Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 5, 568-577
2024
Publisher: Learning Gate
DOI: 10.55214/25768484.v8i5.1718

Microbial diversity and cellulase activity of forest soils and litters

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Abstract: The quantity, composition and cellulase activity of forest soils and litters with deciduous and coniferous tree vegetation on the territory of Natural Park "Vitosha" were investigated. Biogenicity, the rate of decomposition of organic matter and specifically of cellulose decomposition were higher in Fagus sylvatica litters than in Pinus sylvestris. In most soil profiles, the total microflora has higher values in the mixed fermentative and humic layer of the litter compared to the fresh litter. The quantity of microorganisms and cellulase activity decrease in the depth of the soil profiles. In general, non-spore-forming bacteria, followed by mold fungi and bacilli, occupy a major share in the composition of the total microflora in litters and soils, and actinomycetes are less represented. The cellulase activity depends to the highest degree on the non-sporulating bacteria, and the values of the mineralization coefficient on the bacteria assimilating mineral nitrogen. The humidity of the litters and soils has a stronger influence on the development, composition, mineralization and cellulase activity of microorganisms, compared to the altitude.

Keywords: *Cellulase, Forest litters and soils, Microorganisms.*

1. Introduction

Microorganisms in soils and litter participate in the circulation of substances, they are the main destroyers of organic substances in them and play an essential role in the sustainable functioning of forest ecosystems. The quantity, composition and enzymes produced by them are indicators used to evaluate the biogenicity, biochemical activity and fertility of the soil. Soil enzymes catalyze various biogeochemical processes related to the mineralization of nutrients at the ecosystem level.

Microbiological and biochemical soil indicators are considered as sensitive and useful markers of soil quality [1, 2, 3, 4, 5, 6], depending on abiotic and biotic factors, and guidelines in soil resource management [7]. Factors that affect soil microbiological activity also affect soil enzyme production and soil nutrient content [8].

According to some authors, bacteria in forest soils are the most abundant group of microorganisms [9, 10] - the high abundance of Acidobacteria, Actinobacteria and Proteobacteria [11, 12, 13, 14, 15]. Other authors found that microbial communities in forest soils are characterized by a high activity of mold fungi, especially in litter [16]. The transformation of organic matter in forest soils, as well as many other processes, depends on the activity of microorganisms, mainly fungi and bacteria [17]. The main share in the composition of the total microflora in forest soils is occupied by non-spore-forming bacteria, followed by bacilli, and actinomycetes and micromycetes are less represented [6, 18], as at lower pH values (acidic zone) the percentage of molds increases and that of non-sporing bacteria decreases [18].

Soil cellulase is a key enzyme in the carbon cycle [19]. The destruction of cellulose and hemicellulose by forest soil microorganisms occurs under the action of endocellulases, cellobiohydrolases, glucosidases and other glycosyl hydrolases [8, 15]. The rate of cellulose

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degradation in forest soils correlates with the total quantity of microorganisms and with the presence of organic substances in the soil - a decrease in cellulase in depth was found [6]. Kanazawa & Miyashita (1987) [20] in the study of brown forest soils found that the activity of the enzyme cellulase decreases in the lower soil horizons, which depends on the quantity and quality composition of the microorganisms in them. Bacteria actively contribute to the degradation of cellulose and hemicellulose in forest soil and litter, with *Pedobacter* and *Mucilaginibacter* showing complex enzyme systems containing a wide variety of carbohydrate-active enzymes for the degradation of cellulose and hemicellulose [15].

The aim of the study was to investigate the microbiological diversity and cellulase activity of forest soils and litters with deciduous and coniferous woody vegetation, determining the dependence between them and environmental factors (humidity, altitude).

2. Material and Methods

Six soil profiles were analyzed on the territory of the Natural park "Vitosha" - 4 under broad-leaved vegetation and 2 under coniferous vegetation, during the spring season (table 1).

Table 1.
General characteristics of the trial areas.

