# EXPERIMENTAL ARTICLES

# Microbiological and Isotopic Geochemical Research in the Arid Steppe Lakes and Sor Solonchaks of Western Transbaikalia

V. B. Dambaev<sup>a, 1</sup>, G. G. Gonchikov<sup>a</sup>, S. P. Buryukhaev<sup>a</sup>, B. S. Tsyrenov<sup>a</sup>, A. M. Zyakun<sup>b</sup>, and B. B. Namsaraev<sup>c</sup>

<sup>a</sup> Institute of General and Experimental Biology, Russian Academy of Sciences, Siberian Branch, Ulan-Ude, Buryatia, 670047 Russia

<sup>b</sup> Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow oblast, 142292 Russia

> <sup>c</sup> Buryat State University, Ulan-Ude, Buryatia, 670000, Russia Received January 21, 2011

**Abstract**—The rates of microbial processes in the sor solonchaks and the silts of the Western Transbaikalean saline lakes were investigated. The rate of deep  $CO_2$  fixation in the silt of Lake Beloe was as high as 0.19 mg  $C \, dm^{-3} \, day^{-1}$ . The content of cellulose and protein in the sor solonchaks (for air-dry material) were up to 5.0 and 0.42 mg g<sup>-1</sup>, respectively. The rates of cellulose decomposition and proteolysis in the silt were 1.08 and 0.96 % day<sup>-1</sup>, respectively. Sulfate reduction (up to 1.82 mg S dm<sup>-3</sup> day<sup>-1</sup>) prevails at the terminal stages of anaerobic decomposition of organic matter in the silt. During the flooding period, methanogenesis producing isotopically light  $CH_4$  probably prevails in the sor solonchaks and arid soils of the region. This suggestion is supported by the surface foaming of the local arid soils after abundant monsoon rains, an increase in the  $\delta^{13}C$  value for soil carbonates to -5.94% along the solonchak profile, and a decrease in the  $\delta^{13}C$  value for the soil carbonates formed by methane-oxidizing bacteria to -11.41%. The seasonal peaks of isotopically heavier (to -16.60%) organic matter in the bottom sediments of the lake may be explained as the result of activity of heterotrophic sulfate reducers, which exhibited the highest growth rates at the peak sulfate concentrations in the silt.

Keywords: sor solonchaks, bottom sediments of saline lakes, sulfate reduction, methanogenesis, stable carbon isotopes.

**DOI:** 10.1134/S0026261711050031

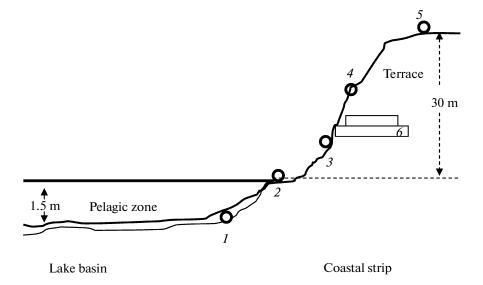
The subordinate landscapes of the Western Transbaikalia dry steppes are occupied by small shallow lakes (sors) [1], surrounded by saline and bogged soils. During the periods of flooding, they become the basis of erosion for the numerous springs and rivers flowing from the branches of the Khamar-Daban ridge at the south and from the branches of the Barguzin ridge at the north. Accumulating organic matter and salt from surrounding soils, dry steppe lakes of this region act similar to the soil lysimeters, where microbial processes of the carbon cycle occur, which are characteristic of the neighboring landscapes [2].

Short-profile, carbonate-rich chestnut and chernozem soils occupy autonomous positions in the dry steppes of the region. Apart from high carbonate content, which is known for all arid soils of the Northern Hemisphere, they have quite low sulfur content: in the profiles of the investigated arid soils gypsum (CaSO<sub>4</sub>) accumulation does not occur. The bulk content of sulfur in these soils and in the pedogenic rocks (washed Quaternary sands, gravel, and pebblestones, which

cover the bottoms of the intermontane troughs) is extremely low [1, 3]. An irreversible deficiency of sulfur and possibly of other biogenic elements irreplaceable in the cycles in these soils is associated with the long history of denudation of the Central Asian upland and salt removal by the ancient Selenga (pra-Selenga) River [4]. According to Polynov [5], the unique gypsum-free anomaly of the arid soils of this region spreads meridionally (North Mongolia and Western Transbaikalia), i.e., in the direction of the Polar Ocean.

Tectonic shifts ~25 Myr ago resulted in the lowering of the Baikal rift zone and elevation of the Selenga highlands territory (the basin of the Selenga River, the major river flowing into Lake Baikal). Dry mountain steppes and dry steppe sors of the Selenga highlands and the Barguzin depression are located 400-800 m above sea level. They possess immense kinetic energy and vary the concentrations of  $S-SO_4^{2-}$  and other elements and compounds along the genetically coupled landscapes and water bodies of the Western Transbaikalia, forming mini- and megatransects [6].

<sup>&</sup>lt;sup>1</sup> Corresponding author; e-mail: slavadmb@rambler.ru



**Fig. 1.** Schematic representation of the minitransect: sor solonchaks and silts of the Selenga highlands (coastal soils and silts of Lake Beloe). Designations: lake silt (silty sand) (1), sandy beach (2), soil profile 1C (3), soil profile 2C (4), soil profile 3C (5), and stone slabs (6).

Sulfur is a component of many hydrolytic enzymes. Chapman [7] and Chowdhury et al. [8] showed that the processes of organic matter decomposition in desalinated carbonate soil are critically dependent on the concentration of sulfate sulfur (S-SO $_4^{2-}$ ). Suppressed growth and hydrolytic activity of cellulose-degrading microorganisms was observed at SO $_4^{2-}$  content in soil below 21  $\mu$ g S g<sup>-1</sup> soil. At 11  $\mu$ g S g<sup>-1</sup> soil and less, microbial utilization of glucose is also suppressed. For sulfate reducers, sulfate ions (SO $_4^{2-}$ ) are thermodynamically more advantageous electron acceptors than CO $_2$ . At SO $_4^{2-}$  excess in soils, sulfate reducers compete for reducing electrons and hydrogen with methanogens and suppress their growth and methanogenesis [9].

