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The Effect of Stand Structure on Soil Physico-Chemical and Biological Properties in a Primary Beech Forest

Ján Židó, Ladislav Šumichrast, Stanislav Kucbel and Erika Gömöryová *

Faculty of Forestry, Technical University in Zvolen, TG Masaryka 24, SK-96001 Zvolen, Slovakia
* Correspondence: gomoryova@tuzvo.sk

Abstract: The study investigates the links and interactions between soil properties, soil microorganisms and the structure of a primary beech forest. The study was performed in the reserve Havešová (Bukovské vrchy Mts., Slovakia). On 40 sampling plots, soil samples from the O-horizon and from the first 10 cm of the organo-mineral horizons were taken to analyze the physico-chemical and biological properties. Moreover, stand structural characteristics (volume of trees, additive stand density index, coefficient of homogeneity, tree influence potential, development stage indices, etc.) were measured and calculated. In general, we did not observe any strong effects of forest structure on the topsoil characteristics. The effect of stand structure was more reflected in the physico-chemical properties than in the biological attributes. We found that the P and K content in the forest floor increased at plots with a higher volume or density of trees per plot. Moreover, a positive correlation was found also between the K content and tree influence potential. The development stages expressed by the indexes based on the diameter structure were reflected especially by the soil reaction in the A-horizon. Within functional groups of microorganisms based on the Biolog assay, significant differences were found, especially in the utilization of D-cellobiose, which positively correlated with the presence of the optimum stage index. The effect of soil physico-chemical properties on biological indicators was more pronounced than the effect of stand structure.

Keywords: topsoil spatial variability; soil ecosystem; microorganisms; functional diversity; primary beech forest; stand structure

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1. Introduction

Positive as well as negative relationships between the soil microbial attributes and plants have been documented from plenty of experiments [1–3]. Trees can distinctly influence the soil physico-chemical and biological properties as they represent a major biomass component in forest ecosystems, and their impact on soil is much longer lasting than the impact of agricultural crops, for instance. Trees significantly contribute to the production of organic matter, which, in the form of litter, dead roots and root exudates, serves as the main source of nutrients for soil biota. Furthermore, trees influence the redistribution of these sources in soil under the canopy by drawing water and through the water-released nutrients by roots [4,5]. Root and soil microorganisms interact directly with each other [6,7].

Trees also influence soil microorganisms indirectly by modifying the penetration of solar radiation and precipitation water input to the soil (crown interception, stemflow and changes in rainwater chemistry) [8]. Moreover, trees affect the composition of the herb and shrub layers, which also interact with soil microorganisms [9].

Most of the primary beech and beech-dominated forest remnants are located in the Carpathians—Slovakia, Ukraine and Romania—and in the mountain ranges of the western Balkans—Slovenia, Bosnia and Herzegovina and Albania [10]. According to Korpeľ [11,12], the primary beech forests of eastern Slovakia belong to the best-preserved primary forests, not only in Slovakia, but also throughout Europe. They represent a unique example of

undisturbed temperate forests and exhibit the most complete and comprehensive ecological patterns and processes of pure stands of European beech across a variety of environmental conditions. Therefore, the selected primary beech forests of Slovakia and Ukraine were inscribed on the UNESCO World Heritage List in 2007 [13].

The structure of primary beech forests in Central Europe is shaped mainly by frequent small-scale disturbance events. Although medium-to-large-sized disturbances can also occur infrequently [14–16], prevailing small-scale gap dynamics generate an intricate heterogenous structure [17], which creates diverse conditions in the understory [18]. This heterogeneity in forest structure (vertical or horizontal) can be distinguished using multiple structural indices [19].

In the past, several authors tried to describe the dynamics of the primary forest structure using the forest cycle and its development stages [12,20,21]. These stages were differentiated based on the various structural characteristics (stand volume, deadwood, diameter distribution, etc.), but distinction among the stages in the field was often based on subjective evaluation. In the last decade, more accurate methods were developed [22,23]. Feldmann et al. [23] introduced an approach that allows to easily determine the extent of overlapping development stages and to describe the complex structure of primary beech forests on relatively small inventory plots.

Most studies performed in the recent past dealt with comparisons of the soil properties between primary and managed forests [24,25]. So far, no information is available about the effects of the stand structure of primary beech stands on forest floor composition and the topsoil. In light of the fact that the forestry sector in a substantial part of Europe currently tends to shift the stand management practices towards close to nature forestry, mimicking processes in natural forests, such information is needed to steer this transition in a meaningful way. Therefore, the objectives of this study were (1) to analyze and find out whether the differences in stand structure of the primary beech forest are reflected in the soil properties and microbial communities; and (2) to evaluate the dependence of the soil biological properties on the stand structure and physico-chemical properties of soil.

2. Materials and Methods

2.1. Site Description

The experimental site was situated in beech-dominated primeval forests in the National Nature Reserve (NNR) Havešová (Bukovské vrchy Mts., Slovakia, 49°00′35″ N 22°20′10″ E). NNR Havešová has been included in the UNESCO World Nature Heritage list since 2007 [26]. NNR Havešová covers 171.32 ha and is situated between 440 and 741 m above sea level. The forest cover is a primary European beech forest (*Fagus sylvatica* L.) with an admixture of sycamore (*Acer pseudoplatanus* L.), ash (*Fraxinus excelsior* L.) and elm (*Ulmus glabra* Huds.). The study site is located in the middle warm to middle cool and very humid regions. The mean annual air temperature is 6.0–6.5 °C, and the annual precipitation ranges between 800 and 850 mm. The main soil type in the study area is Dystric Cambisol developed on Paleocene sandstone and mudstone. The average slope is 15°, with a S or SW aspect.

Within the study area, 40 circular plots were established by stratified random selection; the grid spacing was 140 m with respect to the natural conditions (Figure 1). The research plots were selected by a research team led by Professor Leuschner from the Georg-August University in Göttingen [25].

