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Biomass Amounts of Spontaneous Vegetation on Post-Coal Mine Novel Ecosystem in Relation to Biotic Parameters

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Abstract: The amounts of biomass in ecosystems depends on the efficiency of energy gathering in organic chemical bonds by autotrophs, which are the only organisms that synthesize inorganic compounds into organic compounds structured into biomass. Plant species composition in the vegetation patch and the associated microorganism communities are factors that affect the matter and energy flow in the ecosystem. In human-established or severely transformed novel ecosystems, in particular in mineral-poor, oligotrophic habitats, the knowledge about the biotic parameters related to the biomass amount is very limited. The presented studies were performed on post-black coal mining heaps that provide the mineral material habitat. The following biotic parameters, the vegetation plant species composition of the distinguished vegetation types, soil organic matter, soil enzymatic activity, soil fauna presence, and the functional aspect of soil carbon release concerning amount of biomass are considered. The aim of this research was to analyze the influence of the selected biotic factors on the biomass amount in the vegetation patches of the studied sites. The results showed that the effect of the species composition diversity on the biomass amount is complex. The influences of soil enzyme activity on biomass amount are dependent on the enzyme presence and the vegetation type. Similarly, the impact of the soil organic matter (SOM) on the biomass amount is significantly different and dependent on vegetation type. The relationship between the amount of biomass and respiration showed that the biomass of the dominant plant species is positively correlated with the biomass amount in the studied vegetation types.

Keywords: biodiversity; soil enzyme activity; soil respiration; novel ecosystems; black coal mining heaps

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1. Introduction

The biomass amounts in ecosystems reflect the efficiency of the energy application introduced into those ecosystems. Autotrophic organisms, mostly plants, are the only elements in the ecosystems that are synthesizing the inorganic compounds into biomass [1,2]. The process of the matter—biomass building and circulation, along with energy flow—depends on many factors, including the crucial vegetation species composition, which determines the ecosystem type, and the related microorganisms' communities. Biodiversity has a direct impact on the amount of biomass, and, indirectly, biodiversity affects all ecosystem processes [1,3]. Higher biodiversity, in general, is predicted to increase the biomass parameters and the stability of the ecosystem [4–6]. Two of the upper limits of biomass primary production are the biotic and abiotic environmental factors [3,5]. Whether the amount of biomass influences plant species composition and biodiversity, and the consequent below-ground parameters, or the other way around is a crucial question in ecology [2,7,8]. Some studies show a unimodal (hump-shaped) relationship between biomass and plant species biodiversity [7,9]. The plant biomass amount increases when diversity increases, but, after a certain point, a biomass decrease is recorded. The decrease might occur because vegetation communities with high biomass are frequently dominated by low species numbers (frequently a single species) of highly productive competitive species [10,11]. The question remains open as to whether the high community biomass is causing the decrease in biodiversity or the opposite: the decreasing diversity increases the biomass amount [3]. Some researchers favor the opposing viewpoint, emphasizing that the community and ecosystem diversity and biomass are dependent on the below-ground and above-ground conditions, resource supply, and the biotic processes of the ecosystem functioning [8,12].

Apart from the autotrophic species diversity, the enzyme activity reflects the soil microorganism activity, which is related to the biochemistry of biomass. The biochemistry of the biomass depends on the plant species composition of the vegetation, characterized as the vegetation type [13–15]. The enzyme activity is the catalyst of soil chemical and biological reactions, which directly represent the biomass biochemical composition resulting from the vegetation type plant species composition [16–18]. Vegetation plant species composition, the above- and below-ground parts, affect the rhizosphere root exudates and soil-gas exchange. This influences the soil diversity of microbial, fungi, and soil fauna assemblages and indirectly affects enzyme activity [19–23]. Soil enzyme activity reflects the decomposition, mineralization, and turnover of biomass, particularly soil organic matter (SOM) and the regulating factors of the soil carbon and nitrogen cycles [24–26]. Enzyme activity is used as an indicator to evaluate and assess the fertility status in ecosystems, including agriculture systems [27], due to the microbial status and soil physical and chemical habitat conditions [28,29].

The relation between biomass and soil fauna presence is underlined by some authors [30,31]. The soil parts of the ecosystems are formed of diverse organisms such as invertebrates, which are accountable for the turnover of dead biomass organic matter [30–32]. The soil animals deliver key ecosystem functions, significantly affecting the decay of organic matter, and the energy and matter flow, allowing the recycling of nutrients [30,33].

In natural and semi-natural ecosystems, the biomass should scale with respiration, based on the hypothesis suggested by the metabolic scaling theory [34]. The biomass-respiration relationship is equivalent within and among different species, irrespective of habitat and environmental conditions, and, therefore, might influence the normalization constant but not the exponent [35,36]. The photosynthesized organic carbon is divided into components that accumulate in the biomass (Pn) and form the part that is used by plants themselves to allow the plant's metabolism (R); it is replaced relatively quickly in the atmosphere [37]. Although many eco-physiological studies were undertaken to understand the relationship between Pn and R dynamics during plant and vegetation development, a general understanding is still not agreed upon [38].

The vegetation plant species composition of the spontaneous vegetation is fundamental in terrestrial natural and semi-natural ecosystems. Apparently, in the disturbed (e.g., overgrazed) and human-induced ecosystems [39,40], the biotic factors and biomass parameters are poorly studied. The studies on the role of biotic habitat conditions—e.g., plant species composition, the activity of soil enzymes, soil fauna, and, separately, soil respiration—have been conducted in a few agricultural areas [41], forest ecosystems, and some sites transformed by human activity, such as open cast lignite mining and sites with heavy metals or heaps after brown coal mining [13,42–45]. Fewer studies on single biotic parameters, such as the activity of soil enzymes, have been carried out on heaps after hard coal mining [46].

The mineral material habitats of coal mine heaps provide novel unknown habitat types and examples of newly established poor oligotrophic novel ecosystem habitats that differ significantly from the ecosystems present in the surrounding landscape [15,47–49]. Previous studies have revealed that the poor, mineral, oligotrophic post-coal mining sites are colonized by different living organisms in the process of spontaneous succession, providing novel non-analogous species compositions of flora and fauna [21,30,50,51]. The vegetation composed of non-analogous species is assembled in response to the extraordinary habitat conditions defined as novel ecosystem sensu [52]. In the novel ecosystem sites, the changes in conditions and availability of resources are so fundamental that the given bio-geo-chemical habitat threshold is crossed. As a result, returning to the initial species composition is impossible. The habitats with vegetation appearing de novo are examples of novel ecosystems [52]. The processes and functions of the systems are not known.

The study aimed to determine the relationship between the biomass amount and the biotic habitat parameters of the spontaneously established novel ecosystems on the coal mine heaps. Among the biotic habitat parameters, the following will be considered: (i) the diversity of the plant species composition of the spontaneous vegetation types; (ii) the soil organic matter (SOM); (iii) the soil enzyme activity; (iv) the selected soil substrate fauna; and (v) the soil substrate respiration parameters.

We hypothesized that, with regard to the novel ecosystems on post-coal mines heaps, (1) The vegetation types that develop in habitats of higher SOM will provide more biomass compared to vegetation types that develop in habitats of lower SOM; (2) The vegetation types of higher diversity have lower biomass; (3) The vegetation types of higher biomass (dominant/rest) amounts are characterized by higher soil enzyme activity, higher fauna presence, and higher CO_2 release; (4) The vegetation types of the higher biomass amount will support a higher number of soil substrate fauna; and (5) The vegetation types of higher biomass amount will respire more intensively.

2. Materials and Methods

2.1. Study Site Description

The research was carried out in the area of the Upland Katowicka, which is part of the Silesian Upland macro-region (Figure 1). The research covered four post-black coal mining heaps, i.e.,: "Makoszowy" (Zabrze, Sośnica; 50°16′22″ N, 18°44′43″ E); "Kostuchna" in Katowice (50°11′04″ N, 19°00′33″ E); Murcki Boże Dary" in Murcki (50°11′21″ N, 19°02′07‴ E); and "Wesoła" in Mysłowice (50°10′28″ N, 19°05′44″ E). The heaps were created in the years 1900–1906 as a result of intensive coal mining by KWK Sośnica-Makoszowy, KWK Wesoła, KWK Murcki-Staszic, and Murcki Boże Dary [53]. On the studied heaps, mineral material is stored as a result of the sorting process, during which the coal is divided from the other accompanying geological minerals' admixture, which has been also mined. The heaped mineral material is a specific by-product of the mining exploitation. This material consists of carboniferous rock, or pit stone, with a very small admixture of carbon [47,54]. The mineral material stored on the heaps is varied in texture and can be divided into two types: coarse material, 20–200 mm, falls into the lower parts at the bottom of the heap, and the spaces between the stones are filled with air and fine-grained textured material of up to 20 mm, which predominates in the upper layer of the heap [47,54]. With time, aligned with the age of the heap, changes in the textural composition of the waste material is observed. Newly created and unburned heaps represent mostly a stone skeleton, while in older heaps the number of parts in which the soil formation process has begun increases [47]. In the stored material, we can distinguish between mudstones and, in smaller quantities, sandstones. Limestone is rare. The petrographic composition of the geological layers present in the heaped material varies depending on the location of the coal mine. The layer formation is influenced by the geological structure and the geological origin of the rocks that are accompanying the coal layers [54].

