Diversity and activity of microorganisms in Antarctic polar soils

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Academic editor: Carla-Leanne Washbourne

Abstract

The study is focused on microbiological analyses in polar soils in selected monitoring sites in Livingstone Island, Antarctica region. The analyses include determination of the quantity and qualitative composition of the heterotrophic block of soil microflora (non-spore-forming bacteria, bacilli, actinomycetes, micromycetes, bacteria absorbing mineral nitrogen), insofar as it plays a major role in the element cycling and soil formation processes. Aerobic (rapidly and slowly growing) and anaerobic groups of soil microorganisms were investigated and the biogenicity (total microflora) and the rate of mineralisation processes (mineralisation coefficient) were determined. Mostly non-spore-forming aerobic bacteria, followed by actinomycetes, are dominant in determining the biogenicity of the studied polar soils. The rearrangement of the microorganisms in the composition of the total microflora by degree of dominance indicates the participation of all the studied groups of microorganisms in most sites in the initial and final stages of the decomposition of organic matter. The mineralisation of soils is most active in sites with vegetation cover. The established pigmentation in aerobic microorganisms is probably due to their good adaptation and protection under extreme polar conditions, while the absence of oxygen impedes the formation of pigments.

Keywords

polar soils, non-spore-forming bacteria, bacilli, actinomycetes, micromycetes, bacteria absorbing mineral nitrogen

Introduction

The soil microflora in Antarctica have been intensively studied by a number of scientists in recent decades. A general trend of the studies is the heterogeneity of microbial communities from different parts of the continent. Dominant bacterial genera were isolated and less common, including extremophiles, bacterial biomass and structure of their communities were analysed (Cowan et al. 2014Vincent 1988, Vincent 2004, Saul et al. 2005, Aislabie et al. 2006, Aislabie et al. 2008, Aislabie et al. 2009, Aislabie et al. 2013). The microbial presence and biochemical activity of isolated strains have been studied in the Peninsula region, taking into account reduced biogenicity of vegetation-free soils and at higher altitudes (Yergeau et al. 2007a, Yergeau et al. 2007b, Yergeau et al. 2006, Yergeau et al. 2011). Microbial diversity in different parts of the continent and in different habitats has been determined, with most researchers reporting specificities in the diversity of microbes depending on the specific physicochemical parameters of the environment, the presence of vegetation and altitude (Chong et al. 2012, Chong et al. 2011). Bacteroidetes species dominate in mossy areas and have been isolated from the soils of Livingston Island (Ganzert et al. 2011).

An evolution in understanding the microbial diversity of soil habitats in Antarctica has been observed during the last two decades. Early studies have shown the presence of microbial endemism at species level (and to a lesser degree of the genus level), but soil isolates from the continental sites have been relatively restricted to a narrow range of families dominated, in particular, by Gram-positive bacteria Firmicutes (Vishniac 1993). The introduction of up-to-date molecular phylogenetic and metagenomics methods that provide much more comprehensive analysis has shown that the microbial diversity of the Island is much broader, with more than psychrophilic bacteria occurring (Chan et al. 2013). Some authors have also discovered thermophilic species of microorganisms that can produce glutamate dehydrogenase (Flores et al. 2018). Microbial diversity has also been found not to decrease as a function of latitude change (Howard-Williams et al. 2010). According to studies by Dennis et al. (2012), the soil fungal composition does not vary widely as the latitude changes. According to a study by Lysak et al. (2018), the total microbial biomass in Antarctica is low, with mushrooms predominating (77-99%).

The number of microbiological studies of the “Ice continent” is still relatively small and too early to fully understand the microbial diversity model in extreme conditions. It is a fact that conflicting results have been reported - for example, in Antarctica, Howard-Williams et al. (2010) and Dennis et al. (2012) and in the Arctic, Neufeld and Mohn (2005) do not report a clear trend for the dependence of microbial diversity on latitude, whereas Yergeau et al. (2008), Yergeau et al. (2007a), Yergeau et al. (2007b)report decreases in bacterial diversity in Antarctica by this indicator.