Soil profile/ Type	Soil horizon	Exposure	Slope	Altitude (m)	Tree vegetation	Base rock
SP1 Distric-eutric cambisols	LFH	Southeast	Upper part	1234	Pinus sylvestris L.	Monzonite
	A					
	Bw					
	C					
SP2 Regosols	L	West	Lower part	1223	Fagus sylvatica L.	Monzonite
	FH					
	A					
	C					
SP3 Distric-eutric cambisols	L	Southeast	Middle part	1195	Fagus sylvatica L.	Monzonite
	FH					
	A					
	Bw					
	C					
SP4 Distric-eutric cambisols	A	South	Lower part	1150	Pinus sylvestris L.	Andesite
	Bw					
	C					
SP5 Distric-eutric cambisols	L	West	Middle part	1370	Fagus sylvatica L.	Monzonite
	FH					
	A					
	Bw					
	C					
SP6 Distric-eutric cambisols	L	North	Lower part	1092	Fagus sylvatica L.	Monzonite
	FH					
	A					
	Bw					

Sampling was carried out along soil horizons with a sterile instrument in sterile paper bags. The method of limiting dilutions and triplicate inoculation of solid nutrient media was used with

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 5: 568-577, 2024
DOI: 10.55214/25768484.v8i5.1718

subsequent counting of colony-forming units (CFU) in 1 g of absolutely dry soil/litter [21, 22, 23]. . Systematic and physiological groups of microorganisms were defined - bacilli and non-spore-forming bacteria (on meat-peptone agar), micromycetes (mold fungi) - on Chapek-Dox agar, actinomycetes and bacteria assimilating mineral nitrogen (on Actinomycetes isolation agar). The total microflora was determined. The mineralization coefficient was calculated according to the formula: bacteria assimilating mineral nitrogen / (non-spore-forming bacteria+bacilli) [24, 25].

Cellulase activity was determined spectrophotometrically according to the method of Gradova et al. (2004) [26].

Soil moisture was determined on a moisture balance, brand DBS.

A correlation analysis is presented to establish interrelationships between the studied indicators. The MS-Excel 2010 program was used for statistical processing.

3. Results and Discussion

Microbial diversity in soils and dead forest litters has different trends depending on the soil horizon (sampling depth) and the type of vegetation (table 2).

Table 2.
Quantity, composition and activity of microflora in soils and forest litters (cfu/g).

Variant	Total microflora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	Bacteria assimilating mineral nitrogen	Mineralization coefficient
SP1-LFH	608678.88	456509.16	0.00	152169.72	0.00	1673866.92	3.67
SP1-A	903747.02	95131.26	237828.16	142696.90	428090.69	856181.38	2.57
SP1-Bw	117904.53	33212.54	24909.41	18266.90	41515.68	157759.58	2.71
SP1-C	16250.62	5644.95	5987.07	684.24	3934.36	23948.28	2.06
SP2-L	451145.04	300763.36	0.00	0.00	150381.68	5112977.10	17.00
SP2-FH	3282341.83	149197.36	1491973.56	895184.14	745986.78	20887629.84	12.73
SP2-A	459705.41	79259.55	142667.20	63407.64	174371.02	1870525.48	8.43
SP2-C	34562.89	17921.50	8800.74	1280.11	6560.55	69765.83	2.61
SP3-L	2828318.58	2199803.34	157128.81	157128.81	314257.62	4085349.07	1.73
SP3-FH	7460903.73	3968565.82	793713.16	952455.80	1746168.96	4762278.98	1.00
SP3-A	2067879.18	1730565.55	146658.10	131992.29	58663.24	1847892.03	0.98
SP3-Bw	223798.48	188630.15	7992.80	6394.24	20781.29	441202.72	2.24
SP3-C	20693.66	16683.26	1443.74	160.42	2406.24	41708.15	2.30
SP4-A	1532786.43	968075.64	161345.94	96807.56	306557.29	2178170.19	1.93
SP4-Bw	316395.21	261228.87	16225.40	0.00	38940.95	147651.10	0.53
SP4-C	22473.65	17092.64	2215.71	791.33	2373.98	51594.44	2.67
SP5-L	6101930.50	5423938.22	508494.21	0.00	169498.07	2372972.97	0.40
SP5-FH	4572984.52	3181206.62	397650.83	99412.71	894714.36	994127.07	0.28
SP5-A	851213.75	491084.85	130955.96	81847.48	147325.46	1636949.52	2.63
SP5-Bw	176445.53	44111.38	19409.01	54698.11	58227.02	105867.32	1.67
SP5-C	38691.67	28589.91	5908.58	1143.60	3049.59	114359.62	3.31
SP6-L	1573404.26	349645.39	699290.78	174822.70	349645.39	12062765.96	11.50
SP6-FH	3489956.33	1919475.98	697991.27	348995.63	523493.45	1919475.98	0.73
SP6-A	906140.35	588991.23	181228.07	75511.70	60409.36	0.00	0.00
SP6-Bw	180409.44	83396.82	54463.23	18721.73	23827.66	612711.31	4.44