Figure 1. The novel ecosystems on post-coal mines heaps present environmental islands in the urban industry landscape in Silesia.

These sites are very specific habitats (Figure 2), after establishing without soil and vegetation cover, linked to the absence of soil or seed banks and have a very low number of nutrients in the substratum. In addition, the post-coal mining heap novel ecosystem sites are characterized by high temperatures, salinity, and significant fluctuations in moisture. The instability of the coarse grain substrate increases the heap's susceptibility to water runoff and/or mass erosion [47,55], and these factors might contribute to the difficulty of colonization of these areas by plant and animal species.

Figure 2. The habitat conditions of novel ecosystems present on black post-coal mine heaps are very specific.

2.2. Vegetation Data Collection

The varied physiognomy of vegetation patches has been identified. Due to the non-analogous species composition and structure of the varied vegetation patches, it is possible to cover the whole vegetation diversity by tracking the dominant plant species [47,49,55]. The vegetation samples were recorded in plots established in circles (6 m in diameter) in order to assess the relationship between the biomass and the biotic parameters, including vegetation plant species composition and diversity. In all established plots, the vascular plant species, listed together with all species abundance, were recorded. In this way, all the identified distinctive vegetation patches, dominated by particular plant species, that are occurring on the studied heaps were taken for analysis. Collected soil samples were also used to determine soil organic matter content using the Loss-on-Ignition (LOI) method. The soil samples for analysis were preprocessed upon arrival at the laboratory, including the removal of any visible plant residues and stones. Subsequently, the soil samples were dried at a temperature of 105 °C to eliminate moisture. After drying, the samples were weighed. From each sample, 20 g of soil was measured, and the samples were then subjected to combustion in a furnace at a temperature of 550 °C for a specified duration. After combustion, the samples were weighed again to determine the weight of the inorganic components. The content of soil organic matter was calculated using the following formula: Soil Organic Matter (%) = [(Initial weight of the soil sample—Final weight of the inorganic residue) / Initial weight of the soil sample] × 100. The content of soil organic matter was expressed as a percentage (%).

2.3. Vegetation Diversity Measurements

To identify the diversity of the vegetation type plant species composition, several indexes that characterize varied aspects of diversity were calculated for the sampled vegetation types within the studied post-coal mining heaps. The Shannon-Wiener index (H'), Simpson's dominance index, and evenness uniformity were used to evaluate the species richness and composition of the vegetation. The Shannon-Wiener index was used to measure the diversity of plant species in the studied patches, taking into account both the number of species and their evenness. Simpson's index was used to calculate the probability that two individuals selected at random from a given area would belong to the same species. The results of these diversity indexes can provide valuable information on the state and health of plant communities in the study area.

2.4. Biomass Collection

Biomass collection was carried out on previously designated research plots, on which a representative square was indicated (containing the best representation of the entire patch). The sampled subplot of 0.25 m^2 had a side length of 0.5 m. The above-ground parts of the plant were collected from a given area with garden shears, precisely determining the dominant species and overall plant species. Biomass samples were placed in string bags and appropriately labeled for later identification. After collection, the samples were immediately weighed using a field scale, indicating the mass of fresh biomass of the dominant species and other plants. The biomass of each individual from the dominant plant species in each sample was weighed separately from the biomass of the rest (non-dominant individuals) of the species. The samples were then transported to the laboratory for further analysis, where their species and biomass were identified, and the data was recorded for later use.

2.5. Enzyme Activity Measurement

The measurement of dehydrogenase [EC 1.1] activity involved the utilization of the Schinner method developed in 1996, which relied on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) [56]. Urease [EC 3.5.1.5] activity was assessed using the Alef and Nannipieri protocol, which involved incubating soil substrate samples with a urea solution to measure the enzymatic reaction. The acid phosphatase and alkaline phosphatase activities were evaluated by quantifying the release of p-nitrophenol (PNP) resulting from the enzymatic reactions. This was achieved through soil incubation with a buffered sodium p-nitrophenyl phosphate (115 mM) solution at pH 6.0 for acid phosphatase and pH 11.0 for alkaline phosphatase, following the protocol described by reference [56].

2.6. Soil Fauna Samples

The plots were delineated on the heaps and under homogeneous conditions, which was necessary due to the different age of the heaps, size, method of dumping, nature of the surroundings, and high variability of abiotic factors. In order to present the fullest possible range of variability, a network of systematically delineated study plots was used [47]. Three soil samples (approximately 1.5 kg each) were taken at the test plots. By taking soil from different locations, a proper picture of the abundance of soil fauna species was obtained, i.e., the average number of vascular flies occurring on the study plot. The material was stored in string bags at low temperature (6 °C). The nematode and vascular nematode dispersal procedure was carried out using a Tullgren apparatus. Quantitative analysis of the biological material obtained was carried out using a Delta Optical SZH-650T stereo microscope. The material was poured into a Petri dish and additionally washed with a Phalcon of water.

2.7. Respiration Measurement

Soil respiration was measured in each established plot once per year in the phenological optimum (flowering and the optimum of biomass) in mid-August. The system consisted of a gas sampling chamber, a temperature and humidity sensor, and a CO_2 analyzer. The gas sampling chamber was placed on the soil surface, and CO_2 efflux from the soil was measured over a 10 min period. The results were recorded and averaged over three replicate measurements. The respiration rate was calculated as the amount of CO_2 released per unit time and expressed as mg CO_2-C. These measurements were important for determining the relationship between soil moisture and soil respiration, which can help to better understand the dynamics of soil carbon cycling in different ecosystems. Soil respiration was measured using a Targaz—1 analyzer automated soil CO_2 flux system.

2.8. Data Analysis

All statistical analyses were performed using R software (ver. 4.2.2, R Core Team 2022) language and environment. In order to show diversity of vegetation data, non-metrical multidimensional scaling (NMDS) (Manhattan distance) was performed. Only vegetation patches that had at least 5 repetitions (plots) for dominant species were included (the number of replicates varied between 7 and 61). Of 324 plots, a final 312 plots were subjected to further analyses. Indicator species analysis (ISA) was conducted using labdsv::indval() function to indicate species that significantly occur more frequently in a distinguished dominant vegetation patch.

For each plot species richness (S), Shannon—Wiener index (H), species evenness €, and Simpson's dominance index (D) were calculated by means of vegan::diversity() and abdiv:dominance() functions. PERMANOVA with 999 iterations of permutation test using vegan::adonis() function was performed to examine significance of differences among dominants. The passive projection of explanatory variables was performed (vegan::envfit()) to check whether total cover, the biomass of dominant species, the biomass of remaining species, and biodiversity indexes significantly explained species variation. A Kruskal-Wallis test, followed by Conover test for multiple comparisons, was used to assess significance of differences among dominant groups in biodiversity indexes, cover of biomasses, and the studied biotic data.

The Spearman rank correlation analysis was performed to check mutual relationships among biomass of dominant and remaining species, SOM, soil enzymes, and SRL and biotic data, i.e., abundance of nematodes and *Enchytraeidae*.

3. Results

Plant species that occur in the recorded vegetation patches with the highest indicator value and strongest significance are listed in Table 1. The following vegetation type coding of the distinguished vegetation types is used: CE-1 *Calamagrostis epigejos*; CR-2 *Centaurea rhenana*; CP-3 *Chamaenerion palustre*; DC-4 *Daucus carota*; EP-5 *Eupatorium cannabinum*; F-6 *Festuca* group; LC-7 *Lotus corniculatus*; MA-8 *Melilotus albus*; PA-9 *Phragmites australis*; PC-10 *Poa compressa*; SG-11 *Solidago gigantea*; and TI-12 *Tripleurospermum inodorum.* TF-13 *Tussilago farfara* is used in the performed analysis of the result section.

Table 1. The list of (indicator) plant species with a significantly higher indicator value from the species composition of the recorded vegetation patches communities.

