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Analysis of the Microbiological Characteristics of Soils from the Territory of Vitosha Nature Park, According to the Differences in the Environmental Conditions

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Abstract. An analysis of the microbiological status of 13 soil profiles from the territory of Vitosha Nature Park was carried out. Eight of the studied soils were defined as *Dystric* Cambisols (Brown Forest soils), and the other five as Umbrisols (Mountain Meadow soils). In order to assess the biogenicity and the prevailing processes of transformation of soil organic matter, the total microbial number was determined, as well as the percentage distribution of the main microbiological groups (spore-forming bacteria, non-spore-forming bacteria, actinomycetes and micromycetes) of the *A* horizon of the studied soils. The highest microbial abundance was observed in the altitude range from 1000 m a.s.l. up to 1600 m a.s.l. In higher altitudes there was a decrease in the total microbial number. At an altitude of over 2000 meters, soil microorganisms are strongly suppressed. According to the data received in the process of the study it has been found that at such altitudes, higher levels of organic carbon and total nitrogen do not result in greater microbial abundance. Altitude dynamics affects the values of the total microbial number, but no relationship has been established with the distribution of microbial groups. The results show a higher microbial abundance in the tested areas under deciduous vegetation compared to the ones under coniferous and grass vegetation. It was found that the lower pH values cause a greater abundance of the micromycete group.

Key words: microorganisms, total microbial number, forest soil, vegetation cover, altitude.

Introduction

Vitosha Mts. is among the few Bulgarian mountains that are subjected to immediate anthropogenic pressure, especially in summer, when there is a mountain-valley circulation, typical of the northern slopes of the mountain, where slope winds carry polluted air masses from the nearby capital – Sofia (Kadinov, 2021a). However, according to a study by Kadinov (2019), the presence of fine dust particles has a background status, with less than 1% due to transport from Sofia and does not indicate exceedances of particulate matter (PM) deposition rates on the soil. This is confirmed by the seasonal dynamics of the PM, whose maximum is in the summer, while in the capital it is in the winter (Kadinov, 2021b).

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This is a prerequisite for analysis of the territory of Vitosha Nature Park as an environmentally friendly ecosystem.

Soil microorganisms are an essential part of the forest food network. They play a major role in the transformation of soil organic matter and the preparation of organic component transformation for inclusion in the biogeochemical cycle. A large number of studies are focusing on the important role of microorganisms as predictors of environmental change (Baldrian et al., 2012; Trivedi et al., 2013; Jansson & Hofmockel, 2020). Due to their sensitivity and adaptability, microorganisms can serve as an indicator of soil health in forest areas and from there they can provide timely information on the state and dynamic changes occurring in forest ecosystems as a result of global climate change. It is the rapid response to changes in the environment that allows microorganisms to be used as an indicator of changes in soil status (Kennedy et al., 1995; Pankhurst et al., 1995).

The abundance and distribution of microbial groups are important indicators of soil health and soil quality (Kennedy et al, 1995; Kibblewhite et al., 2008; Sharma et al., 2011).

The presence of high biogenicity of forest soils implies the processes of transformation of organic matter at a rate that provides the necessary nutrients for plants and maintains a regular cycle of substances.

Soil microorganisms are a key element of forest soils. They are involved in the transformation of plant materials into humus (Vanmechelen et al., 1997).

A number of studies have examined the influence of soil parameters on the abundance, activity and dynamics in the distribution of soil microorganisms. The main components influencing the microbial soil biota are considered to be the organic matter content, the content of total nitrogen and organic carbon and the acidity of the soil habitat (Fierer & Jackson, 2006; Lauber et al., 2009; Romanowicz et al., 2010).

Shen et al. (2013) point out that bacterial diversity and community composition are influenced by soil pH. The pH(H_2O) in the range of 5 and 7 was found to be optimal for microbiological activity and nutrient availability (Vammechelen et al., 1997). Although pH is an important factor in the development of microorganisms, Cho et al. (2016) and Lauber et al. (2009) established that it is not fundamental to soil biogenicity. Vegetation, and in particular the combination of tree species, influences soil microbiological activity (Algusto et al., 2015; Snajdr et al., 2013). The microbial diversity and percentage distribution of the main microbiological groups are influenced by the different dominant plant species (Prescott & Grayston, 2013).

