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THE COMPLEX RESEARCHES OF STRUCTURE AND FUNCTION OF ANTARCTIC TERRESTRIAL MICROBIAL COMMUNITIES

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Complex research of structure and function of Antarctic terrestrial microbial communities. O. Tashyrev.

Abstract. Complex researches on studying structure and functions Antarctic microbial communities and formation of plural mechanisms of adaptation of microorganisms to extreme environmental factors of Antarctic are carried out. As model ecosystems subpolar thermostatic oases on the islands located in passages Lemejr and Penola along coast of Antarctic peninsula (a zone 30 x 60 km) are used. For the first time presence in environments of subpolar thermostatic Antarctic oases (including on vertical rocks) microorganisms, resistant to UV- radiation, antibiotics and toxic metals is shown. Obtained data testify to an opportunity of development on the basis of extremophilic microorganisms the express-indicator systems for an estimation of influence of climatic factors on functioning of Antarctic biota. With use of GIS-systems and 3D-models the integrated models of microbial communities' adaptation to extreme climatic factors are developed. Original positions about functioning ice algal-bacterial communities which apparently, play an essential role in formation of global vector streams of biogenic elements and balance of hotbed gases are formulated.

Key words: structure, function, microbial communities, model ecosystems.

Комплексные исследования структуры и функций антарктических наземных микробных ценозов. А.Б. Таширев.

Реферат. Проведены комплексные исследования по изучению структуры и функций антарктических микробных ценозов и формированию множественных механизмов адаптации микроорганизмов к экстремальным факторам окружающей среды Антарктики. В качестве модельных экосистем использованы субполярные термостатированные оазисы на островах, расположенных в проливах Лемейр и Пенола вдоль побережья Антарктического полуострова (зона 30 х 60 км). Впервые показано наличие в биотопах субполярных термостатированных антарктических оазисов (в т.ч. на вертикальных скалах) микроорганизмов, резистентных к УФ излучению, антибиотикам и токсичным металлам. Полученные данные свидетельствуют о возможности создания на основе экстремофильных микроорганизмов экспресс-индикаторных систем для оценки влияния климатических факторов на функционирование антарктической биоты. С использованием GIS-систем и 3D-моделей разработаны интегрированные модели адаптации микробных ценозов к экстремальным климатическим факторам. Сформулированы основные положения о функционировании ледовых водорослево-бактериальных ценозов, которые, по-видимому, играют существенную роль в формировании глобальных векторных потоков биогенных элементов и балансе парниковых газов.

Ключевые слова: структура, функция, микробные ценозы, модельные экосистемы.

Комплексні дослідження структури і функцій антарктичних наземних мікробних ценозів. О.Б. Таширев.

Реферат. Проведено комплексні дослідження по вивченню структури і функцій антарктичних мікробних ценозів та формуванню множинних механізмів адаптації мікроорганізмів до екстремальних факторів антарктичного довкілля. У якості модельних екосистем використано субполярні термостатовані оазиси на островах, розташованих у протоках Лемейр та Пенола вздовж узбережжя Антарктичного півострова (зона 30 х 60 км). Вперше показано наявність у біотопах субполярних термостатованих антарктичних

оазисів (у т.ч. на вертикальних скелях) мікроорганізмів, резистентних до УФ-випромінювання, антибіотиків та токсичних металів. Отримані дані свідчать про можливість створення на основі екстремофільних мікроорганізмів експрес-індикаторних систем для оцінки впливу кліматичних факторів на функціонування антарктичної біоти. З використанням GIS-систем та 3D-моделей розроблено інтегровані моделі адаптації мікробних ценозів до екстремальних кліматичних факторів. Сформульовано основні положення щодо функціонування льодових водоростево-бактеріальних ценозів, які, можливо, відіграють істотну роль у формуванні глобальних векторних потоків біогенних елементів та балансі парникових газів.

Ключові слова: структура, функція, мікробні ценози, модельні екосистеми.

From the beginning of XXth century to the present time microbiological researchers in Arctic and Antarctic in general have been accented to discovering and analyses of extremophilic microorganisms.

Classical methods, generally extended in geological, aquatic and soil microbiology, unfortunately were not used in investigation of Antarctic microorganisms. Therefore, in 2001 we started a complex researching of structure and functions of Antarctic microbial cenoses. The main object of investigation is a biogeographical polygon on is. Galindez. The polygon is situated in 20 min walk from the station "Academik Vernadsky".

