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EXPERIMENTAL ARTICLES

Microbiological and Isotopic–Geochemical Investigations of Meromictic Lakes in Khakasia in Winter

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Abstract—Microbiological and isotopic-geochemical investigations of the brackish meromictic lakes Shira and Shunet were performed in the steppe region of Khakasia in winter. Measurements made with a submersed sensor demonstrated that one-meter ice transmits light in a quantity sufficient for oxygenic and anoxygenic photosynthesis. As in the summer season, in the community of phototrophic bacteria found in Lake Shira, the purple sulfur bacteria *Amoebobacter purpureus* dominated, whereas, in Lake Shunet, the green sulfur bacteria *Pelodictyon luteolum* were predominant. Photosynthetic production, measured using the radioisotopic method, was several times lower than that in summer. The rates of sulfate reduction and production and oxidation of methane in the water column and bottom sediments were also lower than those recorded in summer. The process of anaerobic methane oxidation in the sediments was an exception, being more intense in winter than in summer. The data from radioisotopic measurements of the rates of microbial processes correlate well with the results of determination of the isotopic composition of organic and mineral carbon ($\delta^{13}C$) and hydrogen sulfide and sulfate ($\delta^{34}S$) and suggest considerable seasonal variations in the activity of the microbial community in the water bodies investigated.

Key words: meromictic water bodies, photosynthesis, sulfate reduction, microbial production and oxidation of methane, stable isotopes of carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$).

Meromictic water bodies are characterized by pronounced stratification of the water mass all year round. This phenomenon, which is due to the high mineralization of the hypolimnion layer, is reinforced in summer by thermal stratification. Due to the absence of vertical circulation of the water mass, which occurs in spring and autumn in other water bodies in temperate climates, stable anaerobic conditions are formed in the hypolimnion of meromictic water bodies and an abrupt change of the redox potential occurs at the boundary between the oxidized and reduced waters [1].

For microbiologists, meromictic water bodies are an ideal model for investigation of the anaerobic processes of, above all, sulfate reduction and methanogenesis; for estimation of the role of photo- and chemoautotrophic organisms, whose massive development occurs at the redox potential jump, in the production of organic matter; and investigation of the contribution of microbial organic matter to the trophic webs and general carbon cycle in water bodies [1].

For two meromictic water bodies (the Black Sea and Lake Mogil'noe), it has been shown that the process of assimilation of carbon dioxide by photo- and chemoautotrophic organisms is accompanied by fractionation of stable carbon isotopes. This phenomenon leads to noticeable changes in the value of $\delta^{13}C$ for both organic and inorganic carbon in the contact zone of the oxygen and hydrogen sulfide waters of meromictic water bodies [2, 3].

Intensive investigation of the microbial processes in the meromictic lakes Shira and Shunet, situated in a local depression in the steppe region of Khakasia, was started several years ago [4–6]. However, until the present study, all the field investigations had been carried out in the summer period, which is obviously insufficient, especially for water bodies where massive development of photosynthetic microorganisms occurs. Moreover, knowledge on microbial processes in ice-covered water bodies is extremely scarce [7].

Therefore, the principal task of the present study, performed in February and March 2003, was quantitative investigation of the key the microbial processes of sulfur and carbon cycles—sulfate reduction (SR), autotrophic and heterotrophic fixation of CO_2, methanogenesis (MG), and methane oxidation (MO)—under

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hydrological winter conditions and investigation of the impact of microbial processes on the fractionation of stable carbon and sulfur isotopes in the meromictic lakes of Khakasia.

MATERIALS AND METHODS

The studies at lakes Shira and Shunet were performed from February 24 to March 3, 2003. Samples of water and bottom sediments were taken at the station with the greatest depth, and the sampling was performed from the ice surface through a drilled hole. Sampling, bottling, determination of the principal chemical parameters, introduction of substrates, and fixation of the samples were performed in a specially equipped tent on the ice surface directly above the hole. The air temperature in the tent during the investigations varied from –5 to 4°C; outside the tent, the temperature was from –15 to –25°C. Flasks containing the samples were incubated in situ using a capron rope fixed on the ice surface.

The main hydrochemical parameters were determined with a DateSonde 4a submersible multichannel sound (HYDROLAB, United States) equipped with temperature, conductivity, turbidity, pH, and Eh sensors. Underwater radiation (photosynthetically active radiation (PAR)) was measured with a Li-Cor PAR sensor (United States) and expressed in μmol quanta/(m^2 s), which is equivalent to μE/(m^2 s). The sensor was dipped under the ice through a hole 20 cm in diameter, and the hole was shaded with an opaque screen. The samples of water were taken with a 1-l glass bottle, while the bottom sediments were sampled by means of a limnological stratometer with a glass tube 6 cm in diameter. The contents of oxygen and hydrogen sulfide and the value of total alkalinity were determined immediately after the sampling using Aquamerck sets of reagents (Germany).

