

## LOW TEMPERATURE AND VEGETATION EFFECTS ON THE SOIL BACTERIAL COMMUNITIES STRUCTURE IN HIGH MOUNTAINOUS AND COLD BIOTOPES IN KYRGYZSTAN

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**Abstract.** It is well known that soil microorganisms play essential roles in the biogeochemical cycling of biogenic elements and soil-forming processes. However, little is known about the effect of the vegetation type on the bacterial community structures in soils from cold regions. For these reasons, we have analysed the bacterial communities of eight biotopes covered with different plants and two biotopes without vegetation in the Son-Kull Valley as the coldest corner in Kyrgyzstan. Using the culture-dependent and culture-independent (16S rRNA gene sequencing) methods, we found 4 phylum (Actinobacteria- 55.0%, Proteobacteria- 30%, Firmicutes- 13%, and Bacteroides- 2%) and 5 classes of the bacterial community, with dominant classes of Actinomycetia (60.03%), Gammaproteobacteria (25.0%), Bacilli (10.0%), Bacteroidia (3.0%) and Alphaproteobacteria (2.0%). The dominant generalist genera were *Arthrobacter*, *Pseudomonas*, *Actinobacter*, *Dermacoccus*, *Brevibacterium*, and *Micrococcus*. The results have confirmed that bacterial community structures were significantly affected by the vegetation type and environment factor, such as temperature. The diversity of the bacterial community was higher in the rhizosphere of succulent vegetation with a short lifespan, that is, in ephemerals, and with a high content of organic matter, like manure, in soil. The soil under the snow harboured the highest proportion of uncultured bacteria, representing Actinobacteria phylum.

**Keywords:** *environment factor, soil-forming bacteria, 16S rRNA gene of bacterial diversity, dominant soil bacterial phylotypes and classes*

### Introduction

In some regions on Earth, the temperature can reach 30 °C or more in summer. However, in areas with a temperate climate, the soil temperature never reaches 20 °C, and the temperature drops below freezing in winter in many regions. In these cold regions, harsh climatic conditions, such as sudden changes in temperature, strong winds, ultraviolet radiation and an acute lack of moisture, significantly reduce the primary production of organic matter and thereby determine soil formation characteristics (Brambilla et al., 2001; Smith et al., 2006; Olubukola et al., 2009; Yang et al., 2015; Zhang et al., 2016). Cold ecosystems are susceptible to climate change, and microorganisms play a critical ecological role in these habitats; therefore, understanding their role and potential has a significant environmental and scientific importance (Margesin and Collin, 2019; Collins and Margesin, 2019).

These cold environments are colonised by cold-adapted, psychrophilic and psychrotolerant microorganisms, which can grow at temperatures of 0 °C and below (Gray and Williams, 1971; Neufeld and Mohn, 2005; Anesio et al., 2009; Mackelprang

et al., 2011; Harding et al., 2011; Ghiglione et al., 2012; Crump et al., 2012; Lee et al., 2013; Larose et al., 2013; Cuthbertson et al., 2017).

These cold-adapted soil fungi and bacteria take a leading role; they perform biochemical processes, drive the cycling of biogenic elements (e.g., carbon, nitrogen, phosphorus, potassium) necessary for plant nutrition in the soil, decompose plant residue and form organic matter in the ground (Goodfellow and Williams, 1983; Holmalahti et al., 1994; Wynn-Williams, 1996; Hill et al., 2011; Bottos, et al., 2014; Lysak et al., 2018).

Bacteria contribute to the decomposition of plant residue by producing a range of extracellular hydrolytic enzymes that can degrade animal and plant polymers, including lignin, cellulose, chitin and other organic compounds (Eisenlord and Zak, 2010).

Psychrotrophic bacteria are usually found in temperate climate soils, and, as a rule, they grow in a wide temperature range (Ingram, 1965; Druce and Thomas, 1970). To survive and thrive in harsh conditions, they have developed a series of adaptations in particular cellular components and biochemical pathways involved in metabolism to compensate for the negative effects of low temperatures (Larose et al., 2013; Boetius et al., 2015).

Soil type, soil pH and cover vegetation can influence the distribution of different bacterial species population in soil habitat (Matsukawa et al., 2007; Han et al., 2007; Hayakawa, 2008; Xu et al., 2014). Assessing and measuring the biodiversity of soil bacteria in relation to local vegetation can reveal the ecological role and function of bacteria in soil formation and ecosystem integrity (Babalola et al., 2007; Xu et al., 2014). Extreme cold environments are mainly dependent on microbial activities because this climate restricts higher plants and animals (Dhakar and Pandey, 2020).

The high-altitude Son-Kul valley is a unique and not yet explored corner of the globe for microbiological biodiversity, which determines the processes of decay and transformation of plant organic matter at low-temperature conditions. This territory is highly elevated; it includes the high-mountainous basin of Son-Kul Lake surrounded by mountains and is located at more than 3000 m above sea level. The average annual air temperature is minus 2 °C. In July, summer is rainy and cold with an average monthly temperature of 7–10 °C. The Son-Kul basin soils are subalpine, alpine, meadow-steppe and meadow (Mamytov, 1974, 1996).

In this work, we aimed to study the quantitative content and biodiversity structure of the heterotrophic bacterial block responsible for organic decomposition residues in different biotopes of the Son-Kul valley, focusing on various altitudinal belts. The overall goal was to understand the functioning of this coldest corner of the globe.

## Materials and Methods

### *The soil types in the Son-Kul valley of Kyrgyzstan, described by A. Mamytov (1974, 1996)*

On the one hand, the Son-Kul valley soils have chestnut and chernozem features, and on the other hand, they have subalpine and alpine mountain-steppe features. Due to the high elevation of the territory, the soil-forming process takes place during a short growing season. These soils are well-sod, humus-rich and have a light alkaline reaction.

**Mountain meadow subalpine soils** are formed under subalpine meadow vegetation, which is dominated by meadow timothy grass, bluegrass, fescue, sedge and so on. Such soils contain significant humus (8–15%). They are leached from carbonates and have a pH of 6.5–7.0. These soils are well structured.

**Mountain meadow steppe alpine soils** are formed under alpine meadow-steppes. They are characterized by a dark grey colour of the humus horizon, a lumpy-granular structure. Meadow-steppe-alpine soils are rich in humus (10-11%). The grounds are carbonate from the surface, the amount of CO<sub>2</sub> in the humus horizon does not exceed 1.5-3.0%. The reaction of the soil solution is neutral or slightly alkaline. These soils belong to medium and heavy loams according to the mechanical components.

**Mountain meadow alpine soils** are formed under alpine meadows on northern (shaded) slopes of ridges in the alpine belt zone. They are characterised by a dark grey and black colour, granular structure and good sodding. The soils are characterised by a high content of total nitrogen (0.6-0.8%), gross phosphorus (0.25-0.40%) and potassium (2.6-4.0%). Mountain meadow alpine soils are containing a humus of 6.7-8.13%.

**Alpine semi peaty soils of cobresia barrens** exhibit strong turfiness at the upper horizon, which has a dark colour, and resemble peat. They are rich in humus (up to 20%), with deep penetration along with the profile. They are characterised by a neutral reaction of the soil environment (pH = 7.0-7.4) or a weakly alkaline reaction (pH = 7.7-8.4).

### **Sampling site description and sample collection**

The study area was located in the Son-Kul valley. The valley includes the alpine lake Son-Kul, the Son-Kul Too ridge to the north and the Borbor Alabas and Moldo Too mountains to the south (Fig. 1). The valley lies at an altitude of 3,016 m and has an area of about 270 km<sup>2</sup> and a volume of 2.64 km<sup>3</sup>. The lake's maximum length is 29 km, its breadth is about 18 km and the deepest point is 13.2 m. It is the second-largest lake in Kyrgyzstan, after Issyk Kul, and the largest freshwater lake in Kyrgyzstan. The mean temperature in the lake basin is -3.5 °C (25.7 °F), with a mean temperature of -20 °C (-4 °F) in January and 11 °C (52 °F) in July.