Margestin et al. (2008) found that microbial activity decreases with increasing altitude and with decreasing temperature in soils of alpine and subalpine zones. Opposite data were also obtained in the study of Siles et al. (2016), in which an increase in abundance with an increase in altitude was reported in the study zones due to higher amounts of organic carbon, total nitrogen and other nutrients. The same study states that there are no stable trends in the composition and distribution of microorganisms with increasing altitude. In a large number of studies (Fritze et al., 2000; Taylor et al., 2002; Eilers et al., 2012), it has been stated that microbial abundance is highest in the *A* horizon. In order to have comparability of the results in the analysis of different soil units, the present study focuses on the biogenicity reported in *A* horizon.

Heterogeneity in forest soils determine various parameters for the development of soil microbial biota. The main aim of the present study is to analyze some of the microbiological parameters of forest soils from the territory of Vitosha Nature Park and their interrelations with soil parameters and the altitude.

Materials and Methods

The subject of the study is soils from the territory of Vitosha Nature Park. The studied territory covers almost the entire territory of Vitosha Mountain. According to Sabev & Stanev (1963) the territory of the Park falls into two climatic regions - the temperate continental climatic subregion - Climatic region of the hills and mountainous parts of Western Central Bulgaria and Mountain climatic region. Frequent short-term precipitations are observed under the influence of intramass convective clouds. As a result, summer precipitation significantly exceeds winter ones (Koleva-Lizama, 2018). The duration of the period with air temperature above 10 °C varies from 93 to 144 days, and the temperature sum above 10 °C is between 1130 °C and 2030 °C (Koleva-Lizama, 2018).

Vitosha Nature Park is characterized by the presence of a vertical band in relation to the vegetation cover. According to Velchev (2002) on the territory of the Park were expressed all plant belts except the alpine. Vitosha Nature Park is characterized by a rich and diverse flora. Of the higher seed plants, 1500 species have been identified, which represents half of the flora of Bulgaria (Tsavkov & Dimova, 2001). Research by Stefanov (1939) shows that among the deciduous species in Vitosha mountain pure beech (*Fagus sylvatica* L.) forests are with the highest prevalence. The current area of beech forests is 40.5% of the territory (Management Plan of Vitosha Nature Park, 2014). According to the data of the Management Plan of Vitosha Nature Park, 2014 the coniferous tree species occupy 34.7% of the afforested area, and with the largest share is *Pinus sylvestris* - 18.4%.

According to Ninov (2002) the territory of Vitosha mountain falls into 2 soil provinces. The first is Vitosha-Srednogorian mountain province, which is dominated by *Dystric – Eutric* Cambisols and Umbrisols, and the second - Mediterranean soil region, High Vitosha-Rila-Pirin-Rhodope province. Cambisols and Umbrisols are widespread in it.

The most widespread are the *Dystric – Eutric* Cambisols, which occupy the range between 600 m - 800 m above sea level as the lower limit and 1800 m above sea level as the upper limit (Petkov et al., 1983).

Soil samples were taken in November 2021, to analyze the peak development of microbial communities at the end of the autumn season. Microbiological samples were collected using sterile instruments. The collected soil samples were placed in sterile paper bags according to all sterility guidelines. Thirteen soil profiles were surveyed. Soil material for analysis was collected from *A* horizon. Laboratory analyzes were performed to determine the main soil characteristics, as follows - soil acidity (pH(H_2O)) - ISO 10390; the organic carbon content - modified method of Turin (Filcheva & Tsadilas, 2002), and the Kjeldahl digestion (ISO 11261) method was carried out to determine total nitrogen content.

