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EXPERIMENTAL ARTICLES

Factors Controlling the Activity of the Microbial Community of the Alkaline Lake Beloe (Transbaikal Region)

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Abstract—In this work, the main environmental factors determining the functioning of the microbial community of the alkaline low-mineralized Lake Beloe during the annual cycle were studied. High numbers of phototrophic and heterotrophic microorganisms (up to 10^7 cells/mL) and high rates of bacterial processes of organic matter (OM) production and destruction were observed. The highest rate of dark CO_2 assimilation (up to 0.43 mg C dm^{-3} day^{-1}), as well as the peak intensities of the terminal processes of sulfate reduction and methanogenesis (up to 1.81 mg S dm^{-3} day^{-1} and 0.96 μL CH_4 dm^{-3} day^{-1}, respectively), detected at the end of summer, were comparable to the rates of these processes detected in the bottom sediments of most soda lakes of the Transbaikal Region. Principal Component Analysis (PCA) allowed us to estimate the effect of environmental factors on the functioning of the microbial community of the alkaline Lake Beloe. Four main components, explaining 98% of variations, were detected. The first one (PC1) explained 63.5% of the seasonal variations and represented the temperature factor consisting of the temperatures of air, water, and bottom sediments. Water temperature and pH were the main contributors to the second component (PC2) and determine 26.2% of the seasonal variations. The PC3 (silt temperature and the concentration of organic matter) and PC4 (salt concentration) components were less important and explained only 6.5 and 2.2% of the variations, respectively.

Keywords: soda lake, microbial community, multivariate analysis, Principal Component Analysis

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Traditional statistical methods, such as dispersion/regression analysis, or the methods which can be described as statistical in all but name, including factor analysis, cluster analysis, and multidimensional scaling, are often used for analysis of the multidimentional data obtained in the course of microbial survey studies of natural ecosystems. However, the application of traditional methods for analysis of the results of ecological observations (ecological monitoring) is problematic due to some rigid assumptions (randomness of values and their Gauss distribution, uniformity of estimated sampling variances, zero or negligible errors during determination of the independent variables, etc.) [1] on which these methods are based. Analysis of the results of microbial survey studies demonstrates that none of these assumptions are applicable to them. Moreover, statistical models, including regression ones, cannot be used as evidence of cause-effect relationships within a studied system. The standard correlation analysis was proved to be ineffective when studying the variables with a non-normal distribution. In addition, traditional statistical approaches are usually not suitable for processing of the data combining both numerical and non-numerical (qualitative) variables, for simultaneous analysis of data on objects of different nature and different levels of description (water and sediments), and for analysis of nonlinear relationships between variables, even though nonlinear relationships prevail in natural ecosystems. These limitations of traditional statistics are uncharacteristic of modern methods of multidimensional data processing, including Principal Component Analysis. This method is based on the assumption that each measured parameter of each specimen is affected by a multitude of other independent parameters [2]. The goal of such studies is calculation, screening, and graphic representation of vast amounts of multivariable data [3]. In our work, we attempted to determine the main factors that control the seasonal changes in the extreme microbial community of an alkaline low-mineralized lake.

Low-mineralized brackish alkaline lakes are unique aquatic ecosystems characterized by relatively low salt concentrations and alkaline pH, which provides favorable conditions for growth of prokaryotic organisms in the water column and bottom sediments [4–6]. The study of the diversity of microbial communities is of considerable scientific interest, since it provides insight into the functioning of soda lakes as a unique type of ecosystem [7–9].

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In the steppe and forest-steppe zones of the Transbaikal Region with a sharply continental climate, shallow drying lakes with water mineralization ranging from several grams of salt per one liter of water to the saturation level are common [10]. Brackish lakes with the total water mineralization of 1–25 g/dm^3 are most widespread. The sharply continental climate and interannual fluctuations of the total humidity result in significant changes in the lake hydrological regime, as well as in variations in the cation-anion balance.

The goal of the present work was to reveal the main properties of the ecosystem of the low-mineralized alkaline Lake Beloe by determining the seasonal changes in the functioning of the extreme microbial community of the lake.