**Figure 1.** A) A map of the Son-Kul valley (Latitude: 41° 49' 59.99" N, Longitude: 75° 09' 60.00" E) and (B) a view of the lake

Annual precipitation averages 300–400 mm from April to October and 100–150 mm from November to March. Snow cover in the lake basin persists for 180 to 200 days a year. In winter, the lake surface freezes, and the ice can be up to 1–1.2 m thick. The ice on Son-Kul begins to thaw in mid to late April and completely disappears by late May (Ramsar Sites Information Service, 2011).

Samples were taken from the rhizosphere soil and the soil without vegetation in the summer (middle of July) from 10 biotopes. Samples were taken at every 100 m, moving

from the lakeshore to the top of the subalpine and alpine belts of the Kondoy Too mountain range, which has permafrost in the summer. A detailed description of the investigation sites is given in *Table 1*.

**Table 1.** Soil sampling sites in the Son-Kul valley

| Site and sample numbers | Description of localities and covered vegetation   | Soil type                           | Air temperature °C | Soil temperature °C | Altitude, meter above sea level and GPS coordinates | pH soil |
|-------------------------|--|-------------------------------------|--------------------|---------------------|---|---------|
| SK-1                    | The coast of Lake Son-Kol (10 m from the beach). Vegetation - low-grass meadows dominated by the common skullcap ( <i>Scutellaria galericulata</i> ) | Alpine semi-peaty soils             | 18.1               | 8.3                 | 3027; 41°50' 45.620" N and 75°9'07.877" E           | 7.77    |
| SK-2                    | Terrain 100 m from the coast of Son-Kul. Meadow undersized Forbes associations. Dominated by edelweiss ( <i>Leontopodium fedschen-kaanum</i> ).      | Mountain-meadow-steppe-alpine soils | 18.1               | 10.0                | 3031; 41°50' 45.611" N and 75°9'07.890" E           | 7.17    |
| SK-3                    | The depression between the foothills of the ridge "Suuk Kolot" 1.5 km from the lake, meadow forb vegetation, yellow geraniums and tulips dominate.   | Mountain-meadow-steppe-alpine soils | 15.3               | 8.0                 | 3095; 41°50' 45.286" N and 75°9'07.974" E           | 7.67    |
| SK-4                    | The Suuk Kolot ridge. Vegetation meadow, peat-bog cereal communities dominate  | Mountain-meadow alpine soils        | 9.3                | 7.3                 | 3200; 41°50' 45. 123" N and 75°9'07.995" E          | 8.28    |
| SK-5                    | Floodplain banks of the Uzun-Bulak River. Meadow vegetation, feather grass and geranium prevail  | Mountain-meadow-steppe-alpine soils | 9.4                | 9.5                 | 3088; 41°50' 42. 279" N and 75°9'04. 410" E         | 8.115   |
| SK-6                    | Uzun-Bulak. Heavily used for livestock, the soil is fertilised with manure, dominated by dandelion ( <i>Taraxacum officinale</i> ) and cinquefoil.   | Mountain-meadow-steppe-alpine soils | 9.0                | 8.6                 | 3103; 41°50' 42. 224" N and 75°9'04. 465" E         | 7.93    |
| SK- 7                   | Foothill area "Kondoy Too"southern ridge, low-growing mountain-valley vegetation, labiate associations dominate                                      | Mountain-meadow alpine soils        | 8.1                | 8.4                 | 3141; 41°50' 42. 0.19" N and 75°9'03. 636" E        | 7.59    |
| SK-8                    | Top of the Kondoy Too mountain, near a mountain glacier. Alpine meadows. A dense thicket of wild onion association and yellow tulips.                | Mountain-meadow alpine soils        | 11.16              | 8.9                 | 3222; 41°50' 41. 929" N and 75°9'03.407" E          | 6.32    |
| SK-9                    | Top of the Kondoy Too mountain, soil under a glacier, soil moisture 100%. No vegetation.   | Mountain-meadow alpine soils        | 11.7.              | 0.0                 | 3243, 41°50' 41. 937" N and 75°9'03.380" E          | 6.59    |
| SK-10                   | Top of the mountain "Kondoy Too", soil under a melting glacier, humidity soil - 100%, without vegetation   | Mountain-meadow alpine soils        | 10.7               | 3.0                 | 3244; 41°50' 41. 963" N and 75°9'03.310" E          | 6.50    |

Three repeating plots were randomly selected for each type of vegetation. The size of each plot was  $\sim 20 \text{ m} \times 20 \text{ m}$ . A soil sample was taken from the depth of the root system (5–20 cm) under the dominant plant species in each plot with a soil drill (5 cm in diameter). Before sampling, the top 2–3 cm of sod was removed. Selection was carried out in good weather, in the morning before the onset of heat or at the end of the day, so the sample was as dry as possible. The resulting samples were mixed and formed the average specimen. Each mixed piece was composed of 5 individual samples taken from 5 points. The mass of the sample for analysis was 300–400 g. Notes were attached to all samples, which contained all their characteristics: the exact place of taking and the plot area.

The samples were stored in double sterile plastic bags, labelled and transported to the laboratory. The samples were conserved in an incubator with ice bags during transportation. The soil samples were frozen at  $-80^\circ\text{C}$  until nucleic acid extraction. The samples were air-dried at room temperature, separated from roots and debris and passed through a 2-mm plastic sieve before chemical and microbiological analysis. The pH was determined using a pH meter (Thermo Scientific, Orion Laboratory Products) and exchangeable and hydrolytic acidity were measured by titration with KCl and  $\text{CH}_3\text{COONa}$ , respectively (soil: solution ratio of 1:2.5). Carbon and nitrogen content was measured using a soil elemental analyser (ElvaX Plus spectrometer, Elvatech Ltd., Canada). All physical-chemical and biological parameters were analysed in triplicate at a minimum ( $n = 3\text{--}6$ ). Soil classification was performed according to Mamytov (1971, 1996).

### ***Isolation of bacteria from soil by cultivation-dependent approach***

To isolate bacteria species from the soil, soil samples were analyzed using the acetate selection protocol of Travers et al. and the methods of soil microbiology and biochemistry (Zvyagintsev, 1991) with some modifications. Samples of 10 g were prepared from each soil sample and ground in a sterile porcelain mortar for 5 min in aseptic conditions. After grinding, the soil sample was washed in sterile water. Ten milliliters of Luria-Bertani broth, 1 g from each soil sample, was added and buffered with sodium acetate (0.25 M, pH 6.8) in a 125-ml flask. The broth was incubated in a shaker at 200 rpm for 4 h at  $28^\circ\text{C}$ . A 1-ml aliquot was spread on nutrient agar plates (NA), and incubated at  $15^\circ$ ,  $20^\circ$  and  $28^\circ\text{C}$  for 48–72 h. The colonies were subcultured on new NA plates until pure cultures were obtained, and they were kept at  $4^\circ\text{C}$  for further identification.

The colonies were subjected to Gram staining, and the results were analysed along with colony shape and bacterial movement. The isolated bacterial cultures were studied for their ability to grow on meat-peptone broth (MPB), meat-peptone agar (MPA), oxidative-fermentative (OF) medium and catalase. Conventional tests were performed, such as protein hydrolysis, reduction of nitrates to nitrites, reduction of nitrates to nitrogen, indole production (tryptophane), fermentation (glucose), arginine dihydrolase, gelatin hydrolysis and the urea breath test. The phenotypic and biochemical characteristics of the isolates were established according to the determinants (Bergey's Manual of Determinative Bacteriology, 2004). Isolated bacteria were grouped on the basis of their morphological, biochemical and physiological characteristics.

