FOREST SOIL SCIENCE

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AUTHENTIC SOIL MICROBIAL COMMUNITIES IN PRIMEVAL FOREST ECOSYSTEMS OF UZHANSKYI NATIONAL NATURE PARK

Abstract. The article presents the modern aspects of studying and evaluating the biodiversity of soil microbiocenoses in the Carpathian region. The purpose of the work was to investigate the soil microbiota of virgin ecosystems, namely the structure of microbial communities, the number of major ecological trophic groups; to analyze the successional processes occurring in the soil microbiocenosis due to the influence of endogenous and exogenous factors. Anthropogenic impact: soil compaction, sanitary felling has led to changes in the groups of soil microorganisms, reduced their numbers and functional diversity. The primeval forests as etalon ecosystems better combine above resistance and stability with high productivity of biomass. Influence of ecological factors caused changes in the community of soil organisms, varied their abundance and functional diversity. 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.

Key words: *microbial diversity, soil, primeval forest, ecosystem.*

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АУТЕНТИЧНЫЕ СООБЩЕСТВА ПОЧВЕННЫХ МИКРООРГАНИЗМОВ ПРАЛЕСОВЫХ ЭКОСИСТЕМ УЖАНСКОГО НАЦИОНАЛЬНОГО ПРИРОДНОГО ПАРКА

Аннотация. В статье представлены современные аспекты изучения и оценки биоразнообразия микробоценоза почвы в Карпатском регионе. Пралесы как эталонные

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экосистемы прекрасно сочетают в себе хорошую сопротивляемость и стабильность вместе с высокой продуктивностью биомассы. Целью работы было исследовать микробиоту почвы пралесовых экосистем, а именно структуру микробных сообществ, численность основных эколого-трофических групп. Проанализировать сукцессионные процессы, происходящие в микробоценозе почвы вследствие влияния эндогенных и экзогенных факторов. Антропогенное воздействие (уплотнение почвы, санитарные рубки) привело к изменениям в сообществах почвенных микроорганизмов, снижению их численности и функционального разнообразия. Вопрос о сохранении микроорганизмов почвы сейчас по большей части игнорируется. Однако их роль в биогеохимических процессах и их потенциал в качестве резервата ценной генетической информации и продуктов обмена веществ делают их такими же важными, как животных и растений. Изучение микробиоты почвы создает необходимые предпосылки для сохранения микробного разнообразия и формирования базы эко-микробиологического мониторинга.

Ключевые слова: *микробное разнообразие, почва, пралесы, экосистема.*

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АВТЕНТИЧНІ УГРУПОВАННЯ ГРУНТОВИХ МІКРООРГАНІЗМІВ ПРАЛІСОВИХ ЕКОСИСТЕМ УЖАНСЬКОГО НАЦІОНАЛЬНОГО ПРИРОДНОГО ПАРКУ

Анотація. У статті представлено сучасні аспекти вивчення та оцінки біорізноманіття мікробоценозів ґрунту в Карпатському регіоні. Праліси як еталонні екосистеми краще поєднують у собі хорошу опірність та стабільність разом із високою продуктивністю біомаси. Метою роботи було дослідити мікробіоту ґрунту пралісових екосистем, а саме структуру мікробних угруповань, чисельність основних еколого-трофічних груп. Проаналізувати сукцесійні процеси, що відбуваються в мікробоценозі ґрунту внаслідок впливу ендогенних і екзогенних факторів. Антропогенний вплив (ущільнення ґрунту, санітарні рубки) призвів до змін в угрупованнях ґрунтових мікроорганізмів, зниження їх чисельності і функціонального різноманіття. Питання щодо збереження мікроорганізмів ґрунту нині досить ігнорується. Проте їх роль у біогеохімічних процесах і їх потенціал як резервата цінної генетичної інформації і продуктів обміну речовин роблять їх такими ж важливими, як тварин і рослин. Вивчення мікробіоти ґрунту створює необхідні передумови для збереження мікробного різноманіття і формування бази еко-мікробіологічного моніторингу.

