The structure of the prokaryotic communities of the initial stages of soil formation in Antarctic Peninsula

E A Ivanova1*, G V Gladkov2, A K Kimeklis2, A A Kichko2, D V Karpova3, E E Andronov1,2 and E V Abakumov4

1Dokuchaev Soil Science Institute, Pyzhevsky lane, 7 b.2, Moscow 119017, Russian Federation
2All–Russia Research Institute for Agricultural Microbiology, Podbelsky chaussee, 3, Pushkin, Saint Petersburg 196608, Russian Federation
3Faculty of Soil Science, Lomonosov Moscow State University, Leninskiye gory, GSP–1 12, Moscow 119991, Russian Federation
4Biological Faculty, Saint Petersburg State University, 16th line VO, 29, Saint Petersburg 199178, Russian Federation

*E-mail: ektrnivanova@gmail.com

Abstract. The study of living organisms in the Antarctic soils is one of the most priority areas of polar research. The essential role of microorganisms in soil-formation and biospheric processes implies the relevance of the study of the microbiome adaptations and its ecological patterns in a variety of Antarctic soils. The use of Illumina sequencing technique was applied for investigation of prokaryotic communities in soils not covered and covered by moss-lichen cover, and in soils in conditions of different degree of ornithogenic impact. The results obtained showed that Actinobacteriota were generally dominated on the places without moss-lichen cover, Bacteroidota – in the soils with well-developed organogenic horizon, including the group of ornithogenic soils. The studied microbiomes were also characterized by a significant proportion of Cyanobacteria – prokaryotic phylum, typical for the initial stages of soil formation. Biodiversity analysis showed that the main factor that formed the composition of prokaryotic community was the presence and amount of organic matter in the soil, while ornithogenic influence was of subordinate importance. The results obtained make a contribution to the study of the microbial ecology of the primary soils of Antarctica and the adaptive trends of the microbiome to the harsh conditions of ecosystems of polar regions.

1. Introduction
Study of the state of Antarctic environment components is an urgent task in connection with the global climate regulation role of the polar regions. Despite the fact that the continent was previously considered sterile, studies over the past decades have demonstrated relatively high levels of biodiversity in the microbial community of Antarctic soils and soil-like bodies [1].

The almost full absence of higher plants combined with the dominance of moss-lichen associations and cyanobacteria creates favorable conditions for the development of soil microbial community, which plays a leading role in soil formation processes in extreme conditions of polar regions [2]. Antarctic Peninsula (Maritime Antarctica) is the region with relatively most favorable (in terms of south polar biome) environmental conditions, in comparison to other Antarctic regions, and is characterized by the
highest variety of pedogenic factors combinations resulting in implementation of relatively high diversity of soils and soil-like bodies. The key diagnostic feature of soil differentiation in Antarctica is generally the presence or absence of the organogenic horizon [3]. The following groups of soils can be distinguished: 1) soils without macroscopically distinct organogenic horizon (so-called Ahumic soils) – this group is generally referred to soil-like bodies and characterized by relative lack of moisture and inhibited rates of functioning of organisms engaged in organic matter primary production; 2) with surface organogenic – moss, algae and lichen cover, under which the formation of histic like horizons is possible. The last are often favorable for nesting places for penguins (penguin rookeries), thus these soils undergo different degrees of ornithogenic influence. Thus, the aim of the study was to investigate the soil microbiomes in these three types of objects. Since the application of metagenomic approaches (involving extraction and analysis of total soil DNA) to the analysis of terrestrial microbiomes allows investigation of the structure and biodiversity of microbial communities, including uncultivated forms of microorganisms, this work involved the study of microbiomes using Next–generation sequencing technique.

2. Materials and methods

2.1. The study site

King George Island is the largest in the South Shetlands archipelago with the area of ~1400 km². However, only about 5% of its area is free of ice [4]. The Fildes Peninsula and Ardley Island together (around 33 km²) comprise the second largest ice-free area of the South Shetland Islands and the largest on the King George Island. Gentle topography dominates the Fildes Peninsula with a wide central plain and several others at different altitudes. It is a tableland made up of old coastal landforms with numerous rocky outcrops and an average height of 30 m above sea level (a.s.l). The climate is cold, moist, and oceanic with a mean annual air temperature of −2.2 °C and mean summer air temperatures above 0 °C up to 4 months [5]. The mean annual precipitation is 350–500 mm/yr. In the South Shetland Islands, permafrost is sporadic or non-existent at altitudes below 20 m a.s.l., and occurs more or less discontinuously at altitudes from 30 to 150 m a.s.l. Mosses, lichens, and algae are widespread, along with two vascular plants (Deschampsia antarctica and Colobanthus quitensis). Penguins, seals, and seabirds are common in coastal areas.

