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To cite this article: I V Volkov *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **232** 012017

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Influence of the temperature regime on the diversity of microorganisms and plants on moraines of the Maly Aktru glacier (Russia)

I V Volkov, I I Volkova, V V Muhortov, E G Nikitkina, V A Nikitkin, E D Popova, I V Lushchaeva, R Cazzolla Gatti and S N Kirpotin

Bio-Clim-Land Centre of Excellence, National Research Tomsk State University, 36, Lenina Pr., Tomsk, 634050, Russia

E-mail: volkovhome@yandex.ru

Abstract. The studies on the moraine complex of the Maly Aktru glacier (Altai, Russia) revealed multidirectional correlations of microbial functional diversity and microorganisms population density depending on the temperature regime of their habitats on the moraines. The closer to the glacier, the bigger functional diversity of microorganisms and the less the density of their populations are. The decrease in the higher plants diversity and the projective cover of plant communities on the whole is linearly related to proximity to the glacier.

1. Introduction

Research in the spatial and temporal dynamics of a periglacial biota is a topical issue of environmental studies. The prevailing current trend towards a decrease in the size of mountain glaciers and their influence on the surrounding environment against the background of advancing climate change provides the conditions for an accelerated transformation of the periglacial ecosystems. One of the index zones to study these processes is the glaciers in the Aktru valley (the Republic of Altai, Russia) (Figure 1) that have been in the focus of attention of the scientific community over the years. Their being of interest is due to the geographical location (in the centre of the Eurasian continent), as well as the long-term systematic scientific observations that allow us to widely assess the current trends in the glaciers' dynamics [1].

High-towering peaks in the Aktru valley (up to 4,000 m above sea level), extensive plateaus, steep slopes and their combinations are the most common forms of the relief in the area under study. The basin area was composed of monotonous and highly dislocated sericite-chlorite shales and other.

Devonian rocks that are prone to weathering processes and are easily destroyed, which contributes to the accumulation of debris at the foot of the slopes, the craters and glacier tongues [2].

The moraine cover of the basin varies in thickness and age and determines a typical moraine landscape of high mountains [3]. The existing vegetation cover of the investigated area was formed under the influence of ancient glaciations. The current glaciation has a decisive effect both on the nature of the plant communities distribution and on the climate of the area as a whole. It is this factor that affects the vegetation of the periglacial zone, growing on the complex of moraines, which release gradually because of the glaciers' shrinking. Comparative observations of the temporal dynamics of the vegetation in the Maly Aktru glacier (2000–2011), which rapidly reduces its size and the mass of ice in the ablation zone, showed that the transformation rate of the vegetation on the moraine is higher



than that of alpine plant communities outside the zone of glaciers' impact. It is caused by glacier thawing, which results in the shrinking of the zone in which the glacier "controls" the habitat climate making it drier and colder [4]. These results were obtained by means of analyzing the vegetation on a glacier moraine with the help of the method of ecological scales. However, there are still no reliable data demonstrating the dependence of the vegetation and other biota components on the thermal regime on the glacier moraines. Therefore, the aim of this study is to investigate the dynamics of the surface air temperatures of the habitats on the glacier moraines and their effect on the diversity of plants and microorganisms. In accordance with the aim, we set the following tasks: 1) to assess the temperature impact of the glacier on various parts of the moraine and reveal the boundaries of the zone of its influence on the ecosystems in the periglacial area; 2) to assess the diversity and population of the plants and microorganisms inhabiting the substrates of moraine parts and to carry out a comprehensive assessment of the nature of the effect of the glacier on functional diversity of microbiota and plants in the periglacial zone.