On the polygon the complex biological and geodesic investigations have been carrying out for 9 years. They are: selection of samples, mapping of biotopes and other. The polygon is a rock cliffs, the height is 58 m. At the top rocks die away with relict glacier and transform to moss fields with soil and bird's nestings and further to rocky cliffs, the height of which is 10-15 m. Below the polygon, pond with powerful sludge accumulations is situated.

With the help of GPS and GIS-methods the topographic map and 3D-model of the polygon were developed. They show basic landscape elements, typical biotopes and a net of positioned points for long-term monitoring – near 150 points (Fig.1). Geochemical analyses on contain of 20 elements, concentration of total carbon and soil humic acids were carried out.



Fig. 1. Biogeographical Polygon (Is. Galindez), 3D-Model.

The polygon is a subpolar termostatic oasis, which includes all types of biotopes – ice and algal-bacterial films, crustose lichens, soil, moss, grass and other (Fig. 2).



A main result and achievement of microbiological researches is that for the first time on a representative object, subpolar oasis, a structure and functions of Antarctic microbial cenoses were investigated (Fig. 3).



Fig. 3. Complex characteristics of microbial communities of the polygon.

Complex researches were conducted with usage classic methods of general, soil and geological microbiology in combination with modern methods of stereometrical design. Characteristic features for the Antarctic Region are low temperatures and small amount of organic compounds in biotopes. It is considered that psychrophilic oligocarbophilic bacteria dominate there, i.e. microorganisms, growing at low temperatures and small amount of organic matters. It was indeed confirmed at the quantitative account of physiological groups of microorganisms. However, we showed that in soil of polygon at high concentration also present microorganisms, growing at the middle and high concentration of carbon (i.e. mesotrophilic and copiotrophic bacteria) [Таширев О.Б., Таширева Г.О., 2004].

In addition, not only psychrophilic bacteria have a wide spread in soil, but also mesophilic, and even thermophilic microorganisms (i.e. growing at temperatures 25 and 45°C accordingly). Presumably, wide occurrence oh copiotrophic and thermophilic microorganisms on the polygon is related to avifauna. A new and important result is discovering similarity between microbial cenoses's structure of subpolar oasis and regions with a temperate climate (conditionally - «European soils»). Put another way, all mentioned ecological groups, such as r-strategical and other, are present both in Antarctic and in «European» soils (Fig. 4).



Fig. 4. Ecological-trophic characteristics of microbial communities of the polygon.

A concentration of microorganisms of the indicated groups is on the average in a range $10^5 - 10^6$ cells in 1 g of sample. It was shown that a concentration of chemo-organotrophic microorganisms in Antarctic soil is 2-3 orders less than in soil of Europe. Also appropriately, that in soil the quantity of cellulose-fermenting microorganisms is low because vascular plants do not form a continuous cover.

In Antarctic soil all physiological groups of microorganisms, which provide functioning of complete redox-cycle of carbon, from CO_2 to CH_4 , are present (Fig. 5). Their high concentration, $10^4 - 10^6$ cells/g of sample, testifies to potentially high biogeochemical activity of microorganisms of carbon cycle.



Fig. 5. Complete Redox-Cycle of Carbon (from CO_2 to CH_4): Physiological Groups of Microorganisms.

Similar regularities are obtained for the microbial cycle of nitrogen. The cycle of nitrogen is also complete (from nitrogen⁵⁺ to nitrogen³⁻). Concentration of cells is high, on the average $10^4 - 10^6$ cells/g of a sample (Fig. 6).



Fig. 6. Complete Redox-Cycle of Nitrogen (from NO^{3-} to NH_4+): Physiological Groups of Microorganisms.

Diversification of physiological groups of carbon and nitrogen cycles, and also high concentration of microorganisms, allows to suppose that they have substantially influence on the integral vectors of elements in Antarctic environment. And foremost they have influence on engaging of CO_2 and atmospheric nitrogen to biogeochemical cycles in Antarctica. An important characteristic of microbial ecosystems is their homoeostasis, i.e. ability to save stability at influence of extreme, or stress factors. An UV-radiation, organic xenobiotics, antibiotics and heavy metals belong to such factors. We have chosen for determination of quantitative parameters of the Antarctic microorganisms homoeostasis as an indicator stress-factor a copper in form of bivalent cation Cu^{2+} , because it is a strong inhibitor. It was expected that at the concentration 5–10 ppm would cause complete suppression of microbial growth, because in this range a copper is bactericidal for swingeing majority of chemoorganotrophic microorganisms.

