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EXPERIMENTAL ARTICLES

Activity and Metabolic Regulation of Methane Production in Deep Peat Profiles of Boreal Bogs

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Abstract—The potential activity of methane production was determined in the vertical profiles of the peat deposits of three bogs in Tver oblast, which were representative of the boreal zone. In the minerotrophic fen, the rates of methane production measured throughout the profile did not change significantly with depth and comprised 3–6 ng CH_4-C g^{-1} h^{-1}. In ombrotrophic peat bogs, the rate did not exceed 5 ng CH_4-C g^{-1} h^{-1} in the upper layer of the profile (up to 1.5 m) and increased to 15–30 ng CH_4-C g^{-1} h^{-1} in the deep layers of the peat deposits. The distribution of fermentative microorganisms and methanogens in the profiles of peat deposits was uniform in all the studied bogs. In bog water samples, the presence of butyrate (up to 14.1 mg l^{-1}) and acetate (up to 2.4 mg l^{-1}) was revealed throughout the whole profile; in the upper 0.5-m layer of the ombrotrophic bogs, formate (up to 8.9 mg l^{-1}) and propionate (up to 0.3 mg l^{-1}) were detected as well. The arrangement of local maxima of the fatty acid content and methanogenic activity in the peat deposits, as well as the decrease in the acetate concentrations during summer, support the hypothesis that the initial substrates for methanogenesis come from the upper peat layers. It was established that the addition of sulfate and nitrate inhibits methane production in peat samples; the changes in the concentrations, recorded in situ, may also influence the methane content in peat layers.

Keywords: methanogenesis, peat bogs.

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Bog soils are the largest basin for long-term accumulation of carbon on Earth. At the same time, they are the largest natural source of methane (CH_4), which is the second most important greenhouse gas after carbon dioxide (CO_2). Despite the recent intense studies, the regulatory mechanisms of methane production and emission to the atmosphere are still poorly understood. A number of works by Russian and foreign scientists have demonstrated that the rate of methane flux depends significantly on the water level, temperature, pH, and the composition of the vegetation coverage [1, 2]. However, the patterns of temporal and spatial variation of these fluxes cannot be exhaustively explained by the effect of physicochemical factors; further investigations of the biological processes responsible for methane production and transformation in bog deposits are therefore required.

The process of methane production (methanogenesis) occurs due to microbial activity. Two metabolic pathways of methane production exist: aceticlastic, in which acetate is an immediate precursor of CH_4, and autotrophic (hydrogen-dependent), in which methane is produced from hydrogen (H_2) and CO_2 [3]. In bog soils of the Northern Hemisphere (boreal zones and tundra), the hydrogen pathway of methanogenesis prevails, whereas the role of the aceticlastic pathway is insignificant [4]. It has been suggested that the role of acetate as a methane precursor diminishes as temperatures decrease and the bogs become more oligotrophic. This may cause accumulation of acetate in peat deposits [5].

The methane from deep layers of peat has traditionally been considered as inert and not mobile. This corresponded well with the common paradigm regarding the division of a peat deposit into two layers, "active" and "inert" [6], or "acrotelm" and "catotelm" [7] with a boundary along the minimum level of bog waters. However, the recent data on the hydrochemistry [8], water cycle [9], dynamics of dissolved gases [10], and the temperature regime of the peat deposit [11] have seriously undermined this concept. Radioisotope studies have demonstrated that, in the deep layers of peat deposits, a considerable portion of methane is produced from "fresh" organic matter. As a consequence, the ^{14}C values of the methane released to the atmosphere are

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close to those of the atmospheric CO_2 [12]. Using the ^{14}C isotope balance, the amount of methane produced in the deep layers of peat deposits was estimated at 1.2 g CH_4 m^{-3} $year^{-1}$. It was suggested that newly formed organic matter arriving at the deep layers from the upper ones serves as the main carbon source for methanogenesis [13]. The input of methanogenesis substrates can be due to the hydrologic processes of the movement of bog water [9], as well as to thermal convection [11].

