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Research Article

Biodiversity of microbial populations as the indicator of biogenicity of soil under ashes and agricultural soil

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Abstract

The number, activity and diversity of microorganisms define the level of biogenicity and indicate the quality and health of the soil. The abundance and structure of microbial communities vary in different types of soils, so in order to preserve and protect natural and agroecological systems, in addition to physical-chemical analyses, monitoring of dynamics of microbiological activity in agricultural and non-agricultural soil is also necessary. Each type of soil has its own characteristic micro biocenosis, and different methods of soil use can have a positive or negative impact on microbiological activity, which directly affects the fertility of the soil. Soil bacteria are very important in biogeochemical cycles, and biological nitrogen fixation plays an important role in nitrogen cycling by transferring atmospheric dinitrogen into the soil. It is performed by symbiotic and asymbiotic nitrogen-fixing microorganisms, called nitrogen fixators. Amonificators are bacteria that play a very important role in soil ecosystem, as well as nitrogen fixators. This group of bacteria participates in the processes of protein decomposition and transformation. The aim of the paper is to indicate, based on the presence of nitrogen fixators and amonificators in soil under ashes, the biogenicity of this soil at four different localities: pure ashes, soil under ashes covered with herbaceous vegetation, soil under ashes covered with wood vegetation and agricultural soil, and to give advice on future activities regarding recultivation of these types of soil under ashes. The results of the research showed that microorganisms are mostly represented in different numbers in the tested soils, which indicates variations on the soil biogenicity and quality.

Keywords: amonificators; biogenicity of soil; microbial activity; nitrogen fixators

Introduction

Microorganisms are one of the most important chain in the biological phase of soil and terrestrial ecosystems, most physical, chemical and biological processes in the soil (80-90%) are going on due to

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microorganisms (Dennis *et al*., 2010; Anđelković *et al*., 2011). Microorganisms are found in huge quantities in the soil, however, their exact number has not yet been precisely determined (Jarak and Hajnal, 2006). The latest research, using epifluorescent microscopy, has experimentally proved that one gram of soil can contain up to 10 billion different types of microorganisms (Van elsas Dirk *et al.*, 2007). Among soil microorganisms, bacteria, actinomycetes and fungi are the most numerous (Chen *et al.*, 2003; Islam and Wright, 2005; Lupwayi, 2010).

The distribution and activity of microorganisms are influenced by the physical-chemical characteristics of the soil properties, climatic conditions, agrotechnical measures, plant species, the presence of heavy metals and pollutants, and biotic interactions between the microbial populations (Sivojiene *et al.*, 2021). In order to get high yields and ecologically acceptable food it is necessary to provide optimal conditions for the functioning of microbiological processes.

The presence and abundance of different genera and species of microorganisms and their activity represent one of the indicators of general microbiological activity and biogenicity of the soil (Roljević Nikolić *et al.*, 2022). Reduced abundance, diversity and enzyme activity of microorganisms may indicate changes in the physical-chemical properties of the soil, the negative impact of pollutants and soil degradation (Jarak *et al.*, 2005). Agricultural soil in urban and rural zones is at greater risk of pollution, due to the frequency of industrial plants, highways, landfills, groundwater, etc. (Tintor *et al.*, 2008; Marinković *et al.*, 2010; Marinković *et al.*, 2015).

Microorganisms with their life activities, such as mineralization of organic matter, which is their basic role in nature, enable life for today's biocenoses. Organic matter represents the main substrate and energy source for microorganisms (Xu *et al.*, 2013), as well as the limiting factor for the abundance and diversity of the microbial population (Fließbach *et al.*, 2007). All functions of microorganisms are related to the exchange of matter and energy - metabolism, formation and action of ferments, i.e. enzymes as bioactivators or biocatalysts of biochemical reactions.

Microorganisms play a key role in the mineralization of organic compounds to inorganic, in mobilizing hard soluble inorganic compounds in the soil, creating humus aggregates and transforming biogenic elements from one form into another, thus providing the necessary nutritious to plants. For these reasons, soil is now considered a biological reactor.

The tolerance of microorganisms to pesticides and heavy metals allows certain genera and species to be used in soil bioremediation. One of the most dangerous soil pollutants are heavy metals. They significantly affect the number, composition of species and life activity of soil microorganisms, inhibit the processes of mineralization and synthesis of various substances in the soil, suppress the quantity of air in the soil, have a micro biostatic effect and also can produce a mutagenic effect (Roane and Pepper, 2000). The main reasons why heavy metals are considered one of the main pollutants of soil are their enormous impact on the ecological balance (Sastre *et al.*, 2002), as well as their relatively strong connection to soil particles and poor mobility that prevents their removal (Sieghardt *et al.*, 2005).

Although it is common to diagnose contaminated soils by chemical analysis (Bojovic et al., 2019; Popovic *et al.*, 2011; 2016; 2020a; 2020b; Jovanović-Todorović *et al*., 2020; Kolarić *et al*., 2021; Ljubičić *et al.*, 2021; Lakić *et al*., 2020), the abundance and activities of certain groups and types of microorganisms can also serve as good indicators of soil quality and health (Milošević *et al.*, 2007). Therefore, in order to preserve and protect natural and agroecological systems, in addition to physical-chemical analyses, the dynamics of abundance and microbiological activity in agricultural and non-agricultural soils are monitored.