Regarding the total microflora in the dead forest litter, the total quantity of microorganisms is higher in the mixed fermentation and humus layer (FH) of SP3 and the fresh fall (L layer) of SP5, followed by FH-layers of SP5, SP6 and SP2 - sites with deciduous tree vegetation – *Fagus sylvatica*. In general, the degradation of conifer litter is more difficult due to the phenolic compounds contained in

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 5: 568-577, 2024
DOI: 10.55214/25768484.v8i5.1718

conifers [6]. The rate of litter mineralization depends on temperature, humidity (climatic conditions), as well as the chemical composition of the plant material – coniferous litter decomposes more slowly due to the presence of phenolic compounds [27]. The mineralization coefficient has the highest values for both layers at SP2 and L layer at SP6, and the lowest values for both layers at SP5, i.e. the quantity of microorganisms is not an independent factor for their activity, which is also established in other microbiological studies of forest soils [6]. The total quantity of microorganisms in the litter of SP1 (*Pinus sylvestris*) was the lowest. At this site, at the time of sampling, the three layers of the dead forest litter were not distinguished, therefore it was studied as a mixed LFH layer. No dead forest litter was found at site SP4 (*Pinus sylvestris*).

For the L layer of the dead forest litter, non-spore-forming bacteria occupy a major share in the composition of the total microflora at all sites, except for the L layer of SP6, where spore-forming bacteria (bacilli) are more than non-spore-forming bacteria. No bacilli and micromycetes are found in the dead forest litter of SP1, in the fresh litter of: SP2 – bacilli and actinomycetes, SP5 – actinomycetes. For the L layer of SP3 and SP6, all studied groups of microorganisms were found with the following distribution in the composition of the general microflora: for SP3 – non-spore-forming bacteria 77.8%, bacilli and actinomycetes each 5.6%, micromycetes (mold fungi) 11.1% ; for SP6 – non-sporing bacteria and molds 22.2% each, bacilli 44.4% and actinomycetes 11.1%. For the FH layer of the dead forest floor, the main share in the composition of the total microflora is again occupied by non-spore-forming bacteria at all sites, except for the FH layer of SP2, where the quantity of mold fungi is higher than that of non-spore-forming bacteria. At all objects in the FH layer, all studied groups of microorganisms were found. The L-layer is dominated by epiphytes that came with the plant residues - non-spore-forming bacteria and yeast-like fungi (mineralization processes begin - simple carbohydrates, pectin and proteins are broken down); in the F-layer, cellulose-degrading fungi prevail, accompanied by bacteria and yeasts (cellulose, chitin, lignin are degraded; humic substances are synthesized); The H-layer is dominated by bacilli and actinomycetes (they complete the processes of degradation and synthesis of humic compounds) [27].