The chemical factors that controls methanogenesis in pure cultures and in some natural communities are nowadays relatively well understood. In marine sediments, the competitive inhibition of methanogenesis by the products of sulfate reduction had been previously detected [14]. In tundra soils, the advantage of homoacetogenic bacteria over methanogens in their competition for C-substrates resulted in the accumulation of low-molecular-weight fatty acids [15]. However, the patterns found in these ecotopes cannot be automatically applied to boreal bogs and require further experimental analysis.

The goal of this work was to assess the intensity of methane production in deep layers of peat deposits of three bogs in Tver oblast, as well as to study the biological and chemical regulatory factors of this process.

MATERIALS AND METHODS

Peat samples were collected in June 2002 in Tver oblast, at experimental sites located at the Western Dvina Forest and Bog Station of the Institute of Forest Science, Russian Academy of Sciences. The following bogs of various types characteristic of the boreal zone were selected: the ombrotrophic Petrilovo bog on moraine loam, the ombrotrophic Sosvyatskoe bog on sands, and the minerotrophic fen Zailov'e with groundwater upwelling. The peat deposits are 7, 5, and 4 m thick, respectively. The detailed characteristics of these sites are reported in [9, 11]. Peat samples were collected in the form of whole cores (60 × 150 mm) with a peat drill TBG-1. The samples were taken every 25 cm to the depth of 1 m and every 50 cm in the deeper layers. After sampling, the peat cores were placed in PVC cylinders of the same size. The cylinders were hermetically sealed with butyl rubber stoppers and transferred in an isothermal bag to the station laboratory. Within 2–4 h after sampling, a number of samples (10–11 g) were obtained from the central part of the cores under argon flow and placed in Hungate tubes. The tubes were then vacuum-degassed twice and filled with argon (at a rate of 50 ml min^{-1}, 5 min). Prior to incubation, the peat-containing tubes were stored at 4°C for not more than two days. The ratio between the volume of peat and of the gas phase in the tube was approximately 3 : 1. The exact weight of the peat and the volume of the gas phase in each tube were measured on completion of the incubation experiments. The peat humidity was 94–97% (wt./wt.).

A number of bog water samples were collected both simultaneously with peat sampling and also on April 21 and August 18, 2002. The sampling was carried out with stationary piezometer systems installed with the vertical step of 0.25–1 m; the construction of these systems provides for the independent study of various horizons of peat deposits [9]. To determine the content of dissolved gases immediately after sampling, 5-ml water samples were transferred into 10-ml vacuum-degassed tubes with a syringe. The procedure was performed in triplicate. To determine the chemical composition of bog waters, 10-ml water samples were fixed with sodium merthiolate (0.001%).

Incubation experiments. Prior to the incubation, the tubes with peat samples were vacuum-degassed twice and filled with argon. In order to assess the rates of methanogenesis, the obtained peat samples were incubated at 25°C for 14 days. From each tube, 0.5-ml samples of the gas phase were taken with a syringe in order to determine the methane content on a Model 3700 gas chromatograph (Russia) equipped with a flame-ionization detector. The accumulation of methane followed the zero-order kinetics; the rate of methanogenesis was calculated from the following equation: $d[CH_4]/dt = k_o$ per gram of peat at the natural humidity level. At the end of the incubation experiment, the integral accumulation of carbon dioxide in the gas phase was determined on a Model 3700 gas chromatograph (Russia) equipped with a katharometer detector.

The effect of additional sources of carbon and inorganic ions on the rate of methanogenesis was studied using the peat samples from Sosvyatskoe bog (sampling depth 2.0 m) as an example. Organic compounds (glucose, potassium acetate, sodium formate, methanol, and methylamine; all as water solutions) were injected with a syringe into the tubes containing peat samples so that the initial concentration was 10 mg C g^{-1} dry peat (10 mM); alternatively, the headspace in the tubes was filled with the gas mixture consisting of H_2 and CO_2 (20 : 80 vol./vol.). The initial concentration of potassium nitrate was 10 mg N g^{-1} of dry peat.