For isolation and cultivation of bacteria, semi-differential media compositions were used: NutriSelect™ Plus (Merck KGaA) nutrient agar for microbiology ( $15 \text{ g L}^{-1}$  agar;  $1 \text{ g L}^{-1}$  meat extract;  $5 \text{ g L}^{-1}$  peptone;  $5 \text{ g L}^{-1}$  sodium chloride;  $2 \text{ g L}^{-1}$  yeast extract); King medium (20 g protease peptone #3 (Difco);  $1.5 \text{ g K}_2\text{HPO}_4$ ;  $1.5 \text{ g MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 10 mL

glycerol; 15 g agar; 1 L distilled water); and starch ammonium agar medium or ISP Medium No. 4 (10 g L<sup>-1</sup> soluble starch; 1 g L<sup>-1</sup> dipotassium phosphate; 1 g L<sup>-1</sup> magnesium sulphate heptahydrate; 1 g L<sup>-1</sup> sodium chloride; 2 g L<sup>-1</sup> ammonium sulphate; 2 g L<sup>-1</sup> calcium carbonate; 0.001 g L<sup>-1</sup> ferrous sulphate heptahydrate; 0.001 g L<sup>-1</sup> manganous chloride 7H<sub>2</sub>O; 0.001 g L<sup>-1</sup> zinc sulphate 7H<sub>2</sub>O; 20 g L<sup>-1</sup> agar; pH 7.2 ± 0.2; 25 °C).

To identify the bacteria species, modern special keys were used (Bergey's Manual of Systematics of Archaea and Bacteria, 2015). Pictures of bacterial cells were obtained using a microscopy camera (Motic Images Plus, Version 2.0 ML, Quick Start Guide, 163 Series Compound Biological Microscopes).

### ***Determination of the colonies united form (CUF) of bacteria on nutrient medium per g soil***

To calculate the number of microorganisms in soil using the inoculation method, two conditions must be met: the soil suspension from which the dilution is carried out must contain only single free-floating cells, and each cell, once on the nutrient medium, must produce a colony (Zvyagintsev, 1991).

The following method was used to determine the number of bacterial colony-forming units (CFU) per gram of soil. Under aseptic conditions, 1 g of soil was moistened to a paste and ground with a rubber pestle for 5 min. After preliminary dispersion of the ground, dilutions of the soil suspension were prepared. Plating was carried out at dilutions from 1:2 to 1:100,000, depending on the group of microorganisms, type of soil, season and soil moisture. The most accurate calculation is obtained when 50–200 CFU develop on a plate. The seeded cups were turned upside down and placed in a thermostat at 15<sup>0</sup>, 20<sup>0</sup> and 28 °C.

Bacterial colony counting on NA in Petri dishes was conducted at 7 days for the r-strategies group and at 10 days for the K-strategies group. Colonies were usually counted using a magnifying glass device without opening the Petri dishes. For convenience, the positions of the counted colonies were marked with dots on the underside of the dish using a glass marker. Having counted the number of colonies on all parallel plates, the average number of colonies per plate was determined and then recalculated per 1 g of air-dried and dried soil, according to the *formula (1)*:

With a large number

$$N = C / [V \times (n1 + 0,1 \times n2) \times d] \quad (\text{Eq.1})$$

where,

N = Number of CUF microorganisms in 1 g

C = Total number of CUF in all counted Petri dishes

V = Volume transferred to Petri dishes (1 ml)

n1 = Number of Petri dishes counted from the first dilution

n2 = Number of Petri dishes counted from the second dilution

d = Dilution factor of more concentrated than the 2 consecutive dilutions from which the calculation is made (10<sup>-2</sup>). Based on the results obtained, the number of microorganisms per gram soil was calculated.

## ***Isolation of microorganisms from soil by cultivation-independent methods***

### ***DNA extraction, PCR amplification and sequencing of bacteria***

DNA was extracted from the enrichment cultures during the active phase of microbial growth, using the Ultraclean Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) and alternative protocol developed by the Mo Bio Laboratories. To process soil samples, 5 g of soil was mixed with 10 to 30 mL of phosphate-buffered saline (PBS) to create homogenized slurry. Samples were mixed for 1 hour at room temperature and then centrifuged for 5 minutes at 123×g. The supernatant was removed and centrifuged at 20 000×g for 15 minutes. The supernatant was then carefully discarded, and the pellet was resuspended in 1 mL of PBS. To extract DNA, 700 uL of the resuspended soil extraction pellet was processed. The purified bacteria were incubated in meat peptone medium (MPM) for 2 days at 25°C. Cells were harvested at the early exponential growth phase, and their DNA was then extracted by the alternative protocol of the Mo Bio Laboratories. Successful DNA extraction was determined by agarose gel electrophoresis (1.0% agarose). Amplification was performed with a Multi Gene Thermal Cycler (TC9600-G/TC, Labnet International, Edison, New Jersey, USA), using a 25 uL mixture containing 15 uL of PCR MasterMix (Taq DNA polymerase, MgCl<sub>2</sub>, deoxyribonucleotide triphosphate, and reaction buffer), 2 uL of each primer, 1 uL of template DNA, and 1 uL of H<sub>2</sub>O. The amplification program was used as the follows: 94°C for 5 minutes, 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 60 seconds, and 72°C for 7 minutes. PCR products were electrophoresed in a 1.0% agarose gel and visualized using the BioDoc-It Imaging Systems (Ultra-Violet Products Ltd) after ethidium bromide staining. To control contamination, we used a negative control reaction, and sterile water was added as a matrix. Almost full-length fragments of 16S rRNA genes were amplified using the primers 16S-27F (27f 5'-AGAGTT TGA TCC TGG CTC AG-3') and 16S-1492 R (5'-GGTTAC CTT CTT ACG ACT T-3'). Sequence analysis was performed by the Macrogen Company (10F World Meridian Center, Seoul, Korea), and sequences were edited with Applied Biosystems 3730XL sequencers. Only sequences with more than 700 nucleotides were used for diversity analyses. The phylogenetic relatedness among different sites was determined using the cluster environment. The 16S ribosomal RNA (rRNA) gene sequences were deposited in the GenBank and DB of the National Center for Biotechnology Information nucleotide sequence databases.

### ***Statistical analysis***

The Shannon index was used to determine the complete species composition of the bacterial community, including the abundant rare species at the studied sites. The Simpson index was used to characterise the community by the dominant group of species.

The obtained data were statistically processed using SPSS version 25 (IBM, USA).

## **Results**

### ***The ecological features of the Son-Kul valley that serve as habitats for plants and soil microorganisms***

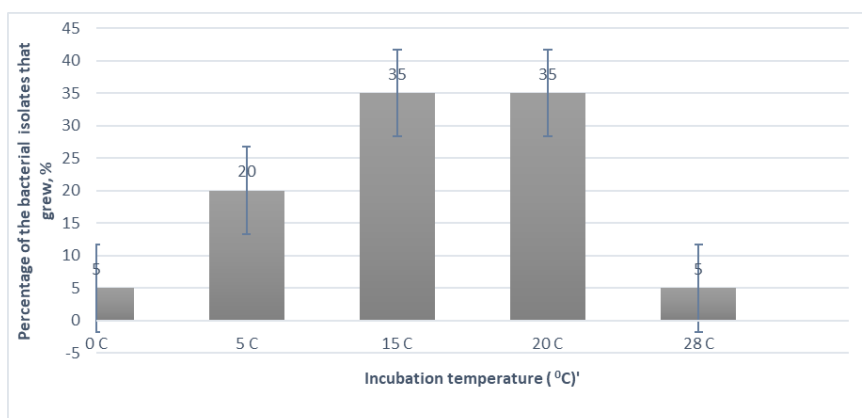
Seven altitude zones are conventionally distinguished in Kyrgyzstan. Son-Kul is located in subalpine and alpine areas, where subalpine and alpine meadows are

widespread. The regional vegetation types vary with altitude (Tsekanov, 1979; Golovkova, 1990) in the Son-Kul basin. Soil organic carbon density ranged from 9.73 to 35.21 kg m<sup>-2</sup> at 0–60 cm at the hill scale (Mamytov, 1974). The climate and vegetation change with every 100–200 m of ascent into the mountains. Only a few plant species are found in the glacial belt. The conditions here are very harsh, as they are in the tundra, and the plants that grow here do so in response to this natural and climatic zone. There is permafrost under the topsoil at the mountaintops. There are famous high-mountain pastures; in the alpine belt, they are covered with turf grasses, feather grass, fescue and grasses. Some plants grow only at certain altitudes; for example, edelweiss is not found below 2,000 m. Alpine tundra is found above 3,000 m, where the vegetation is poorer and more monotonous. Plants cover the soil, but not entirely. They are found in spots, ribbons and rings.

