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Influence of pH, Organic Carbon and Total Nitrogen Content on the Amount and Distribution of Different Microbial Groups in the Organic Layers of Luvisols

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Abstract. The influence of altitude, pH, organic C and total nitrogen content on the Quantity and Distribution of soil microflora in the organic layers of Luvisols was investigated. The experimental plots include A horizon of 5 soil profiles. Aiming to determine the biogenity of analyzed plots, the total microbial number and the percentage share of different microbial groups has been examined. The samples were taken from forest soil according to microbiological requirements. The standard microbiological method for analyses was used. The obtained results showed different biogenity and percentage share of microbial groups according to the different environmental conditions – pH, org. C and total N. Strong correlation between total microbial number and org. C and total N has been found. Soil with the largest total microbial number (6.7 lg CFU/g dry soil) was the soil with the highest account of org. C (49.98 g.kg^{-1}) – tested plot (TP) 2. pH as a parameter of soil environment has been correlated with the distribution of microbial groups.

Key words: microorganisms, total microbial number, forest soil, pH, org. C, total N.

Introduction

Soil environment is one of most complex habitat for microorganisms due to the different condition (Horner - Devine et al., 2004). Microorganisms are one of most important biological components of the soil (Bhatt et al., 2015). They are the main agent responsible for decomposition of organic compounds (Schloter et al., 2018). Microorganisms have fundamental role in the transformation of substances in the soil according to their metabolism (Jacoby et al., 2017).

The development, diversity and abundance of microbial communities in the soil are influenced by the main characteristics of the soil - its acidity, organic carbon content and total nitrogen (Jha et al., 1992). A number of studies are focused on the change of the microbial community in the event of a change in the nutrient base and environmental conditions (Willey et al., 2008), but do not consider the overall effect that the individual parameters have on the soil microflora. The humus-accumulating soil horizon is characterized by a greater microbial abundance, as a result of the increased content of nutrients and the presence of more suitable water-air and temperature conditions of the environment

(Fritze et al., 2000), which is the main prerequisite for its analysis in the present study.

Bhatia (2008) points out that the physical properties and the amount of humus is directly related to the abundance of microflora. In their studies, Farrell et al. (2014) and Khatoon et al. (2017) emphasize the fact that the content of organic carbon and total nitrogen are directly related to the activity of soil microflora.

Lauber et al. (2009), cite the soil reaction as one of the main soil factors that influence the growth and development of microorganisms. Many soil chemical and biological reactions are controlled by the pH of the soil (Hendershot et al, 1993). pH range for optimal microbial activity and nutrient availability has been considered to be between 5.0 and 7.0 (Vanmechelen et al., 1997). Differences in acidity suggest different conditions for the development of different microbial groups (Fierer & Jackson, 2006). Other authors have shown that pH cannot be used as a stand-alone indicator of the abundance and distribution of microbial biota (Cho et al., 2016).

However, there is only a few studies have attempted to analyze and verify the combinative connection between soil microbial community and pH, org. C and total N.

According to this, the main aim of the present study is to investigate the connection between the quantity and distribution of the microbial community on the one hand and pH, the content of organic C and total nitrogen on the other, in soils, including statistic relationship between the analyzed parameters.

Materials and Methods

The subject of the study is soils from part of the territory of the Western Stara Planina Mts. - lower belt with the northern slopes exposition. The studied territory covers the part of the Stara Planina Mts. between the western border of Bulgaria and the Iskar Gorge. According to the climatic zoning, the Western Stara Planina Mts. falls into two climatic regions: Fore mountain and mountain region. Both regions are part of the temperate continental climate subregion. The studied area is characterized by precipitation from 750 to 1000 mm (Velev, 2002; Koleva–Lizama, 2006). According to Bondev (2002) the studied part of the Western Stara Planina is part of the European region of deciduous forests and the Illyrian province.

The territory of the Western Stara Planina Mountain falls in the Moesian forest growing area, subregion Northern Bulgaria.

In the monograph Soils in Bulgaria (1960), the main type of soils for the territory of the Western Stara Planina Mountain in the lower forest belt are Luvisols. These forest soils are typical in the study area and they have a more intensive biological cycle, more pronounced processes of mineralization of organic matter, deeper leaching, textural differentiation between the surface horizon and the underlying agric horizon (Koinov et al., 1998)..

