Symochko, L., Hoxha, E., Hamuda, H. B. (2021): Mapping hot spots of soil microbiome using GIS technology. Agriculture and Forestry, 67 (1): 191-203

DOI: 10.17707/AgricultForest.67.1.16

Lyudmyla SYMOCHKO[1], Edmond HOXHA[2], Hosam Bayoumi HAMUDA[3]

MAPPING HOT SPOTS OF SOIL MICROBIOME USING GIS TECHNOLOGY

SUMMARY

Virgin forests are unique ecosystems, which can be used as etalon for basic monitoring investigation. The paper presents original results of research. The aim of this study was long term investigation of soil microbiome in primeval forest ecosystems of Carpathian Biosphere Reserve, namely the structure of microbial communities, the number of major ecological-functional groups and determining hot spots.

The Hotspots mapping is realized through GIS (Geographical Information System) technology. It was found that the structure and functional activity of soil microbiome change with altitude. Rebuilding structure of soil microbiome was fixed at altitude 555m; 776m; 1040m. The soil at altitude of 1,040 meters above sea level was characterized by minimum content of ammonifiers.

At the altitude of 555 meters content of ammonifiers increased at six times, which indicates accumulation of organic matter in the soil. Similar changes occurred with the number of bacteria which are using mineral forms of nitrogen for their nutrition. Their maximum quantity was in the soil of biotope disposed at altitude of 555 meters above sea level. After 10 years, fluctuations of soil microbiota at different altitudes were the same. Long term monitoring during 2008-2018 years allowed determining hot spots in structural successions of soil microbiome.

***Keywords**:* Soil, microbiome, primeval beech forest, hotspots, GIS.

INTRODUCTION

A primeval beech forests are the ecosystems which were created during phylocoeno-genesis in corresponding to soil-climatic conditions and landscapes.

[1]Lyudmyla SYMOCHKO, (1) Uzhhorod National University, Faculty of Biology, Uzhhorod, Ukraine (2) Institute of Agroecology and Environmental Management, Kyiv, UKRAINE

[2]Edmond Hoxha (corresponding author: ehoxha63@gmail.com), Polytechnic University of Tirana-Faculty of Geology and Mining, Tirana, ALBANIA

[3]H. B. Hamuda Obuda University, Faculty of Light Industry and Environmental Engineering, Budapest, HUNGARY

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Recieved:10/11/2020 *Accepted:16/03/2021*

Autotrophic and heterotrophic elements of primeval forest ecosystems and pedosphere have no indications of such anthropogenic influence which could change their natural status and sylvagenesis. Hence, they function as homeostasis ecosystems (Stoyko and Kopach, 2012). As etalon ecosystems, they better combine above resistance and stability with high productivity of biomass. Therefore, the virgin forests reliably indicate the direction of restoration of disturbed ecosystems (Hamor *et al.*, 2008; Debeljak, 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 (Symochko & Kalinichenko, 2017). 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.

The few remnants of natural forests which could be potentially investigated are not larger than 50-100 ha, while continuous forest areas of more than 1,000 ha are very rare. 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 (Commarmot *et al.*, 2005; Commarmot *et al*., 2013) and almost never directed to the ecological study of soil microbial communities. 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 *et al*., 2007; Rout and Southworth, 2013; Minz, *et al*., 2013). However, the extent to which both abiotic and biotic factors contribute to microbial communities is not fully understood (Turner *et al*., 2013; Zolla *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 *et al*., 2015). Primeval forests are ideal ecosystems to study the interaction of bacteria, fungi, and archaea with their abiotic environment (Grayston and Rennenberg, 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. Moreover, since most European forest stands have been managed for centuries (Bengtsson *et al.*, 2000),

very little is known about the diversity, ecology, and distribution of soil microorganisms in natural, undisturbed forest ecosystems in Europe (Baldrian, 2017; Symochko *et al.*, 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 and Symochko, 2013). The primeval forests as etalon ecosystems better combine above resistance and stability with high productivity biomass (Symochko and Hamuda, 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. Due to this fact, the purpose of the research was to determine the number of different ecological-trophic groups of soil microorganisms, to estimate successional processes of authentic soil microbiota and determine hot spots of soil microbiome during long-term microbial monitoring.

