ASSESSMENT OF SOIL AND LITTER PARAMETERS IN YUNDOLA STATIONARY SAMPLE PLOT FOR INTENSIVE MONITORING OF FOREST ECOSYSTEMS

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Abstract

Litterfall is an essential part of forest ecosystems. We assessed obtained data about aboveground litterfall (as input for nutrients in the ecosystem) and chemical composition of soil waters (as an output of the ecosystem). In addition, total microbial number of heterotrophic microflora and the structure of microbial communities were investigated. The aboveground litterfall had an average pH_{H20} value 4.2. Concentrations of Ca, Mg and P were high in the foliar fraction. The C/N ratio in the Litter fraction was high – 65 and in the Wood fraction – 60. In contrast to the L layer, FH layer showed an increase in microorganisms' activity. The total microbial number decreased in the A horizon compared to that in the foliar litter. The total microbial number for layer L was 1.2×10^7 cfu/g DsM (dry soil mass), increased in the fragmented FH layer to 1.4×10^7 cfu/g DsM and sharply decreased to 9.0×10⁵ cfu/g DsM in A horizon. The dominance of non-sporeforming bacteria was established in the litter and A horizon of the soil profile. Actinomycetes increased significantly in the soil depth. In the L layer they were only 1 % and in A horizon - they reached 16 %. The percentage of micromycetes was 1 % and increased up to 3 % in A horizon. The group of Spore-forming bacteria was 9 % of the total microflora. The reaction of soil solution sampled by tension lysimeters was assessed as slightly acidic to neutral. The electrical conductivity varied between 21 and 140 µS/cm, which characterized waters as slightly mineralized. From 2011 to 2019, there was a balanced ratio of cations with basic and acidic functions. There was a slight tendency for the removal of basic cations outside the soil profile and this process should be observed over time.

Key words: aboveground litterfall, lysimeter, microflora, soil solution chemistry.

Introduction

The concentration of nutrients in soils are determined by rock weathering, amount of litterfall (depending on tree species), forest management practices, chemical composition of the atmospheric deposition and others (Zimmermann et al. 2002, Starra et al. 2003, Berg et al. 2006). Litterfall introduces org. C into the soil (Sayer 2006), which allows forecasting the retention of org. C in the soils (Liski et al. 2005). The parameters of aboveground litterfall (total mass and chemical composition) are important for assessing the nutrients in forest ecosystems (Schleppi et al. 2007). Soil microorganisms as a part of forest ecosystems play a key role in the transformation of organic matter (Schloter et al. 2018). The decomposition of litterfall is a main process of biogeochemical cycles in the forest (Bani et al. 2018). The degradation of the litterfall is influenced by the type of plant litter, the quantitative and qualitative composition of the microbial community, as well as external factors, such as temperature, humidity and others (Li et al. 2017).

The 'input' of substances influences the pH of soils due to the formation of organic acids (Máthé-Gáspár et al. 2005). The chemical composition of litterfall of deciduous trees changes during the vegetation period Finér (1996). In autumn, the concentrations of nitrogen, phosphorus and potassium are lowest, and the concentrations of calcium and manganese are highest. In recent decades, the natural cycle of the elements has been disrupted by the influence of atmospheric depositions, as reported by several authors. Increased return of nitrogen, calcium, magnesium and potassium with the wood fall in soils was observed, in the range of 3.8-5.1 times more than the concentrations of the natural cycle (Zimmermann et al. 2002). According to Asi et al. (2008), increased return was also found for sulphate sulphur, magnesium and other elements.

The export of nutrients from the forest ecosystem is driven by biomass removal (logging) and leaching (Smidt et al. 2012).