In the area of Livingston Island, microbiological studies performed by Bulgarian researchers date from 1996. The microflora of penguin excrement has been investigated (Gushterova et al. 1999a, Gushterova et al. 1999b), establishing the dominance of

mesophiles and psychrophiles. The same team examined the microbiological characteristics of glaciers and reported the dominance of bacterial microflora (Gushterova et al. 1999b, Gushterova et al. 1999a, Gushterova et al. 2005). Savova studies the taxonomy of yeast and identified some of the isolated strains as rare (Savova 1999). Bogoev and Kenarova examined the total biomass and amount of heterotrophic microflora and reported the dominance of psychrophiles and micromycetes and variation in bacterial diversity, as well as the microbial diversity of mosses communities and coastal zones (Bogoev et al. 1999, Kenarova and Bogoev 2002). Noustorova et al. 2002 studied the heterogeneity of soils from different zones by microbiological indicators. A thermophilic strain was studied as a producer of secondary metabolites and identified as Microbispora aerata subsp. nov. (Gushterova et al. 2002) The production of three antibiotics by *Actinomyces flavovirens* 6^7 has been demonstrated (Ivanova et al. 2002). Five yeast strains were identified in soils, mosses and lichens and, for all, the biosynthesis of exopolysaccharides has been demonstrated (Pavlova et al. 2004a, Pavlova et al. 2004b).

When examining the microbial soil communities on Livingston Island in Antarctica, Raykovska et al. (2005) determined that soils have biodegradable potential with respect to phenol and its derivatives. The biodegradation potential is mainly due to the bacterial component of the microbiocenoses, with the actinomycetes being the least prevalent. However, some authors have isolated strains of actinomycetes from Antarctica soils for the production of antimicrobials - for example, the strain *Streptomyces anulatus* 39 LBG09 synthesises an extracellular (86%) broad-spectrum antibiotic complex, active against gram-positive and gram-negative bacteria, including phyto-pathogenic bacteria (Dimitrova et al. 2013). Most of the isolated actinomycetes from these areas are streptomycetes, but some other species have also been identified, including new species (Gushterova et al. 1999a, Gushterova et al. 1999b,Noustororva et al. 2001), some of which produce antibiotics (Noustorova et al. 1999).

The purpose of this study was to determine microbial indicators by analysing the biogenicity of polar soils, establishing the activity of aerobic and anaerobic, "slow growing" and "fast growing" microorganisms, as well as the part of pigmented microbes as an expression of good adaptation and protection of the species under the extreme environmental conditions.

Materials and methods

Polar soils (Gelisols) from different sites located in the region of Livingstone Island were sampled in December 2018. A total number of thirty three (33) samples were collected from sites differentiated according the vegetation cover: without vegetation, under mosses, lichens and grass vegetation. The superficial soil layers were sampled – 0-7 (10 cm) and 7 (10) - 15 cm, depending on the depth of soil profile. Soil samples were air–dried in the laboratory room of the Bulgarian Antarctic Basis on Livingstone Island and transported in plastic bags in January 2019. The description of sites with an indication of the sampling design are presented in Table 1.

Microbiological analyses were realised in March-July 2019 and include determining the amount and composition of the heterotrophic block of soil microflora, insofar as it plays a major role in the processes of soil formation and the elemental cycles in ecosystems. The amount of aerobic microbial communities (total microflora) and the quantitative presence and composition of anaerobes in microbocenosis were investigated. In addition, representative enzymatic activities of soil microbial inhabitants have been determined as an expression of their biochemical activity and role in the transformation of organic compounds. The content of organic carbon in microbial biomass was also investigated as an expression of the relative participation and role of microorganisms in the composition of soil organic matter.