Collection of sampling. Soil samples for microbiological and soil analyses were taken from 5 soil profiles on the territory of Stara Planina Mountain. The soil is Luvisols. Soil analyses were performed by standard methods and includes determination of soil acidity in aqueous extract ($pH_{(H2O)}$), using a glass pH electrode in a 1:5 (V/V) suspension of soil in water (ISO 10390); determination the organic carbon were found according modification method of Turrin (Filcheva & Tsadilas, 2002) and total nitrogen content was determined by Kjeldahl digestion (ISO 11261).

Microbiological analyses included determination of total microbial number, determination of percentage distribution of different microbial groups, as bacilli, non-spore forming bacteria, actinomycetes and micromycetes. Total number of microorganisms was determined by Koch's method, involving successive dilutions and subsequent inoculation on appropriate elective agar medium. The count was made as colony forming units per gram dry soil mass (CFU/gram dry soil mass) under logarithm (lg). The statistical analyses of data were carried out by StatSoft Statistica 12 program under significance thresholds 95%.

Results

Five soil profiles were studied and analyzed. The morphological characteristics of the soil profiles of the studied soils define as Luvisols the soils from all Tested Plots (TP) – TP1, TP 2, TP 3, TP 4 and TP 5 according to the requirements of the Basic classification of soils in the country (Penkov et al., 1992).

The studied soils have a characteristic profile of the type 0ABtC. On the surface of some of the profiles there is accumulated litter. For the purpose of the present study, the soil characteristics of the A horizon were considered. As emphasized above, the humus-accumulating soil horizon (A horizon) is characterized by a greater abundance of microbial communities and is the main soil horizon in which the transformation of soil organic matter takes place.

The considered soil profiles are set at an altitude of 185 m to 651 m in order to cover the variety of specific environmental factors in the study of soil microflora. Table 1 presents the characteristics of the tested soils.

Table 1. Main characteristics of soil profiles.

TP №	Location	Altitude (m)	Exposure	Vegatat ion	pH	Humus (%)	org. C ($g.kg^{-1}$)	Total N ($g.kg^{-1}$)	C:N
1	N 43 23 09 E 23 14 51	185	NW	*Quercus frainetto* *Pinus nigra* *Fraxinus ornus*	5.10	2.42	14.03	2.06	7
2	N 43 31 26 E 22 41 56	390	NW	*Quercus cerris* *Carpinus betulus*	7.40	8.62	49.98	6.66	8
3	N 43 30 42 E 22 38 17	510	N	*Carpinus betulus* *Quercus robur*	4.90	5.49	31.87	3.60	9
4	N 43 36 41 E 22 31 54	610	NW	*Fraxinus ornus*	6.20	4.53	26.29	3.77	7
5	N 43 10 30 E 23 09 12	615	N	*Carpinus betulus* *Fagus sylvatica*	4.60	3.84	22.26	2.85	8

There is no relationship between the increase in altitude and the amount of humus and org. C in the A horizon of the studied soils, as evidenced by the low correlation coefficient (r=0.13). As a consequence, we have a low correlation between altitude and the amount of total nitrogen (r=0.093).

Obtained results for the studied soils show that the A horizon is characterized by a pH in the range of 4.6 at an altitude of 615m to 7.4 at an altitude of 390 m. These differences in acidity are due to the large diapason of altitude and influence of different vegetation. The acidity in the considered profiles varies from very strongly acidic in TP5 to alkaline in TP2. Profiles № 1 and № 3 are characterized by pH 5.1 for TP1 and 4.9 for TP3 in the humus-accumulative horizon, which in accordance with the adopted classification means that in this profiles soil has a strong acidic reaction. This acidity indicates that the aggressive fraction of humic acids is related. pH values at TP5 indicate very strong acidic reaction, for which is characterized by the presence of free organic acids - fulvic acids (Ganev, 1990). TP4 is characterized by pH 6.2 or the soil in this plot has a slightly acidic reaction. With neutral reaction of pH 7.4 has been determined TP2. There is no correlation between soil pH and altitude change (r=0.10).

