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SOIL MICROBIOME: DIVERSITY, ACTIVITY, FUNCTIONAL AND STRUCTURAL SUCCESSIONS

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ABSTRACT

The article presents original results of research. The aim of this study was long term investigation of soil microbiome in primeval forest ecosystems, namely the structure of microbial communities, the number of major ecological-functional groups, functional parameters such as: enzymatic activity of the soil by the level of catalase and invertase. To analyze impact of endogenous and exogenous factors on soil microbial community and succession processes. Estimate biodiversity of soil microbiome by Shannon index in different edaphotopes of primeval forest ecosystems. Microbiological studies of soil were carried out according to generally accepted methods in soil microbiology. Enzymatic activity of the soil: catalase – was determined by gasometric method and invertase - by colorimetric method. Statistical analyses were performed by using Statistica 10 software. Basic descriptive statistics was calculated, that is, the arithmetic mean and standard deviation. The influence of endogenous and exogenous factors in primeval forest ecosystems of Shyrokoluzhansky massif of the Carpathian Biosphere Reserve caused changes in microbial community. For ten years changed diversity and functional activity of soil microbiome. Based on long-term studies of changes in soil microbial communities of natural ecosystems, a succession concept of soil microbiome has been proposed.

Keywords: ecosystem, succession, soil, diversity, microbiome, enzyme activity.

INTRODUCTION

Forests are specific ecosystems comprising a multitude of microbial habitats with specific roperties, such as foliage, the wood of living trees, the bark surface, ground vegetation,roots and the rhizosphere, litter, soil, deadwood, rock surfaces, invertebrates, wetlands orthe atmosphere that are dynamic on a broad temporal scale with ecosystem processes ranging from short-term events over seasonal ones to long-term stand development where fungi, bacteria and other organisms composing the forest microbiome play important roles (Baldrian P., 2017*; Symochko L., 2018*). Soil microorganisms are responsible for most biological transformations and drive the development of stable and labile pools of carbon, nitrogen and other nutrients, which facilitate the subsequent establishment of plant communities (Banning N.C., et all 2011). Over half a century ago, Odum identified mechanistic linkages between the successional dynamics of natural communities and the functioning of natural ecosystems (Odum E. P., 1969).

Ecologists have documented the process of plant succession for centuries, yet the successional patterns exhibited by microbial communities have received relatively little attention. Specifically, as communities progress through succession, diversity is expected to increase and nutrients will become 'locked-up' in the biota, with consequences for the build-up of soil organic matter and closure of the mineral cycles. More recently, the interplay between aboveground and belowground biodiversity has emerged as a prominent determinant of the successional dynamics in biological communities. However, little is known about how changes in the soil biota contribute to the associated changes in ecosystem functioning. In the complex and dynamic plant root interaction with the microbiome, both biotic and abiotic factors play critical roles for microbiome composition, richness, and diversity. Biotic factors, such as host genotypes, developmental stages and abiotic factors, such as temperature, soil pH, seasonal variation, and the presence of rhizospheric deposits, act as chemical signals for microbes and influence the microbiome community structure and function (Walker L.R. 2007; Rout ME., Southworth D., 2013; Minz D., Ofek M., Hadar Y., 2013). However, the extent to which both abiotic and biotic factors contribute to microbial communities is not fully understood (Turner TR., James EK., Poole PS., 2013; Zolla G., et al 2013). Forests represent one of the largest and most important ecosystems on Earth, covering more than 40 million km^2 and representing 30% of the total global land area (Keenan RJ, Reams GA, Achard F, et el, 2015). Primeval forests are ideal ecosystems to study the interaction of bacteria, fungi and archaea with their abiotic environment (Grayston S.J., Rennenberg H., 2006). Virgin forests are essential for the conservation of biological and genetic diversity. They reserve the relict and endemic species of flora and fauna. The study of primeval forest is a unique opportunity to explore the natural structure, diversity and genetic structure of unmodified forest and ecosystem dynamical processes and relationships that occur in them under the influence of ecological factors. Despite of the intensive exploitation of forests in the last ten centuries, its area decreased by 3.5 times, and virgin forest ecosystems which have special value remained only in the Carpathian Mountains. Moreover, since most European forest stands have been managed for centuries, very little is known about the diversity,ecology, and distribution of soil microorganisms in natural, undisturbed forest ecosystems in Europe (Bengtsson, J., Nilsson S., 2000; Symochko L., 2015). Soil microorganisms have been largely ignored by conservation efforts. However, their role in biogeochemical processes, their diversity and abundance, and their potential as repositories of valuable genetic information and metabolic products make them as important as animals and plants to the biosphere and human welfare. Study of authentic soil microbiota creates the necessary prerequisites for the conservation of microbial diversity and forming the base of the eco-microbiological monitoring (Patyka V., Symochko L., 2013).