To study the relations between methanogenesis and sulfate reduction, the peat suspension (10 g of wet peat plus 2 ml of water) was prepared under anaerobic conditions, as described above, and incubated at 25°C for 15 days. Potassium acetate (10 mg C g^{-1} of dry peat) was added as a methanogenesis substrate; potassium sulfate (10 mg g^{-1} g of dry peat, which corresponds to 0.7 mM SO_4^{2-}) served as a sulfate reduction substrate. Sodium molybdate (1.0 mM) and chloroform (0.1 mM) were used as inhibitors of sulfate reduction and methanogenesis, respectively [16]. During the incubation period, a series of measurements were made to determine the dynamics of methane concentration in the gas

phase; the contents of inorganic anions and organic acids in peat were determined at the end of the experiment.

Determination of the titer of viable cells of anaerobic microorganisms was carried out by inoculation of serial tenfold anaerobic dilutions of peat samples onto specific media in Hungate tubes. Microorganisms were cultivated on a modified mineral medium (pH 4.5) [4] containing (mg l^{-1}) NH_4Cl, 200; KH_2PO_4, 200; $MgCl_2$, 10; $CaCl_2 \times 6H_2O$, 10; NaCl, 200; and resazurin, 4. Before inoculation, the tubes were gassed with nitrogen. Then, for enumeration of fermentative bacteria, sterile solutions of sodium dithionite ($Na_2S_2O_4$, 0.2 mM) and glucose (5 g l^{-1}) were added; for enumeration of aceticlastic methanogens, the tubes were supplemented with sodium acetate (5 g l^{-1}); and for enumeration of autotrophic methanogens, the gas phase was replaced by a mixture of H_2 and CO_2 (20 : 80 vol./vol.).

The total number of bacteria in the peat samples was determined by fluorescence microscopy. To desorb bacterial cells from the surfaces of soil particles, the samples supplemented with detergent Tween 80 (Serva, Germany) and the M-30 silicone anti-foam emulsion (Serva, Germany) were processed using a UZDN-A ultrasonic device (Russia) (3 min; 22 kHz). Then the flocculating mixture containing $Ca(OH)_2$ and $MgCO_3$ (2 : 5) was added. The resultant mixture was left for 5 min to let the particles and colloids precipitate. The supernatant containing bacterial cells was filtered through non-fluorescent polycarbonate filters (Poretix, United States), stained with 4% fluorescein isothiocyanate solution (Serva, Germany), and examined in a Lumam I2 epifluorescence microscope (Russia).

The chemical analysis of bog waters was performed in order to determine the contents of C_1–C_4 volatile fatty acids (VFA) and anions NO_3^-, PO_4^{3-}, and SO_4^{2-}. For the removal of suspended solid particles and hydrophobic humus compounds, the samples were centrifuged at 5000 *g* for 15 min and passed through a column (15 cm) packed with activated charcoal and then through a 0.2 μm membrane filter (BioRad). The concentrations of VFA and anions were determined by high-performance gas-liquid chromatography on an IC–1000 ionic chromatograph (Biotronic, Germany), from the retention time and peak areas of the standard solutions and studied specimens.

RESULTS AND DISCUSSION

It has been previously demonstrated that the content of dissolved methane in deep peat layers undergoes a gradual depletion during the period from spring to fall and increases in winter [10]. It has been assumed that the revealed patterns are associated with de novo methane production. The results of the experiments with peat samples incubated without the addition of any supplementary substrates confirmed the possibility of methane production in the deep layers of the peat deposits. Significant differences were found between the distributions of dissolved methane and methanogenic activity along the profile in bogs of various types (Fig.1). In Zailov'e fen, the rates of methane production were relatively uniform along the entire profile and ranged from 4 to 10 (7.7 ± 3.0) ng CH_4-C g^{-1} h^{-1} (Fig. 1a). The profiles of methanogenesis in the two ombrotrophic bogs were similar and differed significantly from that of the minerotrophic fen. In the upper part of the profile (to 1.5 m), the rates of methanogenic