Microbiological studies of the collected soil samples were performed immediately after their collection. Basic microbiological parameters related to soil biogenicity were analyzed - total microbial number, determination of percentage distribution of different microbial groups (spore forming bacteria, non-spore forming bacteria, actinomycetes and micromycetes). Koch's method was used to determine the total microbial number. The method includes successive dilutions and subsequent inoculation on appropriate elective agar medium (Davis et al., 2005). For the determination of spore-forming bacteria, the soil extract was previously pasteurized. Nutrient agar was used for the cultivation of spore-forming bacteria and non-spore-forming bacteria. Actinomycete isolation agar was used to isolate actinomycetes. Capek Dox agar is used for the isolation of micromycetes (Parks & Roland, 1997). The count was expressed as colony forming units per gram dry soil (CFU/g dry soil) under logarithm (lg). Software was used to perform the statistical data processing - StatSoft Statistica 12 under significance thresholds 95%.

Results

Thirteen soil profiles were studied and analyzed. The morphological characteristics of the soil profiles are shown in Table №1. The soils from tested areas (TAs) 1-8 are determined as *Dystric* Cambisols and the soils form tested areas 9-13 as Umbrisols, according to the WRBSR (2006,2007).

The considered soil profiles are set at an altitude of 1032 m to 2261 m in order to cover the variety of altitude gradient of the Vitosha Nature Park (Fig. 1).

The soils of all tested areas are acidic, which is due to the leaching of the bases from the profile. The more acidic reaction of the soil is due to the increased amount of precipitation and the decrease in temperatures with increasing altitude. This trend is clearly expressed when considering the reported acidity of soils in the range 1500-1800 m altitude compared to soils in the range 1000-1500 m altitude. This acidification is also due to the fact that at higher altitudes mainly coniferous vegetation predominates, which determines the course of acidification processes in soils.

Table 1. Main characteristics of soil profiles.

TA	**Soil Unit**	**Location**	**Altitude (m)**	**Exposure**	**Vegetation**	**Soil horizons**	**pH**	**Humus (%)**	**Org. C g.kg^{-1}**	**Total N g.kg-1**	**C:N**
1.	Dystric Cambisols	42°38'29.73"N 23°13'4.56"E	1032	N	*F. sylvatica*	A	5.90	3.08	18.20	1.28	14
2.	Dystric Cambisols	42°38'17.00"N 23°13'9.27"E	1057	W	*Q. petraea* *F. sylvatica*	A	5.50	3.20	18.72	1.49	13
3.	Dystric Cambisols	42° 27' 16'' N, 23° 13' 14'' E	1151	S	*Q. petraea* *Q. cerris* *Q. frainetto*	A	5.40	3.30	19.50	1.33	15
4.	Dystric Cambisols	42° 29' 53'' N, 23° 12' 51'' E.	1163	S	*C. betulus* *C. monogyna* *C. avellana* *Q.s petraea*	A	5.90	5.83	33.80	3.83	9
5.	Dystric Cambisols	42° 31' 11'' N, 23° 21' 9'' E	1171	S	*P. nigra* *Q. cerris*	A	5.30	7.60	44.10	2.42	18
6.	Dystric Cambisols	42°36'28.37"N 23°17'50.53"E	1289	N W	*F. sylvatica*	A	5.30	4.46	25.90	2.00	13
7.	Dystric Cambisols	42°35'50.79"N 23°14'12.26"E	1565	N W	*P. abies*	A	4.30	9.39	54.50	4.74	12
8.	Dystric Cambisols	42°35'20.81"N 23°18'37.25"E	1598	SE	*P. sylvestris*	A	5.00	10.56	61.40	5.26	12
9.	Umbrisols	42°35'17.79"N 23°14'47.25"E	1788	SE	*J. communis*	A	4.60	6.78	38.17	5.85	7
10.	Umbrisols	42°35'25.36"N 23°17'24.96"E	1793	E	*P. peuce*	A	4.70	6.98	39.98	5.93	7
11.	Umbrisols	42°35'26.48"N 23°17'24.99"E	1793	E	*F. valida*	A	4.60	7.18	41.04	5.60	7
12.	Umbrisols	42°35'14.17"N 23°17'19.88"E	1845	SE	*P. mugo*	A	5.00	12.01	69.8	5.31	13
13.	Umbrisols	42°33'49.28"N 23°16'48.22"E	2261	S	*N. stricta,* *Vaccinium sp,* *Juniperus sp.*	A	4.50	20.02	117.4	9.4	12