### ***Density of bacterial colonies found in the studied biotopes of the Son-Kul valley***

#### ***Number of bacterial species found growing at different temperatures***

A total of 320 bacterial isolates belonging to various morphological groups and genera were isolated from the biotopes. They were cultivated in MPA at 0, 5, 15, 20 and 28 °C for 15 days. As *Figure 2* shows, the growth ability of the bacterial populations isolated from the 10 biotopes under the range of temperature conditions was significantly different. Of the isolates, 5.0% could grow at 0 °C, and these were found in the SK-9 and SK-10 site samples, which were permafrost and snow. When tested at 5 °C, 20% of the isolated bacterial populations grew, and they were isolated mainly from sites SK-8 and SK-9. About 30% of the natural isolates grew at 15 °C, and 35% established colony growth at 20 °C. These isolates were from locations other than SK-8, SK-9 and SK-10. At 28 °C, about 5.0% of the isolates could grow, representing species from sites SK-6, SK-5, and SK-2 (*Fig. 2*).

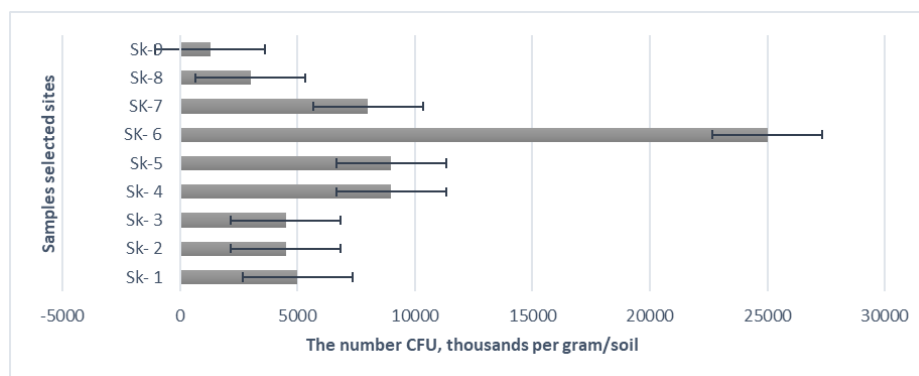


**Figure 2.** The proportion of bacterial isolates found to grow at different temperatures. Values are given as mean ± SD, n = 3, significantly different at  $P \leq .05$

#### ***The number of bacterial colony-forming units (CFU) able to grow at a temperature of 15-20 °C in the studied soils of the Son-Kul Valley***

The number of ammonifying bacteria able to grow at 15–20 °C was examined in this study. We focused on this temperature range because these are the optimal temperatures

at which heterotrophic, ammonifying bacteria are actively involved in the decomposition of fresh plant residue in the short summer period. The number of bacteria capable of decomposing organic residue in the different biotopes ranged from  $4.3 \times 10^3$  to  $25.3 \times 10^3$  CFU/g of soil, indicating a generally low bacterial biomass in this high-mountain region. The Uzun-Bulak soils were found to have the highest content of ammonifying bacteria:  $25.3 \times 10^3$  CFU/g of soil. The lowest concentrations were found in the SK-9 and SK-10 samples, which were taken after the glacier melted (3,244 m.a.s.l.), with  $3 \times 10^2$  CFU/g of soil (Fig. 3).



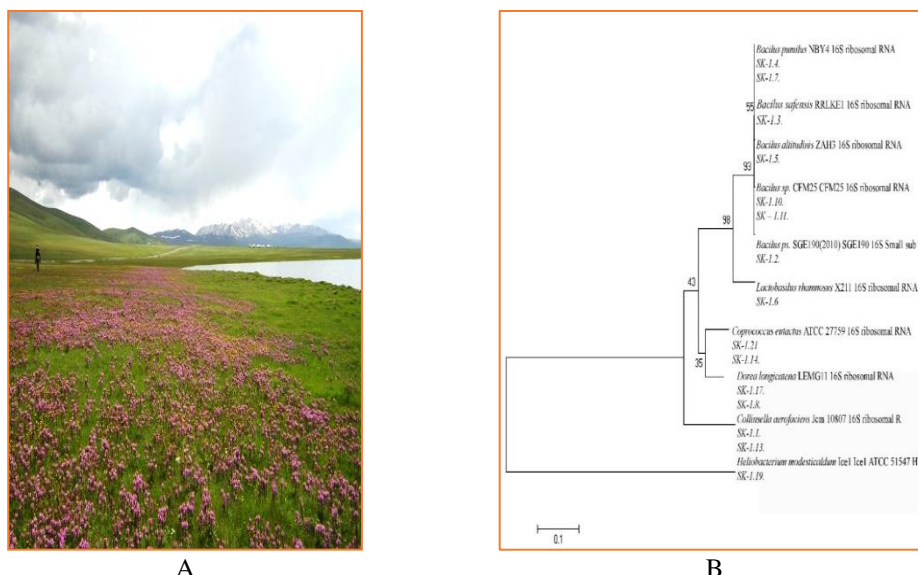
**Figure 3.** The number of colony-forming units (CFU) of heterotrophic bacteria in the studied soil biotopes of the Son-Kul Valley. Values are given as mean  $\pm$  SD,  $n = 3$ , significantly different at  $P \leq .05$ .

Rhizosphere bacteria living around plant root systems and directly involved in the transformation of plant root exudates into mineral compounds were studied. However, the findings suggest that these bacterial communities have insignificant biomass due to low temperatures and sharp daily fluctuations. Significantly, high numbers of bacteria were found at the Uzun-Bulak site, where the soil was enriched with animal manure; a stall of sheep and a cow that grazed daily in the pasture were housed here at night each year. The annual introduction of animal protein into the soil promoted the activation of ammonifying bacteria and an increase in their biomass in this biotope.

### Soil bacterial biodiversity determined using molecular identification

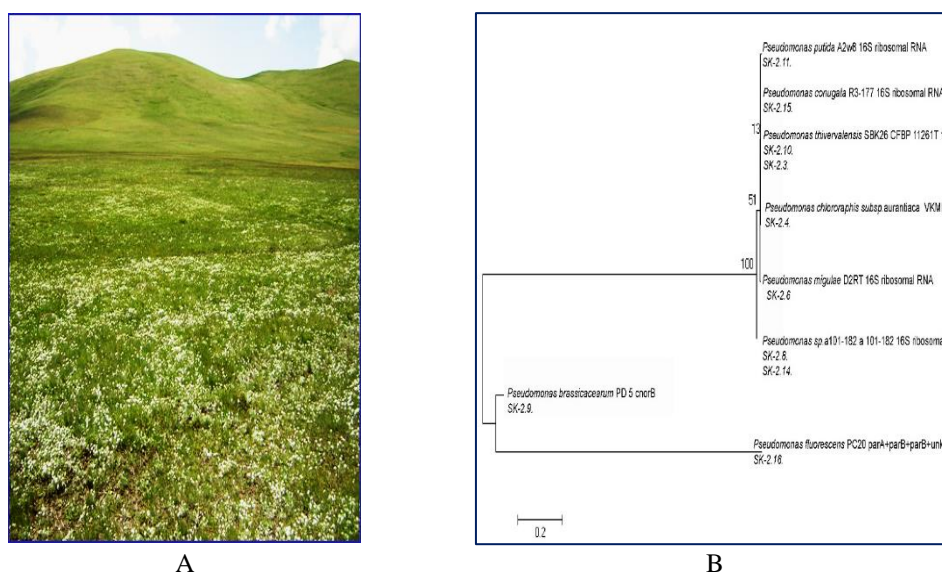
The molecular identification results show that during the summer, in high-altitude soils of the Son-Kul valley were dominated by the Firmicutes, Actinobacteria, Gammaproteobacteria, Betaproteobacteria genetic groups. The findings show that bacterial species composition differs in soils under different vegetation cover according to the terrain's altitude.