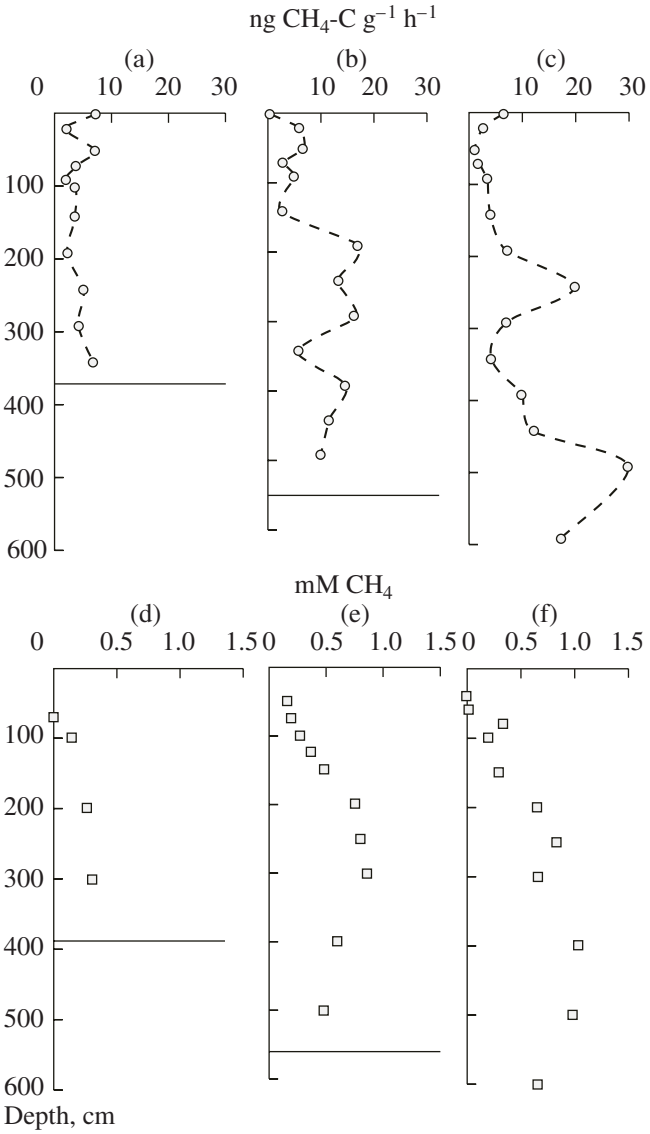

Fig. 1. Profile distribution of the methanogenesis rates in peat samples and of the dissolved methane concentrations in the peat deposits of the three bogs of the Tver oblast: Zailov'e fen (a, d), ombrotrophic Sosvyatskoe bog (b, e), and ombrotrophic Petrilovo bog (c, f). Experimental points on the plot are an average of five measurements.

Table 1. Integral accumulation of methane and carbon dioxide during incubation experiments with peat samples

Bog	Fen		Ombrotrophic			
	Zailov'e		Sosvyatskoe		Petrilovo	
	Concentrations in the gas phase, μg C g^{-1} day^{-1}					
Depth, cm	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2
5	0.47	18.37	0.03	13.29	0.43	3.23
20	0.14	11.90	0.37	2.2	0.19	2.83
50	0.47	9.30	0.45	3.94	0.07	4.63
70	0.24	6.46	0.26	2.89	0.08	5.75
90	0.13	4.48	0.33	4.96	0.23	4.62
100	0.25	6.30	N/D*	N/D	N/D	N/D
140	0.23	6.99	0.20	4.43	0.33	4.61
190	0.22	5.26	1.11	7.44	0.43	5.98
240	0.49	6.66	0.87	6.53	1.52	5.46
290	0.28	5.17	1.05	4.99	0.49	3.21
340	0.45	8.06	0.39	3.70	0.27	5.93
390			0.99	7.16	0.68	6.07
440			0.76	4.15	1.39	6.44
490			0.63	2.79	1.85	9.14
590					1.02	2.50

Note: * N/D stands for "no data".