Main idea of this work is to study soil microbiome and its diversity, activity, functional and structural successions in natural ecosystems. As a model ecosystem was investigated virgin forests of Shyrokoluzhansky massif of the Carpathian Biosphere Reserve The primeval forests as etalon ecosystems better combine above resistance and stability with high productivity biomass (Symochko L. Hamuda H.B., 2015). In the Transcarpathian region of Ukraine the Carpathian Biosphere Reserve offers a unique opportunity for studying the biodiversity and natural processes of primeval forest ecosystems, i.e. forests that have never been significantly modified by human activity.

MATERIALS AND METHODS

Materials of research were soil samples, which had been collected from natural ecosystems: virgin forests of Shyrokoluzhansky massif of the Carpathian Biosphere Reserve at the deep 0-25cm. CBR (Carpathian Biosphere Reserve) offers a unique opportunity for studying the biodiversity and natural processes of virgin or primeval forest ecosystems. The region covers an area of about 53,650 ha and became part of the World Network of Biospheres Reserves of UNESCO in 1992. The total area of the Shyrokoluzhansky massif is about 15,033 ha. The massif consists of two contiguous areas (foresteres): Uholka and Shyrokyi Lug. It lies within the Krasnyanskyi physical-geographic area of the Middle maountain-Polonyny region and Uholka physical-geographic area of the Low mountain-Rocky region. It is located between the rivers Tereblya and Teresva. The massif is separated by the mountain range Krasna from the Mokryanka river valley and lies within the Duklyanska, Prokuletska, Rakhiv and Maramorosh tectonic zones. The Duklyanska zone covers the northeastern part of the massif and is represented by sandy and clay-sandy flysch. The southwestern part of the massif is occupied with the formations of the Prokuletska zone, which is represented by massive diverse-grained sandstones. The southern part of the massif is made up of the Maramorosh rocky zone sediments, which are represented by cretaceous sediments, palaeogene sandstones, gridstones, aleurolites, marlstones and argillites, and also small-grained greenish-grey flysch with some stratums of

grey small-grained sandstones. The soils are very stony, mostly midloamy with good water and air penetration ability. Climate conditions change from mild-warm to cold. The massif belongs to three different climatic zones with annual average temperatures ranging from 0 to +7 °C and annual average precipitation varying between 1,000 mm and 1,500 mm. The temperature in July elevates from +17 °C to +12 °C, and in January from -3 °C to -10 °C. The sum of active temperatures changes with the altitude from 2,300 °C to 800 °C. Researches were conducted from 2008 to 2018 years. Sampling was carried out in depth of 0-25 cm at different altitudes from 500 m to 1,100 meters (Table 1).

Table 1. Characteristics of the soil sampling location in virgin beech forests

№	Vegetation	Coordinates (latitude; longitude)	Altitude above sea level, m
1	*Fagetum (silvaticae)*	48°18.671´ 23°44.388´	500
2	*Fagetum (silvaticae)*	48°21.087´ 23°43.398´	600
3	*Fagetum (silvaticae)*	48°18.450´ 23°43.227´	700
4	*Fagetum (silvaticae)*	48°19.928´ 23°42.879´	800
5	*Fagetum (silvaticae)*	48°21.562´ 23°43.425´	900
6	*Fagetum (silvaticae)*	48°19.344´ 23°45.620´	1000
7	*Fagetum (silvaticae)*	48°21.810´ 23°44.532´	1100