Soil bacteria are very important in biogeochemical cycles and have been used for crop production for decades. Biological nitrogen fixation plays an important role in nitrogen cycling by transferring atmospheric dinitrogen to the soil. It is performed by symbiotic and asymbiotic nitrogen fixing microorganisms, called nitrogen fixators. Nitrogen fixation is the process of biological binding of nitrogen by microorganisms thus enriching soil with nitrogen compounds (Bashan, 1998). In addition, microorganisms release some of the nutrients that are found in organic matter (N, C, P, S). Nitrogen fixators present a specific group of organisms in the soil that have the ability to reduce elemental nitrogen from the atmosphere by transforming it into ammonia and then into organic form. Species from the genus *Azotobacter* are one of the most important free, aerobic nitrogen fixators, and their abundance is adversely influenced by the physical and chemical properties of the soil (Milošević, 2008). The representation of this group of nitrogen fixators is a good indicator of soil fertility, while the absence of this bacteria may indicate primarily an unfavorable reaction of the soil solution (acidic or strongly alkaline), lack of nutrients or soil degradation.

Free nitrogen fixators live in the soil, rhizosphere or on the surface of the roots of plants, without causing changes in plants. The root–soil interface, or rhizosphere, is the point of greatest activity within the soil biogeocenosis (Bertin *et al.*, 2003). In numerous interactions in the soil-plant system, microorganisms are one of the most important factors. Abundance and microbiological activity in the rhizosphere depends on the amount and composition of root secretions, habitus and age of the roots (Dobbelaere *et al.*, 2003). Plants and microbes have been coevolving for several millennia, conferring fitness advantages that expand the plant's own genetic potential. These beneficial associations allow the plants to cope with abiotic stresses such as nutrient deficiency across a wide range of soils and growing conditions (Pantigoso *et al.*, 2022).

Microorganisms colonizing plant surfaces and internal tissues provide a number of life-support functions for their host (Cordovez *et al*, 2019). Free-living soil bacteria beneficial to plant growth, usually referred to as plant growth promoting rhizobacteria, are capable of promoting plant growth by colonizing the plant root. This rhizobacteria are also termed plant health promoting rhizobacteria or nodule promoting rhizobacteria (Cvijanovic *et al.*, 2012). They are associated with the rhizosphere, which is an important soil ecological environment for plant–microbe interactions. Symbiotic nitrogen fixing bacteria include the cyanobacteria of the genera *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium* and *Mesorhizobium*. These bacteria are most common associated with plants from family Fabaceae (Jarak and Colo, 2007). Free-living nitrogen fixing bacteria or associative nitrogen fixers, for example bacteria belonging to the genera *Azotobacter*, *Azospirillum*, *Enterobacter*, *Klebsiella* and *Pseudomonas*, have been shown to attach to the root and efficiently colonize root surfaces (Hayat *et al.*, 2010).

Amonificators include a large group of bacteria, fungi and actinomycetes that transform proteins and other organic nitrogen compounds, releasing ammonia. Amonificators are the most represented group of microorganisms in the soil (Rasulić *et al.*, 2021). Amonificators are very important in soil ecosystem, as well as nitrogen fixators. These groups of bacteria participate in the processes of protein decomposition and transformation, and converting it into the organic ammonia form, using very small quantities of mineral nitrogen content from soil (Rasulić *et al.*, 2021). Their abundance is an indicator of the content of organic nitrogen compounds. Based on the abundance and activity of the amonificators, it can be determined whether this nitrogen is accessible to plants or is bound in the cells of microorganisms.

The aim of the paper is to estimate, based on the presence of nitrogen fixators and amonificators in soil under ashes, the biogenicity of this soil at four different localities and provide guideline on future activities regarding recultivation of these types of soil under ashes.

Materials and Methods

Research in the field for agrochemical analyses of soil under ashes and agricultural soil, and its microbiological activity were performed during May and June in 2022, in the region of Branicevo district, munipicality of Kostolac.

Soil samples were taken from four localities: pure ashes, soil under ashes covered with herbaceous vegetation, soil under ashes covered with wood vegetation and agricultural soil, in the first layer of soil by depth of 0-30 cm. From each locality the samples were taken in three different micro localities, in three repetitions.

The coordinates of every investigated locality were taken, and are presented in Table 1 and locality in Figures 4 -6.

Table 1. Sample origin

No.	Locality	Soil depth, cm	Date of sampling	GPS coordinates
1.	Ashes	0-30	May, June 2022	40,74102^{0}N 21,18406^{0}E
2.	Soils under ashes covered with herbaceous vegetation			44,73621^{0}N 21,16878^{0}E
3.	Soil under ashes covered with wood vegetation			44,73684^{0}N 21,16887^{0}E
4.	Agricultural soil			44,72390^{0}N 21,21083^{0}E

Agrochemical soil analyses were carried out in the laboratory of the Agricultural, Expert and Advisory Service of Pozarevac, according to the following methods: determination of pH value (potentiometric) - method SRPS ISO 10390: 2007; determination of carbonate content (volumetric) - method SRPS ISO 10693:2005; determination of humus content by the Kotzmann method (volumetric) - VDM 01; determination of easily accessible potassium by AL method according to EngerRiehm (flamephotometric) - VDM 02 and determination of easily accessible phosphorus by AL method according to EngerRiehm (spectophotometrically) - VDM 03 (Table 2).

Microbiological studies included basic microbiological properties and the presence of bacteria which perform processes of nitrogen fixation and ammonification in the soil. The abundance of these tested groups of microorganisms was determined indirectly by the method of dilution, on the appropriate nutrient substrates.

On the agarized soil extract, the total number of microorganisms was determined, and the number of amonificators was morphologically determined on mesopepton agar (MPA plates) (Pochon and Tardieux, 1962). Seeding was carried out with 0.5 ml of suspension of dilution 10^{-6} in an empty petri box after which it is poured with 20 ml of molten MPA substrate. Incubation lasts for 3 days at a temperature of 28 °C. After incubation, the number of colonies was counted and the number of amonificators per 1 g of absolutely dry land was determined (Figure 1a).