The biogenicity of the studied soils decreases in the depth of the soil profile, with the highest quantity being found in the A soil horizon of SP4, followed by the surface soil horizons of SP6 and SP1. The lowest is the total microflora in the C-horizon of SP1. For the A-soil horizon, non-spore-forming bacteria occupy a major share in the composition of the total microflora in soil profiles: SP3, SP4, SP5 and SP6, and micromycetes, followed by bacilli, in SP1 and SP2. For the B-soil horizon, non-spore-forming bacteria have a major role in SP2, SP3, SP4 and SP6, and micromycetes in SP1 and SP5. For the C-soil horizon, the main share in the composition of the total microflora is occupied by non-spore-forming bacteria at all sites, followed by bacilli and micromycetes, except for the C-horizon of SP1, where the bacilli are more than the non-spore-forming bacteria, and at SP6 no C- soil horizon. The least represented in the soils are the actinomycetes, except in the A-soil horizon of SP6, where the micromycetes are less numerous. The mineralization rate is the highest in the A-soil horizon of SP2, which is dependent on the ratio of bacteria assimilating mineral nitrogen (high quantity) and the sum of ammonifying bacteria (non-spore-forming bacteria and bacilli) – a lower quantity compared to the other sites.

A major share in the composition of the total microflora in forest soils is occupied by bacteria [9, 10, 11, 12, 13, 14, 15, 18, 28, 29], and at lower pH values (acidic zone) the percentage of mold fungi increases, and that of non-spore-forming bacteria decreases [18]. Similar trends are also found for agrogenic soils [30, 31, 32]. Other authors found that microbial communities in forest soils are characterized by a high activity of mold fungi, especially in litter [16].

Cellulose degradation occurred to a higher extent in litters, especially in the FH-layer, than in soil horizons. Cellulase activity by soil profiles and horizons is presented in the following figure 1.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 5: 568-577, 2024
DOI: 10.55214/25768484.v8i5.1718

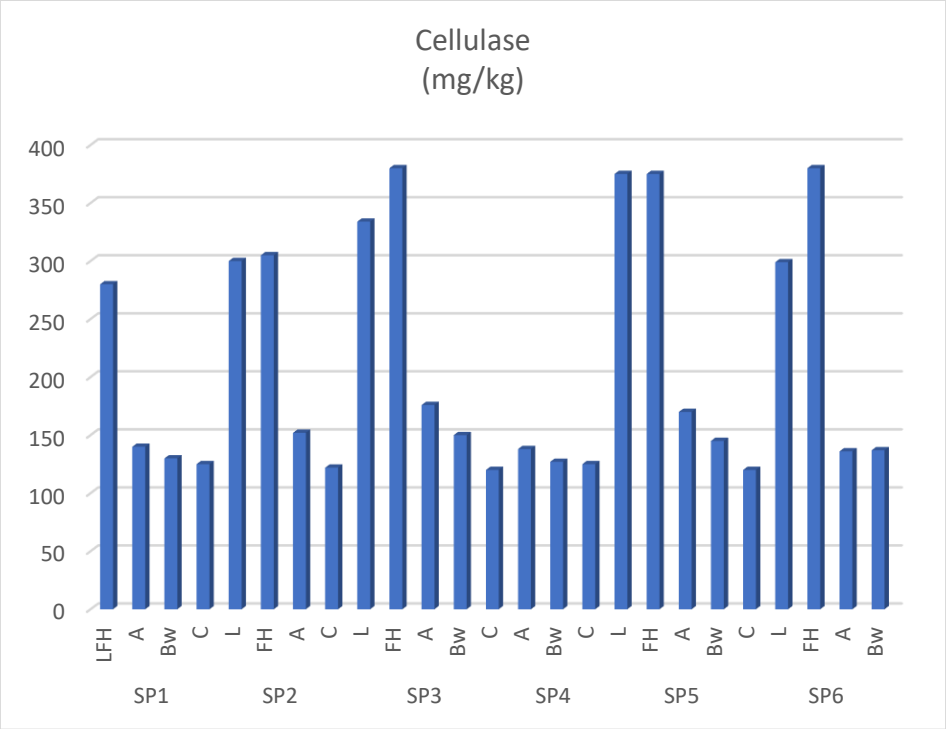

Figure 1.
Cellulase activity (mg/kg) of microorganisms in litters and soils.