The goal of the present work was to investigate the rates of microbial processes of production and decomposition of organic matter in a minitransect including the sor solonchaks and silts of Western Transbaikalia and to determine the stable isotope distribution in organic and inorganic carbon.

#### MATERIALS AND METHODS

#### Objects of Investigation

The object of investigation was Lake Beloe with the surrounding solonchaks (Selenga highlands). The plant formations of these landscapes include the lithophilic Agropyron crisratum Gaertn., Stipa krylovii Roshev., Serratula centauroides L., Scabiosa comose Fisch., Artemisia frigida Willd., Chamaerhodos altaica Bge., Arctogeron graminearum DC, Arenaria capillaris Poir., etc., often together with fescues and bunchgrass groups.

The samples of the solonchak soils and silts were collected at the northeastern part of the saline Lake Beloe, where the aquatic and terrestrial elements of the relief are separated by a narrow strip of the coastal beach. The water and silt were sampled 10 m from the waterside, and the sand was sampled from the upper 0–3 cm of the beach. The soil profile 1 (1C) was taken 10 m from the beach edge, profile 2 (2C), at the slope of the lakeside terrace, and profile 3 (3C), at the terrace's top, where the salt crust is visible as a whitish coating (Fig. 1). The sors, sor solonchaks, and the dominant vegetation are described in our previous work [6].

#### Investigation Techniques

Research was carried out from 2000 to 2002 and additionally in 2008. Samples of water, soils, and silts were collected aseptically into paper bags or hermetically sealed glass vials. They were delivered to the laboratory in a cooled Thermos jar. The temperature of the lake water and its pH, redox potential (Eh), and salinity were determined at the sampling time. Temperature was measured with a Prima sensor electric thermometer (Portugal), pH was determined with a pHep2 portable pH meter (Portugal), Eh was measured with an ORP portable redox meter (Portugal), and salinity was measured with a TDS-4 portable tester—conductometer (Singapore).

In the laboratory dissolved oxygen was determined by the Winkler method [10], carbon dioxide and bicarbonate in water and soil extracts were measured by titrimetry, and sulfate was determined by turbidimetry [11, 12]. Organic matter (OM) in the silts, water, and soil were determined by wet incineration according to Tyurin [13, 14].

The rates of decomposition of protein and cellulose were determined in situ by the application method, with the gelatin of the photographic paper as a protein substrate and cotton tissue as a cellulose one [13]. The plates with the substrates were placed in the upper 5-to 10-cm layer of the silt. The net rate of OM decomposition was determined by the Winberg method [14].

The rates of sulfate reduction, methanogenesis, and dark  $\mathrm{CO}_2$  assimilation were determined with the radioactive isotopes of carbon and sulfur [15, 16]. The silt and soil samples were collected into glass vials, sealed with rubber stoppers, and covered with aluminum caps. The labeled compounds were injected with a syringe. The radioactivity was measured on a Rackbetta liquid scintillation counter (Sweden) in the Institute of Microbiology, Russian Academy of Sciences (Moscow).

The rate of sulfate reduction was measured in a fashion similar to Ivanov's method [17]. The sample was supplemented with 0.1 ml  $\mathrm{Na_2}^{35}\mathrm{SO_4}(0.1-1~\mathrm{mCi})$  and incubated for 0.4–2 days. The samples were then fixed with 10–25% solution of cadmium acetate.

Methanogenesis rates were determined with NaH<sub>14</sub>CO<sub>3</sub>. The samples were fixed with 10 N NaOH. Labeled methane was collected and burnt to CO<sub>2</sub> at 700°C. After combustion CO<sub>2</sub> was captured in the scintillation liquid. For determination of the rates of dark CO<sub>2</sub> fixation, the radioisotope method was also used [18]. The samples were fixed with 10 N NaOH and incubated for 0.5–2 days. The bottom sediments and soil were sonicated for 2 min (UZDN-2, 22 kHz, 0.4 A). After filtration through 0.2-μm membrane filters, the samples were dried and the radioactivity was measured.

The rates of the processes were calculated from the equation A = r[C]/Rt, where A is the process rate, C is the substrate concentration, r is the radioactivity of the product, [R] is the radioactivity of the labeled substrate, and t is the incubation time.

## Determination of the <sup>13</sup>C/<sup>12</sup>C Ratios

The carbonate content and the <sup>13</sup>C/<sup>12</sup>C ratios in the carbonates and organic matter were determined on a Varian mass spectrometer (Germany) in the Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences.

The carbon isotopic composition of the samples was presented as the  $\delta^{13}$ C values (‰) calculated from the accepted expression:

$$\delta^{13}C_{\text{sample}} = ((R_{\text{sample}}/R_{\text{st}}) - 1) \times 1000(\%e),$$

where  $R_{\text{sample}}$  and  $R_{\text{st}}$  are the ratios of the levels of the carbon isotope [ $^{13}$ C]/[ $^{12}$ C] in the sample and in the PDB standard (PD formation belemnite), respec-

tively. The "+" sign indicates that the sample is isotopically heavier than the standard; the "-" sign indicates an isotopically lighter sample. Carbon dioxide gas for the analysis was obtained by treating the carbonate minerals with orthophosphoric acid followed with freezing out of the resultant CO<sub>2</sub> in liquid nitrogen. Prior to analysis of the isotopic composition of organic matter, carbonates were removed from soil by treatment with 10% HCl. Combustion was carried out at 550°C with CuO as an oxidizer. The carbonate content was determined according to Khitrov [19].

All the calculations were carried out per volume (for water and silts) or for an air-dried sample (for soil). The data presented are averages of three repeats.