Species	Dominant Cluster	Indicator Value	Probability
Chamaenerion palustre	2	0.1882	0.008
Echium vulgare	3	0.1974	0.003
Arrhenatherum elatius	5	0.1221	0.04
Trifolium arvense	6	0.2004	0.002
Symphytum officinale	6	0.1263	0.005
Trifolium pratense	7	0.2591	0.001
Taraxacum officinale	7	0.2397	0.001
Erigeron acris	7	0.1095	0.02
Crepis biennis	8	0.1371	0.013
Poa palustris	9	0.3227	0.001
Bidens frondosa	9	0.1333	0.007
Lathyrus sylvestris	9	0.1333	0.011
Phalaris arundinacea	9	0.1125	0.012
Urtica dioica	9	0.1081	0.034
Agrostis gigantea	9	0.1043	0.042
Plantago major	9	0.0988	0.047
Erigeron annuus	10	0.2691	0.001
Achillea millefolium	10	0.2657	0.001
Plantago lanceolata	10	0.1828	0.003
Tanacetum vulgare	11	0.167	0.005
Hypericum perforatum	11	0.1565	0.006
Carex spicata	11	0.1333	0.011
Astragalus glycyphyllos	11	0.1325	0.015
Deschampsia caespitosa	11	0.1151	0.025
Melandrium album	11	0.1	0.044
Tripleurospermum inodorum	12	0.8519	0.001

Table 1. *Cont.*

Species	Dominant Cluster	Indicator Value	Probability
Polygonum aviculare	12	0.5694	0.001
Cirsium vulgare	12	0.1842	0.005
Senecio viscosus	12	0.1603	0.011
Capsella bursa pastoris	12	0.1364	0.012
Solanum nigrum	12	0.1364	0.011
Lepidium ruderale	12	0.1297	0.014
Stachys palustris	12	0.1214	0.012
Echinochloa crus galli	12	0.1108	0.027
Conyza canadensis	12	0.1059	0.043
Chenopodium album	12	0.0861	0.044
Tussilago farfara	13	0.8582	0.001

In order to find the relationship between the biomass (dominant species individuals and the rest of the biomass amounts and vegetation cover in the patch) of the identified vegetation types and the biotic parameters in the post-coal mine novel ecosystems, the non-metric dissimilarity analysis NMDS was performed. The non-parametric monotonic relationship between the dissimilarities in the analyzed matrix, the Euclidean distances between the plots, and the location of each of the plots in the low-dimensional space revealed the presence and identification of 13 different vegetation groups. Most of the plots (vegetation patches) were in well-distinguished clusters. The thirteen separated clusters presented a high degree of floristic integrity and were characterized by separated vegetation types (communities), mostly after the species occurring as the dominant plant. In this way, the following vegetation types groups were distinguished: *Calamagrostis epigejos*-dominated (CE Cluster 1), *Centaurea rhenana*-dominated (CR Cluster 2), *Chamaenerion palustre*-dominated (CP Cluster 3), *Daucus carota*-dominated (DC Claster 4), *Eupatorium cannabinum*-dominated (EP Cluster 5), *Festuca* sp.-dominated (F Cluster 6), *Lotus corniculatus*-dominated (LC Claster 7), *Melilotus albus*-dominated (MA Cluster 8), *Phragmites australis*-dominated (PA Cluster 9), *Poa compressa*-dominated (PC Cluster 10), *Solidago gigantea*-dominated (SG Cluster 11), *Tripleurospermum inodorum*-dominated (TI cluster 12), and *Tussilago farfara*-dominated (TF Cluster 13) (Figures 3 and 4 and Table 1). According to NMDS (Figures 3 and 4), vegetation patches on the studied heaps differed significantly from each other, which was confirmed by PERMANOVA (pseudo-F = 15.149, R^2 = 0.3781, $p < 0.001$) and the goodness of fit of grouping factor (r^2 = 0.0321 p= 0.006) on to ordination.

The most distant plots are some of those dominated by *Solidago gigantea* (SG Cluster 11, (Figure 5)), *Calamagrostis epigejos* (CE Cluster 1,(Figure 6)), *Daucus carota* (DC Cluster 4), and *Tripleurospermum inodorum* (TI cluster 12). The initial stage vegetation plots dominated by *Centaurea rhenana* (CR Cluster 2), *Tussilago farfara* (TF Cluster 13, (Figure 7)), *Chamaenerion palustre* (CP, (Figure 8)), and *Poa compressa* (PC) are grouped closely in the middle of the graph (Figures 3 and 4).

The plant community patches representing the distinguished vegetation types are primarily divided by the second axis. There are some vegetation types that are present only on the right side of the dissimilarity space. The patches of *Tripleurospermum inodorum*, *Tussilago farfara*, *Lotus corniculatus,* and most of the *Phragmites australis* (Figure 9) plant communities are on the right part of the matrix. On the left side are the *Solidago gigantea* and *Poa compressa* (Figure 10) plant community patches. In the center of the matrix, though more on the left side, are the *Centaurea rhenana*, *Melilotus albus*, and *Chamaenerion palustre* patches. Along the vertical axis are the *Daucus carota*, *Calamagrostis epigejos*, and *Festuca* group patches. The only plant community patches that are limited to the upper part, above the horizontal axis, are the *Eupatorium cannabinum* community patches.

In Table 1, the indicator value and the probability are calculated for plant species frequency and abundance for the identified vegetation community groups. The names of the groups are derived from the vegetation types most physiognomically distinctive during the field study observation.

In Figure 4 (NMDS with passively projected bioindicator values), it is possible to detect which of the recorded plant species are mostly related to bioindicator values (Figure 4). The distribution of the plant species illustrates which species are related to the increasing value of the diversity of indexes of the vegetation type associations.

The first axis is shaped by the diversity gradient from the high *H′* value (on the left) to low *H′* (high dominance index D (on the right)). However, the second axis is determined by the biomass of the dominant plant and the biomass amount of the rest of the accompanying (non-dominant) plant species present in the analyzed vegetation community patch. The diversity and biomass factors presented in Figure 4 show a close relationship between the dominant plant biomass and the overall vegetation cover value in the patch, while the biomass amount of the rest of the plant species present in the community patch are closely related to the species richness.

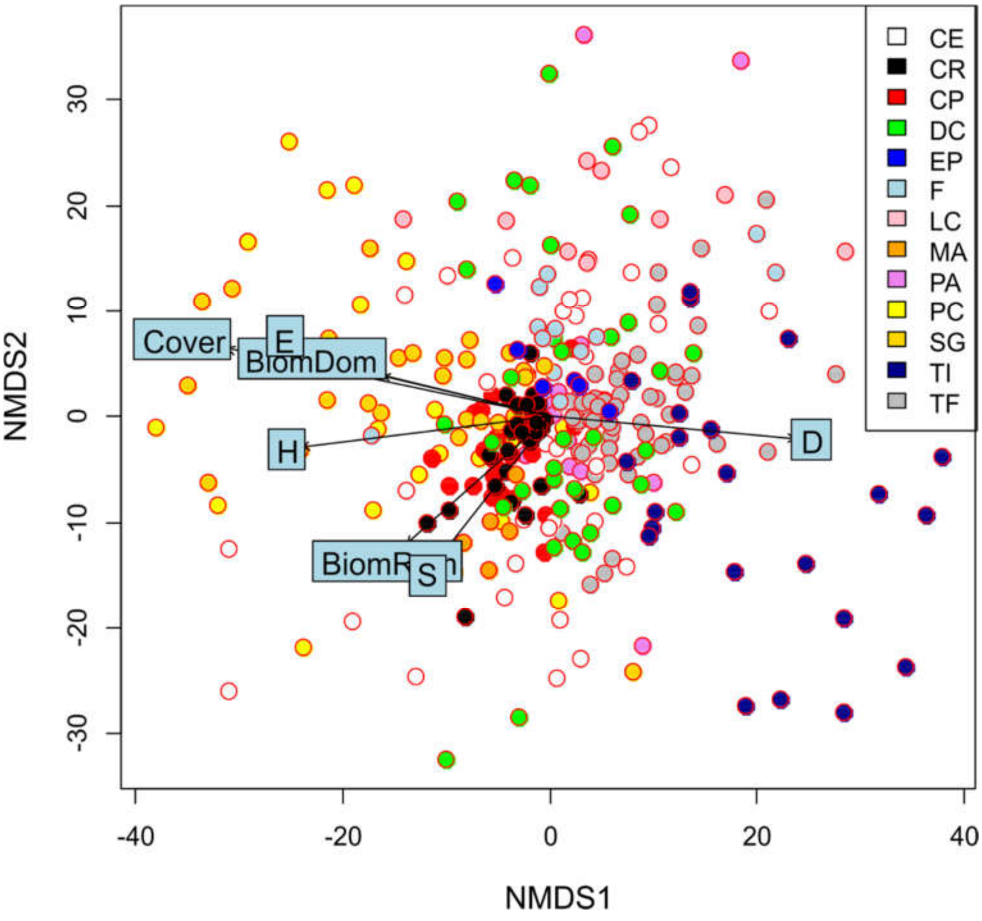

Figure 3. The biplot of NMDS with passive projection of explanatory variables (all are significant, $p < 0.05$) based on species abundances of the recorded vegetation growing on coal mine heaps. Abbreviation explanation: Shannon-Wiener-*H′*; Evenness -E; species richness -S; Simson dominance index -D. The coding of the distinguished vegetation types: CE-1 *Calamagrostis epigejos*; CR-2 *Centaurea rhenana*; CP-3 *Chamaenerion palustre*; DC-4 *Daucus carota*; EP-5 *Eupatorium cannabinum*; F-6 *Festuca* group; LC-7 *Lotus corniculatus*; MA-8 *Melilotus albus*; PA-9 *Phragmites australis*; PC-10 *Poa compressa*; SG-11 *Solidago gigantea*; TI-12 *Tripleurospermum inodorum*; TF-13 *Tussilago farfara*.