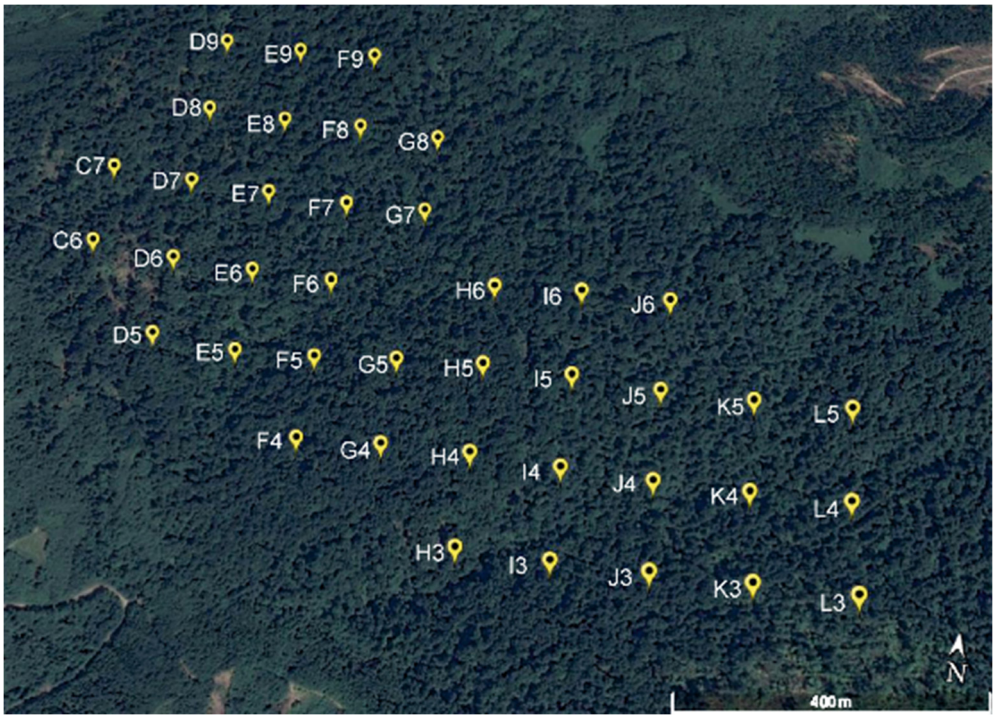


Figure 1. Position of the 40 research plots (Google Earth, Google LLC).

2.2. Stand Structure Characteristics

The data for this study were collected in the abovementioned network of 40 research plots. To assess the stand structure, each plot was divided into three concentric circles of the size 500 m^2, 100 m^2 and 25 m^2. According to the original methodology of Professor Leuschner, living trees with a diameter at breast height (DBH) $\geq$ 8 cm were measured in the largest circle; trees with a DBH of 1–8 cm were added in the middle-size circle; and trees with a DBH of <1 cm (but with height $\geq$ 20 cm) were also measured [25]. For the trees with a DBH $\geq$ 8 cm, the diameter, distance and azimuth referenced to the plot center were recorded. In addition to this, in the 100 m^2 circles, we recorded the DBH of the recruitment (DBH = 1–8 cm), and in the 25 m^2 circles, the height of the regeneration individuals (DBH < 1 cm) was measured, to an accuracy of 5 cm.

In the largest circle, the DBH of the standing and lying dead trees (DBH $\geq$ 8 cm) was measured, too, provided that the stump was located within the plot. Moreover, we applied the classification system of Meyer et al. [27] to assign a decay class to every dead tree trunk.

We characterized stand structure via different indicators. First, we used allometric equations to quantify the regeneration and recruitment aboveground biomass (AGB) [28]. Though only an approximate measure, AGB can be used as an estimate of how beech regeneration fills the space, integrating information on the stem number and height in a single parameter [29]. Second, the volume (V) of trees with a DBH $\geq$ 8 cm was calculated. Tree heights needed to fit the stand height curve [30] were obtained from previous measurements in Havešová (TUZVO—Department of Silviculture) and V was calculated for living trees according to the volume equations of Petráš and Pajtík [31].

Subsequently, we computed two structural indices to better capture the various components of stand structure. Stand density was estimated using the additive stand density index (ASDI) by Long and Daniel [32].

$$ASDI = \sum_i 20\left(\frac{DBH_i}{25}\right)^{1.6} \quad (1)$$

where DBH_i is the diameter of the i^{th} tree (cm).

ASDI is a modification of the stand density index by Reineke [33] and can be used in uneven-aged stands. To characterize the DBH heterogeneity, we utilized the coefficient of homogeneity, H [34], which represents the relationship between the stem number and its volume in diameter classes.

$$H = \frac{\sum_{i=1}^{n-1} SN_i}{\sum_{i=1}^{n-1} SN_i - SV_i} \quad (2)$$

where SN_i is the cumulative relative frequency of the stem number (%) in the i^{th} diameter class; SV_i is the cumulative relative frequency of the stand volume (%) in the i^{th} diameter class; and n is the number of diameter classes.

H values range from 1 to theoretical infinity, where lower values signify more heterogenous and higher values a more homogenous stand structure. In addition to stand structural characteristics, tree influence potential, IP [24,35], was quantified. IP considers the size and distance impact of individual trees on a sample plot.

$$IP = \sum_i BA_i e^{-d_i} \quad (3)$$

where BA_i is the basal area of the i^{th} tree (m^2); and d_i is the distance from the research plot center of the i^{th} tree (m).

Finally, we employed the development stage index (I_{DS}) designed by Feldmann et al. [23]. I_{DS} allows to quantify the relative extension of three development stages at the plot level using empirical data on stem number and tree size. To calculate it, trees were classified into diameter classes, whose thresholds should indicate a transition between three ontogenetic development phases [36]. Based on that classification, premature trees ($7 \leq$ DBH $<$ 40 cm), mature trees ($40 \leq$ DBH $<$ 70 cm) and over-mature trees (70 cm $\leq$ DBH) were assigned to the initial, optimum and terminal development stage, respectively. Dead trees were also incorporated into the computation because of the impact they have had on the structure in the recent past. However, reduction factors specific for each decay class (DC) were applied for calculating the stem number and basal area (DC1 = 1, DC2 = 0.95, DC3 = 0.85, DC4 = 0.7, DC5 = 0.5). Subsequently, the stem number and basal area of a given development stage (DS: ini—initial; opt—optimum; ter—terminal) were expressed in relative terms using the research plot with the highest value as a reference (maximum N_{DSi}/BA_{DSi} in the study site). The I_{DS} value in an individual research plot p was then computed as

$$I_{DSp} = \left(\frac{N_{DSp}}{N_{DSref}} + \frac{BA_{DSp}}{BA_{DSref}} \right) \quad (4)$$

where N_{DSi} is the actual stem number (n); N_{DSref} is the maximum stem number (n); BA_{DSi} is the actual basal area (m^2); and BA_{DSref} is the maximum basal area (m^2).

The relative proportions of individual I_{DS} in plot i were computed as well (I_{DS}%). I_{DSi} along with I_{DS}% shed more light on the structural composition and the mixing of development stages inside a research plot, while the development stage with the highest I_{DSi} value is considered dominating in that plot. Finally, we computed the evenness (E_{Ids}) of the I_{DS}% for each research plot to quantify its structural heterogeneity. It was calculated by dividing the Shannon diversity index by its maximum, which represents equal relative abundance of the developmental stages (I_{DS}%) at the plot.

2.3. Soil Properties and Microbial Analysis

Soil samples for the analyses of the physico-chemical and microbial properties were collected in the center of each plot in July 2020. Samples were taken from the O-horizon (surface organic layer) from the area of 0.0625 m^2 and from the uppermost A-horizon, 500 g each sample, from a depth of 0–10 cm. After bringing the samples to the laboratory, each sample was divided into two parts, and a part of the samples used for the physico-chemical

analyses was air-dried. The remaining part of the samples was stored in a refrigerator until microbial analyses were performed.

Soil water content was estimated based on weighing a soil sample before and after the oven-drying at 105 °C to constant weight. Soil reaction (pH-KCl) was determined in soil-1M KCl solution (1:2.5) after 24 h potentiometrically. Elementary analysis with thermal conductivity detection was used for the determination of total carbon and nitrogen content. Plant available phosphorus and exchangeable potassium, calcium and magnesium were measured in Mehlich III soil extracts employing the ICP-AES (inductively coupled plasma atomic emission spectrometry); all analyses were performed in the laboratories of the National Forestry Centre in Zvolen, Slovakia.

