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Analysis of some soil microbial characteristics under *Picea abies* (L.) Karsten stands in stage of decline

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Abstract

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The study investigated some soil microbial characteristics under *Picea abies* (L.) Karsten in decline. Seven soil profiles were analyzed – six under plantations in decline, and one control. The pH, humus (%), total nitrogen and C/N ratio of the soils were determined. The amounts of bacteria, actinomycetes and microscopic fungi per horizon were recorded for each soil profiles. The biogenicity of the soils was determined. The percentage participation of the microbiological groups in relation to the microbial community was analyzed. The results show significantly lower values of the microbial biota in the soils under *Picea abies* in decline – between three and nineteen times. The total microbial number (TMN) of the studied soils for the A horizon ranges between 12.36 -17.32*10^{-4}(CFU/g soil), compared to the control – 44.78*10^{-4}(CFU/g soil). The data show an atypical distribution of the microbial groups in part of the studied soil profiles. There are no correlations between soil parameters and the studied microbiological characteristics. No correlation was found between the investigated soil parameters and the (TMN). Only a weak correlation was reported between the content of humus (%) and the TMN of the Bw horizon of the studied soils (R =.62). The present study found that when growth depression occurs, we have a decline in soil microbial abundance and an atypical distribution of the participation of microbial groups in soil microbial communities. Potential relationships between growth depression in *Picea abies* (L.) Karsten and changes in soil microbial communities need to be further investigated.

Keywords: soil microorganisms; soil microbial communities; *Picea abies* (L.) Karsten; decline; permanent growth depression

Introduction

Projected shifts in global climatic conditions are anticipated to significantly impact the productivity of terrestrial forest ecosystems, thereby influencing their carbon sequestration capabilities and altering carbon cycle dynamics within these ecosystems (Vinogradova and Titkova, 2021; Zhao et al., 2019). Climatic models project exacerbation of drought phenomena across Europe, even under scenarios positing significant climate change mitigation efforts (Ionita et al., 2021; Masson-Delmotte, 2021). In order to predict the extent of such an impact, long-term observations and interdisciplinary studies on the main factors that lead to permanent growth depression (decline) of forest plantations are needed. To achieve this goal, it is important to study the main factors that lead to permanent growth depression in *P. abies* (L.) Karsten plantations. These factors are divided into two groups – primary (abiotic) and secondary (biotic). One of the primary factors are the soils, in which forest ecosystems are formed, as they represent the main nutrient and growth medium for the fine roots (<2 mm) of woody vegetation. When considering biotic factors, special attention should be paid to the soil microbial biota. Reed and Martiny (2007) and Strickland et al. (2009) established that composition and abundance of soil microbial communities is an important determinant of the rate of ecosystem processes. Due to the adaptability of microorganisms and their speed of response to changes in environmental conditions, identification of microbial community composition, is an essential component for predicting ecosystem responses to environmental changes (Baldrian et al., 2012). Soil microorganisms are the main drivers in the biochemical circulation of substances. They play a major role in the nitrogen cycle, and are key to forest health (LeBauer and Treseder, 2008; Hayatsu et al., 2008; Wang et al., 2018). Soil microorganisms are directly related to the provision of growth elements available to tree vegetation.

In recent years, a number of studies have looked for stable interrelationships between soil characteristics, the abundance and activity of soil microorganisms and the condition of tree stands. Certain studies have shown that variations in soil moisture under *P. abies* have led to the differentiation of spatially defined bacterial communities that are tolerant to soil moisture stress (Wilkinson and Anderson, 2001). Other studies have focused on "hot zones" around tree roots, where microbial communities can reach abundances up to 20 times greater than those at the periphery (Baldrian et al., 2010). On the other hand, as was pointed out by Urbanova et al. (2015), trees, through leaf litter, qualitatively and quantitatively influence both the soil microbial community, and the microorganisms generated in the different layers of the litter.

The main aim of the present study was to analyze the quantity and some of the characteristics of soil microbial communities under *Picea abies* (L.) Karsten in a state of growth depression (decline). Achieving the main goal will contribute to a deeper understanding of the reasons that led to the deterioration of the condition of the studied tree plantations.