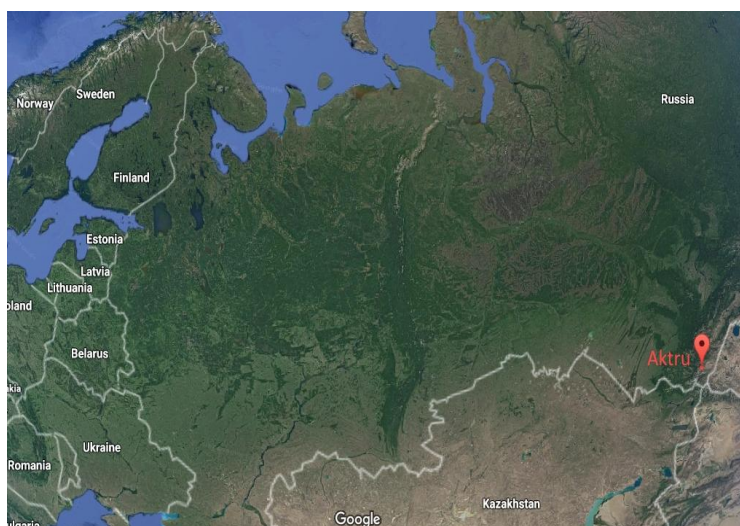


Figure 1. Study area location

2. Materials and methods

To study the dynamics of the surface air temperatures we used temperature logger TP-1, which is a highly economical microcontroller consisting of a temperature sensor, a lithium battery and memory up to 8,192 measurements, hermetically sealed in a disk case made of strong stainless steel. This equipment has a wide temperature range from -45°C to $+85^{\circ}\text{C}$. The loggers were simultaneously installed in the moraines in the Maly Aktru glacier (Fig. 2) next to the glacier tongue end on a bottom moraine – (№ D3) and at a distance of 593 m – (№ 8C), on the lower part of the slope formed by the left lateral moraine at a distance of 754 m from the glacier – (№ 53), on the lower part of the slope formed by the left lateral moraine at a distance of 528 m from the glacier – (№ 35) and at a distance of 321 m from the glacier – (№ 69). The scheme for placing temperature loggers is given in Fig. 3. The location of temperature loggers makes it possible to analyze the temperature patterns in various parts of the moraine complexes and the impact rate the glacier has on the habitat climate. The temperature values were recorded with an interval of 15 minutes.

A study of vegetation (species diversity and projective cover) was carried out and soil samples were taken for microbiological analysis in the locations the loggers were installed with an area of 100 m^2 (named by logger numbers).

The soil samples were taken with sterile instruments that were sterilized in a drying oven at 160°C for 120 minutes and placed in sterile containers: paper bags. Before sampling, the soil was cleaned of grass and large objects (branches, etc.). Point samples were selected by the envelope method from the depth of 1.0–5.0 cm. The combined sample was made by mixing point samples taken at one test site.

The combined sample of volume necessary for the analysis (150-200 g) was placed in sterile containers and labeled (the date, time and place of sampling). The date, time and place of sampling were put down in a worksheet.



Figure 2. The moraine complex of the Maly Aktru glacier – the research site



Figure 3. The scheme for placing temperature loggers on the moraine complex in the Maly Aktru glacier (on the left there is the end of the glacier's tongue, the numbers of temperature loggers are given in brackets)

We carried out the microbiological analysis of the samples in the microbiological laboratory of BioClimaLand Center, TSU. We used the method of multisubstrate testing (MST) and determination of the total microbial number (TMN) in the ground substrate using a set of fluorescent dyes LIVE/DEAD® BacLight™ Bacterial Viability Kits L-7012 (Molecular Probes, Eugene, Oreg.).

As a result, the quantitative (number of microorganism cells per gram of substrate) and qualitative indicators in the substrate samples taken on the moraines at the registration sites were obtained.

MST method is based on the analysis of the spectra of substrate consumption (SCS) by natural microbial communities. Such substrates may be the sources of basic biophilic elements (carbon, nitrogen, sulfur, phosphorus), physiologically active substances (vitamins, hormones, growth inhibitors, including antibiotics, etc.), substances that affect the osmotic properties or acidity of the medium, intermediates of the main metabolic pathways, etc. The tested substrates are arranged in panels based on standard 96-well microplates. During sample preparation, a 3-gram portion was taken and ground in a small amount of water, and then the sample was adjusted to 50 ml and placed in a sterile polypropylene tube Falcon. The contents of the tube were thoroughly mixed in the shaker for 15 minutes at a speed of 3,000 rpm. The suspension was centrifuged for 2 minutes at 2,000 rpm. The supernatant was carefully (without stirring up) taken with a pipette with a sterile tip into a sterile polypropylene 50-ml tube. 22 cm³ of supernatant was placed into a sterile tube and used for multisubstrate testing.