#### RESULTS AND DISCUSSION

#### Geochemical Characterization of Lake Beloe

Lake Beloe is located within the contour of the gypsum-free chestnut soils upon the ancient alluvium of the left shore of the Selenga River. The lake is protected against eolian processes by the branches of the Khamar-Daban ridge. The southwestern shore is swampy, while the other shores are covered with solonchak soils. In water-rich years, a small intermittent spring flows out of the lake. The soils surrounding Lake Beloe are characterized as cold ones. In April 2002 the thawing depths at the key sites 1C, 2C, and 3C (Fig. 1) were 49, 55, and 52 cm, respectively, while the air temperature was 10°C. At the depth of 15 cm, the soil temperature was 5° C. In May 2002 the thawing depths were 61, 90, and 76 cm, respectively, while the water temperature was 18.8°C, pH 6.2, salinity 0.16 g l<sup>-1</sup>, and Eh. 236 mV. In July the soil was heated to 24°C, while in autumn the temperature decreased to 3°C. In July, the water temperature was 25°C, pH 8.8, salinity  $1.9 \text{ g l}^{-1}$ , and Eh +335 mV. During the first sampling (October 2000), the water temperature was 2°C, ice thickness was 3 mm, pH 8.5, salinity  $1.4 \,\mathrm{g}\,\mathrm{l}^{-1}$ , Eh 251 mV, and  $O_2$  concentration was  $4.7 \text{ mg ml}^{-1}$ .

The concentrations of O<sub>2</sub>, CO<sub>3</sub><sup>2-</sup> HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, and SO<sub>4</sub><sup>2-</sup> in Lake Beloe were 8.2, 180, 760, 1700, and 500 mg l<sup>-1</sup>, respectively. Lake Beloe has carbonate-bicarbonate salinization.

According to Ochirov [20], frost penetration in the soils of Transbaikalia occurs 15 or more days earlier than in European Russia and Western Siberia. The freezing period is over nine months long. The short warm season remains for a complete vegetation cycle and for at least partial (sufficient for renewal of the cycle of matter) destruction of organic detritus by the microbial biota of soils and silts.

In order to assess the production branch of the microbial carbon cycle, we determined the rate of dark

**Table 1.** Rates of dark CO<sub>2</sub> fixation in the sandy beach of Lake Beloe in 2002

Sampling site	Dark CO <sub>2</sub> fixation, mg C kg <sup>-1</sup> day <sup>-1</sup>					
Sampling site	May August		October			
Beach (sand), 0-5 cm	0.07	0.21	0.002			
Silt, 0–5 cm	0.06	0.43	0.19			

CO<sub>2</sub> fixation in the silt of Lake Beloe and the protein and cellulose content in the silts and sor solonchaks.

From May to October 2002, the rate of dart  $CO_2$  fixation by the benthic microbial community of the lake varied from 0.06 to 0.43 mg C kg<sup>-1</sup> day<sup>-1</sup>, with a maximum in late summer (August) (Table 1).

The content of cellulose in the solonchak soils and silt of Lake Beloe was generally higher than the protein content (Table 2). In the lake silt, the contents of cellulose and protein were 1.3 and 0.31 mg ml<sup>-1</sup> wet silt.

At the key sites of the shore (1C, 2C, and 3C), the content of cellulose varied from 1.7 to 5.0 mg g $^{-1}$  soil and the protein content was from 0.21 to 0.42 mg g $^{-1}$ . The highest levels of cellulose and protein were observed at the site 2C and the lowest at the site 1C. At the sandy beach, the values were 4.6 and 0.03 mg g $^{-1}$ , respectively (Table 2).

#### Cellulolysis and Proteolysis

The rates of cellulolysis and proteolysis in different seasons were studied for several years (from October 2000 to October 2002). Independent of time, the rate of proteolysis in all sors exceeded the rate of cellulolysis (Fig. 2).

The rates of these processes exhibit seasonal variation. The lowest rates of cellulolysis in the silt of Lake Beloe were observed in May 2001 and April 2002 (0.43 and 0.08% substrate consumption per day, respectively). The highest rates occurred in August 2001 and

August 2002 (0.64 and 1.08% substrate consumption per day, respectively) (Fig. 2).

In Lake Beloe the highest proteolysis rates of 1.27 and 1.47% substrate consumption per day were observed in June 2001 and 2002, respectively (Fig. 2). The lowest rates of proteolysis were recorded in May 2001 and April 2002 (0.57 and 0.5% substrate consumption per day, respectively). At the onset of the proteolysis experiments (June 2002), the absorbed moisture in the A1 surface layer was 0.52, 0.73, and 1.65% for sites 1C, 2C, and 3C, respectively.

The rates of protein decomposition were high both in aquatic (lake silt) and terrestrial (solonchaks) environments. In the silt it was 0.77% substrate consumption per day, while in soil it reached 0.73% substrate consumption per day (Fig. 3). The proteolysis rate varied along the solonchak profile, decreasing at the lower horizons. Its highest rate was observed in the A1 horizon of site 3C and in the A1B horizons of sites 2C and 3C.

The sandy beach (Fig. 1) is covered by a thick slat crust. The rates of dark  $CO_2$  fixation (Table 1) and decomposition of biopolymers there are very low.

The dynamics of biopolymer decomposition in the silt of Lake Beloe was typical, with a single peak characteristic for the factors of climatic (temperature) control of these processes. The peaks of cellulolysis and proteolysis in the solonchak soils and silts were therefore associated with the warm summer season (July to August), and the recessions occurred in the cold season (autumn and winter). The effects of limitation of these processes by S–SO<sub>4</sub><sup>2-</sup>, which have been revealed in freshwater bogs of the Selenga delta as two seasonal peaks [21], were not found in the biotopes under study.