For determination of the total concentration of suspended matter, the water samples were filtered through preliminarily incinerated and weighted (with an accuracy of ±0.05 mg) GF/F glass-fiber filters (47 mm in diameter) and then dried at 60°C.

The intensities of the microbial processes—light and dark assimilation of carbon dioxide, SR, MO, and MG—were determined by the radioisotopic method using $NaH^{14}CO_3$, $Na_2{}^{35}SO_4$, $^{14}CH_4$, and $^{14}CH_3COONa$. For the water samples, 30-ml glass flasks were used; for the bottom sediment, 5-ml plastic syringes with a rubber piston and a cut off edge were employed. After being filled with a sample of undisturbed sediment, with no air access, the syringes were plugged with a gas-impermeable rubber stopper.

The abundance of anoxygenic phototrophic bacteria was determined by the end-point dilution method under field conditions on an agarized (0.8%) modified Pfennig nutrient medium. The medium variant used to grow Lake Shira microorganisms was composed of (g/l of distilled water) KH_2PO_4, 0.7; NH_4Cl, 0.7; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2$, 0.1; NaCl, 20; KCl, 0.33; $NaHCO_3$, 1.5; Na acetate, 0.5; Na pyruvate, 0.5; yeast extract, 0.1; $Na_2S \cdot 9H_2O$, 0.5; $Na_2S_2O_3 \cdot 5H_2O$, 1; a solution of microelements, 1 ml; and vitamin B_{12}, 20 μg. The pH was 6.8 for green sulfur bacteria and 7.5 for purple bacteria. The medium variant for Lake Shunet microorganisms was as follows (g/l of distilled water): KH_2PO_4, 0.5; NH_4Cl, 0.5; Na_2SO_4, 21; $MgSO_4 \cdot 7H_2O$, 14.8; NaCl, 5.3; $MgSO_4 \cdot 7H_2O$, 4.3; KCl, 0.33; $CaCl_2$, 0.1; $NaHCO_3$, 1; Na acetate, 0.5; Na pyruvate, 0.5; yeast extract, 0.1; $Na_2S \cdot 9H_2O$, 0.5; $Na_2S_2O_3 \cdot 5H_2O$, 0.5; a solution of microelements, 1 ml; and vitamin B_{12}, 20 μg. The pH was 6.8 for green sulfur bacteria and 7.5 for purple bacteria.

The pigment composition of the obtained cultures of phototrophic bacteria was investigated in suspensions of intact cells in 50% glycerol and in acetone–methanol (7 : 2) extracts. The absorption spectra were taken with a LOMO SF 56 spectrophotometer (St. Petersburg) in the wavelength range 350–1000 nm.

For determination of the light and dark assimilation of CO_2, 2 light and 1 dark flasks were used for samples taken at each water level. After the addition of 0.2 ml (20 μCi) of a solution of $NaH^{14}CO_3$, the flasks were fastened to a capron rope and lowered to the corresponding water levels through separate ice holes, which were then shaded by a piece of ice from above. This in situ exposure lasted 1 day, after which the contents of the flasks were fixed with diluted HCl and filtered through capron membrane filters with a pore size of 0.2 μm. In order to remove the residual radiolabeled carbonate, the filters were rinsed with a threefold volume of filtered slightly acidified lake water.

The flasks and syringes intended for determination of the intensity of the microbial processes after the addition of labeled compounds were also incubated in situ. After completion of the incubation, the samples were fixed with 1 ml of 0.1 M KOH. The samples were then treated according to the methods described in [7, 8].

The content of methane in water and sediments was determined by the head-space method in accordance with the phase-equilibrium degassing technique [9]. The methane content was determined on a Chrom-5 gas chromatograph equipped with a flame-ionization detector. Sulfate, chloride, and acetate were registered on a Biotronik ion chromatograph (Germany). The isotopic composition of the carbon from organic matter ($\delta^{13}C_{org}$) and from bicarbonate ion $\delta^{13}C\text{-}HCO_3^-$ was determined on an MI-1201B mass spectrometer (Ukraine) equipped with an SNG-3 three-channel system of gas injection. The accuracy of measurements was ±0.2‰ [10].

