The results of 5- year experiment of methane production from frozen soils

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ABSTRACT
The question of the origin of methane and activity of methanogenesis at low temperatures remains unclear. Methane production was observed at low temperatures in the laboratory during a long-term experiment. Samples of frozen soils were taken from Yakutsk (Russia), Fairbanks (USA) and Tomakomai (Japan) and incubated at – 5 °C in order to study methane production. The soil was incubated in glass flasks for 4.5 years under anaerobic conditions. The measurement of methane content in the air of the flasks was conducted at various intervals, from one week to a month. During the experiment some samples were thawed and frozen again. There was little observed methane emission from the Yakutsk samples. The emission from the Alaskan and Tomakomai samples was slow. However, methane concentration in the air of flasks increased significantly at thawing.

RÉSUMÉ
L’origine du méthane et de l’activité des micro-organismes méthanogènes à basses températures demeure incertaine. La production de méthane a été observée à basses températures lors d’une expérience à long terme en laboratoire. Des échantillons de sols gelés ont été récupérés à Yakutsk (Russie), à Fairbanks (USA) et à Tomakomai (Japon). Ils ont été incubés dans des flacons en verre pendant une période de 4 ans et demi dans des conditions anaérobiques et à une température de - 5 °C afin d'observer une possible production de méthane. La mesure du contenu en méthane dans l’air des flacons a été effectuée à différents intervalles allant d’une semaine à un mois. Au cours de l'expérience, quelques échantillons ont été dégelés et gelés. À partir des échantillons de Yakutsk, peu d’émissions de méthane ont été observées. Les émissions produites à partir des échantillons d’Alaska et de Tomakomai ont été lentes. Cependant, la concentration de méthane dans l’air des flacons a augmenté de façon significative lors du dégel.

1 INTRODUCTION

A significant portion (20 - 50%) of ecosystem C and N cycling and soil-atmosphere trace gas fluxes can occur during winter. However, the nature, extent and regulation of many biogeochemical processes during winter are poorly characterized (Edwards and Cresser 1992).

Permafrost contains large deposits of methane (CH4) and other gases. Studies about the distribution and content of CH4 in frozen ground have been carried since 1980s - early 1990s [Solomatin, 1986; Kvenvolden, 1988; Arkhangelov, Novgorodova, 1991; Rivkina et al., 1992]. Methane concentrations in permafrost at the Bovanenkovo gas field are 3500 mlkg in gas-saturated layers (probably as a gas hydrate) and 35 ml/kg in others (Yakushev and Chuvilin, 2000). Edoma deposits on the Arctic coast of Siberia contain up to about 10,000 ppm (Moriizumi et al., 1995). Average methane content in permafrost could be estimated as 0.05-10 ml/kg in Eastern Siberia, near Yakutsk (Brouchkov, Fukuda 2003) and up to 40 ml/kg north-East Siberia (Rivkina et al, 2007). Because permafrost is nearly impervious, gases can be trapped in ice and empty pores for a long time

The main issues requiring research are: 1) whether there is large-scale gas accumulation in the upper permafrost, 2) why sudden gas blowouts occur in permafrost during well-drilling operations in different regions 3) what will happen to the accumulated reserves of methane in climate change, because CH4 is a potent greenhouse gas with ~25 times the radiative effect of carbon dioxide (CO2) on a 100-y time scale (Boucher et al. 2009). The composition, content and genesis of gas migration in frozen soils is still poorly known, making definitive conclusions difficult. Freeze-thaw cycles are now also thought to contribute to the seasonal cycle of emissions of CH4 [Mastepanov et al., 2008], but the mechanisms remain uncertain.. It was found that methane content in the active layer is much lower at the beginning of winter than at the end. Methane collection in the active layer in the winter period is about 100 ml/kg, and could be from 4 to 9 times more then in summer (Glotov, 1991). Methane emission in tundra is a few mg/m2 a day in summer, and one third of the total emission in winter season (Inque et al., 1997). There is a methane flux at negative temperatures up to 0.05 g/m2 a day (Ota N. et al., 2000).

Some recent studies (Graham D. et al, 2011) show that microbial activities are largely responsible for whether the permafrost will be a net source or sink of greenhouse gases in the coming decades.