Ключові слова: *мікробне різноманіття, ґрунт, праліси, екосистема.*

INTRODUCTION

The role of soil microorganisms is of high interest, since they 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 et al., 2011). Primeval forests are ideal ecosystems to study the interaction of bacteria, fungi and archaea with their abiotic environment (Grayston, Rennenberg, 2006). Microbial communities can be considered as architects of soils and many ecosystem services that are linked to terrestrial ecosystems, including plant production, safeguarding of drinking water or carbon sequestration, are closely linked to microbial activities and their functional traits (Hillebrand, Matthiessen, 2009).

Our main idea is to study and estimate biodiversity of the authentic microbiocenoses of soil of Uzhanskyi National Nature Park. As model ecosystem we investigated primeval forest. The primeval forests as etalon ecosystems better combine above resistance and

stability with high productivity biomass (Magurran, 2004). In the Transcarpathian region of Ukraine (south-west), the Uzhanskyi National Nature Park (Uzhanskyi NNP) 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 by envelope method from the virgin forests of Uzhanskyi National Nature Park. Uzhanskyi National Nature Park is located in the western part of Transcarpathia in the basin of the river Uzh and extends from the southwest of the village Zabrod (226 m above sea level) to north-east to Uzhotskyi pass (852 m above sea level).

In 2007 primeval beech forests of Uzhanskyi National Nature Park were included into UNESCO World Heritage List «Primeval Beech Forests of the Carpathians and the Ancient Beech Forests of Germany» Researches were conducted during 2014–2017 years. Sampling was carried out by squares method in depth of 0–25 cm at different altitudes from 450 m to 650 m. Microbiological studies of soil were carried out at the Scientific Research and Educational Center of Molecular Microbiology and the Immunology of Mucous Membranes (Uzhhorod National University) following the standard protocol (Goldman, Green, 2012).

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 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: Starch-ammonia agar, Meat peptone agar, Soil agar, Agar-Agar and Czapek agar in 4 repetitions. Petri dishes with study material were incubated in the thermostat at 37 ºC for 48 hours in aerobic conditions. Petri dishes with Czapek agar were incubated in the thermostat at 28 ºC for 96 hours Toxicity of soil samples was determined by the standart method (Bitton, Rossel, 1997). All statistical calculations were performed with Statistica v. 10.0 and Excel for Windows-2010.

RESULTS AND DISCUSSION

Soil microorganisms represent a significant portion of any terrestrial ecosystem and they are highly sensitive to anthropogenic pressure, so changes of qualitative and quantitative composition of the soil microbiota – is an indicator of the environmental changing (Cao Rui et al., 2016). Due to the high sensitivity to changes in environment microorganisms serve as a convenient object of observation. They are in a close contact with habitat and they are characterized by high rate of growth and reproduction. Extremely important aspect is the study of homotypic and heterotypic types of cocci, which involved soil microorganisms. Biocenotic relations of trophic and topical types are decisive in edaphotope shaping of different type of ecosystems (Reynolds et al., 2003). Due to this fact, the purpose of the research was to determine the number of different ecological-functional groups of soil microorganisms, phytotoxicity of soil. Studies of the soil taken from primeval ecosystems revealed general regularities of distribution of main ecological-functional groups of microorganisms, their population dynamics in different habitats. The most favorable conditions for the development and functioning of microorganisms were recorded in edaphotop located at an altitude of 450 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 (Fig. 1).

The number of ammonifiers at an altitude of 450 m amounted 5.33 million CFU/gr.ab.d.s. and at an altitude of 650 m – 3.01 million CFU/gr.ab.d.s., what indicate a significant enrichment of soil by organic matter of plant origin. Anthropogenic impact, in particular soil compaction, negatively influenced on the structure of soil microbiota. The content of oligotrophic microbiota significantly increased in the samples of this soil, but the

number of ammonifiers and pedotrophes microbiota was minimal compared to the other surveyed edaphotope (Fig. 2).