RESULTS

Lake Shira: physicochemical characteristics. During our investigations, the ice on Lake Shira was

Table 1. Vertical distribution of hydrochemical parameter values in Lake Shira

Depth, m or cm	*T*, °C	pH	Eh, mV	PAR, μE/(m² s)	*Alk*, mg-equiv/l	O_2, mg/l	SO_4^{2-}, g/l	H_2S, mg/l	CH_4, μl/l
Water column under ice									
1.0	–0.71	8.4	130	15.3	14	7.3	9.5	0	0.57
10.0	–0.73	8.3	120	0.97	17	6.6	10.0	0	0.58
14.0	–0.65	8.3	120	0.27	18	5.8	10.3	0	0.60
14.5	–0.51	8.3	120	0.24	18.5	5.0	10.4	0	0.64
15.0	–0.35	8.3	–140	0.20	16	4.5	9.75	0.25	4.63
15.5	–0.08	8.27	–150	0.17	9.0	0	5.61	2.7	3.70
16.0	0.3	8.27	–150	0.15	5.5	0	2.64	6.2	5.68
16.5	0.8	8.23	–180	0.10	9.5	0	7.9	7.5	6.60
18.0	1.64	8.17	–210	0.03	19.5	0	9.78	10.0	7.65
19.0	1.63	8.18	–220	0.01	19.5	0	9.42	14.0	9.73
21.5	1.51	8.17	–240		19.5	0	8.13	12.5	11.4
Bottom sediments									
0–2	1.8	8.0	–290	–	18.0	0	12.4	n.d.	40.7
2–5	1.8	8.1	–305	–	19.0	0	12.0	n.d.	31.3
12–17	1.7	8.0	–280	–	20.5	0	11.7	n.d.	31.8
25–30	1.8	8.2	–300	–	22.0	0	16.2	n.d.	34.4

110 cm thick. The water layer under the ice cover was characterized by a homogeneous distribution of temperature, conductivity, pH, and Eh values to a depth of 15.5 m. The temperature of the layer under the ice was –0.7°C and remained negative to 15.5 m; below this, the temperature increased and attained 1.5 to 1.6°C in the bottom layer (Table 1).

Determinations of the concentration of oxygen and hydrogen sulfide revealed a narrow redox zone at a depth of 15.0–15.5 m. An abrupt jump in the values of the redox potential was recorded within the depth range 14.5–15.5 m, which corresponded to the upper horizon of the redox zone. The maximum content of oxygen was recorded in the water layer under the ice (7.3 mg/l), and that of hydrogen sulfide was registered in the bottom layer (12.5–14.0 mg/l (see Table 1)).

The pH values decreased from 8.4 in the layer under the ice to 8.17 in the bottom layer, and the values of total alkalinity increased from 14 to 19.5 mg-equiv/l, with a noticeable local trough (to 5.5 mg-equiv/l) observed in the thermocline layer (Table 1). The content of suspended matter increased from 18 mg/l in the layer under ice to 33.5 mg/l in the redox zone and 37–44 mg/l in the bottom layer (Table 2).

The bottom sediments gave off a strong smell of hydrogen sulfide and were rather uniform with respect to the temperature, pH, and redox potential values to a depth 30 cm.

The intensity of CO_2 assimilation and the isotopic composition of particulate organic carbon and dissolved mineral carbon. Direct measurements by means of a submersible sensor showed that the 1-m-thick ice transmitted a sufficient quantity of light for photosynthesis (Table 1). Just as we expected, the maximum intensity of light assimilation of carbon dioxide was recorded in the layer under the ice (20.9 μg C/(l day)). A second maximum (16.1 μg C/(l day)) coincided with the zone in which there was a concomitant presence of oxygen and hydrogen sulfide (Table 3) and seems to be accounted for by the activity of anoxygenic phototrophic bacteria. The profile of the dark assimilation

Table 2. Quantity and isotopic composition of particulate organic carbon and the isotopic composition of dissolved mineral carbon in the water column of Lake Shira

Depth, m	Suspended matter, mg/l	$\delta^{13}C_{org}$, ‰	$\delta^{13}C_{HCO_3^-}$, ‰
1.0	18.4	–24.7	–6.4
10.0	22.9	–25.7	–6.2
14.0	25.2	–25.6	–15.7
14.5	23.4	–25.2	–10.9
15.0	30.0	–25.4	–4.5
15.5	33.5	–25.6	–3.4
16.0	33.7	–25.9	–6.4
16.5	37.2	–25.5	–8.8
19.0	44.3	–24.9	–9.7
21.5	39.6	–24.5	–7.3