The amount of CH4 emitted from an ecosystem is the balance between CH4 production (methanogenesis) and CH4 oxidation (methanotrophy). It is widely believed that the primary mechanism for determining the presence of biogenic CH_4 in the permafrost is its formation in sediments at positive temperatures and further cryopreservation during freezing. But there is some possibility for methane production in cold environments. Methanogens start to produce methane at positive temperatures. There has been recent interest in biogeochemical processes during winter in temperate ecosystems. Studies have demonstrated that root and microbial processes are surprisingly active in cold (0 - 5 °C) and even frozen soils (Vogt et al. 1986, Coxson and Parkinson 1987, Taylor and Jones 1990, Sommerfeld et al. 1994, Clein and Schimel 1995, Melloh and Crill 1995, Brooks et al. 1996). Many isolates showed psychroactive growth at -2.5 °C and acetotrophic methanogenesis continued between 5°C and -17°C. (Vishnivetskaya et al., 2006; Gilichinsky and Rivkina, 2011).

Thus, there is no doubt that methane is formed at low temperatures, but direct experimental confirmation is still needed. This work was carried out to assess the contribution of biogenic methane emissions at low temperatures. Samples of frozen soils were incubated at -5 °C in order to study possible methane production for 4.5 years.

2 METHODOLOGY

Experimental studies were carried out upon frozen Yakutsk (Russia), Alaska (USA) and Tomakomai (Japan) soil. The Yakutsk soil sample was from the Neleger site at a depth of 0.5 m in the active layer. This is mostly a larch forest area of the high Lena River terrace. The sample was a sandy loam and had a natural salinization of about 0.08%. The Alaskan sample was taken from near Fairbanks, Alaska, from an active layer consisting of eluvium deposits. The Tomakomai sample was taken from the experimental forest of Hokkaido University, in a floodland of a creek from the depth of 0.3 m in over watersaturated silt soil.

All samples were over saturated by a concentrated solution of NaCl. The soil was put into glass-flasks that were covered by a rubber plug of diameter of about 2 cm. The flasks were placed in a refrigerator in a vertical position and frozen at the temperature of -5°C for three days. After freezing the air in the flasks was exchanged to nitrogen to create anaerobic conditions. Some samples were sterilized at 120°C for 20 min (Table 1). There were used samples of different weight. The difference in weight of the samples is caused by necessity to study the relationship between weight and the amount of gas for the same samples. For example, the weight of the same samples P7 and P4 is 500 and 1300 grams. Table 1 shows main characteristics of the samples used in the experiment.

Table 1. Some characteristics of the samples used in the experiment

Sample site	Sample №	Sample weight, g	Water content:
Yakutsk	P1	500	1
Yakutsk (sterilized)	P2	500	1
Tomakomai	P4	500	7.5
Tomakomai (sterilized)	P5	500	7.5
Tomakomai	P7	1300	7.5
Alaska	P8	1300	0.3

Then, the flasks were placed in the refrigerator at the temperature of -5°C for incubation (Fig.1).

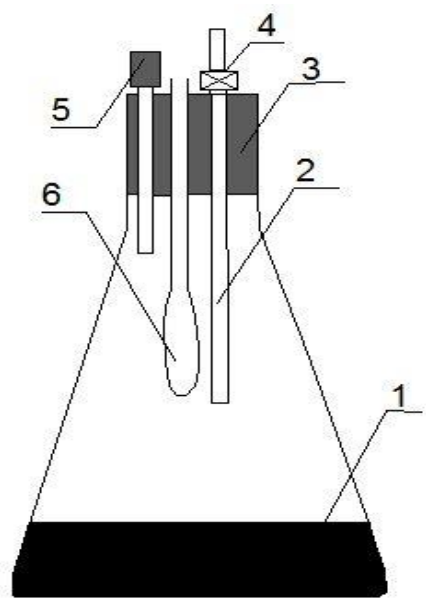


Figure 1. Incubation flask : 1- soil sample; 2- pipe nitrogen input; 3- rubber plug; 4-tap; 5- sampling plug; 6- plastic bag for maintenance of the constant pressure (Brouchkov et al., 2003)

Measurement of methane content in the air of the flasks was conducted through the rubber plug by a syringe at various intervals from a week to a month. The samples were incubated for 4.5 years. During the experiment thawing the samples was held at a temperature of 20 °C after 6 months (day 163) and 1.5 year (day 542). After melting the samples were repeatedly frozen at -5°C. The gas chromatograph Shimadzu GC-8A that was used had an accuracy of the methane content measurements of 0.002 ppmv.