MATERIAL AND METHODS

Description of sites and soil sampling

Materials of research were soil samples, which had been collected from natural ecosystems: virgin forests of Shyrokoluzhansky massif of the Carpathian Biosphere Reserve (CBR). The total area of the Shyrokoluzhansky massif is about 15,033 ha. The massif consists of two contiguous areas (foresters): Uholka and Shyrokyi Lug. It lies within the Krasnyanskyi physical-geographic area of the Middle mountain-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 north eastern 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°C to +7°C, and annual average precipitation varying between

1,000 mm and 1,500 mm. The sum of active temperatures changes with the altitude from 800 °C to 2,300 °C. The temperature in January elevates from -3°C to -10°C., and in July from +12°C to +17°C.

Researches were conducted from 2008 to 2018 years. Sampling was carried out in depth of 0-25 cm at different altitudes from 555m to 1040m (Table 1). The soil sampling was carried out by standard methods (ISO 10381-6 : 1993). All samples were prepared using the unified procedure: they were air dried and grounded to < 3 mm in size; visible plant and mesofauna residues were removed. Experiments were performed in fivefold repetition. Studies of soils were carried out at the Scientific Research and Educational Centre 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 project "Eco-microbiological monitoring of various types ecosystems of the Carpathian region" №0116U003331 (state registration number).

Microbiological analyses of soil

Microbiological study of soil was performed in sterile conditions following the standard protocols (Tepper *et al*., 2004; Shyrobokov, 2011; Goldman and Green, 2015). 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: Meat peptone agar, Agar-Agar, Eshbi agar, Soil agar, Starch agar in 4 repetitions. Petri dishes with study material were incubated in the thermostat at 29-37ºC for 48-72 hours in aerobic conditions. The colonies which grew in these media were calculated on the assumption that one colony is formed from one vital cell. The results of measuring the number of microorganisms grown on the nutrient media were expressed in colony-forming units (CFU) per 1 g of dry soil. For this purpose, we determined the moisture of the soil samples for the experiments using the thermostat-gravimetric analysis and recalculated the obtained number of colonies taking into consideration the coefficient of moisture and solution of the soil suspension. The inoculations were repeated three times, the obtained data were analysed using mathematical statistics, calculating the confidence interval in the number of microorganisms.

Using GIS Technology on Hot Spots analyses

Spatial database development involved conversion of the data on spreadsheet to GIS shape file in ArcGIS 10.4 software. The shape file was further linked to elevation and other data. The coordinates were collected by GPS, the Coordinate System ETRS 1989 UTM.

The mapping process and hotspots analysis is carrying out by Geographical Systems Information (GIS) technology through ArcGIS 10.4.1 program. As it is

known the ArcGIS analyses are very helpfully not only showing the hotspots but also creating a clear visual view of the distribution of the elements in the region. In our case the hotspots analyses have been carrying out using two techniques: (1) Spatial Statistic, Hot Spot Analyses (Getis-Ord Gi*); and (2) IDW (Inverse Distance Weighted). The Satellite image is taken from Base Map.

For the detailed analyses there were built separate thematic maps for each element as: Ammonifiers, Oligotrophes, Pedotrophes and Bacteria using mineral form of Nitrogen. These thematic hotspots maps are built using the IDW method (Inverse Distance weighted) (Fig.1 and Fig.2).

The final General Map presented on Fig.3 shows a comparative Hotspots analyses for the year 2008 and 2018 in the form of graphics bar.