Materials and Methods

The object of the present study are some soil microbiological characteristics under *Picea abies* (L.) Karsten in a state of permanent growth depression. The permanent growth depression of the forest massifs was established by an ecological dendrochronological method (Schweingruber, 1996), and subsequent processing of the data according to standard methodology (Stokes and Smiley, 1968). For the purpose of the study, seven soil profiles (SP) were formed in different mountainous parts of Bulgaria with spruce plantations (Table 1). The first six profiles are in plantations with established growth depression. SP7 is a control with no established decline in the plantations. Basic soil parameters related to the microbiological indicators of the soil such as pH H_2O – ISO 10390, Total nitrogen – Kjeldahl method, Humus and organic carbon content – modified Tyurin method (Filcheva and Tsadilas, 2002) were reported. The soil samples for the microbiological analyzes were collected using sterile instruments. Each soil sample was stored in a sterile container at a temperature of 4 degrees until the beginning of the analyzes (not later than 24 h after the collection of the material needed for the research). At the start of the microbiological analyses, each of the soil samples is pre-prepared – unwanted impurities are cleaned and the soil material is homogenized. Microbiological analyzes include: Determination of bacteria – by the Koch method, involving serial dilutions and subsequent inoculation on Meat-Peptone Agar (MPA). Cultivation is at a temperature of 28°C, for 48 h; Determination of actinomycetes – by the Koch method and using a selective medium for actinomycetes. Cultivation was carried out at 28°C for 10 days; Determination of micromycetes – by the Koch method and using Capek Dox Agar medium. Cultivation was carried out at 28°C for 7 days; Determination of soil biogenicity (BP) for each sample – by determining the total microbial number (TMN).

Microbiological parameters are reported for each individual horizon. Data are provided in column forming units (CFU) per gram of absolute dry soil. StatSoft Statistica 12 program, with a significance threshold of 95%, was used for statistical data processing.

Results

The studied soil parameters are shown in Table 1.

The analyzes carried out, show an acidic reaction of all studied soil profiles. Soil acidity decreases with depth in all soil profiles except SP 7, where we have a very slight increase in acidity in the Bw horizon relative to A and then again, a decrease in acidity and reaching pH = 5.3. The humus accumulative horizon of all studied soils, with the exception of SP 5, was defined as very strongly acidic with pH values from 4.1 to 4.7. These acidity levels are an indicator of free organic acids. High levels of acidity are also maintained at depth in the Bw horizon of SP 3, SP 4 and SP 7, where the soils are again determined to be very highly acidic. In the remaining soil profiles, a decrease in the acidity scale is observed and the soils are defined as highly acidic, but nevertheless the values are in the range of pH = 4.9 to 5.2. This is an indicator of an increase in the intensity of destructive changes and the potential presence of exchangeable aluminum. The lowest lying C horizon of PP3 remains in the range of highly acidic soils. In all other soil profiles, the acidity in the lower lying soil horizon decreases, reaching values equal to pH =5.3 (SP 1, SP 2, SP 5, SP 7).

Humus (%) in all studied soil profiles decreases with depth. The decrease in the percentage content of humus from A to Bw is from 1.7 to 2.9 times. The most serious decrease in the organic content was observed in the PP7 control,