The obtained data on the content of org. C in the studied soils show, that it varies from medium to very high (on the scale of Vanmechelen, 1997). In TP1 and TP5 its quantity is respectively 24.20 $g.kg^{-1}$ and 38.38 $g.kg^{-1}$. TP3 and TP4 are characterized by high org. C content. The highest content of org. C is reported at TP2 – 49.98 $g.kg^{-1}$.

Similar to org. C, the content of total N in the humus-accumulating horizon varies from medium to very high (on the Vanmechelen scale, 1997). The soil from TP1 is determined with average content of total nitrogen (2.06 $g.kg^{-1}$). According to the received data TP3, TP4 and TP5 have a high content of total nitrogen, and in TP2 it is very high (6.66 $g.kg^{-1}$).

The ratio org. C/total N in the studied soils is in the range of 7-9. These ratios are rated as very low on the Vanmechelen scale and show an advanced degree of transformation of organic matter to the formation of stable humic substances.

A large number of factors such as active reaction, stock of soil with org. C and nitrogen, altitude and others affect the number of microorganisms in the soil and the microbial activity. The quantitative characteristic of the general microflora is one of the microbiological indicators that can be used to assess the biogenicity of the soil.

Table 2 presents the reported microbiological indicators of the studied soils in colony forming units per gram dry soil mass under logarithm (lg CFU/g dry soil).

The total microbial number of the tested plots is reported, as well as the percentage of the individual microbial groups in the microbial community. The total microflora is presented as lg of the sum of CFU (colony forming units) of non-spore-forming bacteria, bacilli, actinomycetes and micromycetes in Table 2. The influence of the pH, org. C and the total nitrogen was statistically analyzed both in relation to the formation of the general microflora and in relation to the influence of the parameters on the distribution of the microbial groups.

Table 2. Total microbial number lg CFU/g dry soil.

TA	Total microbial number	*Bacillus* sp.	Non-spore forming bacteria	Actynomicetes	Micromycetes
1	6.12 ± 0.22	6.05 ± 0.38	4.25 ± 0.90	5.09 ± 0.34	4.90 ± 0.34
2	6.70 ± 0.22	5.01 ± 0.38	6.68 ± 0.90	5.28 ± 0.34	4.41 ± 0.34
3	6.45 ± 0.22	5.75 ± 0.38	6.15 ± 0.90	5.60 ± 0.34	5.71 ± 0.34
4	6.26 ± 0.22	5.41 ± 0.38	5.77 ± 0.90	5.97 ± 0.34	4.35 ± 0.34
5	6.24 ± 0.22	5.68 ± 0.38	5.64 ± 0.90	5.63 ± 0.34	5.59 ± 0.34

The total microbial number of microorganisms in the tested accumulative horizons –varied from 6.12 lg CFU/g dry soil to 6.70 lg CFU/g dry soil. The soil with the highest total microbial number was found in the TP2 on 390 m altitude and northwest exposure.

According to the "r" factor no correlation was found between altitude and total biogenicity (r= 0.06). In contrast, other studies prove a high correlation between soil biogenicity and altitude change, but at higher altitude gradient ranges (Grigorova-Pesheva, 2019).

Soil pH determines the solubility of many nutrients and their availability to plants and soil microflora (Mohammed & Zigau, 2016; Fierer & Jackson,2006). In contrast, other authors argue that the degree of development of soil microflora cannot be evaluated only on the basis of effect of acid reaction on the soil microbial biota (Cho et al., 2016).

Based on the obtained results, we believe that the process of soil acidification in the considered forest ecosystems is natural and the autochthonous soil microflora is adapted to it. This is proved by the performed correlation analysis of the two parameters. The measured active reaction of the soil and the amount of the total soil microflora show a relatively weak correlation (Fig. 1.).

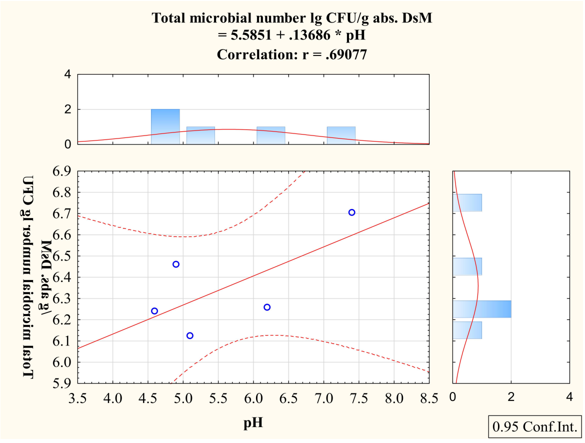

Fig. 1. Statistical relationship between total microbial number and pH.