**The SK-1 site** (the coast of Son-Kul, 10 m from the beach) is located at 3,027 m.a.s.l. This site was covered with low-grass meadows dominated by the common skullcap (*Scutellaria galericulata*). At this site, in the skullcap's rhizosphere, spore-forming bacteria were dominant, namely representatives of the phylum Firmicutes: *Bacillus* sp, *Bacillus pumilus*, *Bacillus safensis*, and *Bacillus altitudinus*. Also, non-spore-forming representatives of the same phylogenetic group were present: *Lactobacillus rhamnosus*, *Coprococcus eutactus*, *Dorea longicatena* and *Heliobacterium modesticaldum* Icel. Phylum Actinobacteria representatives made up an insignificant share: *Collinsella aerofaciens*, *Dermacoccus* sp. and *Micrococcus* sp. (Fig. 4).



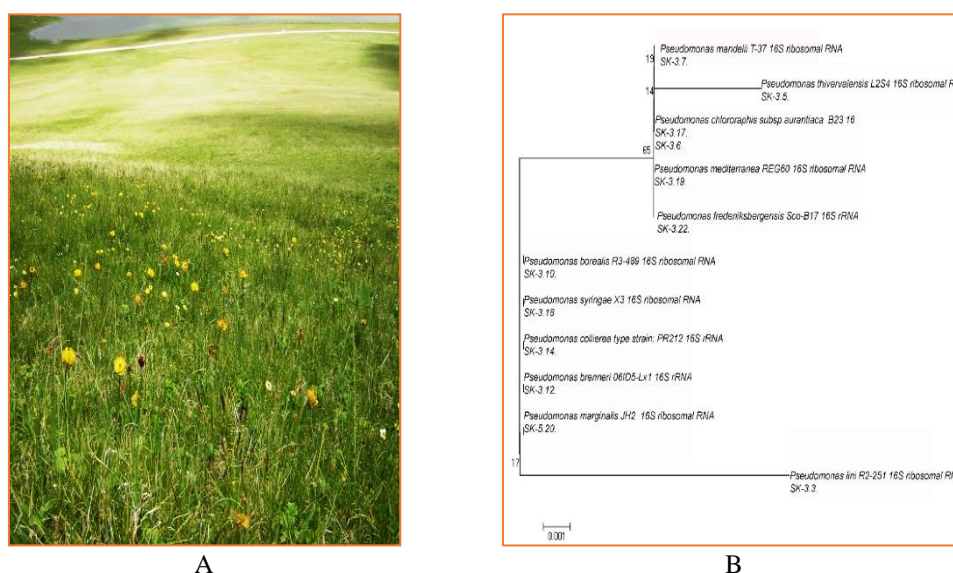
**Figure 4.** A- SK-1 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-1 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the GenBank database

**The SK-2 site** (100 m from the coast of Son-Kul) is located at 3,031 m.a.s.l. This site was covered with vegetation of meadow undersized Forbes associations and dominated by edelweiss (*Leontopodium fedschen-kaanum*). At this site, under the edelweiss population and in the rhizosphere of the alpine flowers, only non-spore-forming bacteria from the class Gammaproteobacteria (phylum Proteobacteria) were found: *Pseudomonas putida*, *Pseudomonas fluorescens*, *Pseudomonas migulae*, *Pseudomonas tolaasii*, *Pseudomonas corrugata*, *Pseudomonas thivervalensis*, *Pseudomonas chlororaphis* subsp. *aurantiaca*, *Pseudomonas brassicacearum* and *Pseudomonas* sp. (Fig. 5).



**Figure 5.** A- SK-2 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-2 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the GenBank database

**The SK-3 site** (the depression between the foothills of the Suuk Kolot ridge, 1.5 km from the lake) is located at 3,095 m.a.s.l. This site is covered with meadow forb vegetation, and yellow geraniums (*Geranium maculatum*) and tulips (*Tulipa kaufmanniana*) dominate. At this site, under the geranium and tulip populations, in the rhizospheres of the alpine flowers, the same bacteria of the genus *Pseudomonas* were found as at SK-2, namely *P. thivervalensis* and *P. chlororaphis*. In contrast to SK-2, other species were found at SK-3: *Pseudomonas mandeli*, *Pseudomonas mediterranea*, *Pseudomonas frederiksbergensis*, *Pseudomonas borealis*, *Pseudomonas syringae*, *Pseudomonas collierea*, *Pseudomonas brenneri*, *Pseudomonas marginalis* and *Pseudomonas lin*. It is well-known that non-spore-forming bacteria, as active ammonifiers, decompose fresh plants. Therefore, their complete dominance in these two biotopes indicates their active involvement in the process of ammonification in high-mountain areas (Fig. 6).

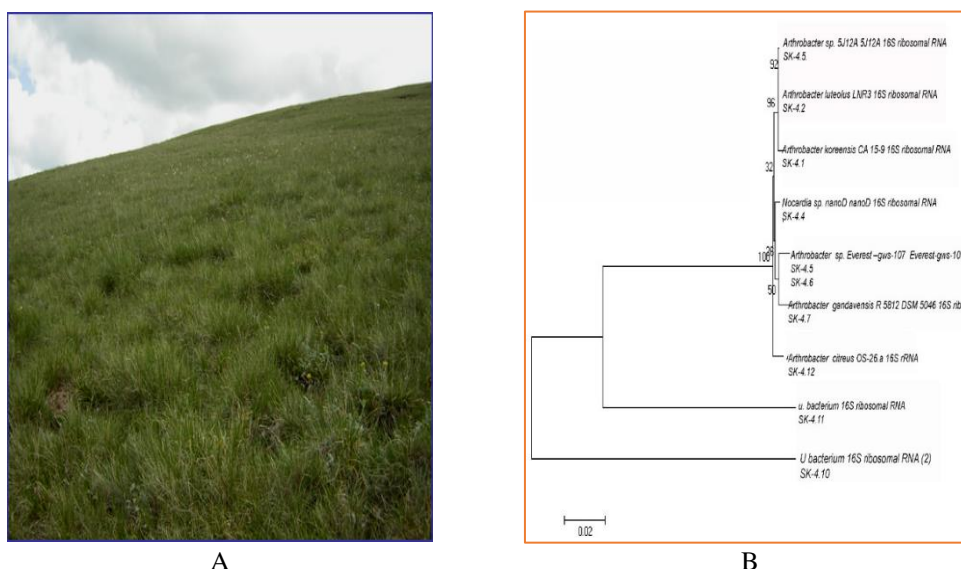


**Figure 6.** A- SK-3 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-3 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the GenBank database

**The SK-4 site** (the Suuk Kolot ridge) is located at 3,200 m.a.s.l. This site is covered with meadow vegetation, and peat-bog cereal communities are dominant. At this site, in the peat-bog cereal rhizospheres, it was found that representatives of the Actinobacteria phylum were dominant: *Arthrobacter* sp., *Arthrobacter luteolus*, *Arthrobacter koreensis*, *Nocardia* sp., *NanoD*, *Arthrobacter* sp. *everest*, *Arthrobacter gandavensis* and *Arthrobacter citreus*, as well as uncultivated bacteria: *Uncultured bacterium* (Fig. 7).