However, unexpectedly we found out the phenomenon of metal-resistance. The Antarctic soil microorganisms appeared very resistant to this stress factor. At the concentrations 200 ppm of copper the number of cells decreased by an order [Matveeva, 2006]. However, the further 5-multiple increase of copper concentration (from 200 to 1000 ppm) practically did not influence on microorganisms; their concentration even at 1000 ppm of Cu^{2+} was tens of million CFU/g of soil. This fact testifies to super-resistance of Antarctic soil microorganisms to copper (Cu^{2+}). Systemic quantitative calculation of Cu^{2+} -resistant microorganisms in biotopes of the polygon were carried out. Obtained data were entered in stereometrical 3D-model of the polygon (Fig. 7). Copper-resistant microorganisms are widely occurred on the biogeographical polygon. They are revealed in the whole of biotopes, such as rocks, crustose lichen, moss, grass, soil, sludge. Their quantity is $10^5 - 10^6$ CFU/g of a sample [Tashyreva, 2006].



Fig. 7. Structural-Functional 3D-model of Cu^{2+} resistan Microorganisms Distribution in biotops of the Polygon.

The phenomenon of co-resistance to several toxic metals is a characteristic feature of microorganisms. We chose in as an indicator 3 the most toxic metals as Hg^{2+} , Cd^{2+} and CrO_4^{-2-} (Fig. 8).



Fig. 8. 3D-model of Distribution in Biotopes (Soil, Moss, Sludge) Resistant to Hg^{2+} , Cd^{2+} and CrO_4^{2-} Microorganisms.

They are the most powerful metals-substituter and oxidative metals. They inhibit the growth of microorganisms at the concentration of 10 ppm. It is emerged that for microorganisms of the polygon poly-resistance is a characteristic feature. In such biotopes as moss, sludge and soil microorganisms, resistant to Hg^{2+} , Cd^{2+} and CrO_4^{2-} , are widely occurred [Таширев, Матвеева, 2007]. The quantity of microorganisms was $10^2 - 10^5$ cells/g of a sample in presence of ultrahigh concentrations of metals as 50 ppm Hg and 500 ppm Cd and Cr. Thus, microorganisms, resistant to 4 the most toxic metals, are widely occurred. The concentrations of these metals exceed bactericidal ones on 1-2 orders. Consequently, explored ecosystem is characterized by high level of homeostasis – i.e. resistance to metals as to stress factors.

For the estimation of resistance of Antarctic microorganisms to super-high concentrations of mercury, chromium and copper let take a good look at theoretical aspects of interaction of microorganisms with metals [TaIIIIµpeB, MarBeeBa, 2007]. 15 years ago we developed a conception of thermodynamic prognosis of interaction of microorganisms with metals. The main idea of the conception is that microorganisms can reduce any metal if the standard redox-potential of metal placed in a zone of thermodynamic stability of water. A top limit of water stability is defined by reaction of reducing of O_2 to water (E_o '=+814 mV), a bottom limit is defined by reducing of proton to hydrogen (E_o '=-414 mV). For example, redox-potential of the reducing of chromium(VI) is inside a zone of water stability and equal to +555 mV. Therefore, chromium(VI) reduces to chromium(III) by microorganisms. Further microbial reducing of chromium(VI) to chromium(0) is impossible, because potential of this reaction is beyond of water stability(-926 mV). Competence of the conception was proved on wide spectrum of microorganisms and metals. However, one "dark spot" has been existing for 15 years in this conception.

We theoretically proved that toxicity of any metal-oxidaser is proportional to the concentration of metal and magnitude of its standard potential. Hg, Cr and Cu form a line of toxicity, proportional to the magnitude E_o' It is obviously on example of Cr(VI), that its toxicity determined in the first place by the value of standard potential E_o' , and very little depends on the concentration of metal. The increase of concentration of Cr(VI) on 8 orders, from 1×10^8 M/l to 1,0 M/l, the value E_o' increase only on 145 m/V, and at the 1 M/l concentration of Cr(VI) the potential is equal to +555 mV.