Table 3. Rates of the microbial processes in Lake Shira

Depth, m or cm	Total count, cells/ml	CO_2 assimilation, μg C/(l day)		MG		MO	SR, μg of acid-soluble sulfur/(l day)
		light	dark	nl/(l day)	from CO_2, %	nl/(l day)	
Water column under ice							
1.0	7.3×10^5	20.9	16.9	n.d.	n.d.	1.36	–
10.0	7.9×10^5	8.3	5.9	n.d.	n.d.	1.75	–
14.0	8.0×10^5	3.1	8.9	0.68	35	2.28	12.2
14.5	6.0×10^5	7.4	9.7	0.03	70	2.49	13.3
15.0	14.0×10^5	16.1	15.2	8.5	94	13.98	16.1
15.5	12.0×10^5	4.0	14.9	8.2	97	10.84	7.9
16.0	9.0×10^5	0	13.5	4.1	97	18.72	2.2
16.5	7.6×10^5	0	13.0	8.9	90	20.62	6.3
18.0	13.0×10^5	0	10.3	12.5	72	25.83	14.9
19.0	4.0×10^5	0	9.1	13.8	79	32.74	14.4
21.5	7.0×10^5	0	22.5	26.9	89	51.58	14.0
Bottom sediments							
0–2	n.d.		67.4	184	85	360	1780
2–5	n.d.		20.3	349	96	173	722
12–17	n.d.		8.2	409	85	179	460
25–30	n.d.		8.6	612	97	272	68

of carbon dioxide exhibited three maximums: one under ice, a near-bottom one, and an intermediate maximum, coinciding with the peak activity of the phototrophic bacteria in the redox zone. The total value of the dark fixation of CO_2 significantly surpassed the value of CO_2 fixation in the process of photosynthesis.

Determination of the concentration of pigments in the water samples revealed the presence of bacteriochlorophyll *a*, which peaked at depths of 15 and 19 m (23.3 and 23.5 μg/l, respectively).

The isotopic composition of the particulate organic carbon (Table 2) hardly changed in different horizons of the water column ($\delta^{13}C_{org}$ was from –24.5 to –25.9‰). Conversely, the isotopic composition of mineral the carbon varied within a wide range ($\delta^{13}C\text{-}HCO_3^-$ was from –3.4 to –15.7‰). The light isotopic composition of the mineral carbon corresponded to depths of 14.0 and 14.5 m. Lower down, within the redox zone, the isotopic composition became abruptly heavier, reaching –3.4 to –4.5‰, and, again, became lighter in the bottom layers (–7.3 to –9.7‰).

Bacterioplankton from the water column of Lake Shira. The total abundance of bacteria in the water column of Lake Shira varied from 4×10^5 to 14×10^5 cells/ml (Table 3). The abundance maximum at a depth of 15 m coincided with the chemocline. As in the summer season, the dominant phototrophic bacterium was a purple sulfur bacterium that we identified as *Amoebobacter purpureus* according to its morphology and pigment composition. At the depth of 15.0 m, its abundance was 1.65×10^4 cells/ml; at 15,5 m, 6.7×10^3 cells/ml; at 16.0 m, 5.5×10^3 cells/ml; and at 16.5 m, 6.7×10^3 cells/ml. It is noteworthy that deeper than 15 m, no light fixation of CO_2 was recorded.

From an enrichment culture obtained from a depth of 16 m, a monoculture of green anoxygenic phototrophic bacteria, whose presence has not previously been noted in Lake Shira, was isolated. The immotile cells were rods 0.7–1.1 μm in width and 0.9–1.5 μm in length. They did not contain either gas vacuoles or sulfur granules. Most of the cells occurred in filaments consisting of 2–20 or more (10 on average) cells (Fig. 1).The absorption spectrum of pigments of the living cells revealed the presence of bacteriochlorophyll *c* (753 nm) and the carotenoid chlorobactin (459 nm) (Fig. 2). By the complex of morphological features and the characteristic absorption spectrum of the pigments, the obtained bacterial culture was identified as *Chlorobium limicola.*

Intensity of generation and oxidation of methane. Occurrence of the process of methane generation was demonstrated in the water column beginning from a depth of 14 m. The intensity attained its maximum value in the bottom layer (Table 3). Generally, the profile of methane generation rates correlated with the methane concentration profile. An abrupt increase in the activity of methane generation was observed in the bottom sediments. The highest activity occurred in the lower horizons.