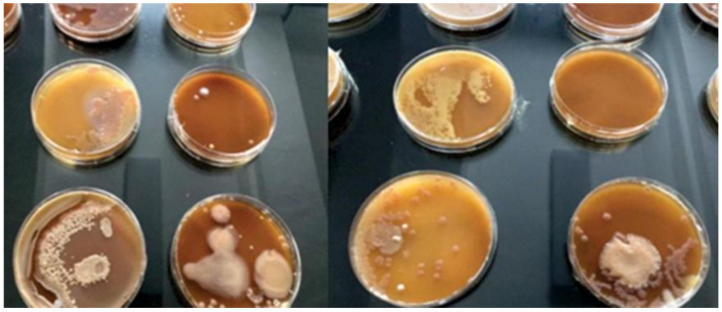
The number of nitrogen fixators was determined by the method of agar plates on Fyodor's substrate (Sarić, 1989). In the empty Petri box, 0.5 ml of suspension of the soil of grading to 10^{-5}, and poured with 20 ml of molten Fyodor's substrate. After incubation of 5 days at 28 °C, the grown colonies were morphologically determined, counted and the number is converted to 1 g of absolutely dry soil (Figure 1b).

After drying 1 g of soil from each locality: pure ashes, soil under ashes covered with herbaceous vegetation, soil under ashes covered with wood vegetation and agricultural soil, to absolutely dry measure the result was 0,933 g (Picture 1). According to this result and this equation

$$H = \frac{a \cdot b \cdot c}{d}$$

where H = the number of bacteria in 1 g of absolutely dry soil, a = average number of colonies grown on seeded Petri boxes, b = mass of soil taken for analysis, c = dilution by which the seeding was carried out and d = mass of absolutely dry land from which seeding was carried out, the total number of nitrogen fixators and amonificators was counted.

(a)



(b)

Figure 1. Process of drying soil in the laboratory, (a.), Grown colonies in Petri boxes with MPA and Fyodor's substrate, after incubation (b.)

Results and Discussion

The presence of certain systematic groups of bacteria indicates the flow and intensity of microbiological processes in the soil. In the tested soil samples, the abundance and activity of microorganisms depended on the locality and the method of soil use.

According to the obtained results, all tested samples showed an alkaline pH reaction. The values varied from strongly alkaline for pure ashes, to medium alkaline for soil under ashes covered with herbaceous vegetation, soil under ashes covered with wood vegetation and agricultural soil.

The process of soil humification is the decomposition of organic matter and the synthesis of humus (Stokić *et al.*, 2022). Based on the results in Table 2, it can be concluded that the tested soils are mostly well supplied with humus.

The tested samples of soil differed in the procentage of humus, from pure ashes which is medium humus soil, to the strongly humus soil which are soil under ashes covered with herbaceous vegetation, soil under ashes covered with wood vegetation and agricultural soil As for pure ashes, where humus essentially represents unburned organic matter of coal, we can only conditionally call it humus. Humus in the soil is not only an important source of nutrients for higher plants, but also a food source for soil microorganisms (Bjelić *et al.*, 2014).

The results for $CaCO_3$ show that the soil of the Branicevo district is generally carbonate-free or weakly carbonated, which is a consequence of the absence of $CaCO_3$ in the parent substrate (Mitić *et al.,* 2021), which is also shown in our results.

Regarding the procentage of nitrogen based on humus content, all the tested samples are well supplied with nitrogen content.

The agrochemical analyses of determination of easy-access potassium in the soil, showed that each type of tested soils are well supplied with potassium, so this indicates that the presence of K_2O is very high.

The analyses of determination of easily accessible phosphorus in the soil, showed that all of the tested soils are weakly supplied with phosphorus.

Table 2. Basic agrochemical analyses of soil

Sample	pH in H_2O	pH in KCl	$CaCO_3$ (%)	Humus (%)	N (%)	K_2O	P_20_5
						(mg 100 g^{-1})	
Pure ashes	8.84	8.29	0.47	3.18	0.159	19.68	8.17
Soil under ashes covered with wood vegetation	8.20	7.54	1.91	4.41	0.220	23.76	7.45
Soil under ashes covered with herbaceous vegetation	8.26	7.44	1.90	4.29	0.214	34.32	11.50
Agricultural soil	7.20	6.26	0.10	4.33	0.216	31.44	18.68

Species of the genus *Azotobacter* are one of the most important free, aerobic nitrogen fixators, and their presence or abundance is in the soil ecosystem or rhizosphere is affected by physical and chemical characteristics of soil. In addition to oxygen, soil moisture can be a limiting factor for the growth and activity of associative nitrogen fixing microorganisms. Species of genus *Azotobacter* reacts vigorously by changing the number to the adverse effects of environmental factors. Bacteria from the genera *Bacillus* and *Azotobacter* can also synthesize organic acids and phosphatases to the inaccessible phosphorus which will be translated into plants (Cherr *et al.,* 2006; Wilhelm *et al.*, 2007).

The presence of nitrogen fixators is an indicator of soil fertility, while their absence may indicate certain degradation processes in the soil. The presence of nitrogen fixators was observed in all tested localities, and the number of these species differed within micro localities (Table 3, Figure 2).