### Bacterial Sulfate Reduction and Methanogenesis

The SO<sub>4</sub><sup>2-</sup> content in Lake Beloe exhibited seasonal variations with a peak of 1.4 g l<sup>-1</sup>. The rates of sulfate reduction in the silt also exhibited seasonal

**Table 2.** Cellulose and protein content in the sor solonchaks, sandy beach, and silt of Lake Beloe

Experimental objects (key sites)	Sampling depth, m	Cellulose, mg ml <sup>-1</sup> wet silt or g <sup>-1</sup> soil and sand	Protein, mg ml <sup>-1</sup> wet silt or g <sup>-1</sup> soil and sand		
1C	0-19	1.7	0.21		
2C	0-12	5.0	0.42		
3C	0–9	3.5	0.37		
Beach (sand)	0-3	4.6	0.03		
Silt	0-20	1.3	0.31		

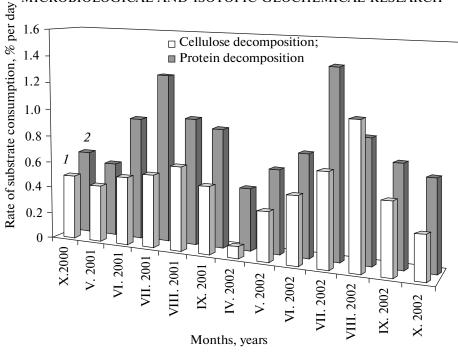


Fig. 2. Dynamics of cellulose and protein decomposition in the silt of Lake Beloe in 2000–2002.

peaks. For example, while the peak value in May was 0.003 mg S dm<sup>-3</sup> day<sup>-1</sup>, in August it was as high as 1.280 mg S dm<sup>-3</sup> day<sup>-1</sup> (Table 3). The peak of sulfate reduction rates coincided with both the peak of sulfate reducer abundance  $(6.0 \times 10^5 \text{ cells cm}^{-3})$  and the peak of sulfate (170 mg S dm<sup>-3</sup>), while the minimum of sulfate reduction rates coincided with the minimum of the relevant cell population  $(2.2 \times 10^5 \text{ cells cm}^{-3})$  and the minimum of sulfate (156 mg S dm<sup>-3</sup>) [6]. The rate of methanogenesis was an order of magnitude or more lower than the rate of sulfate reduction. The highest peak of methanogenesis  $(0.321 \,\mu\text{l CH}_4 \,\text{dm}^{-3} \,\text{day}^{-1})$  was observed in August 2002.

In the freshwater biotopes of the Baikal region, the rate of methanogenesis calculated per carbon consumption was, on the contrary, higher than the rate of sulfate reduction. According to Namsaraev and Zemskaya [22], the methanogenesis rate in the bottom sediments of Lake Baikal (Frolikha Bay, depth 430 m) reached 534.7  $\mu$ l CH<sub>4</sub> kg<sup>-1</sup> day<sup>-1</sup>, while the rate of sulfate reduction was 0.025 mg S kg<sup>-1</sup> day<sup>-1</sup>. Up to 99.9%

of CH<sub>4</sub> was produced by autotrophic methanogens, while the rest was formed by methylotrophic methanogens. The same picture was observed in freshwater bogs of the near-delta depression of the Selenga river: the methanogenesis rate in the silt water of these bogs (0.331–12.818 μl CH<sub>4</sub> kg<sup>-1</sup> day<sup>-1</sup>) was much higher than the rate of sulfate reduction (0.21 mg S kg<sup>-1</sup> day<sup>-1</sup>) [21]. Methanogenesis probably also prevails in the arid soils of this region. After abundant summer rains, when warm weather is established, the open surface of the gypsum-free soils of the Selenga highlands begins to "foam," forming microbubbles [1, 23].

According to Tsybzhitov et al. [3], this phenomenon, which is as yet unclear, is due to extrusion of the air of the soil pores with rainwater. The soil air removed from the soil pore volume by the downpouring water moves upward, resulting in foaming of the wet soil surface.

This effect, however, has not been observed in other arid soils [24]. The reason for foaming of the gypsumfree soils of this region is, therefore, probably methanogenic, rather than hydraulic. In these soils methanogenic

**Table 3.** Rates of the terminal stages of anaerobic decomposition of organic matter in the sandy beach and silt of Lake Beloe, 2002

Sampling site	Sulfate reduction, mg S dm <sup>-3</sup> day <sup>-1</sup> at different months			Methanogenesis, mg CH <sub>4</sub> dm <sup>-3</sup> day <sup>-1</sup> at different months		
	May	August	October	May	August	October
Silt	0.003	1.820	0.530	0.002	0.321	0.026
Beach (sand)	0.0002	0.042	0.003	0.0001	0.085	0.0001

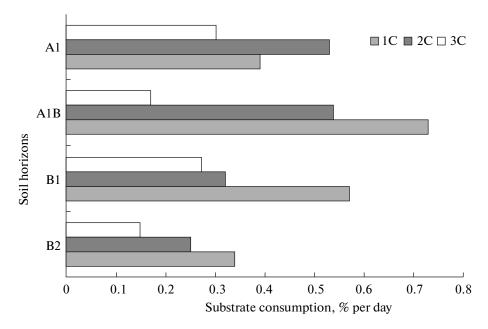


Fig. 3. Proteolysis rates in the soil profiles 1C, 2C, and 3C, % of protein consumption per day.

nogens a priori do not compete with sulfate reducers. Moreover, they have renewable (and therefore inexhaustible) sources of oxidized carbon (CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>) and protons (H<sup>+</sup>) generated in the course of soil calcification [25] by CaCO<sub>3</sub> formation from Ca<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup>. The proposed "arid" methanogenesis should manifest itself externally, on the wet surface of the arid soils of this region as small-scale CH<sub>4</sub> exhausts due to increased CH<sub>4</sub> pressure in the soil water and pose volume. Internally, in the relevant soil layers, it should be manifested by the heavier stable isotope composition of the carbon of soil carbonates (δ<sup>13</sup>C<sub>carb</sub>).

#### Soils of Sor Solonchaks

Ryskov et al. [26], Landi et al. [27], and other authors demonstrated that the  $\delta^{13}C_{carb}$  distribution in the upper 50–60 cm of the oxic layers of arid soils, where air penetrates freely during the dry periods is the same as the distribution of the stable isotope composition for organic carbon ( $\delta^{13}C_{org}$ ). This pattern, however, does not apply to the lower, anoxic layers, where  $\delta^{13}C_{carb}$  becomes heavier, i.e., enriched with the  $^{13}C$  isotope. Ryskov et al. [26] attribute this effect to the so-called lithogenic carbonates, comprising the marine inclusions in soils, since  $\delta^{13}C$  of these carbonates is heavier (from -2 to +2%).

