*Bulgarian Journal of Agricultural Science, 30 (No 5) 2024, 769–776*

# Microflora of Luvisols from the territory of Western Stara Planina Mountain

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## Abstract

Grigorova-Pesheva, B. (2024). Microflora of Luvisols from the territory of Western Stara Planina Mountain. *Bulg. J. Agric. Sci., 30*(5), 769–776

The aim of the present study is to provide basic information on the biogenicity of the studied soils, to track the change in the microbial populations of A and B horizons, as well as to determine the impact of environmental factors on the microbial abundance of forest soils in the light of ecological forest management. The present study examines six soil profiles in Western Stara Planina Mountain. Soils are defined as Luvisols. For determination of total microbial number and the amount of individual microbiological groups (spore-forming bacteria, non-spore-forming bacteria, actinomycetes and fungi) the standard method of serial dilutions and subsequent inoculation was used. The results are reported in Colony-forming unit (CFU). Standard laboratory analyzes were used to measure the physical and chemical parameters of the soil. A horizon has a greater microbial abundance (from 13.32 and 50.69 × $10^5$ CFU g/ dry soil) than B horizon (1.81 to 6.24 69 × $10^5$ CFU g/ dry soil). In relation to the A horizon, the most strongly influencing factor is the content of total nitrogen (r = 0.76), followed by pH (0.72) and organic carbon content (0.55). According to the B horizon, these indicators show no correlation. In the A horizon, mechanical composition and density do not correlate with total microbial numbers. In the B horizon mechanical composition and soil density have an impact on biogenicity. With highly compacted soil (bulk density above 1.3 g/$cm^3$), suppression of the soil microbiota is observed. There are no clear dynamics in the redistribution of the percentage participation of microbial groups at depth.

*Keywords***:** soil microorganism; soil; Luvisols; forest ecosystems; total microbial number

## Introduction

Forest ecosystems are of immense importance for the Earth's biosphere, covering over 30% of the land area (Keenan et al., 2015). Additionally, forest ecosystems provide habitat for countless species of flora and fauna. They are home to about 80% of the world's terrestrial biodiversity, which includes many endangered and threatened species (Butchart et al., 2010). Forests also have significant cultural and socio-economic value, providing livelihoods for millions of people around the world who depend on them for their livelihoods and traditional ways of life.

Forest ecosystems face numerous threats from human activities, including deforestation, fragmentation, and degradation. Deforestation, in particular, is a major driver of biodiversity loss and climate change, as well as a contributor to soil erosion, flooding, and water scarcity (Gibbs et al., 2010).

To address these threats and promote sustainable forest management, many countries and organizations have established conservation and restoration initiatives, such as protected areas, reforestation programs, and sustainable forestry practices. These initiatives are crucial for ensuring the long-term health and resilience of forest ecosystems and the many benefits they provide to humans and the planet as a whole. The study of forest ecosystems is essential from an ecological perspective as they play a fundamental role in carbon dioxide sequestration (Lladó et al., 2017) and per-

form vital water conservation functions (Pavlova-Traykova et al., 2021). A number of studies in forest areas have drawn attention to the potential danger of soil erosion in forests and the need for designing and implementing anti-erosion measures (Pavlova-Traykova & Marinov, 2021; Pavlova-Traykova, 2022). Such information confirms the necessity of ecologically sound and targeted forest management.

Microbial communities are directly linked to the functions of forest ecosystems (Graham et al., 2016). Soils and soil microorganisms, as their components, are an essential part of these ecosystems. Forest soils are key to providing the ecosystem services offered by forests. In recent years, forest soils have been heavily threatened, not only due to increased anthropogenic pressure but also as a result of improper cultivation of crops, reforestation mainly with monocultures, and the use of heavy machinery, which leads to soil compaction. This necessitates the creation of strategies for ecologically sound active soil management aimed at reducing the loss of essential soil functions and their restoration (Wilpert, 2022). Microorganisms are a key biological component of forest soils that need to be thoroughly studied. In a global context, most studies of soil microorganisms are related to the study of fungi, neglecting the important role that bacteria play (Lladó et al., 2017).

