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Analysis of Soil microbial carbon metabolic function, functional diversity and community similarity of six profiles from Luvisols

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Abstract. The study investigated the microbial carbon metabolic function, functional diversity, and community similarity of six profiles from Luvisols. The Community Level Physiological Profiling (CLPP) technique and the Biolog EcoPlate method based on it were used to determine microbial carbon metabolic function and functional diversity. Soil microbial carbon metabolic function (SMCMF) was found to differ among the six soils, despite all being Luvisols. The sample TA3, under mixed broadleaf vegetation and pH 7.4, showed the highest SMCMF, while the lowest values were observed in sample TA6 under mixed broadleaf and coniferous vegetation and pH 5.1. Carbohydrates were the preferred carbon source for the microbial communities in the studied Luvisols, while the amine/amide group was poorly utilized (only in TA5). The results for functional diversity ranged from 19.35% to 38.71%, with the highest functional diversity found in samples under broadleaf vegetation (TA2, TA3, and TA5). There was a positive correlation (r=0.74) between soil microbial functional diversity and soil organic carbon content, but no such correlation was observed with soil pH. The Simple Matching Coefficient was calculated for the studied soils. TA1 and TA6, with the highest % functional diversity, showed the most similarity finger print (SMC=77%). TA3 and TA5, with the lowest % functional diversity, showed the least similarity (35%). The results of this study confirm the need for further analysis of soil microbial communities, and the importance of considering soil microbial carbon metabolic function, functional diversity and community similarity as separate parameters of the soil microbial community in forest ecosystems.

Key words: soil microbial carbon metabolic function, functional diversity, community similarity, Luvisols.

Introduction

Soil and soil microorganisms are key components of forest ecosystems (Baldrian, 2016). Understanding the mechanisms of organic matter transformation and the complex relationships of the soil microbial community is essential for proper forest management. Microorganisms are responsible for the stability and function of soil ecosystems (Schulz et al., 2013). Forest ecosystems rely on soil microbial communities because they transform plant biomass and soil organic matter into forms suitable for plant uptake (Rogers & Tate, 2001). Biochemical processes in soils are largely dependent on the activity and diversity of microorganisms present in the soil (Li et al., 2013). Studying the dynamics of microbial communities and their ability to utilize different sources of carbon, depending on environmental conditions, is a key aspect of understanding soil microbial communities as

a whole (Bhattacharyya et al., 2022). Community Level Physiological Profiling (CLPP) is one of the methods used to analyze the functional diversity of soil microorganisms. Based on this technique, Biolog EcoPlate have been developed, which provide rich information on the microbial community and can be used to analyze soil microbial carbon metabolic function and functional diversity in soil microbiomes (Gałązka & Furtak, 2018). Biolog Eco Plates contain a set of carbon substrates used by microorganisms for their growth and metabolism. By measuring the ability of microorganisms to utilize these substrates, we can gain insight into the functional diversity of soil microorganisms and their potential ecological roles (Sofo & Ricciuti, 2019).

In their study, Lan et al. (2019) utilized Biolog Eco Plates to analyze the differences in carbon source utilization capacity with changes in forest age. The specific management practices of forests and reaching a certain age of tree stands result in different patterns of carbon substrate utilization by soil microorganisms. This highlights the potential of Biolog Eco Plates for monitoring the effects of different forest management practices on soil microbial communities.

In their study, Zheng et al. (2005) used Biolog Eco Plates to assess the effects of regenerating forest cover on soil microbial communities. The study revealed that different management practices and regeneration with different types of forests or secondary natural forests lead to changes in the functional diversity of soil microorganisms, indicating potential changes in ecosystem functioning. These results emphasize the need for further in-depth research on the functional diversity of soil microorganisms to understand the impact of human activities on soil microbial communities and the resulting consequences for ecosystem services.

The analysis of functional diversity of forest soil microorganisms is vital, and Biolog Eco Plates, a microbiological tool, play a crucial role in this analysis. The use of these plates provides a better understanding of soil microbial communities, and this understanding can guide management practices that support the maintenance of healthy forest ecosystems.

In a study conducted by Louisson et al. (2015), the effects of different land use on the functional diversity of soil microbial communities in the Brazilian Cerrado were evaluated. The authors found that when a change in land use occurs, microbial communities respond by transitioning from communities that reflect their previous land use to those that reflect their current land use.