We revealed a similar pattern in the sor solonchaks of Western Transbaikalia [28]. However, unlike the steppe soils of the lowland nondrainable landscapes, which were formed on ancient marine deposits, the arid soils of the Central Asian upland do not contain marine inclusions and did not contain them in the past [29].

The results of investigation of the solonchak soils of the region are presented in Table 4. The content of organic matter found in the solonchaks was low. It was 1.13–1.54% of the air-dry sample in the upper 5– 10 cm and decreased gradually to 50–60 cm, where the conventional border between oxic and anoxic soil layers is located. OM content in the latter increased up to 0.50–0.54%. These soils had a high total alkalinity. It was especially high in the profile 1C, up to 0.34% HCO<sub>3</sub> of the air-dry sample or 5.6 mg-eq per 100 g soil in the oxic 20-cm layer. Lower in the profile, the total alkalinity decreased and then increased slightly in anoxic layers. The profiles of the other investigated solonchaks (2C and 3C) exhibited a similar pattern. The profiles of total alkalinity were similar to the profiles of the distribution of soil carbonates. Carbonate accumulation was especially pronounced in the solonchak 1C, 10 m from the shoreline of Lake Beloe. The content of soil carbonates and sulfates in the water extract from soils decreased along the minitransect. The soil of site 3C, located at the upper edge of the coastal terrace, was especially carbonate-impoverished. The peaks of carbonate accumulation in the profiles of these soils were associated with the location of the oxic—anoxic layers. For example, in profile 1C, a relatively high carbonate peak (12.9% of the air-dry sample) was located at the oxic 45-cm layer, while another peak, the maximum (18.6% of the air-dry sample), was located at 70 cm in the anoxic layer. A similar carbonate distribution was found in the profile 2C. The 3C profile was somewhat different. The highest carbonate content (6.2% of the air-dry sample) was

**Table 4.** Content of  $SO_4^{2-}$ ,  $HCO_3^-$ , organic matter (OM), soil carbonates (SC), and stable carbon isotopes ( $\delta^{13}C_{org}$  and  $\delta^{13}C_{carb}$ ) in the sandy beach and sor solonchaks of Lake Beloe

Soil profile	Average depth of soil layers, cm	HCOO <sub>3</sub> , %/mg-eq per 100 g soil	SO <sub>4</sub> <sup>2-</sup> , mg pre 1 g of air-dry sample	OM, % of air- dry sample	SC, % of air- dry sample	$\delta^{13}$ Corg,	$\delta^{13}C_{carb}$ ,	$\Delta = \delta^{13} C_{carb} - \delta^{13} C_{org}, \% o$
Beach (sand)	0-3	0.004/0.07	147.0	0.54	4.5	-22.48	-7.68	14.80
1C	10	0.02/0.3	27.75	1.13	2.9	-22.98	-6.54*	16.44
	20	0.34/5.6	5.85	0.71	2.4	-21.94	-6.95	14.19
	45	0.23/3.8	3.87	0.68	12.9	-23.65	-7.60	16.05
	55	0.07/1.2	0.81	0.38	2.7	-23.94	-8.88*	15.06
	70	0.05/0.9	2.40	0.50	18.6	-26.52	-6.29*	20.23*
	90	0.19/3.0	11.85	0.42	7.1	-27.54	-8.50	19.04*
2C	5	0.02/0.3	0.02	1.12	4.6	-23.86	-8.48	15.38
	30	0.11/1.8	0.77	1.05	0.7	-26.67	-10.92*	15.75
	50	0.05/0.9	0.39	0.29	9.6	-25.03	-5.94*	19.06*
	60	0.06/1.0	1.25	0.31	4.2	-23.76	-7.59	16.17
	80	0.05/0.9	0.99	0.41	7.3	-24.60	-6.24	18.36
3C	5	0.02/0.4	3.25	1.54	6.2	-24.24	-6.15*	18.09
	15	0.004/0.11	5.70	1.0	4.7	-25.59	-9.0	16.59
	35	0.03/0.4	5.37	0.69	1.1	-25.12	-8.77	16.35
	55	0.07/0.12	2.33	0.22	0.3	-24.64	-11.41*	13.23*
	70	0.03/0.5	1.80	0.54	0.3	-24.19	-9.64	14.55
	90	0.02/0.3	4.87	0.59	1.1	-26.86	-8.38	18.48

<sup>\*</sup> Pronounced differences of  $\delta^{13}C_{carb}$  and  $\Delta$  from the averaged values found on the sandy beach.

found in the uppermost 5-cm oxic layer. It decreased with depth and increased slightly only in the 90-cm anoxic layer.

In general the distribution of <sup>13</sup>C<sub>OM</sub> and <sup>12</sup>C<sub>OM</sub> along the soil profiles was uniform, with a slight trend to an increase in  $\delta^{13}C_{OM}$  in the upper oxic layers [28]. However, the distribution of  $\delta^3$ C of the soil carbonates in the profiles exhibits significant variations. Along the solonchak profile, δ<sup>13</sup>C<sub>carb</sub> changes stepwise, with decreased  $\delta^{13}$ C values in the oxic layers and its increase in some oxic layers, although mainly in the anoxic ones (Table 4). Thus, the difference ( $\Delta$ ) expressing the gap in the genetically conjugated relation between  $\delta^{13}C_{carb}$  and  $\delta^{13}C_{org}$  was in some horizons, e.g., the 70to 90-cm anoxic layers of site 3C, higher ( $\Delta = 19.04$ – 20.23%) and in some horizons, e.g., in the 55-cm layer of 3C soil, lower ( $\Delta = 13.23\%$ ) than in the sandy beach ( $\Delta = 14.80\%$ ,  $\delta^{13}C_{carb} = -7.68\%$ ), where carbonate accumulation was allochthonous, i.e., averaged in composition.