Table. 1. Basic soil parameters

SP	Altitude	Coordinates	Soil Type	Horizon	pH $_{(H2O)}$	Humus, %	Org. C, g/kg^{-1}	Total N g/kg^{-1}	C/N
1	1744	N 41 66 28 E 23 41 28	Umbrisols	A	4.5	2.46	14.28	1.573	9
				Bw	5	0.95	5.46	0.887	6
				C	5.3	0.49	2.88	0.616	5
2	1795	N 41 40 00 E 23 25 17	Cambisols	A	4.4	4.38	25.38	2.153	12
				Bw	5.2	2.31	13.44	1.106	12
				C	5.3	1.01	5.87	0.978	6
3	1735	N 42 34 28 E 23 18 23	Umbrisols	A	4.1	11.37	65.95	5.782	11
				Bw	4.5	6.35	36.84	2.570	14
				C	4.7	6.20	36.00	2.258	16
4	1647	N 43 11 02 E 23 04 30	Cambisols	A	4.3	20.59	119.35	10.255	12
				Bw	4.4	9.96	57.81	3.983	15
5	1765	N 41 44 55 E 23 32 31	Cambisols	A	4.9	5.59	32.49	1.530	21
				Bw	5.2	1.96	11.38	0.668	17
				C	5.3	0.74	4.31	0.359	12
6	1611	N 43 26 47 E 22 38 16	Cambisols	A	4.7	14.31	83.00	5.058	16
				Bw	4.9	8.56	49.64	2.780	18
7	1820	N 42 05 12 E 23 20 31	Cambisols	A	4.7	4.96	28.84	3.710	8
				Bw	4.6	2.96	17.16	1.799	10
				C	5.3	0.23	1.34	0.288	5

where the amount of humus in the C horizon compared to Bw decreased by 13 times (from 2.96% to 0.23%).

In terms of organic carbon content, the studied soils vary in their indicators within wide range. The soil of SP 1 for all horizons is defined as poor in organic carbon content according Vanmechelen scale (Vanmechelen et al., 1997). The soils of the A horizon of SP2 and SP 7 are defined as soils with an average content of organic carbon. In these soils, the Bw horizon is defined as poor in organic carbon, and the C horizon as very poor. With a rich content of organic carbon, are defined A horizons of SP 3 (65.59 g/kg^{-1}) and SP 6 (83.00 g/kg^{-1}). With the highest content of organic carbon is the A horizon of SP 4. Here the organic content is 8 times higher compared to the lowest values reported for A horizon (SP1 – 14.28 g/kg^{-1}). There is no relationship between soil type and organic carbon content. The specific influence of environmental factors in the formation of soil characteristics is visible. This necessitates the need for additional complex studies of environmental factors.

The content of total nitrogen in all studied profiles decreases with increasing depth. Again, similar to humus content, there is a dynamic in values. The A horizon of SP 1, SP 2, SP 5 are defined as moderately rich in total nitrogen according to the Vanmechelen scale. In these profiles, the lower lying soil horizons are poor in nitrogen, while in the C horizon of PP5 its values correspond to class 1 – very low. The soil of the A horizon of the Control (SP 7) is rich in total nitrogen, in the Bw horizon the total nitrogen levels are medium, and in the C horizon the soil is defined as very poor in nitrogen. Again, total nitrogen content levels from the Bw to the C horizon drop by more than 6 times in the control. A similar drop down is not found in the other profiles. The A horizon of SP 4 (10.255) stands out with the highest total nitrogen content. The C/N ratio was assessed as very low on the Vanmechelen scale in SP 1, SP 2, SP 3, SP 4 and SP 7 for all soil horizons. For soil profile 5, the ratio in both soil horizons was assessed as low. SP 1 and control PP7 stand out with the lowest ratio.

Table 2 presents the microbiological characteristics of the studied soils.

The investigated soil profiles show significant dynamics when considering the values of the TMN. The obtained results shows that the microbial abundance is highest in A horizons, with the exception of SP 4. A horizon of SP1 is characterized by the lowest amount of TMN, decreasing in depth (the TMN is 2.36 $*10^{-4}$ for the A horizon). The results for the distribution of TMN in A and Bw horizons show greater scatter in the data in the soil profiles under decline. C horizons have the lowest microbial biogenicity in all soils. An analysis of the obtained results for TMN in the individual horizons of the studied soils was performed. The analysis showed that the standard distribution of values of A and Bw horizons is above the average, while in C horizon, most of the values are distributed below the average (Figure 1).