In another study, Li et al. (2021) used Biolog Eco Plates to investigate the effects of sulfuric and nitric acid rain on the metabolic functions of soil microbial communities. The authors found that the two types of acid rain had different effects on the function of soil microbial communities. Changing the type of acid rain can influence ecosystem stability and there is a risk of disrupting the ecosystem. In another study by Wang et al. (2019), using Biolog plates, it was demonstrated that microbial community function is more easily influenced than microbial biomass when adding nitrogen to soil.

These studies demonstrate the influence of environmental factor changes on the functional diversity of microorganisms. In addition, they demonstrate the usefulness of Biolog Eco Plates in assessing the impacts of human activities, such as changes in land use, nitrogen deposition, forest management, and others, on soil microbial communities and the resulting consequences for ecosystem functioning.

The aim of the present study is to compare the soil microbial carbon metabolic function, functional diversity and community similarity of six profiles of Luvisols from the forest territory of Western Stara Planina Mountain.

Materials and Methods

The subject of this study is the A horizon of Luvisols in a part of the Western Stara Planina Mountain region. Soil samples were collected using sterile instruments and stored in sterile pouches for microbiological analysis. The samples were kept at a temperature of four degrees Celsius until analysis. Prior

to analysis, the soil samples were cleaned of impurities and homogenized. Biolog EcoPlates were used to determine the soil microbial carbon metabolic function and functional diversity of the soil microbial community. These plates contain 96 wells, each one containing 31 different carbon sources in triplicate and the redox dye tetrazolium blue in triplicate. The first well of each triplicate serves as a control without a carbon source. To perform the analysis, serial dilutions and preparation of each sample were carried out. Inoculation into the Eco-Plates was performed in triplicate using a channel pipette. A single reading was taken after cultivation for 96 hours at 26°C without light. A positive reaction for the ability to utilize a given carbon source present in the well was recorded based on a change in color from clear to purple due to the production of formazan (Preston-Mafham et al., 2022).

In this study, we examined soil microbial carbon metabolic function as the ability of soil microbial communities to utilize specific carbon sources from the groups of Carbohydrates, Polymers, Carboxylic and ketonic acids, Amino acids and Amides/amines. Soil microbial carbon metabolic function is related to the diversity index, which represents % functional diversity and reflects the percentage utilization of carbon sources in the plate. Functional diversity was calculated using the following formula according to Puterbaugh & Edenborn (2007):

$$\textbf{\% functional diversity} = \frac{100 \times (\text{number of positive carbon source wells})}{31}$$

A comparison of the results obtained from the analyzed samples was performed, and the Simple Matching Coefficient was calculated using the following formula:

$$\textbf{Simple Matching Coefficient (SMC) \%} = \frac{(a+d)}{(a+b+c+d)} \times 100$$

where:
a = Number of carbon sources used by both sample A and sample B
b = Number of carbon sources used by Sample B but not by Sample A
c = Number of carbon sources used by Sample A but not by Sample B
d = Number of carbon sources not used by bacteria in either sample

Organic carbon content was determined according to the modified Tyirin method (Filcheva & Tsadilas, 2002). Soil acidity ($pH_{(H2O)}$) was determined using the ISO 10390 standard.

Connection between functional diversity and Organic carbon content, as so between functional diversity and soil pH were underwent statistical analysis using the StatSoft Statistica 12 program, with a significance threshold of 95%.

Results and Discussion

The positive reactions reflecting Soil microbial carbon metabolic function observed after incubation are shown in Figure 1.

8 kinds of sources were observed in TA1. The predominant sources used are from the group of Carboxylic and Ketonic acids (*Galactonic Acid-Lactone, Dgalacturonic Acid; 4-Hydroxy Benzoic Acid; DGlucosaminic Acid*). Three of the sources are Carbohydrates (*D-Xylose, D-Mannitol; N-Acetyl-Dglucosamine).* Only one source from the group of Polymer was used by the microbial community – Tween 80. There is no positive reaction for absorption of the group of Aminoacids and Amines/Amides. Unlike TA1 in TA2 10 kinds of sources were observed, and they fall into all Compound groups with the exception of Amines/amides group. From the Carbohydrates group *Pyruvic Acid Methyl Ester; D-Mannitol. Tween 40, Tween 80* and *Glycogen* were used by the Polymer group. From the group of Carboxylic and ketonic acids, *DGlucosaminic Acid, 4 - Hydroxy Benzoic Acid; Itaconic acid* were used. - *L-Arginine; L-Phenylalanine; L-Serine* from