The methods of analysis followed Cai et al. (2011) as following:

Heterotrophic block of microbocenosis - by methods of dilution and three times inoculation of solid nutrient medium with subsequent reading of colony-forming units (CFU) in 1 g abs. dry soil:

Aerobic component of microflora - systematic and physiological groups of germs - bacilli and non-spore-forming bacteria (ordinary agar), micromycetes (mould fungi) - of Chapek-Dox agar, actinomycetes and bacteria that absorb mineral nitrogen (Actinomycete isolation agar) have been identified.

Anaerobic component of microflora - the cultures were cultivated on the same nutrient medium in anaerobic pods.

Statistical processing of the data from the microbiological indicators include calculating the average value of three repetitions and coefficient of variation by the use of Excel 2010.

Results and discussion

The biogenicity (quantitative presence of microorganisms) of the studied polar soils as expected was low (up to about 10,000 times lower in the upper layer compared to forest and cultivated soils) and of varying intensity at individual sites, depending on the presence of vegetation and sampling depth. The aerobic heterotrophic block of soil microflora is presented in Table 2.

The total microflora, respectively the most active microbiological processes, have the highest values at 4 sites - soils under moss (HPP 2 Skua, JD 1, PB 2, HPP B2 Bay), followed by the soils under: no vegetation (CA 4), lichen (HPP 2 Skua), grass (CA 1) and in organogenic soil under nest (CA 2). Depending on the location of the sites, the physicochemical and chemical characteristics of the tested samples, different biogenicity was found for soils under the same vegetation cover. When comparing individual sites with the same vegetation cover, the microbial population is found to be lower by 4.6 times in soil under grass (CA 1> JD 1), 3.6 times in soil under mosses (HPP 2 Skua > SV 1), 2.3 times in soil under moss + grass (PB 3´> SV 2), 2.1 times in soil under no vegetation

cover (SV 1> CA 3), 1.4 times in soil under lichen compared to soil under black lichen (HPP 2 Skua > PUN-H2). In depth, the total amount of microorganisms decreases 1.5 times in soil under moss + grass (PB 3 ') and increases 1.1 times in soil under mosses (PB 3). This tendency for the same site shows the importance of both vegetation, chemical and physico-chemical soil properties for the development of microorganisms in depth. A similar effect on the reduction of biogenicity was observed in the other two sites - PUN-H2 (1.5 times less germs in moss - top layer than moss - bottom layer) and CA 4 (1.4 times in no vegetation soil - top layer compared to the bottom layer).

The highest percentage (34 ÷ 99.4%) in the total microflora is formed by non-spore-forming bacteria, except for: PB 2 – soil under mosses (bacilli), PB 1 - soil (actinomycetes dominate), HPP 1 Skua - soil under lichen (actinomycetes and micromycetes dominate), PB 3 – soil under mosses > 7 cm (actinomycetes dominate) and HAN 1 (actinomycetic microflora dominate). The amount of non-spore-forming bacteria in these sites was lower both in comparison with some of the other groups of microorganisms and in general compared to the other sites tested. This tendency to regroup microorganisms in the composition of the general microflora at these sites shows the participation of all groups of microorganisms in the initial and final stages of decomposition of organic matter substances. On the other hand, development of actinomycetes is not observed in some of the sites (HPP B2 Bay - moss, CA 2 - nest, PB 2 - moss, HPP B1 Bay - soil without vegetation) and micromycetes (JD 1 - grass, CA 2 - nest, PB 2 - moss, PB 3 - moss 0-7 cm, HPP B1 Bay - soil without vegetation). Lower development of these two groups of microorganisms has been established in previous studies (Gushterova et al. 1999a, Gushterova et al. 1999bNoustorova et al. 2002). In other sites, the development of bacilli - PB 2 and SV 3 (moss) is increasing. Particularly in PB 2, it is indicative that bacilli, as spore-forming microorganisms, can develop predominantly under extreme conditions. These trends are indicative of the specific climatic and soil conditions of the polar environment.