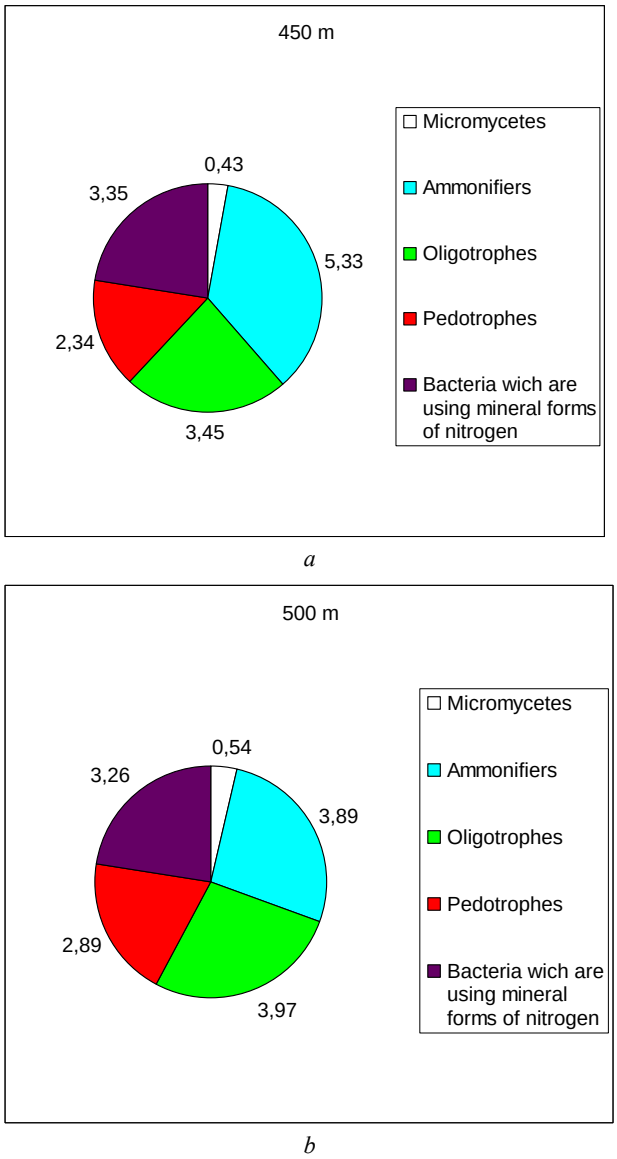
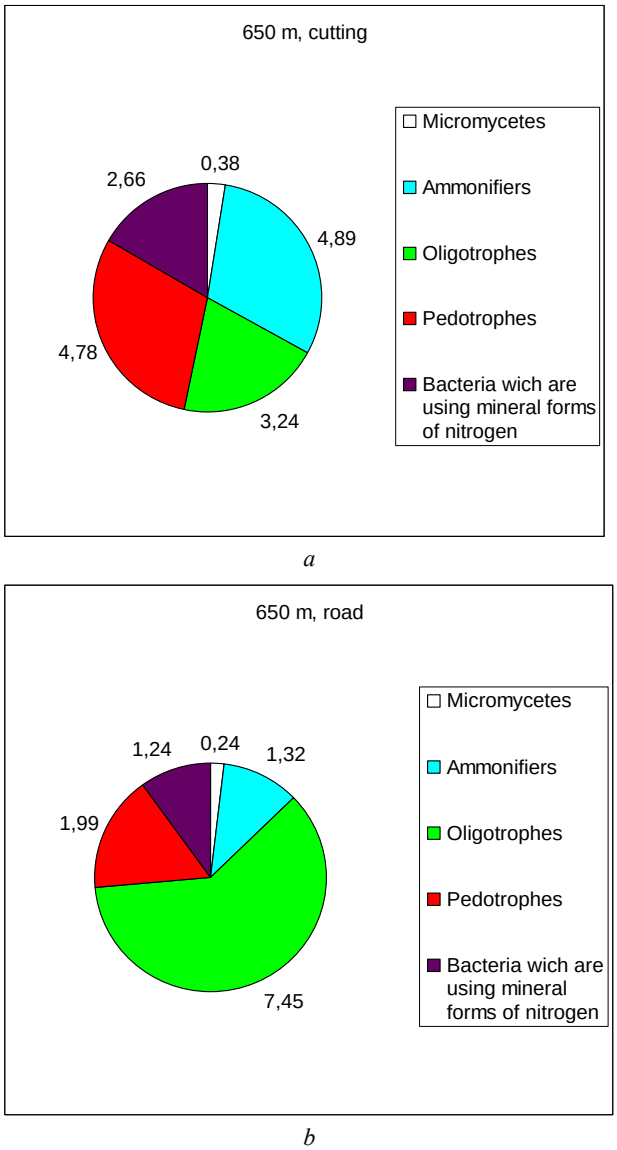