the amino acids were used as a carbon source. The microbial community of sample TA3 assimilated the largest number of carbon sources – a total of 13. In this soil sample, the main sources of digestible carbon were from the Carbohydrates group (*D-Cellobiose; B - Methyl – D-glucoside; D-Xylose; D-Mannitol; N-Acetyl-D-glucosamine and Glucose-1-Phosphate*). No source was used from the polymer group. From the group of Carboxylic and ketonic acids were used - *2 - Hydroxy Benzoic Acid; 4 - Hydroxy Benzoic Acid, A-Keto Butyric Acid, D-Malic Acid*. *L-Arginine, L-Phenylalanine, and L-Serine* were used from the group of Amino acids. In TA4, ten carbon sources were assimilated. Again, similar to sample TA3, those from the Carbohydrates group prevail (*Pyruvic Acid Methyl Ester; D-Cellobiose; -β - Methyl-D-glucoside; D-Mannitol and D, L-a-Glycerol Phosphate*). Tween 40 and L-Threonine were the only sources used from the group of Polymers and the group of Amino acids, respectively. From the group of Carboxylic and ketonic acids, there was a positive reaction in the wells responsible for *Γ-Hydroxybutyric Acid; Itaconic Acid; D-Malic Acid*. The microbial community of sample TA5 tested positive in eleven wells. Three of the wells were from the group of Carbohydrates (*Pyruvic Acid Methyl Ester; D-Xylose; N-Acetyl-D-glucosamine*), three were from the group of Amino acids (*L- Aspargine, L- Threonine, Glycyl - Lglutamic Acid*). From the group of polymers, there was again only one positive reaction for absorption of *Tween 40*. Only one was the absorbed source and from the group of Amines/amides - *Putriscine*. *Γ-Hydroxybutyric Acid, Itaconic Acid and D-Malic Acid* were used as carbon sources absorbed by the microbial community from the group of Carboxylic and ketonic acids. Sample TA6 stands out with the least number of assimilated carbon sources. This sample yielded only six positive wells. All carbon sources used were from the group of Carbohydrates - *B -Methyl-D-glucoside; D-Xylose; D-Mannitol; N-Acetyl-D-glucosamine, Pyruvic Acid Methyl Ester; D-Cellobiose.*

TA1				TA2				TA3			
A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
D1	D2	D3	D4	D1	D2	D3	D4	D1	D2	D3	D4
E1	E2	E3	E4	E1	E2	E3	E4	E1	E2	E3	E4
F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4
H1	H2	H3	H4	H1	H2	H3	H4	H1	H2	H3	H4
TA4				**TA5**				**TA6**			
A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
D1	D2	D3	D4	D1	D2	D3	D4	D1	D2	D3	D4
E1	E2	E3	E4	E1	E2	E3	E4	E1	E2	E3	E4
F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4
H1	H2	H3	H4	H1	H2	H3	H4	H1	H2	H3	H4

A1 - Water; **A2** -B -Methyl-D-glucoside; **A3** - Galactonic Acid-Lactone; **A4** - L-Arginine;
B1 - Pyruvic Acid Methyl Ester; **B2** - D-Xylose; **B3** - Dgalacturonic Acid; **B4** - L-Aspargine;
C1 - Tween 40; **C2** - I - Erythritol; **C3** - 2 - Hydroxy Benzoic Acid; **C4** - L-Phenylalanine;
D1 - Tween 80; **D2** - D-Mannitol; **D3** - 4 - Hydroxy Benzoic Acid; **D4** - L-Serine;
E1 - Cyclodextrin; **E2** - N-Acetyl-D-glucosamine; **E3** - Γ-Hydroxybutyric Acid; **E4** - L- Threonine
F1 - Glycogen; **F2** - DGlucosaminic Acid; **F3** - Itaconic acid; **F4** - Glycyl - Lglutamic Acid;
G1 - D-Cellobiose; G**2** - Glucose-1-Phosphate; **G3** - A-Keto Butyric Acid; **G4** - Phenylethylamine;
H1 - D-Lactose; **H2** - D,L-A-Glycerol Phosphate; **H3** - D-Malic Acid; **H4** - Putrescine.

Fig. 1. Positive reaction of utilization of carbon sources.

After conducting the analysis, a clear tendency of the absence of bacteria that can metabolize *I - Erythritol*, *D-Lactose*, and *Putrescine* was observed in the studied soils. In all soils except TA4, microbial communities used *D-Mannitol* as a carbon source. After *D-Mannitol*, the most commonly used carbon sources were *D-Xylose* (TA1, 3, 5, and 6) and *N-Acetyl-D-glucosamine* (TA1, 3, 5, and 6).

Figure 2 Illustrates the percentage utilization of carbon sources by the different groups.