The highest amount of bacteria utilising mineral nitrogen was found in CA 1 (grass), CA 2 (nest) and HPP 2 Skua (moss) and decreased 19.9 times in the site with the lowest development of this group of microorganisms - HAN 1 (moss). The varying development of both the bacteria-absorbing mineral nitrogen and the non-spore-forming bacteria and bacilli, regardless of the vegetation, determine the different values of the mineralisation coefficient (MC). The lowest degradation rate was found in CA 4 (no vegetation – top layer), HPP B2 Bay (moss), HPP 2 Skua (black lichen), PUN-H2 (moss- top layer), PB 2 (moss) and JD 1 (moss) - MC values up to 0.50. Higher values of MC (1.00 ÷ 1.50) for the sites: SV 1 (moss), HPP B2 Bay (soil without vegetation), HPP 1 Skua (moss), SV 3 (moss), SV 2 (moss + grass), PUN-H1 (moss), HPP 2 Skua (moss) and at the highest rate of mineralisation (MK 1.50 ÷ 2.00) the samples at PB 3 '(moss + grass > 10 cm)) and HPP 1 Skua (lichen). The remaining sites (at most) have an average rate of mineralisation of organic matter - MC values from 0.51 to 1.00.

Amongst actinomycetes and micromycetes, species with white, brown and grey-green air micelles occur. The presence of pigment-forming species is also found in mineral nitrogen-absorbing bacteria to a greater extent than in ammonia-producing bacteria. The

pigments are of the carotenoid type. Chromogenesis was determined in all studied sites except for HPP B1 Bay - soil and CA2-soil under nest. Probably pigmentation is an expression of good adaptation and protection of the species from the extreme environmental conditions. Vaz et al. (2011) found that, of the yeast isolates they studied, 41.7% produce pigments and/or mycosporins and can be considered adapted for survival in Antarctica. According to these authors, the production of photoprotective compounds (carotenoids and mycosporins) from yeast can be a strategy for survival in extreme environments. Carotenoids function mainly as antioxidants and their UV protective role has also been demonstrated in yeast (Moliné et al. 2009, Moliné et al. 2010). The pigment formation in our study reached up to 98% of the amount of all groups of microorganisms tested, which confirms the above-mentioned assumption (Fig. 1).

The different growth rate of the microorganisms indicates their distribution in each sample, since the determination of the R-strategists shows rapid growth in response to the presence of nutrients, unlike the K-strategists (presented above - Table 2), which are defined as slowly growing (Shilev et al. 2008). In the present study, bacteria forming visible colonies at 28°C on the culture medium after 30 h incubation were identified as "fast growing" (R-strategists), while the other colonial-forming bacteria in the later period as "slowly growing" (K- strategists). The development of R-strategists follows that of K-strategists. Colonial ordinary agar counts reported at 30 hours after incubation - R-strategists (non-spore-forming bacteria + bacilli) represent about 55-60% of K-strategists in the same culture medium at all sites. The rapid development of bacteria-absorbing mineral nitrogen - R-strategists reaches 25-30% of the amount of slowly growing - K-strategists at all sites (Fig. 2).

Anaerobic microorganisms are also present in the composition of microbocenosis, but with a lower amount of aerobes and without pigmentation (Table 3).

The total amount of anaerobic microorganisms is highest in CA 2 (under nest), PB 3 (moss > 7 cm), CA 1 (grass) and HPP B2 Bay (moss), falling from 13 to 26 times in PUN-H2. (lichen), HPP 2 Skua (black lichen) and PB 3 '(moss + grass > 10 cm). Non-spore bacteria (50 ÷ 97.9%) and bacilli (2.1 ÷ 50%) are included in the total anaerobic microflora. Anaerobes were not established for sites: SV 2 (moss + grass), PB 3 (moss top layer 0-7 cm), PB 3 '(moss + grass 0-7 (10) cm) and PUN-H2 (moss – top layer). Bacteria-absorbing mineral nitrogen in the absence of oxygen are best represented in CA 2 (under nest) and CA 1 (grass), their amount decreasing from 20 to 66.7 times in HPP B1 Bay (soil without vegetation), SV 1 (soil without vegetation), PB 3 '(moss + grass > 7 (10) cm), PB 1 (soil without vegetation), PUN-H2 (lichen), HPP 1 Skua (lichen). The poorer development of this group of microorganisms at all soil sites correlates with the lowest mineralisation rate of organic matter - MC values from 0.00 to 20.00. The highest mineralisation coefficient (2.58) was calculated for the soil under black lichen (HPP 1 Skua), despite the low amount of microorganisms at this site. It is therefore not always and not only the amount of micro-organisms that is a prerequisite for their activity. It is influenced by humidity, temperature, nutrients, soil pH, climatic conditions, vegetation type and other factors.