activity ranged from 1 to 10 (6.1 ± 3.4) ng CH_4-C g^{-1} h^{-1} (for Sosvyatskoe bog) (Fig. 1b) and from 1 to 8 (4.7 ± 2.6) ng CH_4-C g^{-1} h^{-1} (Petrilovo bog) (Fig. 1c). In the deep layers of the deposits, the rates of methanogenesis increased and reached 20–30 ng CH_4-C g^{-1} h^{-1}. In the profile of Petrilovo bog, two local maxima were observed at depths of 2.5 and 5.0 m, while in Sosvyatskoe bog, the distribution of methanogenic activity at the same depths was relatively uniform.

The obtained data coincide with the profile of dissolved methane. In the ombrotrophic bogs Petrilovo and Sosvyatskoe, the methane content gradually increased from the surface down to the depths of 2–3 m (Figs. 1e, 1f), while in Zailov'e minerotrophic fen, the methane distribution along the profile was relatively uniform (Fig. 1d). The above-mentioned patterns of methane distribution in the peat deposits are in agreement with the mass-exchange indices, which depend on hydrologic [9] and temperature [11] factors.

The studied bog soils are characterized by low rates of methanogenesis as compared to other organogenic waterlogged soils. The high rates of microbiological processes of anaerobic transformation of organic matter by fermentative microorganisms may be a possible cause of this phenomenon. The measurements of integral accumulation of methane and carbon dioxide in the peat samples (Table 1) revealed that the amount of CO_2 carbon exceeded the amount of CH_4 carbon in all the studied layers of the deposit. The maximum differences, by one order of magnitude or more, were discovered in the upper 0.5-m layer of Zailov'e minerotrophic fen, as well as in the surface layer (up to 10–15 cm) of the ombrotrophic Sosvyatskoe bog. These are the horizons that are located above the bog water level and which often become partially aerated in summer. Comparable amounts of the CO_2 and CH_4 carbon were detected only in the underlying horizons of the ombrotrophic bogs Petrilovo and Sosvyatskoe, at depths of 6 and 5 m, respectively.

The distributional patterns of the potential methanogenic activity in various bogs and peat layers can be due to the following factors: (1) distributional patterns of methanogens; (2) competition or cooperation between

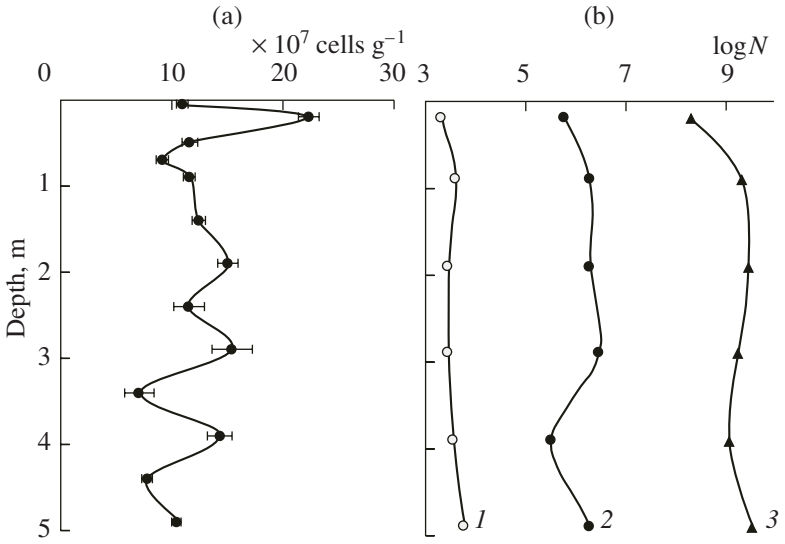

Fig. 2. Distribution of the total numbers of microbial cells determined by direct microscopic count (a), and the most probable numbers of microorganisms determined by inoculation (b), in the profile of the peat deposit of Sosvyatskoe bog: (*1*) aceticlastic methanogens; (*2*) autotrophic methanogens; (*3*) fermentative bacteria.

methanogens and other anaerobic microorganisms; and (3) differences in the major fluxes of methanogenesis substrates and/or methanogenesis inhibitors.