The relationship between litter chemical composition of deciduous trees, soil and chemical composition of soil waters is contradictory because it is associated with a wide variation in their values. It has been found that the values of chemical composition of soil waters are most dynamic, captured right below the litter (Merila and Derome 2008). In Bulgaria, the parameters of aboveground litterfall and soil solution sampled by tension lysimeters are measured at 3 stationary sample plots for intensive monitoring – 'Yundola', 'Vitinya' and 'Staro Oryahovo' (Kolarov et al. 1997– 1998, Kolarov et al. 1999–2002, Malinova et al. 2003, Pavlova et al. 2004–2008), as part of the European Forest Ecosystem Monitoring Network (ICP Forest Manuals 1986–2016).

The aim of the study was to evaluate the amount of aboveground litterfall, the concentration of nutrients in the litterfall and chemical composition of soil waters from the intensive monitoring stationary sample plot in Yundola area, obtained for 2019. Further, we investigated their annual dynamics for the period of 2013 to 2019. In addition, we characterized the soil microbial community in the litterfall and in *A* horizon (upper mineral horizon) of the soil.

Subject and Method

Yundola stationary sample plot is in the Western Rhodopes, on the territory of Yundola Training and Experimental Forest Range (41°55'34" N and 23°53'40" E). The experimental site is located in the Middle Mountain Subarea (Koleva-Lizama 2002) and is characterized by a mountain climate.

It was selected as representative for the Norway spruce (*Picea abies* (L.) Karst.) forests in the country (Kolarov et al. 2002). The altitude is 1550 m. The soil type is *Dystric* Cambisols, formed on granite.

Aboveground litterfall was collected in 5 traps, each with an area of 1 m². It was collected every two weeks. After the collection, aboveground litterfall is sorted into

different fractions – foliar litter fraction (L), wood fraction (WF, bark, branches, twigs and others) and fruits and seeds fraction (FS) (ICP Forests Manual 2016a).

Eight sites were used to collect soil solution samples, in which lysimeters were pre-installed. Soil solution was sampled by tension lysimeters. The collected samples from A horizons in each site were mixed into one pooled sample. Same applies to the samples collected for Bw horizons. The samples of lysimeters were collected every two weeks according to ICP Forests Manual (2016b) requirements. Sampling and laboratory analyses of the observed parameters were carried out by the Executive Agency for the Environment at the Ministry of Environment and Waters in connection with the implementation of 'The International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests under the Convention on Longrange Transboundary Air Pollution of the United Nations Economic Commission for Europe' (ICP Forests 1986-2016). The obtained data are compared to the indicated minimum and maximum values of the different parameters of litterfall in ICP Forests (1986–2016). These parameters are monitored in the ICP Forests intensive Programme across Europe.

Microbiological assays perwere formed for L, FH layers and A horizon. The assays were performed under indirect cultural method. This method includes successive dilutions of the initial soil suspension (Koch method), subsequent inoculation on elective solid media. Nutrient agar for non-spore forming bacteria and spore forming bacteria (after pasteurization), actinomycete isolation agar for actinomycetes and Czapek-Dox Agar for micromycetes) were used. Germinated colonies were counted (Bonnet et al. 2020). The microbiological assays included determination of spore-forming bacteria and non-spore-forming bacteria, micromycetes and actinomycetes and subsequent determination of total microbial number. The measurement was carried out in colony forming unit under Ig logarithm per gram dry soil mass (cfu Ig/DsM soil).

Results and Discussion

The mass of litterfall has been observed for the period 2010–2019 (Fig. 1). The obtained data showed that annual total biomass of aboveground litterfall varies widely between 200 and 488 g/m². Similar results have been described by some other authors (Berg et al. 2006). The amount of different litter fractions determines the value fluctuation of aboveground litterfall total biomass.

The amounts of wood fraction (WF) and fruit and seeds fraction (FS) varied much less due to the almost equal amounts of bark, twigs, reproductive organs, and others that fall into the litter traps every year.

The maxima of aboveground litterfall for the coniferous tree species are in different seasons. For example, most abundant needle litterfall of the Norway spruce (*Picea abies*) is in the spring (Zimmermann et al. 2002). In Yundola stationary sample plot the maxima of the aboveground litterfall were measured in October.

