Microbial Monitoring of Soil as Additional Tools for Conservation Biology

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Abstract: *The designation the primeval beech forests of the Carpathians as strictly protected areas is a continuing conflicting issue in Ukraine. As they represent an outstanding example of undisturbed, complex temperate forests and exhibit the most complete and comprehensive ecological patterns and processes of pure, they can serve as indispensable reference areas for scientific research and monitoring. Microbiological investigations may be used as an additional tool for conservation practitioners to evaluate the anthropogenic impacts on ecosystems. A strict non-intervention management is required to protect virgin forests as a treasurer of bidiversity in Ukraine.*

Keywords: microorganisms, soil, primeval forest, conservation

1 Introduction

Soil microorganisms have been largely ignored by conservation efforts. Yet 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 [1]. Microorganisms are rarely considered by conservation biologists and yet they form the base of most food chains and accomplish biogeochemical transformations of critical importance to the biosphere.

Microorganisms have high instrumental value, where instrumental refers to their uses to the biosphere and to humans. They carry out important biogeochemical transformations in most environments on which multicellular organisms depend. From an instrumental point of view, one reason to conserve and protect microorganisms is that we depend on them. They are not merely useful, our survival depends on them. From a more pragmatic perspective, microbial biological diversity also harbours huge resources of value for the pharmaceutical, biotechnology and food industries [2].

The loss of microbial diversity implies a loss of potentially valuable resources analogous to existing concerns about loss of potential medicinal compounds associated with plant biodiversity. The conservation of microorganisms on account of their instrumental value is not a controversial motive. The importance of microorganisms in biogeochemical processes and human industries is understood by most conservationists, who agree that from an instrumental perspective, their protection is merited [1]. However, we still know little about which microorganisms carry out important biogeochemical functions. From a biodiversity perspective, microorganisms dominate life on Earth and it is estimated that, 10% of the Earth's microbial diversity has been characterized [3].

Our main idea is the study, estimation and conservation the biodiversity of the authentic microbiocenoses of soil in the Carpathian region. As model ecosystem we investigated primeval beech forest.

The primeval beech forests as etalon ecosystems better combine above resistance and stability with high productivity biomass. Therefore the virgin forests reliably indicate the direction of restoration of disturbed ecosystems [4]. 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. In the Transcarpathian region of Ukraine (south-west), the CBR (Carpathian Biosphere Reserve) offers a unique opportunity for studying the biodiversity and natural processes of virgin or primeval forest ecosystems, i.e. forests that have never been significantly modified by human activity. The region covers an area of about 53,650 ha and became part of the World Network of Biospheres Reserves of UNESCO in 1992. However, it should be noted that the attention of researchers focused mainly on studies of flora and fauna biodiversity [5, 6] and almost never directed to the ecological study of soil microbial communities. Due to this fact, the purpose of the research was to determine the number of different ecological-trophic groups of soil microorganisms, biological activity and phytotoxicity of soil, intensity of microbiological processes by index of pedotrophity, oligotrophity, fermentative activity of soil in primeval beech forest.

2 Materials and Methods

Samples of soil for study were taken in the virgin forests of Shyrokoluzhansky massif of the Carpathian Biosphere Reserve, Ukraine. The total area of the massif is about 15033 ha. The massif consists of two contiguous areas (foresteres): Uholka and Shyrokyi Lug. It lies within the Krasnyanskyi physical-geographic area of the Middlemaountain-Polonyny region and Uholka physical-geographic area of the Lowmountain-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 north-eastern part of the massif and is represented by sandy and clay-sandy flysch. The south-western 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 mid-loamy with good water and air penetration ability [7]. 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 to -10°C. The sum of active temperatures changes with the altitude from 2,300 to 800°C. Researches were conducted from 2008 to 2014 years. Sampling was carried out by squares method in depth of 0-25 cm. at different altitudes from 500 m to 1,100 m. Microbiological analysis of soil has been carried by standard methods [8, 9, 10]. Samples of soil were collected in a sterile container from all investigated plots (Fig. 1).