MATERIALS AND METHODS

Sampling site. The low-mineralized soda Lake Beloe is located in the Orongoi Depression of the Selenga River, 47 km south-east of Ulan-Ude (Buryatia) (51°32′40″N, 107°02′42″E). The depth and area of the lake depend on the weather conditions. The maximum area of the lake is 0.63 km^2; the maximum depth is 2.1 m. The samples were collected from the littoral zone of the lake once a month, from November 2006 to December 2007.

Physicochemical parameters. The temperatures of water, air, and sediments were measured with a Prima electric sensor thermometer (Portugal); pH was determined potentiometrically using a pHep2 pH meter (Portugal). The concentration of the major ions and the content of organic carbon were determined using traditional techniques [11, 12]. Mineralization (M, g/L) was calculated from the concentrations of the major ions:

$$M = [Na^+] + [K^+] + [Mg^{2+}] + [Ca^{2+}] + [Cl^-] + \left[SO_4^{2-}\right] + \left[HCO_3^-\right] + \left[CO_3^{2-}\right].$$

Water and sediment samples were collected into sterile bottles. To determine the species composition of cyanobacteria, as well as the total number of microorganisms, the samples were fixed with 4% formalin. Microscopic examination of cyanobacteria was carried out under an Axiostar plus microscope (Carl Zeiss, Germany). Determination of the taxonomic position of cyanobacteria was carried out according to their morphological properties [13].

The total number of microorganisms (TNM) was determined on membrane filters by the method of Razumov [14]. The numbers of aerobic and anaerobic degraders were determined on selective media at 30°C. The rates of sulfate reduction, methanogenesis, and dark CO_2 assimilation were determined by the radioisotope method [15, 16].

Statistical analysis. To elucidate the main environmental factors responsible for the seasonal fluctuations in the ecosystem of Lake Beloe, Principal Component Analysis (PCA) was applied using the MathLab10 and Microsoft Excel software packages.

RESULTS AND DISCUSSION

Physicochemical parameters. The atmospheric temperature during the study varied from –21.7°C (winter) to 26.4°C (summer) (Fig. 1). The annual temperatures of water and bottom sediments depended on changes in the atmospheric temperature and ranged from –1.15 to 23.2°C and from 0 to 23.2°C, respectively. The pH values ranged from 8.3 to 9.2. Salinity varied from 0.3 to 3.4 g/L; chloride and carbonate anions prevailed; the cation composition was of a mixed type. The highest mineralization level (3.4 g/L) was observed in early November. In spring, due to ice thawing and subsequent desalination, the concentrations of almost all studied ions decreased and reached their lowest values, which were detected in April, with the exception of CO_3^{2-} and HCO_3^-, for which the lowest concentrations were detected in summer. In summer, the concentrations of K^+ and Mg^{2+} ions increased. The seasonal dynamics of the content of organic matter in the water column showed two peaks, at the end of summer/beginning of autumn and in the early spring.

Diversity of cyanobacteria and the numbers of microorganisms. Cyanobacteria inhabited the water column and the surface of bottom sediments of the studied lake. In the course of our study, four species of cyanobacteria were detected in Lake Beloe, namely: *Anabaena variabilus* f. *tenuis* Popova, *Jaaginema woronichinii* (Anissimova in Elenkin) Anagnostidis et Komárek, *Leptolyngbya fovelarium* (Rabenchorst et Gomont) Anagnostidis et Komárek, and *L. komarovii* (Anissimova) Anagnostidis et Komárek. When determining the taxonomic position of the studied cyanobacteria, special attention was paid to the majority of characteristics fitting the description of one or another species, as well as to their variability, since the morphological properties of many species varied within a wide range and were often found to be typical of several species.

The total number of microorganisms varied from 0.2×10^6 to 1.8×10^7 cells/mL and reached its peak between the end of July and the middle of August. In the winter season, the numbers of bacteria belonging to various physiological groups decreased ($<1 \times 10^6$ cells/mL), except for anaerobic saprophytes in February, when their numbers reached 6.3×10^6 cell/mL (Fig. 2). Low numbers (up to 4×10^4 cells/mL) of sulfate-reducing bacteria were detected throughout the year.