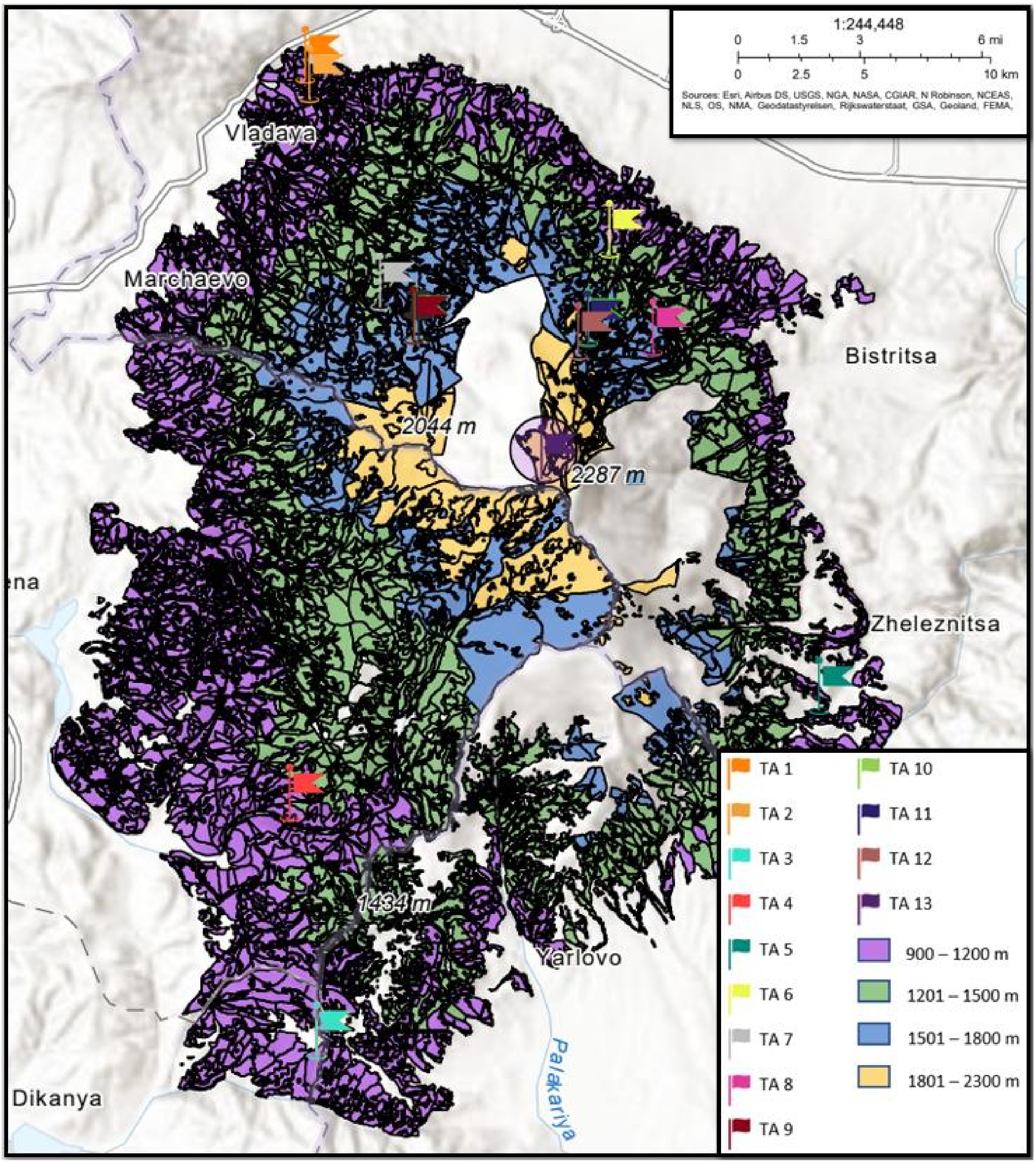

Fig. 1. Location of tested areas (TAs) on the territory of Vitosha Nature Park.