The obtained correlation coefficient has a value of r = 0.69 at pH values in the range from 4.6 to 7.4. Other authors also come to similar conclusions, showing that soil pH is not a sufficient indicator for the development of the microbial biocenosis, but a complex approach is needed (Cho et al, 2016). However, the data show a much higher proportion of micromycetes compared to other microbial groups in the studied soils, where the acidity is lower, namely TP3 (5.71 lg lg CFU/g dry soil) and TP5 (5.59 lg CFU/g dry soil), which shows the influence of the soil reaction on the redistribution of microbial groups.

In contrast to pH, the amount of org. C significantly affects the biogenicity of the soil and this is evidenced by the high correlation coefficient between the two parameters: R= 0.98 (Fig. 2.).

The correlation between the content of org. C and the biogenicity of the soil has been proven and by other authors Khatoon et al. (2017). Org. C is a source of nutrients and plays a key role in maintaining the activity of soil microbial biota.

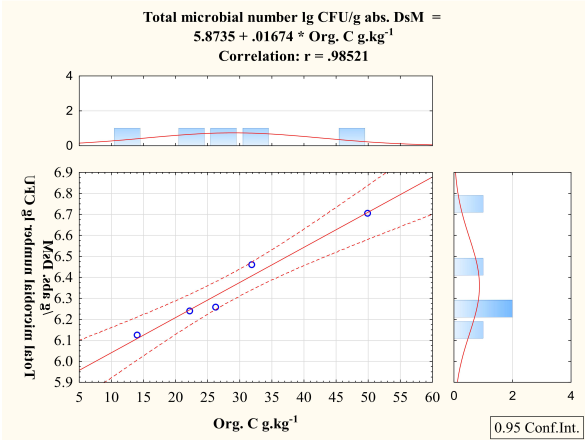

Fig. 2. Statistical relationship between total microbial number and Org. C.

The obtained results show a high value of the correlation coefficient when considering the statistical relationship between the amount of total nitrogen and the total microbial number (r=0.94), (Fig. 3).

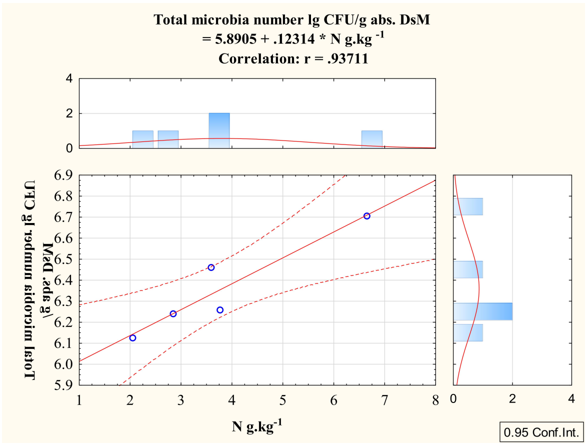

Fig. 3. Statistical relationship between total microbial number and total N.

This relationship is not surprising, given that soil microbial biota is involved in all aspects of the nitrogen cycle. The strong correlation between the two parameters is mainly related to the transformation of organic matter by microorganisms, i.e. the use of total nitrogen as a source of energy and as a material for building the bodies of microorganisms. With the increase of the content of total nitrogen in the soil, the exponential development of the soil microflora begins, which is responsible for the transformation of the various forms of nitrogen. Our results correspond to the results reported in other scientific studies (Li at al., 2018).

Fig. 4 shows the composition of the microbial communities by groups as a percentage of the total microflora for the individual TPs.

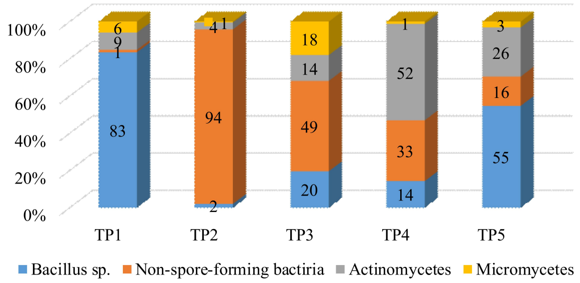

Fig. 4. Percentage share of microbial groups in the total microbial number, (%).