Bacterial production and destruction of organic matter. One of the integral indicators of activity of an anaerobic microbial community is dark CO_2 assimilation. In the course of dark CO_2 assimilation, the microbial community inhabiting the bottom sediments of Lake Beloe was able to fix from 0.06 to

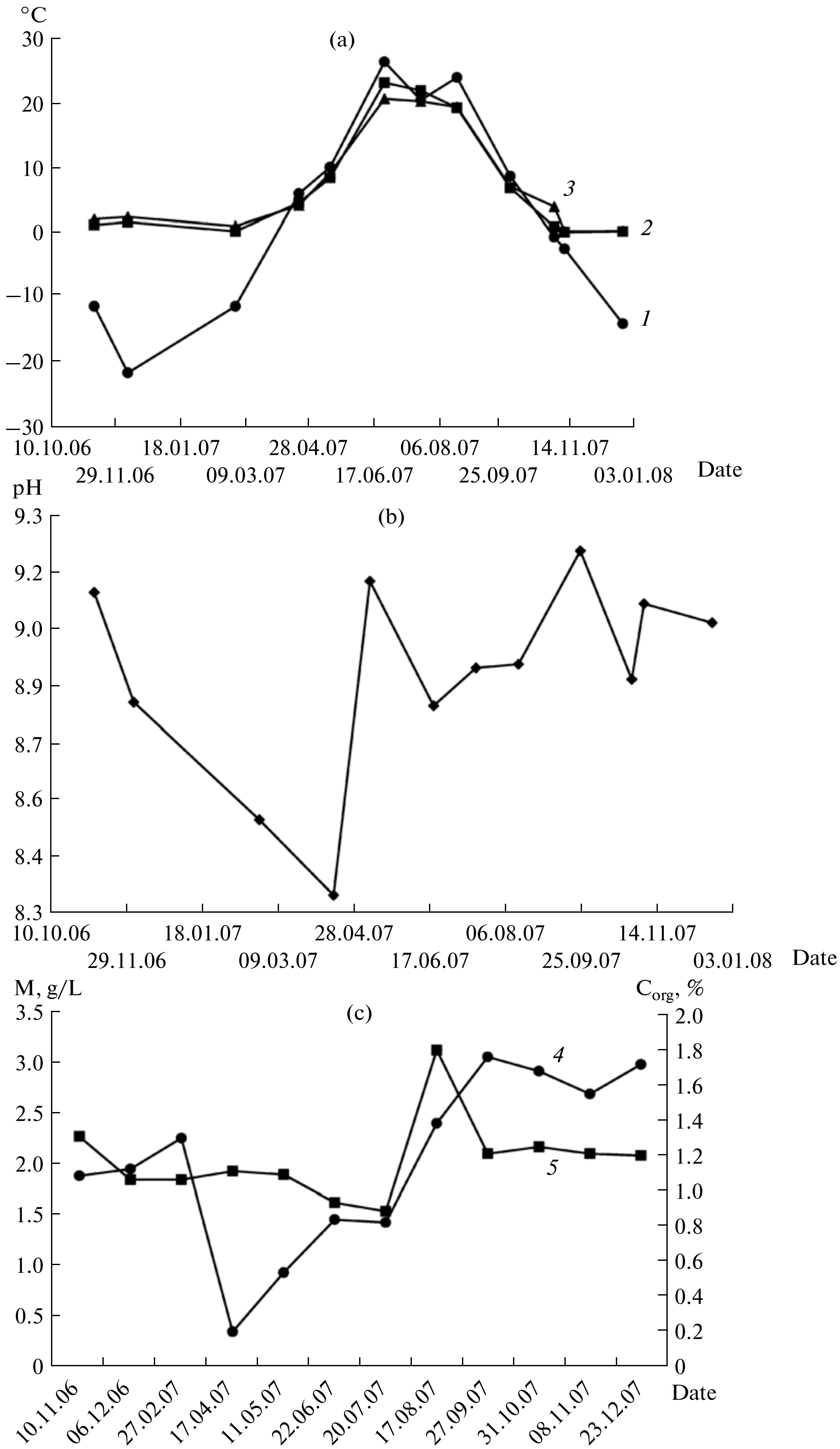

Fig. 1. Seasonal changes in temperature (a), pH (b), and mineralization and the organic matter content (c) in Lake Beloe from November 2006 to December 2007. Designations: atmospheric temperature (*1*), water temperature (*2*), silt temperature (*3*), mineralization (*4*), and C_{org} content, % of the total C content (*5*).