Table 3. Total abundance of nitrogen fixators

Parameter	Number of microorganisms (CFU/g^{-1} of absolutely dry land) Number of nitrogen fixators sp. x 10^{-5}		
Depth, 0-30 cm	**Micro locality no. 1.**	**Micro locality no. 2.**	**Micro locality no. 3.**
Ashes	4.3	5.4	46.1
	3.2	2.1	37.5
Average value	3.75	3.75	41.8
Soil under ashes covered with herbaceous vegetation	25.7	no grown colonies	11,3
	23.6	1.03	26.7
Average value	24.65	1.03	19.00
Soil under ashes covered with wood vegetation	56.4	80.9	24.5
	no grown colonies	79.9	32.2
Average value	56.40	80.40	28.35
Agricultural soil	2.1	47.7	79.5
	no grown colonies	60.4	45.5
Average value	2.10	54.05	62.50

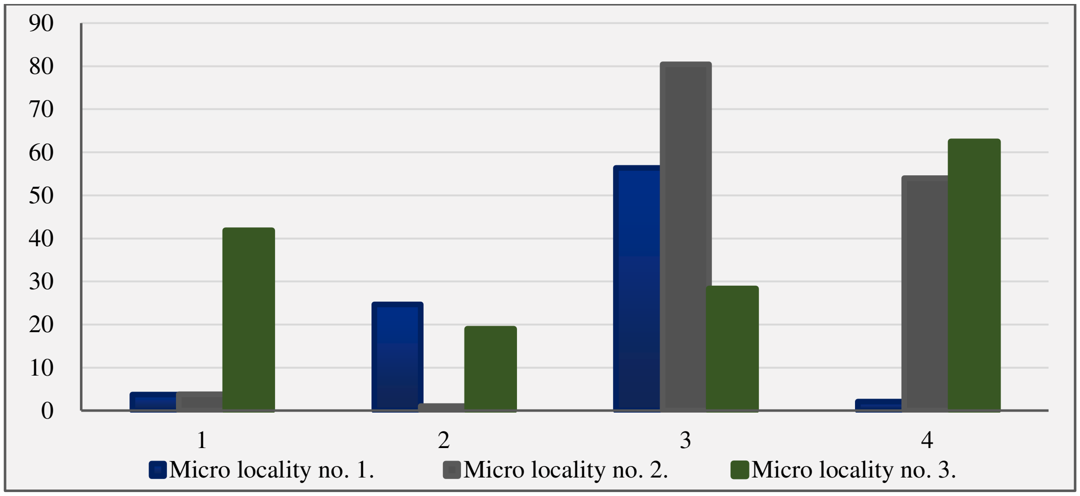


Figure 2. Average value of abundance of nitrogen fixators in each micro locality on: 1. Pure ashes; 2. Soils under ashes covered with herbaceous vegetation; 3. Soil under ashes covered with wood vegetation; 4. Agricultural soil

The abundance and activity of the amonificators, is a good indicator for accessibility of nitrogen to plants or it shows it is bounded in the cells of microorganisms. Mineralization of organic compounds produces ammonium ions (NH_4^+) which are very important for plant nutrition and amino autotrophic microorganisms, as well as for the process of humification (Cvijanović, 2002).

In the process of ammonification, a very heterogeneous group of microorganisms participates, from aerobic to anaerobic, sporogenic to asporogenous, acidosensitive to acidoresistant fungi, bacteria and actinomycetes (Ditch *et al.*, 2007).

In all the tested samples of soil under ashes, an average high abundance of amonificators was detected (Table 4, Figure 3). Given that the soils under ashes covered with herbaceous and wood vegetation is a microlocality where fertilization is carried out due to the symbiotic relations with this bacteria and roots of higher plants species, this result was expected.

Table 4. Total abundance of amonificators

Parameter	**Number of microorganisms (CFU/g^{-1} of absolutely dry land) Number of amonificators sp. x 10^{-6}**		
Depth 0-30 cm	**Micro locality no. 1.**	**Micro locality no. 2.**	**Micro locality no. 3.**
Ashes	79.3	1.07	18.2
	37.5	1.07	no grown colonies
Average value	58.4	1.07	18.20
Soils under ashes covered with herbaceous vegetation	10.7	3.1	25.7
	36.0	57.6	57.6
Average value	23.35	30.35	41.65
Soil under ashes covered with wood vegetation	no grown colonies	43.4	58.6
	18.1	30.1	26.6
Average value	18.10	36.75	42.60
Agricultural soil	4.2	37.1	24,4
	47.7	60.4	22.2
Average value	25.9	48.8	23.3

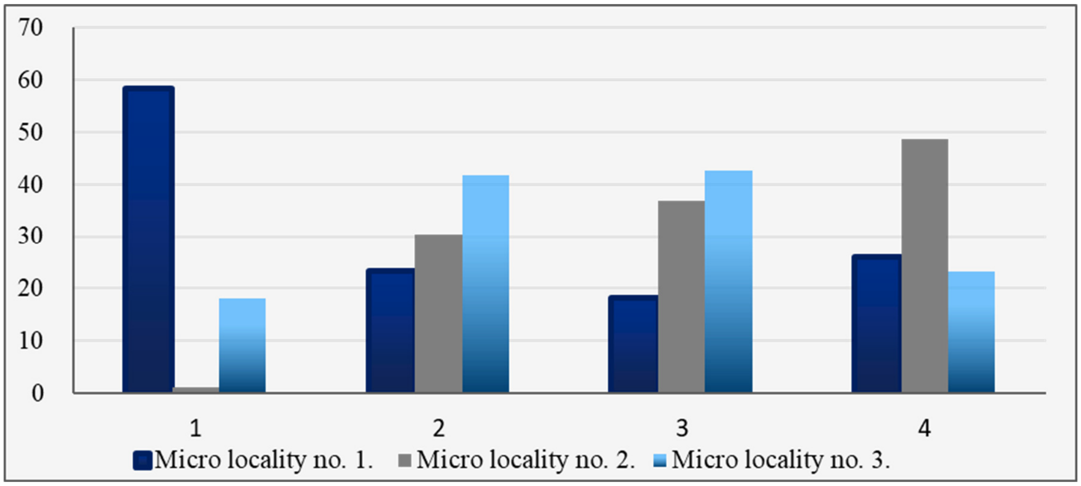


Figure 3. Average value of abundance of amonificators in each micro locality on: 1. Pure ashes; 2. Soils under ashes covered with herbaceous vegetation; 3. Soil under ashes covered with wood vegetation; 4. Agricultural soil.