The percentage participation of different groups of microorganisms in the soil is an indicator of the course of the individual stages of transformation of soil organic matter (SOM). Various studies have focused on the relationship between the dominant group of microorganisms and the different soil characteristics (Zhang et al., 2013). As pointed out by Perfanova et al. (2015) the distribution of microbial groups in the soil microbial community is one of the main indicators of the degree and rate of transformation of SOM.

The graph clearly shows the differences in the dominant microbial group for the individual tested plots. In the tested plots with lower pH, the percentage of micromycetes is increased. This group of microorganisms prefers more acidic media than bacilli and non-spore-forming bacteria (Mohammed & Zigau, 2016). The obtained data show a strong statistical correlation between the percentage participation of the group of non-spore-forming bacteria and the amount of org. C (Fig.5).

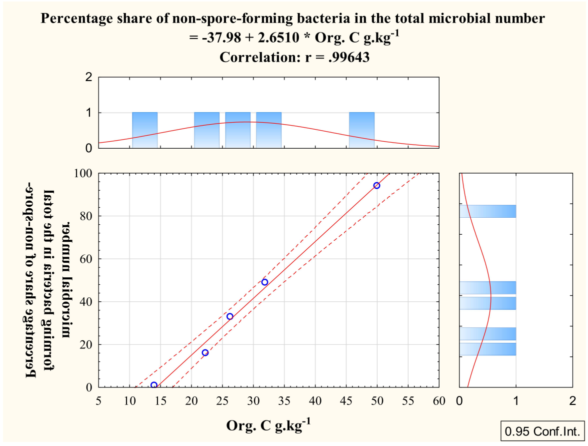

Fig. 5. Statistical relationship between % share of non-spore-forming bacteria and the quantity of Org. C.

With increasing content of org. C increases the percentage of non-spore-forming bacteria. Respectively in TP1, where the content of org. C is almost twice as low, the percentage of non-spore-forming microorganisms is only 1%.

The large percentage share of the group of bacilli in the A horizon of TP1 shows predominant processes of advanced transformation of SOM and the presence of more stable forms of organic matter. The situation is similar in TP5, where the bacilli are 55% and the actinomycetes 26% of the total microflora. The more active phase of mineralization of the hardly degradable organic substances in TP5 is further confirmed by the increased percentage of actinomycetes compared to the other tested areas.

In TP2 and TP3, the obtained data on the distribution of microbial groups are an indication of the predominance of the processes of transformation of more easily degradable SOM.

In the TP4, given the percentage distribution of the microbial groups, it can be concluded that the processes of transformation of complex SOM and simple SOM run in parallel, and their activity is similar.

Discussion

One of the main tasks of the present study was to analyze the influence of individual environmental factors (pH, org. C and total N) on the total microbial number and the distribution of microbial groups in the microbial communities of the studied forest soils. Previous studies have demonstrated the influence of pH, org. C and total N on soil microflora, but this environmental factors have been considered separately and not as a whole (Cho et al., 2016; Lauber et al., 2009; Khatoon et al., 2017). In the present study we discuss the complex influence of the environmental factors on soil microorganisms. The current scientific study shows a statistically significant relationship between environmental factors and changes in the total microbial number and the redistribution of microbial groups in forest microbiological communities. Here we provide evidence about the importance role of organic carbon and total nitrogen content during the development of the soil microorganisms.

Conclusions

We concluded that amount of organic C and total N were the environmental factors most strongly related to total microbial number. Soil pH was not correlated to the total microbial number, but was one of the most important factors related to the composition of the soil microorganisms – micromycetes have great abundance in profiles with acid pH. The percentage of different microbial groups shows a relationship between the amount of organic carbon and total nitrogen relative to the dominance of non-spore-forming bacteria. In this study, no relationship was found between the microbial abundance of the studied soils and altitude.

The research and the obtained data can be used in future projects as a basis for comparison and long-term monitoring of the studied sites. Further research is needed to link and analyze additional environmental conditions, such as exposure, specific vegetation, detailed mechanical composition of the soil, etc. to enrich the knowledge of the complex effects on the soil microbial community.

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