Table 1. Characteristics of the soil samples

Nr.	Vegetation	Coordinates	Altitude above sea level, m
1	*Fagetum (silvaticae)*	48°17.663′ 23°44.389′	800
2	*Fagetum (silvaticae)*	48°17.659′ 23°45.523′	910
3	*Fagetum (silvaticae)*	48°19.349′ 23°45.628′	1010
4	*Fagetum (silvaticae)*	48°20.126′ 23°45.390′	655
5	*Fagetum (silvaticae)*	48°20.069′ 23°44.026′	650
6	*Fagetum (silvaticae)*	48°20.595′ 23°45.115′	1020
7	*Fagetum (silvaticae)*	48°21.292′ 23°44.595′	700
8	*Fageto (sylvaticae) Abietum (albae)*	48°21.817′ 23°45.557′	885
9	*Fagetum (silvaticae)*	48°21.805′ 23°44.529′	1040
10	*Fagetum (silvaticae)*	48°18.454′ 23°43.223′	773
11	*Abieto (albae) Piceeto (abietis) Fagetum (silvaticae)*	48°19.203′ 23°43.658′	776
12	*Fagetum (silvaticae)*	48°20.226′ 23°43.498′	683
13	*Fagetum (silvaticae)*	48°19.928′ 23°42.879′	800
14	*Fagetum (silvaticae)*	48°19.832′ 23°42.119′	844
15	*Fagetum (silvaticae)*	48°20.980′ 23°41.826′	970
16	*Fagetum (silvaticae)*	48°21.089′ 23°43.399′	645
17	*Fagetum (silvaticae)*	48°18.673′ 23°44.389′	555
18	*Fagetum (silvaticae)*	48°21.568′ 23°43.422′	925
19	*Fagetum (silvaticae)*	48°21.730′ 23°41.997′	890
20	*Fagetum (silvaticae)*	48°19.455′ 23°44.547′	770

Statistical analysis of data

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 AND DISCUSSION

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. Soil microbiome as a part of forest ecosystems plays an important role in sustainable development of forestry. Each of microbial 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. 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 555-776 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 (Table 2).

As shown in Table 2, at the altitude of 555 meters content of ammonifiers was at six times higher and amounted to 6.24 million CFU/gr.d.s., what 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 - 4.32 million CFU/gr.d.s. was in the soil at the altitude of 555 meters above sea level (Figure 1). At the highest point of sampling (1040 m.) their number was 2.65 times lower. Succession, dynamic changes of microbial communities of soil related primarily from the impact of abiotic factors such as temperature and humidity.

Rebuilding the functional structure of soil microbial cenosis due to the influence of exogenous factors, as evidenced not only by changing the number of specific ecological-trophic groups of soil microorganisms (Symochko, 2020; O'brien *et al*, 2016; Demyanyuk *et al*, 2020), but also from direction of microbiological processes in soil of virgin ecosystems. It should be noted that at altitudes of 776 meters above sea level, significant changes occur in the structure of microbial community.

The content of pedotrophes and oligotrophes in the structure of soil microbiome increased. 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. Because bacteria inhabit small niches, the properties of their immediate environment rather than the mean soil properties affect the local bacterial community.

Long term investigations showed significant changes in the structure of soil microbiome, increased in twice the quantity of oligotrophic $4{,}97*10^6$ CFU/gr.d.s. (2008); $8.98*10^6$ CFU/gr.d.s. (2018) and pedotrophic bacteria $7.89*10^6$

CFU/gr.d.s. (2008); 4.95*10^6 CFU/gr.d.s. (2018). Number of ammonifiers wasn't changed significantly.

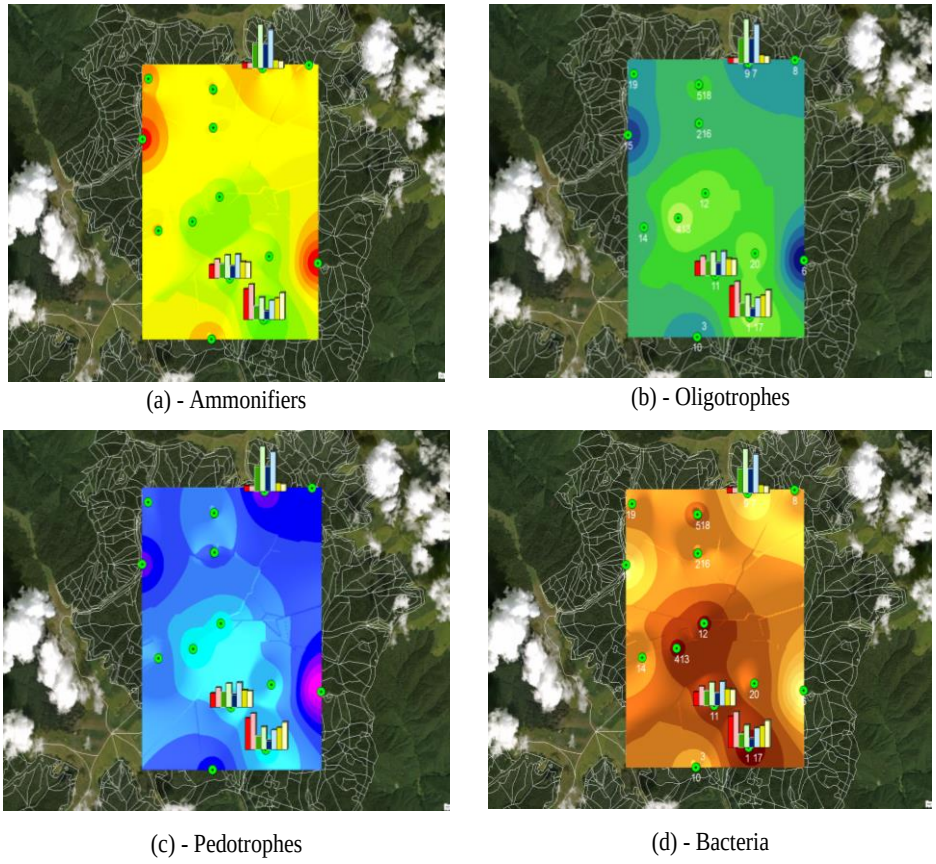
Table 2. Soil microbiome of primeval forest ecosystems (2008 - 2018) (CFU/gr.d.s.)