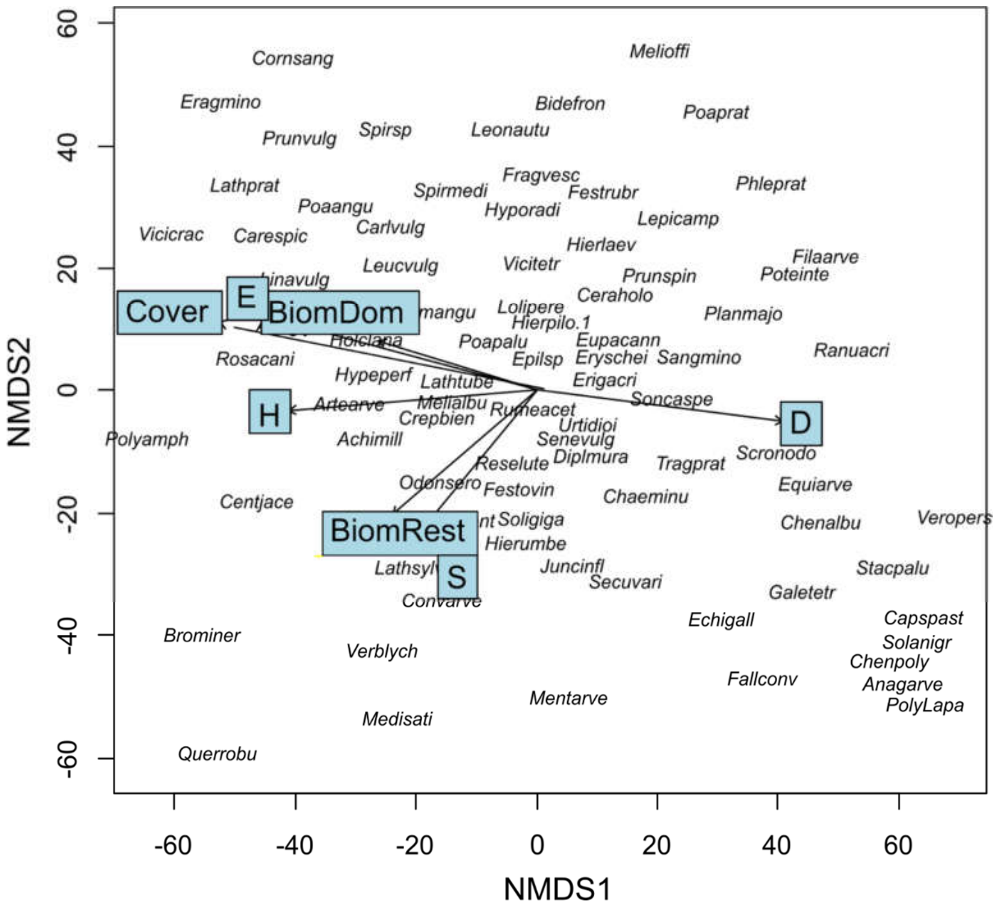

Figure 4. The biplot of NMDS with passive projection of explanatory variables (all are significant, $p < 0.05$) based on species occurrence of the recorded vegetation growing on coal mine heaps. The first four letters of names denote genus names and next four denote species names. Abbreviation explanation: Shannon-Wiener-*H′*; Evenness -E; species richness -S; Simson dominance index -D.

Figure 5. The vegetation patch dominated by *Solidago gigantea* on coal mine heap.

Figure 6. The vegetation patch dominated by *Calamagrostis epigejos* on coal mine heap generally creates more mono-specific patches.

Figure 7. The vegetation patch dominated by *Tussilago farfara* on a slope of coal mine heap, with accompanying *Phragmites australis* and *Scirpus lacustris*.

Figure 8. The pioneer vegetation patch dominated by *Chamaenerion palustre* on coal mine heap, accompanied by single individuals of *Calamagrostis epigeios* and single *Pinus sylvestris* juvenile individuals.

Figure 9. The pioneer vegetation patch dominated by *Phragmites australis* on coal mine heap.

Figure 10. The vegetation patch dominated by *Poa compressa* on coal mine heap.

The cover (percentage cover) of the recorded plant species, the Evenness diversity indicator, and the dominant plant biomass value are very close together in the dissimilarity analysis (Figure 4). The other side of the X axis shows the biomass of the rest of the accompanying plant species, which are not the dominant ones, and the H′ and the S diversity index are present. However, the dominance diversity index seems to be on the opposite end, as it is on the other right side of the Y axis. The distribution of the plant species shows that the species like *Solidago gigantea*, *Hypericum perforatum*, and *Carex spicata* are related to the cover (percentage cover) of the recorded plant species, indicated by the Evenness diversity indicator. Some other species are related to neither the dominant biomass nor the rest of the species that are not dominant, as indicated by the H′ and S diversity indexes (Figures 3 and 4).

The plant species distribution, based on the results obtained from the NMDS with passive projection of explanatory variables, revealed the impact of the vegetation patch diversity indexes in which the cover and biomass of dominant plant species are correlated with the first axis of NMDS. The biomass of the rest of the species is correlated with species Irichness along the second axis of NMDS (Figures 3 and 4); the comparison of biodiversity indexes among the distinguished vegetation types was performed. Some significant differences were recorded when the biodiversity indexes among patches with dominant vegetation were compared.

The comparison of the value of the diversity H′, E, D, and species richness (S) indexes calculated for the distinguished plant community vegetation types were performed. In terms of the species richness, the *Eupatorium cannabinum* and *Festuca* G\group and *Tussilago farfara* vegetation type are significantly different from *Poa compressa* and also partially different from *Solidago gigantea* and *Tripleurospermum inodorum.* The value of the H′ diversity index of *Daucus carota* was significantly different from those of the *Festuca* group, *Phragmites australis*, and *Tripleurospermum inodorum*. The value of the H′ diversity index of the *Tussilago farfara* vegetation type was significantly lower. The values of the E diversity indexes of the *Centaurea rhenana* and *Daucus carota* groups were significantly different from those of *Melilotus albus*. The values of the E diversity indexes of *Tussilago farfara* and the *Tripleurospermum inodorum* vegetation types were significantly lower. In terms of the Simson dominance index (D), the *Daucus carota* vegetation type was significantly lower. The *Calamagrostis epigejos*, *Centaurea rhenana*, *Chamaenerion palustre*, *Eupatorium cannabinum*, *Lotus corniculatus*, *Melilotus albus,* and *Poa compressa* groups were similar in terms of the D index (Figure 11 and Table 2).

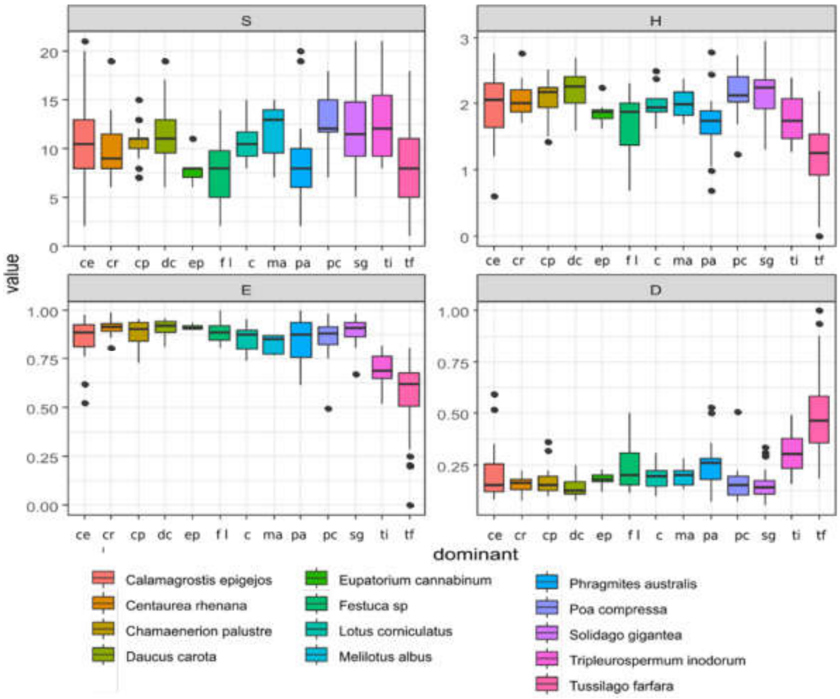

Figure 11. The comparison of biodiversity indexes among distinguished dominant vegetation patch groups (significance of differences with the indicators of the post-hoc are in Table 2). Abbreviation explanation: Shannon-Wiener—*H*′, Evenness—E; species richness—S, Simson dominance index—D.