The microflora of forest soils is the main driving force in both the processes of organic matter decomposition in forest areas and the processes associated with humus synthesis and transformation of organic components into forms suitable for plants (Milosevic et al., 2003). A number of ecosystem functions are associated with the abundance of soil microorganisms and the structure of microbial communities (Strickland et al., 2009). Recently, more and more scientific research from around the world has focused on the important role that soil microorganisms play in the conservation and management of forest ecosystems (Fonseca, 1990; Staddon et al., 1999; Zhang et al., 2020). In their study, Barreto-Garcia et al. (2021) pointed out that different forest management methods may have different effects on soil microbial communities and thus on ecosystem functioning, highlighting the need for further research to identify effective management practices. In Bulgaria, separate studies have been conducted on the soil microflora in forested areas. Some of them are focused on the enzymatic activity of soil microorganisms (Malcheva, 2020), as well as on changes in soil microorganisms after fires (Malcheva & Velizarova, 2021). Other research focuses on the seasonal dynamics of soil communities (Grigorova-Pesheva, 2020) and changes in microbial abundance depending on environmental factors (Grigorova-Pesheva & Hristov, 2021). Within the confines of the Western Stara Planina region, specifically in the non-urbanized forested area, a series of partial soil microbiological investigations were conducted.

These studies aimed to examine the influence of altitude (Grigorova-Pesheva & Petrova, 2022) and evaluate the impact of environmental factors on the humus-accumulative A horizon of soils classified as Luvisols (Grigorova-Pesheva & Hristov, 2021). Notably, the present study represents the pioneering attempt to comprehensively analyze the microflora of Luvisols across various depths of the soil profile within the forested expanse of the Western Stara Planina region.

The aim of the present study is to provide basic information on the biogenicity of forest soils of the Luvisols type in the forested areas of the Western Stara Planina Mauntain, to trace changes in microbial populations in the A and B soil horizons, and to determine the impact of environmental factors on the microbial abundance of forest soils in light of ecologically sustainable forest management.

## Materials and Methods

The subject of the study are forest soils from the territory of Western Stara Planina. The Western Balkan Range encompasses the segment of the Stara Planina Mountain range located between the western border of Bulgaria and the Iskar Gorge. According to climatic zoning, the Western Balkan Range falls within two climatic regions of the Temperate Continental Climatic Subregion (Velev, 2002) with precipitation levels ranging from 750 to 1000 mm - the Submoutain and Mountain climatic zones (Koleva-Lizama, 2006). It is also a part of the European region of deciduous forests and the Illyrian province (Bondev, 2002). The study was conducted in May 2021. According to climate data for the area, spring is relatively cool. The stable transition of the air temperature through 10°C in spring in the lower parts of the Stara planina is in the middle of April. Precipitation is characterized by a well-defined maximum in May. The average amount of precipitation during the three spring months in the lower parts of the region is about 170 mm – 240 mm (Koleva-Lizama, 2006). For the purpose of the study, six soil profiles (SP) were examined in Luvisols at an altitude ranging from 185 m to 615 m. A and B horizons were investigated. Sampling was performed using sterile tools and soil samples were collected in sterile paper bags.

- ***Soil analyses***

The basic soil parameters related to soil microflora were determined. Modified Tyurin method (Filcheva & Tsadilas, 2002) was used to determine organic carbon content. Total

nitrogen was measured using the Kjeldahl digestion method (ISO 11261). Soil acidity ($pH_{(H2O)}$) was determined using the ISO 10390 standard. The mechanical composition was determined in the field using field methods (Donov et al., 1974). Soil bulk density was determined using the Kachinski method (Donov et al., 1974).