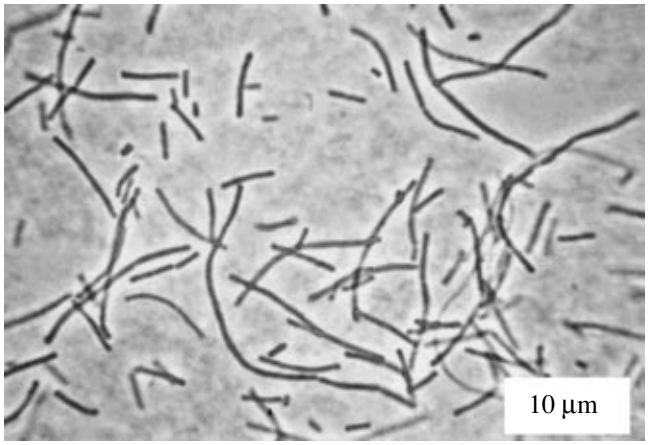

Fig. 1. The green sulfur bacterium *Chlorobium limicola* isolated from Lake Shira in winter 2003.

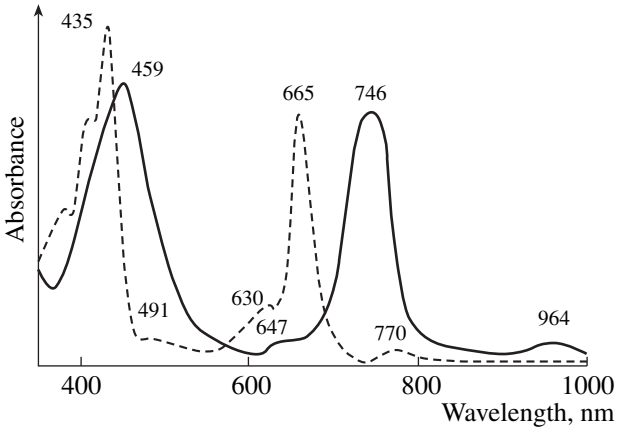

Fig. 2. Absorption spectrum of the pigments in living cells of the green sulfur bacterium *Chlorobium limicola* isolated from Lake Shira in winter 2003: the thick line is the spectrum of the living cells in glycerol and the thin line is the spectrum of an acetone–methanol extract of the pigments.

The intensity of methane oxidation in the aerobic part of the water column was very low. It did not exceed 2.5 nl CH_4/(l day). In the bottom sediment, the peak methane oxidation activity occurred in the surface horizon (360 nl CH_4/(l day). Deeper in the sediment, the intensity of the process decreased.

Thus, during the investigation period, the balance of methane generation in the bottom sediments was positive (except in the surface layer). In the water column, the intensity of the methane oxidation surpassed its generation.

The intensity of sulfate reduction and the isotopic composition of sulfur compounds. The process of sulfate reduction was active throughout the anaerobic layer of water. A noticeable decrease in the intensity of SR was characteristic of the 15.5- to 16.5-m horizon immediately under the redox zone (Table 2). In the bottom sediments, a high intensity of SR was recorded in the thin surface layer (1780 μg S/(l day)). Deeper in the sediment, the intensity of this process noticeably decreased, specifically, to 68 μg S/(l day).

The isotopic composition of hydrogen sulfide in the anaerobic water layers showed little variation: –37.3 to –41.2‰ (Table 4). The isotopic composition of sulfate varied within a wider range: 28.4‰at 16 m to 14.0‰ at 19 m.

The heaviest isotopic composition of hydrogen sulfide (–0.1‰) was recorded in the upper layer of the sediments, where the intensity of SR was at its highest. In the lower layers of the sediment, where the intensity of sulfate reduction decreased (Table 2), the isotopic composition of hydrogen sulfide was lighter.

Lake Shunet: Physicochemical characteristics. Lake Shunet is a lake without an outflow. Its depth reaches no more than 6.2 m. Vertical stratification of the water column is clearly pronounced (Table 5). During our investigations, the ice cover was 80 m thick. The temperature of the water under the ice was −1°C and changed little to a depth of 4 m. Lower down, at depths of 4 to 5.5 m the temperature abruptly increased to 7.7°C. To a depth of 4 m, the water contained dissolved oxygen. From the depth of 4 m, hydrogen sulfide was present in the water. Its content attained 120 mg/l in the near-bottom layer. Within the same depth range, the value of total alkalinity increased from 10 to 78 mg-equiv/l, and the content of dissolved methane increased from 1.9 to 765 μl/l. There was no clear boundary between the water layer and bottom sediment. The intermediate layer was represented by black liquid silt with a strong odor of hydrogen sulfide.