Table 2. The results of the post-hoc test indicate the significance of differences between the extinguished groups of studied vegetation types in relation to the diversity indexes. The same letter indicates the lack of differences. The significance of differences in biodiversity indexes, total cover, and biomasses among groups (Kruskal-Wallis test and post-hoc Conover test) indicates that the dominant species that share the same letters do not differ significantly at $p < 0.05$.

Species	CE	CR	CP	DC	EP	F	LC	MA	PA	PC	SG	TI	TF
S	bcd	cde	bcd	abc	e	e	abcd	abc	de	a	ab	ab	e
H	bc	bc	ab	a	cd	d	bcd	bcd	d	ab	ab	d	e
E	bc	a	abc	a	ab	abc	cd	d	bcd	cd	ab	e	e
D	def	efg	efg	g	cdef	cd	cdef	cde	bc	efg	fg	b	a

Explanations of abbreviations: CE—Calamagrostis epigejos; CR—Centaurea rhenana; CP—Chamaenerion palustre; DC—Daucus carota; EP—Eupatorium cannabinum; F—Festuca group; LC—Lotus corniculatus; MA—Melilotus albus; PA—Phragmites australis; PC—Poa compressa; SG—Solidago gigantea; TI—Tripleurospermum inodorum; TF—Tussilago farfara.

The comparison of the value of vegetation cover, the biomasses of the dominant plant species, and the biomasses of the non-dominant accompanying plant species among the distinguished vegetation types of patches was performed. Some significant differences were recorded. The *Lotus corniculatus, Phragmites australis*, and *Solidago gigantea* vegetation types were significantly different (higher diversity) compared to patches of the *Tussilago farfara* vegetation type (Figure 12 and Table 3).

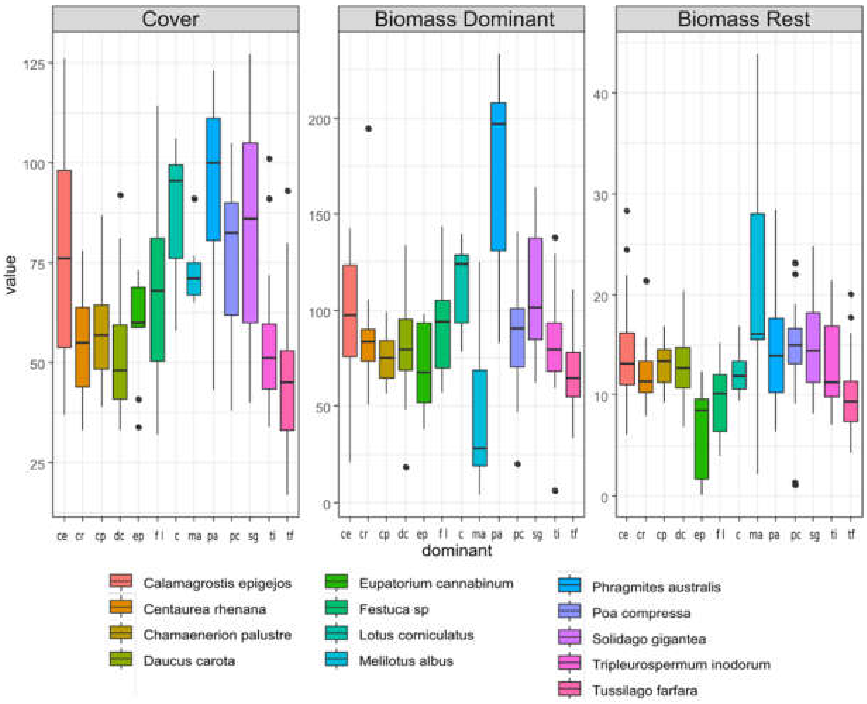

Figure 12. The comparison of the vegetation cover, biomasses of the dominant plant species, and the biomasses of the rest of the species in vegetation patches not being the dominant among the distinguished vegetation patch groups (significance of differences in Table 3).

Table 3. The results of the post-hoc tests indicate the significant differences between the analyzed groups of studied vegetation patches and the listed biotic parameters. The same letter is indicating the lack of differences. The significance of differences in biodiversity indexes, total cover, and biomasses among groups (Kruskal-Wallis test and post-hoc Conover test) show that the dominant species that share the same letters do not differ significantly at $p < 0.05$.

Species	**CE**	**CR**	**CP**	**DC**	**EP**	**F**	**LC**	**MA**	**PA**	**PC**	**SG**	**TI**	**TF**
Cover	ab	cd	cd	de	bcd	bc	a	ab	a	ab	a	de	e
Biomass Dom	bc	de	e	de	ef	bcd	ab	f	a	cde	b	de	f
Biomass Rest	abc	cd	abc	bc	e	de	bcd	a	abc	ab	ab	bc	e

Explanation of abbreviations: CE—Calamagrostis epigejos; CR—Centaurea rhenana; CP—Chamaenerion palustre; DC—Daucus carota; EP—Eupatorium cannabinum; F—Festuca group; LC—Lotus corniculatus; MA—Melilotus albus; PA—Phragmites australis; PC—Poa compressa; SG—Solidago gigantea; TI—Tripleurospermum inodorum; TF—Tussilago farfara.

The vegetation types of higher diversity have been identified. The Daucus carota, Poa compressa, and Tripleurospermum inodorum vegetation types presented the highest diversity in terms of species richness and the Shannon-Weiner diversity index. Different diversity measures revealed varied values in particular diversity indexes. Species richness does not consider the abundance (Cover) or population size of each species within the community. The Shannon's index, on the other hand, considers both species richness and species abundance. It takes into account not only the number of different species present in a given area but also the relative abundance of each species. The abundance of species can be measured using various methods, allowing for a more comprehensive understanding of the ecosystem [57,58].

The Evenness index (E) is influenced by the overall number of species present within a specific community [59]. When the values of this index are elevated for a cluster of patches, it indicates that those patches harbor species with a more balanced distribution of abundance, such as coverage or biomass. Analysis of the value of the Shannon-Wiener (H′) diversity index was undertaken. The highest H′ value of this indicator was found in patches dominated by Daucus carota, Poa compressa, and Solidago gigantea. However, the highest average value of this indicator was recorded in patches dominated by Solidago gigantea. The analysis of the value of the uniformity index E in the examined patches showed differences. The highest average values of this indicator were found in patches dominated by Daucus carota. On the other hand, the lowest average value of the uniformity index E was found in patches dominated by Tussilago farfara. The purpose of this index is to depict the proportion between the real diversity and the maximum potential diversity.

The analysis of the value of the dominance index (D) of the examined patches showed differences. The highest average values of this indicator were found in the areas dominated by Tussligao farfara. On the other hand, the lowest values of this indicator were found in patches dominated by Daucus carota individuals.

Calculating the dominance index provides additional insight into the diversity of the examined patches. The index represents the likelihood that an individual chosen at random will belong to any of the numerous species present in the community. The values of the index increase when the community is characterized by a greater dominance of one or a few species.

In terms of the dominant plant species biomass amount recorded in identified vegetation types, the CP Chamaenerion palustre-dominated plant communities were significantly lower compared to the PA Phragmites australis, SG Solidago gigantea, TF Tussilago farfar„a and MA Melilotus albus vegetation types (Figure 12 and Table 3).

While the amount of biomass of the remaining species was not dominant, the accompanying plant species among the distinguished vegetation patches were significantly different. (Figure 4 and Table 1). The more detailed analysis performed for each of the distinguished vegetation type groups separately revealed that Eupatorium cannabinum, Melilotus albus, and Tussilago farfara were significantly different (Figure 12 and Table 4).

Table 4. The significance of differences in soil organic matter SOM, soil enzyme activity (Dehydrogenase DH, Urease UR, Acid Phosphatese AP, Basic phosphatase BP, soil fauna (Enchytridae—EN, Nematodes—N), and the respiration CO_2 (SRL) among distinguished vegetation types (Kruskal-Wallis test and post-hoc Conover test, the vegetation types that share the same letters do not differ significantly at $p < 0.05$).