№	Altitude above sea level m	Number of soil microorganisms (CFU-colony forming units/ per 1 gram of dry soil)							
		Ammonifiers *10^6		Oligotrophes *10^6		Pedotrophes *10^6		Bacteria using mineral forms of nitrogen *10^6	
		2008	2018	2008	2018	2008	2018	2008	2018
1	800	2,77±0,03	2,90±0,05	3,53±0,03	6,72±0,04	2,78±0,01	6,03±0,01	3,45±0,01	3,04±0,01
2	910	1,64±0,02	1,88±0,03	3,68±0,01	4,90±0,03	2,96±0,04	6,45±0,03	2,18±0,04	2,90±0,02
3	1010	1,15±0,05	1,22±0,02	4,45±0,02	8,32±0,02	4,68±0,02	7,33±0,02	2,12± 0,03	2,34± 0,05
4	655	4,10±0,04	4,89±0,01	2,65±0,06	3,48±0,04	1,85±0,05	4,55±0,05	3,52±0,05	3,54±0,03
5	650	4,12±0,01	4,66±0,02	2,44±0,01	3,44±0,01	1,82±0,02	4,46±0,02	3,52±0,02	3,88±0,02
6	1020	1,13±0,01	1,33±0,05	4,90±0,01	8,56±0,03	4,42±0,01	7,77±0,04	1,85±0,01	1,97±0,01
7	700	3,12±0,02	3,67±0,02	2,87±0,02	5,02±0,02	2,35±0,01	4,56±0,01	3,95±0,07	4,56±0,03
8	885	2,56±0,08	2,64±0,08	3,38±0,07	4,39±0,06	2,68±0,01	3,70±0,03	2,84±0,02	2,84±0,02
9	1040	1,07±0,03	1,15±0,02	4,97±0,02	8,98±0,01	4,95±0,02	7,89±0,01	1,63±0,01	1,45±0,03
10	773	2,84±0,01	3,84±0,04	2,98±0,03	4,65±0,02	2,45±0,03	4,75±0,02	3,44±0,04	3,53±0,02
11	776	2,80±0,03	3,91±0,03	3,00±0,04	4,76±0,03	2,55±0,04	4,90±0,01	3,40±0,03	3,26±0,05
12	683	4,03±0,03	4,77±0,07	2,78±0,01	4,11±0,05	1,96±0,02	4,43±0,02	3,50±0,01	4,72±0,01
13	850	2,96±0,07	3,68±0,03	3,79±0,01	6,96±0,01	2,90±0,02	6,88±0,03	3,12±0,01	3,12±0,03
14	844	2,78±0,08	3,23±0,08	3,55±0,02	4,23±0,03	2,35±0,01	6,62±0,01	2,86±0,02	3,04±0,01
15	970	1,33±0,02	1,33±0,02	3,72±0,03	4,78±0,02	2,84±0,08	7,32±0,08	2,33±0,04	2,33±0,04
16	645	4,30±0,02	5,30±0,06	2,64±0,03	3,67±0,03	1,85±0,09	3,99±0,03	3,64±0,06	4,74±0,02
17	555	6,24±0,03	7,13±0,05	2,65±0,07	4,50±0,02	1,77±0,05	3,85±0,05	4,32±0,01	5,43±0,02
18	925	1,7±0,01	2,07±0,03	3,69±0,05	7,97±0,03	2,63±0,02	5,52±0,02	2,68±0,01	2,89±0,01
19	890	2,34±0,01	2,56±0,01	3,34±0,01	7,81±0,01	2,46±0,06	6,80±0,06	2,96±0,03	2,71±0,03
20	770	3,90±0,04	3,97±0,09	2,85±0,02	5,12±0,04	2,11±0,04	5,41±0,03	3,26±0,02	3,18±0,04