Studies of soils were carried out at the Scientific Research and Educational Center of Molecular Microbiology and the Immunology of Mucous Membranes (Uzhhorod National University), Research Laboratory Monitoring of Water and Terrestrial Ecosystems of department entomology and biodiversity conservation (Uzhhorod National University) and in Laboratory of Microbial Ecology (Institute of Agroecology and Environmental Management, Kyiv Agrarian Academy of Sciences of Ukraine). The research was carried out within the framework of the complex theme „Eco-microbiological monitoring of various types ecosystems of the Carpathian region" №0116U003331 (state registration number), following the standard protocol (Tepper, 2004; Shyrobokov, 2011; Goldman, Green, 2015) All soil samples were analyzed within 24 hours. Microbiological study of soil was performed in sterile conditions. The method of serial dilution was used to obtain the suspension where microorganisms titre were 10^{-3} CFU/ml. - 10^{-5} CFU/ml. 100 µl (CFU-Colony Forming Units) of the soil suspension was evenly distributed on the surface of the medium with a sterile spatula. For the study we used the following media: Endos agar, Meat peptone agar, Strepto agar and Entero agar, Agar-Agar, Eshbi agar, Soil agar, Chapek agar**,** Starch agar, Fedorova Agar, Vinogradsky Agar, Ashbys agar in 4 repetitions. Petri dishes with study material were incubated in the thermostat at 37ºC for 48 hours in aerobic and anaerobic (Wilson-Blair Agar, Vinogradsky Agar) conditions. Petri dishes with Czapek agar were incubated in the thermostat at 28ºC for 96 hours. Enzyme activity of the soil: catalase - by gasometric method, invertase - by colorimetric method (Guan S.M., 1986; Dick W.A., 2011). All isolated microorganisms were identified by applying of appropriate biochemical test-systems LACHEMA according to the instructions. Biodiversity of soil microbiome was calculated according to the Shannon index (Magurran, 1988).
Shannon's diversity index (H):

$$\mathbf{H = -\sum P_i \ln (P_i)}$$

p_i – is the proportion of individuals belonging to species *i*.

The results of the experimental studies were statistically analyzed using the Microsoft Excel program package. Results were expressed as means (±) standard deviation (SD) and (SSD_{05}) smallest significant differences of experiments conducted in quadruplicating. The level of significance selected for the study was $P < 0.05$ (Bailey, 1995).

RESULTS

Soil microbiome as a part of forest ecosystems plays an important role in sustainable development of forestry. Forest ecosystems provide a broad range of habitats for bacteria, including soil and plant tissues and surfaces, streams, and rocks, among others, but bacteria seem to be especially abundant on the forest floor, in soil and litter (Hardoim PR., et all, 2015) Usually five phyla, *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*, appear to be abundant in most soils. In addition to pH, which seems to be the most important driver of the bacterial community composition in soils, organic matter content, nutrient availability, climate conditions, and biotic interactions (especially the effect of vegetation) affect the composition of bacterial communities (Lauber C., Hamady M., Knight R., 2009). The spatial variation of these parameters is responsible for the presence of hot spots of microbial activity with increased abundance and activity in the soil, such as in and on plant debris, including litter and deadwood, or on and around plant roots (Kuzyakov Y., Blagodatskaya E.,. 2015). Each of these niches has specific properties and, consequently, a specific bacterial community. Biocenotic relations of trophic and topical types are decisive in edaphotope shaping of different type of ecosystems. Due to this fact, the purpose of the research was to determine structure and functions of soil microbiome. Studies of the soil were taken from primeval ecosystems revealed general regularities of distribution of main ecological-functional groups of microorganisms, their population dynamics in different habitats. The most favourable conditions for the development and functioning of microorganisms were in an edaphotops which were located at an altitude of 500-700 meters above sea level. It is highly connected to local temperature and water regime, as well as reserves of nutrients (organic origin) in the soil (Tab. 2).

Table 2. Soil microbiome of primeval forest ecosystems (2008) (CFU/gr.d.s.)

№	Biotopes, altitude above sl, m	Ammonifiers*10^6	Spore forming bacteria *10^6	Micromycetes*10^3	Actinomyces*10^3	Bacteria wich are using mineral forms of nitrogen*10^4	Anaerobic bacteria*10^3	Aerobic nitrogen fixing bacteria, %	Anaerobic nitrogen fixing bacteria *10^3	Oligotrophic bacteria *10^6	Oligonitrophic bacteria*10^4	Pedotrophic bacteria*10^6
1	500	6.56	2.65	26.22	12.80	4.89	30.20	79.30	5.89	2.59	4.50	1.74
2	600	4.20	2.88	30.30	12.94	4.09	33.42	72.00	7.45	2.78	4.00	2.01
3	700	3.12	3.25	33.60	16.97	3.95	40.67	69.20	7.90	2.87	3.81	2.35
4	800	2.77	4.89	35.72	30.90	3.45	96.34	51.50	14.50	3.53	3.43	2.78
5	900	1.56	5.35	45.70	40.24	2.54	97.18	42.00	15.56	3.91	1.43	3.23
6	1000	1.44	5.60	50.78	42.30	2.31	113.60	40.60	22.76	4.10	1.31	3.60
7	1100	1.15	5.76	53.21	44.00	2.12	120.56	40.00	23.10	4.45	1.12	4.68

Note: the data are statistically significant, $P < 0.05$, $x \pm SD$, $n = 4$.