For the determination of basal respiration (BR), we used the alkali absorption approach. CO_2 released from samples during 24 h at 22°C were absorbed in 0.05 N NaOH and its amount was estimated by the titration with 0.05 N HCl after the precipitation of carbonates by $BaCl_2$. Catalase activity (Cat) was estimated using the method of Khaziev [37], which is based on the measurement of oxygen volume released during 10 min. after 3% hydrogen peroxide was added to a fresh soil sample. N mineralization (Nmin) was determined according to Kandeler [38]. Briefly, soil samples were incubated under anaerobic conditions at 40 °C for 7 days and the released NH_4–N was quantified using a colorimetric procedure.

Microbial biomass carbon (C_{mic}) was determined using the procedure described by Islam and Weil [39]. Soil samples were microwave-irradiated to kill the soil microorganisms and extracted with 0.5 M K_2SO_4. Carbon concentration in the extract was quantified by the oxidation with potassium dichromate in the presence of H_2SO_4 and titrimetrically by ammonium sulfate (Mohr's salt). The same procedure was used for non-irradiated samples. C_{mic} was then determined as

$$C_{mic} = \frac{TOC_i - TOC_{ni}}{K_{ME}} \quad (5)$$

where TOC_i is the total organic carbon in the irradiated sample (μg C g^{-1}), TOC_{ni} is the total organic carbon in the non-irradiated sample (μg C g^{-1}) and K_{ME} is extraction efficiency factor (equal to 0.213; [39]).

Two approaches were used for the characterization of the community-level physiological profiles (CLPPs) of the microbial communities. The first one employed Biolog® EcoPlates [40] that contain 31 different carbon sources and the redox dye tetrazolium. A 150 μL extract, prepared by resuspending the soil in 0.85% NaCl and diluted to 1:1000 and 1:10,000 for samples from the O- and A-horizon, were put in each well of the EcoPlate and incubated at 27 °C for 5 days. The absorbance rate at 590 nm was recorded at regular intervals using the Tecan's Sunrise absorbance microplate reader (Tecan, Salzburg, Austria). The metabolic activity was calculated as the area below the time–absorbance curve and was used as a measure of the abundance of the respective functional group.

The second approach, which is according to Campbell et al. [41], is based on the colorimetric quantification of carbon dioxide evolved from 300 mg of soil samples placed in the deep wells of microtiter plates and amended by 25 μL of solution with a carbon source concentration of 30 mg of C g^{-1} of soil water. We used the following twelve substrates: α-ketoglutaric acid, L-arginine, asparagine monohydrate, cellulose, L-glutamine, DL-malic acid, malonic acid, D-mannose, D-(−)-methylglucamine, L-phenylalanine, L-serine and D-(+)-xylose. Immediately after the amendment with C source, the plate was covered by a microtiter plate that contained 1% Noble agar gel with a dye—cresol red pH indicator (12.5 μg g^{-1}), potassium chloride (150 mM) and sodium bicarbonate (1.5 mM). The CO_2 evolved from the soil samples was absorbed into the alkali, which was set in a gel, causing a color change. The plate with agar was read immediately before incubation and after 6 h of incubation at 25 °C with a Tecan's Sunrise absorbance microplate reader (Tecan, Salzburg, Austria) at 590 nm. The absorbance after 6 h was normalized for any differences recorded at the first reading before exposure. The absorbance values were converted to the CO_2 amount using the calibration curve.

2.4. Statistical Analysis

All values of the microbial characteristics were converted per unit of dry matter of the soil. For the calculation of the diversity of soil microbial functional groups in the Biolog® approach, we used the Hill's index [42]:

$$N_2 = \frac{1}{\sum_i p_i^2} \quad (6)$$

where p_i is the frequency (relative abundance) of the i-th functional group.

Basic statistical characteristics (average, standard deviation, coefficient of variation, minimum and maximum value) were calculated for the individual soil characteristics within the individual horizons. To analyze our data, we used the statistical and analytical software STATISTICA developed by StatSoft. We used one-way analysis of variance and subsequently Tukey's post-hoc tests to determine whether the individual soil characteristics differ significantly between the individual horizons.

To assess how the soil properties and composition of the functional groups of microorganisms are related to the stand structure, Pearson's correlation coefficients were calculated and direct gradient analysis (redundancy analysis; RDA) was performed using CANOCO 5, Centre of Biometry, Wageningen, NL [43]. The significance of the environmental variables and RDA axes was tested using the Monte-Carlo permutation test (999 runs). To account for multiple comparisons, the significance levels of the individual environmental and vegetation factors were adjusted using the Bonferroni correction.

3. Results

3.1. Spatial Variability of Stand Structure and Soil Biological Properties

The mean values and variability in stand structure characteristics are given in Table 1. We found the highest variability in the case of tree influence potential, which depends on the size of the trees and their proximity to the plot center. Other characteristics that varied markedly were the aboveground woody biomass of the regeneration and recruitment individuals. In contrast, the characteristics representing structural heterogeneity (coefficient of homogeneity—H; evenness of the developmental stages—E_{Ids}) varied little among research plots. Consistently, the low H and considerably high E_{Ids} values indicate markedly heterogeneous stand structures, which are typical for beech primary forests. Other characteristics, such as volume and additive stand density index, had a slightly higher variability than H and E_{Ids}. We also found differences between the distributions of the development stage indexes. Index values of the terminal stage were relatively low and variable, in contrast to the index values of the almost ubiquitous initial stage.

Table 1. Overview of the measured stand structure characteristics.

Stand Structure Characteristics	Mean ± S.D.	Min.	Max.	Coefficient of Variation (%)
Volume (V) ($m^3 \cdot ha^{-1}$)	658 ± 257.14	156.00	1168.00	39.10
Aboveground woody biomass of regeneration (AGB_{reg}) (kg ha^{-1})	335 ± 460.80	0.00	2131.83	137.61
Aboveground woody biomass of trees DBH 1–4 cm ($AGB_{d1–4}$) (kg ha^{-1})	756 ± 782.41	0.00	3613.79	103.45
Aditive stand density index (ASDI)	509.28 ± 144.34	221.00	833.00	28.34
Coefficient of homogeneity (H)	1.46 ± 0.20	1.21	2.16	13.75
Tree influence potential (IP)	121.16 ± 226.62	0.73	1315.48	187.05
Initial stage index (I_{ini})	0.82 ± 0.38	0.27	2.00	46.45
Optimum stage index (I_{opt})	0.77 ± 0.44	0.00	2.00	57.19
Terminal stage index (I_{ter})	0.55 ± 0.41	0.00	2.00	75.44
Evenness (E_{Ids})	84.79 ± 15.41	40.88	99.73	18.18

A basic overview of the investigated physico-chemical and biological variables is given in Table 2. The soil characteristics, except for C_{mic}/C, differ significantly between the forest floor and the organo-mineral horizon. Most physico-chemical and biological variables achieve higher values in the O-horizon than in the A-horizon, except the C_{mic}/C ratio. We observed a considerable spatial variability in the soil parameters in the O- and A-horizon. The coefficient of variation ranges from 4.28% to 106.24% in the O-horizon and from 6.61% to 75.1% in the A-horizon. Some of the soil properties exhibited higher variability in the O-horizon (e.g., catalase activity, microbial biomass, N-mineralization) while other in the underlying A-horizon (all chemical properties except C/N and most of the biological attributes).

