers area, following the multiyear period of cultivation in anaerobic conditions reached 40 %, which expressly indicated the presence of active methane-producing archaea in these deposits, however after repeated inoculation, the microorganisms would not grow. The metagenomic analysis of the microcosm alone allowed to determine the presence of methanogens that belong to the genus Methanosarcina [*Vishnivetskaya et al., 2018*]. We have demonstrated that a thin methane-containing sandy horizon penetrated by BH 4-95, from which the process of methane formation was reported, marked the ancient lacustrine sediments whose age was determined to be about 15,000. Later, as Miers lake shrank in size, the exposed lake sediments experienced freezing and were subsequently overlain by the dominantly aeolian deposits.

Similarly, methane marked the lacustrine-lagoon deposits in the Larsemann Hills Oasis area (Progress Station), overlain by a moraine [*Demidov et al., 2013*].

Analysis of the three Antarctic – Dry Valleys, King George Island and the Larsemann Hills Oasis – has therefore provided a compelling evidence that methane and methane-producing microorganisms act as indicators of the past depositional environments. Sometimes methane is interpreted to be the only indicator that can be used to reconstruct the conditions extant at the time of deposition. Thus, a methane-containing soil horizon within the deposits penetrated by BH 4-95 (Miers Valley), was no different from the overlying sediments. Inasmuch as the content of organic carbon was a hundredth of a percent throughout the well, it is the presence of methane that could provide insights about the history of this area evolution. As a rule, methane is found in epicriogenic strata [*Rivkina et al., 2007*], however, the fact that conditions favorable for methanogenesis may also exist during syncriogenesis is not ruled out.

CONCLUSION

Our research results have shown that methane would mark sediments formed under hydromorphic conditions, where oxygen was either non-abundant or nonexistent, which subsequently created a redox situation that favored generation of biogenic methane.

Direct measurements of methane concentration in permafrost sediments of the Antarctic oases revealed its presence in the amount up to 330 μmol/kg. Methane-containing sedimentary rocks were formed under anaerobic hydromorphic conditions.

Methane-producing archaea are shown to be viable in the studied sediments and capable of producing methane when the sediment temperature increases. This inference is particularly relevant for analysis of climate change impact on the state of Antarctic permafrost, when the warmest permafrost sedimentary rocks (about –1 °C, as in sediments in the vicinity of Bellingshausen Station) become involved in the modern process of methane generation by methanogens, while the emission of newly formed methane into the atmosphere can, in turn, constitute an additional factor stimulating air temperature rise and thereby implementing the “feedback” scenario.

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