Anaerobic microorganisms occupy a significant part of the microbial communities in soils, except for 4 sites where their development is completely limited: SV 2 (soil under moss + grass), PB 3 (soil under moss top layer 0-7 cm), PB 3´ (soil under moss + grass 0-7 (10) cm), PUN-H2 (soil under moss – top layer). For other sites, the total amount of anaerobes with regard to the amount of aerobes varies from site to site.

The results show that the ratio of aerobes to anaerobes is highest in soils under lichen, ie. the amount of anaerobes decreases most strongly at these sites - from 29 times in soil under lichen to 48 times in soil under black lichen, as well as in sites with "moss + grass" vegetation cover - from the complete absence of anaerobes to a reduction of 24 times that of the aerobes. In most sites without vegetation, the development of anaerobes decreases 7 times, while in soils under mosses, it varies from 1 to 7 times at individual sites. The total microflora developing in the absence of oxygen decreases the least in all objects under grass (1-2 times), as well as in organogenic soil under nest (2 times). Oxygen concentration in soils is an important factor for the structure of the microbial community (Liebner et al. 2008) and is closely linked to the saturation of soil with water, which determines the development of both aerobic and anaerobic forms of metabolism (Bell et al. 2013, Bell et al. 2013). The water content is also related to the structure of bacterial and archaeal communities of polar soils (Hoj et al. 2006, Stomeo et al. 2012, Bell et al. 2013), although it may have a greater impact on mycromycetes and other microeukaryotes (Fell et al. 2006, Bridge and Newsham 2009, Arenz and Blanchette 2011).

As indicated, the bacteria occupy a major part in the composition of common microflora, for both aerobes and anaerobes. Similar results have been obtained by other authors (Vincent 1988, Vincent 2004, Gushterova et al. 1999b,Noustorova et al. 2002, Saul et al. 2005,Raykovska et al. 2005, Aislabie et al. 2009, Aislabie et al. 2013, Aislabie et al. 2008 , Aislabie et al. 2006,Ganzert et al. 2011), while according to a study by Lysak et al. (2018), micromycetes are predominant in the total microbial biomass of Antarctica.

Conclusions

Soil biogenicity varies from site to site, depending on presence of vegetation cover and sampling depth. It is highest in soils with vegetation cover or dead plant residues (in descending order - moss, lichen, black lichen, grass, organogenic residues from nest). Different biogenicity in soils under the same vegetation is found, which shows the strong influence of the edaphic and climatic conditions of the environment. On the other hand, at the same site (JD 1), the lowest biogenicity of soils under grass and a significantly higher amount of microorganisms under moss were reported, which also indicates the influence of vegetation cover type on the development of microorganisms.

The predominantly non-spore-forming aerobic bacteria (in 81.8% of the samples) followed by actinomycetes (in 12.1% of the samples) are dominant in the composition of the total microflora. The rearrangement of the microorganisms in the composition of the

total microflora by the rate of dominance indicates the participation of all studied groups of microorganisms in the initial and final stages of decomposition of organic matter.

Established R-strategists (fast-growing microorganisms) - ammonifying bacteria and bacteria-absorbing mineral nitrogen, as well as K-strategists (slow-growing microorganisms from all study groups) play a major role in the initial (R-strategists) and final (K-strategists) stages of organic matter decomposition in soils.