Methanogens (MG) are known to preferentially use  $^{12}$ C forming isotopically light methane with  $\delta^{13}$ C to -100%, which results in the heavier  $\delta^{13}$ C of the remaining  $CO_2$  and  $HCO_3^-$ . Galimov et al. [30] found

that, in the Sea of Kara bottom sediments, methanogenic archaea involved in the  $\delta^{13}C$  collisions of the carbonate sediments develop under anoxic conditions at low  $SO_4^{2-}$  content. They demonstrated that  $\delta^{13}C_{org}$  is monotonously distributed along the profile of marine sediments, while the profiles of  $\delta^{13}C_{carb}$  have a broken, peak-shaped configuration. The peaks of heavier and lighter  $\delta^{13}C_{carb}$  occur in the anoxic and oxic layers of marine bottom sediments where methanogens and methane-oxidizing bacteria (MOB) occur, respectively. Since MOB consume the isotopically light  $CH_4$ , they accumulate isotopically light carbonates.

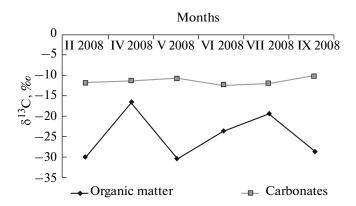
It should be noted that the investigated solonchaks, being semihydromorhic soils, have practically the same properties as the above-mentioned marine sediments [3] (Table 4). In the solonchak layers where  $SO_4^{2-}$  content was low, the difference between  $\delta^{13}C_{carb}$  and  $\delta^{13}C_{org}$ , expressed by the module  $\Delta$ , became more noticeable. It, however, was more dependent upon the oxic or anoxic nature of the soil horizons. In the potentially oxic soil layers, the  $\Delta$  values decreased due to the lighter  $\delta^{13}C_{carb}$ , and vice versa. If the  $\Delta$  modules expressed the fluctuations of  $\delta^{13}C_{carb}$  resulting only

from precipitation of CaCO<sub>3</sub> from the soil solution saturated with carbonic acid [31], their distribution along the soil profiles would have been monotonous. In reality, it is intermittent, indicating the action of some external factor specifically affecting the soil carbonate system.

Among the known factors of soil formation and diagenesis, only MG and MOB exhibit specific affinity to the components (CO<sub>2</sub>) and products (CH<sub>4</sub>) of the carbonate system [30]. Thus, only MG and MOB are able to decouple the genetically determined (due to microbial mineralization of  $C_{\rm org}$ ) relation between  $\delta^{13}C_{\rm carb}$ , and  $\delta^{13}C_{\rm org}$ , which in other soils and diagenesis sites is observed as copying the  $\delta^{13}C_{\rm org}$  profile.

In the dry years and seasons, the soil under study experiences drastic changes in humidity,  $SO_4^{2-}$  content, redox potential, alkalinity, and other factors. During draughts, the oxic soil layers expand to the lower horizons, where the development of obligately aerobic MOB is probably initiated. Thus, MOB were probably responsible for the effects of  $\Delta$  decrease and, therefore, for the lightening of the  $\delta^{13}C$  fractions of soil carbonates occurring, for example, at the 55-cm interface of the anoxic layer in profile 1C (which became anoxic by the time of investigation), where  $\delta^{13}C_{carb} = -8.88\%$ , and at the 55-cm interface of the anoxic layer in profile 3C, where  $\delta^{13}C_{carb} = -11.41\%$ .

During the wet periods, which are established due to the effect of the Asian monsoon, the profiles of these soils, including the uppermost layers, become overwetted [3] and, therefore, anoxic. Moreover, the cells of MG and sulfidogenic bacteria are localized in soil microaggregates, which occur both in the lower and in the upper soil horizons [24]. Thus, the effects of increasing  $^{13}$ C content in the carbonates ( $\Delta =$ 18.09‰) in the uppermost 5-cm oxic layer of the 3C profile and in the transition 50-cm layer of the 2C profile ( $\Delta = 19.06\%$ ) are associated with the growth of MG in soil microaggregates humidified by atmospheric precipitation (the profile 2C is located above the stone plates and is less subject to ascending humidification by the waters of Lake Beloe) (Table 4). Methanogenesis induced in the 50-cm transition layer of the profile 2C probably resulted in unmetabolized isotopically heavy carbonate fractions ( $\delta^{13}C_{carb} = -5.94\%$ ), while the methane diffusing into the oxic 30-cm layer was oxidized by methanotrophic bacteria, resulting in decreased  ${}^{13}C$  content in this horizon ( $\delta^{13}C$  = -10.92%). The methane produced in the uppermost 5-cm layer of the profile 3C was not oxidized by methanotrophs, so that the isotopic composition of the carbonates did not change ( $\delta^{13}C_{carb}=-6.15\%$ ) and the escaping  $CH_4$  caused the foaming of soil (formation of microbubbles on the wet surface of this gypsum-free, weakly solonchak chestnut soil.



**Fig. 4.** Dynamics of  $\delta^{13}C_{org}$  and  $\delta^{13}C_{carb}$  in the silt of Lake Beloe in different months of 2008, ‰.

The  $\delta^{13}$ Cvalue is a reliable and objective record of long-past natural climatic events. The  $\delta^{13}C_{carb}$  data supplemented with the  $^{14}$ C age determination for the carbonates formed in the gypsum-free soils of this region will make it possible to reconstruct a reliable history of the periods of flooding and draughts in the Central Asian upland. Moreover,  $\delta^{13}C_{org}$  may be used to reconstruct events in the recent past, e.g., in the months and seasons of a year.

For example, accumulation of  $C_{org}$  and  $C_{carb}$  in the bottom sediments of Lake Beloe in different months and seasons of 2008 was accompanied by the fluctuations in the  $\delta^{13}C_{org}$ , but not in the  $\delta^{13}C_{carb}$  (Fig. 4). A peak of accumulation of isotopically heavy  $C_{org}$  ( $\delta^{13}C_{org}=-16.60\%o$ ) in the silt sand was revealed in April 2008, and another peak ( $\delta^{13}C_{org}=-19.48\%o$ ) was revealed in July 2008, while no fluctuations on the same scale were found for  $\delta^{13}C_{carb}$ , which varied within a narrow range from -10.29 to -12.45%o (Table 5).