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 are likely to be the primary resources limiting biomass accumulation. Long term monitoring of authentic soil microbiome allowed determining hot spots (Figure 1). Estimating the size of hotspots and the proportion of the total soil volume that they represent is a major challenge in soil microbial ecology.

The occurrence of hotspots in soil, which is the most heterogeneous and complex component of the biosphere (Young and Crawford, 2004), is a result of soil development. The increasing variability of soil properties is a key

characteristic of soil formation, structuring the environment. Such properties include local density and pore volume, soil acidity and redox potential, organic matter and nutrient contents, microbial biomass and composition of microbial communities, and enzyme activities. The heterogeneity of the soil environment is responsible for huge diversity not only of microorganisms but also of various processes ongoing at close distances that would be not possible in a homogeneous system (Kuzyakov and Blagodatskaya, 2015). The range of hotspot sizes is very broad, individual microbial cells are insufficient to be accepted as hotspots because they functions are not relevant on the higher scales.



(a) - Ammonifiers (b) - Oligotrophes

(c) - Pedotrophes (d) - Bacteria

Figure 1: IDW GIS Hotspots Map (a) Ammonifiers; (b) Oligotrophes; (c) Pedotrophes; (d) Bacteria using mineral forms of nitrogen.

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).

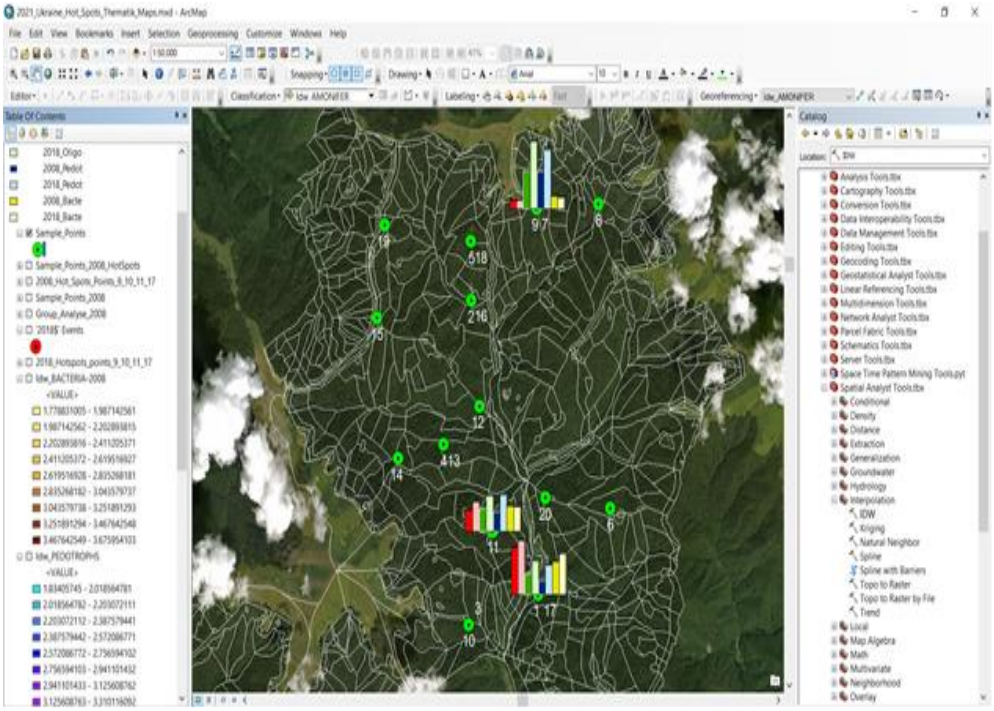


Figure 2: Hot Spot Analyses (Getis-Ord Gi*). A Comparative Map 2008 – 2018
(a) Ammonifiers; (b) Oligotrophes; (c) Pedotrophes; (d) Bacteria

Endogenous heterotrophic succession cause increasing biomass of oligotrophic bacteria and decreasing phylogenetic diversity. Diversity is indicate, how changed microbial communities during succession (Symochko, 2020). It should be noted that at altitudes of 555, 776, 1040 meters above sea level, significant changes occur in the structure of microbial community (Figure 2). The content of pedotrophes and oligotrophes in the soil increases.