The pigmentation of air micelles in actinomycetes and micromycetes (brown, grey-green), as well as the carotenoid type of chromogenesis in bacteria-absorbing mineral nitrogen and ammonifying bacteria, is probably due to the good adaptation and protection of the species under extreme polar conditions. The formation of pigments is only found in aerobic species of micro-organisms. Probably the absence of oxygen impedes the formation of pigments in anaerobes.

Anaerobic microflora are also better developed in soils with vegetation cover and occupy a significant part of the microbial communities in soils. Again in the composition of the total amount of anaerobic microorganisms, non-spore-forming bacteria followed by bacilli occupy the highest percentage.

In most sites, the rate of organic matter decomposition is average in terms of the mineralisation coefficients and the quantitative development of the individual groups of microorganisms. The most active mineralisation is found in the soils with vegetation cover.

Acknowledgements

The authors thank Prof. Pimpirev and the members of the Bulgarian Antarctic Institute for the logistical support during the participation in the XXVII and XVIII polar expeditions to the Bulgarian Polar Base at Livingstone Island, Antarcica, realised in 2018 and 2019.

Funding program

The projects were funded by Bulgarian Antarctic Institute under the National Programme for Polar Researches 2017-2021 with CM Decision No. 718 / 29.08.2016. and by the National Science Fund under the Programme for BILATERAL PROJECTS – BULGARIA – RUSSIA 2018 -2019

Conflicts of interest

The authors declare no conflict of interests.

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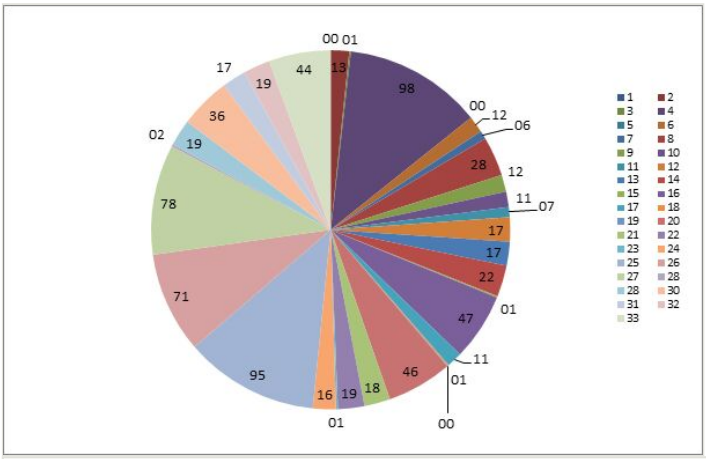

Figure 1.

Pigment bacteria (% from the total amount of all groups of microorganisms).

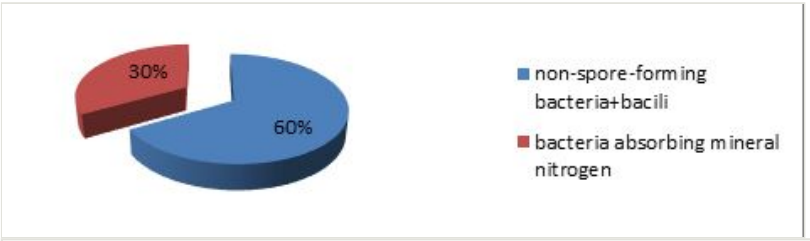

Figure 2.

Participation of R-strategists and K-strategists in %.

Table 1.

Site locations and sampling of soils.