The number of ammonifiers at an altitude of 500 m was 6.56 million CFU/gr.ab.d.s., and at altitude of 1100 m – 1.15 million CFU/gr.d.s., which indicate a significant enrichment of soil by organic matter. The content of oligotrophic microbiota significantly increased, practically in twice at altitude 1000-1100 meters above sea level, the same as sporeforming bacteria (2.65-5.76. million CFU/gr.d.s). Process of nitrogen fixation was more active in edaphotops at altitude 500-600m.a.s.l. Percentage of content in the soil aerobic nitrogen fixing bacteria was 79.30%-72.00 %. It should be noted that at altitudes of 700-800 meters above sea level, significant changes occur in the structure of microbial community. The content of anaerobic microbiota, pedotrophs and oligotrophs in the soil increases.

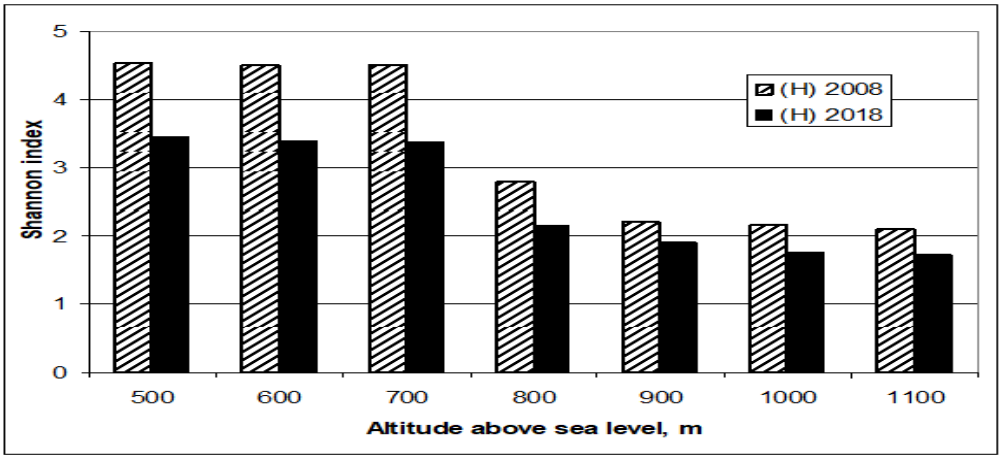
The number of micromycetes and actinomyces is increasing. Instead, the number of ammonifiers, aerobic nitrogen fixing bacteria is decreasing. The changes in the structure of soil microbiome indicate the realization of structural and functional successions and the presence of hot spots at these altitudes. For linking of the presence of bacteria or their activity to soil properties, it is important that soil is a complex of microniches with heterogeneous physicochemical properties on various scales. Because bacteria inhabit small niches, the properties of their immediate environment rather than the mean soil properties affect the local bacterial community. This spatial heterogeneity has been shown to result in the heterogeneity of bacterial communities on small scales. Furthermore, local dispersal limitations can also remarkably influence the bacterial community composition (O'Brien SL, Gibbons SM, Owens SM., 2016). Considering the high level of spatial variation of forest C stocks on the same scale, the occurrence of individual taxa in forest soil may actually be highly variable on a small scale and may differ among activity hot spots (Martiny JB, Eisen JA, Penn K., 2011; Kuzyakov Y, Blagodatskaya E, 2015).
The same dynamic of different functional and trophic groups of soil microorganisms were saved for 10 years.
In 2018 were fixed hot spots at the same altitudes (Table 3).

Table 3. Soil microbiome of primeval forest ecosystems (2018) (CFU/gr.d.s.)