**The SK-5 site**, floodplain banks of the Uzun-Bulak River is located at an altitude of 3088 m.a.s.l., meadow vegetation, feather grass (*Nassella tenuissima*) and geranium (*Geranium maculatum*) are prevailed. At the SK-5 site, species of class Gammaproteobacteria (phylum Proteobacteria) predominate: *Stenotrophomonas rhizophila*, *Stenotrophomonas maltophilia*, *Stenotrophomonas* sp. and uncultured *Stenotrophomonas*. There are also significant representatives of the genus *Xanthomonas*: *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas bacterium* *IK1*. Representatives of other phyla were found in small amounts. For example, from Actinobacteria:

*Arthrobacter* sp., from Firmicutes: *Brevibacterium* sp. and from *Bacteria*: *Uncultured bacterium* sp. (Fig. 8).



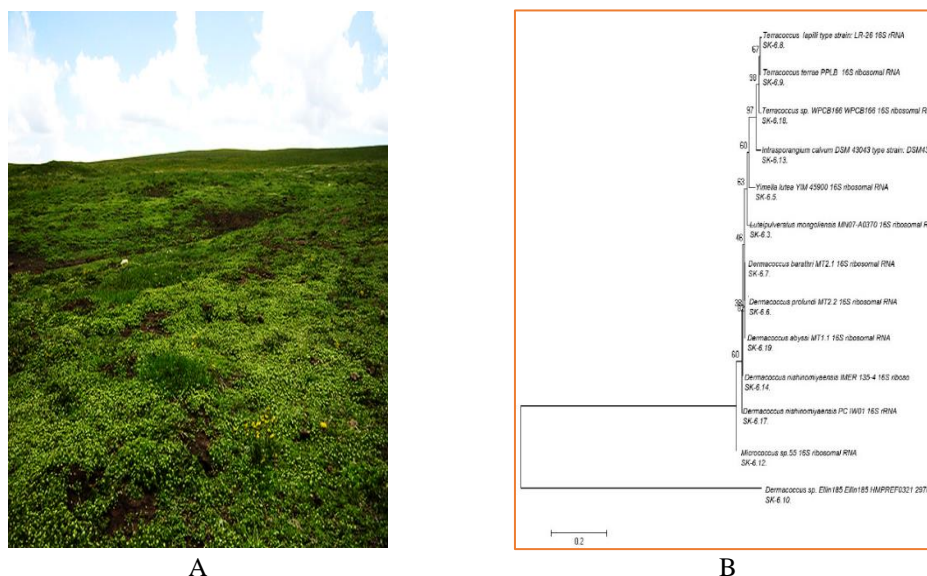
**Figure 7.** A- SK-4 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-4 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the GenBank database



**Figure 8.** A- SK-5 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-5 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the GenBank database

**The SK-6 site** (Uzun-Bulak) is located at 3,103 m.a.s.l. This site is heavily used for livestock. The soil is fertilised with manure, and the vegetation is dominated by dandelion (*Taraxacum officinale*) and cinquefoil (*Potentilla reptans*). At this site, almost 95% of the bacterial community was found to consist of representatives of the Actinobacteria

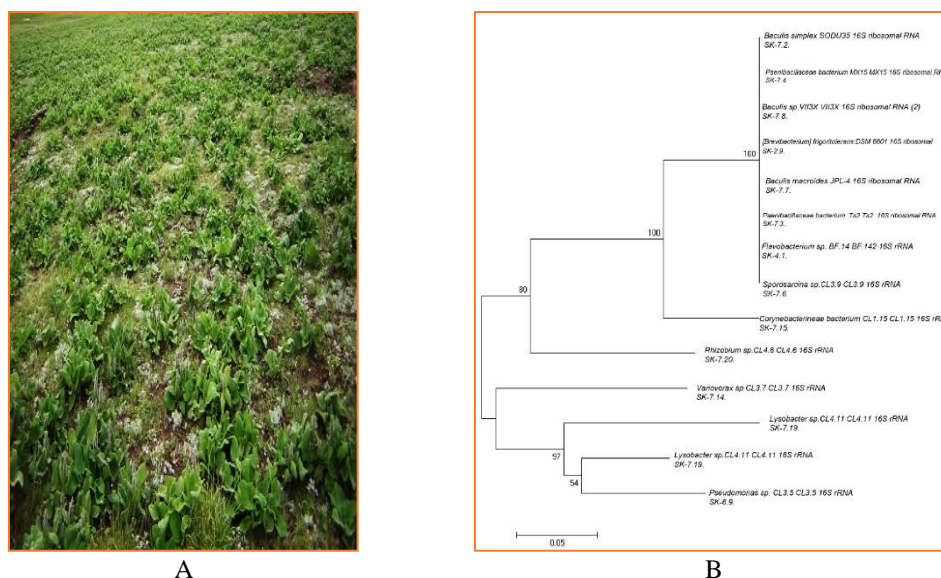
phylum. However, within this phylogenetic group, rich genera and species diversity were found. The following six genera were found to make up the given proportions of the bacterial community: *Dermacoccus* (46.15%), *Terracoccus* (23.076%), *Janibacter* (7.6%), *Luteipulveratus* (7.6%), *Intrasporangium* (7.6%) and *Yimella* (7.6%). Genus *Dermacoccus* was represented by the following species: *Dermacoccus profundi*, *Dermacoccus barathni*, *Dermacoccus abyssi*, *Dermacoccus nishinomiyaensis* and *Dermacoccus* sp. Ellin. The following species were found in the genus *Terracoccus*: *Terracoccus* sp. WPCB166, *Terracoccus lapilli* typestrain and *Terracoccus terrae*. The genera *Intrasporangium*, *Janibacter*, *Luteipulveratus* and *Yimella* were all represented by one species each: *Intrasporangium calvum*, *Janibacter* sp. BSi20546, *Luteipulveratus mongoliensis* and *Yimella lutea*, respectively (Fig. 9).



**Figure 9.** A- SK-6 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-6 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the GenBank database

**The SK-7 site** (the Kondoy Too southern ridge foothills) is located at 3,141 m.a.s.l. It has low-growing mountain-valley vegetation, and labiate associations (*Labiatae*) are dominant. A rich diversity of bacteria from phyla Firmicutes, Actinobacteria, Proteobacteria (classes: Gammaproteobacteria and Alphaproteobacteria) and Bacteria was identified at this site. The Firmicutes phylum was found to be the richest in terms of species diversity and number, with the following species found: *Paenibacillaceae bacterium* Ts2, *Brevibacterium frigoritolerans*, *Sporosarcina* sp. CL3.9, *Eubacterium* sp. 11–12 and *Firmicutes bacterium*. The second richest phylum was found to be Actinobacteria, represented by: *Micrococcineae bacterium* BF.10, *Corynebacterineae bacterium* CL1.15 and *Arthrobacter* sp. 210\_15. This was followed by the Bacteria phylum with: *glacial ice bacterium* G500K-17, *Antarctic bacterium* L2 and *marine sponge bacterium* plate OTU5. The rest of the phylogenetic groups were represented by one species each. From the Gammaproteobacteria class was *Lysobacter* sp. CL4.11, and from the Alphaproteobacteria class was *Rhizobium* sp. CL4.3. At this site, bacteria were represented mainly by species associated with plant root systems (e.g., *Paenibacillaceae*)

and species related to plants living in peaty-soddy soils (e.g., *Sporosarcina* sp., *Micrococcineae* bacterium and *Corynebacterineae* bacterium) (Fig. 10). These species have been found in similar peat soils in other countries (Cousin et al., 2010).



**Figure 10.** A- SK-7 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-7 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the Genebank database

**The SK-8 site** (the top of the Kondoy Too mountain) is located at 3,222 m.a.s.l. and near a mountain glacier. The alpine meadow vegetation consists of a dense thicket of wild onion association (*Allium stellatum*) and yellow tulips (*Tulipa sylvestris*). At this site, up to 99% of the detected microbial community consisted of representatives of the phylum Actinobacteria: *Rhodococcus* sp. RE 59, *Rhodococcus groberulus*, *Rhodococcus gingshengii* L-10, *Rhodococcus bonitorelans*, *Rhodococcus erythropolis*, *Rhodococcus* sp., *Nocardia coeliaca*, *Nocardia globerula*, *Nocardia bacterium* PhyCEM, *Nocardia bacterium* KDV, *Nocardia smegmatus* str. actinobacterium CH21i and uncultured actinobacterium, and phylum Bacteroidetes (marine bacterium WP02-3-63) (Fig. 11).