Correlation analysis was performed at 95% significance thresholds to assess the relationship between elevation increase and changes in humus percentage. The reported correlation coefficient is 0.83. As a result of this relationship, there is a direct proportionality between changes in altitude and organic carbon content (r = 0.82). Soils located at higher altitudes contain more organic carbon. The obtained data on the content of organic carbon in the studied soils show that it varies from low to very high (according to the Vanmechelen scale, 1997).

The strongest correlation was found between the increase in altitude and the change in total nitrogen content. The correlation is positive, in which the content of total nitrogen increases with increasing altitude (r = 0.96). The nitrogen content was assessed as average in TA1,2,3,5 and 6, as high in TA4 and TA7 and as very high in TA 8,9,10,11,12,13 according to the scale of Vanmechelen (1997). The organic carbon/total nitrogen ratios are in the range of 7-18. They are rated as very low to value 16 and as low to value 18 on the Vanmechelen scale. The results obtained show that the decomposition prosses are in an advanced stage.

Soil biogenicity is a dynamic indicator that is related to the parameters of the soil. The dynamics in the number and percentage distribution of soil microorganisms is an indicator of the activity of the transformation of soil organic matter. The results for the total microbial number and the distribution of the microbial community reflect the biogenicity of the studied soils. The results of the analyzed microbiological indicators are shown in Table 2.

Table 2. Total microbial number and number of main microbial groups in lg CFU/g dry soil.

TA	Total microbial number	Spore forming bactiria	Non-spore forming bacteria	Actynomicetes	Micromycetes
1.	6.32 ± 0.15	5.58 ± 0.16	5.72 ± 0.19	4.88 ± 0.23	5.13 ± 0.37
2.	6.36 ± 0.15	5.62 ± 0.16	5.85 ± 0.19	4.62 ± 0.23	5.09 ± 0.37
3.	6.40 ± 0.15	5.96 ± 0.16	6.11 ± 0.19	5.00 ± 0.23	5.26 ± 0.37
4.	6.41 ± 0.15	5.88 ± 0.16	6.18 ± 0.19	5.10 ± 0.23	5.28 ± 0.37
5.	6.32 ± 0.15	5.88 ± 0.16	6.00 ± 0.19	5.17 ± 0.23	5.27 ± 0.37
6.	6.31 ± 0.15	5.90 ± 0.16	6.00 ± 0.19	5.06 ± 0.23	5.08 ± 0.37
7.	6.30 ± 0.15	5.84 ± 0.16	5.90 ± 0.19	5.49 ± 0.23	5.30 ± 0.37
8.	6.26 ± 0.15	5.73 ± 0.16	6.04 ± 0.19	4.93 ± 0.23	4.88 ± 0.37
9.	6.10 ± 0.15	5.71 ± 0.16	5.74 ± 0.19	5.26 ± 0.23	4.43 ± 0.37
10.	6.11 ± 0.15	5.51 ± 0.16	5.65 ± 0.19	5.15 ± 0.23	5.59 ± 0.37
11.	6.18 ± 0.15	5.69 ± 0.16	5.76 ± 0.19	5.42 ± 0.23	5.21 ± 0.37
12.	6.00 ± 0.15	5.54 ± 0.16	5.69 ± 0.19	5.15 ± 0.23	4.30 ± 0.37
13.	5.96 ± 0.15	5.51 ± 0.16	5.59 ± 0.19	5.17 ± 0.23	4.72 ± 0.37

The total microbial number of microorganisms in the tested accumulative horizons –varied from 5.96 lg CFU/g dry soil to 6.41 lg CFU/g dry soil.

The tested area (TA) 4 of Dystric Cambisols at an altitude of 1163 m, under mixed deciduous vegetation, stands out with the highest biogenicity. The tested area with the lowest total microbial count is at the highest altitude (TA 13). The obtained results show a greater microbial abundance under deciduous or mixed vegetation, compared to conifers and grasslands. This is a result of both greater diversity and reduced competition in soils under deciduous forests, and of the reduced temperature and reduced activity of microbial communities at higher altitudes, where coniferous vegetation predominates.