Londry and Des Marais [32] demonstrated that the sulfate reducers Desulfotomaculum acetoxidans growing heterotrophically increased the  $\delta^{13}$ C value of metabolized acetate by 8-9%. It is interesting to note that the increase in the  $\delta^{13}C_{\text{org}}$  in the silt of Lake Beloe was also by 8-9%. The mentioned increase in the  $\delta^{13}C_{org}$  in the lake silt may, therefore, be explained by the activity of heterotrophic sulfate reducers similar to D. acetoxidans. Importantly, the periods of  $\delta^{13}C_{org}$ increase in the lake silt were close to the periods of increases in SO<sub>4</sub><sup>2-</sup> content and abundance of sulfate reducers [6]. The isotopically light carbon dioxide produced by sulfate reducers is probably involved in photosynthesis, resulting in the isotopically light OM. This is confirmed by the periods of carbonate decrease to 19.2 and 18.1% of the air-dry silt sample during spring and summer, while the  $\delta^{13}$ C value for the silt carbonates changed insignificantly (Table 5). Another part of the isotopically light OM in the silt is allochthonous. It has averaged  $\delta^{13}$ C values.

•	Sampling date	OM, % of the mineral part of the sample	C <sub>org</sub> , % of the mineral part of the sample	C, % of the air-dry sample	C <sub>carb</sub> , % of the air-dry sample	δ <sup>13</sup> C <sub>org</sub> ,	δ <sup>13</sup> C <sub>carb</sub> , %0	$\Delta = \delta^{13} C_{carb} - \delta^{13} C_{org}, \%o$	
,	27.02	25.6	1.55	52.3	3.18	-30.12	-11.67	18.45	
	10.04	10.8	0.66	26.1	1.58	-16.60	-11.28	5.32	
	29.05	2.2	0.13	19.2	1.16	-30.36	-10.77	19.59	
	30.06	7.3	0.44	27.8	1.69	-23.73	-12.45	11.28	
	16.07	29.0	1.76	39.5	2.40	-19.48	-12.05	7.43	
	25.09	1.9	0.11	18.1	1.10	-28.65	-10.29	18.36	

**Table 5.** Content of organic matter (OM), carbonates (C), and organic ( $C_{org}$ ) and inorganic ( $C_{carb}$ ) carbon and their stable isotopes ( $\delta^{13}C_{org}$  and  $\delta^{13}C_{carb}$ ) in the silt of Lake Beloe in different months and seasons of 2008

Thus, the production branch of the cycle of organic carbon in the dry steppe Lake Beloe is highly intense. The rate of dark CO<sub>2</sub> fixation in the silt is as high as 0.19 mg C dm<sup>-3</sup> day<sup>-1</sup>, and the content of cellulose and protein in the sor solonchaks is 5.0 and 0.42 mg per 1 g of air-dry sample, respectively. During the warm season, the rates of cellulose decomposition and proteolysis in Lake Beloe silt are 1.08 and 0.96% substrate consumption day<sup>-1</sup>. At the terminal stages of anaerobic OM degradation in lake silt, sulfate reduction predominates, with the maximal rate of 1.82 mg S dm<sup>-3</sup> day<sup>-1</sup>. The distribution of carbonates in the solonchak soils generally mirrors the distribution of total alkalinity and oxic-anoxic layers. However, the difference between the  $\delta^{13}$ C values for carbonates and OM changes significantly, indicating heavier or lighter values for  $\delta^{13}C_{carb}$  along the soil profile. Accumulation of the isotopically heavier carbonates in soil and foaming of the arid soils of this region after abundant summer rainfall are both probably associated with the activity of methanogens, which produce isotopically light CH<sub>4</sub>. Accumulation of the isotopically light soil carbonates may be associated with the activity of methane-oxidizing bacteria, which always accompany methanogens. Seasonal peaks of heavier  $\delta^{13}C_{OM}$  found in the silts of Lake Beloe result probably from the activity of heterotrophic sulfate reducers.

#### **ACKNOWLEDGMENTS**

The work was supported by grants of the Russian Foundation for Basic Research (projects nos. 10-04-01185 and 10-04-93169), Presidium of the Russian Academy of Sciences, Siberian Branch (nos. 38 and 95), Ministry of Education of the Russian Federation (no. RNP 2.1.1/12153), and Baikal Scientific Educational Center.

#### REFERENCES

- 1. Nogina, N.A., *Pochvy Zabaikal'ya* (Soils of the Transbaikal Region), Moscow: Nauka, 1964.
- Zavarzin, G.A., Lektsii po prirodovedcheskoi mikrobiologii (Lectures in Naturalistic Microbiology), Moscow: Nauka, 2003.
- 3. Tsybzhitov, Ts.Kh., Tsybikdorzhiev, Ts.Ts., and Tsybzhitov, A.Ts., *Pochvy basseina ozera Baikal. T. 1. Genezis, geografiya i klassifikatsiya kashtanovykh pochv* (Soils of the Lake Baikal Basin. Vol. 1. Genesis, Geography, and Classification of Chestnut Soils), Novosibirsk: Nauka, 1999.
- 4. Bazarov, D.B., Chetvertichnye otlozheniya i osnovnye etapy razvitiya rel'efa Selenginskogo srednegor'ya (Quaternary Deposits and Major Stages of Development of the Relief of the Selenga Highlands), Ulan-Ude: Bur. Kn. Izd., 1968.
- 5. Polynov, B.B., Landscapes of the Northern Gobi, in *Geograficheskie raboty* (Geographical Works), Moscow: Gos. Izd. Geograf. Liter., 1952.
- Dambaev, V.B., Gonchikov, G.G., and Namsaraev, B.B., Distribution of Saprotrophic Microorganisms in Sor Solonchaks of the Transbaikal Region, *Pochvovedenie*, 2004, no. 11, pp. 1360–1374 [*Eur. Soil Sci.* (Engl. Transl.), no. 11, pp. 1205–1218].
- 7. Chapman, S.J., C Substrate Mineralization and S Limitation, *Soil Biol. Biochem.*, 1997, vol. 29, pp. 115–122.
- 8. Chowdhury, M.A.H., Kouno, K., and Ando, T., Critical Sulfur Concentration and Sulfur Requirement of Microbial Biomass in a Glucose and Cellulose-Amended Regosol, *Biol. Fertil. Soils*, 2000, vol. 32, pp. 310–317.
- 9. Chapelle, F.H., The Significance of Microbial Processes in Hydrogeology and Geochemistry, *Hydrogeol. J.*, 2000, no. 8, pp. 41–46.
- Alekin, O.A., Semenov, A.D., and Skopintsev, B.A., Rukovodstvo po khimicheskomu analizu vod sushi (Manual for Chemical Analysis of Terrestrial Waters), Leningrad: Gidrometeoizdat, 1973.
- 11. Arinushkina, E.V., *Rukovodstvo po khimicheskomu analizu pochv* (Manual for Chemical Analysis of Soils), Moscow: Mosk. Gos. Univ., 1970.