The same results were obtained for Hg^{2+} and Cr(VI). So, we got a paradoxical, but certain conclusion. If the standard potential of the reaction of metal reducing is in the field of redox-stability of water, it is thermodynamically acceptable that some microorganisms can exists (i.e. grow) at any high concentrations of oxidizer-metal, till to 1 M/l.

For 3 years we have been researching a wide spectrum of natural and anthropogenic communities, including zones of ecological catastrophes. Their maximal resistance to metals did not exceed 50-100 ppm.

Who could think that after 15 years in Antarctica, the most ecologically clean region, the "theoretically counted" over-resistant to metals microorganisms would be found?!

Unadapted to metals microorganisms were isolated from soil of the polygon. They showed very high resistance to chromium(VI) (Fig. 9).



Concentration Cr(VI), ppm (in form of CrO₄²⁻)

Fig. 9. Antarctic Microorganisms: Ultrahigh Resistance to Chromates, Growth at the Concentration > 1,0 M Cr(VI) - 60000 ppm of Cr(VI).

3 strains grew almost at the concentration 1 M of Cr(VI), and 2 – at higher concentration - 60 grammas per litre of Cr(VI). We did not obtain a limited concentration of Cr(VI), because it was not dissolving in a medium. In addition, 10 poly-resistant strains were isolated from soil samples of the polygon. These strains resistant to super-high concentrations of 6 the most toxic metals. The strains are able to grow at the concentration (ppm): Hg²⁺ - 500, Co²⁺ - 1000, Ni²⁺ - 2000, Cu²⁺ - 2500 and Cr(VI) - 60000 [TaumpeB, MatbeeBa, 2007]. It is on some orders higher than bactericidal concentrations of metals for chemo-organotrophic microorganisms. Phenomenon of multiple resistant of chemo-organotrophic microorganisms to the most toxic metals in a concentration range from a few grammes per litre and to tens of grammes was described for the first time. We supposed that Antarctic microbial cenoses participate «in vivo» in the biogeochemical cycles of metals.

However, for this purpose is necessary a simultaneous combination of followings conditions:

- 1. Presence of organic compounds and metals in biotopes (donor-acceptor pair).
- $2.\,High\,concentration\,of\,metal-resistant\,microorganisms\,in\,biotopes.$
- 3. Resistance of microorganisms to wide spectrum of metals.
- 4. And capability to interaction of microorganisms with metals.

We have shown <u>3 types of interaction</u> of the Antarctic microorganisms with metals:

- 1. <u>Reduction ang formation of insoluble compounds:</u>
- -reduction of chromat to chrome(III) hydroxide;
- -reduction of iron(III) and sedimentation in the form of sulphide;
- -reduction of cupper(II) to insoluble cuprous oxide.
- 2. Mobillization of metals:
- -dissolving of copper hydroxide by microbial metabolites.

3. <u>Immobilization of metals</u>: – accumulation of copper(II) and iron(III) in colonies of growing microorganisms.

Consequently, there are all reasons to suppose that metal-resistant microorganisms have a wide spread in biotopes of the polygon and play a substantial role in biogeochemical cycles of metals.

Further, appropriately following questions about metal-resistance of Antarctic microorganisms were arisen.



1. Why the resistance of microorganisms to metals is thousands times higher, than their concentration is in biotopes – is it a high level of adaptation or we deal with the retro-forms of microorganisms of times of neotectonic activity?

2. What is the limit of the resistance of microorganisms to metals on other islands and on the Continent - the Antarctic Peninsula? We have studied metal-resistant microorganisms on 19 positioned objects in the area of the inner shelf in size 30x60 km (Fig. 10).

Fig. 10. Screening of Metalresistant Microorganisms (on 19 objects – Islands and Capes of Antarctic peninsula Seaboard)

Results:

- 1. Metal-resistant microorganisms were observed on all 19 objects.
- 2. Microorganisms grow at high limiting concentrations of 6 toxic metals: 5000 ppm Cr(VI), 2000 ppm Ni²⁺, 1000 ppm Cu²⁺ and Co²⁺, 500 ppm Cd²⁺ and 100 Hg²⁺.
- 3. Polyresistance (co-resiastance) of microbial communities was discovered.