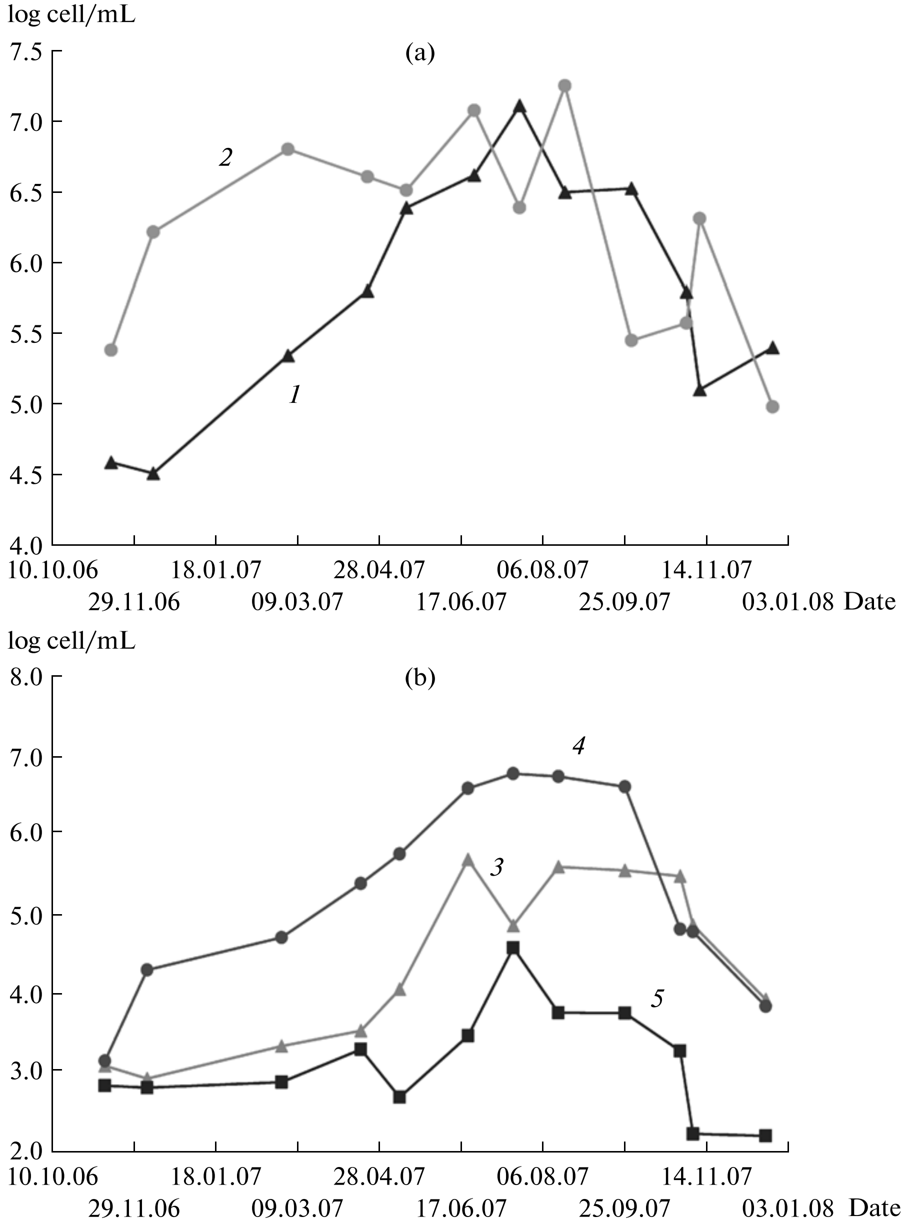

Fig. 2. Seasonal changes in the numbers of microorganisms. Designations: aerobic saprophytes (*1*), anaerobic saprophytes (*2*), aerobic cellulolytics (*3*), anaerobic cellulolytics (*4*), sulfate reducers (*5*).