The bottom sediment was reduced, rather homogeneous, saturated with hydrogen sulfide and methane, and contained a considerable quantity of slightly decomposed terrigenous vegetative material.

Intensity of CO_2 assimilation. The light fixation of carbon dioxide was recorded in the aerobic layer under the ice and in the zone exhibiting a concomitant presence of oxygen and hydrogen sulfide (Table 6). The first maximum was caused by the development of phytoplankton, while the second one was obviously due to photosynthetic bacteria. A local peak in the intensity of

Table 4. Isotopic composition of sulfur compounds in the water column and bottom sediments of Lake Shira

Depth, m	$\delta^{34}S$-H_2S, ‰	$\delta^{34}S$-SO_4^{2-}, ‰	Sediment horizon, cm	$\delta^{34}S$-H_2S, ‰
16.0	–37.3	28.4	0–3	–0.1
16.5	–40.9	17.4	3–8	–3.9
19.0	–41.0	14.0	10–15	–11.6
21.5	–41.2	20.3	17–23	–7.5
			25–30	–10.2

Table 5. Vertical distribution of hydrochemical parameter values in Lake Shunet

Depth, m or cm	*T*, °C	pH	Eh, mV	PAR, μE/(m_2 s)	*Alk*, mg-equiv/l	O_2, mg/l	SO_4^{2-}, g/l	H_2S, mg/l	CH_4, μ/l
Water column under ice									
1.0	–1.0	7.88	160	6.5	12.5	4.5	4.5	0	1.74
4.0	–0.93	7.85	160	1.9	11.5	0.3	4.89	0.3	1.85
4.25	–0.06	7.82	–40	1.4	10.0	0	5.61	1.2	24.3
4.5	1.75	7.78	–190	0.95	15.5	0	9.54	3.5	161
4.75	3.41	7.40	–320	0.25	22.5	0	13.0	25	324
5.35	6.81	6.51	–420	0.01	63.5	0	29.0	120	684
5.50	7.7	6.53	–440	0	78.5	0		n.d.	765
Bottom sediments									
0–2	7.7	6.7	–440	0	85	0	33.0	n.d.	626
5–7	7.7	6.6	–440	0	n.d.	0	27.0	n.d.	688
12–15	7.7	6.7	–440	0	n.d.	0	45.0	n.d.	720

Table 6. Rates of the microbial processes in Lake Shunet

Depth, m or cm	Total count, cells/ml	CO_2 assimilation, μg C/(l day)		MG		MO, nl/(l day)	SR, μg of acid-soluble sulfur/(l day)
		light	dark	nl/(l day)	from CO_2, %		
Water column under ice							
1.0	2.7×10^5	43.6	22.7	26.8	91	27.8	26.9
4.0	6.0×10^5	29.8	29.3	39.5	94	9.3	26.6
4.25	5.0×10^5	0	69.2	28.1	73	86.1	27.9
4.5	31.0×10^5	0	57.1	42.3	91	540	57.2
4.75	10.0×10^5	0	66.4	68.3	88	2220	105
5.35	28.0×10^5	0	130	207	88	3750	191
Bottom sediments							
0–2	n.d.	0	158	11850	85	4610	205
5–7	n.d.	0	173	2720	86	5550	659
12–15	n.d.	0	114	2490	92	3930	1600

the dark assimilation of CO_2 was located slightly below, at a depth of 4.25 m. In this layer, no photosynthetic activity was recorded. The maximum intensity of dark CO_2 assimilation was observed in the near-bottom layer. The activity of carbon dioxide fixation at various horizons was 2–5 times higher in Lake Shunet than in Lake Shira. The isotopic composition of the particulate organic carbon in the water column grew somewhat heavier from the layer under the ice (–24.6‰) to the near-bottom layer (–23.3‰) (Table 7).

Bacterioplankton from the Lake Shunet water column. The total abundance of bacteria in the water column of Lake Shunet varied from 2.7×10^5 to 31×10^5 cells/ml (Table 6). The highest number of cells was found at a depth of 4.5 m and in the near-bottom layer. Among the phototrophic bacteria, similarly to the summer season, green sulfur bacteria, identified as *Pelodictyon luteolum* dominated. At a depth of 4.5 m, their abundance was 7.4×10^4 cells/ml, and, at a depth of 4.74 m, 1.7×10^6 cells/ml. In addition to green bacteria, purple bacteria were also present. At the depths of 4.5 m and 5.35 m, purple bacteria were mainly represented by *Amoebobacter* sp.; the purple bacterium that we previously identified as *Lamprobacter* sp. was also present. The abundance of purple bacteria at these depths was 7.4×10^4 and 3.0×10^5 cells/ml, respectively. In contrast to the summer season, no nonsulfur purple bacteria were found.