The cellulase activity is highest in the FH-layer of SP3 and SP6, followed by L and FH of SP5. For the soils, the cellulase activity is higher in the A and B soil horizons of SP3 and SP5, but in depth - for the C horizon, the cellulase decreases to a higher degree compared to the other soil profiles. In general, cellulase activity in dead forest litter correlates with the total quantity of microorganisms, while for soils such a dependence is not fully established. The cellulase activity of the microorganisms in the dead forest litter depends on the type of accumulated litter – in deciduous woody vegetation (*Fagus sylvatica*), the enzyme values are higher than in coniferous woody vegetation (*Pinus sylvestris*). Similar trends were found for catalase activity in the same objects [33]. Malcheva et al. (2015) [34] reported that catalase activity was highest in the L layer and cellulase in the H layer of Pinus sylvestris forest litter, compared to the other two litter layers. According to these authors' study, catalase activity was higher in mineral horizons, increasing slightly with depth relative to litters, while cellulase activity was lower in mineral soil layers, also with a slight increase with depth. Cellulase activity decreases with depth and depends on the temperature and humidity of soils and litters, on the content of organic substances in them, on the quantity and composition of microorganisms [6, 20, 34].

To recalculate the quantity of microflora to an absolutely dry substrate and as the main factor for the development and activity of microorganisms, the humidity of the soil and litter was determined (Figure 2).

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 5: 568-577, 2024
DOI: 10.55214/25768484.v8i5.1718

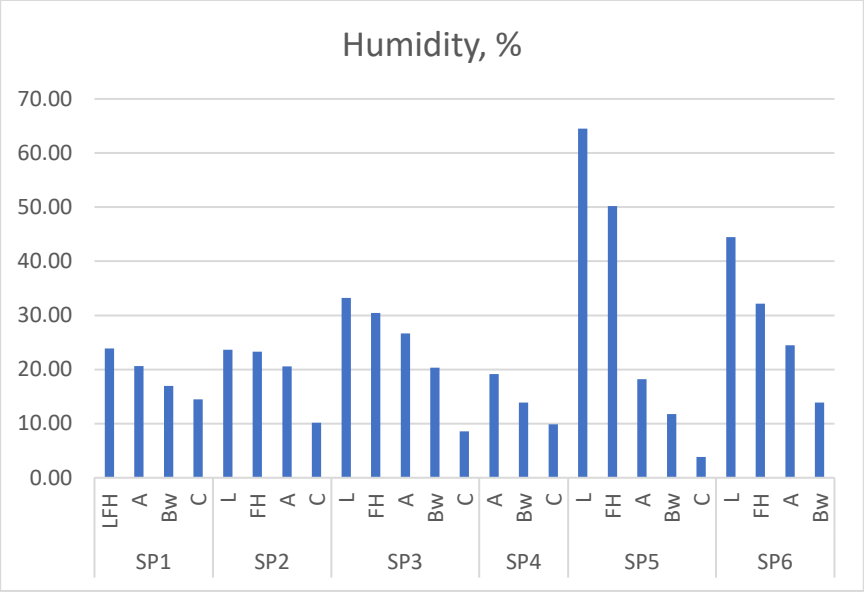

Figure 2.
Humidity (%) of litters and soils.

In general, mold fungi require higher humidity for their development, but no clear tendency to increase their quantity with increasing substrate humidity is established. Their quantity is higher in the litters, where the humidity is higher compared to the soil layers, but this decrease in the depth of the soil profile is characteristic of all studied groups of microorganisms. The biogenicity and enzymatic activity of soil microorganisms depend on the soil moisture (to a higher degree for mold fungi), the irrigation regime, the period of research after irrigation, the amount and composition of the microflora, the type of irrigation water (fresh, borehole, pool), fertilization, presence of vegetation and type of vegetation [35]. The correlation dependences between the individual indicators are presented in the following table 3.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 5: 568-577, 2024
DOI: 10.55214/25768484.v8i5.1718

Table 3.
Corelation coefficients.