- ***Microbiological analyses***

Microbiological analyses were conducted within 24 h of sample collection. Prior to analysis, samples were stored at 4°C. Microbiological analyses included determination of total microbial number as a basic indicator of soil biogenicity (Redžepović et al., 2012). The amount of major microbial groups in the two investigated horizons was also determined to trace potential trends in the dominant groups. Microbiological analyses were conducted through successive dilutions and subsequent plating on suitable solid nutrient media in Petri dishes, followed by cultivation. Nutrient agar was used for culturing non-spore-forming bacteria, and the cultures were incubated for 48 h at 24°C (Küsel et al, 1999; Davis et al, 2005). The samples were pasteurized for culturing spore-forming bacteria, followed by plating on Nutrient agar and incubation for 48 h at 24°C (Küsel et al, 1999; Davis et al, 2005). Actinomycetes were cultured using Actinomycete isolation agar for 7 days at 35°C, and fungi were cultured on Czapek-Dox agar for 7 days at 30°C (Küsel et al, 1999; Davis et al, 2005). Colony development was recorded for all investigated microbial groups in terms of colony forming units (CFUs) per gram of absolutely dry soil.

- ***Statistical data processing***

Results were statistically analyzed using the StatSoft Statistica 12 software program at 95% significance thresholds.

## Results and Discussion

The obtained data for the studied basic soil parameters, as well as some additional characteristics, are shown in Table 1.

The studied soils are located at an altitude ranging from 185 m to 615 m. Based on their morphological features, they are classified as Luvisols according to WRBSR (Schad et al., 2014). The results for the recorded acidity of the studied soils indicate the presence of both strongly acidic soils (SP4 and SP6) and weakly alkaline soils (SP2). The acidity of the analyzed samples is dynamic and varies from 4.6 to 7.5 for the A horizon. The data for the B horizon show pH values ranging from 4.2 to 6.9. In four of the profiles, an increase in acidity with increasing depth is observed (SP1, SP3, SP4, and SP6). In soil profiles 2 and 4, the A horizon is more acidic compared to the B horizon. In conclusion, the mineral soil is dominated by acidic products of soil-forming processes.

The content of organic carbon and total nitrogen decreases with depth in all studied soils. The content of organic carbon in the A horizon varies from class 3 (medium) for SP1, SP3, and SP6 to class 4 (high) for SP2, SP4, and SP5 according to the Vanmechelen scale (Vanmechelen et al., 1997). Soils with a high content of total nitrogen (SP3, SP4, SP5, SP6) prevail - class 4 on the Vanmechelen scale (Vanmechelen et al., 1997). SP 2 has a very high nitrogen content, while SP 1 has a medium content of total nitrogen. The studied soils are well stocked with both organic carbon and total nitrogen as a whole. These data are not surprising considering the natural nature of the studied area. The data from the conducted microbiological analyses are shown in Table 2.

When analyzing the microbiological indicators, a standard deviation analysis was performed. At the A horizon, the values are dispersed in a wide range, while at the B horizon, the values are grouped within closer limits. The quantity of total

**Table 1. Characteristics of the soil profiles**

| | Altitude | $pH_{(H2O)}$ | Org. C, $g.kg^{-1}$ | Total N, $g.kg^{-1}$ | Mechanical composition | Density, $g/cm^3$ | Vegetation |
|---|---|---|---|---|---|---|---|
| I A | 185 | 5.8 | 24.2 | 2.06 | Sand-dusty | Low density (0.75) | *Q. frainetto, P. nigra, F. ornus* |
| IB | | 4.6 | 11.695 | 1.30 | clay-sandy-loamy | Very High density (1.52) | |
| II A | 390 | 7.4 | 73.67 | 5.98 | Sand-dusty | High density (1.11) | *Q. cerris, C.betulus, F. ornus* |
| IIB | | 7.5 | 38.1 | 3.87 | clay-loamy | High density (1.29) | |
| III A | 480 | 5.8 | 35.43 | 3.53 | loamy | Low density (0.96) | Meadow vegetation |
| III B | | 5.5 | 12.445 | 1.77 | clay-loamy | High density (1.22) | |
| IV A | 510 | 4.9 | 54.95 | 3.60 | Sand-dusty | High density (1.30) | *C.betulus, Q. robur, F. ornus* |
| IV B | | 4.6 | 18 | 2.35 | Clayey-sandy | Very High density (1.69) | |
| V A | 610 | 6.2 | 45.33 | 3.77 | loamy | Low density (0.64) | Meadow vegetation |
| V B | | 6.9 | 20.84 | 2.26 | clay-loamy | High density (1.28) | |
| VI A | 615 | 4.6 | 38.38 | 2.85 | Clayey-sandy | Low density (0.89) | *C. betulus, F. sylvatica* |
| VI B | | 4.2 | 7.725 | 1.34 | Sandy-clayey | High density (1.19) | |