Intensity of methane generation and oxidation. Methane generation in Lake Shunet was observed throughout the entire water column, beginning from the aerobic layer under the ice (Table 6). The intensity of this process increased from the surface to the near-bottom layer, with a local maximum at a depth of 4 m and a decrease in activity at 4.25 m. An abrupt increase in the activity of methane generation was recorded in the surface layer of the bottom sediments. Deeper in the sediments, the activity of this process decreased. Autotrophic methanogenesis was superior to aceticlastic methane generation.

The process of methane oxidation was recorded both in the aerobic water layer and under anaerobic conditions in the water and bottom sediments. The maximum activity of this process was observed in the near-bottom water and in the sediments. A noticeable maximum of methane oxidation activity corresponded to the 4-m horizon, i.e, to the zone in which there was a concomitant presence of oxygen and hydrogen sulfide. Probably, the conditions in the contact zone were unfavorable both for classical methanotrophs and for a community of microorganisms performing methane oxidation under strictly anaerobic conditions.

Intensity of sulfate reduction. SR occurred throughout the entire water column and in all of the sediment layers sampled (Table 6). The intensity of the process did not change from the layer under the ice to the 4.25-m horizon, but it increased in the deeper layers, attaining its maximum value in the near-bottom layer. In the bottom sediments, the intensity of SR increased with depth, attaining a value of 1.6 mg S/(dm^3 day).

In the water column of Lake Shunet, the heaviest isotopic composition of hydrogen sulfide sulfur (–19.3‰) was recorded in the near-bottom water layer (Table 8), where the rate of SR was at its highest. The variations in the isotopic composition of sulfate sulfur were smaller (15.4–16.6‰). An extremely heavy isotopic composition of hydrogen sulfide (–3.0‰) was recorded in the surface layer of the bottom sediments; in the deeper layers, the isotopic composition abruptly became much lighter (–19.3‰).

DISCUSSION

Table 9 summarizes the summer [4] and winter data on the rates of microbial processes and on the content of methane and hydrogen sulfide under 1 m^2 in the central basin of Lake Shira. As was expected, the rates of all the processes are lower in winter. The rate of the photosynthetic processes, which provide all the groups of heterotrophic bacteria with newly formed organic matter, decreased by 5 times in winter. As a result, there was a significant decrease in the activity of the terminal processes of anaerobic decomposition of organic matter in the water column: SR decreased 12 times, and MG decreased more than 40 times. The rate of MO in winter only decreased 4 times. In combination with the abrupt decrease in the MG rate, there was a noticeable decrease, by almost two times, of the methane content in the water column (Table 9).

Table 7. Quantity and isotopic composition of particulate organic carbon and the isotopic composition of dissolved mineral carbon in the water column of Lake Shunet

Depth, m	Suspended matter, mg/l	$\delta^{13}C_{org}$, ‰	$\delta^{13}C_{HCO_3^-}$, ‰
1.0	16.3	–24.6	–5.8
4.0	15.8	–24.5	–7.2
4.25	18.0	–23.7	–8.3
4.5	63.0	–23.9	–7.2
4.75	98.5	–23.1	–10.7
5.35	130	–23.3	–19.5

Table 8. Isotopic composition of sulfur compounds in the water column and bottom sediments of Lake Shunet

Depth, m	$\delta^{34}S$-H_2S, ‰	$\delta^{34}S$-SO_4^{2-}, ‰	Sediment horizon, cm	$\delta^{34}S$-H_2S, ‰
4.5	–36.4	16.6	0–5	–3.0
4.75	–32.2	15.0	5–10	–16.9
5.35	–19.3	15.4	12–15	–19.3

Hydrogen sulfide oxidation in Lake Shira occurs with the participation of both anaerobic photosynthetic bacteria and aerobic thionic bacteria. The rate of anaerobic oxidation, calculated from the intensity of light fixation of CO_2 in the anaerobic zone (Table 9), decreased in winter, similarly to SR, by 10 times. However, the oxidizing activity of the thionic bacteria, estimated from dark fixation of CO_2, changed very little (Table 9). It seems that the imposition of these processes should result in a decrease in the content of H_2S in the water column. Nevertheless, the sum content of H_2S under 1 m^2 did not change. Obviously, in winter, an additional source of hydrogen sulfide in the water column comes from the bottom sediment, where the rate of SR was found to be higher in winter (Table 9). The increase in the flux of hydrogen sulfide from the bottom in winter is indicated, inter alia, by the data on the isotopic composition of H_2S sulfur in the water column. The average value of $\delta^{34}S$-H_2S is –43.1‰ in summer [4] and –40.1‰ in winter (Table 4). Given the considerably lower rate of SR in winter, the heavier isotopic composition of H_2S in the water column can be explained only by an inflow of isotopically heavy hydrogen sulfide from the bottom sediments (Table 4). The value of the fractionation of sulfur isotopes during SR is known to be inversely proportional to the rate of