Ardley Island is located on a south-west coast of King George Island, 500 m east of the Fildes Peninsula coast in Maxwell (Fildes) Bay. Mean annual air temperature (MAAT) at King George and Ardley Island is about −2.3 °C; mean annual wind speed is 9.3 m/s; annual precipitation – 729 mm. The area is represented mainly by andesite-basalt lavas and tuffs belonging to the Tertiary. Several elevated terraces-beaches are also described. Due to high biodiversity of Ardley Island it is designated as Antarctic Special Protected Area (ASPA). A wide variety of seabirds arrive in the area during the breeding period (11 species) or molting. Ardley Island is also characterized by a highly developed and noticeable flora, including several species of vascular plants, lichens and mosses. The most common lichens at studied plots are Usnea gen. and Himantormia lugubris, as well as Xanthoria sp., Rinodina sp., Placopsis sp., Buellia sp., Haematomma sp., and Caloplaca sp., which occupied mostly the higher parts of Ardley Island.

A previous investigation of morphological structure of soil profiles, their geomorphological position and their physicochemical properties allowed us to specify six major reference soil groups [6] that form the soil cover of the Fildes Peninsula and Ardley Island (in the order of total area decreasing): Cryosols > Leptosols > Arenosols > Fluvisols > Technosols > Histosols.

2.2. Laboratory methods

2.2.1. Soil description and sample analysis. Soil identification was carried out using the «Classification and Diagnostics of Soils of Russia» [7] and the World Reference Base for Soil Resources [8]. Both classification schemes can be applied to the soils of Antarctica, but are clearly insufficient to describe
the entire diversity of soils in the region. Characteristic of studied soil and their horizons are given in table 1. The routine chemical soil analyses have been performed according to standard methods [9].

| Plot | Depth, cm | Coordinates / description | Soil |
|------|-----------|----------------------------|------|
| Bel4 | 0–2       | 62°13’46.13”S 58°58’36.04”W | Histic Spodic Cryosol |
|      |           | Fildes Strait southwest coast |     |
|      |           | A vast (150x8 m) area is covered with moss and reaching along the coast |     |
| Bel16| 0–2       | 62°12’00.4”S 58°59’41.0”W | Grey–Humus Lithic Cryosol |
|      |           | CHN (Chinese Antarctic Program) monitoring site on top of a rocky highwall on the shore of the Drake Strait at the Biologists Bay. Deschampsia antarctica grows by curtains, moss cover grows sporadically. Fine detrital material is on the surface |     |
| Bel20| 0–2       | 62°12’46.3”S 58°56’05.6”W | Cryosol Turbic Stagnic |
|      |           | Ardley Island, Top Hill, without vegetation |     |
| Bel21| 0–3       | 62°12’55.1”S 58°55’58.6”W | Cryosol Orhnigenic Stagnic |
|      |           | Overmoisted bare covered by mosses |     |
| Bel22| 0–5       | 62°12’48.3”S 58°55’36.1”W | Cryosol Turbic |
|      |           | Central part of the Ardley Island, patterned grounds, plogonal microrelief |     |
| Bel23| 0–1       | 62°12’44.4”S 58°55’36.1”W | Post–ornithogenic Grey–humus Lithosol |
|      |           | A mild hillslope in the central part of Ardley Island. A penguin nesting site is on a nearby rocky highwall. The surface is covered by alga Prasiola crispa |     |
| Bel25| 0–1       | 62°12’34.9”S 58°55’33.7”W | Post–ornithogenic Grey–humus Lithosol/Post–ornithogenic grey–humus Lithozem |
|      |           | East coast of Ardley Island. The surface is covered by moss vegetation (projective cover is about 70%). Feathers of penguins and other birds are on the surface |     |
| Bel26| 0–1       | 62°12’38.1”S 58°55’44.4”W | Histic Spodic Cryosol/Peat Cryoturbated Podbur (underlain by permafrost) |
|      |           | Bellingshausen Station. Top of a flat hill. Moss–lichen cover. There are a lot of detrital material on the surface (40–50 cm) |     |
| Bel27| 0–5       | 62°11’44.3”S 58°57’41.3”W | Hyperskeletal Turbic Technosol/Cryoturbated Technozem underlain by permafrost |
|      |           | Vicinity. Bellingshausen Station. The area between residential buildings and diesel power plant. Waterlogging. Permafrost is at a depth of 4 meters. |     |
The studied ornithogenic soils are distinguished by a specific morphological structure of the soil profile, which is often represented by the following set of soil horizons: peat horizon – organic dark gray or dark-brown horizon and median horizon.