Methane can be present in the soil water and also adsorbed on the surface of soil particles. It is not possible to exclude completely presence of small air bubbles in soil suspension over-saturated by water; however, we assume it is negligible small. The solubility of methane in water is low: at 20 °C it is 0.033 (v/v) (Yaws and Yang, 1992). After freezing the air in the flasks was exchanged to nitrogen; however, an amount of methane (0.61–1.43 ppmv) was present in the flasks (Table 2)

Table 2. Value of methane concentration 1 day after the samples were frozen

Sample №	P1	P2	P4	P5	P7	P8
methane concentration, ppmv	0.61	0.98	1.09	1.43	0.87	0.62

3 RESULTS

In Yakutsk samples (P1-P3) observed change in the concentration of methane in the flask is negligible, and practically do not differ from each other for three samples. The maximum difference of concentrations in the flask was 1,57 ppmv, that turns out 16.96 µl /kg of soil. So description will focus more on the results of the other samples.

For Tomakomai and Alaska samples there is concentration variation observed in the flask. Sampling the gas content was held in 3 phases. Constant temperature in the flask was -5°C, at the end of the first phase (day 163) and at the end of the 2nd phase (day 542) thawing the samples produced at 20 °C (Fig.1). Table 3 below shows some values of the concentration of methane in the flask during the experiment.

Table 3. The concentration of methane in the flask (ppmv) for samples Tomakomai (P4-P5-P7) and Alaska (P8)

day	79	163	164	326	542	659	1177
P4	1.96	9.48	14.81	16.1	404.26	555.62	396.42
P5	1.38	1.63	2.12	2.4	5.09	5.57	4.63
P7	1.50	27.26	42.44	37.42	1536.79	1002.25	1266.6
P8	0.74	0.89	1.57	1.65	89.8	5.35	7.91

As a result of thawing produced at day 163 had released some gas. Thus, the concentration of the sample in a flask of Alaska (P8) was 0.89 ppmv, and Tomakomai samples (P4, P5 and P7) 9.48 ,1.63 and 27.26 respectively. Thus it can be seen that during the first part of the experiment in some samples there was some slow process of methane formation at a temperature of - 5 degrees After freezing to -5 degrees was immediately measured the concentration at 164 day and there was increase in the concentration in all the samples, after this there was a trend to decreasing the concentration till the 2nd melting. And at the end of the second stage, before re-thawing concentration value was the same or smaller than at the 164 day, but still more than released during thawing.