Instead, the number of ammonifiers and bacteria using mineral forms of nitrogen is decreasing. At the altitude 555 meters above sea level content of ammonifiers was $6.24*10^6$ CFU/gr.d.s.(2008); $7.13*10^6$ CFU/gr.d.s. (2018) and bacteria using mineral forms of nitrogen $4.32*10^6$ CFU/gr.d.s.(2008); $5.43*10^6$ CFU/gr.d.s. (2018) , at the altitude 1040m content of these microorganisms decreased and number of ammonifiers was $1.07*10^6$ CFU/gr.d.s. (2008); $1.15*10^6$ CFU/gr.d.s. (2018) , bacteria using mineral forms of nitrogen - $1.63*10^6$ CFU/gr.d.s. (2008); $1.45*10^6$ CFU/gr.d.s. (2018).

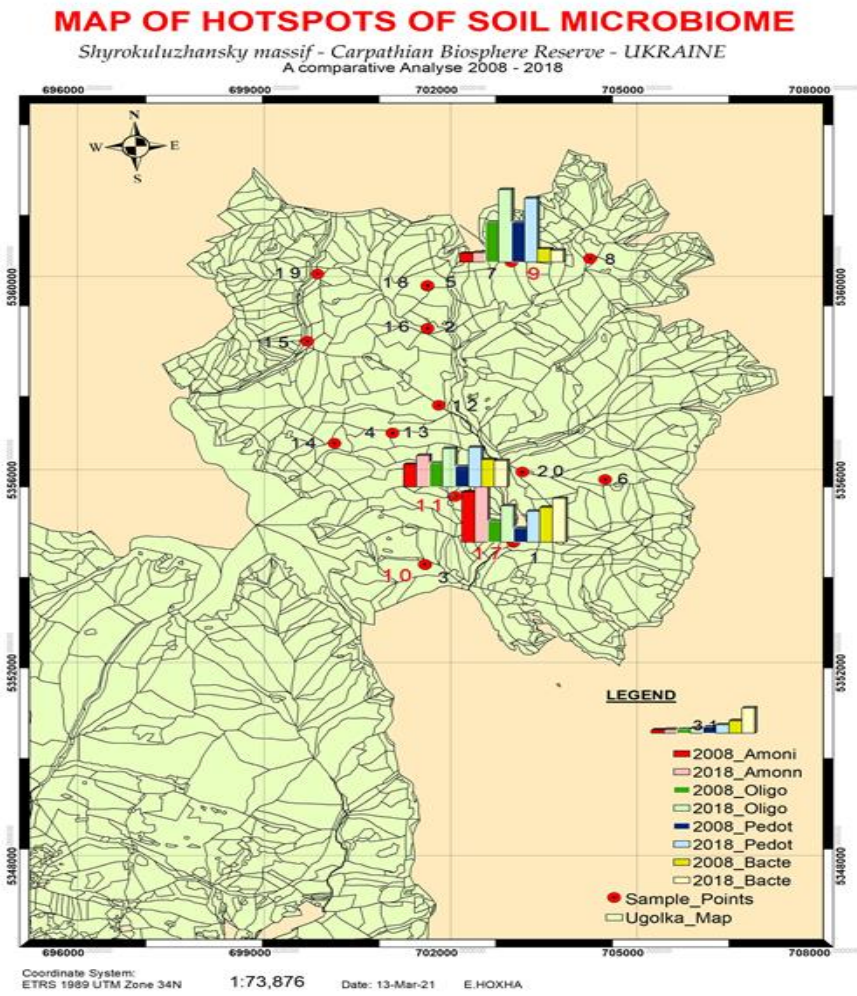


Figure 3: GIS Map of the Hotspots of soil microbiome of Shyrokoluzhansky massif in Carpathian Biosphere Reserve in Ukraine.
Comparative Map 2008 – 2018

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 *et al*., 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 et al., 2011; Kuzyakov and Blagodatskaya, 2015). The same dynamic of different functional groups of soil microorganisms were saved for 10 years (Figure 3).