The results by Li *et al.* (2019) suggested that soil N availability and rhizodeposition strongly modified the microbial communities of nitrogen fixing bacteria. Thus, dissemination of certain groups of microorganisms assimilating mineral forms of nitrogen in the soil indicates the active course of processes of mineralization of organic substances (Alieksieiev and Patyka, 2016).

The results of our research showed the high abundance of colonies of bacteria of nitrogen fixators and in soil under pure ashes only in the third micro location (41.8×10^{-5}) (Figure 4a).

Also, the high abundance of amonificators was detected in pure ashes in the first micro location (58.4×10^{-6}) and on third (18.20×10^{-6}). This is a possible consequence of the strong intensity of the local south-eastern wind "Kosava", which is constantly present and dominant type of wind in this area. This has caused the colonies of microorganisms to be scattered over longer distances. "Kosava" is the wind characteristic for the Branicevo district, whose intensity increases in open surfaces such as ashes.

The results also showed the high abundance of colonies of bacteria of nitrogen fixators and amonificators in soil under ashes covered with wood vegetation. On the first micro location the average value of nitrogen fixators was 56.40×10^{-5}, and on the second 80.40×10^{-5}. On the second micro location the average value of amonificators was 36.75×10^{-6}, and on the third 42.60×10^{-6}.


(a) (b)

Figure 4. (a) Pure ashes; (b) Soil under ashes covered with wood vegetation

The wood plants vegetation is self- sprouting and mostly represented by the species of acacia (*Robinia, Fabaceae*) and poplar (*Populus, Salinaceae*), which is shown in Figure 4b.

The number of nitrogen fixing bacteria in the rhizosphere changes under the influence of species and varieties of plants. This indicates that the symbiotic nitrogen fixing bacteria have associated in rhizosphere with the roots of plants, mainly from species of family *Fabaceae*. Symbiotic bacteria from genus *Mesorhizobium* and *Sinorhizobium* are characteristic nitrogen fixing bacteria associated with species form genus *Robinia* (Wei *et al.,* 2009). Furthermore, because of the capability of these bacteria to uptake and fix the N from air (dinitrogen N_2), the presence of N in this soil is high.

The results also showed average abundance of colonies of bacteria of nitrogen fixators and amonificators in soil under ashes covered with herbaceous vegetation. The highest abundance of nitrogen fixators was in the first and third micro location (24.65×10^{-5}; 19.00×10^{-5}). On the other hand, the abundance of amonificators was higher than the nitrogen fixators. On the first, second and third micro location the numbers were: 23.35×10^{-6}, 30.35×10^{-6} and 41.65×10^{-6}. The herbaceous vegetation in this location is also self- sprouting, and mainly represented by family *Poaceae* and *Cyperaceae*, but also species from genera *Ranunculus, Chenopodium, Amaranthus* and *Trifolium* can be spoted (Figure 5). The large presence of amonificators in the soil under ashes covered herbaceous vegetation is an indicator of the abundance of reserves of nitrogen organic matter, which remains on the surface and in the soil after the end of vegetation period. Since the dominant grass vegetation is from the family of *Poaceae*, large amounts of organic matter are cyclically renewed every year.

Figure 5. Soils under ashes covered with herbaceous vegetation

The use of microorganisms and the exploitation of beneficial plant–microbe interactions offer promising and environmentally friendly strategies for conventional and organic agriculture worldwide (Berg, 2009). Our results showed the highest abundance of colonies of bacteria of nitrogen fixators and amonificators in agricultural soil under species of corn *Zea mays* (Figure 6). On the second micro location the average value of species of nitrogen fixators was 54.05×10^{-5}, and on the third 62.50×10^{-5}. On the second micro location the average value of amonificators was 48.75×10^{-6}. Maize, as an agricultural crop, with its roots can significantly affects the composition and number of microorganisms not only in the rhizosphere, but also in the surrounding land. The corn plant stimulates the development of a large number of microorganisms: amonificators, proteolytic bacteria, cellulolytic bacteria, fungi and species from genus *Azotobacter* (Mrkovački *et al.*, 2012). However, the representation of these microorganisms in the rhizosphere is different, which depends on numerous ecological factors.

Figure 6. Agricultural soil

For the development and activity of the genus of nitrogen fixing bacteria, and also amonificators, the presence of organic matter in the soil greatly affects its abundance. This result was expected, due to intensive application of fertilizers, so the soil is rich in nitrogen, which is also shown in agrochemical analysis. The population of microorganisms that inhabit the soil rhizosphere affects the environment and the state of health of the crop and yield (Welbaum *et al.*, 2004, De Vita *et al.*, 2007a). Rhizobium can directly promote both plant growth and plant health, and modulating root architecture and growth via the release of plant phytohormones (Sun and Shahrajabian, 2022).

Microorganisms allow the movement of the processes of humification and dehumidification, thus occupying a central place in the circulation and biogeochemical cycles of the necessary macro and micro nutrients. According to the detailed analysis of all overall obtained results, the following conclusions were drawn: The distribution, diversity and activity of microorganisms are influenced by the physical-chemical characteristics of the soil type, climatic conditions, agrotechnical measures, plant species that inhabit soil, the presence of heavy metals and other organic pollutants, as well as the relationship between the microbial population.