Table. 2. Analyzed microbiological parameters

	Horizons	TMN	Bacteria	Microscopic fungi	Actinomycetes
	A	2.36 ± 13.37	0.91 ± 12.24	0.18 ± 1.51	1.27 ± 3.84
SP1	Bw	2.42 ± 6.36	0.74 ± 5.80	0.37 ± 0.72	1.30 ± 1.35
	C	2.17 ± 7.25	0.54 ± 4.91	0.54 ± 0.88	1.09 ± 1.86
	A	14.03 ± 13.37	12.87 ± 12.24	0.19 ± 1.51	0.96 ± 3.84
SP 2	Bw	4.82 ± 6.36	4.11 ± 5.80	0.00 ± 0.72	0.71 ± 1.35
	C	3.62 ± 7.25	3.44 ± 4.91	0.00 ± 0.88	0.18 ± 1.86
	A	17.72 ± 13.37	16.20 ± 12.24	0.76 ± 1.51	0.76 ± 3.84
SP 3	Bw	14.71 ± 6.36	12.64 ± 5.80	1.13 ± 0.72	0.94 ± 1.35
	C	12.69 ± 7.25	8.04 ± 4.91	2.14 ± 0.88	2.50 ± 1.86
SP 4	A	9.81 ± 13.37	4.28 ± 12.24	0.36 ± 1.51	5.17 ± 3.84
	Bw	16.12 ± 6.36	14.72 ± 5.80	0.35 ± 0.72	1.05 ± 1.35
	A	12.96 ± 13.37	2.32 ± 12.24	0.39 ± 1.51	10.25 ± 3.84
SP 5	Bw	11.67 ± 6.36	8.44 ± 5.80	2.15 ± 0.72	1.08 ± 1.35
	C	6.60 ± 7.25	5.63 ± 4.91	0.39 ± 0.88	0.58 ± 1.86
SP 6	A	13.83 ± 13.37	4.36 ± 12.24	4.36 ± 1.51	5.12 ± 3.84
	Bw	16.39 ± 6.36	15.27 ± 5.80	0.56 ± 0.72	0.56 ± 1.35
	A	44.78 ± 13.37	35.53 ± 12.24	0.56 ± 1.51	8.70 ± 3.84
SP 7	Bw	19.50 ± 6.36	14.68 ± 5.80	0.37 ± 0.72	4.46 ± 1.35
	C	19.76 ± 7.25	13.49 ± 4.91	1.52 ± 0.88	4.75 ± 1.86

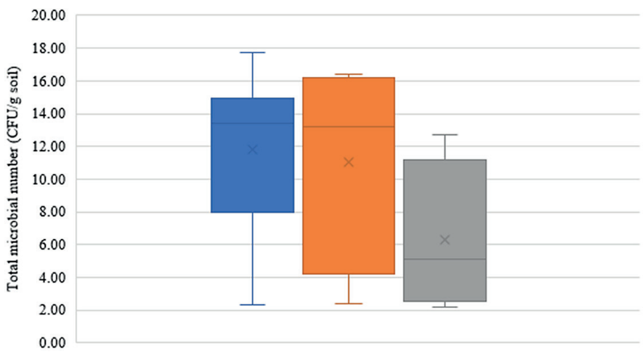

Fig. 1. Total microbial number of soils with decline of *P.abies*

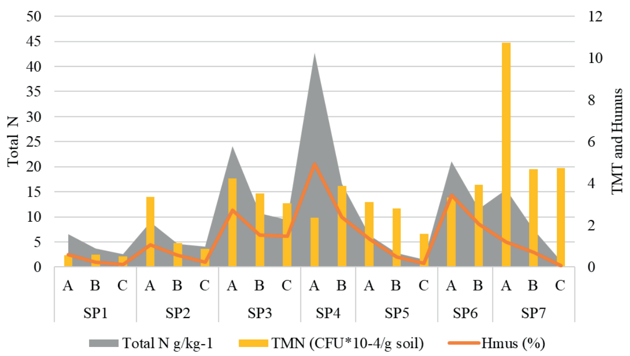

Fig. 2. Comparison between the amount of total nitrogen, humus and TMN

All profiles under *Picea abies* (L.) Karsten in decline, have reduced biogenicity relative to the control. In SP 7, the TMN was 19 times greater than the lowest reported values of the investigated profiles (SP 1). Even with the highest total microbial number reported in SP 3, again the control showed values over 2.5 times greater.

