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STUDY OF MICROBIOLOGICAL STATUS OF SOILS IN BEECH PLANTATIONS

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Abstract

The main objective of the study is to investigate the microbiological status of soils in beech plantations of the first and second site index. Ten soil profiles were established in ten test areas with beech plantations. The study includes an analysis of the main physico-chemical and microbiological parameters of the ten soil profiles. In each test area, the site index, relative stocking and the average volume (m^3/ha) of the plantation was determined. The studied soils are of the Cambisols, Regosols and Rendzinas type. Soil samples were taken from the A and B (C) horizons. Basic indicators related to soil microorganisms were studied - humus (%), org.C mg/kg^{-1}, pH and mechanical composition. For determination of total microbial number and the amount of individual microbiological groups (bacteria, actinomycetes and fungi), the standard method of serial dilutions and subsequent inoculation was used. The results are reported in Colony-forming unit. A horizon has a greater microbial abundance than the underlying soil horizons, regardless of the considered soil type. There are no clear dynamics in the redistribution of the percentage participation of microbial groups at depth. There is no clear correlation between the microbial abundance and site index of the plantations. The highest microbial abundance was observed at an altitude above 1300 m (TA9 and TA10). Brown forest soils stand out with the highest average biogenicity. It was found that the percentage of microscopic fungi increases in acidic soils, while their amount in Rendzinas decreases below 1.0 × 10^5 CFU/g dry soil.

Key words*: soil, soil microorganism, forest ecosystems, total microbial number.*

INTRODUCTION

Forest ecosystems are of great importance for the Earth's biosphere, covering more than 1/4 of the land (Keenan et al., 2015). When determining the quality (site index) of the plantation, a number of factors are taken into account: the biological characteristics of the tree species, the rank of the tree in the plantation, the rate of growth, soil conditions, soil microorganisms, climatic conditions, growth space - expressed by the density and fullness of the stand, etc. (Callesen et al., 2006; Uroz et al., 2007; Packham et al., 2012; Kirchen et al., 2017). Tree species have specific requirements for environmental conditions and for soils. Growth and productivity of tree species depend on soil fertility, on the activity and abundance of soil microorganisms (Graham et al., 2016). Microorganisms participate in a number of complex processes of organic decomposition and humus synthesis, which determines their important role in the development and stability of forest ecosystems (Paul & Clark, 1989). Without their active participation, the circulation of substances and providing the ecosystem services by the forests is impossible (Strickland et al., 2009). There are studies that highlight the possibility of microorganisms being used as bioindicators for the state of forest ecosystems, given their rapid response to environmental changes. (Turco et al., 1994; Kennedy & Papendick, 1995; Dick, 1997.) Although there are several studies that consider ecosystem services considering the activity and abundance of soil microorganisms (Fonseca, 1990; Staddon et al., 1999; Zhang et al., 2020), there are still no sufficiently in-depth studies on the interrelationships between the condition of the plantation as reflected by its site index and the microbiological status of the soils, as their main characteristic.

The present study represents the pioneering analysis of main characteristics and microbiological status of the soils and the site index of beech plantations from the Vitosha Nature Park region.

MATERIALS AND METHODS

For the study, ten test areas (TA) were laid out on the territory of Vitosha Nature Park, Sofia

region, Bulgaria. The selection of Vitosha Natural Park was prompted by the available information on its background levels of pollution despite its proximity to the capital Sofia, making it a suitable site for studies in an ecologically clean environment (Kadinov, 2019; Kadinov, 2021a; Kadinov, 2021b).

In each test area, the tree stand was surveyed, and its site index, relative stocking (RS) and the average volume (AV) (m^3/ha) were determined was determined. Characterization of the soil type was carried out by making a soil profile. The main microbiological parameters of the soil were studied. The TAs are located at an altitude of 910 to 1445 m.

Soil samples were collected from each of the soil profiles for analysis. The samples were collected from the average depth for each of the investigated horizons. An average sample was formed from five points taken horizontally, at the selected depth of the soil horizon. An approximate amount of 1.5 kg was collected for each sample taken.

The soil analysis includes the study of soil parameters related to the microbiological characteristics of the soil and the assessment of site index of the tree plantations. The following soil parameters were studied: org. C content (kg/mg^{-1}) and humus (%) - according to Turin's method; Mechanical composition - Kaczynski's method; pH - potentiometric.