**The SK-9 site** (the top of the Kondoy Too mountain) is located at 3,243 m.a.s.l. and consisted of soil under a glacier. The soil moisture level was 100%, the soil pH was 6.5 and the temperature was 0.0 °C. There is no vegetation. At this site, only microbial communities from the Actinobacteria phylum and *Paenarthrobacter* genus (Syn. *Arthrobacter* genus) were found: *Paenarthrobacter ilicis*, *Arthrobacter oxydans*, *Paenarthrobacter histidinovorans*, *Arthrobacter boritolerans*, *Paenarthrobacter nicotinovorans*, *Arthrobacter aurescens*, *A. luteolus*, *Paenarthrobacter nitroguajacolicus*, *A. citreus* and *Pseudarthrobacter chlorophenolicus*. Among the identified species, a glacier-dwelling species was found: *Glacial ice bacterium* (Fig. 12).

**The SK-10 site** is located at the top of the mountain "Kondoy Too", at an altitude of 3244 m.a.s.l, soil under glacier, soil moisture - 100%, no vegetation. Soil type is mountain-light brown, with pH -6.5 and temperature 3.0 °C. There is no vegetation. At this site, the same microbial communities from the Actinobacteria phylum as at the SK-9 site were identified (Fig. 13).



**Figure 11.** A- SK-8 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-8 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the GenBank database

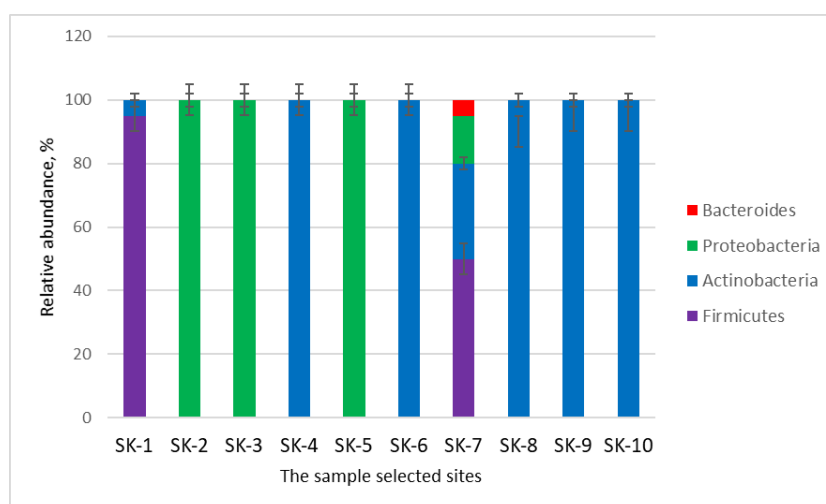


**Figure 12.** A- SK-9 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-9 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the GenBank database

Analysis of 16S rRNA revealed the DNA sequences and identities of the bacterial species within the communities directly after soil extraction. It also allowed us to examine the biodiversity of the species and their distribution among the different ecological niches in the studied biotopes. Representatives of four phyla were identified in total, with the diverse *Actinobacteria* phylum dominating (about 55% of the total), then the *Proteobacteria* (30%), *Firmicutes* (13%) and *Bacteroides* (2%) phyla (Fig. 14).

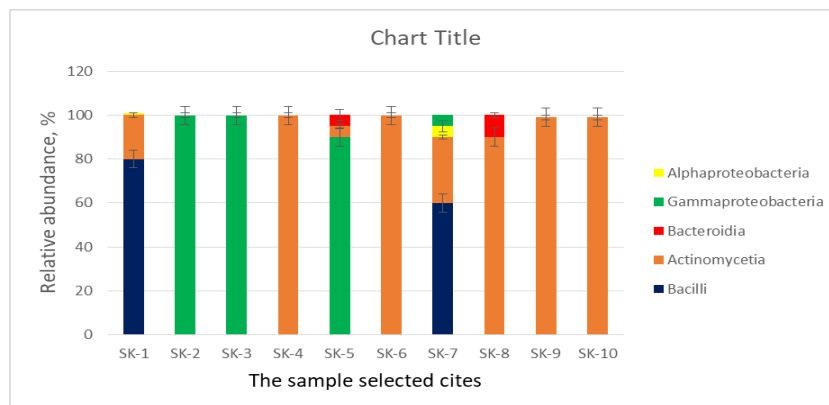


**Figure 13.** A- SK-10 site view; B- Phylogenetic tree of bacteria found in the soils of the Son-Kul Valley at the SK-10 site based on the analysis of 16SrRNA sequences. Each group contains at least > 97% sequence similarity available in the GenBank database



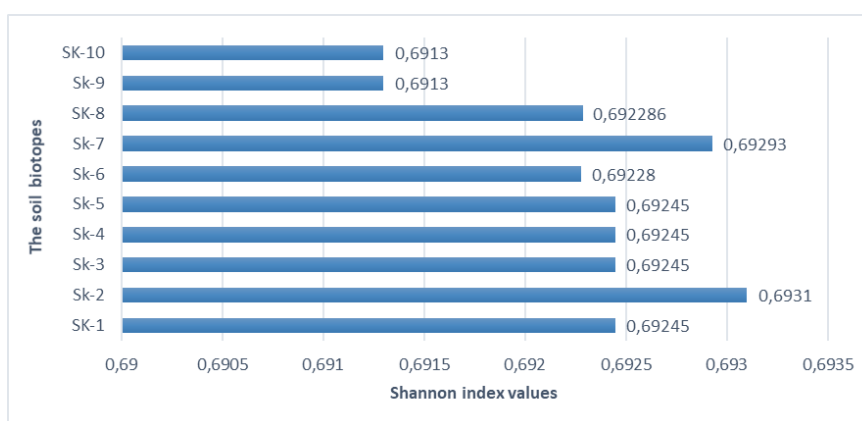
**Figure 14.** The bacterial community composition at the phylotype level for the 10 sample sites. Values are given as mean  $\pm$  SD,  $n = 3$ , significantly different at  $P \leq .05$

When we analysed the obtained bacterial communities at the class level, each studied biotope under a specific plant species had distinctive bacterial biodiversity. The proportion of *Actinomycetia* class representatives was found to be high compared to the other classes identified. Species from the *Gammaproteobacteria* class were the second most frequently occurring, and species from the classes *Alphaproteobacteria* and *Bacteroidia* were found in insignificant proportions under the vegetation of alpine steppe meadow biotopes (Fig. 15).

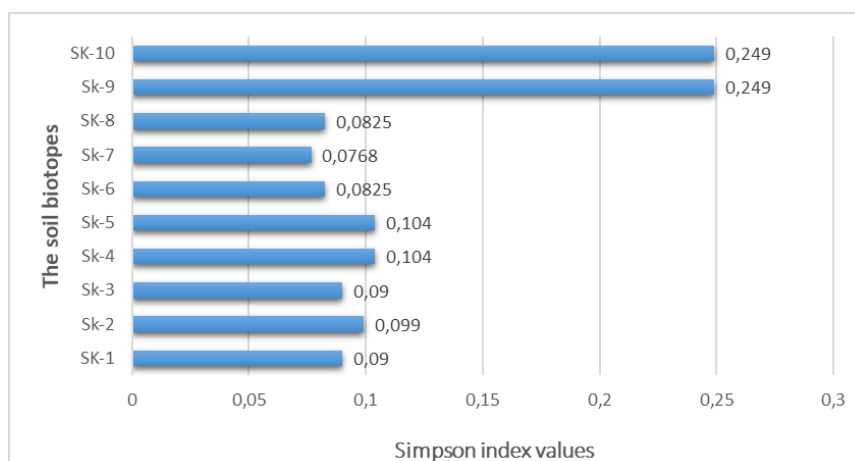


**Figure 15.** The bacterial community composition at the classes level for the 10 sample sites. Values are given as mean  $\pm$  SD,  $n = 3$ , significantly different at  $P \leq .05$

As the results of the analysis of the Shannon and Simpson index showed that the richness and diversity occurring species was low in this study region (Figs. 16 and 17).