In most of the profiles, the amount of humus and total nitrogen is related to the total microbial number of the particular profile – the total microbial number is highest in the A horizon and decreases in depth (Figure 2). An exception is profile SP 6, where a disturbance in the amount of microflora in the humus-accumulative horizon is observed. In this profile, the amount of reported total microflora is lower compared to the B horizon. This implies a specific transport of nutrients in depth.

There is no correlation between the content of humus in all studied soils and the total microbial number in the A horizon (r = -1.6). The correlation coefficient values for Bw show r = 0.62. These data suggest a greater dependence of microbial communities on the nutrient content of the underlying B horizon. In depth, the relationship between the two parameters strongly decreases again (r = 0.21). Such data require additional analysis of the soil organic matter composition in the B horizon in future studies.

No significant correlation was found between the abundance of microorganisms and the rest of the studied soil parameters. When analyzing the obtained results, it is perceived that the process of soil acidification in forest ecosystems is natural and the autochthonous soil microflora is adapted to it.

There is also no correlation between the amount of individual groups of microorganisms and the studied parameters of the soil.

Figure 3 shows the percentage participation of each of the studied microbiological groups for each soil profile by horizon.

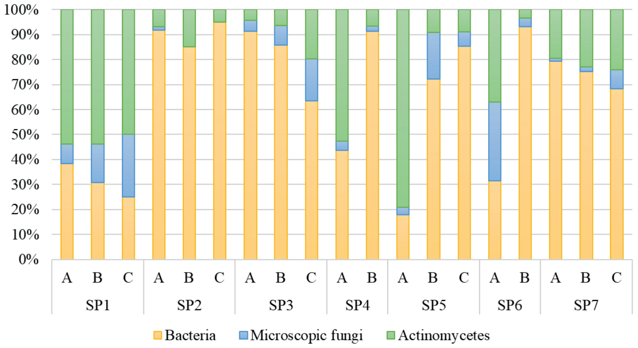

Fig. 3. Percentage share of microbial groups in the total microbial number, (%)

The graph shows heterogeneity in the percentage participation of the individual studied microbiological groups in the microbial communities of the individual soil profiles. These differences cause different dynamics in the transformation of the soil organic matter and in the processes taking place in the soil. The group of bacteria in profiles SP 2, SP 3 and SP 7 is dominant in all soil horizons. In SP 1, the participation of actinomycetes is high, in all soil horizons. At SP 4 in the A horizon, the amounts of actinomycetes and bacteria are equal, while the percentage participation of microscopic fungi is very low. In the depth of this soil profile, the percentage participation of bacteria increases at the expense of actinomycetes and fungi. In SP 5, the actinomycete group again has the highest percentage participation in the A horizon, followed by bacteria and fungi. In the lower horizons of this soil profile, the largest share of the microbial community is bacteria. In a study of other forest soils, it was found that the group of bacteria prevailed in terms of percentage participation (Llado et al., 2017; Grigorova-Pesheva and Hristov, 2021). The obtained results are related to the available forest growth depression. Bacteria is extremely sensitive to a sudden change in the environment conditions, and her small percentage participation is an indication of atypical processes in the soils. Their reduced percentage participation in profiles 1 and 4 suggests that abrupt changes in the environment occurred, potentially associated with reduced moisture along the depth of the entire soil profile. Bacteria as a group of microorganisms, prefer slightly acidic to alkaline environments. However, we believe that their reduced percentage participation is not related to soil pH. Acidification of forest soils is a constant process, to which soil microbial communities are adapted. Of interest is the preponderance of the percentage participation of actinomycetes in SP 1, SP 4 and SP 5. Their dominant presence in these soil profiles suggests more complex processes of decomposition of soil organic matter. The high percentage of actinomycetes is associated with an advanced phase of destruction, in which undegraded substances such as cellulose, lignin, chitin and proteins remain.

With a typical distribution of microbial groups in the microbial community, SP 2 and the control stand out. These data again impose the need for in-depth studies covering a larger number of soil profiles to establish the interrelationships between the changes occurring in the microbial communities, and the available decline of *Picea abies* (L.) Karsten plantations.