Statistical processing of the results was performed, and a correlation between the microbiological indicators and the environmental parameters was found.

Statistical evaluation of the results shows the presence of a high statistical coefficient between the biogenicity of the studied soils and the increase in altitude at 95% significance thresholds. The correlation is negative. The high correlation coefficient (r = - 0.92) determines the strong influence of altitude on the amount of soil microflora (Fig. 1). As a redistributor of the other factors of the relief, the changes in the altitude lead to changes not only in the temperature and water regime, but also to changes in the vegetation cover. The obtained results clearly show that at altitudes above 1600 meters, the soil microbial abundance is reduced, and above 2000 meters, the soil microbial biota is strongly suppressed (Fig. 2).

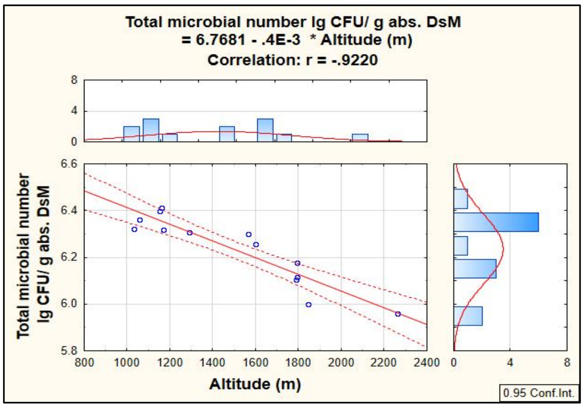

Fig. 2. Statistical relationship between total microbial number and altitude.

A correlation was made between the pH values and the reported biogenicity of the tested areas (Fig. 3). The correlation coefficient is relatively low (0.67), which shows a weak relation between pH values and differences in the microbial abundance of the studied soil profiles. The data obtained correspond to other studies in which it was found that soil acidity is not a major factor in its biogenicity (Cho et al., 2016; Lauber et al., 2009).

Although Khatoon et al. (2017) established that organic carbon is a source of nutrients and plays a vital role in maintaining the activity of soil microflora, its content and impact on soil microbial biota should be considered comprehensively together with other factors of the environment.

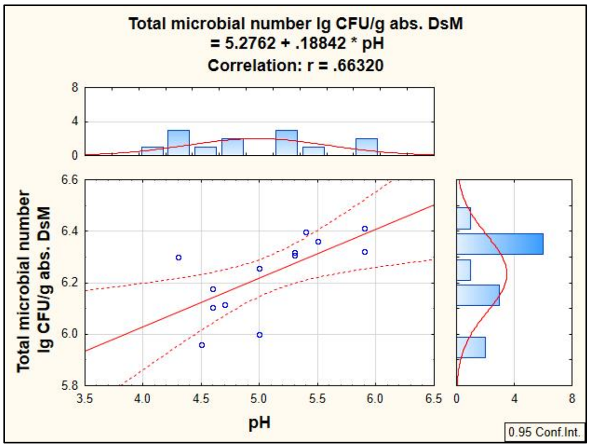

Fig. 3. Statistical relationship between total microbial number and pH.

The results of the correlation analysis show that there is an inverse relationship between the content of organic carbon and the abundance of soil microorganisms in the studied soil profiles (Fig. 4). This negative correlation is related to the positive correlation between the increase in altitude and the increase in the content of organic carbon. The obtained relationships show that although the carbon content increases with increasing altitude, the microbial biota decreases, especially above 1800 m. This decrease in the total microbial number in the high parts of Vitosha Nature Park is associated with an increase in the suppressive effect of environmental factors - mainly the temperature decrease.

When looking for a correlation between the total biogenicity of the studied soil profiles, relative to the content of total nitrogen, we have found once again an inverse relationship (Fig. 5). Although the total nitrogen content increases with increasing altitude, this does not lead to an increase in microbial abundance. Again, the strong influence of the height gradient on the microbial abundance of forest soils stands out.