Fig. 1.**The structure of soil microbial community at altitude of 450 (*a*) and 500 meters (*b*) above sea level (CFU/gr.ab.d.s.)**

The growth in the number of oligotrophes indicates a decrease in the supply of nutrients in the soil. Similar changes were recorded by us and our colleagues in the study of biodiversity of soil microorganisms in primeval ecosystems of the Carpathian biosphere reserve (Symochko et al., 2015).

With the creation of favorable conditions for competitive species of microbiota we can see changes in microbial cenoses, owing to the active competition of microorganisms. In edaphotope that was not changed by direct human impact the dominate type of microorganisms was organotrophic microbiota. It should be noted that their percentage in the structure of groups was reduced by 36 % with increasing of the height. At the same time

the number of oligotrophes also increased with altitude. Significant negative changes in the structure of microbiocenosis of the soil can be the effect of anthropogenic influence. Violation of the integrity of the phytocenosis as a result of deforestation has led to the increase in the content of oligotrophes and pedotrophes, what indicates disruption of the normal flow of microbiological processes in the soil. It also influenced the decline in biodiversity of soil microorganisms. The most negative changes in the structure of soil microbial community were observed due to compaction. Nearly 80 % of the studied ecological-functional groups of microorganisms were oligotrophes, which indicates a significant deterioration of the ecological state of the soil. Toxic substances produced by microorganisms enter the plant directly from the soil, and they are concentrated mainly in the overground organs, and almost not observed in the roots of plants. Soil in virgin forest ecosystems was characterized by relatively low levels of phytotoxic activity (Table 1).



a

b

Fig. 2. **The structure of soil microbial community at altitude 650 meters above sea level (CFU/gr.ab.d.s.) (*a*) – biotope with cutting and (*b*) – biotope with compacted soil**

Table 1

Phytotoxical activity of the soil in virgin beech forests of Uzhanskyi National Nature Park

№	Altitude above sea level, m	Phytotoxity of the soil, % spring	Phytotoxity of the soil, % summer	Phytotoxity of the soil, % autumn
1	450	5,25±0,95	8,75±1,20	12,10±0,84
2	500	8,75±0,74	10,50±0,86	15,75±1,15
3	650	15,45±1,12	22,25±1,10	28,15 ±0,68

Studies have shown that the toxicity of the soil varies seasonally. The highest level of phytotoxic activity of the soil – 28,15 % was observed at an altitude of 650 meters. It should be noted that phytotoxicity of the soil in primeval ecosystems in the spring was twice lower than in the autumn, this pattern was observed in all studied biotopes. Identical changes in the level of soil toxicity were established by us in the forest ecosystems on the territory of the Carpathian Biosphere Reserve (Symochko et al., 2015). Such changes in the level of soil toxicity are associated with succession processes occurring in the structure of microbiocenosis due to the influence of exogenous factors.

CONCLUSIONS

On the territory of Uzhanskyi National Nature Park forest ecosystems of the particular value are preserved. The study of the soil microbiota of beech forests showed that microorganisms are sensitive reagents to the influence of external factors and can be used as a good indicator of the condition of ecosystem. It was established that the ratio of different ecological-functional groups of soil microorganisms varies depending on the height of the habitat above sea level, what is caused by the influence of abiotic factors. Violation of the integrity of the phytocenosis in the result of deforestation and soil compaction led to significant negative changes in the structure of soil microbial community. It was observed the decreasing in the functional biodiversity of the authentic groups of microorganisms where dominated groups of microorganisms were oligotrophes and pedotrophes.

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