Microbial communities are most often applied to improve the supply of plants nitrogen and phosphorus and mineralize organic matter, and as a result, there is an increase in the amount of affordable assimilatives of plants. Microorganisms affect the degree of supply plants by phosphorus through mineralization of organic phosphorus compounds, by immobilizing affordable phosphorus, as well as the dissolution of insoluble mineral forms of phosphorus. Microbial phosphorus biomass is a small, but very labile reservoir potentially affordable for plant root assimilation. Based on the present findings it can be concluded that, all tested soil samples showed weak supply of phosphorus content. In order to increase microbial activity through mineralization of organic phosphorus compounds, the use of fertilizers should be applied. Rhizobacteria also help in solubilization of mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates, and improve soil structure and organic matter content. They retain more soil organic N, and other nutrients in the plant–soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients.

Conclusions

According to the results obtained in this paper, the number, activity and diversity of individual groups microorganisms define the level of biogenicity and indicate the quality and health of the soil.

- Variations in the number of tested groups of microorganisms: nitrogen fixators and amonificators in all four localities indicate a different state of the soil: the processes of mineralization and humification do not take place on pure ashes, but are represented on the soil under ashes covered with herbaceous and wood vegetation. Future activities for further improvement of this soil should be planned, such as recultivation, or certain methods of phytoremediation, eco-friendly and sustainable technology, and nowadays it represents commercial and sustainable phyto management;
- Plant–bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. Plant growth promoting rhizobacteria have the potential to contribute to sustainable plant growth promotion.
- The number and diversity of microorganisms in agricultural soil, in this study under corn field, depends on the physical-chemical characteristics: the climate factors, methods of tillage and cultivation, hybrids, the root scavengers etc. The number of selected group of bacteria differed in this type of soil, but on average the number of both amonificators and nitrogen fixators was high. Binding atmospheric nitrogen (N_2) in the process of biological fixation of nitrogen is the most important component in the nitrogen cycle in nature with a special significance for agriculture, so high abundance of these microorganisms in the fourth locality of this research is of great significance for biogenicity of this type of soil.
- Large reserves of organic phosphorus in the soil are not available for the plants, so the role of microorganisms in its decomposition is irreplaceable.
- Measures on improvement of agriculture efficiency and recovery of fertility of soil under ashes should necessarily be connected with microorganisms activity.

Authors' Contributions

Conceptualization, T.S., V.S., A.S., Z.Ž., V.P.; methodology, M.S., M.B.; software, V.P.; validation, T.S.; formal analysis, T.S., V.S., V.P.; investigation, V.P.; resources, T.S., V.S.; data curation, V.P.; writing—original draft preparation, T.S., V.S., A.S., V.P.; writing—review and editing Z.Ž., M.S., M.B., V.P.; visualization, A.S., T.S., V.S., V.P.; supervision, T.S., N.S.; project administration, V.P.; funding acquisition, T.S., M.S. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Alieksieiev OO, Patyka VF (2016). Influence of biological products on the microbiom soil in the rhizosphere of *Glycine max* (L.) Merr. Science and World. Ukraina 12 (40):54-58.

Anđelković S, Jarak M, Vasić T, Radović J, Lugić Z, Anđelković B (2011). Brojnost mikroorganizama u agroekosistemima [The number of microorganisms in agroecosystems]. Ecologica 18 (63): 481-484.

Bashan Y (1998). Inoculants of plant growth–promoting bacteria for use in agriculture. Biotechnology Advances 16:729-770. *https://doi.org/10.1016/S0734-9750(98)00003-2*

Berg G (2009). Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied Microbiology and Biotechnology 84:11-18. *https://doi.org/10.1007/s00253-009-2092-7*

Bertin C, Yang X, Weston AL (2003). The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil 256:67-83. *https://doi.org/10.1023/A:1026290508166*

Bjelić M, Nešić Lj, Ćirić V (2014). Praktikum iz pedologije. Univerzitet u Novom Sadu, Poljoprivredni fakultet. [Practicum of pedology. University of Novi Sad, Faculty of Agriculture].

Bojovic R, Popović V, Ikanović J, Živanović Lj, Rakaščan N, Popović S, Ugrenović V, Simić D (2019). Morphological characterization of sweet sorghum genotypes across environments. The Journal of Animal and Plant Sciences 29(3):721-729.

Chen G, Zhu H, Zhang Y (2003). Soil microbial activities and carbon and nitrogen fixation. Research in Microbiology 154:393-398. *https://doi.org/10.1016/S0923-2508(03)00082-2*

Cherr CM, Scholberg JMS, McSorley R (2006). Green manure approaches to crop production: A synthesis. Agronomy Journal 98:302-319. *https://doi.org/10.2134/agronj2005.0035*

Compant S, Samad A, Faist H, Sessistsch A (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. Journal of Advanced Research 19:29-37. *https://doi.org/10.1016/j.jare.2019.03.004*

Cordovez V, Dini-Androete F, Carrion JV, Raaijmakers MJ (2019). Ecology and evolution of plant microbiomes. Annual Review of Microbiology 8(73):69-88. *https://doi.org/10.1146/annurev-micro-090817-062524*

Cvijanović G (2002). Uticaj diazotrofa na prinos i mikrobiološku aktivnost u zemljištu kod kukuruza, pšenice i soje. Doktorska diseratacija. Poljoprivredni fakultet Novi Sad. Univerzitet u Novom Sadu. [Influence of diazotr of on yield and microbiological activity in soil in maize, wheat and soybeans, PhD Thesis, Faculty of Agriculture Novi Sad, University of Novi Sad].