There were also periods when the amount of aboveground litterfall was steady – for 2 months, mainly October and November. The data show that wood fraction was in high amounts at the beginning of the vegetation season – April, and in the end – October. For the fruits and seeds fraction, this period was in September–October.

Table 1 presents the chemical char-



Fig. 1. Total biomass of the aboveground litterfall for the period 2010–2019. Note: L – litter, WF – wood fraction, FS – fruits and seeds.

acteristics of the aboveground litterfall by fractions in 2019.

The obtained data (Table 1) show that pH_{H20} of the aboveground litterfall for 2019 is in strongly acidic spectrum. Over time, values continue to show stability (Fig. 2). The pH of fraction *L* changes very slightly.

The reaction of the *FS* fraction showed a wider range of variation but is in the acidic spectrum throughout the observed period.

The nitrogen content of the aboveground litterfall for 2019 was assessed as average - it was in the range between the minimum and maximum values specified in the ICP Manual for Norway Spruce litterfall (Table 1). Its quantity was relatively equal in the observed period, which forms a lasting trend (Fig. 3). The org. C/N ratios differed in the individual fractions. The highest value was in WF - 86, which corresponds to the slow decomposition of organic matter. It was also high in fraction L = 65. Fraction FS was subject to the fastest decomposition - org. C/N = 51.

The concentrations of sulphur in deciduous trees are monitored in the forest ecosystem monitoring system (ICP Forests 1986–2016) due to increased air pollution in the recent past and associated acidic atmospheric depositions (Koptsik et al. 1998, Michel and Seidling 2017). In some areas, soil acidification also extends to deeper soil layers (Iwald 2016). After 2000, recovery processes have been registered in some parts of Europe (Vanguelova et al. 2008). For Eastern Europe, however, these processes are reported to be delayed due to the later development of industrialization (Lorenz et al. 2007).

The available data show that the sulphur concentration in the aboveground litterfall from Yundola stationary sample plot in 2019 was low (Table 1). Figure 4 shows the decreasing trend of sulphur concentrations.

In 2019 the concentrations of macroelements – calcium, potassium and magnesium were assessed as high and as av-