To validate the first hypothesis, the total number of bacterial cells and titers of anaerobic fermentative microorganisms, as well as of autotrophic and aceticlastic methanogens, were determined in samples from the peat deposit of the ombrotrophic Sosvyatskoe bog. Our studies have shown that the numbers of hydrogen (autotrophic) methanogens were two orders of magnitude higher than the numbers of aceticlastic methanogens, which is in accordance with the results obtained by other authors [4]. The distribution of bacterial cells in the peat layers was relatively uniform with a slight tendency to increase downward to the lowest layers and without pronounced local maxima (Fig. 2). Hence, the differences in the rates of methanogenesis may be due to the factors that regulate the methanogenic activity of microorganisms, rather than their cell numbers.

In our experiments with samples from the ombrotrophic Sosvyatskoe bog with high methanogenic activity, we studied the effect of additional carbon sources on the methanogenic activity. Substrates that can be utilized by methanogens either directly (H_2 + CO_2, acetate, methanol, and methylamine) or via the activity of syntrophic microorganisms (formate and glucose) were used. The obtained results (Fig. 3) demonstrate that the maximum effect resulted from the incubation in H_2 + CO_2 atmosphere. After 14 days of incubation, the methane content in the gas phase was ten times higher than in the case when no additional substrates were introduced; the rate of methanogenesis increased from 10.4 to 165 ng CH_4-C g^{-1} h^{-1}. The addition of acetate and formate stimulated methanogenesis to a lesser degree; the rates of methanogenesis were 47 and 38.8 ng CH_4-C g^{-1} h^{-1}, respectively. The addition of methanol and methylamine did not appreciably change the methanogenic activity. The addition of glucose enhanced the methanogenic activity significantly, up to a value of 91.7 ng CH_4-C g^{-1} h^{-1}. This fact may point to the presence in bog soils of syntrophic associations between methanogens and other obligate anaerobic

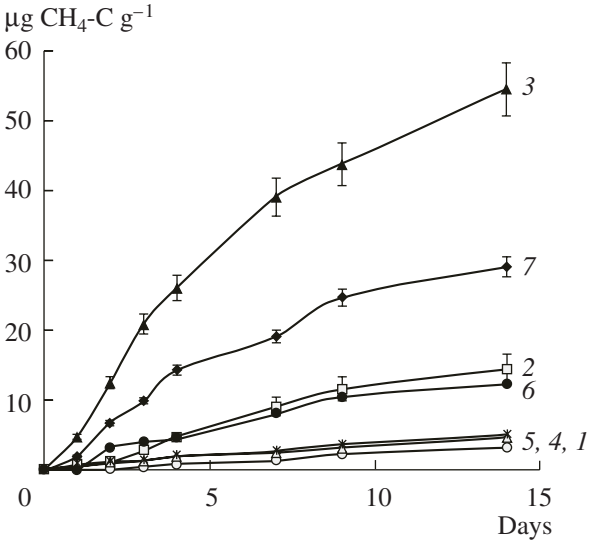

Fig. 3. Dynamics of CH_4 accumulation in the course of anaerobic incubation of the peat samples collected from the ombrotrophic Sosvyatskoe bog at the depth of 2 m: (*1*) without additions; (*2*) acetate; (*3*) H_2 + CO_2; (*4*) methanol; (*5*) methylamine; (*6*) formate; and (*7*) glucose.