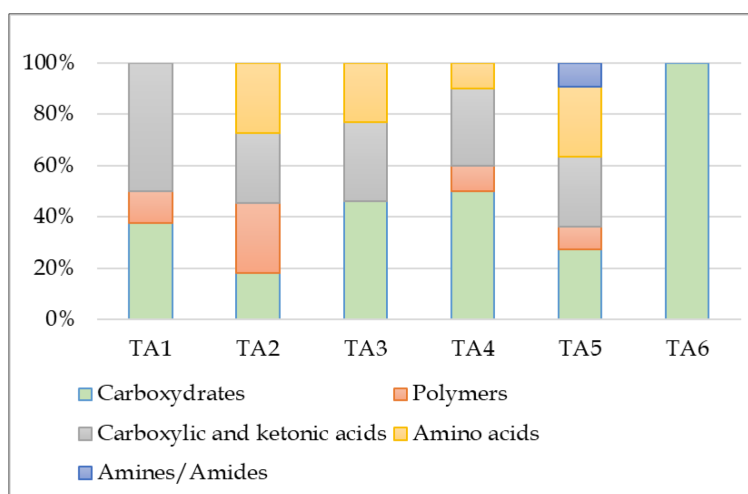

Fig. 2. The percentage use of carbon sources by the different groups.

The microbial communities from all the samples investigated utilized carbon sources from the group of Carbohydrates. TA6 is of interest, as all positive samples tested with Biolog were from the group of Carbohydrates. For the investigated microbial communities, the least attractive sources of carbon were from the group of Amines/amides (phenylethylamine and putrescine). Only the microbial community of TA5 showed the ability to utilize carbon from *Phenylethylamine*. After carbohydrates, carbon sources from the group of Carboxylic and ketonic acids were most commonly utilized. There was no relationship between the ability of microbial communities to utilize certain carbon sources and the vegetation cover. There was also no detected correlation with soil parameters.

The results of the functional diversity (%) of the studied soils, as well as some key soil characteristics, are presented in Table 1.

Table 1. Functional diversity (%) and some main soil parameters

TA	Altitude (m)	Vegetation	pH	Org. C $g.kg^{-1}$	Functional diversity (%)
TA1	610	*Meadow*	6.2	45.53	25.81%
TA2	510	*C. betulus, Q. robur, F. ornus*	4.9	54.95	35.48%
TA3	390	*Q. cerris, C. betulus, F. ornus*	7.4	73.67	38.71%
TA4	480	*Meadow*	5.8	35.43	32.26%
TA5	615	*C.betulus, F. sylvatica*	4.6	38.38	35.48%
TA6	185	*Q. frainetto, P. nigra, F. ornus*	5.1	24.20	19.35%

The results for functional diversity, expressed as a percentage, varied from 19.35% to 38.71%. This clearly demonstrated that despite investigating soils of the same type, their functional activity varied depending on the environmental conditions.

The results indicated a low functional diversity under mixed forest with coniferous species and under meadow vegetation. On the other hand, the sample taken under broadleaf vegetation showed a high functional diversity. These findings are consistent with other similar studies that emphasize the influence of vegetation on the functional diversity of soil microorganisms (Hu et al., 2010).

A correlation analysis was conducted between functional diversity (%) and soil organic carbon content (Fig. 3). The results showed a positive relationship between soil microbial functional diversity and soil organic carbon content (r=0.74). Similar findings of a direct proportional relationship between the two parameters have also been reported by other authors such as Gomez et al. (2006), Li et al. (2015) and Galieva et al. (2018).

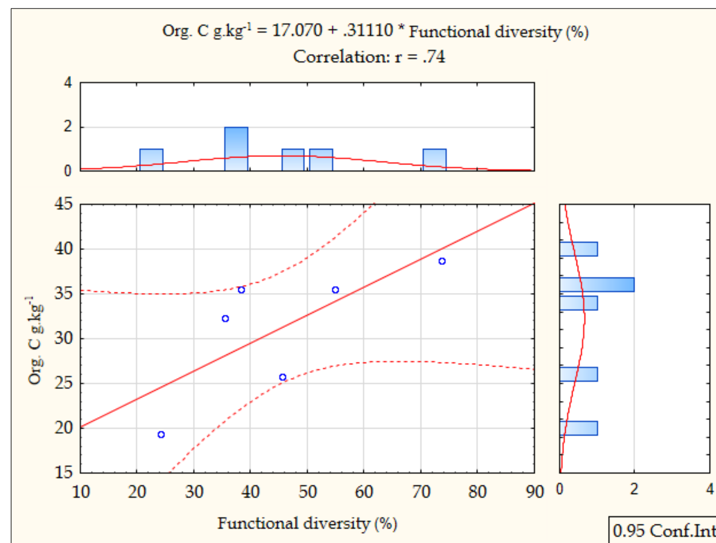

Fig. 3. Correlation between functional diversity (%) and Organic carbon content ($g.kg^{-1}$).