**Table 2. Microbiological characteristics of soil horizons**

| | Total microbial number | Sporforming bacteria | Non-spore forming bacteria | Actinomycetes | Fungi |
|---|---|---|---|---|---|
| I A | 17.33 ± 15.80 | 4.77 ± 3.87 | 4.40 ± 19.37 | 4.31 ± 2.99 | 3.85 ± 1.39 |
| I B | 3.25 ± 1.51 | 0.15 ± 1.38 | 2.45 ± 1.33 | 0.41 ± 0.49 | 0.23 ± 0.08 |
| II A | 50.69 ± 15.80 | 1.03 ± 3.87 | 47.51 ± 19.37 | 1.89 ± 2.99 | 0.26 ± 1.39 |
| II B | 5.01 ± 1.51 | 0.16 ± 1.38 | 4.52 ± 1.33 | 0.26 ± 0.49 | 0.07 ± 0.08 |
| III A | 40.01 ± 15.80 | 1.94 ± 3.87 | 30.51 ± 19.37 | 6.13 ± 2.99 | 1.42 ± 1.39 |
| III B | 3.72 ± 1.51 | 0.39 ± 1.38 | 1.76 ± 1.33 | 1.45 ± 0.49 | 0.13 ± 0.08 |
| IV A | 18.11 ± 15.80 | 2.59 ± 3.87 | 5.91 ± 19.37 | 9.39 ± 2.99 | 0.22 ± 1.39 |
| IV B | 1.81 ± 1.51 | 1.09 ± 1.38 | 0.54 ± 1.33 | 0.15 ± 0.49 | 0.03 ± 0.08 |
| V A | 13.88 ± 15.80 | 7.60 ± 3.87 | 2.18 ± 19.37 | 3.67 ± 2.99 | 0.44 ± 1.39 |
| V B | 4.04 ± 1.51 | 0.48 ± 1.38 | 3.05 ± 1.33 | 0.50 ± 0.49 | 0.00 ± 0.08 |
| VI A | 13.32 ± 15.80 | 11.11 ± 3.87 | 0.18 ± 19.37 | 1.23 ± 2.99 | 0.79 ± 1.39 |
| VI B | 6.24 ± 1.51 | 3.74 ± 1.38 | 2.25 ± 1.33 | 0.19 ± 0.49 | 0.06 ± 0.08 |

microflora in the investigated soils ranges from 13.32 to 50.69 × $10^5$ CFU/g dry soil for the A horizon. For comparison, lower microbial abundance has been established for the Cambisols in other studies (Grigorova-Pesheva, 2020). For the B horizon, the values are much lower, ranging from 1.81 to 6.24 × $10^5$ CFU/g dry soil, indicating a strong decrease in soil microflora with a depth of over 10 times. Similar results for the decrease in microbial biota in the soil profile have been obtained for different types of forest soils worldwide (Fierer et al., 2003; Tripathi et al., 2018; Taylor et al., 2002; Eilers et al., 2012). These data demonstrate the difficulty in developing the microbial biota in the lower soil horizons. These results highlight the need for ecologically sound management of forest stands and minimizing the entry of heavy machinery. The use of heavy machinery leads to strong soil compaction and changes in the density and structure of the A horizon. This, in turn, leads to the disruption of soil gas exchange, the reduction of oxygen content, and even more difficult development of soil microorganisms (Wilpert, 2022; Hildebrand & Schack-Kirchner, 2002).