Microbiological analyzes were performed according to all sterility rules. The microbial abundance of each of the investigated samples was determined by determining the total microbial number (TMN) for all horizons. Microbiological analyzes include determination of the main microbiological groups in the soil related to its biogenicity and to the site index of tree plantations - bacteria, actinomycetes and fungi (micromycetes). Inoculation was carried out on appropriate nutrient media: (Nutrient agar for bacteria, Starch ammonia agar for actinomycetes, Čapek dox agar for fungi). Incubation was carried out at a certain temperature and duration for each of the studied groups (for bacteria 48 hours at 24°C, for actinomycetes 7 days at 35°C and for micromycetes - Czapek-Dox agar for 7 days at 30°C. The number of sprouted colonies was determined. The data are presented in colony forming units (CFUs) per gram of dry soil.

Results were statistically analyzed using the StatSoft Statistica 12 software program at 95% significance thresholds. The program was used to compare and search for correlations between the individual parameters studied, based on direct and inverse correlations.

RESULTS AND DISCUSSIONS

All of the studied tree stands have a 100% participation of *Fagus sylvatica*, with the exception of TA1, in which there is 94% participation of *Fagus sylvatica* and a minimum participation of *Carpinus betulus* - 6%. The stand relative stocking (RS) and the average volume (AV) (m^3/ha) were determined, as follows:

- TA1-1.49 relative stocking; 628 m^3/ha average volume;
- TA2-0.90 relative stocking; 375 m^3/ha average volume;
- TA3-0.99 relative stocking; 493 m^3/ha average volume;
- TA4-1.19 relative stocking; 580 m^3/ha average volume;
- TA5-0.82 relative stocking; 422 m^3/ha average volume;
- TA6-1.07 relative stocking; 619 m^3/ha average volume;
- TA7-1.26 relative stocking; 569 m^3/ha average volume;
- TA8-1.29 relative stocking; 565 m^3/ha average volume;
- TA9-1.11 relative stocking; 472 m^3/ha average volume;
- TA10-1.09 relative stocking;618 m^3/ha average volume.

Table 1 and Table 2 present the results of the conducted research. A total of 10 soil profiles were investigated. Of them, TA7 and TA1 were defined as Regosoli, TA 2 and TA 6 as Rendzini, and TA3, TA4, TA5, TA8, TA9 and TA10 as Cambisols. The main microbiological groups - and the total microbial number was calculated (Table 2).

Data was statistically processed, and the standard deviation is presented. A statistical analysis of the relationship between the TMN of the upper soil horizon and the underlying soil horizon with respect to altitude was performed

without considering soil type (Figure 1 and Figure 2).

Correlation was performed to follow the dynamics of microbial communities in height. Statistical processing of results was also performed to detect potential interrelationships between microbial abundance and environmental factors.

Table 1. Main characteristics of the studied soils and the determined sites index

TA	Altitude(m)	Soil type	Coordinates	Horizon	Humus %	Org. C g.kg^{-1}	Mechanical composition	pH	Site Index
1	910	Regosols	N 42.644786 E 23.241678	A	6.5	37.72	finely dusty	5.4	II
				C1	2.84	16.46	dusty	5.5	
2	1005	Rendzinas	N 42.488373 E 23.195845	A	4.14	23.99	finely dusty	7.2	II
				AC	4.67	27.06	finely dusty	7.3	
3	1092	Cambisols	N 42.552401 E 23.192019	A	6.14	35.62	dusty	6	I
				B	2.85	16.54	dusty	6.2	
4	1153	Cambisols	N 42.604438 E 23.202027	A	4.26	24.69	sandy dusty	4.5	II
				B	1.19	6.93	sandy dusty	5.1	
5	1195	Cambisols	N 42.474051 E 23.258257	A	3.56	20.63	finely dusty	5.2	II
				B	1.14	6.62	clay dusty	5.9	
6	1218	Rendzinas	N 42.468242 E 23.232454	A	3.77	21.86	finely dusty	7.5	I
				AC	1.14	6.60	finely dusty	7.8	
7	1223	Regosols	N 42.46923 E 23.25111	A	4.57	26.52	dusty	4.8	II
				C	0.48	2.25	dusty	4.9	
8	1270	Cambisols	N 42.608444 E 23.298922	A	3.71	21.53	finely dusty	5.8	II
				B	2.8	16.22	finely dusty	5.4	
9	1370	Cambisols	N 42.572043 E 23.205129	A	2.56	15.40	sandy dusty	5.4	II
				B	1.36	7.88	sandy dusty	5.3	
10	1455	Cambisols	N 42.61697 E 23.240181	A	6.5	37.68	sandy dusty	5	I
				B	4.09	23.74	sandy dusty	5	