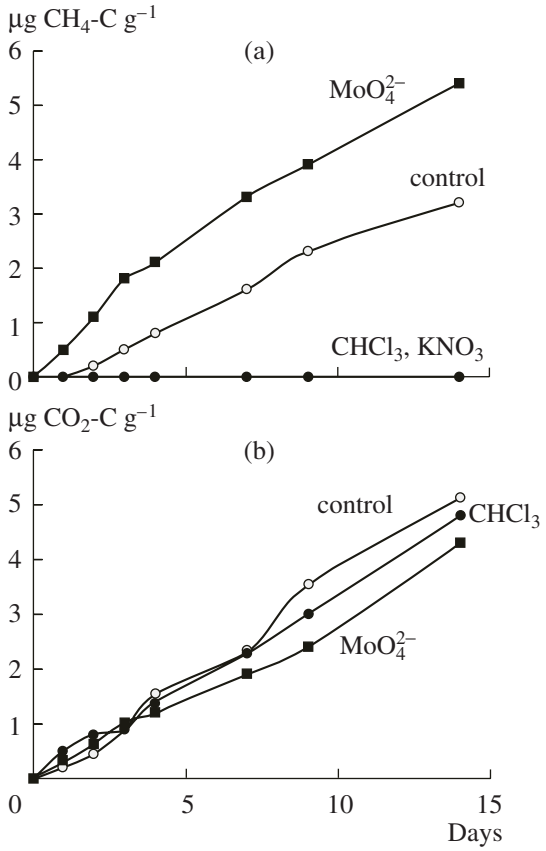

Fig. 4. Effect of the inhibitors of methanogenesis and sulfate reduction on (a) methane and (b) CO_2 production in the peat samples collected from the ombrotrophic Sosvyatskoe bog at the depth of 2 m.

microorganisms, which produce CO_2 and H_2, the main substrates of methanogenesis. These results demonstrate the presence of high numbers of fermentative microorganisms in bog soils (Fig. 2).

In order to test the possibility of competition between microorganisms for organic matter, a series of incubation experiments with the peat samples collected from Sosvyatskoe bog were carried out using inhibitors of methanogenesis and sulfate reduction. The addition of sodium molybdate as a sulfate reduction inhibitor led to a substantial increase in the methanogenic activity, from 8.8 to 15.8 ng CH_4-C g^{-1} h^{-1} (Fig. 4a), and had no noticeable effect on the CO_2 production (Fig. 4b). In this case, the rate of sulfate consumption during the incubation was five times lower than that in inhibitor-free control cultures (data not shown).

The addition of chloroform and potassium nitrate inhibited methane production by 80–90%; however, it did not affect CO_2 accumulation (Fig. 4b). This fact may be considered as a supporting evidence that, in this case, chloroform is a specific inhibitor of methanogenesis. The addition of chloroform did not affect the sulfate consumption rates (data not shown). These results suggest that the competition between methanogens and sulfate reducers for reducing equivalents may occur in bog soils under certain conditions.

In order to assess the applicability of the results obtained during our laboratory experiments to the real bog ecosystems, we have analyzed the content of volatile fatty acids (VFA) and inorganic anions in bog water samples. The results are shown in Table 2.

The contents of both VFA (acetate and butyrate) and anions (nitrate and sulfate) varied substantially within the peat deposits, including the deep horizons. A tendency towards an increase in the butyrate content during the period from spring to fall was observed. On the contrary, the acetate concentration decreased, especially in the layers characterized by high methanogenic potential (Table 2). During spring, high contents of formate (up to 8.9 mg l^{-1}) and propionate (up to 0.3 mg l^{-1}) were observed in the upper (0–50 cm) layers of the peat deposits of ombrotrophic bogs. These compounds, however, were not detected in the underlying layers, nor during the fall period.

The results of determination of sulfate and nitrate present some interest. The local minima of these anion contents in oligotrophic bogs approximately corresponded to the local maxima of methanogenic activity; this allows us to conclude that their content may be one of the factors that determine the in situ regulation of methanogenesis.

The results obtained allow us to conclude that both the rate of methanogenesis and methane accumulation in the bog soil profile depend on a complex of hydrologic, chemical, and microbiological processes. Nitrate and sulfate ions act as methanogenesis inhibitors, and changes in their concentrations may regulate the methane content in peat layers. For example, this may occur when the anaerobic conditions in the upper part of the profile give way to aerobic conditions, as well as when the rates of nitrification and sulfide oxidation increase. The fact that the highest methanogenesis intensity was detected in situ in the peat layers with low nitrate and sulfate concentrations may be attributed to this phenomenon. The mechanism of methanogenesis inhibition by nitrate is still poorly understood. The inhibitory effect of sulfate is probably the result of competition between microorganisms for carbon sources and reducing equivalents, its efficiency being dependent on the ratio between the carbon substrate and sulfate concentrations. Thus, competitive interactions between methanogens and sulfate reducers may occur both in ecosystems with high sulfate concentrations (marine sediments) and in low-mineral, oligotrophic bog ecosystems.