- Kuznetsov, S.I. and Dubinina, G.A., Metody izucheniya vodnykh mikroorganizmov (Methods for Investigation of Aquatic Microorganisms), Moscow: Nauka, 1989.
- Gel'tser, Yu.G., Biologicheskaya diagnostika pochv (Biological Diagnostics of Soils), Moscow: Mosk. Gos. Univ., 1986.
- 14. Romanenko, V.I. and Kuznetsov, S.I., *Ekologiya mikro-organizmov presnykh vodoemov* (Microbial Ecology of Freshwater Basins), Leningrad: Nauka, 1974.
- 15. Belyaev, S.S. and Ivanov, M.V., Radioisotope Method for Determination of Intensity of Bacterial Methanogenesis, *Mikrobiologiya*, 1975, vol. 44, pp. 166–168.
- Volkov, I.I. and Zhabina, N.N., Metody opredeleniya razlichnykh soedinenii sery v morskikh osadkakh, in Khimicheskii analiz morskikh osadkov (Methods for Determination of Various Sulfur Compounds in Marine Sediments), Moscow: Nauka, 1984, pp. 172– 180.
- 17. Ivanov, M.V., Application of Isotopes for Investigation of Intensity of the Process of Reduction of Sulfates in Lake Belovod', *Mikrobiologiya*, 1956, vol. 25, no. 3, pp. 305–310.
- 18. Sorokin, Yu.I., Role of Bacterial Dark Carbon Dioxide Assimilation in the trophics of Water Bodies, *Mikrobiologiya*, 1964, vol. 23, no. 5, pp. 880–887.
- 19. Khitrov, B.N., Ionic and Salt Composition of Soils in One Sample, *Pochvovedenie*, 1984, no. 5, pp. 119–127.
- 20. Ochirov, B.R., Freezing and Thawing of Forest-Steppe and Steppe Soils of the Selenga Highlands, in *Fizicheskie i khimicheskie svoistva pochv Buryatskoi ASSR* (Physical and Chemical Properties of Soils of the Buryat ASSR), Ulan-Ude, 1966.
- 21. Buryukhaev, S.P., Namsaraev, B.B., Korsunov, V.M., and Gonchikov, G.G., The Abundance of Microorganisms and the Dynamics of Decomposition Rates in Low-Moor Bogs of the Baikal Region, *Pochvovedenie*, 2003, no. 1, pp. 81–89 [*Eur. Soil Sci.* (Engl. Transl.), no. 1, pp. 76–84].
- 22. Namsaraev, B.B. and Zemskaya, T.N., Microbiological Processes of the Carbon Cycle in Lake Baikal Bottom

- Sediments, Gorlenko, V.M., Ed., Novosibirsk: Nauka, SO RAN, 2000.
- Tsybzhitov, Ts.Kh., Pochvy lesostepi Selenginskogo Srednegor'ya (Forest-Steppe Soils of the Selenga Highlands), Ulan-Ude, 1971.
- Kovda, V.A., Osnovy ucheniya o pochvakh (The Fundamentals of Soil Science. General Theory of Soil Formation), Moscow: Nauka, 1973.
- 25. McConnaughey, T.A. and Whelan, J.F., Calcification Generates Protons for Nutrient and Bicarbonate Uptake, *Earth Sci. Rev.*, 1997, vol. 42, pp. 95–117.
- 26. Ryskov, Ya.G., Borisov, A.V., Ryskova, E.A., Oleinik, S.A., and Demkin, V.A., On the Relationship between Pedogenic and Lithogenic Carbonates and Their Dynamics in the Profile of Steppe Soils During the Last 4000 Years, *Pochvovedenie*, 1999, vol. 32, no. 3, pp. 293–300 [*Eur. Soil Sci.* (Engl. Transl.), vol. 32, no. 3, pp. 263–270].
- 27. Landi, A., Mermut, A.R., and Anderson, D.W., Origin and Rate of Pedogenic Carbonate Accumulation in Saskatchewan Soils, Canada, *Geoderma*, 2003, vol. 117, pp. 143–156.
- 28. Gonchikov, G.G., Namsaraev, B.B., Zyakun, A.M., and Dambaev, V.B., Stable Carbon Isotopes in the Profile of the Transbaikalean Sor Solonchaks, *Sibir. Ekol. Zh.*, 2007, no. 3, pp. 375–380.
- 29. Chernousenko, G.I. and Yamnova, I.A., About the Genesis of Soil Salinity in the Western Part of the Transbaikal Region, *Pochvovedenie*, 2004, no. 4, pp. 399–414 [*Eur. Soil Sci.* (Engl. Transl.), no. 4, pp. 341–355].
- 30. Galimov, E.V., Isotopic Organic Geochemistry, *Organic geochemistry*, 2006, vol. 37, pp. 1200–1260.
- 31. Mook, W.G., Bommerson, J.C., and Staverman, W.H., Carbon Isotope Fractionation Between Dissolved Bicarbonate and Gaseous Carbon Dioxide, *Earth Planet. Sci. Lett.*, 1974, vol. 22, pp. 169–176.
- 32. Londry, K.L. and Des Marais, D.J., Stable Carbon Isotope Fractionation by Sulfate-Reducing Bacteria, *Appl. Environ. Microbiol.*, 2003, vol. 69, no. 5, pp. 2942–2949.