0.43 mg C dm^{-3} day^{-1}. In March, the activity of the microbial community of the bottom sediments was low due to low ambient temperatures. An increase in temperature resulted in an increase in the rate of dark CO_2 assimilation, which reached its first peak (0.31 mg C dm^{-3} day^{-1}) in May (Fig. 3a). From the beginning and middle of summer, the rate of dark CO_2 assimilation was slightly lower. The second peak of activity was detected in August. The rate of the process reached its peak (0.43 mg C dm^{-3} day^{-1}) and remained high (0.4 mg C dm^{-3} day^{-1}) until September. In November, as the ambient temperature decreased further, the rate of CO_2 assimilation dropped to its lowest level (0.06 mg C dm^{-3} day^{-1}).

The curve of sulfate reduction rates was similar to that of the rates of dark CO_2 assimilation. During the year, two peaks of activity of sulfate-reducing bacteria were detected: in May and August. In August, the ambient temperature was high enough and the influx of the newly formed autochthonous and allochthonous organic matter occurred. The distribution of the peaks of activity of sulfate-reducing bacteria correlated well with the content of organic matter in the bottom sediments detected during this period. The lowest rate of sulfate

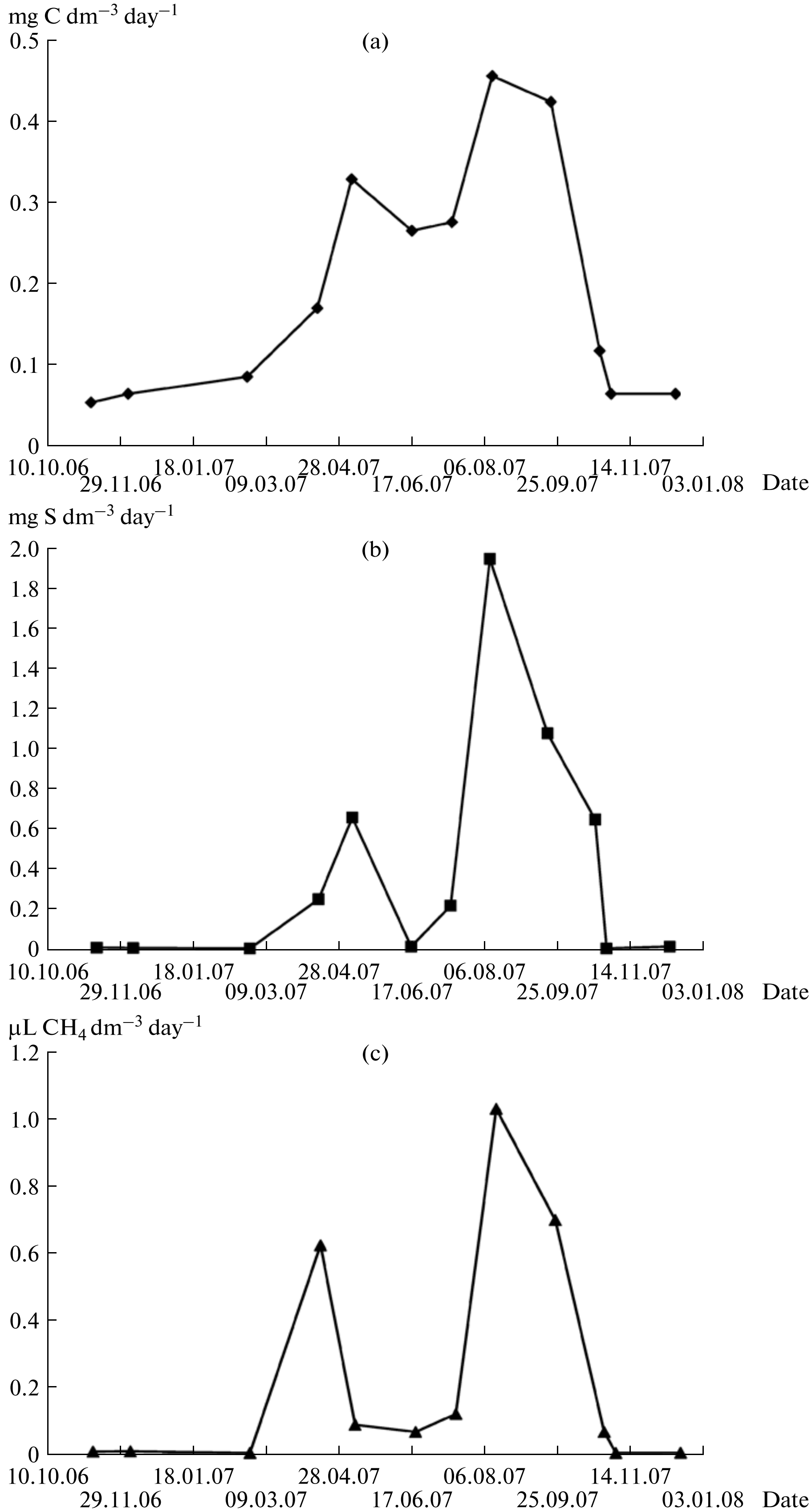

Fig. 3. Seasonal changes in the rates of the microbial processes of dark CO_2 assimilation (a), sulfate reduction (b), and methanogenesis (c) in Lake Beloe.

reduction (0.001 mg S dm^{-3} day^{-1}) was detected in November (Fig. 3b). The low rate of sulfate reduction at the end of autumn, despite the rather high organic matter concentration, was due to low ambient temperatures.

The rate of methanogenesis was also subject to seasonal fluctuations (Fig. 3c). It reached its peak in August and was lowest in March.

The revealed fluctuations of microbial activity depended on the ambient temperature and organic matter concentrations in the environment. A significant positive correlation with the temperature conditions was observed for dark CO_2 assimilation, sulfate reduction, and methanogenesis (r_s = 0.81, 0.52, and 0.52, respectively). The concentration of organic matter correlated well with the rates of the terminal processes of sulfate reduction and methanogenesis (r_s = 0.75 and 0.64. respectively). The highest rates of microbial processes corresponded to the periods when the ambient conditions were most favorable (high temperatures and organic matter concentrations). A decrease in one of these parameters resulted in a decrease in the activity of the microbial community.