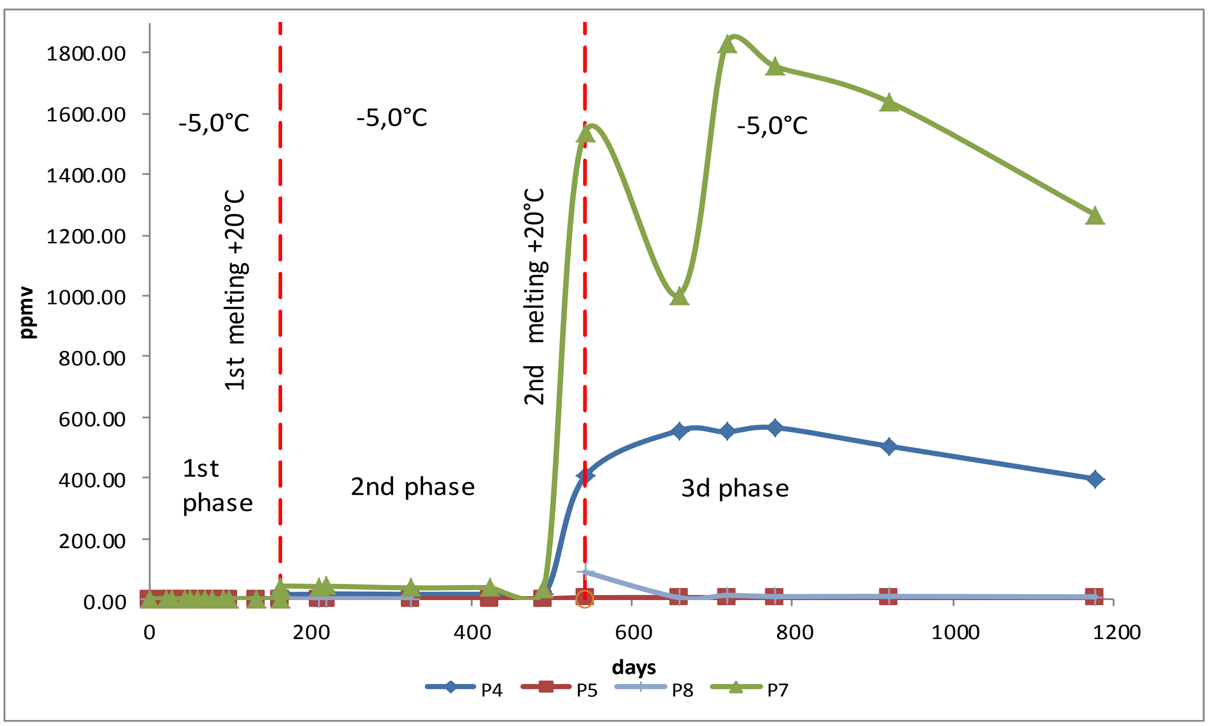


Figure 2. Changing the concentration of methane in the sample flask P4 (Tomakomai), P5 (Tomakomai sterilized),P8 (Alaska) for the whole time of experiment (1177 days)

During the second thawing there was a significant methane release in all samples except P5. For the sterilized sample P5 changes were minor: the concentration of released gas was 5.09 ppmv in the flask, which is slightly different from the indications during the course of the experiments. For Alaska and Tomakomai samples the values are higher: 89.8 for P8 (Alaska), 404.26 for P4 (Tomakomai) and 1536.79 for P7 (Tomakomai). Thus, the concentration of methane in flasks is hundreds or even thousands of times greater than the initial value and tens of times greater than the amount released during the first thawing. For the initial value of the methane content we accept the concentration in the flask measured on the first day of freezing of the samples (Table 2 above).

Unfortunately, there is no measurement data immediately after freezing samples at the 3rd stage of the experiment. The next measurement is carried out in 117 days. At this point all samples except P4 (there was a sharp decrease and then sharp increase even after 59 days to greater amounts than at the second thawing) observed a decrease in methane concentration in the flasks, small fluctuations of gas amounts with decreasing trend.

The quantities of methane (in microliters) for the samples recalculated per kilogram of soil are shown in Table 4.

Table 4. Methane content in the flask per kilogram of soil (μl /kg)

day	1	162	163 (melting)	491	542 (melting)
P4	70.22	162.76	609.33	952.36	25977.45
P5	91.94	100.24	104.92	120.03	327.15
P7	21.51	36.72	673.86	836.37	37982.42
P8	2.33	3.06	3.36	6.25	339.48

Here, we see that during this part of the experiment, which lasted for 1.5 years (542 days) there was accumulated a huge amount of methane (Table 5).

Table 5. The amount of methane accumulated 542 days (the difference between the methane released in the second thaw and initial content)

sample №	concentration, ppmv
P4	25907.23
P5	235.21
P7	37960.91
P8	337.16

Significant differences in the dynamics of methane in samples of Alaska and Tomakomai caused, in our opinion, the differences in the composition and activity of the microbial community. Within this experiment, we did not made the identification of methanogenes, which were possibly responsible for the methane production. The type of soil, organic material and water content are also essential for the microbial activity. So we can only make general conclusions about the capabilities and rates of gas accumulation and production at low temperatures without specifying types of microorganisms responsible for the process.

Tomakomai samples P4 and P7 were identical in composition and genesis but the difference was in their weight - 700g and 1300 g, respectively. Nevertheless, in per kilogram of soil amount of released gas was not the same : 25977,5 (μl /kg) and 37982,42 (μl /kg). So we may say that the relationship between the size of the sample and the gas content is not linear.