Table 2. Main microbiological characteristics of the soils

TA	Horizon	TMN	Bacteria	Actinomycetes	Fungi
1	A	17.58 ± 0.34	11.94 ± 0.77	1.61 ± 0.01	4.04 ± 1.12
	C1	2.30 ± 1.22	1.75 ± 0.92	0.31 ± 0.13	0.24 ± 0.17
2	A	19.58 ± 2.48	17.59 ± 5.22	1.01 ± 3.00	0.99 ± 0.26
	AC	2.29 ± 0.58	2.23 ± 0.37	0.05 ± 0.21	0.01 ± 0.07
3	A	18.09 ± 10.74	16.13 ± 10.19	0.60 ± 0.89	1.36 ± 1.89
	Bw	7.01 ± 2.61	5.35 ± 2.95	1.65 ± 0.54	0.02 ± 0.22
4	A	14.24 ± 10.74	8.92 ± 10.19	0.18 ± 0.89	5.14 ± 1.89

	Bw	10.87 ± 2.61	10.81 ± 2.95	0.02 ± 0.54	0.04 ± 0.22
5	A	17.42 ± 10.74	13.55 ± 10.19	1.70 ± 0.89	2.18 ± 1.89
	Bw	4.74 ± 2.61	3.63 ± 2.95	0.65 ± 0.54	0.45 ± 0.22
6	A	16.07 ± 2.48	10.20 ± 5.22	5.25 ± 3.00	0.62 ± 0.26
	AC	3.12 ± 0.58	1.70 ± 0.37	0.34 ± 0.21	0.11 ± 0.07
7	A	18.06 ± 0.34	10.84 ± 0.77	1.59 ± 0.01	5.63 ± 1.12
	C	0.58 ± 1.22	0.44 ± 0.92	0.13 ± 0.13	0.01 ± 0.17
8	A	18.60 ± 10.74	15.75 ± 10.19	1.53 ± 0.89	1.32 ± 1.89
	Bw	4.47 ± 2.61	3.72 ± 2.95	0.59 ± 0.54	0.17 ± 0.22
9	A	39.91 ± 10.74	32.22 ± 10.19	2.62 ± 0.89	5.07 ± 1.89
	Bw	4.53 ± 2.61	3.12 ± 2.95	0.85 ± 0.54	0.56 ± 0.22
10	A	35.02 ± 10.74	33.12 ± 10.19	0.73 ± 0.89	1.17 ± 1.89
	Bw	8.44 ± 2.61	7.24 ± 2.95	1.01 ± 0.54	0.19 ± 0.22

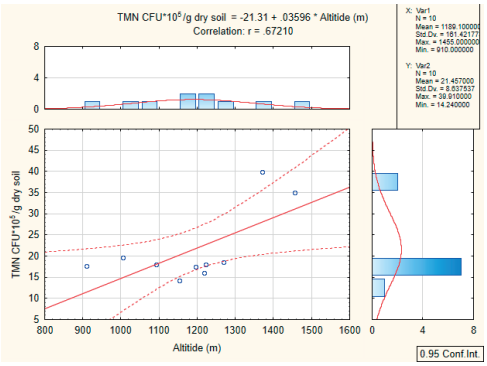

Figure 1. Correlation TMN of A horizon and altitude

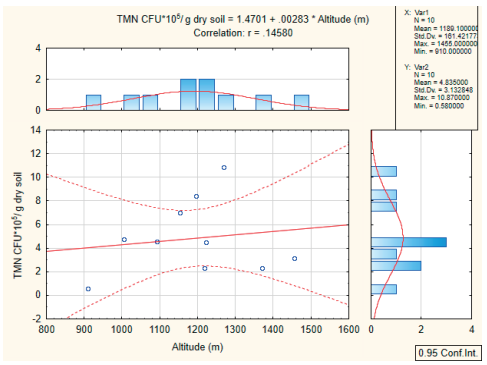

Figure 2. Correlation TMN of B horizon and altitude

Soils from TA1 and TA7 are defined as Regosols. The soil of TA7 is highly acidic, and that of TA1 is moderately acidic. According to the specified levels of Org. C, the considered A horizon of soils of the Regosols type are defined as moderately stocked with organic matter. With an increase in the depth of the soil profile, however, at TA7, a sharp drop in the humus content, respectively, in the amount of org. C and the stock is determined to be very low. In contrast to TA7, in TA1 in the depth of the soil profile, the amount of organic matter does not decrease so sharply, although it falls to class II - low stocking according to the Vanmechelen scale (Vanmechelen et al., 1997). In terms of mechanical composition, both considered profiles have a predominant dust fraction. The soils have a reduced volume of pore spaces and a lack of well-formed aggregates, which leads to a lack of an optimal water-air regime.