Table 2. Overview of the basic statistical characteristics of the measured soil parameters for individual soil horizons and the Tukey tests of the differences in means between the O- and A-horizons.

Soil Properties	Mean ± S.D.		Min.		Max.		Coefficient of Variation (%)	
Horizons	O-Hor	A-Hor	O-Hor	A-Hor	O-Hor	A-Hor	O-Hor	A-Hor
Soil biological properties								
Catalase activity (mL O_2.min^{-1} g^{-1})	16.48 ± 4.54a	3.23 ± 0.70b	6.84	1.34	31.12	4.23	27.55	21.64
Basal respiration (μg CO_2.g^{-1} h^{-1})	23.17 ± 6.41a	0.51 ± 0.24b	8.89	0.16	42.74	1.34	27.65	47.86
Microbial biomass (μg C g^{-1})	6,617 ± 3,615a	735 ± 269b	990	329	17,395	1364	54.63	36.64
N-mineralization (μg NH_4^+–N g^{-1} d^{-1})	26.17 ± 11.01a	1.25 ± 0.40b	8.16	0.67	63.98	2.17	42.09	32.26
Hill's index	13.55 ± 2.01a	10.55 ± 1.82b	9.29	5.71	17.18	13.67	14.84	17.21
Richness	23.40 ± 2.49a	19.00 ± 2.29b	19.00	14.00	28.00	25.00	10.64	12.04
C_{mic}/C	179.89 ± 96.85a	200.84 ± 76.74a	30.37	107.97	413.19	505.53	53.84	38.21
BR/C_{mic}	0.50 ± 0.54a	0.07 ± 0.02b	0.12	0.03	3.43	0.14	106.24	32.38
BR/C	0.63 ± 0.17a	0.14 ± 0.05b	0.22	0.05	1.04	0.50	27.51	55.49
Physico-chemical properties								
Soil Moisture (% *w*/*w*)	243.72 ± 73.47a	44.58 ± 10.06b	32.43	28.59	415.04	75.54	30.15	22.57
pH-KCl	5.46 ± 0.23a	4.80 ± 0.32b	4.81	4.15	6.09	5.54	4.28	6.61
N (%)	1.51 ± 0.25a	0.26 ± 0.08b	1.04	0.12	1.91	0.45	16.82	30.98
C (%)	37.22 ± 4.55a	3.86 ± 1.33b	28.20	1.78	45.90	8.37	12.23	34.45
Mg (mg kg^{-1})	574 ± 86a	77 ± 44b	400	23	776	229	15.11	56.93
Ca (mg kg^{-1})	3,525 ± 385a	673 ± 420b	2,849	79	4528	1,593	10.93	62.35
K (mg kg^{-1})	906 ± 312a	122 ± 59b	471	44	1805	282	34.51	48.44
P (mg kg^{-1})	81.12 ± 22.11a	4.87 ± 1.57b	49.00	2.25	132.00	8.21	27.26	32.16
C/N	25.11 ± 3.73a	15.10 ± 2.08b	17.91	11.98	32.46	19.27	14.87	13.75
Community level physiological profiles (CLPPs)								
α-Ketoglutaric acid (Ket) (μg CO_2 -C g^{-1} h^{-1})	4260 ± 1360a	403 ± 184b	1176	138	7861	928	31.94	45.73
Arginine (Arg) (μg CO_2 -C g^{-1} h^{-1})	1786 ± 801a	131 ± 65b	402	18	3777	319	44.88	49.82
Asparagine (Asp) (μg CO_2 -C g^{-1} h^{-1})	2682 ± 1347a	109 ± 63b	455	24	6385	265	50.24	57.98
Cellulose (Cel) (μg CO_2 -C g^{-1} h^{-1})	1051 ± 791a	50 ± 20b	156	23	3129	115	75.30	40.99
Malic acid (Mal) (μg CO_2 -C g^{-1} h^{-1})	2358 ± 678a	385 ± 114b	930	186	3680	758	28.76	29.70
Methylglucamine (Metg) (μg CO_2 -C g^{-1} h^{-1})	2105 ± 928a	122 ± 50b	252	32	4440	234	44.11	41.33
Phenylalanine (Phe) (μg CO_2 -C g^{-1} h^{-1})	1094 ± 414.44a	80 ± 34b	242	10	1939	148	37.88	42.97
Serine (Ser) (μg CO_2 -C g^{-1} h^{-1})	3550 ± 1716.59a	163 ± 88b	1139	45	11,215	412	48.35	54.30
Mannose (Man) (μg CO_2 -C g^{-1} h^{-1})	3154 ± 1392.23a	176 ± 91b	752	40	8036	524	44.14	52.10
Glutamine (Glu) (μg CO_2 -C g^{-1} h^{-1})	1215 ± 394.65a	157 ± 66b	345	37	1988	349	32.49	42.36
Malonic acid (Maln) (μg CO_2 -C g^{-1} h^{-1})	1969 ± 1174a	106 ± 79b	327	6	6115	358	59.61	75.10
Xylose (Xyl) (μg CO_2 -C g^{-1} h^{-1})	3926 ± 1390a	121 ± 69b	692	13	6606	316	35.41	57.24

Mg, Ca and K—exchangeable magnesium, calcium and potassium, respectively; P—available phosphorus; different letters designate homogeneous groups based on Tukey´s HSD post-hoc tests.

3.2. Stand Structure as a Factor Influencing Soil Physico-Chemical and Biological Properties

In the case of the effect of stand structure on physico-chemical properties (Tables 3 and 4), we found differences between the horizons. In comparison to the organo-mineral horizon,

the volume and stand density index (ASDI) had a positive effect on the soil reaction, N, K and P content within the forest floor, indicating that the soil pH, N, P and K content was higher at plots where trees had a higher volume or higher density per plot. The effect of the other structural characteristics was less pronounced. Nevertheless, correlation coefficients showed that the effect of tree influence potential (IP) also had a positive effect on the pH and K content within the forest floor, but in the organo-mineral horizon it had a negative effect or was indifferent to these parameters, which means that soil pH and K content was higher in the vicinity of bigger trees. Moreover, within the A-horizon we found a negative correlation between the optimum stage index, pH and Ca content and a positive correlation between the initial stage index and pH. On the other hand, within the forest floor, a positive correlation between the optimum stage index (I_{opt}) and P content, terminal stage index (I_{ter}) and Ca content, and a negative correlation between the terminal stage index and C/N ratio were found; in the organo-mineral horizon, they were non-significant. Thus, diameter structure significantly influenced the soil properties; e.g., the dominance of trees in the initial growth stage ($7 \leq$ DBH < 40 cm) contributed to higher values of soil pH, the dominance of mature trees ($40 \leq$ DBH < 70 cm) caused lower values of soil pH and Ca content but higher values of P content, while the plots with a dominance of over-mature trees (70 cm $\leq$ DBH) exhibited a lower C/N ratio and a higher Ca content.