Moments increasing concentration alternated with some moments of reducing the concentration of methane, with a maximum variation occurs during the third phase, after the second thawing

Also we estimated the rate of methane production and emisson (Table 6,7).

Table 6. The average rate of methane production in frozen samples during the 2 phases of the experiment (μl / kg * day)

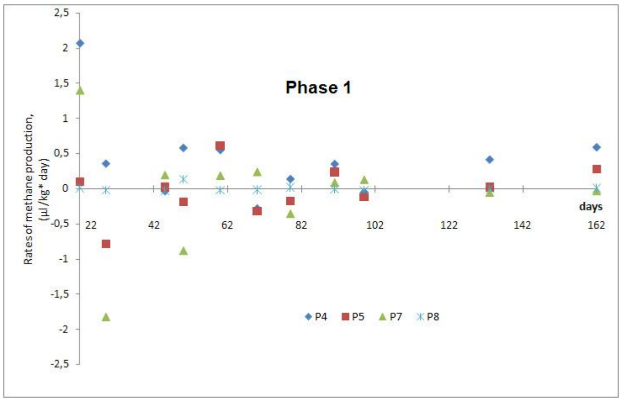
	P4	P5	P7	P8
Phase 1	3.33	0.08	4.03	0.01
Phase 2	66.76	0.58	98.18	0.88

Table 7. The rate of methane emissions during the 3 phases of the experiment (maximum speed uptake and emission of methane (μl / kg * day)

Sample №	phase 1		phase 2		phase 3	
	Max uptake rate	Max rate of emission	max uptake rate	Max rate of emisiion	max uptake rate	max rate of emission
P4	-0.28	2.07	-1.37	171.09	-27.42	82.43
P5	-0.79	0.62	-0.59	15.78	-0.44	0.26
P7	-1.82	1.40	-1.64	187.53	-111.96	340.99
P8	-0.02	0.14	-0.05	1.28	-2.71	0.46

The released methane on 163 day and 542 day was used to estimate the amounts of accumulated gas and average speeds of possible production in frozen samples. The estimation of methane emission and uptaking rates from frozen soils was provided using the changes in concentrations in the air of flask under below zero conditions

Figure 3 below shows changing the rates of methane emissions in the samples P4,P5 P7,P8 during 3 phases of experiment between thawing provided.



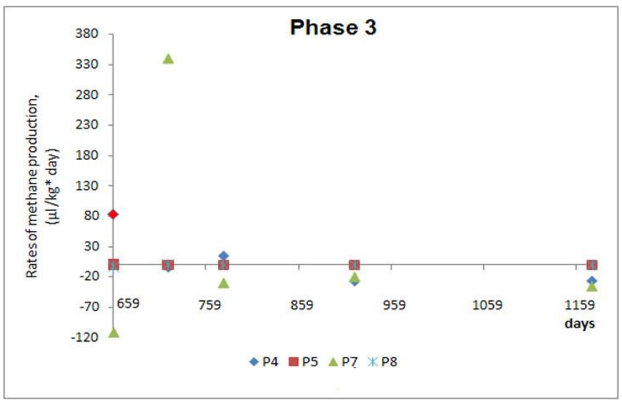
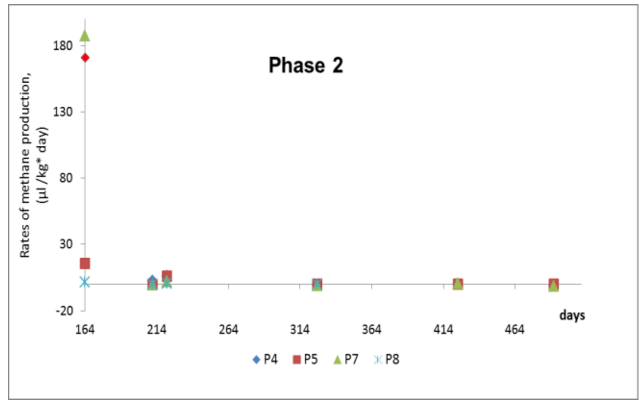


Figure 3. Changing the rates of methane emissions in the samples P4,P5 P7,P8 for the 3 phases of the experiment.