Vegetation Type	**SRL**	**SOM**	**DH**	**UR**	**AP**	**BP**	**EN**	**N**
Calamagrostis epigejos	cd	ef	cd	bc	d	de	ab	bcd
Centaurea rhenana	ab	ab	g	a	e	h	e	cde
Chamaenerion palustre	d	bcd	ef	bc	abc	cde	de	abc
secondary Daucus carota	e	e	cd	d	f	fg	bc	e
Eupatorium cannabinum	bc	fgh	cdef	bc	bcd	gh	a	cde
Festuca sp	d	gh	def	bc	e	ef	ab	ab
Lotus corniculatus	de	h	bc	cd	de	def	cde	de
Melilotus albus	cd	a	cdef	a	abcd	ab	cde	e
Phragmites australis	a	cde	a	bc	abcd	abc	e	bc
Poa compressa	cd	ef	cde	bc	ab	bcd	abc	bc
Solidago gigantea	c	fg	def	b	a	a	bcd	cde
Tripleurospermum inodorum	de	de	f	c	cd	bcd	cde	ab
Tussilago farfara	cd	bc	b	bc	f	fg	bc	a

The similarities between the analyzed vegetation patch groups revealed in Table 2 partially explain the patch distribution reflected in Figure 3.

Each of the biotic parameters presented in Figure 13 provides a different picture of the relationship between species. The highest value of CO_2 was recorded in *Phragmites australis* and *Centaurea rhenana*. The lowest values of CO_2 release were revealed in *Daucus carota*. The higher values of CO_2 release were revealed in *Tussilago farfara* and *Solidago gigantea*. The difference between the *Phragmites australis* group and *Daucus carota* group was significant. The values revealed for *Melilotus alba* were outstanding in terms of urease activity, basic phosphatase activity, and soil organic matter.

The analysis of soil organic matter (SOM) content revealed the highest levels in vegetation types dominated by *Daucus carota, Lotus corniculatus*, and *Melilotus albus*. Conversely, the lowest SOM content was observed in the *Calamagrostis epigejos* and *Poa compressa* vegetation types. Importantly, the differences in SOM content among these vegetation types were found to be statistically significant. However, no significant difference in SOM content was observed between *Calamagrostis epigejos* and *Poa compressa* vegetation types. Regarding acid phosphatase activity (AP), the highest levels were recorded in the *Solidago gigantea* vegetation type, while the lowest AP activity was observed in the *Daucus carota* and *Tussilago farfara* vegetation types. These differences in AP activity were found to be statistically significant. Basic phosphatase activity (BP) was found to be highest in the *Solidago gigantea* vegetation type and lowest in the *Centaurea rhenana* vegetation type. Statistical analysis confirmed the significant differences in BP activity between these two vegetation types. Dehydrogenase activity (DH) was notably higher in *Lotus corniculatus*, while the *Phragmites australis* vegetation type exhibited the lowest DH activity. Statistically significant differences in DH activity were observed between *Phragmites australis* and *Centaurea rhenana*, as well as the *Tripleurospermum inodorum* vegetation type. Urease activity (UR) was significantly higher in *Melilotus albus,* compared to the *Daucus carota* vegetation type, which displayed the lowest UR activity. Additionally, the *Melilotus albus* and *Centaurea rhenana* vegetation types exhibited significant differences in UR activity when compared to the *Daucus carota* vegetation type. Specific root length (SRL) was found to be significantly different in the *Phragmites australis* vegetation type when compared to the *Chamaenerion* *palustre*, *Festuca*, and *Daucus carota* vegetation types. The *Daucus carota*, *Melilotus albus*, and *Tussilago farfara* vegetation types displayed significant differences in nematode (N) populations compared to other vegetation types. Nevertheless, the biomass of the dominant plant species compared to the rest of the species that were not the dominant in the patches of the particular vegetation types was revealing, with outstanding values for *Melilotus albus* and *Lotus corniculatus* vegetation types. The highest fauna (*Nematode* and *Enchytraeidae*) presence was found in *Euaptarium cannabinum*, *Festuca sp.* Group, and *Calamagrostis epigejos*, and also in the *Poa compressa* and *Melilotus albus* vegetation types. In turn, the highest nematode presence was observed in *Tripleurospermum inodorum*, *Melilotus alba*, and *Lotus corniculatus*, as well as in *Phragmites australis*.

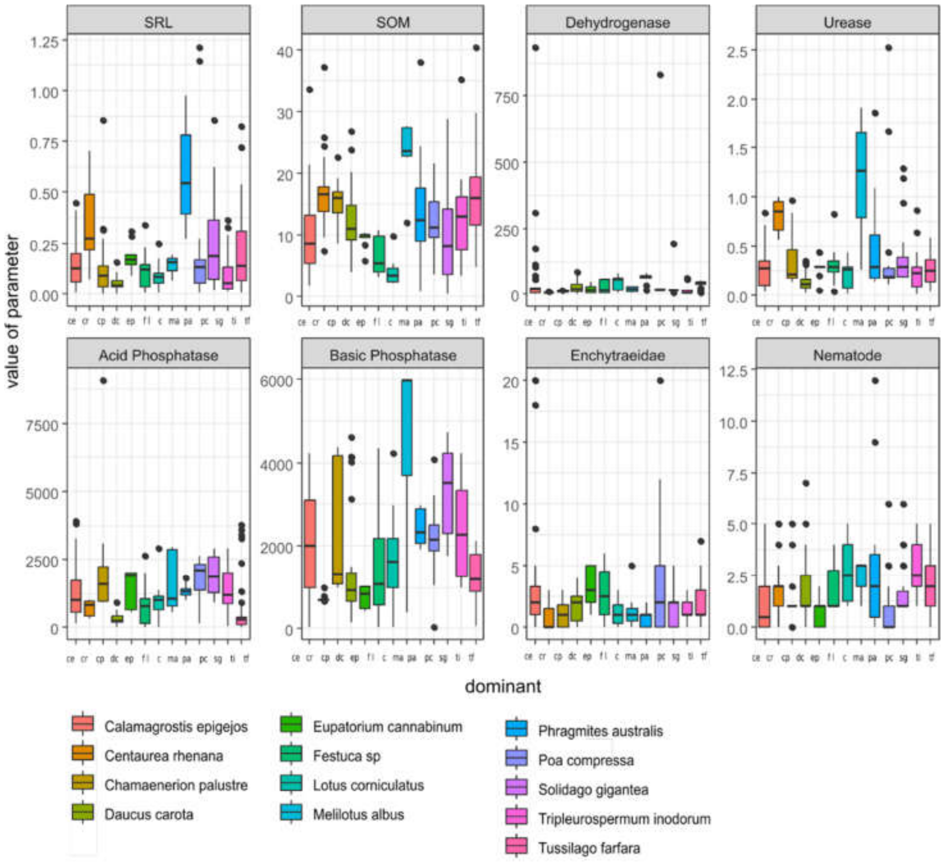

Figure 13. The comparison of soil organic matter SOM, soil fauna, soil enzyme activity, and the respiration of CO_2 among dominant vegetation patches (the significant differences are indicated in Table 4).

The correlation matrix revealed the positive relationship between the biomass of dominant species and some soil enzyme activity, e.g., acidic phosphatase and basic phosphatase. Figure 14 shows that the strongest linear relationship exists between BiomDom and BiomRem (0.45). A weak positive relationship is presented for soil respiration. There is a negative correlation between SOM and the biomass of dominant species (−0.32).

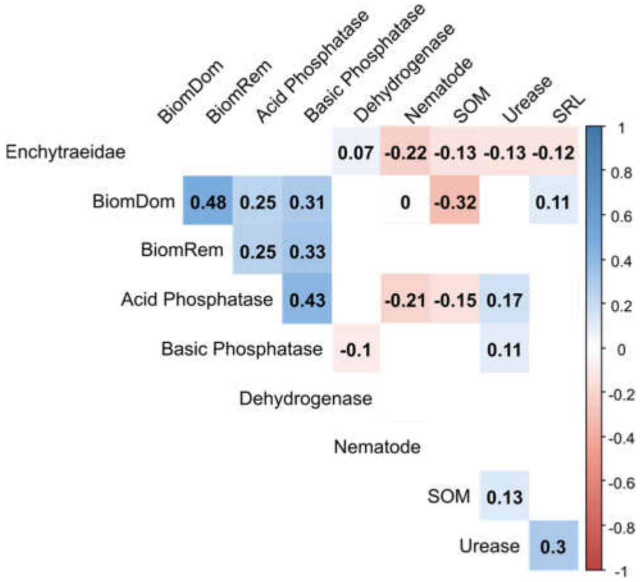

Figure 14. The Spearman correlation matrix of the biomass of dominant and remaining species and biotic data including SOM, soil enzymes, SRL *Nematodes* and *Enchytraeidae*.

