Experimental modeling of the bacterial community translocation during freezing and thawing of peat permafrost soils of Western Siberia

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Abstract. The stratification of microorganisms along the depth of the column occurs during the processes of freezing and thawing. Two processes – sedimentation and freezing – are sufficient for the formation of a concentrated layer of microorganisms. By contrast, vertical transfer and concentration of nutrients from the surface layers or from the thawing underlying layer of permafrost are not required. The spatial distribution of soil microflora can serve as a marker of climatic changes in the studied region.

1. Introduction

Permafrost rocks are widespread on Earth and their age in some regions reaches hundreds of thousands or millions of years. Polar regions and other constantly cold areas form 80% of the terrestrial biosphere. These regions are a natural repository of the oldest natural communities of microorganisms on Earth, a bank of ancient genes and biomolecules. It is known, that Arctic ecosystems are the most affected by both global (climate warming) and local (pollution) environmental factors. Further, they play an important role in defining the climate of the entire planet, which is primarily related to their impact on the biogeochemical carbon cycle and, consequently, the concentration of carbon dioxide in the atmosphere. A temperature increase in permafrost, accompanied by thawing and release of buried organic carbon, is one of the most important factors of global climate change [1-3].

A rise in summer temperatures leads to an increase in the thickness of the active layer and the inclusion of deeper horizons of permafrost peat in the metabolic activity of bacterial communities. However, the formation mechanisms of such layers are not fully understood. Existing literature presents several hypotheses concerning their formation, including the “burying” of seasonal water reservoirs due to the collapse of coastal hills or hummocks.

In her analysis of the wide ranging literature, Karaevskaya [4] has shown that aerobic and anaerobic bacteria, archaea, algae, micromycetes and protozoans have been studied in the Arctic permafrost. In comparison to pure ice, permafrost deposits are the best natural environment that enables the long-term preservation of viable microorganisms. Cells stored in permafrost can survive for a long geological time up to several million years [5].
The soils of Arctic and Antarctic house an abundant microflora. In fact, the microflora in the soil above the permafrost layer is often no less abundant than the microflora of soils in more temperate climates. In frozen sedimentary rocks, the number of microorganisms is only slightly lower (one order of magnitude) than in the permafrost soils [6,7].

Pure ice in the cryosphere is almost sterile and no viable cells have been found in the fossil ice of the Arctic [6-8]. One explanation for the relative sterility of fossil ice is that the crystals of ice mechanically destroy the cells from the outside. An alternative explanation would be the death of cells due to their own metabolism as cell products accumulate in the absence of transport systems [6,7]. In addition, A V Mironov [9] has found evidence of natural cryopreservation of thermophilic and hyperthermophilic microorganisms. Exiting literature has shown that the number of viable cells has decreased when they have been stored in a frozen state using the methods of cryopreservation. This is partly due to damage caused by the growth of ice crystals inside the cells during freezing or ice recrystallization [9].

In cryobiosphere (a habitat with negative temperatures at which the rates of biochemical reactions and biological processes become extremely low), in comparison to pure ice, frozen rocks are more favorable for living and thus the most populated with microorganisms. This is because the unfrozen moisture in the frozen rocks functions as the basis of the econiche, which ensures the preservation of cellular structures in the cryobiosphere. Preservation of cell structures at low temperatures is possible due to the internal cryoprotectors, the role of which can be played by the cytoplasm [10]. Another factor preventing the formation of ice inside the cells is the relatively low freezing rate, which gives cell water time to squeeze into the extracellular space [11-13]. “ Explosive growth” is characteristic for bacterial communities in thawing frozen rocks and soils. This is the case especially for the psychrotrophic bacteria, which do not have specialized dormant forms. However, recently such active growth in the thawing of frozen samples has also been shown for yeast [14]. The proportion of hypometabolic cells to deeply dormant cells is higher in cryogenic soils than in modern soils. The proportion can be taken as a criterion for assessing the adaptation of microbes to extreme conditions.

The existing data indicate a smaller ratio of the total number of bacteria and their cultivated number (from 17 to 104 times depending on the layer) in peat soils of permafrost bogs than that found in mineral soils of Siberian tundra (200–20000 times) [15,16]. A common pattern is the decrease in the number of bacteria with increased depth. However, there are deviations from this regularity, for example, a significant increase in this ratio in the soil above permafrost [17].

The following possible mechanisms for the accumulation of bacteria in the deep horizon of sandy soils are suggested: (1) the mechanical removal of bacteria from the upper part of the porous soil column by the downward migrating soil solutions and their accumulation in the layer of frozen sea clays or (2) the burial of the biologically active upper soil horizons as a consequence of soil collapse caused by thermokarst processes [16]. Another option is the top-down filtration of bacteria with soil moisture from the layer of seasonal thawing towards the permafrost layer. Simultaneously with the entry and accumulation of sediments, the permafrost boundary rises and the material freezes.

Another possible process that can cause a local maximum of microbial cells inside the soil profile (often at a depth of 20-45 cm) is the accumulation of biomass and organic matter in pockets formed by cryoturbation processes, as has been observed in the mosses of the Taimyr Peninsula tundra [16]. However, such findings remain hypothetical and require further study.