Table 3. Correlation coefficients between the soil physico-chemical and biological properties, as well as the stand structure characteristics, of the forest floor (O-horizon).

	V	**AGB_{reg}**	**$AGB_{d1–4}$**	**ASDI**	**H**	**IP**	**I_{ini}**	**I_{opt}**	**I_{ter}**	**E_{Ids}**
Soil biological properties										
Cat	−0.163	0.130	0.095	−0.180	−0.071	0.022	0.025	−0.197	−0.059	−0.023
BR	0.158	0.168	−0.107	0.256	0.340*	−0.005	0.169	0.234	−0.065	−0.153
Cmic	0.104	0.404 *	−0.026	0.121	−0.016	−0.032	−0.037	0.137	−0.056	0.166
Cmic/C	0.061	0.420 **	−0.010	0.089	0.018	−0.030	−0.014	0.167	−0.107	0.139
BR/Cmic	−0.218	−0.179	0.058	−0.129	0.281	−0.037	0.314	0.079	−0.160	−0.238
BR/C	−0.031	0.114	−0.052	0.104	0.470 **	−0.008	0.284	0.270	−0.201	−0.226
N−min	0.137	0.340 *	−0.093	0.176	0.024	−0.017	−0.104	0.248	−0.129	0.001
Hill index	−0.088	−0.027	0.251	−0.085	−0.175	0.262	−0.128	0.052	−0.020	0.113
Richness	0.110	0.010	0.150	0.108	−0.143	0.322 *	−0.068	0.101	0.047	0.150
Physico-chemical properties										
Moisture	0.099	0.114	−0.116	0.150	0.232	−0.010	0.133	0.135	0.001	−0.008
pH	0.325 *	−0.305	0.019	0.392 *	0.046	0.323 *	0.170	0.095	0.055	0.089
N	0.366 *	0.142	0.159	0.332 *	0.055	0.105	−0.205	0.229	0.250	0.229
C	0.194	0.166	0.217	0.225	0.253	0.311	−0.081	0.301	−0.081	0.038
C/N	−0.289	−0.059	−0.028	−0.219	0.143	0.123	0.190	−0.019	−0.365 *	−0.245
Mg	0.291	−0.077	0.021	0.268	−0.134	0.095	−0.162	0.067	0.203	0.332 *
Ca	0.243	−0.164	−0.102	0.187	−0.117	−0.003	−0.088	−0.142	0.367 *	0.240
K	0.401 *	−0.071	−0.025	0.446 **	0.030	0.454 **	−0.081	0.295	0.067	0.256
P	0.437 **	0.205	−0.012	0.444 **	0.092	0.225	−0.266	0.392 *	0.031	0.085

* $p < 0.05$; ** $p < 0.01$; Mg, Ca and K—exchangeable magnesium, calcium and potassium; P—available phosphorus; Cat—catalase activity; BR—basal respiration; N-min—nitrogen mineralization; Cmic—microbial biomass carbon; Richness—functional group richness; Hill's index—diversity of functional groups; V—volume of trees; $AGB_{reg/d1–4}$—aboveground biomass of regeneration/trees with a DBH of 1–4 cm; ASDI—stand density index; H—coefficient of homogeneity; IP—tree influence potential; I_{ini}—initial stage index; I_{opt}—optimum stage index; I_{ter}—terminal stage index; E_{Ids}—evennes.

Table 4. Correlation coefficients between the soil physico-chemical and biological properties, as well as the stand structure characteristics, of the organo-mineral A-horizon.

	V	AGB_{reg}	$AGB_{d1–4}$	ASDI	H	IP	I_{ini}	I_{opt}	I_{ter}	E_{Ids}
Soil biological properties										
Cat	0.238	0.149	−0.294	0.199	−0.163	−0.250	−0.049	−0.092	0.046	0.076
BR	0.279	0.128	0.356 *	0.248	−0.042	0.051	−0.161	0.070	0.018	0.018
Cmic	0.244	0.148	0.217	0.158	−0.017	0.286	−0.287	−0.019	0.181	0.107
Cmic/C	0.166	0.089	0.271	0.120	0.158	0.098	−0.071	−0.046	0.149	0.137
BR/Cmic	0.186	0.026	0.167	0.219	−0.033	−0.051	0.072	0.098	−0.106	−0.067
BR/C	0.184	0.104	0.399 *	0.169	0.094	−0.036	−0.018	0.021	0.030	0.071
N−min	0.175	−0.035	−0.097	0.125	0.007	0.109	0.045	−0.310	0.194	−0.042
Hill index	−0.193	0.031	0.293	−0.108	0.098	0.204	−0.006	0.250	−0.365 *	0.061
Richness	0.082	0.124	0.199	0.195	0.333 *	0.026	0.027	0.361 *	−0.305	−0.034
Physico-chemical properties										
Moisture	−0.012	0.099	0.055	−0.125	−0.090	0.361 *	−0.185	−0.288	0.200	0.025
pH	−0.304	−0.192	−0.203	−0.287	0.072	−0.400 *	0.420 **	−0.441 **	−0.001	−0.074
N	0.106	0.051	−0.026	0.145	0.099	0.014	0.025	0.071	0.062	0.238
C	0.018	−0.006	0.002	0.094	0.200	−0.004	0.082	0.106	−0.046	0.124
C/N	−0.167	−0.187	0.057	−0.070	0.232	−0.039	0.207	0.028	−0.197	−0.223
Mg	−0.026	−0.187	−0.150	−0.001	0.060	−0.241	0.291	−0.278	0.116	0.095
Ca	−0.208	−0.155	−0.070	−0.213	0.110	−0.228	0.300	−0.400 *	0.083	0.010
K	0.150	−0.203	−0.186	0.199	−0.050	0.116	0.160	−0.074	0.090	0.152
P	0.252	0.115	−0.013	0.194	0.023	−0.055	−0.102	−0.116	0.233	0.183