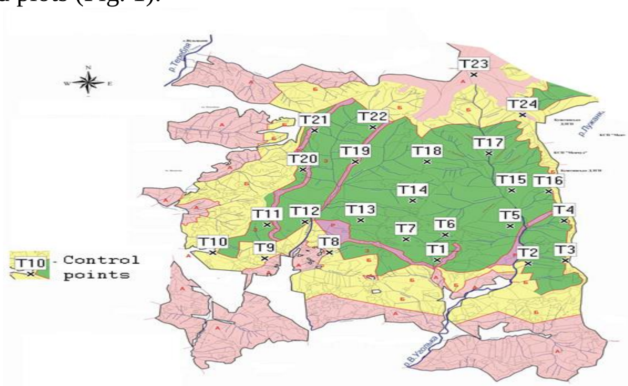

Figure 1

Map of the soil sampling in virgin beech forests

The biological activity of the soil was determined by V. Shtatnov [11]. The direction of microbiological processes in the soil by Andreyuk and methods described by Volkohon [12, 13].

Mineralization - immobilization calculated by the formula:

$K_{m\text{-}i} = C_{KAA}/C_{MPA}$, where C_{KAA}, C_{MPA} - the number of microorganisms that grew on ammonia agar and meat peptonic agar.

Oligotrophity of soil using the formula:

$K_{ol} = C_{PA}/(C_{KAA} + C_{MPA})$, where C_{PA} - the number of microorganisms grown on pure agar.

Pedotrophity of soil:

$K_{ped} = C_{S.A} / C_{MPA}$, where $C_{S.A}$-the number of microorganisms grown on soil agar. Toxicity of soil samples was determined by the Berestetsky method [14]. Statistical analyses were conducted using software Statistica 7.0.

3 Results and Discussion

There are preserved virgin ecosystems of particular value in the Carpathian Biosphere Reserve. The beech forest's soil microbiota studies are very important, they are sensible reagents to the impact of external factors, ecosystem indicators and succession processes that occur in them [15, 16]. We have established that the number of different ecological-trophic groups of soil microorganisms varies with the height of the habitat above sea level. The number of ammonificators with increasing height decreased, minimum content organographies 1.22 (colony forming units per 1gr. absolutely dry soil) was characterized soil at altitude of 1.100 meters above sea level (Table 1).

As shown in Table 1, at the altitude of 500 meters content of ammonificators was at six times higher and amounted to 7.07 million CFU / gr.ab.d.s., indicating a significant enrichment of soil organic matter of plant origin. The similar changes in the bacteria content, in the case of bacteria that used mineral nitrogen were observed. The maximum number of these microorganisms in the soil was 4.32 million CFU / gr.ab.d.s., at the altitude of 500 meters above sea level.

At the highest point of sampling (1.100 m.) their number was 2.82 times lower. Fluctuations of number micromycetes were not as significant as bacterial flora, but in edaphotops located within 500-800 meters their biodiversity was higher than at other sampling points.

The content of microscopic fungi in the soil of virgin ecosystems was 17-28 thousand CFU / gr.ab.d.s. The number of oligotrophic and pedotrophic microbiota with increasing altitude increased, indicating a decrease in nutrients necessary for life of the soil microbiocoenosis (Table 1).

Table 1
Number of different ecological-trophic groups of soil microorganisms in the virgin beech forests

№	Altitude above sea level, m	(CFU-colony forming units/ per 1 gram of absolutely dry soil)					%
		Micro-mycetes, $*10^3$	Ammoni-ficators, $*10^6$	Oligo-trophes $*10^6$	Pedo-trophes $*10^6$	Bacteria which are using mineral forms of nitrogen $*10^6$	*Azotobacter*
1	500	17	7.07	2.33	1.68	4.32	80.23
2	600	20	4.30	2.61	1.88	3.64	68.44
3	700	20	3.46	2.87	2.00	3.22	60.29
4	800	21	2.93	3.24	2.26	3.14	58.56
5	900	25	1.66	3.70	2.96	2.18	54.67
6	1.000	26	1.30	3.80	3.12	1.96	50.13
7	1.100	28	1.22	3.94	3.65	1.83	41.34
SSD_{05}	-	0.32	0.14	0.41	0.18	0.21	1.28

SSD_{05}-the smallest significant difference

In order to assess the direction of microbiological processes in the soil of beech forests the calculation of coefficients of oligotrophity, pedotrophity and mineralization-immobilization were carried out (Table 2).