In the distinguished vegetation types, the highest SOM amount was recorded in the *Melilotus albus* vegetation type, while the highest biomass amount was recorded in the *Phragmites australis* and *Solidago gigantea* vegetation types.

The highest fauna (*Enchytridae*) presence was found in *Eupatorium cannabinum*, *Festuca sp.* group, and *Calamagrostis epigejos*, and also in the *Poa compressa* and *Melilotus albus* vegetation types. In turn, the highest presence of nematodes was observed in *Tripleurospermum inodorum*, *Melilotus alba,* and *Lotus corniculatus,* as well as in the *Phragmites australis* vegetation type.

4. Discussion

In the presented study, we analyzed some biotic habitat parameters concerning the relationship of vegetation types to the amount of biomass. Among the biotic site characteristics, the following parameters were considered: species composition diversity of the studied vegetation types, the amount of SOM, the enzyme activity, and the soil respiration quantity record for the studied vegetation types. The plant species composition of the vegetation patch mosaic reflects the nutrient composition, as mainly the organic carbon distribution and the biotic status of the habitat profoundly influences the composition of plant communities. In turn, the biochemical status of the system and the stability and health of below- and above-ground ecosystem functions depend on species composition of the vegetation mosaic [60]. This creates a feedback system of mutual dependence.

4.1. Plant Species Composition Diversity and the Amount of Biomass

In our study, we revealed that there are significant differences between the distinguished vegetation type clusters in terms of vegetation diversity and abundance measurement. For the abundance measurement and the vegetation percentage cover of each of the vegetation patches, both the percentage cover of dominant plant species and the percentage cover of the non-dominant plant species, i.e., the accompanying species, were measured. The amount of biomass was divided into the dominant plant species biomass amount and the accompanying species biomass amount.

Many studies conducted in natural and semi-natural ecosystems have revealed the positive relationship between biodiversity measured by different indexes and the biomass amount. Regardless of the convincing interpretation of the unimodal (hump-shaped) relationship between biomass amount, the vegetation type, and the vegetation plant species composition biodiversity (alfa and beta diversity), according to some study results, the relationship between plant biomass measurements and diversity does not always follow the hump-shaped model [61,62]. More recent studies have presented primary autotroph biomass as the dependent rather than the independent variable [3,63], usually by demonstrating that the biomass amount (measured by vegetation percentage cover or vegetation biomass weight) generally increases with plant diversity in newly established experimental communities [62,64].

The majority of studies conducted in this field are by experimental research, i.e., not conducted in the field, and, as a result of artificially simplified setup factors, the main explanations for the positive relationship between biodiversity and biomass amount are obtained [65]. One of the possible reasons for the observed phenomenon is the so-called sampling or selection effect. According to this concept, the more diverse the community, and consequently the ecosystem, is, the more likely it is to include highly productive species [65,66]. A different approach is presented in the complementarity effects understanding. The space and time factors cause differences in species resource acquisition, thereby allowing for more efficient resource use and resulting in the increased biomass amount when the vegetation communities in an ecosystem are more diverse [3]. The facilitative effects concept assumes that some co-occurring plant species are able to positively influence the establishment of biomass by supporting the establishment and survival of other species [67–69]. Some research has been conducted to compare the presented explanations [3,65–68,70,71], comparing sampling effects with complementarity effects, and found that the sampling effects are more important in explaining the relationship between biodiversity and biomass production than complementarity effects [70]. The sampling effects do not completely explain how the biodiversity affects the biomass amount. The sampling effects occur far less generally than previously suspected [72,73]. The most reliable explanation is the merging of sampling, complementarity, and facilitative effects, which are mutually non-exclusive, and the net effect of biodiversity on biomass amount can be understood as the sum of these three effects.

The few studies conducted in the field, which were focused on the relationship between vegetation diversity and above-ground biomass, include the comparison of the tree species composition in three different forest types was undertaken [74]. It has been hypothesized that the tree species diversity in different habitats in the variety of studied wetlands, in terms of soil and environment factors, will cause differences in the biomass amount [74]. In this study, estimates of above-ground biomass was used as an essential prerequisite for studies on carbon stocks, e.g., the effects of forest vegetation type and deforestation on carbon sequestration and the carbon balance [74–76].

In this study, the recorded vegetation types were analyzed in terms of the vegetation patches and plant species composition similarity in relation to the vegetation cover and biomass of both the dominant plant species and the accompanying plant species. The dissimilarity analysis of all the vegetation types recorded during the field studies was performed. The spontaneous vegetation developing on the black mineral post-coal mine heaps were determined based on the distinctive physiognomy of the dominant plant species. The dissimilarity tests distinguished 13 vegetation types, merging some of the field-determined vegetation types based on the abundance measurements.

The research conducted over hundreds of post-coal mine heaps has revealed that the herbaceous spontaneous vegetation types can be characterized by the abundant occurrence of one dominant species, often accompanied by single individuals of numerous other species [15,21,47–49,51,77]. The mineral post-black coal mining sites provide a stony gravel soil substrate for the colonizing organisms [30,48,49]. The vegetation species composition dynamics, including transformation and development on mineral resource excavation sites, influences the soil biomass biochemistry, SOM, nutrient cycling, soil nitrogen loss, and enzyme activities [78–86] and the resistance of the habitat and soil substrate to environmental stresses [84].

4.2. The Relationship between the Amount of Biomass and the Soil Organic Matter (SOM) in the Studied Vegetation Types of Novel Ecosystems on Post-Black Coal Mine Heaps

Most of the studies conducted on the relationship between soil organic matter and the biomass amount consider arable land and the impact of the supply of fertilizers, including fertilizer supply in different forms. In this respect, our study presents a significantly different approach. Soil organic matter (SOM) has a fundamental role in maintaining soil productivity. This soil parameter is also crucial in the protection of the soil environment. Two main SOM fractions can be distinguished, one fraction being inert and the other being decomposable. It has been reported that the ranges for the optimal participation of the decomposable organic carbon (OC) is between 0.2 and 0.6% [87]. When considering the current state of knowledge consistently, it can be concluded that the use of mineral fertilizer has a positive effect on the environment and supports the cultivated crops' biomass. However, too high humus participation in soil can lead to environmental pollution.

The approach to quantify SOM by assessing the field and model experiments, and the soil processes simulation, resulted in the theoretical range of values provided in other studies [88]. However, there is still divergence between the field study and theoretical modeling results. The evaluation of field experimental data does not provide an explanation of the relationship between the soil organic carbon content and the biomass amount. Nevertheless, the results allow us to derive useful conclusions for potential sustainable soil management [87].

The study conducted on the arable land revealed a linear regression relationship between the clay particle content and the C_{org} content in the soil of study sites in the plots of European long-term field experiments [87]. The C_{org} in agricultural soil on the long-term field experiment sites has been considered as inert organic carbon C_{org}. For comparison, the C_{org} present in the black earth is 1.5 to 1.6%. In the differently structured light sandy soils, in which only 3% is clay particle content, the C_{org} occurrence in comparable habitat conditions is about 0.3%. The whole organic carbon presence in the fertilized plots varies between 0.7 and 0.8% C in the studied soils, which could be considered as the optimum concentration. The black earth with a C_{org} content of 1.5% is optimal, whereas sandy-textured soil with 0.8% C_{org} can be considered as very well supplied. The presented study revealed that the assessment of the organic carbon (OC) content in soil is not enough for the optimal organic matter content in relation to the biomass amount. The local habitat conditions, particularly in relation to the soil texture, is necessary. The decomposable part of the carbon content in soil increases with the clay particle content. The humification in soils with high clay content is thus generally twice as high as in sandy-textured soils [87].

As a result of the European long-term field experimental study, it has been calculated that the difference between the soil parameter, which is the whole soil organic carbon presence in the plots supplied, and the close-to-optimum mineral and organic fertilization is on average 0.33% C_{org} (ranging from 0.14 to 0.66%) [87,89]. The results of the cited experiments, and the experience gathered during the last decades, show that 2.0 to 2.1% C_{org} in the soil, 0.5% of which is mineralizable, can be considered as the optimum [87,89]. The exceeded nutrient input (during fertilization) results in increased C content in the soil, leading to harmful N-losses and an unfavorable change of the C-balances [87,89], and also to increasing organic C participation, hygroscopicity, water holding capacity, and porosity.

Discussion about the relationship between SOM and the biomass amount requires information about inert (hardly involved in mineralization processes) and decomposable SOM. The inert SOM is strongly correlated with the clay content in soil and in the agricultural conditions and is, to a large extent, stable. The decomposable SOM depends on the changeable local conditions [87]. Changes in the C_{org} content in soil occurs almost exclusively in its decomposable part. The hot water-extractable carbon has proved to be an appropriate criterion for the characterization of the decomposable carbon.