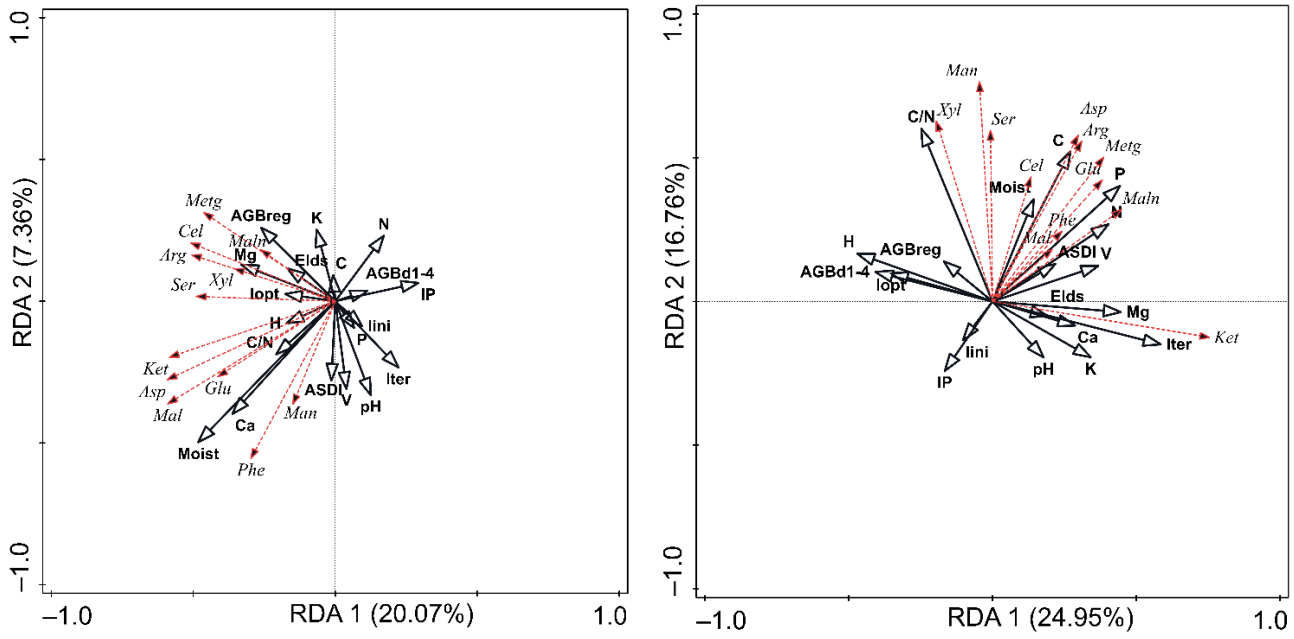
* $p < 0.05$; ** $p < 0.01$; Cat—catalase activity; BR—basal respiration; N-min—nitrogen mineralization; Cmic—microbial biomass carbon; Richness—functional group richness; Hill's index—diversity of functional groups; V—volume of trees; $AGB_{reg/d1–4}$—aboveground biomass of the regeneration/trees with a DBH of 1–4 cm; ASDI—stand density index; H—coefficient of homogeneity; IP—tree influence potential; I_{ini}—initial stage index; I_{opt}—optimum stage index; I_{ter}—terminal stage index; E_{Ids}—evenness.

When evaluating the effect of stand structure on the soil microbial community descriptors, we found that some of the correlation coefficients showed a weak but significant effect of the structural characteristics on microbial attributes at the level $p < 0.05$ (e.g., IP in the forest floor, $AGB_{d1–4}$, H, I_{opt}, and I_{ter} in the A horizon) or at the level $p < 0.01$ (AGB_{reg} and H in the forest floor). Specifically, in the O-horizon we found a significant positive correlation between the tree influence potential (IP) and richness of the functional groups (Rich), coefficient of homogeneity (H), basal respiration and BR/C, and between the aboveground biomass of regeneration (AGB_{reg}) and microbial biomass (C_{mic}), C_{mic}/C ratio and N-mineralization. This indicates that different aspects of stand structure have different effects on the microbial community: the richness of functional groups increases with the increasing size of trees in the vicinity of the sampling plot; in turn, basal respiration was higher where trees had a homogenous structure, and microbial biomass and N-mineralization were higher where regeneration was more abundant. In the A-horizon, we found only a weak positive effect of aboveground biomass of trees with a DBH of 1–4 cm ($AGB_{d1–4}$) on BR and the BR/C ratio. In comparison to the forest floor, the coefficient of homogeneity (H) and optimum stage index had a positive effect on the richness of functional groups in the organo-mineral horizon. We also found a negative correlation between the terminal stage index and Hill's index of functional diversity, indicating that while the richness of the functional groups was higher in the stands with dominance of mature trees, diversity decreased with the increasing dominance of over-mature trees.

3.3. Stand Structure and Soil Physico-Chemical Properties as Factors Influencing Community-Level Physiological Profiles

Within the forest floor of NNR Havešová, the first two ordination axes explained 27.42% of the total variation of CLPP based on the MicroResp™ approach, while in the organo-mineral horizon, the first two ordination axes explained up to 41.71% of the total variation (Figure 2); however, none of the effects was significant after Bonferroni correction.

According to correlation analysis, we found a significant positive correlation at $p < 0.05$ between the coefficient of homogeneity and the activity of the functional group utilizing serine (Ser), aboveground biomass of regeneration (AGB_{reg}) and the activity of functional groups malonic acid (Maln) and arginine (Arg) within the forest floor (Table S1). In the organo-mineral horizon, negative correlations between H and activity of the functional group utilizing α-ketoglutaric acid (Ket) ($p < 0.05$), tree influence potential (IP) and activity of the functional group methylglucamine (Metg) ($p < 0.05$), and optimum stage index and consumers of malic acid (Mal) ($p < 0.05$) were found (Table S2). Moreover, within the organo-mineral horizon, we also found positive correlations between the terminal stage index and activity of the functional group α-ketoglutaric acid (Ket) ($p < 0.01$) between the volume of trees and the groups utilizing asparagine (Asp) ($p < 0.05$). In the case of physico-chemical properties, in the forest floor, especially soil moisture influenced the activity of the functional groups, while in the organo-mineral horizon, the C and P content and C/N ratio also showed a distinct effect (Tables S3 and S4). Soil moisture is a property that can change quite rapidly. However, as water is a basic resource for the soil microbiota, the physiological processes of soil microorganisms are expected to react sensitively to changes in water availability; therefore, we suppose that the observed correlations reflect real causal relationships. Generally, increased moisture and nutrient contents led to a higher activity of functional groups in both horizons, except the effect of the K content. On the other hand, activity of the functional groups was negatively related to the C/N ratio.