No.	Site	Soil sample
1	HPP B1	Without vegetation, soil 0-7 cm
2	HPP B2-1	Mosses, soil 0-7 cm
3	HPP B2-2	Mosses, soil 7-15 cm
4	CA 3	Without vegetation, soil 0-7 cm
5	CA 2	Organogenic soil under nest 0-7 (10 cm)
6	CA 1	Grass vegetation, soil 0-7 cm
7	CA 4-1	Without vegetation, soil 0-7 cm
8	CA 4-2	Without vegetation, soil 7-15 cm
9	JD 1-1	Mosses, soil 0-7 cm
10	JD 1-2	Grass vegetation, soil 0-7 cm
11	BP 1	Mosses, soil 0-7(10) cm)
12	SV 1-1	Without vegetation, soil 0-7 cm
13	SV 1-2	Mosses, soil 0-7 cm
14	SV 2	Mosses + grass vegetation, soil 0-7 cm
15	SV 3	Mosses, soil 0-7 cm
16	PB 1	Without vegetation, soil 0-7 (10 cm)
17	PB 2-1	Mosses, soil 0-7 cm
18	PB 2-2	Grass vegetation, soil 0-7 cm
19	PB 3-1	Mosses, soil 0-7 cm
20	PB 3-2	Mosses, soil 7-15 cm
21	PB 3´-1	Mosses + grass vegetation 0-10 cm
22	PB 3´-2	Mosses + grass vegetation 10-15 cm
23	HPP 1 Skua-1	Mosses, soil 0-7 (10) cm
24	HPP 1 Skua-2	Lichens, soil 0-7 cm
25	HAN 1 - 1	Mosses, soil 0-7 cm
26	HAN 1 - 2	Mosses (turf), soil 0-7 cm
27	HAN 2	Grass vegetation, soil 0-7 cm
28	PUN-H1	Mosses, soil 0-7 cm
29	PUN-H2-1	Mosses, soil 0-7 cm
30	PUN-H2-2	Mosses, soil 7-15 cm
31	PUN-H2-3	Lichens, soil 0-7 cm
32	HPP 2 Skua -1	Mosses, soil 0-7 cm
33	HPP 2 Skua -2	Lichens (black), soil 0-7 cm

Table 2.

Quantity and qualitative composition of aerobic soil microorganisms (CFU x 10/g abs. dry soil); ±CV.

* CV up to 10% for all variants and each group of microorganisms (low dispersion)

No.	Total microflora (aerobes)	Non-spore-forming bacteria	Bacillus	Actino-mycetes	Micro-mycetes	Bacteria absorbing mineral nitrogen	Mineralisation coefficient
1	7060	6980±2.57	80±0.00	0	0	4700±2.43	0.67
2	3920	2880±4.54	20±0.00	760±6.45	260±3.54	3620±4.06	1.25
3	10220	9980±1.64	220±7.42	0	20±0.00	4100±3.98	0.40
4	3440	1960±4.17	700±2.33	500±6.53	280±3.29	1460±2.24	0.55
5	9780	9720±1.68	60±0.00	0	0	6040±2.16	0.62
6	10140	8900±1.83	200±8.16	640±2.55	400±2.32	6360±2.05	0.70
7	11820	10000±1.31	160±6.15	1600±3.06	60±0.00	4000±3.67	0.39
8	8160	4400±2.23	660±2.47	700±4.67	2400±4.76	3640±3.59	0.72
9	11380	4180±2.34	280±5.83	3520±1.86	3400±2.88	2240±7.29	0.50
10	2200	1920±3.40	180±9.07	100±8.84	0	2020±7.28	0.96
11	5460	3180±3.59	500±3.27	1600±2.04	180±5.05	2220±5.15	0.60
12	7180	3160±3.62	500±6.53	1640±1.99	1880±5.21	2160±5.29	0.59
13	3440	3080±3.71	280±3.45	20±0.00	60±0.00	4780±2.05	1.42
14	2480	2000±4.90	320±5.10	140±6.43	20±0.00	2580±3.80	1.11
15	4940	2800±2.33	1600±4.08	300±5.44	240±3.82	4960±2.30	1.13
16	6860	2100±3.89	360±4.54	2520±2.59	1880±3.47	1980±4.95	0.80
17	10380	2560±3.83	7820±1.88	0	0	4560±2.15	0.44
18	5700	4780±2.39	400±4.08	120±7.44	400±2.40	4800±2.72	0.93
19	6780	6120±1.07	140±6.43	520±6.28	0	2600±3.77	0.42
20	7320	2860±3.43	520±3.14	3040±3.22	900±5.44	2420±4.05	0.72
21	5680	2620±3.12	900±3.63	1400±4.67	760±4.30	2000±3.27	0.57
22	3900	2760±3.55	100±8.84	820±3.98	220±4.16	5320±1.23	1.86
23	8380	3820±2.14	260±6.28	3200±4.08	1100±4.45	4780±1.37	1.17
24	8700	1960±5.00	480±3.40	4020±3.66	2240±5.10	4080±1.60	1.67
25	7380	400±4.08	380±4.30	3200±3.57	3400±3.36	600±2.72	0.77
26	8520	160±5.66	180±5.05	4180±3.13	4000±3.27	320±5.10	0.94
27	3020	1540±2.12	520±1.79	520±1.79	440±3.71	1520±1.07	0.74
28	3760	3240±2.02	180±5.44	300±3.07	40±0.00	3600±1.81	1.05
29	9100	4400±2.60	400±2.40	4160±3.53	140±6.43	2000±4,08	0.42
30	6180	3400±2.40	480±1.94	1300±2.51	1000±4.90	2140±2,29	0.55