Thus, the presence of layers with a high concentration of bacteria can be used for predicting the involvement of bacterial communities “preserved” at different times at different depths in the processes of methanogenesis. The fundamental question concerns the formation of such concentrated layers – whether they are the result of accumulation during previous climatic periods or formed as a result of seasonal freezing in the permafrost layer. The depth of such layers can serve as a marker of climatic trends. To answer this question, we devised a laboratory model for studying the thawing and freezing processes.
2. Methods of research and initial data

Processes of soil freezing and thawing have been widely studied [18-20]. The literature mainly describes the approaches that are used in the artificial freezing of soils in the construction of subways, in the sinking of mine shafts, escalator tunnels, distillation tunnels, the construction of stations in a closed way, and the development of pits for subway structures constructed in an open way. To study such processes, various installations for cyclic freezing-thawing have been developed [21-26]. However, most work focus on changing the structure of the soil and the release of greenhouse gases, while the dynamics of biota remain practically unstudied.

To clarify the patterns of formation of microorganism distribution of the in permafrost columns, we developed a laboratory setup allowing us to study such processes in a course of a short time (5-6 days). This setup enabled the uniform freezing of columns with marker suspensions (unicellular alga Chlorella) and soil bacterial cells, changing the direction of the freezing vector, and simulating the cyclic processes of freezing-thawing of permafrost soils and taking samples in order to analyze the distribution of microbial communities along the depth of the column (Figure 1).

Figure 1. Chlorella concentration profile for sedimentation (A) and double-sided column freezing (B)

Plastic cylinders with a capacity 2 dm$^3$ were used for making the column. For the simulating of the non-melting layer of permafrost, 500 ml of distilled H$_2$O was poured into a cylinder and placed in a thermostat at $t = -8 \, ^\circ C$ for at least 14 hours (until completely frozen). The temperature and freezing time were determined empirically. Then, the samples were placed in the cylinder on the layer of “permafrost”. To prevent the “permafrost” from thawing, the samples were pre-cooled to a temperature of $+ 4 \, ^\circ C$ or less. Subsequently, the cylinder was thermally insulated from the sides and from the top with a 5 mm layer of polyurethane foam to minimize the effect of lateral freezing. The cylinders were left in the thermostat for at least 48 hours at a temperature of $-8 \, ^\circ C$. The thermostat used was MIR-154 with a temperature range ($-10 \ldots + 60$) $^\circ C$ with forced ventilation.

For the freeze-thaw cycles, we designed a thermostatic box. On the top of the box, holes were made for the insertion of the cylinders. Next, the cylinders were placed in the box, which only covered the cylinder partially. The height of the box ($h = 120 \, mm$) was chosen so that the upper boundary of the permafrost zone of the cylinders was below the heat-insulating box. Then the box was filled with pre-frozen refrigerants and the openings made for inserting the cylinders were tightly sealed. Then the box with the cylinders was placed in a climate chamber.

After the experiment, the frozen column was cut into 1.5-2 cm thick slices, which were thawed and measured for the concentration of microorganisms. Unicellular alga *Chlorella vulgaris* was chosen as a marker microorganisms, since i) chlorella cells are similar in size and density (1-6 μm, 1g / cm$^3$) to bacterial cells, ii) chlorella concentration in the column slice can be quickly estimated either optically, or, in the case of turbid samples, by the chlorophyll content. An aqueous suspension of chlorella was prepared with an optical density of 0.265-0.270, which corresponded to a chlorophyll concentration of 2900-3370 μg / L.
The chlorella concentration was measured with a photoelectric colorimeter IPS-03 (Russia) and chlorophyll concentration with a Multiple Excitation Wavelength Phytoplankton & Photosynthesis Analyzer Phyto-PAM, Walz (Germany). The concentration of chlorophyll in the suspension of chlorella was measured in the ALGAE mode.

An aqueous suspension of soil bacteria with a concentration of $1.9-2.0 \times 10^7$ cells/ml was prepared in distilled water. Soil bacteria were isolated from peat taken in the permafrost zone. To accumulate the biomass of peat microorganisms, we used a liquid nutrient medium prepared on the basis of the GRM broth, which is a universal nutrient medium for obtaining accumulative cultures of microorganisms of various taxonomic groups [27].

The total number of microorganisms was determined in the Goryaev chamber. To determine the ratio of living and dead bacteria during freezing and repeated cycles of freezing and thawing of permafrost, we modified the method of direct counting of cells, stained with the LIVE / DEAD™ BacLight™ Bacterial Viability Kit. The modification consisted of using a confocal microscope, both for visually detecting the bacteria and for counting their number and the LIVE / DEAD ratio [28].

Samples of the model column of aqueous suspensions and permafrost soils were prepared under laboratory conditions. For the study, 4 types of model samples were prepared: an aqueous suspension of marker microorganisms (chlorella), an aqueous suspension of soil bacteria, a peat suspension with chlorella, and a peat suspension with soil bacteria.