The largest decrease in the total microbial count is observed in SP3, where the decline is over 10 times. In contrast, in SP6, the decrease in microbial abundance is only twice. This is quite interesting since SP6 has the strongest decrease in the quantity of organic carbon (over 4 times) at depth. In their study, Khatoon et al. (2017) demonstrate that increasing organic carbon leads to an increase in the total number of microorganisms in the soil. These data required an analysis of the relationship between the total microbial number of the investigated soils and its relationship with organic carbon (Figure 1 and Figure 2).

The data indicate a weak positive statistical relationship between the organic carbon content in the soil and microbial abundance in the A horizon (r=0.55). Data for the B horizon show a complete absence of correlation. While organic car-

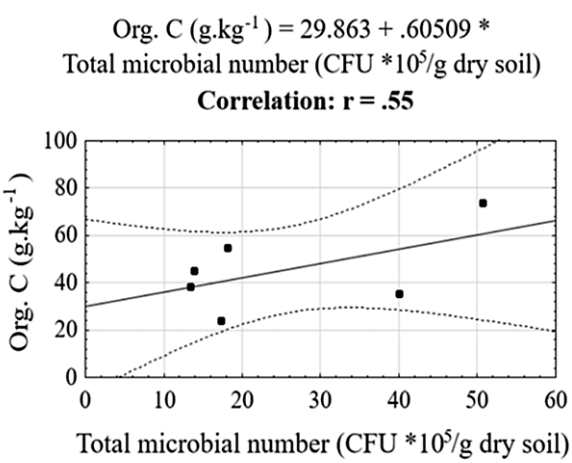


**Fig. 1. Correlation between Total microbial number (CFU*$10^5$/g dry soil) and organic carbon content in A horizon**

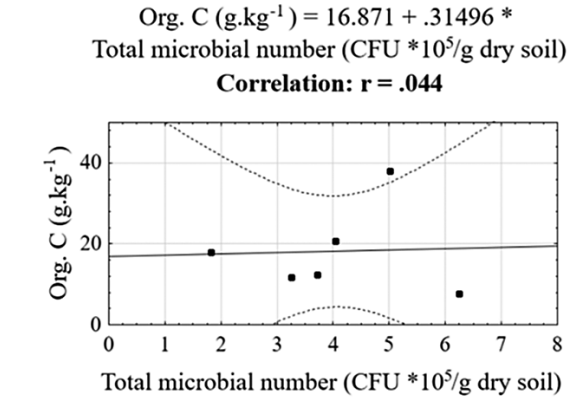


**Fig. 2. Correlation between Total microbial number (CFU*$10^5$/g dry soil) and organic carbon content in B horizon**

bon influences microbial abundance in the A horizon, it is not directly related to the dynamics of the microbial community in the B horizon. These data contradict other studies where the impact of organic carbon content is stronger in lower soil horizons, in Cambisols (Grigorova-Pesheva et al., 2022).

These findings necessitate a multifaceted approach and analysis of the impact of other soil parameters such as total nitrogen content, pH, mechanical composition, and density. In order to perform a comprehensive analysis, a correlation was sought between the aforementioned parameters and changes in the total microbial number. (Figure 3 and Figure 4).

In analyzing the results, it is assumed that the process of soil acidification in forest ecosystems is natural, and the indigenous soil microflora is adapted to it. The statistical analyses show a positive correlation between the microbial abundance in the A horizon and the soil pH (r = 0.77), while such correlation is absent in the B horizon (r = -0.17). These results again demonstrate the need for a comprehensive approach to analyzing the state of forest soil microbial communities. It is important to establish the dynamics of microbial abundance and the main driving factors that affect microbial communities. However, the available literature presents conflicting claims regarding the importance of soil pH as a driving factor for microbial abundance. While some authors consider pH as a key factor (Shen et al., 2013), others have concluded that soil pH is not a sufficient indicator of microbial biocenosis development and a comprehensive approach is needed (Lauber et al., 2009; Cho et al., 2016).