Table 2. Content of organic acids and inorganic anions in the samples of bog water collected in April (IV) and August (VIII), 2002 from the vertical profile of the studied peat deposits, mg l^{-1}

Depth, m	Acetate		Butyrate		NO_3^-		SO_4^{2-}		PO_4^{3-}	
	IV	VIII	IV	VIII	IV	VIII	IV	VIII	IV	VIII
Ombrotrophic Sosvyatskoe bog										
0.8	0.00	0.00	0.15	3.42	0.08	0.18	0.04	0.21	0.07	0.00
1.3	0.03	0.00	0.27	2.66	0.61	0.03	0.45	0.29	0.00	0.66
1.5	0.15	0.00	1.38	3.98	1.85	0.00	2.01	0.23	0.00	0.39
2	0.23	0.00	0.68	1.91	0.17	0.05	0.46	0.36	0.24	0.59
2.5	0.18	0.03	0.58	3.71	3.95	0.00	1.90	0.23	0.00	0.58
3	0.02	0.00	2.44	4.20	1.02	0.83	0.76	0.32	0.34	0.48
4	0.05	0.00	1.06	3.43	0.18	2.94	0.72	0.8	0.13	0.68
5	0.30	0.03	1.63	8.04	2.89	0.00	0.91	0.33	0.00	0.59
6	0.02	0.46	4.42	3.28	1.20	2.68	1.53	1.34	0.00	0.77
Ombrotrophic Petrilovo bog										
0.4	0.01	0.08	1.05	1.06	0.35	0.00	0.47	0.40	0.00	0.42
0.6	0.00	0.00	0.72	0.29	0.34	0.04	0.72	0.26	0.00	0.90
0.8	0.00	0.00	0.76	1.86	0.69	0.06	1.05	0.29	0.00	0.36
1.5	1.07	0.15	0.00	2.12	1.15	0.05	1.99	0.23	0.40	0.33
2	0.05	0.00	1.35	0.53	2.43	0.09	1.28	0.40	0.00	2.07
2.5	0.00	0.00	0.45	4.02	N/D	0.00	N/D	0.18	N/D	0.77
3	0.02	0.03	2.02	1.91	0.13	0.08	0.32	0.76	0.29	1.10
4	0.09	0.00	0.80	4.49	0.09	0.00	0.37	2.63	0.08	1.18
5	0.13	0.08	2.64	1.00	0.56	0.24	0.56	0.19	0.00	0.42
6	0.02	0.00	0.82	3.87	0.74	0.06	1.34	0.51	0.00	1.19
7	0.04	0.01	0.41	3.22	1.29	0.00	2.12	0.23	0.00	0.39
8	0.38	0.03	0.43	2.63	1.09	1.10	1.03	0.79	0.00	0.00
Zailov'e fen										
0.8	0.00	0.46	2.68	3.41	0.08	0.03	0.56	0.45	0.10	0.34
1.2	0.11	0.00	2.55	6.65	0.45	0.12	0.71	0.21	0.00	0.10
1.7	0.04	0.00	3.46	5.00	0.09	0.07	0.53	0.33	0.05	0.90
2	0.01	0.06	1.10	3.98	0.30	0.00	0.30	0.20	0.00	0.30
2.5	0.03	0.00	1.69	3.56	0.10	0.13	0.29	0.49	0.00	0.57
3.1	0.00	0.00	2.63	7.49	1.00	0.02	1.40	0.26	0.00	0.42
4	0.03	0.00	3.11	8.42	4.11	0.04	2.17	0.36	0.38	0.00

Note: N/D stands for "no data".

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