Active layer thickness and dynamics in the studied area have been previously investigated. Permafrost table depths for the area of this study have been previously evaluated by vertical electrical resistivity sounding [10]. It was found that permafrost table lies at the depths of Deschampsia antarctica - uses of physical and chemical (pH, nitrogen, etc.) factors and microbial community structure was determined. Apparently, the microbial community structure is largely influenced by local combinations of physicochemical factors that form

2.2.2. NGS-sequencing data curation. Processing of the sequences of 16S rRNA gene sequences was performed using R packages (using the Rstudio [11, 12]) and QIIME2 [13]. Rstudio [14] was used as the development environment for R. The package dada2 [15] was used for trimming, merging of sequences into phylotypes and further processing. The taxonomic affiliation of the phylotypes was determined using the RDP classifier [16] based on Silva 138 [17]. The phylogenetic tree was constructed in QIIME2 software environment in SEPP package [18]. Data normalization was carried out using a discharge algorithm for the lowest number of reads in the samples (phyloseq [19]) and stabilized by variation (in the Deseq2 package [20]) for comparison of relative representation of phylotypes. Shannon, Faith's phylogenetic index was used for alpha-diversity analysis (Mann–Whitney's paired test was used as a test to compare averages) [21]. Betadiversity analysis was performed by comparing communities with a similarity/difference matrix using weighted unifrac, unweighted unifrac and bray-curtis algorithms. Betadiversity analysis data were visualized by reducing the size of similarity/difference matrices using NMDS [22]. PERMANOVA [23] in the form of adonis2 test incorporated in the vegan package [24] was used as separation statistics for beta-diversity analysis. Post-processing and visualization of the data the packages R phyloseq, ggpubr [25], picante [26], ggforce [27], tidyverse [28], ampvis2 were used as well.

3. Results and discussion

The C content, with regard to mineral horizons, was generally comparable to similar indicators at control plots. Decreasing of organic matter in lower soil horizons was observed both in ornithogenic and non-ornithogenic soils. Ornithogenic factor had a low impact on mineral horizons. Exactly the same picture is in case of N content: average values in upper horizons are 0.7 and 0.3% in ornithogenic and non-ornithogenic soils respectively (table 2).

Accumulation of organic matter of ornithogenic origin is confirmed by both an increased concentration of total organic carbon compared to non-ornithogenic soil samples, and a lower C/N ratio there – the most important indicator of the metabolism of any soil. The C/N ratio decreased two-fold in ornithogenic areas, because bird factor induced the organic residues mineralization. Thus, the ornithogenic factor affects not only soil formation in the traditional meaning [29], but also the formation of soil-like bodies formed in places where cryoconites accumulate.

Analysis of pHH2O values (water extract) in soils showed the predominance of close to neutral values (5.6 < pH < 6.5) in humus horizons and weakly acidic (5.1 < pH < 5.5) in lower horizons (under peat horizons) in case of ornithogenic soils; the predominance of neutral and close to neutral values (5.6 < pH < 7.5) in humus horizons and weakly alkaline and alkaline reactions (7.0 < pH < 8.5) in lower horizons in case of non-ornithogenic soils (table 2).

The studied ornithogenic soils were characterized by significant acidification of the surface layer both due to the organic acids produced by mosses (Deschampsia antarctica) inhabiting these soils and released during the decomposition process of guano organic matter.

The size of the amplicon library was 599151 sequences, the number of sequences per one sample was 10893 in average and included 9162 phylotypes in total. Of these, 67% were identified to the family and 36.2% to the genus taxonomic level.

No joint variation in the values of physical and chemical (pH, nitrogen, carbon, potassium, basal respiration) factors and microbial community structure was determined. Apparently, the microbial community structure is largely influenced by local combinations of physicochemical factors that form
specific ecotone, which in turn led to high heterogeneity of the dataset. At the same time samples are statistically reliably differentiated by the composition of the microbiome according to the analysis of beta-diversity using different metrics (figure 1). One can see two main clusters of the organogenic soils and the more diffuse clusters of the barren soils included samples of Bel16, Bel20, Bel27 sites. This pattern suggests the strongest influence of organic matter on the structure of the microbiome, while its origin and the composition often plays a subordinate role, which is expressed in the fact that groups of samples of ornithogenic soils did not form a separate cluster.