№	Biotopes, altitude above sl, m	Ammonifiers*10^6	Spore forming bacteria *10^6	Micromycetes*10^3	Actinomyces*10^3	Bacteria wich are using mineral forms of nitrogen*10^4	Anaerobic bacteria*10^3	Aerobic nitrogen fixing bacteria, %	Anaerobic nitrogen fixing bacteria *10^3	Oligotrophic bacteria *10^6	Oligonitrophic bacteria*10^4	Pedotrophic bacteria*10^6
1	500	7.32	4.67	40.77	25.89	5.66	50.02	60.30	7.90	4.45	4.34	3.23
2	600	4.90	5.00	45.30	28.14	4.12	55.57	55.53	8.40	4.90	3.80	3.87
3	700	3.67	5.31	46.80	32.91	4.00	70.56	53.00	8.96	5.02	3.72	4.56
4	800	2.90	7.70	70.23	60.80	3.04	100.40	42.40	20.34	6.72	2.07	6.03
5	900	1.65	7.45	78.45	80.42	2.90	110.12	40.60	25.66	6.97	1.43	6.12
6	1000	1.30	8.01	87.76	90.67	2.67	156.22	33.50	30.12	7.89	1.22	6.89
7	1100	1.22	8.12	89.22	90.45	2.34	160.34	32.00	30.52	8.32	1.15	7.33

Note: the data are statistically significant, $P < 0.05$, $x \pm SD$, $n = 4$.

Long term investigations showed significant changes in the structure of soil microbiome, increased in twice the quantity of oligotrophic and pedotrophic bacteria, micromycetes and actinomyces. Number of ammonifiers wasn't changed significantly, the quantity of aerobic nitrogen-fixing bacteria decreased by 17%-22%. Changes in the structure of soil microbiome can be caused by two reasons: the influence of external factors and the availability of resources. Resource availability is also likely to be a fundamental driver of microbial succession, but the limiting resources and environmental factors regulating succession will be more complex given the far greater physiological diversity contained within microbial communities and the breadth of environments in which succession can occur. In autotrophic succession, nutrients and light (or the availability of inorganic electron donors) are likely to be the primary resources limiting biomass accumulation. However, in the earliest stages of autotrophic succession, heterotrophs may also be in relatively high abundance, utilizing trace levels of available carbon (Okabe et al., 2007; Roeselers et al., 2007). During endogenous heterotrophic succession, labile substrates will be consumed first, supporting copiotrophic microbial taxa that are later replaced by more oligotrophic taxa that metabolize the remaining, more recalcitrant, organic C pools in the later stages of succession (Rui et al., 2009). Endogenous heterotrophic succession cause increasing biomass of oligotrophic bacteria and decreasing phylogenetic diversity (Figure 1). Diversity is indicate, how changed microbial communities during succession. After 10 years, fluctuation of microbial diversity at different altitudes was the same. But it should be noted that in 2008 the Shannon index fluctuated within (4.54-2.10), after 10 years the values of this index decreased by an average of 15% and ranged from 3.45 (at altitude 500 m.a.s.l) to 1.72 (at altitude 1100 m.a.s.l).



Note: The data are statistically significant, $P < 0.05$, $x \pm SD$, $n = 4$

Figure 1 Diversity of soil microbial community

Soil enzymes are natural mediators and catalysts of many important soil processes, such as decomposition of organic matter released into the soil during vegetation, reactions of humus formation and decomposition, production of mineral nutrient forms available for plants, nitrogen fixation, as well as the flow of carbon, nitrogen and other basic elements of the biochemical cycle. The process of transformation of organic matter in to the soil takes place with the participation of soil microorganisms and enzymes. Species composition within forest tree stands determines the diversity of microorganisms along with their enzymatic activity (Baldrian P., 2014; Błońska et al., 2017). Tree species and altitude above sea level affect soil pH. The pH value has a significant effect on the activity of microorganisms in the soil, enzymes are highly susceptible to soil reaction. The results of the present study indicate that enzyme activity varies considerably within the studied forest soils (Table 4).