Table 2
Direction of microbiological processes on soil in primeval beech forest

№	Altitude above sea level, m	Coefficient of oligotrophity	Coefficient of pedotrophity	Coefficient of mineralization-immobilization
1	500	0.20	0.23	0.60
2	600	0.31	0.44	0.84
3	700	0.37	0.57	0.93
4	800	0.50	0.77	1.07
5	900	0.94	1.80	1.31
6	1.000	1.18	2.40	1.50
7	1.100	1.29	3.00	1.50

As can be seen from the table 2, the coefficients of oligotrophity and pedotrophity of soil increased with the altitude and their maximum value were at the height of 1,100 meters respectively 1.29 and 3.00. Increasing of the pedotrophity indicates an intensity of decomposition of a soil organic matter, including humus substances. The increasing of the oligotrophity of the soil indicates the reduction of nutrients in the soil. Minimum of these coefficients were at the altitude of 500 meters above sea level: the oligotrophity coefficient -0.20; coefficient of the pedotrophity 0.23 that of 6.4 times and 13 times less than the maximum values of these parameters in the studied ecosystem. Intensity of mineralization processes in the soil also increased in proportion to height of the investigation edaphotopes and maximum values reached at 1,100 m, the rate of mineralization - immobilization was 1.50 which is 2.5 times higher than in edaphotopes at altitude of 500 m succession, dynamic changes of microbial communities of soil related primarily from the impact of biocoenosis abiotic factors such as temperature and humidity.

Rebuilding the functional structure of soil microbial coenosis due to the influence of exogenous factors, as evidenced not only by changing the number of specific ecological-trophic groups of soil microorganisms, but also from direction of microbiological processes in soil of virgin ecosystems.

Toxic substances produced by microorganisms enter the plant directly from the soil, and they are concentrated mainly in the over ground organs, and almost not observed in the roots of plants. Phytotoxines of soil microorganisms cause significant changes in the chemical composition of plant metabolism to break them (impact on nitrogen and carbohydrate metabolism), inhibit seeds germination, growth of sprouts, plant growth and reduce harvest. Toxic forms of microorganisms found in all types of soil.

The genera *Bacillus* and *Pseudomonas* are more often among the bacteria; among micromycetes - *Penicillium, Fusarium*; among streptomycetes - *Streptomyces aurantiacus, S. viridans, S. griseus* [17, 18].

Phytotoxines that formed by soil microorganisms belong to different groups of chemical compounds. There are nitrogen-containing and oxygen-containing heterocyclic compounds and aromatic substances acyclic structure, derivatives of phenols, quinones, and terpenoids among them [19]. More important toxic activity of strains and their inhibitory ability, than in the soil they accumulate in large quantities [20, 21]. Soil in virgin forest ecosystems, characterized by relatively low levels of phytotoxic activity: 14%-21% (Fig. 2). In contrast, anthropogenicaly transformed ecosystems characterized by very high levels of phytotoxic activity on average three times higher than background levels.

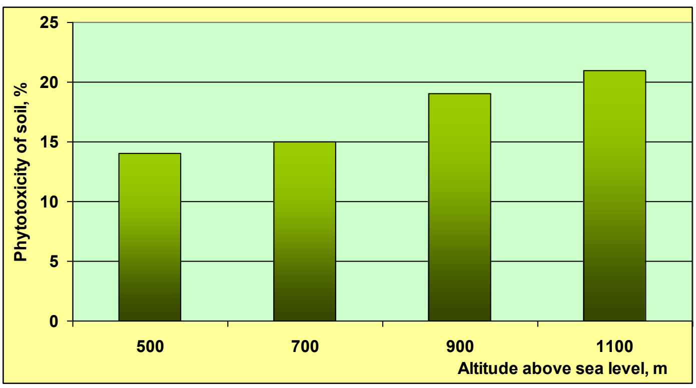

Figure 2
Phytotoxic activity of soil in virgin beech forests of Shyrokoluzhansky massif of the Carpathian Biosphere Reserve