The ready samples were placed into plate wells with a micropipette. The plates were incubated in a thermostat at 28 °C for 72 hours, until the appearance of the visually recorded color of the cells. The

change in the colour in each well is measured as an optical density index at 590 nm using a microplate reader Multiscan FC (Thermo Scientific, China).

Bacterial metabolic activity (for example, the extent to which carbon sources were used) is usually evaluated by calculating the mean value of color development in wells (Average Well Color Development - AWCD) [5]. This is a qualitative indicator of the functional diversity of microbocenosis calculated by means of dividing the sum of the absorption values for 31 carbon sources in Ecoplate™ by the number of substrate cells using the following formula:

$$AWCD = \Sigma ODi/N, \quad (1)$$

where ΣODi is the sum of optical density values in all the wells and N – the number of wells. The quantitative indicator of total bacterial contamination is TMN.

Total microbial number (TMN) was determined by means of dying the specimens prepared from a soil suspension with fluorescent dyes LIVE/DEAD® with subsequent counting of cells using a fluorescent microscope Zeiss Axio Imager Z2 (Germany), according to the manufacturer's protocol. The microorganisms were counted in a special chamber for counting cells. For each sample, 20 fields were viewed. The relative error of this method depends on the number of viewed visual fields and is 10-20% at a significance level 0.05.

Currently, it is not allowed to talk about specific groups of microorganisms without conducting genetic studies.

Studying the vegetation on the model sites at the locations of temperature sensors was carried out using standard geobotanical techniques on an area of 100 m², taking into account the species diversity of flowering plants and the projective cover of the plant community. The table shows the results of studying the vegetation in the moraine complex of the Maly Aktru glacier.

3. Results

Studying the surface air temperature on the moraine in the Maly Aktru glacier resulted in the following (figure 4, 5).

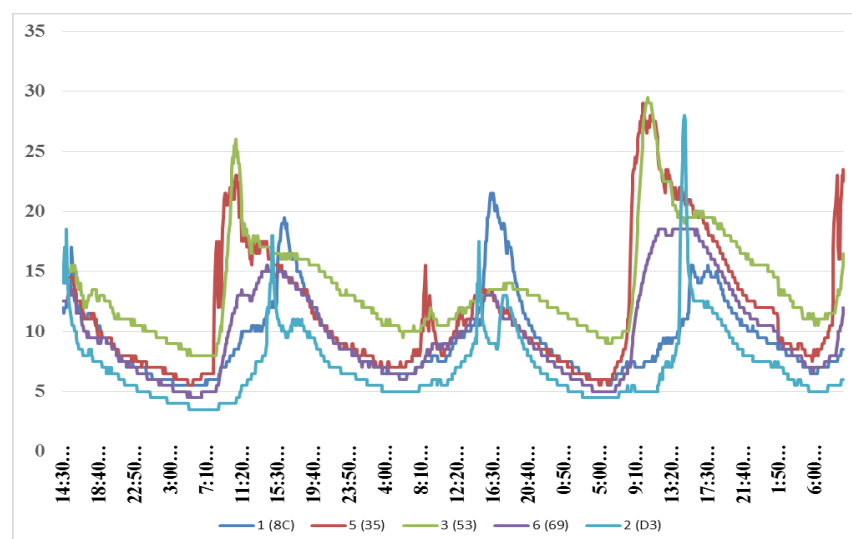


Figure 4. Dynamics of temperature indicators of temperature loggers on the moraine complex of the Maly Aktru glacier (full daily cycles from 15.07.17 to 17.07.17). The abscissa is the time, the ordinate is the temperature. Colours denote numbers of loggers