In 2018 were fixed hot spots at the same altitudes 555, 776, 1040 meters above sea level. This is necessary to identify the mechanisms and factors affecting hotspot origin, formation and functioning. Our long-term investigations confirmed that the hotspots formed 10 years ago saved and continue to function.

CONCLUSIONS

Soil microbial community changed along successional time, but it showed significant difference at altitudes 555, 776, 1040 meters above sea level, which indicate hotspots in edophotopes at this altitude. Consequently, the number of representatives of major ecological-trophic groups of the soil microorganisms varies depending on the altitude of forest's biotopes disposition above sea level.

The number of ammonifiers, and bacteria that use mineral nitrogen decreased with the altitude increasing, the number of oligotrophes and pedotrophes gradually was increasing. Endogenous heterotrophic succession caused increasing biomass of oligotrophic and pedotrophic bacteria.

After 10 years, fluctuations of microbial cenosis at different altitudes were the same. 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. ArcGIS is an appropriate tool to make Hotspots map and different analyses.

Mapping of soil microbiome in primeval beech forest can be used as additional tools for conservation of unique, authentic soil microbiota.

REFERENCES

Bailey N.T.J. (1995): Statistical methods in biology. Third Edition, *Cambridge University Press. New York*, 640p. https://doi.org/10.1017/S0025315400018403

Baldrian P. (2017): Forest microbiome: diversity, complexity, and dynamics. FEMS Microbial Rev, 41, pp. 109–130. doi:10.1093/femsre/fuw040.

Bengtsson, J., Nilsson S., Franc A., Menozzi, P. (2000): "Biodiversity, Disturbances, Ecosystem Function and Management of European Forests." *For Ecol. Manag.* 132: 39-50.

Commarmot, B., Bachofen, H., Bundziak, Y., Burgi, A., Ramp, B., Shparyk, Y., Sukhariuk, D., Viter, R., Zingg, A. (2005): "Structures of Virgin and Managed Beech Forests in Uholka (Ukraine) and Sihlwald (Switzerland): A Comparative Study." *Snow Landsc Res*. 79: 56-45.

Commarmot, B., Brändli, U., Hamor, F., Lavny, V. (2013): Inventory of the Largest Primeval Beech Forest in Europe. A Swiss-Ukrainian Scientific Adventure. *Birmensdorf: Swiss Federal Research Institute WSL*.

Debeljak, M. (2006): "Coarse Woody Debris in Virgin and Managed Forest." *Ecological Indicators* 6: 733-42.

Demyanyuk O., Symochko L., Shatsman D. (2020) Structure and Dynamics of Soil Microbial Communities of Natural and Transformed Ecosystems. *Environmental Research, Engineering and Management (EREM)* Vol. 76 (4), pp. 97-105. doi: https://doi.org/10.5755/j01.erem.76.4.23508.

Goldman, E., & Green, L. H. (Eds.). (2015): Practical handbook of microbiology, *Third Edition*, 527p. doi:10.1201/b17871.

Grayston S.J., Rennenberg H. (2006): Assessing effects of forest management on microbial community structure in a central European beech forest. *Canadian Journal of Forest Research*, pp. 2595–2604.

Hamor, F., Dovhanych, Y. et al. (2008): Virgin Forests of Transcarpathia. Inventory and Management. *Rakhiv: Royal Dutch Society for Nature Conservation*.

ISO 10381-6: (1993). Soil quality. Sampling. Part 6: Guidance on the collection, handling, and storage of soil for the assessment of aerobic microbial processes in the laboratory.

Keenan RJ, Reams GA, Achard F, de Freitas JV, Grainger A, Lindquist E. (2015): Dynamics of global forest area: results from the FAO Global Forest Resources Assessment. For Ecol Manage, 352, *pp.* 9–20. doi:10.1016/j.foreco.2015.06.014.

Kuzyakov Y, Blagodatskaya E . (2015): Microbial hotspots and hot moments in soil: concept & review. Soil Biol Biochem 83*:*pp.184–199. doi:10.1016/j.soilbio.2015.01.025.