Cvijanović G, Dozet G, Djukić V, Đorđević S, Puzić G (2012). Microbial activity of soil during the inoculation of soya bean with symbiotic and free-living nitrogen-fixing bacteria. African Journal of Biotechnology 11(3):590-597. *https:///doi.org/10.5897/AJB11.744*

De Vita P, Di Paolo E, Fecondo G, Di Fonzo N, Pisante M (2007a). No-tillage and conventional tillage effects on durum wheat yield, grain quality and soil moisture content in southern Italy. Soil and Tillage Research 921(2):69-78. *https://doi.org/10.1016/j.still.2006.01.012*

Dennis PG, Miller JA, Hirsch RP (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities. FEMS Microbiology Ecology 72:313-327. *https://doi.org/10.1111/j.1574-6941.2010.00860.x*

Dobbelaere S, Vanderleyden J, Okon Y (2003). Plant growth–promoting effects of diazotrophs in therhizosphere. Critical Reviews in Plant Sciences 22(2):107-149. *https://doi.org/10.1080/713610853*

Fließbach A, Oberholzer HR, Gunst L, Mäder P, Fliessbach (2007). Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agriculture, Ecosystems and Environment 118: 273-284. *https://doi.org/10.1016/j.agee.2006.05.022*

Hayat R, Ali S, Amara, Khalid, R, Ahmed I (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. Annals of Microbiology 60:579-598. *https://doi.org/10.1007/s13213-010-0117-1*

Islam KR, Wright SR (2005). Microbial communities. Encyclopedia of Soil Science, Taylor & Francis.

Jarak M, Čolo J (2007). Soil Microbiology. University of Novi Sad. Faculty of Agriculture. Novi Sad.

Jarak M, Hajnal T (2006). Total number of microorganisms, number of fungi and Azotobacter in compacted and noncompacted soil. Tractors and Power Machines 11(5):37-40.

Jarak M, Milošević N, Milić V, Mrkovački N, Đurić S, Marinković J (2005). Mikrobiološka aktivnost - pokazatelj plodnosti i degradacije zemljišta [Microbiological activity – indicator of fertility and land degradation]. Ekonomika Poljoprivrede 52(4):483-493.

Kolarić L, Popović V, Živanović L, Ljubičić N, Stevanović P, Šarčević Todosijević Lj, Simić D, Ikanović J (2021). Buckwheat yield traits response as influenced by row spacing, nitrogen, phosphorus, and potassium management. Agronomy 11:2371. *https://doi.org/10.3390/agronomy11122371*

Kresović MM, Raičević V (2004). Mikrobiološka aktivnost u zemljištu pod pašnjakom. Zemljište i biljka. [Microbiological activity in soil under pasture]. Soil and Plant 53(1):71-77.

Lakić Ž, Predić T, Đurđić I, Popović V (2020). Recultivation of degraded soil due to mining activity without adding organic layers of soil using alfalfa and mixtures of grass legumes. Agriculture and Forestry 66(4):223-237.

Li Y, Pan F, Yao H (2019). Response of symbiotic and asymbiotic nitrogen-fixing microorganisms to nitrogen fertilizer application. Journal of Soil and Sediments 19:1948-1958. *https://doi.org/10.1007/s11368-018-2192-z*

Ljubičić N, Popović V, Ćirić V, Kostić M, Ivošević B, Popović D, Pandžić M, El Musafah Seddiq, Janković S (2021). Multivariate interaction analysis of winter wheat grown in environment of limited soil conditions. Plants–Basel. 10(3):604. *https://doi.org/10.3390/plants10030604*

Lupwayi NZ, Grant CA, Soon YK, Clayton GW, Bittman S, Malhi SS, Zebarth BJ (2010). Soil microbial community response to controlled-release urea fertilizer under zero tillage and conventional tillage. Applied Soil Ecology 45(3):254-261. *https://doi.org/10.1016/j.apsoil.2010.04.013*

Marinković J, Milošević N, Sekulić P, Tintor B (2010). Microbiological properties of soil in oil refineries. XVIII International Scientific and professional meeting “Ecological Truth” Eco-Ist’ 10. Spa Junaković. Apatin. R. Srbija Proceedings. pp 414-419.

Marinković J, Ninkov J, Tintor B, Bjelić D, Vasin J, Cvijanović G (2015). Mikrobiološke karakteristike zemljišta pod parkovima i pored frekventnih saobraćajnica Novog Sada. Naučni-stručni skup “Održivo korišćenje zemljišta”. Novi Sad. Institut za ratarstvo i povrtarstvo. [Microbiological characteristics of land under parks and in frequent roads of Novi Sad. Scientific and professional conference "Sustainable land use". Novi Sad. Institute of Field and Vegetable Crops. Thematic proceedings pp 75-82].

Milošević N (2008). Microorganisms – bioindicators of health/soil quality. Proceedings Institute of Field and Vegetable Crops. Novi Sad 45(1):205-215.

Milošević N, Tintor B, Dozet D, Cvijanović G (2007). Microbiological properties of soils in natural grasslands. Proceedings of the Institute of Field and Vegetable Crops 44(1):541-546.

Mitić K, Stokić M, Stupar V, Živković Z, Sekulić T, Stojićević D (2021). Sistematska kontrola plodnosti zemljišta u 2017.godini na teritoriji braničevskog okruga. Šesti naučno-stručni skup Politehnika, Beograd, pp 825-829. [Systematic control of soil fertility in 2017 in the territory of braničevo district. Sixth scientific and professional conference Polytechnics, Belgrade, 825-829].