Table 2. Physic-chemical parameters of the soil samples.

| Soil ID | pH_H2O | pH_KCl | Basal respiration, mg/g/hour | C, %    | N, %    | C/N     |
|---------|--------|--------|-----------------------------|---------|---------|---------|
| Bel4    | 5.96   | 5.54   | 3.42                        | 5.32    | 0.76    | 6.97    |
| Bel16   | 5.54   | 5.32   | 2.44                        | 1.32    | 0.31    | 4.27    |
| Bel20   | 8.10   | –      | 2.18                        | 1.97    | 0.06    | 30.43   |
| Bel21   | 5.65   | 5.21   | 1.93                        | 1.25    | 0.14    | 8.77    |
| Bel22   | 5.09   | 4.78   | 3.43                        | 3.12    | 0.65    | 4.78    |
| Bel23   | 5.87   | 5.43   | 4.30                        | 4.31    | 0.53    | 8.10    |
| Bel25   | 5.72   | 5.34   | 3.33                        | 6.43    | 0.43    | 14.89   |
| Bel26   | 5.92   | 5.65   | 0.26                        | 5.02    | 0.79    | 6.33    |
| Bel27   | 5.78   | 5.54   | 2.34                        | 3.31    | 0.65    | 5.08    |

Figure 1. Non-metric multidimensional scaling (NMDS) for different beta–diversity metrics (Bray–Curtis, unweighted and weighted Unifrac). Sampling point, soil horizon type, true/false – ornithogenic effect on the horizon.

Significant difference in alpha-diversity indices was determined (figure 2): maximum for the ornithogenic samples Bel21, Bel25 and a sample with high amount detritus (Bel26), and minimum for Bel20 – Cryosol sampled on the top hill without any moss cover.

Most of the samples differ in a relatively high number of phylotypes, with their rarefaction curves not reaching the plateau (figure 3), except for Bel20 and Bel4 samples. At the phylum level, sites without vegetation differ significantly from soil samples with moss–lichen cover (figure 4). The essential heterogeneity between the sampling points even at high phylogenetic level and general difference of composition of major phyla in comparison with samples of soils of temperate zone [30] was determined. Samples of soils without vegetation were characterized by a higher representation of Actinobacteriota.
This group is generally referred to the l–strategies in soils and are more adaptive to low concentrations of organic matter. These organisms are as well adapted to survive in dry steppe soils characterized by pH values in the alkaline area [31] which can confirm their higher values for samples of Bel20 without any moss cover with the highest value of soil pH (table 2).

**Figure 2.** Faith’s Phylogenetic (PD) and Shannon indices of biodiversity in prokaryotic communities of the samples investigated.

**Figure 3.** Rarefaction curves for the soil samples.
Some samples (Bel22, Bel25) are characterized by a significant proportion of autotrophic microbiota (*Cyanobacteria*). The presence of this group is typical for the primitive initial soil and soil-like bodies as these are organisms are pioneers on weathered rocks – places with weakened species competition, where they are often the only producers of primary organic matter [32].

The high relative abundance of *Bacteroidota* was determined, especially for samples with relatively high C and N content (figure 4, table 2). Some studies indicate the active participation of this group in the cycles of biogenic elements, especially in the processes of organic matter mineralization [33]. Also, this group is generally associated with the vital activity of many animals and birds [34], thus, this result confirms the increase of this group in the part of ornithogenic soils.

The moistened swampy samples (Bel26, Bel21) are characterized by a relatively high abundance of *Acidobacteriota*. Recent studies [35] have shown the association of this bacterial group with microbiomes of tundra soils characterized by high moisture content and low temperatures. The latter, in turn, creates conditions that hinder the mineralization of organic matter and, as a result, leads to a shift towards oligotrophic microbiota, which includes representatives of *Acidobacteriota*.

![Figure 4. Relative abundance of prokaryotes at the phylum level.](image)

### 4. Conclusions

The taxonomic structure and biodiversity of Antarctic soils of the Ardley island and King George Island (Antarctic Peninsula region) has been investigated for the first time with the special reference to key soil attributive characteristics. Despite that in general the set of dominant phyla was similar to that of soils of non–polar regions, the structure was characterized by its uniqueness that was expressed in presence of taxa (representatives of *Cyanobacteria*), typical for initial stages of soil formation. Barren Cryosols and Technosol were characterized by relative increase in abundance of *Actinobacteriota* lineages, *Acidobacteriota* were associated with moist swampy sites. Although the interrelation in soil physico-chemical properties and microbiome structure patterns were not determined, the specificity and some general tendencies were revealed. The most pronounced influence was exerted by the «soil trophicity» (C and N content), while the ornithogenic influence played a subordinate role.
Further studies of microbial communities of Antarctic coastal area will help to broaden knowledge about participation of microorganisms in primary soil formation, specific features of soil microbiome formation in these extreme habitats.

Acknowledgments
This work was supported by Russian Scientific Foundation Grant № 17–16–01030.
Authors thank Ivan Alekseev, Saint–Petersburg State University, for his help in the sampling of the part of the soil collection analyzed.

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