Lee, S.H., Jang, I., Chae, N., Choi, T., Kang, H., (2013): Organic layer serves as a hotspot of microbial activity and abundance in Arctic Tundra soils. Microbial Ecology 65, pp.405-414.

Marschner, P., Marhan, S., Kandeler, E., (2012): Microscale distribution and function of soil microorganisms in the interface between rhizosphere and detritus here. Soil Biology & Biochemistry 49, pp.174-183.

Martiny JB, Eisen JA, Penn K, Allison SD, Horner-Devine MC. (2011): Drivers of b-diversity depend on spatial scale. Proc Natl Acad Sci U S A, 108, *pp.* 7850–7854. doi:10.1073/pnas.1016308108

Minz D, Ofek M, Hadar Y. (2013): Plant rhizosphere microbial communities. The *Prokaryotes: Prokaryotic Communities and Ecophysiology*. Berlin: Springer-Verlag, pp. 57–84.

O'Brien SL, Gibbons SM, Owens SM, Hamptonarcell J, Johnston ER, Jastrow JD, Gilbert JA, Meyer F, Antonopoulos DA. (2016): *Spatial scale drives patterns in soil bacterial diversity. Environ Microbiol.*, 18, pp. 2039–2051. doi:10.1111/1462-2920.13231

Okabe, S., Odagiri, M., Ito, T., Satoh, H. (2007): Succession of sulfur-oxidizing bacteria in the microbial community on corroding concrete in sewer systems. *Appl. Environ. Microbiol*. 73, pp. 971-980.

Patyka V., Symochko L. (2013): Soil microbiological monitoring of natural and transformed ecosystems in the Trans-Carpathian region of Ukraine. *Microbiol Journal*, 75 (2), pp 21-31.

Roeselers, G., Van Loosdrecht, M.C.M., Muyzer, G. (2007): Heterotrophic pioneers facilitate phototrophic biofilm development. *Microb. Ecol*. 54, pp. 578-585.

Rout ME., Southworth D. (2013): The root microbiome influences scales from molecules to ecosystems: the unseen majority. *Am J Bot*., 100, pp. 1689–1691.

Rui, J.P., Peng, J.J., Lu, Y.H. (2009): Succession of bacterial populations during plant residue decomposition in rice field soil. *Appl. Environ. Microbiol*. 75, pp. 4879-4886.

Shyrobokov V. P. (2011): Medical Microbiology, Virology, and Immunologyю. *Nova Knyha, Vinnytsa*, 344p.

Stoyko, M., and Kopach, V. (2012): Centenary of Establishment of the Primeval Forest Reserves in the Ukrainian Carpathians. *Lviv: Prostir* M.

Symochko L. Hamuda H.B. (2015): Microbial monitoring of soil as additional tools for conservation biology. *Obuda University e-Bulletin*, 5(1), pp.177–185.

Symochko, L., Patyka, V., Symochko, V. Kalinichenko, A. (2015): "Soil Microbial Activity and Functional Diversity in Primeval Beech Forests". *Journal of Earth Science and Engineering* 5, pp. 363-371. Doi: 10.17265/2159-581X/2015.06.004

Symochko L. (2020): Soil microbiome: Diversity, activity, functional and structural successions. *International Journal of Ecosystems and Ecology Sciences* (IJEES), Vol. 10 (2), pp. 277-284**.** DOI: https://doi.org/10.31407/ijees10.206

Symochko L.Yu., Kalinichenko A.V. (2017): Soil Microbiome of Primeval Forest Ecosystems in Transcarpathia. *Microbiol Journal,* 80 (3), pp 3-14. doi: https://doi.org/10.15407/microbiolj80.03.003

Tepper, E.Z., Shilnikova, V.K., Pereverzeva, G.I. (2004): *Microbiology practicum, Moscow* 456p.

Turner TR., James EK., Poole PS. (2013): The plant microbiome. *Genome Biol*., (14) pp. 209–219.

Walker L.R., Walker J., Hobbs, R.J. (2007): Linking restoration and ecological succession. Springer.

Young, I.M., Crawford, J.W., (2004): Interactions and self-organization in the soilmicrobe complex. Science 304, pp.1634-1637.

Zolla G, Badria DV., Bakker MG., Manter DK., Vivanco JM. (2013): Soil microbiomes vary in their ability to confer drought tolerance to Arabidopsis. *Appl Soil Ecol*., 68, pp.1–9.