As it can be seen from the figures, during all three phases of the experiment there are going processes such as CH4 production (methanogenesis) and CH4 oxidation (methanotrophy). The rates of emission are very low throughout the first stage (maximum values are 2.07 µl / kg * day for methane production and 1.82 µl / kg * day for methane oxidization). Next, after thawing the samples and repeated freezing (phase2) maximum rates were observed only on the first day freezing (187.5, 171.09 µl / kg, 1 * day for P7 and P4), then the emission rates for all the samples decreased exponentially, reaching approximately the same values as in the first phase. The third phase of the experiment (after a huge release of methane during the 2nd melting) is characterized by the largest spread of emission and uptaking rates of gas . But finally, the speed is also reduced

4 DISCUSSION

It was a number of studies showing that methane production can occur at close to zero temperatures in soils, including recent ones (Shannon&White, 1994; Evgrafova et al., 2010; Tveit et all, 2015). It has being shown the CH_4 production rate at 4 °C was about 25% of that at 25 °C, and at low temperatures syntrophic propionate oxidation as well as electron acceptors and methanogenic biomass were the rate-limiting step for CH_4 production (Tveit et



all, 2015). Our studies can lead to a conclusion that the methane production at -5°C is still possible in soils, however, the rates may vary depending on type of soil. Surprisingly we have got more methane from area which is far from the cold regions unless Hokkaido island might be considered as a northern region. Permafrost soil were less productive perhaps due to lower methanogenic biomass. It is also clear the methane production can be associated with methanotrophy which occurs in the same soils with high water content and at low temperatures. Long term existence of permafrost increase chances for methane collection in frozen soil, and amount of methane trapped in cryolitozene is still an important issue. Preliminary study of methane content in permafrost (Brouchkov&Fukuda, 2002) is an evidence of wide range of rates of the methane production as well as methane consumption at low temperatures.

Possibility of methane producing at low temperatures is established by our experiments in the laboratory during the 4.5 years under anaerobic conditions at – 5 °C. The different rates of methane production are caused, in our opinion, by the differences in the composition and activity of the microbial community. Although the exact number of microorganisms is not known, it is obvious that they are present and exhibit metabolic activity at a negative temperature (-5 °C).

The type of soil, organic material and water content are also essential for the microbial activity. It was observed during the experiment that their activity and consequently the production of methane is significantly increased since the first thawing. The amount of accumulated methane exceed the intial hundreds of times for 1.5 years.

For example methane concentration in permafrost deposits in Yamal peninsula or Edoma deposits in the Arctic coast of Siberia is high (up to 500 ml/kg), which is larger than present atmosphere content in thousands times. Our previous study (Brouchkov & Fukuda, 2003) also shows the large amount of methane trapped in permafrost of the Central Siberia. However these facts do not directly indicate methane production in permafrost, even considering possible production of methane under freezing temperature. Also the study of distribution of methane concentration in frozen soils at different landscapes has shown that the oldest permafrost in the forest is characterized by maximum methane content (Brouchkov & Fukuda, 2003). Deposits of the lower part of the forest contain less methane; probably that part was fired, because it consists of younger trees and permafrost could melt there.

5 CONCLUSIONS

Experiments have shown a slow production of methane in different soils at -5°C. The low permeability of ice and soils and insignificant solubility of methane in water (or particularly in unfrozen water films) strongly limits its possible diffusive transport in the frozen soil. The release of methane by diffusion through the icy soil was expected to be extremely small, and the experiments have taken months before measurable amounts could be found. However, methane released immediately after thawing.Therefore emitted methane is probably a small part of produced amount.

Methanogenes are possibly responsible for the production of methane production in permafrost. The temperature of permafrost is also not stable: it can be argued that methane production is larger at higher temperatures. Finding of a number of methanogenes in permafrost present another evidence of the opportunity

The rates of methane production decrease in time. Thus, there is a possibility that methane is formed at low temperatures. However long-term forecast of methane content in frozen soils is still problematic. The experimental results still do not directly suggest the long-term production of methane in permafrost.

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