Mrkovački N, Jarak M, Đalović I, Jocković Đ (2012). Importance of PGPR application and its effect on microbial activity in maize rhizosphere. Farming and Vegetable Crops 49(3):335-344.

Pantigoso H, Newberg D, Vivanco J (2022). The rhizosphere microbiome: Plant-microbial interactions for resource acquisition. Journal of Applied Microbiology 133(5):2864-2876. *https://doi.org/10.1111/jam.15686*

Pochon J, Tardieux (1962). Techniques d’analyse en microbiologie du sol. Éditions de la Tourelle, St. Mandé (Seine), Paris, France.

Popović V, Glamočlija Đ, Malešević M, Ikanović J, Dražić G, Spasić M, Stanković S (2011). Genotype specificity in nitrogen nutrition of malting barley. Genetika, Belgrade 43(1):197-204. *https://doi.org/10.2298/GENSR1101197P*

Popovic V, Ljubičić N, Kostić M, Radulović M, Blagojević D, Ugrenovic V, Popovic D, Ivosevic B (2020a). Genotype x environment interaction for wheat yield traits suitable for selection in different seed priming conditions. Plants 9(12):1804; *https://doi.org/10.3390/plants9121804*

Popović V, Vučković S, Jovović Z, Ljubičić N, Kostić M, Rakaščan N, Glamočlija-Mladenović M, Ikanović J (2020b). Genotype by year interaction effects on soybean morpho-productive traits and biogas production. Genetika Belgrade 52(3):1055-1073. *https://doi.org/10.2298/GENSR2003055P*

Popovic V, Tatic M, Sikora V, Ikanovic J, Drazic G, Djukic V, … Stevanovic P (2016). Variability of Yield and chemical composition in soybean genotypes grown under different agroecological conditions of Serbia. Romanian Agricultural Research 33:29-39.

Rasulić N, Delić D, Stajković-Srbinović O, Buntić A, Kuzmanović Đ, Knežević M, Sikirić B (2021). Microbiological and basic agrochemical properties of Eutric Cambisols in western and southwestern Serbia. Land and Plant 70(2):1-9.

Roane TH, Pepper IL, Gentry T (2015). Microorganisms and metal pollutants. Environmental Microbiology (Third edition), Academic Press pp 415-439. *https://doi.org/10.1016/B978-0-12-394626-3.00018-1*

Roljević Nikolić MS, Dolijanović, KŽ, Kovačević ĐD, Oljača IS, Majstorović JH (2022). Soil biogenicity in the rhizosphere of different wheat genotypes under the impact of fertilization treatment. Journal of Agricultural Sciences Belgrade 67(4):367-380.

Sarić Z (1989). Praktikum iz mikrobiologije. Naučna knjiga. Beograd. [Practicum of microbiology. Scientific book. Belgrade].

Sastre J, Sahuquillo A, Vidal M, Rauret G (2002). Determination of Cd, Cu, Pb and Zn in 343 environmental samples: microwave-assisted total digestion versus aqua regia and nitric acid extraction. Analytica Chimica Acta 462(1):59-72. *https://doi.org/10.1016/S0003-2670(02)00307-0*

Sieghardt M, Mursch-Radlgruber E, Paoletti E, Couenberg E, Dimitrakopoulus A, Rego F, Hatzistathis A, Barfoed Randrupt T (2005). The abiotic urban environment: impact of urban growing conditions on urban vegetation. In: Konijnendijk CC, Nilsson K (Eds). Urban Forests and Trees: A Reference Book. Springer Verlag, pp 281-283. *https://link.springer.com/chapter/10.1007/3-540-27684-X_12*

Sivojiene D, Kacergius A, Baksiene E, Maseviciene A, Zickiene L (2021). The influence of organic fertilizers on the abundance of soil microorganism communities, agrochemical indicators, and yield in East Lithuanian light soils. Plants 10(12):2648.

Stokić M, Živković Z, Stojićević D, Saulić M, Sekulić T, Stupar V (2022). Substitution Acidity, Phosphorus and Potassium Content in the Land of the Braničevo District in the Republic of Serbia. XIII International Scientific Agriculture Symposium "Agrosym 2022", Jahorina, Faculty of Agriculture, East Sarajevo. Book of Proceedings. pp 841-846.

Tintor B, Milošević N, Marinković J, Pucarević M (2008). Microbiological properties of agricultural land in addition to frequent roads and industrial zones. Proceedings Institute of Field and Vegetable Crops, Novi Sad 45(2):225-232.

Van Elsas D, Jansson JK, Trevors JT (2007). Modern Soil Microbiology. Second Edition, CRC Press.

Wei G, Chen W, Zhu W, Chen C, Young PW, Bontemps C (2009). Invasive *Robinia pseudoacacia* in China is nodulated by *Mesorhizobium* and *Sinorhizobium* species that share similar nodulation genes with native American symbionts. FEMS Microbiology and Ecology 68:320-328. *https://doi.org/10.1111/j.1574-6941.2009.00673.x*

Welbaum G, Sturz AV, Dong Z, Nowak J (2004). Fertilizing soil microorganisms to improve productivity of agroecosystems. Critical Reviews in Plant Sciences 23:175-193. *https://doi.org/10.1080/07352680490433295*

Wilhelm J, Johnson MF, Karlen L, David T (2007). Corn stover to sustain soil organic further constrains biomass supply. Agronomy Journal 99:1665-1667. *https://doi.org/10.2134/agronj2007.0150*

Xu X, Thornton PE, Post WM (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Global Ecology and Biogeography 22:737-749. *https://doi.org/10.1111/geb.12029*

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