Principal Component Analysis (PCA) is one of the most popular methods of multivariate statistical analysis [2, 3]. PCA makes it possible to convert and visualize complex data sets and create a new perspective of main components in which the relevance of factors becomes more obvious. It is difficult to process these data using only simple methods of univariate statistics, due to the vast amount of data and the abundance of multivariate correlations. When using Principal Component Analysis, factors (principal components) are extracted on the basis of the quantitative and qualitative parameters describing the objects under question. PCA includes progressive transformation of the matrix of the initial X values with the $n \times m$ dimension, where n is the number of lines representing the studied specimens or objects of observation and m is the number of columns representing parameters or variables. The structure of the matrix of the initial X values is $X = TP^T$, where T is the T-score matrix with the $n \times m$ dimension and P is the load matrix with the $m \times n$ dimension.

The Principal Component Analysis of the results obtained consists of three important elements, a score component, a loadings component, and the value of the explained dispersion. The scores are the initial specimens projected on the subspace of the principal components within the PC1–PC2 coordinate system. The proximity of two points on the score plot (Fig. 4) indicates their similarity, i.e., a positive correlation. The points lying in diametrical opposition to each other show a negative correlation. The loadings are coefficients between the initial variables and principal components, determining the contribution of each parameter to the principal components. The components (loadings and scores) are determined in increments, i.e., the first principal component (PC1) explains the maximum number of changes (the greatest variation) and is of greater importance for the explanation of the ecosystem condition; the second component (PC2) explains the remaining maximum, etc. Hence, when the number of factors is greater than the number of principal components, it is possible to obtain a complete description of the changes occurring in the ecosystem. After conversion, the majority of data variations will be concentrated in the first positions, which makes it possible to discard the rest of them and study the reduced space. The value of explained variance (ERV) is a relative value describing the importance of each component; it is usually expressed as a percentage or as unit fractions. It is calculated using natural normalization: the sum of squares of all initial values (x_{nm}).

The initial data matrix included both the physicochemical parameters of the water and bottom sediments of Lake Beloe and the main properties of the microbial community, such as TNM, numbers of microorganisms belonging to different physiological groups (aerobic and anaerobic saprophytes and cellulolytics), rates of microbial processes of organic matter production and degradation.