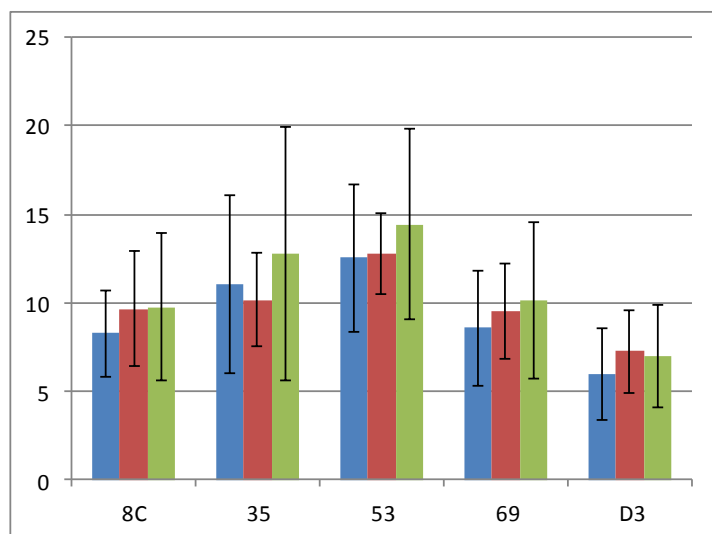


Figure 5. Average daily temperatures characterizing the heat supply of habitats in the points of installation of temperature loggers (colours show the day of temperature studies: blue – 15.07.17, red – 16.07.17, green – 17.07.17). The abscissa is the sensor numbers, the ordinate is the temperature

As expected, the temperature in the immediate vicinity of the glacier (sensor № D3) shows the minimum values of the temperature sums (Fig. 4), while the daily diurnal temperature differences up to 23 °C are recorded here (Fig. 5). The sensor (№ 8C) installed at a distance of about half a kilometer from the glacier (in the hollow along which the cold air flows from the glacier) showed the dynamics of temperature oscillations similar to the sensor installed at the end of the glacier, but with a much higher sum of temperatures and much smaller differences in daily temperatures reaching 14 °C. The peculiarity of the temperature regime in the habitat is the maximum shift in the time of reaching the maximum daily temperatures for a later time of the day, even in comparison with the sensor located near the glacier. On the whole, low heat supply of the habitats on the bottom moraine of the glacier and maximum inertia at temperatures' increasing shows the significant influence of the glacier on the heat supply of the habitats under the influence of cold air from its body. It is typical that sensor № 53, located at a slightly larger distance from the glacier than sensor № 8C, but on the lower third of the slope of the left lateral moraine shows the maximum sum of temperatures (Fig. 4) with a significant dynamics of daily temperatures reaching 20 °C.

An interesting result of the study is the similarity in the course of daily heat supply temperatures for the habitats in the upper part of the moraine at a short distance from the glacier (logger № 69) and a habitat located at a distance of half a kilometer from the body of the glacier on the bottom moraine (sensor № 8C) (Fig. 4, 5). The obtained data make it possible to assume that the influence of the Maly Aktru glacier is almost equally leveled by about two-thirds of the height of the moraine (about 150 meters of height) or a distance of about half a kilometer along the ice sheet of the open flow of cold air from its body. Alongside with this, the temperatures on the crest of the moraine increase while moving away from the glacier (sensor № 35), approaching the background values (i.e. climatically determined temperatures of similar habitats that do not undergo the glacier's influence).

The data of microbial functional diversity in the samples taken from the survey sites on the moraine of the Maly Aktru glacier are shown in Figure 6.

The data on the study of the vegetation in the registration areas are given in the table 1.

The data on the temperature regime on the moraines of the Maly Aktru glacier show significant differences in the dynamics of temperatures and the heat supply of the habitats. The main factors influencing the difference between the indicators is the distance from the glacier and the height of habitat position on a slope formed by a lateral moraine.

The indicators of microbial functional diversity (AWCD) in the places of sampling in the moraine complex in the Maly Aktru glacier display a trend to increase in microbiota metabolic diversity in the most severe conditions of moraine location – on the bottom moraine (D3) next to the glacier tongue (8C) and at the maximum distance from the glacier. The maximum microbial functional diversity is

recorded in habitat 69. It is interesting to note the proximity of indicators of functional diversity of microorganisms in habitats 8C and 69, with similar temperature regime.