Conclusions:

- 1. Obtained appropriateness on resistance of Antarctic microorganisms to metals is the same both for the polygon on is. Galindez and for 19 objects in the area of island shelf.
- 2. Resistance of microbial communities to metals is a widespread phenomenon on the inner insular shelf of the Antarctic Peninsula.
- 3. Antarctic biotopes are stable source of strains for developing of new universal biotechnologies of metal containing waste water purification and for increase of production of colour and precious metals.

Metal-resistant microorganisms are widely occurred in biotopes of the inner insular shelf as soil, moss, lichen, humus and sludge (Fig. 11).



Fig. 11. The High Occurrence of Metal-Resistant Microorganisms in Typical Biotopes of the Inner Insular Shelf

More than 200 samples were studied on presence of microorganisms, resistant to Hg^{2+} , Cr(VI) and Cd²⁺. It was shown, that from 35 to 85% of samples contain microorganisms, resistant to "bactericidal" concentrations of these metals [Таширев А.Б., Романовская, 2008]. On the basis of received data on a structure and functions of Antarctic microbial cenoses we drew next conclusions:

1. Cenoses are diversified on physiological and ecological-trophic groups.

2. Cenoses show the high level of adaptation and resistance to metals, as extreme factors

3. Investigated cenoses of biotopes are similar on a structure and functions.

On these further researches are based, namely:

1. Local microbial cenoses of subpolar oases are representative model ecosystems, which show a reaction of the whole biota on action of extreme factors (including climatic).

2. Resistance of cenoses to metals assumes possible resistance to a complex of other extreme factors, as UV-radiation, antibiotics etc.

3. Model microbial ecosystem should be compact (small area) and as much as possible open to action of a complex of extreme factors.

On these criteria the most suitable are 2 ecosystems:

1 - microbial cenoses of cliffs rocks, and

2 - microbial cenoses of glaciers.

On the polygon two "Skalodromes" are positioned. Rocks in 10-15 m high are the real «vertical oasis»:

- A thick biological layer covers the surface of rocks.

- Rocks during the whole year are open for UV-radiation.

- Uneven distribution of organic matter stimulate competition of microorginisms for substratum, hence – synthesis of antibiotics.

- Finally, microorganisms mobilize toxic metals from rocks.

In rock samples the frequency of occurrence of pigmented forms, their quantity and biodiversity are considerably higher then in other biotopes of the polygon. Bacteria, yeasts and micromycetes, painted in a black, brown, red, pink and yellow colour, were extracted from samples. On the average the quantity of pigmented microorganisms was within the limits $10^4 - 10^6$, but sometimes reached to 10^7 cells/g of a sample. Obviously that pigmented microorganisms of rock biotopes are potential producers of biological active substances as melanin, carotene, flavin etc.

On the basis of typical strains a collection of chemo-organotrophic microorganisms of rock biotopes was created. 10 typical strains were investigated on resistance to UV-radiation and toxic metals. Non-pigmented forms are sensitive to action of UV. Sublethal dose $(LD_{99,99})$ for them was 40 J/m². Pigmented forms, as thought to be, are tens times more resistant to UV. $LD_{99,99}$ for pink Methylobacterium was 300 J/m², for red and black - 1500 J/m² [Романовская, Шилин, 2005].

Typical strains of rocky environments, which were isolated on nutrient medium without metals, showed high resistance to 5 metals. In the line of resistance maximal concentrations of metals, at which microorganisms grow, are shown (Fig. 12).



F i g . 1 2 . Q u a n t i t a t i v e Characteristics of Multiple Resistance of 10 Test-Cultures of Rock Microbial Communities (Skalodrom-2) to Toxic Metals

Following values of concentrations were obtained: $Hg^{2+} - 50$, $Co^{2+} - 150$, Ni^{2+} and $Cu^{2+} - 5000$, Cr(VI) - 20000 [Таширев А.Б., Романовская В.А., 2008]. Despite high resistance of microorganisms to metals, rising of concentrations of metals leads to decreasing of quantity of survived strains. However, for 3 metals Ni²⁺, Cu²⁺ and Cr(VI) limiting concentration were not still obtained. It will be find out in the nearest future.