The number of principal components optimal for this system is four, which was determined on the basis of the values of the explained dispersion. The four principal components (PC) explained 98% of variations; in other words, the noise in the results of the initial data projection on the four-dimensional PC1–PC4 space constituted only 2%. PC1, which is the temperature factor consisting of the temperatures of air, water, and bottom sediments, explained 63.5% of the seasonal variations. Water temperature and pH were the main contributors to the second component (PC2) and determined 26.2% of the seasonal variations.

PC3 (silt temperature and the concentration of organic matter) and PC4 (salt concentration) were less important and explained only 6.5 and 2.2% of the variations, respectively (table). Earlier, seasonal changes in low-mineralized (2.8–31.3 g/L) lakes (pH 8.88–10.89) were described by principal component analysis [6]. The turbidity factor and salinity were of major importance and explained 55–73% of the observed seasonal changes, whereas the temperature factor was less important, unlike the results obtained in the present work. This was obviously due to huge temperature swings in the sharply continental climate of the Transbaikal Region, which affected the lake parameters.

The load plot (Fig. 4a) gives an idea about the role of the studied parameters and their dependence on each other. The child component (PC3–PC4) plot demonstrates that the variables representing the water composition and the rates of microbial processes formed a compact group in the center (group I, Fig. 4a). They almost coincided, which demonstrates