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Litter traps No	Frac- tion	рН _{нго}	Org. C, g/100g	Fe, µg/g						mg	۶,				
-		4.06	51.56	206.81	7.39	0.78	0.61	7.72	1.01	2.48	28.66	1173.13	1.47	1.90	105.36
0	u	4.13	50.21	224.57	7.32	0.95	0.80	7.45	0.98	1.86	39.36	1356.61	3.38	1.90	105.53
ę	ctio	4.14	51.06	256.40	8.38	1.10	0.87	16.65	1.88	2.55	40.76	1394.83	0.75	0.11	106.43
4	ert.	4.19	51.66	161.02	7.81	0.93	0.67	15.09	1.45	2.46	35.57	1672.88	2.14	0.96	107.13
5	tter	4.22	50.97	267.64	8.12	0.91	0.72	9.36	1.17	2.20	39.56	1290.10	2.03	1.82	106.93
Mean	ר!	4.15	51.09	223.29	7.80	0.93	0.73	11.25	1.30	2.31	36.78	1377.51	1.95	1.34	106.28
St. dev.		0.06	0.58	42.47	0.46	0.11	0.10	4.31	0.37	0.28	4.94	185.33	0.97	0.79	0.80
~		5.17	52.56	84.20	4.42	0.63	0.65	<3.00	0.49	3.28	26.72	69.36	5.20	3.08	106.05
2	u	5.16	53.08	65.09	6.66	0.70	0.53	<3.00	0.54	3.31	29.36	66.60	4.64	3.99	107.95
ი	ictic	5.12	52.28	55.45	6.73	0.87	0.74	<3.00	0.58	3.45	27.78	76.04	4.96	2.53	105.61
4	sıt b	5.18	52.46	54.52	4.92	0.73	0.37	<3.00	0.56	2.97	25.25	96.37	3.17	2.64	105.67
5	000,	5.05	54.05	91.90	7.89	0.81	0.45	<3.00	0.57	3.19	35.35	103.47	4.39	1.82	107.11
Mean	M	5.14	52.89	70.23	6.12	0.75	0.55		0.55	3.24	28.89	82.37	4.47	2.81	106.48
St. dev.		0.05	0.72	17.00	1.42	0.09	0.15		0.04	0.18	3.91	16.58	0.79	0.80	1.02
~		4.43	51.27	702.08	8.65	0.80	0.77	4.99	0.71	2.10	52.28	336.53	7.86	9.95	314.32
2	spe	4.43	50.91	617.51	8.66	06.0	0.79	9.13	0.63	1.86	78.57	344.51	7.80	12.08	320.68
ი	uc əəs	4.43	51.27	748.92	8.73	0.88	0.73	5.71	0.71	1.66	43.63	257.85	7.71	6.65	211.26
4	bne bitoe	4.47	51.31	689.85	9.05	0.80	0.69	8.74	0.76	1.79	46.33	464.38	8.81	9.57	429.98
5	s eti srt	4.42	51.03	773.01	10.42	06.0	0.51	5.52	0.69	2.10	55.96	277.43	8.15	12.33	321.60
Mean	μĩ٦	4.44	51.16	706.28	9.10	0.86	0.70	6.82	0.70	1.90	55.35	336.14	8.07	10.12	319.57
St. dev.		0.02	0.18	60.08	0.76	0.05	0.11	1.96	0.05	0.19	13.85	80.76	0.45	2.29	77.38



Fig. 2. Reaction (pH_{H20}) of the aboveground litterfall fractions for the period of 2011–2019. Note: L – Litter, *WF* – wood fraction, *FS* – fruits and seeds.





Note: L – Litter, WF - wood fraction, FS – fruits and seeds.



Fig. 4. Sulphur concentration in aboveground litterfall fractions for the period of 2011–2019 and ICP Forest criteria.

Note: L – Litter, WF – wood fraction, FS – fruits and seeds.

erage for phosphorus (Table 1). They are a continuation of already formed trends in the reviewed period.

The trace elements were high throughout the period. There was an even distribution of their quantities between the different fractions. It should be noted that in 2019 the lead concentration was high in the *WF* fraction.

The degradation of the litter reflects the activity of the microbial community (Golebiewski et al. 2019). The obtained data showed a higher microbiological activity in the litter formed by the litterfall, compared to the *A* horizon, due to the higher total microbial number. This decrease in the total microbial number was a result of a decrease in the amount of organic matter in the *A* horizon.

As pointed by Prescott and Grayston 2013 and Bach at al. (2010), different tree species have different chemical composition and affect the development of soil

microorganisms in different ways.

Due to the slow decomposition of *Picea abies* needles, the organic matter in the surface *L* layer of the litter is in a steady state. This is explained by the presence of persistent substances, such as lignin, resins, and waxes, which are degraded by a smaller spectrum of microorganisms. In contrast to the *L* layer, the *FH* layer shows higher microbial activity. The C/N ratio remained high and determined the slow decomposition of organic matter on the soil surface.

Table 2 presents the reported microbiological indicators of the studied soils (lg cfu/g DsM).

The change of the total microbial number was characterized by a decrease in the *A* horizon compared to litter. In the *L* layer it was 1.2×10^7 cfu/g DsM, increased in the fragmented *FH* layer to 1.4×10^7 cfu/g DsM and sharply decreased to 9.0×10^5 cfu/g DsM in *A* horizon.