An uneven occurrence of organic compounds in Antarctic biotopes leads to a competition of microorganisms for substratum and further to antibiosis. The antibiosis comes out in antibiotics synthesis and in the resistance to these antibiotics [Коцофляк, 2004]. From Antarctic biotopes strain Pseudomonas putida was isolated. This strain suppresses a growth of opportunistic test-cultures of microorganisms. It is interesting to note, that by the optimal temperature for growth (+26 °C), the antibiotic activity was minimal, on the other hand by the unfavourable temperature (+ 4 °C) the antibiotic activity raises steeply. Two Antarctic strains Enterobacter hormaechii and Brevibacterium antarcticum were resistant to 9 broad-spectrum antibiotics. These antibiotics cause damage to cellmembrane and suppress synthesis of protein. Works in investigating of antibiotics synthesis and resistance of Antarctic microorganisms to them started not long ago. However, the received data indicate that antibiosis in Antarctic biotopes exist. The second indicated ecosystem is microbial communities of glaciers. During warm polar summer on a surface of glaciers two-component microbial communities have been actively developing. These communities consist of photosynthetic and chemo-organotrophic microorganisms. Photosynthetic microorganisms fix N_2 and greenhouse gas CO₂, and produce organic compounds (biomass of photosynthetic microorganisms). Heterotrophic microorganisms partially decompose the biomass of photosynthetic microorganisms to biohumus and synthesize greenhouse gas CH₄ and CO₂. Biohumus makes stable vector streams of carbon on glaciers. Thus, on vast territories of the subpolar Antarctic global processes of carbon compounds transformation occur. Thereby, it is obvious that glacial microorganisms on the surface of snow and ice respond to atmospheric and other climatic changes.

Consequently, microbial cenoses of glaciers correspond with indicated ecosystems and can be effectively use for estimation of influence of global climatic factors on Antarctic ecosystems, study of regularity of formation and transportation of organic compounds and estimation of balance of greenhouse gases in the subpolar Antarctic.

We developed environment protecting biotechnologies for fermentation of solid food wastes and for purification of sewage for station "Academic Vernadsky". Technologies based on capability of Antarctic microorganisms adapt to unfavourable environmental conditions and alien organic compounds, including mentioned anthropogenic wastes. Technology of fermentation of solid food wastes was implemented on the station in 2004, and technology of waste water purification was prepared to implementation. These technologies are universal and also could be used in cities for effective decision of strategic goal – utilization of organic wastes of dumps and purification of toxic filtrates to "environmentally clean water" status.

At the present time a problem of correlation of fundamental and applied researchers in Antarctica, - what give preference to?! - are actively being discussed. Which is a sound decision? On this question Frensis Bekon answered in 1620: «Theoretical and applied sciences do not exist, but there is only an application of science to practice!» So, the only right methodologycal approach is phenomena observation and, only after that, its practical application according to established properties. In confirmation, we'll show only one example of our researchers in Antarctica (Fig. 13).





Fig. 13. Fundamental and applied microbiological researches in Antarctica.

During studying structure and function of microbial communities we investigated the influence of extreme factors, including UV-radiation. Than we carried out screening of pigmented strains and developed collection that included N. nigra (T. Beregovaya). Properties of N. nigra were studied, the resistance to toxic metals and synthesis of antiulcer and anticancer compounds were shown. Practical usage of this strain leads to creating of new biotechnologies for extraction of toxic metals and to implementation of new medicines.

On the basis of worded we formulated following strategic perspectives of microbiological researches in Antarctica (Fig. 14).



Fig. 14. Strategic Perspectives of Microbiological Investigations in Antarctica

Researches are planed on 3 levels:

- 1. Microorganisms
- 2. Phenomena
- 3. Processes

On microorganisms' level physiological, ecologo-trophic and other group are being studied, - collections of strains are developed.

On the level of phenomena cenoses homeostasis at the action of a complex of stress factors are being studied.

On the level of processes the role of microbial biota in global biogeochemical and climatic processes is being investigated.

Practical uses of the database reduce to the following scheme:

Microorganisms: collections of strains – producers of biologically active substances (BAS) are being created

Phenomena: environment protecting biotechnologies and biotechnologies of producing BAS are being developed

Processes: models for prediction of biogeochemical and climatic processes are being created.

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