In the habitats with favorable temperature regime (53, 35) one can observe the decrease of microbial functional diversity.

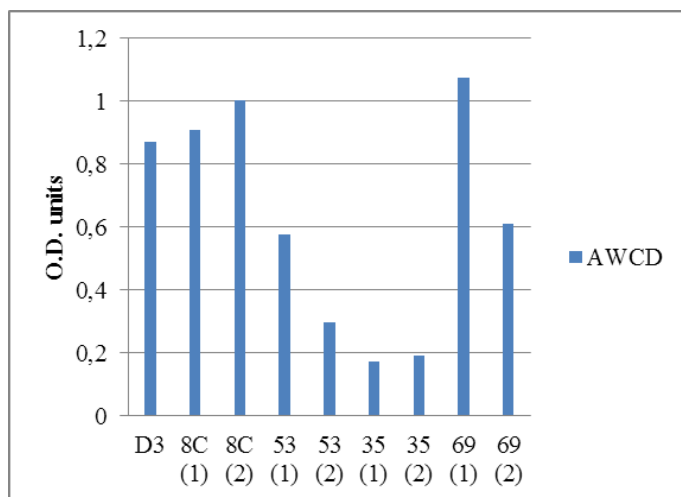


Figure 6. Indicators of microbial functional diversity in the areas of substrate sampling on the moraine in the Maly Aktru glacier. The x-axis shows the numbers of the registration areas where the samples of the substrate for microbiological analysis were selected (1 – soil substrate at a depth 1.0 – 2.5 cm; 2 – soil substrate at a depth 2.5 – 5 cm). The y-axis shows AWCD values. The site numbers are repeated because of analysis duplication at sampling sites. TMN in the samples (millions of cells per gram) are shown in Fig. 6

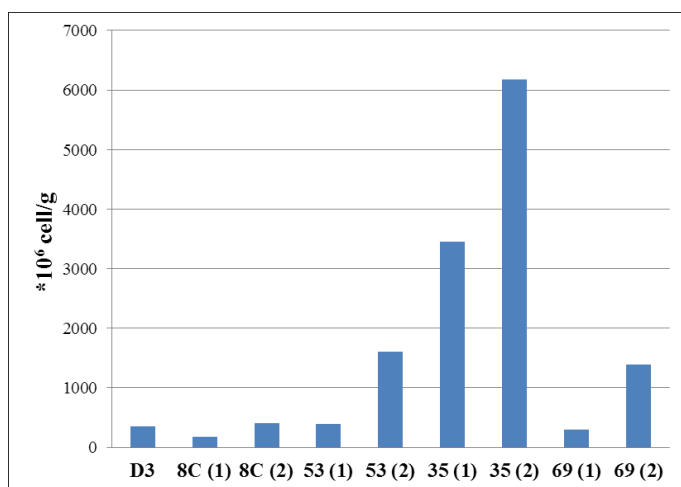


Figure 7. The quantitative indicator the total number of bacterial cell in the plots of sampling substrate on the moraine in the Maly Aktru glacier. The x-axis shows the numbers of the registration areas where the samples of the substrate for microbiological analysis were selected (1 – soil substrate at a depth 1.0 – 2.5 cm; 2 – soil substrate at a depth 2.5 – 5 cm). The y-axis shows TMN in the substrate HPC - HETEROTROPHIC PLATE COUNT. The site numbers are repeated because of analysis duplication at sampling sites.

The quantitative indicators of the presence of microorganisms in the samples show a negative correlation with functional diversity, which more closely corresponds to the generally accepted biota response to the extremalization of living conditions.

Thus, an increase microbial functional diversity on the moraines of the Maly Aktru glacier shows a negative correlation with the habitat conditions extremalization and a positive correlation with a decrease in the density of populations of microorganisms. This phenomenon can be explained by the biological characteristics of microorganisms that have a wider range of tolerance to the dynamics of environmental factors than multicellular organisms.