Table 9. Rates of the microbial processes in lakes Shira and Shunet (February and March 2003, August 2001)

Process rates	*Alk*, g C/m^2	Light CO_2 assimilation	Dark CO_2 assimilation	Methane content,	MG	MO	H_2S content, g S/m^2	SR, mg S/(m^2 day)
		mg C/(m^2 day)		ml/m^2	µl/(m^2 day)			
Lake Shira, winter								
Aerobic zone (1–14.5 m)	2910	177	176	9.2	0.18	24	0	n.d.
Anaerobic zone (14.4–22 m)	1530	6	82	54.8	71	190	64.4	76
Total in the water column	4440	183	258	64	71	214	64.4	76
Sediments (0–30 cm)	n.d.	0	4.5	10.3	133	66	n.d.	146
Lake Shira, summer [4]								
Aerobic zone (1–14.5 m)	2780	866	101	22.3	n.d.	346		n.d.
Anaerobic zone (14.4–22 m)	2200	59	115	96.7	2940	434	71.0	875
Total in the water column	4980	925	226	119	2940	780	71.0	875
Sediments (0–30 cm)	n.d.	0	48	13.0	1480	40	n.d.	48
Lake Shunet, winter								
Aerobic zone (1–4.0 m)	576	110	77	8.7	99	56	n.d.	80
Anaerobic zone (4.0–5.5 m)	564	0	95	472	110	2230	48.3	136
Total in the water column	1140	110	172	481	209	2286	48.3	216
Sediments (0–15 cm)	n.d.	0	22	103	715	714	n.d.	135

the process [12, 13]. Thus, at the lower winter rate of SR, the isotopic composition of H_2S in the water column should be significantly lighter than that in summer, when the rate of SR is 11 times higher.

Significant changes in the isotopic composition of bicarbonate carbon, from 1‰ in the surface waters to –6‰ in the near-bottom water layers, were discovered in the central part of the Black Sea, and it was suggested that this was brought about by anaerobic oxidation of organic matter [14]. The noticeable change in the isotopic composition of dissolved mineral carbon, recorded in the near-bottom water layers of both the investigated lakes (Tables 2, 7), is also likely to be due to the processes of anaerobic mineralization of organic matter.

In the zone in which massive development of autotrophic bacteria was discovered at the boundary between the aerobic and anaerobic waters in Lake Mogil'noe and in the Black Sea, we identified a noticeable change in the $\delta^{13}C$ value of particulate organic matter, indicating active production of organic matter by autotrophic microorganisms [2, 3]. The absence of a similar isotopic effect in the water columns of lakes Shira and Shunet (Tables 2, 7) is due to the low rate of autotrophic CO_2 fixation in these lakes in winter. From Tables 3 and 6, it can be seen that the fixation of CO_2 by photoautotrophic bacteria in lakes Shira and Shunet amounted to 16.1 and 29.8 µg C/(l day) while, in Lake Mogil'noe, where changes in the isotopic composition of particulate organic carbon are especially pronounced, the production of organic matter by autotrophic bacteria may attain 500–800 µg C/(l day) [3].

Thus, a noticeable decrease in the production of organic matter under winter conditions leads to a significant decrease in the intensity of mineralization of organic matter in the water column. In the upper layers of the bottom sediments, which accumulate a dead mass of phototrophic organisms, the SR rate even somewhat increased in winter.

The rates of methane and hydrogen sulfide oxidation by chemoautotrophic aerobes, which use, as substrates, products of the activities of aerobic microorganisms, decreases less than the rates of production and anaerobic consumption of organic matter.

In conclusion, we can say that, in winter, the rates of all the processes, except dark assimilation of CO_2, are significantly lower than in summer. This decrease in activity is due to the low temperature and the lower influx of both allochthonous and autochthonous organic matter. The decrease in the production of hydrogen sulfide and methane is particularly large (by 10 and 40 times, respectively) and can be explained by the connection of these processes with the consumption of newly formed organic matter.

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