31	8160	6720±0.97	20±0.00	660±4.95	760±6.45	4360±1,87	0.65
32	12320	5560±1.76	200±4.88	2400±5.44	4160±3.53	5940±1,10	1.03
33	11500	3900±2.09	880±3.71	3280±2.99	3440±3.80	1940±1,68	0.41

Table 3.

Quantity and qualitative composition of anaerobic soil microorganisms (CFU x 10/g abs. dry soil); ±CV.

* CV up to 10% for all variants and each group of microorganisms (low dispersion)

No.	Total microflora (anaerobes)	Non-spore-forming bacteria	Bacillus	Bacteria absorbing mineral nitrogen	Mineralisation coefficient
1	1000	820±3.98	180±9.07	200±8.16	0.20
2	920	780±2.09	140±7.07	0	0.00
3	3640	3480±2.82	160±5.66	1100±2.97	0.30
4	1220	1000±3.27	220±7.42	0	0,00
5	4200	4000±4.08	200±4.56	4000±3.67	0.95
6	3680	3600±3.63	80±0.00	3900±2.51	1.06
7	1580	1500±2.18	80±0.00	1540±1.06	0.97
8	1200	1120±1.46	80±0.00	1400±2.33	1.17
9	3180	2960±2.21	220±4.16	920±3.55	0.29
10	2880	2820±2.32	60±0.00	3060±1.60	1.06
11	3100	2900±2.82	200±8.16	1040±1.57	0.34
12	1000	800±2.04	200±4.88	100±8.84	0.10
13	3220	3040±4.30	180±5.44	1140±2.86	0.35
14	0	0	0	0	0.00
15	3220	3040±2.15	180±5.05	1180±2.77	0.37
16	980	800±1.19	180±5.44	60±0.00	0.06
17	3080	2880±3.40	200±4.56	1220±2.68	0.40
18	2940	2840±1.15	100±8.84	3100±2.63	1.05
19	0	0	0	0	0.00
20	3700	3480±1.41	220±4.42	1300±2.51	0.35
21	0	0	0	0	0,00
22	160	100±8.84	60±0.00	100±8.84	0.63
23	3120	2940±1.67	180±5.44	1160±2.82	0.37
24	240	160±5.66	80±0.00	60±0.00	0.25
25	1080	960±3.40	120±7.44	720±2.27	0.67
26	980	880±1.86	100±8.84	700±2.33	0.71
27	2880	2760±1.77	120±8.32	2980±1.10	1.03
28	2840	2700±2.42	140±6.43	1020±1.60	0.36
29	0	0	0	0	0.00
30	3260	3060±1.60	200±8.16	920±1.77	0.28
31	280	200±8.16	80±0.00	60±0.00	0.21

32	3400	3240±2.02	160±5.66	1280±2.55	0.38
33	240	120±7.44	120±7.44	620±2.63	2.58