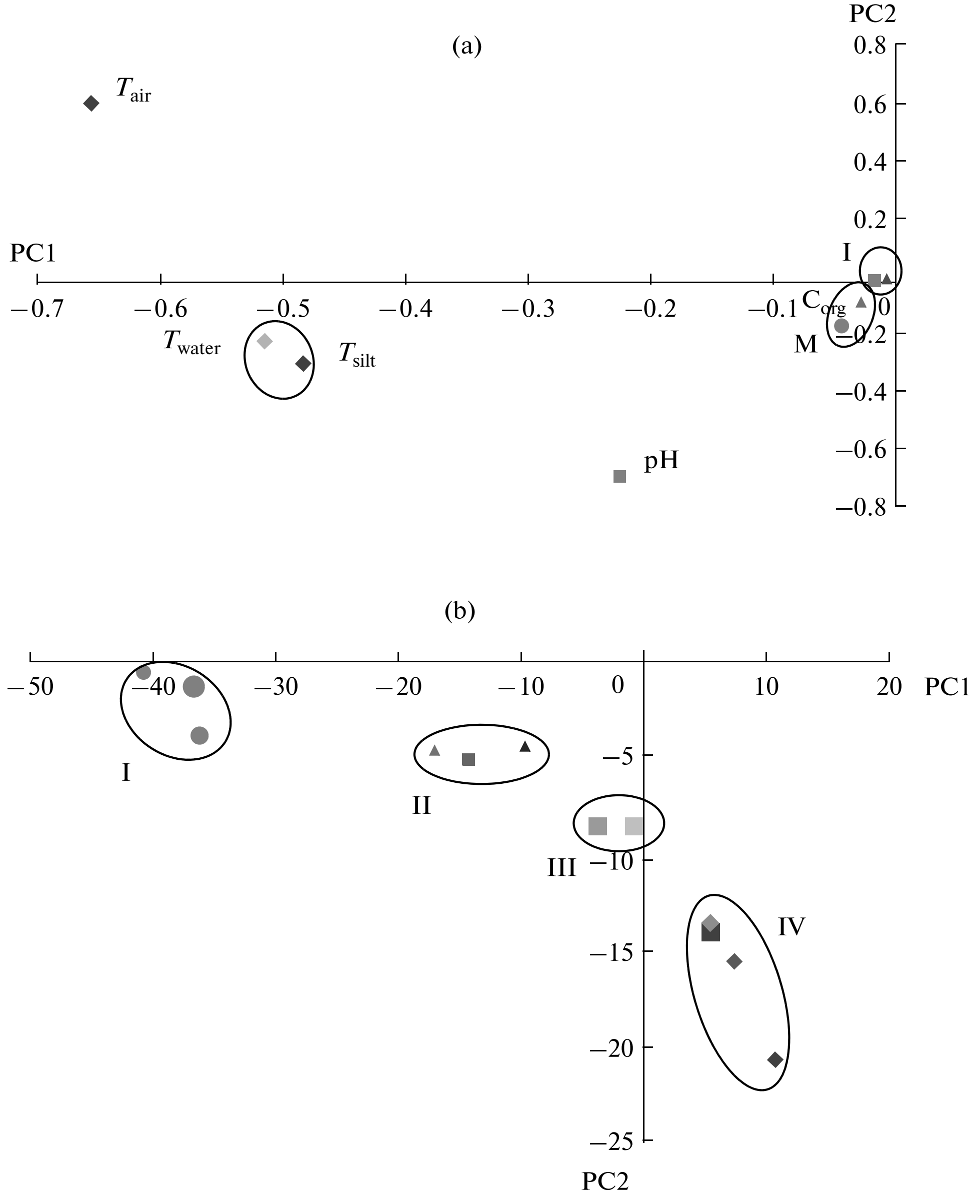

Fig. 4. PC1–PC2 score plot: distribution of factors within the PC1–PC2 coordinate system (I, dark CO_2 assimilation, sulfate reduction, methanogenesis, TNM) (a) and distribution of specimens within the PC1–PC2 coordinate system (cluster I: June, July, August; cluster II: April, May, September; cluster III: October, November; cluster IV: November, December, February) (b).

their close positive correlation and marginal impact on the first two principal components. The variables representing atmospheric temperature, as well as the temperatures of water and silt, exerted the maximum force on the first component. A strong correlation between the water and silt temperatures was observed. The score plot demonstrates the position of the specimens in the projection space of the principal components. The PC1–PC2 score plot demonstrates that the specimens form four distinct seasonal groups scattered diagonally with respect to the axes: summer groups were positioned leftward at the top; winter groups were positioned rightward at the bottom; the specimens obtained in October–November and September–April–May formed separate clusters between them (Fig. 4b). The level of similarity within the clusters is based on all the ecosystem parameters of the initial data matrix. The clustering corresponds to the seasons and not only reflects the seasonal dynamics of the physicochemical parameters, but also demonstrates the real state of the microbial community at a definite time period, i.e., characterizes all the seasonal changes in the ecosystem.

The results obtained indicate that the bacterial processes of heterotrophic production and degradation of organic matter do not contribute significantly to the

Values of the principal components (PC) and their explained variations

Environmental factors	PC1	PC2	PC3	PC4
pH	–	–0.69	–	–
Atmospheric temperature	–0.66	–	–	–
Water temperature	–0.52	0.53	–	–
Temperature of the bottom sediments	–0.49	–	0.57	–
Salinity	–	–	–	0.70
C_{org}	–	–	0.50	–
Total number of microoganisms	–	–	–	–
Numbers of saprophytes	–	–	–	–
Numbers of cellulolytics	–	–	–	–
Dark CO_2 assimilation	–	–	–	–
Sulfate reduction	–	–	–	–
Methanogenesis	–	–	–	–
Explained variation, %	63.5	26.2	6.5	2.2

Note: "–" designates that the loadings of these factors were insignificant (<0.4).

principal components PC1–PC4. At the same time, the rates of these microbial processes are closely related to seasonal changes in the physicochemical properties, including, first of all, the temperature and organic matter content. It was especially true for the terminal processes of organic matter destruction. The studied ecosystem is characterized by the seasonal dependence between fluctuations of some environmental parameters and activity of the microbial community. This is due to the fact that bacterial processes in the bottom sediments depend on several physicochemical parameters (especially the temperature, pH, salinity, and availability of organic matter) at any specific time. Moreover, the reaction of the microbial community to environmental changes manifesting itself in changes in the numbers and activity of microorganisms is of considerable importance. Not only are the absolute values of these parameters of considerable importance, but also the rate and direction of their mutual changes. Hence, at any specific time, bacterial OM decomposition depends on a complex of various, rapidly changing parameters.

Analysis of the data on the ecosystem of Lake Beloe demonstrated that Principal Component Analysis (PCA) could be regarded as an efficient method for assessing relationships between various components of the ecosystem. With the application of PCA we managed (1) to detect the environmental factors responsible for changes in the principal parameters of the microbial community and (2) to reveal the groups corresponding to seasons and associated with each other in their physicochemical parameters and the properties of the studied microbial community within a series of specimans.

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