The analysis of the relationship between total nitrogen and total microbial number shows similar trends to the correlation

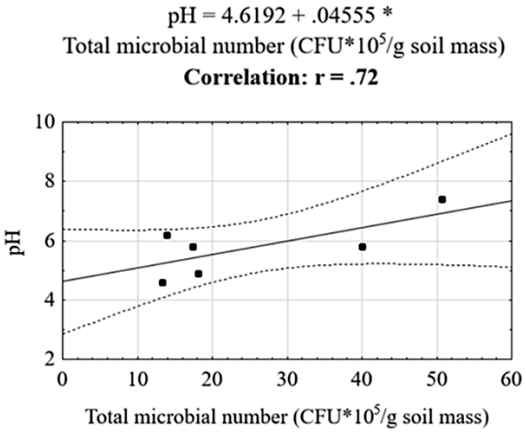


**Fig. 3. Correlation between Total microbial number (CFU*10$^5$/g dry soil) and pH in A horizon**

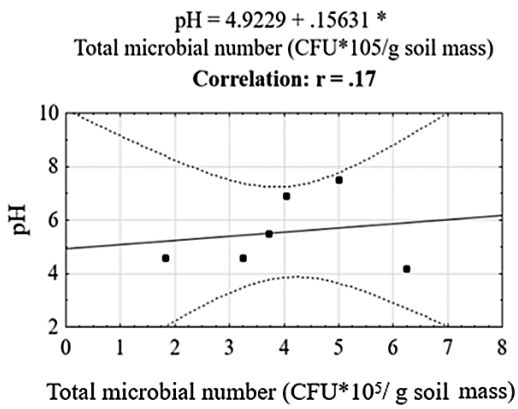


**Fig. 4. Correlation between Total microbial number (CFU*10$^5$/g dry soil) and pH in B horizon**

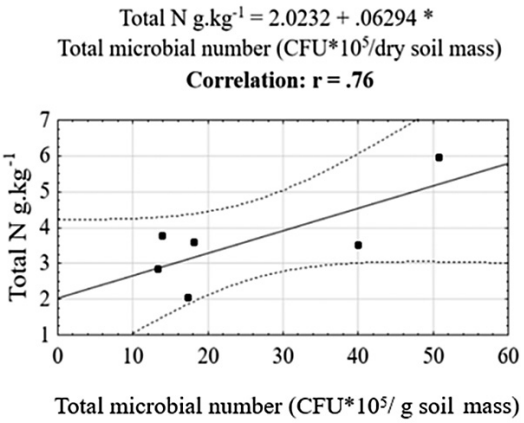


**Fig. 5. Correlation between Total microbial number (CFU*10$^5$/g dry soil) and total nitrogen content in A horizon**

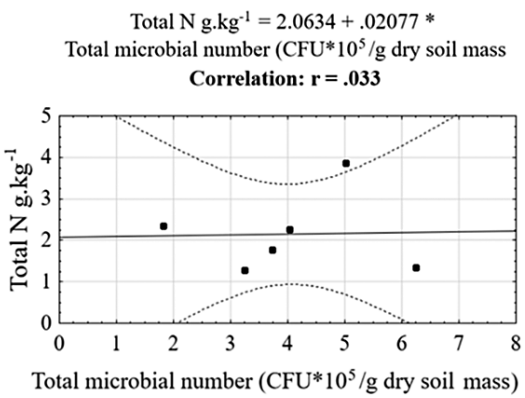


**Fig. 6. Correlation between Total microbial number (CFU*10$^5$/g dry soil) and total nitrogen content in B horizon**

between microbial abundance and organic carbon content. Again, there is a high correlation in the A horizon, while it is completely absent in the B horizon. (Figure 5 and Figure 6).

In her work, Egamberdieva (2011) indicates that changes in the nitrogen cycle are closely related to changes in soil microbial activity. From the results obtained, it can be concluded that the main nitrogen transformation in the studied soils occurs in the A horizon. Higher levels of total nitrogen do not contribute to an increase in soil microbial number in the depth of the soil profile.