Indicators	Total microflora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	Bacteria-N	Minera-lization coefficient	Humidity	Cellulase	Altitude
Total microflora	1									
Non-spore-forming bacteria	0.9155964	1								
Bacilli	0.6805547	0.379539	1							
Actino-mycetes	0.6524458	0.312355	0.842404	1						
Micro-mycetes	0.8145214	0.564371	0.693213	0.833676	1					
Bacteria-N	0.3389611	0.037389	0.838793	0.683453	0.415773	1				
Minera-lization coefficient	-0.162017	-0.3402	0.293463	0.180779	0.01213	0.6665769	1			
Humidity	0.7590702	0.810016	0.487469	0.199854	0.417368	0.2936905	-0.0050845	1		
Cellulase	0.8280404	0.74154	0.644581	0.542404	0.659516	0.4716313	0.1707908	0.804422	1	
Altitude	0.1379763	0.24132	-0.09585	-0.12927	0.008889	-0.1329588	-0.1162809	0.118609	0.081066	1

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 5: 568-577, 2024
DOI: 10.55214/25768484.v8i5.1718

The total microflora, non-spore-forming bacteria and cellulase activity strongly depend on the humidity of bedding and soils, for bacilli and micromycetes this dependence is moderate, and for actinomycetes, bacteria assimilating mineral nitrogen and mineralization rate – a weak dependence. The cellulase activity of microorganisms moderately depends on all studied groups of microorganisms, to the highest extent on the non-spore-forming bacteria, and the dependence of cellulase activity on the total microflora is high. A weak relationship was found between total mineralization activity (mineralization coefficient) and cellulose degradation (cellulase activity). A weak positive correlation was found between the mineralization coefficient and the bacilli, actinomycetes and micromycetes, and a moderate positive correlation with the bacteria assimilating mineral nitrogen. The development and activity of microorganisms are weakly dependent on altitude – all soil profiles are located in the range from 1092 m to 1370 m. At an altitude of 1200 m to 1500 m in forest soils, a high amount of total number of microorganisms is found [18], above 1800 m altitude the distribution of microorganisms in soils is strongly suppressed and above 2000 m altitude the total microbial number drops more than 5 times.

4. Conclusion

The quantity, mineralization activity and cellulase activity of microorganisms were higher in the litter of deciduous woody vegetation (*Fagus sylvatica*) compared to that of coniferous woody vegetation (*Pinus sylvestris*). In most soil profiles, the total microflora has higher values in the mixed fermentative and humic layer compared to the fresh litter. The quantity of microorganisms and the cellulase activity produced by them decrease in the depth of the soil profiles. In general, non-spore-forming bacteria, followed by mold fungi and bacilli, occupy a major share in the composition of the total microflora in litters and soils, and actinomycetes are less represented.

The cellulase activity of the microorganisms moderately, positively depends on all studied groups of microorganisms, to the highest degree on the non-spore-forming bacteria, and the dependence increases to high with the total microflora. Non-sporulating bacteria and cellulase activity depend strongly, positively, and bacilli and micromycetes - moderately, positively, on the humidity of the substrates. A moderate, positive relationship was found between the mineralization coefficient and bacteria assimilating mineral nitrogen. A difference in altitude in a range of about 300 m between individual soil profiles does not have a significant impact on the quantity and activity of microorganisms in soils and litters.

The quantity, composition and cellulase activity in forest soils and litters can serve as sensitive microbiological and biochemical indicators for: assessment of the sustainability of forest ecosystems, health and fertility of soils, the degree of decomposition of organic substances in litters and soils, analysis of the cycle of the carbon.

Acknowledgements:

The current study was performed in the framework of "Study of interrelations in the system "soil - soil microorganisms - tree composition" in forest stands on the territory of „Vitosha" Nature Park." – KP-06-N56/7" funded by Research Fund of Ministry of Education and Science in Bulgaria.

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