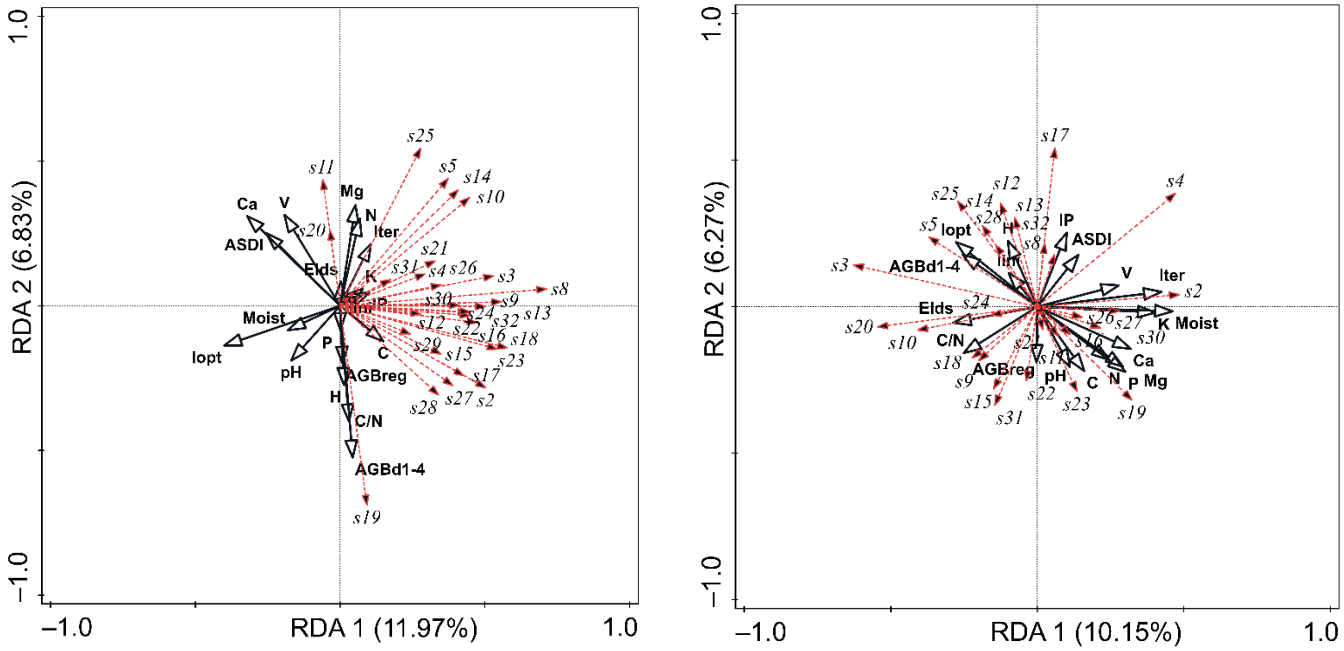


(**a**) (**b**)

Figure 2. Redundancy analysis (RDA) of the community-level physiological profiles of the forest floor (**a**) and the organo-mineral horizon (**b**), based on the MicroResp™ assay: the functional groups positions are represented by dashed lines (Ket—α-ketoglutaric acid; Arg—arginine; Asp—asparagine; Cel—cellulose; Mal—malic acid; Metg—methylglucamine; Phe—phenylalanine; Ser—serine; Man—mannose; Glu—glutamine; Maln—malonic acid; Xyl—xylose), and the stand structure characteristics are represented by solid lines (V—volume of trees; $AGB_{reg/d1–4}$—aboveground biomass of regeneration/trees with a DBH of 1–4 cm; ASDI—stand density index; H—coefficient of homogeneity; IP—tree influence potential; I_{ini}—initial stage index; I_{opt}—optimum stage index; I_{ter}—terminal stage index; E_{Ids}—evenness).

In the case of the effect of stand structure and soil physico-chemical properties on the community-level physiological profiles (Biolog assay), the first two ordination axes

explained 18.80% and 16.42% of the total variation in the forest floor and the organo-mineral horizon, respectively (Figure 3). As in the previous case, none of the effects was significant after Bonferroni correction. Correlation analysis (Tables S5 and S6) revealed a significant positive correlation, at $p < 0.001$, only between the optimum stage index (I_{opt}) and the functional group utilizing D-cellobiose (s25) in the A-horizon. Significant relationships ($p < 0.01$) were observed between the volume of trees (V) and the activity of functional groups utilizing pyruvic acid methyl ester (s5), the aboveground biomass of trees with a DBH of 1–4 cm ($AGB_{d1–4}$), the activity of functional groups utilizing D-mannitol (s14), the terminal stage index (I_{ter}) and the activity of functional group utilizing pyruvic acid methyl ester (s5) in the O-horizon, and between the optimum stage index (I_{opt}) and β-methyl-D-glucoside (s2) in the A-horizon. The other observed relationships were less pronounced ($p < 0.05$).



(**a**) (**b**)

Figure 3. Redundancy analysis (RDA) of the community-level physiological profiles of the forest floor (**a**) and the organo-mineral horizon (**b**), based on the Biolog assay: the functional groups positions are represented by dashed lines (s2—β-methyl-D-glucoside; s3—D-galactonic acid γ-lactone; s4—L-arginine; s5—pyruvic acid methyl ester; s6—D-xylose; s7—D-galacturonic acid; s8—L-asparagine; s9—Tween 40; s10—i-erythritol; s11—2-hydroxybenzoic acid; s12—L-phenylalanine; s13—Tween 80; s14—D-mannitol; s15—4-hydroxybenzoic acid; s16—L-serine; s17—α-cyclodextrin; s18—N-acetyl-D-glucosamine; s19—γ-hydroxybutyric acid; s20—L-threonine; s21—glycogen; s22—D-glucosaminic acid; s23—itaconic acid; s24—glycyl-L-glutamic acid; s25—D-cellobiose; s26—glucose-1-phosphate; s27—α-ketobutyric acid; s28—phenylethylamine; s29—α-D-lactose; s30—D,L-α-glycerol phosphate; s31—D-malic acid; s32—putrescine), and the stand structure characteristic are represented by solid lines (V—volume of trees; $AGB_{reg/d1–4}$—aboveground biomass of regeneration/trees with a DBH of 1–4 cm; ASDI—stand density index; H—coefficient of homogeneity; IP—tree influence potential; I_{ini}—initial stage index; I_{opt}—optimum stage index; I_{ter}—terminal stage index; E_{Ids}—evenness).

In the case of the physico-chemical properties, we observed several correlations; however, they were significant only at the level $p < 0.05$ (Tables S7 and S8). More pronounced relationships ($p < 0.01$) were found between the N content and pyruvic acid methyl ester (s5) and Ca concentration and γ-hydroxybutyric acid (s19) in the forest floor, and between soil pH and D-cellobiose, the C/N ratio, L-threonine, Mg content and L-phenylalanine, and the K concentration and L-arginine in the A-horizon. Surprisingly, increased moisture, and

often also an increased nutrient content, led to lower activity of the functional groups in both horizons. Generally, more significant relationships between the soil physico-chemical properties and biological attributes were found in the A-horizon.

4. Discussion

4.1. The Effect of Stand Structure on Soil Properties

In forest ecosystems, the size and longevity of trees make them important ecosystem engineers that affect both the above- and belowground ecosystem components. The effects of trees on belowground properties are associated with the aboveground deposition of litter and belowground deposition of matter through root exudation and root death [44,45]. Moreover, vegetation structure and composition affect the microclimate, and thus indirectly the soil properties [46] and species-specific plant microbe selection, factors that mutually shape the composition of the litter and rhizosphere microbiome [47,48]. For example, forest gaps exhibit increased solar radiation, soil moisture and soil temperatures compared to a closed forest [49]. Different pathways of precipitation through the forest canopy create a strongly heterogeneous pattern of water input to the soil, with consequences for soil hydrobiochemistry [50].

Microbial communities in rhizosphere soils are not static, differing over time and in space, and in the forest, these communities often vary according to trees and tree gaps, mediated by mechanisms that are likely to change over time and as trees are removed [44]. Spatial patterns in soil microbial communities are often found to be associated with plant species composition, richness and biomass [51,52]. Forest structure was shown to have an effect on soil dynamics and respiration, by influencing nutrient input as well as forest floor light, temperature and water distribution [53]. This study suggested that tree influences may contribute to an exceptional but still weakly explained spatial pedocomplexity of some forests.