Soils from soil profiles TA2 and TA6 are classified as Rendzinas. In these soils, the pH is much higher, and in TA2 soil has a neutral reaction, and in TA6 it has an alkaline reaction. The humus content in the A horizon is lower than the considered Regosols (TA7 and TA1), but in contrast to them, it decreases smoothly in the depth of the soil profile. According to the content of organic carbon, the soils of TA2 are averagely stocked throughout the depth of the soil profile. At TA6, in the humus accumulative horizon, the quantities of organic matter are of class 3 (medium stock), while in depth they pass into class two (low stock). These soils are defined as fine dust. This mechanical composition characterizes the studied Rendzinas, as soils with poor plasticity and stickiness that cannot form stable soil aggregates. In these soils, the pore space is

reduced, leading to a reduction in the air content of the soil.

The remaining soil profiles (TA3, TA4, TA5, TA8, TA9 and TA10) are defined as Cambisols. All soils are defined as acidic, and with increasing altitude there is an increase in acidity due to natural acidification processes. In the overlying humus accumulative horizon, there is an average amount of organic matter for all studied profiles, except for TA9, where the organic matter has a lower content and defines the soil as class two (low amount of organic C) according to Vanmechelen scale. The mechanical composition is diverse. Soil from TA3 is dusty. The soil of TA5 is defined as a fine dusty A horizon and a clayey dusty Bw horizon. The soil of TA8 is defined as fine dust. The soils of TA9 and TA10 are sandy-dusty. The diversity in the mechanical composition of the studied Cambisols shows the influence of the environment and the soil-forming rock on the formation of these soils.

In all soils studied, the microbial abundance decreases in depth of the soil profile. This decrease in total microbial abundance at depth is due to reduced levels of organics as well as altered water–air regimes, as found in the studies of Fierer et al., 2003 and Tripathi et al., 2018. In their study, Taylor et al., 2002 indicates that in the deeper soil horizon there is an absence of microscopic fungi. No such trend was found in the present study. The present study found varying degrees of reduction in microbial abundance in different soil types. Thus, in Regosols and Rendzinas the decrease is much sharper than in Cambisols.

The soils of the Cambisols type stand out as the richest in microorganisms, with an average total microbial number 23 x 10^5. Soils with a sandy-dusty mechanical composition are characterized by greater microbial abundance. No correlation was established between the mechanical composition of the soils and the site index of the stands. There is no clear statistical correlation between humus content and pH. Like the current research, Cho et al., 2016 found that pH, as the only factor considered, did not significantly affect microorganisms' diversity. Furthermore, Lauber et al., 2009 proved that pH is not a sufficient factor to assess the status and diversity of soil microorganisms.

A high correlation coefficient was observed between the increase in total microbial numbers and the increase in altitude ($r = 0.70$), and similar conclusions were generated in the study of Siles & Margesin 2016. In all studied soil profiles, regardless of soil type, the dominant group is bacteria. In highly acidic soils (TA4 and TA7), the percentage participation of the group of fungi increases and reaches over 30% of the total microflora. Looking again at the pH data, the influence of this parameter on the number of microscopic fungi is clearly seen. The neutral and slightly alkaline pH of Rendzinas encourages the development of microscopic fungi, with their levels falling below $1*10^5$. There is no relationship between total microbial number and site index of the stands. No correlation was found between the percentage distribution of microbial groups and the quality site index of the stands.

Further studies of soil parameters are needed to determine which parameters influence the site index of the stands.

CONCLUSIONS

The present study aimed to investigate the microbiological status of soils in beech plantations. There is no clear dynamic between the site index of the stands and the studied soil parameters. No direct correlations were found between stand quality and microbiological characteristics. A decrease in microbial abundance in depth was found in all studied soil profiles. The soils of the Cambisols type stand out as the richest in soil microorganisms. There is no clear correlation between soil characteristics and microbial abundance, regardless of the horizon considered. A positive correlation ($r = 0.70$) was found between increasing total microbial numbers and increasing altitude. Soils above 1,300 meters are characterized by a higher number of microorganisms per gram of soil. The forest and the soil as an element of forest ecosystems are a specific complex, for the understanding and tracking of its functioning, in-depth studies are necessary. We recommend investigating the average content of the total microflora for the entire depth of the soil profile and to look for interrelationships between the microbial abundance and the condition of the stands,

expressed by their site index. We recommend surveying an average sample of the entire stand area, by sampling at least 20-25 points at a depth of 1 m, because soil profiles are likely to reflect the local situation. Microorganisms are a fundamental part of forest ecosystems. Their study and the new information generated can be used to develop strategies and practices for sustainable forest management. Given the important role that soil microorganisms play in the cycling of substances in forest ecosystems, it is necessary to conduct in-depth studies of soil microflora in order to maintain forest massifs in an environmentally friendly manner. Further studies are needed to establish possible interrelationships between environmental factors, soil characteristics, stands site index and soil microbial abundance. The present study can serve as a baseline for future studies.

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