The maximum level of phytotoxical activity (21%) was characterized by samples of soil taken at a height of 1,100 meters above sea level. This is due to the high content of oligotrophes (3.94 mln CFU/g.a.d.s) and pedotrophes (3.65 mln CFU/1g.a.d.s), including the species producing toxic exometabolites. In general, the virgin forest ecosystems are characterized by relatively low levels of phytotoxical activity of soil in comparison with the anthropogenic ecosystems.

Activity of soil microorganisms determines soil fertility, their environmental and phytosanitary status. In addition, soil microorganisms are indicators of contaminants in ecosystems, as reflected on the level of soil biological activity, including enzymatic activity and the intensity of emission of carbon dioxide from the soil surface. A high level of biological activity of the soil positively affects not only its structure, but also on the growth of plants. The results of the biological activity of the soil by the intensity of emission carbon dioxide are presented on Fig. 3.

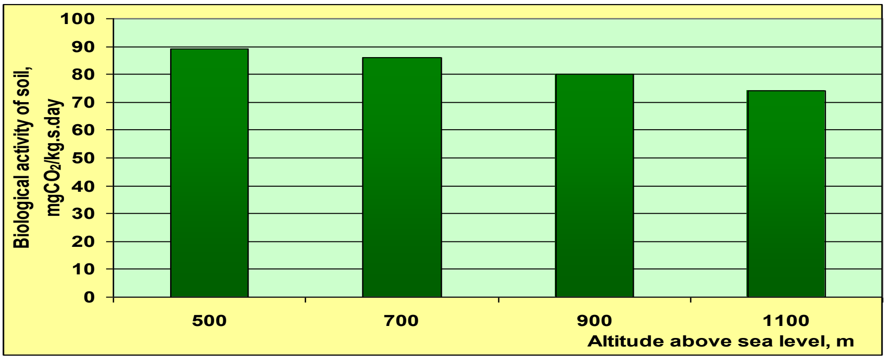

Figure 3
Soil biological activity of virgin beech forests

The high level of biological activity in the soil is characterized by virgin ecosystems. The intensity of emission of carbon ranged from 89-74 mg CO_2/kg soil/day that indicates the favourable environmental conditions for the soil microorganisms. The biological activity of soil decreased with altitude increasing, due to the slowing of microbiological processes and the reduction of the total number of soil microorganisms.

Conclusion

Consequently, the number of representatives of major ecological-trophic groups of the soil microorganisms varies at depends on the altitude of forest's biotopes disposition above sea level.
The number of ammonificators and bacteria that use mineral nitrogen decreased with the altitude increasing, the number of oligotrophes and pedotrophes gradually was increasing. The level of the biological activity in the virgin forest's ecosystems was high. The intensity of emission of carbon ranged from 89-74 mg

CO_2/kg soil/day, it indicates about favourable environmental conditions for the soil microorganisms. Coefficients of oligotrophity and pedotrophity of the soil increased with the altitude and their maximum value were at the height of 1,100 meters respectively 1.29 and 3.00. Increasing of the pedotrophity indicates the intensity of decomposition of the soil organic matter, including humus substances.

Phytotoxicity of the soil is informative parameter that should be used in the implementation of the soil monitoring research to evaluate the anthropogenic impacts on ecosystems. The phytotoxic activity of the soil was characterized by relatively low level in the primeval beech forests. The maximum level of phytotoxical activity (21 %) was in the soil taken at the height of 1,100 meters above sea level. This is due to the high content of oligotrophes and pedotrophes, including the species producing toxic exometabolites.

Thus, the microbiological methods described above may be used as an additional tool for conservation practitioners to evaluate the anthropogenic impacts on ecosystems. A strict non-intervention management is required to protect virgin forests as a treasurer of biodiversity in Ukraine. Furthermore our results demonstrate the need for a detailed study of the unique authentic microbiocenoses. As well as developing tools for their preservation at national level.

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