In the present study, no statistically significant correlation was observed between % functional diversity and soil pH. Similar findings were reported by Weng et al. (2021) who also found no correlation between soil acidity and functional diversity (%).

The Simple Matching Coefficient was calculated for the studied soils (Table 2) as a measure of the overall microbial community similarity. The coefficient compares the overall fingerprint of the microbial communities. The table shows that the most similar fingerprints were found between TA1 and TA6 (SMC=77%). The lowest similarity was observed between TA3 and TA5 (SMC=35%). The remaining samples showed a similarity of around 47%-68%, which can be attributed to the fact that 90% of the samples used D-Mannitol, 66% of the samples used β-Methyl-D-glucoside and 66% of the samples used N-Acetyl-D-glucosamine as sources of carbon.

Table 2. Simple Matching Coefficient of Tested areas (TA).

	TA1	TA2	TA3	TA4	TA5	TA6
TA1	N/A	65 %	58%	48%	65%	77%
TA2	65%	N/A	55%	58%	48%	58%
TA3	58%	55%	N/A	58%	35%	0.71
TA4	48%	58%	58%	N/A	47%	68%
TA5	65%	48%	35%	47%	N/A	65%
TA6	77%	58%	0.71	68%	65%	N/A

The Simple Matching Coefficient analysis did not reveal a clear correlation between the soil samples. The similarity between samples with meadow vegetation was found to be 48%. The highest similarity was observed between TA1 and TA6, which had meadow and mixed woody vegetation, respectively, and both had the lowest % functional diversity. These findings suggest a similar pattern in the soil microbiome under different vegetation and environmental conditions, which warrants further in-depth investigations into the response of microbial commu-nities to changes in the surrounding environment.

Each bacterial community has a specific ability to utilize certain compounds as sources of carbon (Zak et al., 1994). In this study, we aimed to analyze the soil microbial carbon metabolic function, functional diversity, and community similarity of six profiles from Luvisols. Previous research has shown the influence of cover vegetation and soil organic carbon content on the functional diversity of soil microbiota (Gomez et al., 2006; Hu et al., 2010; Li et al., 2015; Galieva et al., 2018), which aligns with our research objectives. Specifically, we examined soil microbial carbon metabolic function as the ability of soil microbial communities to utilize specific carbon sources, and the diversity index as the percentage of functional diversity reflecting the percentage utilization of carbon sources in the plate as individual parameters of soil microbial communities. Our study shows that despite the similar soil type, soil microbial communities exhibit different carbon metabolic function. Our results demonstrate varying functional diversity, with greater similarity in sources that are generally not utilized by communities as opposed to those utilized as carbon sources. The current scientific investigation indicates a positive correlation between soil microbial functional diversity and soil organic carbon content. Our findings demonstrate the significance of employing a more comprehensive analytical approach in investigating the soil microbiome's metabolic capabilities regarding the utilization of specific carbon substrates.

Conclusions

In this paper, we conducted an analysis of the Soil microbial carbon metabolic function, functional diversity, and community similarity of six profiles from Luvisols. Our results demonstrated that regardless of the soil type, soil microbial carbon metabolic function varied. Nevertheless, microbial communities showed greater capacity for utilizing carbon sources from the Carbohydrate group, while the lowest degree of utilization was observed in the Amines/amides group.

Our findings revealed a relationship between vegetation cover and soil microbial functional diversity. We also detected a positive statistical correlation between functional diversity and organic carbon content. The present investigation did not identify a direct effect of soil acidity on microbial functional diversity. There was no clear relationship between the Simple Matching Coefficient values obtained from the analyzed samples. The similarity between samples with meadow vegetation was 48%. The highest similarity was found between TA1 and TA6, located in meadows and mixed forest vegetation. These were the two samples with the lowest reported percentage of functional diversity. TA3 and TA5, with the lowest % functional diversity, showed the least similarity.

The present investigation and the acquired data may be utilized in prospective studies as a fundamental reference for comparative and long-term evaluation of the investigated sites.

Further research is warranted to scrutinize the soil micro-bial carbon metabolic function, the functional diversity and community similarity, as well as the manner in which multiple factors exert influence upon them.

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