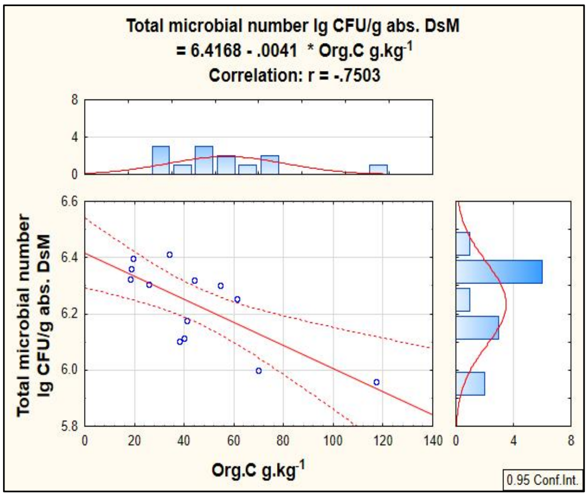

Fig. 4. Statistical relationship between total microbial number and organic carbon content.

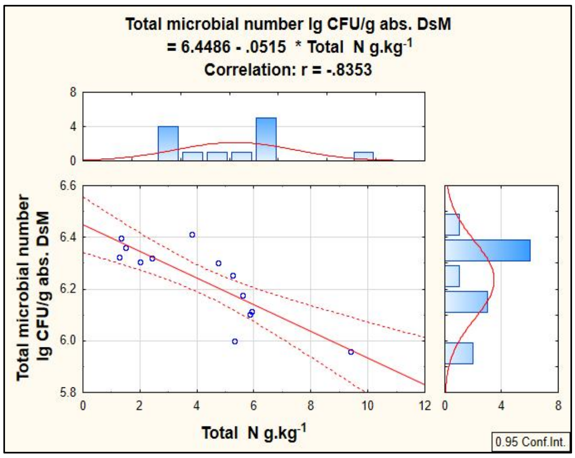

Fig. 5. Statistical relationship between total microbial number and total nitrogen.

In the present study, altitude stands out as one of the main factors related to the total microbial number of the studied soils.

Fig. 6 shows the composition of the microbial communities by groups as a percentage of the total microflora for the individual TAs., including acidity.