**Figure 16.** Histogram of bacterial species probability distribution for the soil biotopes on Shannon index. Values are given as mean  $\pm$  SD,  $n = 3$ , significantly different at  $P \leq .05$



**Figure 17.** Histogram of bacterial species probability distribution for the soil biotopes on Simpson index index. Values are given as mean  $\pm$  SD,  $n = 3$ , significantly different at  $P \leq .05$

## Discussion

The studied soils of the Son-Kul high-mountain ecosystem were found to have different physicochemical and biological characteristics; therefore, these different soil habitats reflected the phenotypic differences of the microorganisms inhabiting the soil. The vegetation found in the Son-Kul basin region varies according to altitude (Mamytov, 1971; Tsekanov, 1979; Golovkova, 1990).

Soil organic matter affects soil functions and is a critical component of the global carbon cycle (Pataki et al., 2003; Houghton, 2007). Our results show that the number of ammonifying bacteria capable of growing at 15–20 °C and participating in the decomposition of plant litter was low in the collected samples, indicating a slower decay rate of new organic matter in soils of this cold ecosystem. The highest numbers of ammonifying bacteria were recorded at site SK-6, where the soil is rich in organic matter and covered under dense meadow vegetation. The lowest numbers of isolated bacteria were recorded in soils under a glacier (sites SK-9 and SK-10). At these sites, the ground has a low organic matter content, there is an absence of vegetation and the temperature is constantly below zero. Many studies have reported intermediate bacterial concentration values from glacier sites (Skidmore et al., 2000; Zhang et al., 2002; Foght et al., 2004). In mid-latitude environments, mountain snow bacterial concentrations have been reported to range from  $3 \times 10^3$  cells/ml (Bauer et al., 2002) to around  $4 \times 10^5$  cells/ml (Alfreider et al., 1996; Sattler et al., 2001; Segawa et al., 2005).

In most cold habitats, nutrient concentrations are low, and temperatures are typically high enough to support microbial growth and activity for only one to two months a year. Under these conditions, psychrotrophic microbes predominate that can withstand extreme temperatures and a lack of moisture for a long time (Larkin et al., 1970; Groudieva et al., 2004). Our results align with these earlier findings—most of the detected bacterial isolates were found to be psychrotrophic. Among the detected isolates, 35% grew well at 15 °C and a further 35% grew well at 20 °C. Thus, 70% of the bacterial species obtained in this study can be considered psychrotrophic. Furthermore, we assume that these bacteria that are capable of growing in a medium temperature range (15–20 °C) are the main bacteria responsible for the degradation and mineralization of plant residue during the short warm summer period. This is a reasonable assumption, given that other studies have proven that bacterial isolates from Arctic snow samples can degrade organic compounds found there at moderate temperature (17 °C) after 24 h of incubation (Amato et al., 2006).

Analysis of 16S rRNA revealed the DNA sequences and identities of the bacterial species within the communities directly after soil extraction. It also allowed us to examine the biodiversity of the species and their distribution among the different ecological niches in the studied biotopes. Representatives of four phyla were identified in total, with the diverse *Actinobacteria* phylum dominating (about 55% of the total), then the *Proteobacteria* (30%), *Firmicutes* (13%) and *Bacteroides* (2%) phyla (Fig. 14). Many other studies have also reported a high diversity of *Actinobacteria* in extreme habitats, such as marine sediments (Duran et al., 2015; Zhang et al., 2019), volcanic caves (Riquelme et al., 2015), deposits of cold springs (Yang et al., 2015), microbial mats of hot springs (Jiang et al., 2012), glacial forelands (Zhang et al., 2016b), lakes (Parveen et al., 2011) and deserts (Ding et al., 2013).

According to available literature, plant communities had different effects on archaea and bacteria in biotopes. Species richness and evenness of the plant community can have direct and indirect impacts on the structure of the soil bacterial community (Lamb et al.,

2011). A more homogeneous plant community increases the abundance of bacteria increases the potential of nitrification processes, here the mechanism of influence of plant evenness in the structural biodiversity of soil bacteria lies in the complementarity of root exudate profiles (Lamb et al., 2011). Other scientists also argue that the genotype of plants is of some importance in creating the species and genetic composition of soil microorganisms (Babalola et al., 2007). Plants and their associated microbial communities have evolved complex adaptations to cold stress. Microbial communities can increase plant resistance to harsh environmental conditions (Compant et al., 2019; Marian et al., 2022).

Our results also confirmed that vegetation cover influences the composition of bacterial biodiversity in the rhizosphere. Each studied biotope under a specific plant species had distinctive bacterial biodiversity. The obtained results showed a certain regularity in the distribution of the bacterial species, genera and classes under the vegetation. For example, under perennial cereals and many other plants, bacteria from the genus *Bacillus* (Bacilli class) dominated as active ammonifiers involved in decomposing plant residue. In another example, under succulent meadow vegetation, which has a short vegetation period, species from the Gammaproteobacteria class dominated, mainly species of *Pseudomonas*. As altitude increased and soil temperature decreased, *Arthrobacter* species from the Actinomycetia class were predominately found under alpine meadow plants (Fig. 15).

Our results align with those of others who have also found that specific phylogenetic groups, such as Betaproteobacteria, Gammaproteobacteria, Firmicutes, Actinobacteria and Alphaproteobacteria, are associated with cold environments (Christner et al., 2001; Zhang et al., 2002; Brinkmeyer et al., 2003; Groudieva et al., 2004; Miteva and Brenchley, 2005; Amato et al., 2006; Chica et al., 2019).

Phylogenetic analysis in several metagenomic studies has shown that Actinobacteria are the most common and predominant phylotype; its populations may be cosmopolitan, dominant in different geographical areas (Buckley and Schmidt, 2002; Smith et al., 2006; Aislabie et al., 2006, 2008). *Arthrobacter* and related organisms differ in that they form coccoid cells during the stationary growth phase or under starvation conditions (Cure and Keddie, 1973). Some soil species of *Arthrobacter* can withstand long periods of in situ starvation, which explains their wide distribution in soils at fluctuating low temperatures (Ensign, 1970).

As the results of the analysis of the Shannon and Simpson index showed that the richness and diversity occurring species was low in this study region (Figs. 16 and 17) compared to other ecosystems where the temperature is more suitable for the activity of soil bacteria, and the natural biodiversity of species is high. For example, when compared with data on the richness and diversity of bacterial communities in ecosystems in typical temperature regions, the highest biodiversity was noted in the grassland ecosystem (4.5-5.0 Shannon Index) and desert (5.0-5.5 Shannon Index), probably reflecting the relationship between community structure and plant species. The diversity in samples obtained from farmland and tree grove ecosystems was narrower than the others (Zhang et al., 2019).

## Conclusion

Results obtained suggest that as one moves up the slope, the soil biogenicity decreases, reaching a minimum in the soils of the Alpine belt. Here, the low temperature of the earth

is already a deterrent to bacterial activity. The decomposition of organic residues is slow with a high plant litter accumulation. The results also suggest that the mineralization of organic residues in the soils of high-mountain intermontane depressions is extremely slow due to the lack of heat and moisture. Here, *Artrubacter* and *Actinobacteria* group bacteria are predominantly distributed, using mineral forms of nitrogen as a source of nitrogen nutrition, indicating soil microflora's weak ammonifying activity.

Finally, our studies have shown although the active layer of alpine high mountain soils is directly exposed to extreme environmental conditions, harboured significant microbial diversity, that has an activity for a short time at moderate temperature, their role in nutrient cycling is essential.

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