To fill the columns, we used peat from the permafrost zone. First, peat was cleared from roots. Then 500 g of peat was placed in a container with the volume of 5.0 dm$^3$ and poured with water in a ratio of 1:10. The container was tightly closed and placed horizontally in a laboratory shaker for 30 minutes. Then, peat was drained through a sieve. After this, peat was again poured with water and placed in a shaker. The procedure was repeated 3 times. Then peat was squeezed, laid out in one layer and placed in an oven for 2 hours at a temperature of 40° C. For sterilization, 450 g of dried peat were placed in paper bags. Sterilization was carried out in an autoclave in conditions of saturated steam, elevated atmospheric pressure and a temperature of 140 °C for 30 minutes. Autoclaving was repeated twice. Peat from the paper bags was transferred to a container, filled with the prepared aqueous suspension of microorganisms with a concentration (in the corresponding series, an aqueous suspension of chlorella with a concentration of chlorophyll of 2900-3370 μg / l or soil bacteria - $1.9-2.0 \times 10^7$ cells / ml), mixed thoroughly and used to fill the columns. All work was carried out in compliance with sterility conditions.

Statistical data processing included standard methods of one-dimensional and multi-dimensional analysis from the STATISTICA package version 10 (Stat-Soft Inc., USA). Parametric criteria were applied if the normal data distribution and the relative equality of variances was established.

3. Results and discussion

Studies with the laboratory model showed that in the process of column freezing, microorganisms are concentrated near the freezing border. Depending on the vector of freezing (top, top and bottom, bottom), the formation rate of the concentration layer of microorganisms can vary from 1.8% (in the freezing from the bottom) to 4.3% (when freezing from the top) of the initial concentration per 1% of the column height. This is associated with the superposition of freezing and sedimentation processes (Figure 1). The sedimentation of cells by the gravity vector is not large (concentration coefficient up to 1.4 times in two days) and can only modify the distribution of cells in a frozen column, not invert it.

The stratification of the distribution of microorganisms along the depth of the column occurs during freezing-thawing and is depends on by the substrate used to fill the column. The highest degree of concentration during freezing is observed in the aqueous suspension (4.50 ± 0.35 times for chlorella and 1.72 ± 0.07 times for soil bacteria). In peat, lower concentrations were observed: up to 2.3 ± 0.09 times for chlorella and up to 1.38 ± 0.08 for soil bacteria.

The formation of a concentrated layer of microorganisms does not occur at the very boundary of the thawed and frozen phases, but higher (10-15% of the column height) in the permafrost zone, which is associated with counter-freezing from the underlying frozen layer.
For the formation of a concentrated layer of microorganisms, processes of the sedimentation and freezing are sufficient; vertical transfer and concentration of nutrients from the surface layers or from the thawing underlying layer of permafrost are not required (Figure 2).

![Figure 2](image)

**Figure 2.** The concentration profile of soil bacteria in the column during a single (A) and repeated (B) freeze-thaw cycles.

The layer of concentrated microorganisms formed and frozen during a season can undergo destruction in the following year. This can take place due to repeated thawing that causes the release of preserved bacteria and involves them in the process of utilization of carbon-containing substrates. The distribution of microorganisms in the peat column could vary depending on temperature trends related to climate. Depending on the depth of seasonal thawing, the formation of a new layer of concentrated microorganisms can occur lower, higher or at the same depth as the previous layer. As a result of cyclic freezing-thawing in the columns of winter frozen soil, two types of profiles can be formed.

The first type presents a monotonic increase in the concentration of microorganisms along the column up to a layer with a sharply increased concentration. Such a type forms during the generally positive trend of climatic changes when each subsequent annual thawing cycle occurs to a greater or the same depth than the previous one, thereby “dissolving” the existing layer of concentrated bacteria and involving them in the processes of substrate utilization. Upon subsequent freezing, a layer of concentrated bacteria forms below or in the same place as the previous one (Figure 3_1).

The second type is formed in the conditions of climatic lowering of temperatures when the thawing zone does not reach the depth of the concentrated layer, and the layers that form in subsequent years freeze at a shallower depth (Figure 3_2).

![Figure 3](image)

**Figure 3.** Scheme of the concentration of microorganisms.

0 - frozen column with a concentrated layer (CL)
1 - phases of summer thawing and autumn freezing with the increasing average annual temperature
2 - phases of summer thawing and autumn freezing with the decreasing average annual temperature. The dashed line is the level of summer thawing; the solid line is the level of the layer of concentrated bacteria.

In this way, layered profiles similar to those shown in figure 4 can be formed.

![Figure 4. Photograph of a frozen column with an aqueous suspension of chlorella after two freeze-thaw-freeze cycles (A) and the concentration profile of soil bacteria in a peat-filled column during repeated freeze-thaw cycles (B).](image)

**4. Conclusions**

The stratification of the distribution of microorganisms along the depth of the column occurs during freezing and thawing. For the formation of a concentrated layer of microorganisms, processes of the sedimentation and freezing are sufficient; vertical transfer and concentration of nutrients from the surface layers or from the thawing underlying layer of permafrost are not required.

The spatial distribution of soil microflora can serve as a marker of climatic changes in the studied region.

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