Table 4. Enzyme activity of soil in virgin beech forests of Shyrokoluzhansky massif of the Carpathian Biosphere Reserve (2008)

№	Altitude above sea level, m	pH	Catalase cm^3O_2/gr. soil per 1 min.	Invertase mg.glucose/gr.soil.
1	500	6.0±0.10	6.73±0.22	27.90± 0.32
2	600	5.8±0.25	6.59±0.14	27,13± 0.26
3	700	5.6±0.15	6.68±0.13	26.10± 0.69
4	800	5.6±0.20	4.92±0.22	20.46± 0.41
5	900	5.1±0.17	4.72±0.18	18.23± 0.33
6	1000	5.1±0.22	4.40±0.32	16.46± 0.75
7	1100	5.0±0.34	4.23±0.17	16.12± 0.43

Determination of enzyme activity along with regulating factors are indispensable to characterize the metabolic potential, soil fertility and are useful in soil assessments with regard to soil microbiome. At the same time, they can be used to study soil biochemical processes and to evaluate soil quality and successions in soil microbial community. The lowest activity of catalase (1.13±0.22 cm^3O_2/gr. soil per 1 min.) was recorded at the altitude 1100 meters. The activity of invertase also was the lowest (12,12± 0.28 mg.glucose/gr.soil) on this edaphotope. Soil invertase deserves special recognition because its substrate, sucros, is one of the most abundant soluble sugars in plants and is partially responsible for the breakdown of plant litter in soils. Catalase activity in soils is considered to be an indicator of aerobic microbial activity and has been related to both the number of aerobic microorganisms and soil fertility. Analysis of functional successions of soil microbiome showed the presence of hot spots in edaphotopes at an altitude of 700-800 meters. In these edaphotopes significantly decreased the level of catalase from 6.68±0.13 to 4.92±0.22 (cm^3O_2/gr. soil per 1 min), and the level of invertase from 26.10± 0.69 to 20.46± 0.41

(mg.glucose/gr.soil). Long-term monitoring of enzymatic activity showed that after 10 years there were slight changes in the level of invertase and catalase, on average by 14 and 18%, respectively (Table 5).

Table 5. Enzyme activity of soil in virgin beech forests of Shyrokoluzhansky massif of the Carpathian Biosphere Reserve (2018)

№	Altitude above sea level, m	pH	Catalase cm^3O_2/gr. soil per 1 min.	Invertase mg.glucose/gr.soil.
1	500	6.1±0.23	5.45±0.12	23.90± 0.36
2	600	5.7±0.35	5.24±0.14	23.03± 0.66
3	700	5.7±0.22	5.02±0.17	22.10± 0.38
4	800	5.6±0.41	3.70±0.26	14.78± 0.35
5	900	5.2±0.12	3.39±0.16	13.66± 0.27
6	1000	5.0±0.34	3.45±0.43	12.46± 0.36
7	1100	5.0±0.11	3.13±0.22	12.12± 0.28

It should be noted that 10 years later, hot spots also were recorded in edaphotopes at altitudes of 700-800 meters above sea level, with a significant decrease in the functional activity of the soil microbiome. Catalase was changed from 5.02±0.17to 3.70±0.26 (cm^3O_2/gr. soil per 1 min), and the level of invertase from 22.10± 0.38 to 14.78± 0.35 (mg.glucose/gr.soil). Together these findings indicate that soil enzyme activities have broad-scale spatial variability depending on the environmental conditions. Due to seasonal and spatial variability, single biological properties cannot be accurate measures of soil quality. Therefore, multiparametric indices are recommended for environmental impact assessment of nonagricultural soils. The statistical results analysis showed that there were highly significant correlations between soil enzyme activities (invertase, catalase) and pH levels. These strong relationships confirm that soil enzyme activities provide a meaningful integrative measure of soil physicochemical properties and biological soil fertility, which thus may play a important role in monitoring soil biological quality.

CONCLUSIONS

Soil microbial community diversity changed along successional time, but it showed significant difference at altitude 700-800 meters above sea level, which indicate hot spots in edophotopes at this altitude. This fact also was confirmed by the quantity of soil microorganism and they functional activity. Endogenous heterotrophic succession caused increasing biomass of oligotrophic and pedotrophic bacteria and decreasing microbial diversity. Diversity indicates, how changed microbial communities during succession. After 10 years, fluctuations of microbial diversity at different altitudes were the same. Multiparametric indices are recommended for environmental impact assessment of soils and monitoring study: microbiological and biochemical. Long term monitoring allowed determining hot spots in structural and functional successions of soil microbiome. Monitoring study database has both theoretical and practical value and can be used for creation of necessary measures to preserve authentic microbial communities and to implement environmental principles of sustainable forestry.

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