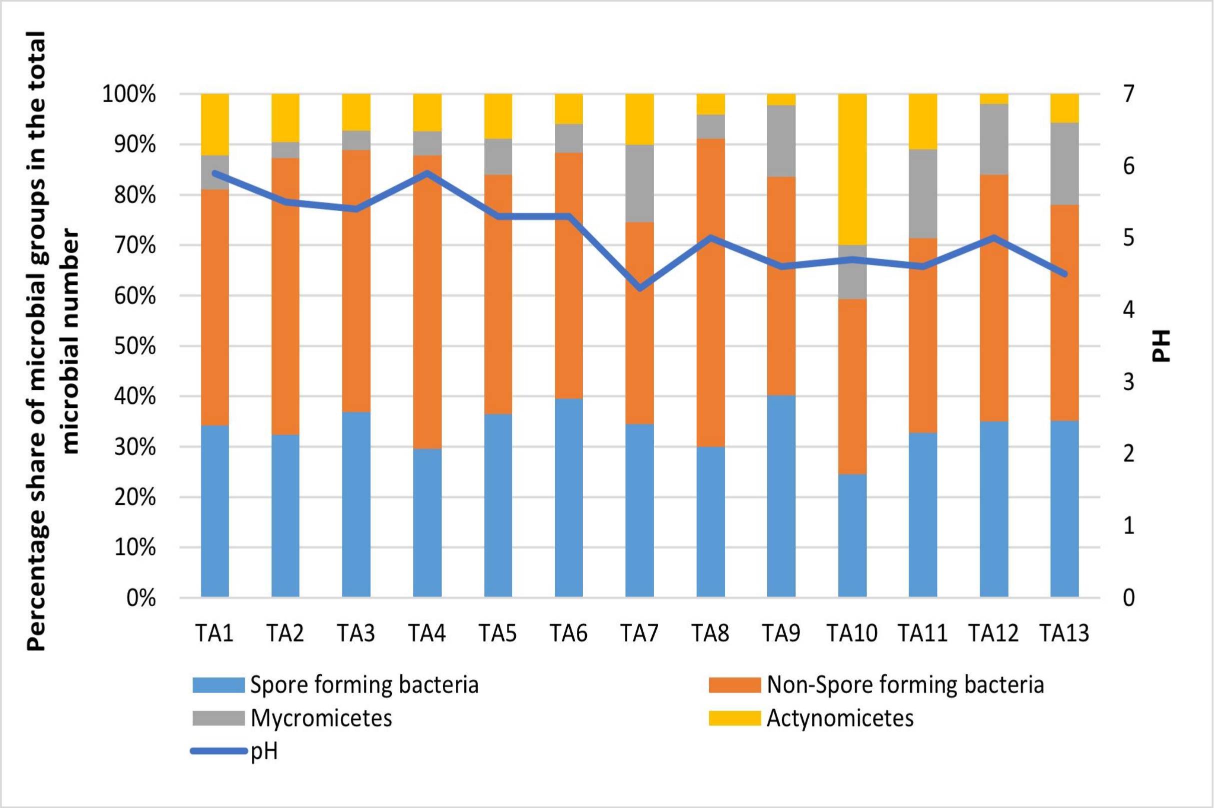

Fig. 6. Percentage share of microbial groups in the total microbial number, (%).

The predominance of a specific group of microorganisms in the soil shows the predominance of certain processes in the transformation of soil organic matter. Non-spore-forming bacteria dominate in all studied tested areas, followed by spore-forming ones. This distribution of microbial groups is an indication of the predominance of the processes of transformation of more easily degradable soil organic matter.

There is no established relationship between the distribution of the main microbial groups and a change in the content of organic carbon, total nitrogen or change in altitude. However, in terms of acidity, there is an increased percentage of micromycetes involved as part of the total microflora in soils with a pH below 5 (TA 7,9,10,11,12,13).

Discussion

One of the main tasks of the present study was to look for relationships between changes in the parameters of the studied soil profiles, changes in altitude and changes in microbial abundance and the relative participation of basic microbial groups in forest soil microbial community.

The study of soil microorganisms is a dynamic process. Their adaptability to the environment, together with their ability to react faster than all other participants in forest ecosystems, makes them an extremely interesting object of analysis. Numerous studies are being conducted around the world to find lasting links between soil microorganisms and the environment. Studies have been carried out to determine soil acidity as a driving factor for microbial abundance. (Lauber et al., 2009; Shen et al., 2013). Conducted are studies that prove the priority impact of the content of organic carbon (Khatoon et al., 2017) and total nitrogen (Egamberdieva, 2011) and others who point out that altitude should be considered as a fundamental factor for the development of soil microflora in forest areas, due to its major role in the distribution of other environmental factors (Margestin et al., 2008; Siles et al., 2016). All these studies demonstrate the need for a comprehensive and in-depth approach involving long-term research.

In the present study, we aimed to cover the maximum altitude range of the Park area, which would cover the diversity of soil microbial habitats. We studied the availability of a statistical relationship between soil parameters, changes in altitude and microbial abundance, including the dynamics of the distribution of basic microbial groups. The present study shows a significant negative correlation between microbial abundance and altitude increase. The results of our study show the main role that altitude plays in the abundance of microorganisms from the soils of Vitosha nature park.

Conclusions

We concluded that altitude is the environmental factor most strongly related to total microbial number. It was found that the greatest microbial abundance, occurs at an altitude of 1000 to 1600 m. The content of organic carbon and total nitrogen, although important for the development of soil microflora are strongly influenced by altitude and its role in redistribution of temperature, humidity and the predominant vegetation cover. Soil acidity is not associated with changes in microbial abundance, but an increased amount of micromycetes is found in more acidic soils, regardless of their altitude. Greater microbial abundance was found in the sample areas under deciduous vegetation compared to coniferous and grassy vegetation.

The present study, as well as all its results, can serve as a basis for conducting a multi-year analysis by seasons of the surveyed areas. This would make it possible to carry out a more in-depth analysis and to look for sustainable relationships over a long-term period of time, covering various environmental factors, potentially affecting soil microbial biota.

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