Moreover, the high diurnal temperature dynamics near the glacier diversifies the ecological conditions of the habitats in accordance with the principle of "ecological carousels". Such dynamics makes various microorganism species find the optimal conditions for their metabolism. Besides, a prerequisite for an increase in functional diversity of microorganisms should probably be considered a decrease in the density of their populations under the most severe conditions of the periglacial zone and as a result of a reduction in the intensity of competition for resources.

Studying vegetation distribution over the sites under study (table 1) resulted in the following. At the site under the glacier (D3) there are no plants. At the site located on the bottom moraine at a considerable distance from the glacier (8C) there are 7 plant species with a projective cover up to 20%. In habitat 53 located at a maximum distance from the glacier in the lower part of the lateral moraine with the most favorable summer temperature conditions there are 6 plant species with a projective cover up to 10 %. This feature suggests that a more favorable temperature regime of the growing season may or may not be a limiting factor in the development of the plant community in the distribution of plants on moraines. Fine earth and better moisture may provide better conditions for plants' development on a bottom moraine.

Table 1. Vegetation distribution

	8C	D3	53	DA	35	69
<i>Salix hastata</i>	+	-			+	
<i>Salix berberifolia</i>	+		+		+	+
<i>Mercuria dahurica</i>	+					
<i>Crepis nana</i>	+		+	+	+	+
<i>Castellea pollida</i>	+		+			
<i>Hamamerium latifolium</i>	+					
<i>Saxifraga oppositifolia</i>	+					+
<i>Dracocephalum imberbe</i>			+		+	+
<i>Salix reticulata</i>			+			
<i>Loidia seriotina</i>			+			
<i>Orastachis spinosa</i>				+		
<i>Selene turgida</i>				+		
<i>Antaxantum alpinum</i>				+		
<i>Empetrum nigrum</i>				+		
<i>Juniperus pseudosabina</i>				+		
<i>Pinus sibirica</i>				+		
<i>Ribes nigrum</i>				+		
<i>Barbera sibirica</i>				+		
<i>Bergenia crassifolia</i>				+		
<i>Salix sapognikovii</i>					+	
<i>Crepis carelina</i>						+
<i>Salix vestita</i>						+
Projective cover (PC), %	20	0	10	35	15	10

Comparing the vegetation in the survey areas in the ridged part of the moraine (69 and 35) showed that in the locality close to the glacier (66) the number of species (6 species) even exceeds the variety of plants in the habitat farther from the glacier, although the projective cover at a distance from the glacier is a little higher. The obtained data showed that in the ridged part of the moraine the influence of the glacier is practically leveled and the actual distance to the glacier is a secondary factor.

Studying the vegetation behind the crest of moraine (site DA) where there is no glacier influence showed maximum species diversity (10 species) with the highest projective cover (35 %). These indicators can be considered background for vegetation, developing on a complex of older moraines, beyond the influence of the glacier.

On the whole, the studies of the vegetation on the Maly Aktru glacier can be considered preliminary because of the absence of replications, which allows us to speak of a static pattern and a high probability in the random distribution of species.

4. Conclusions

The study of the temperature regime of various habitats on the moraine complex of the Maly Aktru glacier showed that the influence of the glacier on the biota is offset by a significant removal along the valley of the cold air flow along the bottom moraine, and by a less significant distance when climbing the lateral moraine, which hinders the penetration of cold air.

The increase in microbial functional diversity on the moraine of the Maly Aktru glacier shows a negative correlation with the extreme conditions of habitats and a positive correlation with a decrease in the density of populations of microorganisms.

On the whole, reducing plant biodiversity and projective cover of plant communities is directly related to habitat extremes when approaching the glacier.

Acknowledgments

This study was partially supported by funds for basic projects of the Russian Academy of Science and by the grant of the Russian Foundation of Basic Research [No 15-29-02599] ‘Integrated study of the spatial and temporal dynamics of species diversity in the extreme habitats of the periglacial zones of Siberia, along latitudinal and altitudinal gradients’. We express gratitude to Large-scale research facilities «System of experimental bases located along the latitudinal gradient» for fruitful cooperation (<http://www.secnet.online/megaustanovka-ru.html>).

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