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Layers/	Total microbial number	Spore-forming bacteria	Non-spore- forming	Micromycetes	Actinomycetes
110112011			lg cfu/g DsM		
L	7.08 ±0.67	6.03 ±0.66	7.03 ±0.72	0 ±1.49	4.99 ±0.43
FH	7.14 ±0.67	6.09 ±0.66	7.07 ±0.72	2.27 ±1.49	5.83 ±0.43
Α	5.95 ±0.67	4.91 ±0.66	5.79 ±0.72	4.39 ±1.49	5.23 ±0.43

Table 2. Microbiological indicators.

The proportion of microbial groups in the soil microbial community is one of the main indicators of the degree and rate of transformation of soil organic matter (SOM) (Perfanova et al. 2015). Amount and variety of substrates available for decomposition influence soil and litterfall microflora (Gomoryova et al. 2013). The percentage share of microbial groups (Fig. 5) shows that the *L*, *FH* layers and *A* horizon were dominated by the group of nonspore-forming microorganisms. Their percentage of the total microflora decreased in depth – from 90 % in the *L* layer to 69 % in the *A* horizon. Contrary to this trend was the percentage of actinomycetes. It increased significantly in depth. In the *L* layer it is 1 %, in the *A* horizon it reaches 16 %. Micromycetes were not reported in *L* layer, which may be due to specific micro conditions of the environment. Their percentage participation in *FH* layer was 1 % and increased up to 3 % in *A* horizon. With a constant amount in the whole studied profile was the group of spore-forming bacteria – 9 % of the total microflora.

The main microorganisms that carried out the initial stages of mineralization of organic compounds were non-sporeforming bacteria, due to which their per-



Fig. 5. Proportion of microbial groups.

centage was highest in the L layer.

The loss of nutrients from the soil was carried out in an acidic environment, as this was monitored by the pH of the soil solution sampled by tension lysimeters in 2019 (Table 3). In the studied period their reaction was characterized by some variation but does not differ significantly.

In comparison to the criteria specified in the ICP Forest Manual (2016b), the values of all indicators were assessed as low. They were close to the lower limit of the established values in soil solution. Soil solution sampled by tension lysimeters had low mineralization. The electrical conductivity of the soil solution varied in a wider range compared to pH but was assessed as low. For example, in the metamorphic horizon it was between 23 and 72 µS/cm.

The conductivity of the solution was also very low. It was dominated by chlorine ions, sulphates, nitrates, phosphates, and ammonium ions. The basic cations were in low concentrations.

Conclusions

The reaction of the aboveground litterfall had stable values for the period 2011-2019. The microbial proportion in the litter, as well as in the A horizon, showed a dominance of the group of non-sporeforming bacteria, which are responsible of the processes of transformation of more easily degradable soil organic matter. The trend in chemical composition of soil waters sampled by tension lysimeters is also stable - they were slightly mineralized. There was a balanced ratio between the cations with basic and acidic functions. The results showed that a small part of the studied elements has been lost in the soil substances cycle process. There is

			-	Table 3. C	Chemi	ical cc	soduuc	sition of	soil wa	aters ir	A an	Id Bw h	orizor	Is for 2	019.				
suozin	огн	Alka- linity,	Electrical conduc- tivitv.	Dis- solved organic matter	¥	Ca	Mg	N-NO ₃	S-SO4	N-NH4	ਹ	Total N	Na	Е	ЧW	Zn	Сц	Pb	cq
он	d	Nomu	hS/cm					mg/dm³							ybrl	dm³			
A	4.61	32	64.35	7.26	2.52	5.50	0.71	0.07	6.24	0.03	4.20	0.71	1.65	421.37	288.00	64.00	13.00	1.00	0.03
ЗW	5.48	32	72.43	5.72	1.84	4.62	1.05	0.16	7.20	0.03	4.00	0.59	2.12	109.60	544.75	29.75	2.00	1.00	0.03

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a slight tendency to export basic cations outside the soil profile, a process that needs to be traced over time.

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