Contrary to our expectations, we did not observe any strong effects of forest structure on the topsoil parameters. The lack of a tight relationships between topsoil features and the structural characteristics of the stand at the fine scale considered here is unexpected and may have different interpretations. It may depend on an inadequate description of the stand structure. The descriptors of stand structure we used may be too rough to account for the variability in topsoil parameters at the fine scale of the study. Additionally, primary beech forests are characterized by an almost ubiquitous structural heterogeneity [17]; therefore, the research plots in our study could vary minimally. Omnipresent heterogeneity in diameter structure is visible on the relatively low values of the coefficient of homogeneity, which fall within the range reported in certain forests known for their diameter variability [54].

Moreover, forest structure and topsoil parameters may be related through nonlinear relationships that cannot be modelled using the (linear) mixed-effect models. Finally, according to Sabatini et al. [55], the response of the soil physico-chemical parameters to changing conditions in the stand structure may be associated with a substantial time lag. Changes in the forest structure may take a relatively short time (from nearly instantaneous disturbance events to decade long growth processes); thus, the microclimatic conditions at the forest floor may change relatively rapidly. Furthermore, in temperate forests, strong mutual interactions exist between the soil properties and the forest composition and vertical and horizontal structure, including canopy gaps [49,56], deadwood [57] and understory [58]. On the other hand, soil characteristics (e.g., C, N and C/N) can require a much longer time (decades to centuries) to show a substantial change, and therefore soil C and N pools can lag behind changes in vegetation structure. Although a direct influence of overstory structure often has been postulated, our study suggests that the present structural features of the canopy, including deadwood and live tree structure, has no or limited ability to predict topsoil parameters.

4.2. Relationships between the Aboveground and Belowground Components

Among the measured structural indices, only the volume of trees, additive stand density index and tree influence potential showed a pronounced effect on the soil physico-chemical properties, especially on the K and P concentration in the O-horizon, and to a lesser extent on the N content. It is not surprising: with an increasing volume of trees and stand density, a higher leaf area index and consequently higher litter amount can be expected, especially in primeval forests exhibiting the presence of several vertical layers [59,60]. A high amount of K and P in soil was found to be related to the aboveground biomass; especially the foliage biomass in comparison to the other plant residues represents the main source of K and P in soils [61,62]. A higher K amount leads to higher pH, as also observed in this study. In the A-horizon, the soil reaction was related mainly to the development stage indexes; a positive correlation was found between pH and I_{ini}, while a negative relationship was found between pH and I_{opt}, indicating the important role of nutrient uptake by the roots. The relationships between the stand structure indices and biological properties were weak in both horizons and therefore difficult to interpret, as no clear pattern was found.

The responses of the respiration rate within individual functional groups of microorganisms based on their ability to degrade particular carbon sources were measured by the MicroResp™ and Biolog® methods. The CLPPs measured by MicroResp™ reflected a bit better the stand structure than the Biolog® method, a potential consequence of the different approaches to the measurements. The Biolog® method is a culture-based method, which provides information only about the culturable fraction of the microbial community and primarily selects for fast-growing bacteria. In contrast, MicroResp™ measures the carbon dioxide evolution from the whole soil sample and does not require the extraction and culturing of organisms [42]. Nevertheless, the relationships between stand structure indices and utilization of carbon sources were weak, similar to the measured biological characteristics. Among the substrates, more distinct differences in utilization were found only in the case of α-ketoglutaric acid, pyruvic acid methyl ester and especially D-cellobiose. Cellobiose is the main product of microbial hydrolysis of cellulose, an important structural component of the cell wall of plants, which represents a major source of carbon for soil microbial communities. Utilization of cellobiose positively correlated ($r = 0.603$, $p < 0.001$) with the optimum development stage (I_{opt}). This is probably associated with the soil reaction, as the catalytic optima of cellobiohydrolases are situated in a narrow pH range, between 4.0 and 5.0 [63], and the decrease in soil pH with an increasing I_{opt} in Havešová only confirms this assumption.

In contrast to the stand structure indices, the physico-chemical properties of soils showed a more pronounced effect on the soil biological properties and CLPPs, which is consistent with the results in previous studies, where the relationship between plants and soil microbial attributes was observed; however, the soil physico-chemical properties were found to be more important drivers of soil microbial properties [64]. Soil water, pH and nutrient status, represented especially by the nitrogen content and the C/N ratio, are regarded as the most influential factors driving the microbial community in forests [48,65]. We confirmed a distinct effect of soil moisture on microbial biomass and activity while the effect of the chemical properties was not so pronounced in comparison to the effect of soil moisture. Soil water seems to limit the decomposer community more than nutrient sources at this locality. Nevertheless, as expected, amounts of organic and inorganic resources in the upper horizons were positively reflected by the microbial biomass and activity. On the other hand, in the case of CLPP, based on the Biolog assay, negative relationships were also found, indicating different optimum conditions for utilization of particular carbon sources.

5. Conclusions

In this study, we investigated the soil properties, including the microbial characteristics, in a primary European beech forest exhibiting heterogeneous structures in both horizontal and vertical directions. Although a direct influence of overstory structure often has been postulated, also in our previous study in an old-growth mixed forest, the

spatial distribution of the microbial activity and microbial resources were linked to tree proximity [24]. In general, this study did not confirm a strong influence of forest structure on topsoil parameters. The lack of a distinct effect indicates that other factors than forest structure can drive the spatial distribution of soil properties and suggests that the present structural features of the canopy, including the deadwood and live tree structure, have no or limited ability to predict topsoil parameters in beech forest. On the other hand, the study demonstrates the distinct effect of soil properties on microbial communities, especially in the upper organo-mineral horizon.

Understanding the spatial distribution of the soil characteristics influenced by forest structure in primary forests is important for forest management practices. Only application of such silvicultural treatments that mimic natural processes, including soil nutrient and water cycles, biodiversity, etc., can offer more resilient stands under changing environmental conditions. Contradictory results regarding the impact of forest structure on soil properties indicate a need for more extensive research in this context.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13091344/s1, Table S1: Correlation coefficients for the community-level physiological profiles (MicroResp^{TM}) and stand structure indices of the forest floor; Table S2: Correlation coefficients for the community-level physiological profiles (MicroResp^{TM}) and stand structure indices of the organo-mineral horizon; Table S3: Correlation coefficients between the soil physico-chemical and microbial properties of the forest floor; Table S4: Correlation coefficients between the soil physico-chemical and microbial properties of the organo-mineral horizon; Table S5: Correlation coefficients for the community-level physiological profiles (Biolog®) and stand structure indices of the forest floor; Table S6: Correlation coefficients for the community-level physiological profiles (Biolog®) and stand structure indices of the organo-mineral horizon; Table S7: Correlation coefficients for the community-level physiological profiles (Biolog®) and soil physico-chemical properties of the forest floor; Table S8: Correlation coefficients for the community-level physiological profiles (Biolog®) and soil physico-chemical properties of the organo-mineral horizon.

Author Contributions: Conceptualization, J.Ž. and L.Š.: methodology, J.Ž., L.Š., S.K. and E.G.; validation, J.Ž., L.Š. and E.G., formal analyses, J.Ž. and L.Š.; writing—original draft preparation, J.Ž.; writing—review and editing, E.G. and L.Š.; visualization, J.Ž. and L.Š.; supervision, E.G.; project administration, J.Ž. and E.G..; funding acquisition, J.Ž. and E.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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