Based on our research and statistical analysis, the correlation between the dynamics of soil microbial total number and the studied environmental factors varies. Regarding the A horizon of Luvisols, the most influential factor is the content of total nitrogen, followed by pH and organic carbon. In contrast, all those parameters show no correlation with the B horizon of Luvisols. These data require additional analyses of the factors that affect and lead to decline in soil microbial communities in the depth of the soil profile.

For this purpose, data on the mechanical composition and density of the soil and their relationship with the total microflora of the studied soils were analyzed. In the A horizon, mechanical composition and density do not have a clear impact and expressed correlation with the total microbial number. However, in the underlying B horizon, the heavier mechanical composition and greater soil compaction have a suppressive effect. This is due to fundamentally deteriorated soil conditions for soil microorganisms at greater depth, primarily due to the lack of easily assimilated organic components. Strong soil compaction leads to a reduction in its pore spaces and a worsened water-air regime. Based on the results obtained and all the conducted analyses, we consider that the mechanical composition in the B horizon is a factor that has a negative impact on soil microbial biomass and hinders its activity, given the lack of correlation with other data.

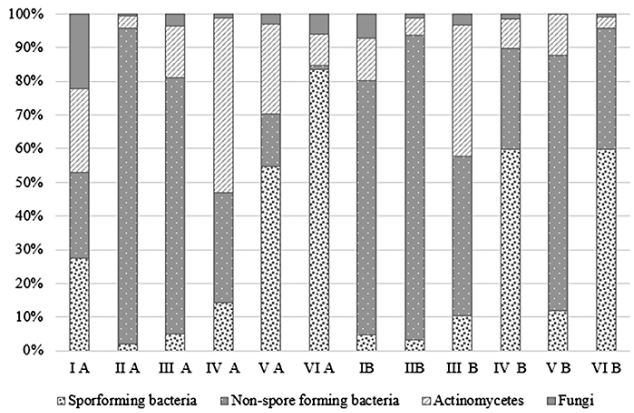


**Fig. 7. Percentage share of microbial groups in the total microbial number, %**

For a more complete characterization of the studied soils, an analysis of the dynamics in the percentage distribution of the basic microbial groups in depth was conducted (Figure 7).

There was no established trend in the redistribution of the percentage share of microbial groups with depth. This necessitates further analyses to determine the mechanism of domination of individual microbial groups, which will allow for tracking the process and dynamics of transformation of organic components in individual soil horizons.

## Conclusion

In this study, we aimed to provide basic information about the microbiological characteristics of forest soils of classified as Luvisols within the Western Stara Planina Mountain. For this purpose, we determined the quantity of individual microbial groups, recorded the total microbial number, and analyzed the main soil parameters related to its biogenicity - organic carbon content, total nitrogen content, pH, mechanical composition, and density. Based on the conducted research, we found that the biogenicity of the A horizon is multiple times higher than that of the B horizon, as in SP2 and SP3 the increase is more than 10 times. For the A horizon, we found that the strongest driving factor associated with increased microbial abundance is total nitrogen ($r = 0.76$), followed by pH($r = 0.72$), and organic carbon ($r = 0.55$). According to the B horizon, all those indicators show no correlation ($r_{(pH)} = 0.17$; $r_{(org.C)} = 0.04$; $r_{(total\ N)} = 0.03$).

Our study found that in the B horizon, the mechanical composition and soil density have an impact on biogenicity. With a heavier mechanical composition (bulk density >1.3 g/cm$^3$) and strongly compacted soil, the number of microorganisms decreases and this decrease is more than 3 times for the soil with the highest value of bulk density (SP4). There was no established trend in the redistribution of the percentage share of microbial groups with depth. The information in this study can be used to develop strategies for sustainable forest management practices that minimize negative impacts on soil microbial communities.

***Acknowledgments***

This study was supported by the Ministry of Education and Science of the Republic of Bulgaria within the National Program Young Scientists and Postdoctoral Students, adopted by Decision No. 206/07.04.2022 of the Council of Ministers.

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*Received:* **July, 20, 2023**; *Approved*: **September, 28, 2023;** *Published:* **October, 2024**