Discussion

One of the main tasks of the present study was to analyze some microbiological characteristics of the different forest soil horizons under *Picea abies* (L.) Karsten plantations decline. The obtained results show significant differences both in the physical and chemical parameters, as well as in the microbiological indicators of the studied soils. The generated results show that the abundance of soil microbial biota is many times higher in the control soil profile, compared to the soil profiles under spruce plantations with established growth depression. Microorganisms are highly sensitive to environmental changes (Llado et al., 2017). It is for this reason that the presence of an indication of sharp transformations in the soil communities, in the case of established growth depression, is expected. Growth depression affects the standard distribution of major groups of microorganisms in the microbial community. Given the atypical distribution of microbial groups, we believe that atypical environmental conditions are present. These conditions, as well as the changed structure of the microbial community, are a prerequisite for increasing growth depression in spruce in the future. The atypical predominance of the actinomycetes group in some of the profiles (SP 1, SP 4 and SP 5) is a clear sign of sudden changes in the environmental conditions. A predominance in the participation of the actinomycete group indicates reduced amounts of simple organic substances, which is related to a potential disturbed flow of transformation of organic matter entering the soil. Additional interrelationships between soil microbial biota and the causes of growth depression in spruce need to be explored, by analyzing enzyme activity and microbial biomass, as well as by analyzing dead forest litter as the main source of organic components. There is no correlation between the abundance of microorganisms and the studied soil indicators, which is an indication of the presence of factors disturbing the microbial biota. In other studies, increased microbial abundance was found at a higher percentage of humus (Hu et al., 2010; Gomez et al., 2006; Li et al., 2015; Galieva et al., 2018), while in the present study no such relationship was found. There are also no clear correlations between the acidity of the environment and the distribution of microbial communities, although a number of studies emphasize the strong correlation between high levels of acidity and the amount of microscopic fungi (Rousk et al., 2009). No correlation was found between total nitrogen and soil microbial abundance. This indicates disturbances in the rate of transformation of nitrogen-containing compounds. A narrow carbon/nitrogen ratio is associated with increased microbiological activity and an advanced degree of organic matter transformation. This trend is clearly evident in the control profile. In the control, we have a low ratio and a high abundance. At SP 1, however, we again have a low ratio, but the microbial abundance is atypically low for forest soils. A very narrow ratio suggests an indication of increased competition between microorganisms and tree species for the supply of nitrogen, in which it is possible that tree species are deficient in this element. It is necessary to investigate the possibility that the increased competition between microorganisms and trees for the available nitrogen to be one of the main reasons for permanent growth depression in the considered forest plantations.

Conclusions

The main objective of the present study was to make a baseline analysis of some of the microbiological soil indicators in *Picea abies* (L.) Karsten plantations. In the present study, basic soil parameters – pH, humus (%) and total nitrogen – were investigated. The performed microbiological analyzes include: determining the total microbial number, amount of individual main microbiological groups and their percentage participation in the microbial communities. A correlation analysis was performed to establish a relationship between environmental factors, microbiological parameters and the presence of growth depression in *Picea abies* (L.) Karsten plantations. The conducted study proved reduced amounts of soil microflora in the profiles under plantations with established growth depression compared to the control. The reduced abundance of microorganisms reached 19 times compared to the control. An atypical percentage participation of the main microbial groups was found in some of the investigated profiles (SP1, SP4 and SP5). Growth depression affects the standard distribution of major groups of microorganisms in the microbial community. The atypical predominance of the actinomycete group in these profiles is a clear sign of sudden changes in environmental conditions. No correlation was found between the studied soil parameters and the total microbial number. An exception is the reported weak correlation between humus content and microbial abundance in the studied B horizons. The typical correlation between increased acidity and percentage participation of the microscopic fungi group was also not reported. The obtained results shows an imbalance in soil microbial communities under *Picea abies* (L.) Karsten plantations in a state of permanent growth depression. The present study needs to be conducted on a larger scale and additional microbiological parameters, such as enzyme activity, enzyme profile and microbial biomass, should be analyzed to look for direct relationships. The present study can serve as a basis for future in-depth studies of growth depression, as well as for the development of different scenarios to predict the biological factors related to growth depression.

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