In arable land, the tendency of SOM changes depends on its initial level. The SOM contributes to the biomass amount of up to 10% on sandy soils, and on loamy soils up to 5% [87]. As the C and N ratio is changing, along with the content of SOM in the habitat, nevertheless, the ranges for the optimal quantities of C and N in soil are reported to be narrow. In the conditions of mean annual temperature between 6 °C and 10 °C and mean annual precipitation of 450 to 800 mm, the optimal content of mineralizable carbon is between 0.2 and 0.6% and that of nitrogen is between 0.02 and 0.06% [87]. The increased humus content in agricultural soil contributes to environmental pollution, especially by influencing the C and N balance in the atmosphere, as well as by groundwater pollution. The "humus balance method" provides the opportunity to control the SOM content in arable soils, to identify the threshold values for optimal C_{org} content, and to avoid environmental pollution [87].

4.3. Soil Enzyme Activities and the Amount of Biomass

The enzyme activity is related to different soil properties, such as soil organic matter SOM [90]. Soil enzyme activity affects the energy supply for microbial growth and enzyme activity. Some studies reported a positive relationship between enzyme activities and SOC and TN in human-disrupted brown coal heap areas [13,42,91,92]. In these studies, it was reported that during spontaneous succession on heaps, established after brown coal extraction, the presence of SOC and TN in the topsoil layer significantly influenced soil enzyme activities. The authors, Zang et al. (2013) [83], found that the direction of vegetation restoration (vegetation diversity) influenced the variety of carbon sources, which in turn influenced the functional diversity and metabolic activity of the microbial assembly in mineral habitats such as sandy-textured soils.

In our research, we did not find significant correlations between SOC n the substrate and dehydrogenase activity or between SOC and the activity of acid or alkaline phosphatases. In our studies, we obtained negative correlations between urease activity and SOC. These results correspond with those obtained by Rodriguez-Loinaz and Šantrůčková [93,94]. It has been stated, for both vegetated and non-vegetated plots, that it is possible that most of the carbon contained in the mineral material habitats is related to organic matter of recent or geogenic origin [95,96]. The geogenic coal present as an admixture in the coal mine heaps material is not available to microorganisms; therefore, despite the high content of organic carbon (10–18%), the substrate in the studied post-coal mine heap plots had few available carbon sources for microorganisms [55,97]. The limited quantity of available carbon (not geogenic in origin) for microorganisms in the total pool of SOC may have contributed to the lack of correlation between SOC and the activity of the studied soil substrate enzymes, i.e., those mainly derived from soil microorganisms. The results of some research have shown that soil pH influences enzyme activity and has an impact on the soil microbial community structure [93,98].

4.4. Soil Fauna Diversity and the Amount of Biomass in the Studied Vegetation Patches

In the natural and semi-natural ecosystems, the relationship between soil fauna and biomass decomposition may differ significantly between developed and undeveloped soils. In undeveloped soils, the soil substratum, as described in the presented paper, has not been previously influenced by soil fauna [99]. The complexity of these processes leads to the conclusion that there is no simple answer to the research problem, i.e., "Does the soil fauna support or decrease the rate of biomass litter and SOM decomposition?" [30].

In encouraging circumstances, soil fauna can digest the entire annual litter in some ecosystems. The soil fauna influence the soil organic matter (SOM) dynamics at many spatio-temporal scales by modifying the soil environment and assimilating the litter. The digestion of litter-feeding fauna modifies the litter, causing pH and redox changes, fragmentation, removal of easily decomposed polysaccharides, increases the proportion of lignin, consumption of associated microorganisms, and a decrease in soluble polyphenols and carbon-nitrogen (C:N) ratios. The soil fauna activity while passing through earthworms' guts covers the biomass, litter, and SOM with clay, reducing the diffusion of nutrients and oxygen, reducing microbial access to the litter, and changing the conditions for microbial activity. Soil fauna also affect the distribution of biomass and organic matter throughout the soil profile. The distribution of fauna determines whether biomass or litter decomposes on the soil surface or deeper in profile, as POM bound to soil particles. The changes in distribution, in turn, influence the composition of the microbial community and the decomposition process. Soil fauna affect the amount of biomass and, subsequently, the soil organic matter entering the range of the SOM pools, thereby determining the litter quality and the degree of soil C saturation and, consequently, the soil profile. The plant species' community composition reflects the soil habitat conditions, and, in turn, the root exudate influences the soil microbial activity and the biomass, litter, and SOM decomposition processes. The soil fauna can affect the biomass, litter SOM as potential soil C storage, and decomposition, either positively or negatively. Soil fauna impact seems to be highest in transformed ecosystems or ecosystems under transition, e.g., ecosystems reestablishing after disturbances in the processes of primary or secondary succession.

The process of litter decomposition and the subsequent stabilization of soil organic matter (SOM) can be influenced by the specific identities of the soil organisms involved in decomposition [100–103]. While soil microorganisms are primarily responsible for decomposing organic matter [33,104], numerous studies have acknowledged the significant impact of soil fauna on decomposition rates, often through their effects on microbial activity [33,104–110]. The influence of soil fauna on litter decomposition is multifaceted and involves various interactions occurring at different spatial and temporal scales. Soil fauna encompass organisms of different sizes and feeding modes. Their consumption of litter, nutrient assimilation, and production of feces occurs over hours to days and spans millimeters to centimeters in spatial scale. Concurrently, soil fauna impact the microflora present in the uneaten portions of litter, creating a dynamic interplay between fauna activities and microbial processes. The activities of microorganisms within faunal excrement (feces) can vary significantly depending on the age of the excrement [110,111].

Beyond their influence on microbial activity, soil fauna can also affect decomposition by facilitating the movement of organic matter within the soil profile through bioturbation, leaching, and other mechanisms [112]. Additionally, soil fauna can create long-lasting structures that accumulate within the soil profile, ultimately influencing soil profile development [33]. Notably, the invasion of North American forests by earthworms serves as a prime example of how these changes in soil properties can alter plant communities and the overall functioning of ecosystems [113]. Consequently, such alterations in plant communities and ecosystem functioning can subsequently impact the rate of decomposition [33,113]. In our study, neither *Nematode* nor *Enchytraeidae* influenced the amount of biomass in the studied vegetation types. The analysis of the separated dominant biomass and non-dominant biomass did not reveal any relation with the studied soil fauna (*Nematode* and *Enchytraeidae*).

In summary, the identities of soil organisms involved in decomposition play a crucial role in the processes of litter decomposition and SOM stabilization. While soil microorganisms are the primary drivers of decomposition, soil fauna significantly influence decomposition rates through their interactions with microbial activity. The complex effects of soil fauna encompass various spatio-temporal scales and involve interactions with microflora, the creation of fecal microenvironments, the movement of organic matter, and the development of soil profiles. Understanding the role of soil fauna in decomposition processes is vital for comprehending the intricate dynamics of carbon cycling in ecosystems and the broader functioning of soil ecosystems.

4.5. The Amount of Biomass and Soil Respiration

It has become increasingly clear that soil and vegetation processes are strictly linked. About 50% of bulk soil respiration is related to the transfer of recent, current, and fresh assimilation into the soil [114,115]. The assimilates present support the microbial decomposition of biomass and the subsequent SOM. Soil scientists are trying to solve the dilemma that a varied supply of low-molecular-weight compounds can mask the decomposition of more recalcitrant compounds, which are most important in long-term processes. In addition to the mentioned dilemma, the temperature sensitivity of the biomass and the subsequent SOM decomposition is highly variable. The biomass decomposition sensitivity to temperature is related to many factors, such as the vegetation type, the litter quality, the history of soil cultivation, and other site-specific factors, e.g., soil developmental stage [116]. The soil developmental stage may be crucial in our study.

Biomass and sub-consequent SOM encounter chemical processes at different levels of structure and composition. These chemical processes result in a dynamic series of progressively more recalcitrant SOM and turnover times. The time can range from days to millennia. Only about 5–15% of total soil respiration (Rs) is related to decomposing the more recalcitrant old carbon fraction with slow turnover rates (i.e., decades to centuries). Most soil carbon belongs to the recalcitrant SOM fraction. However, the fate of old carbon reservoirs cannot be predicted based on biomass biochemistry and quality and soil respiration measurements alone [117]. The short-term warming experiments are not precise enough to predict the long-term effects of warming on soil respiration in the old, recalcitrant carbon stocks. Many carbon balance models assume a fixed temperature sensitivity for respiration (e.g., setting $Q10\frac{1}{4}2$), including shifts in soil water status or substrate supply that may change the temperature-soil respiration relationship or dependence [118].

4.6. Specific Habitat Conditions of Mine Heaps (The Low Albedo of Mineral Deposits)

Albedo alludes to the reflectivity of a surface, particularly the division of sun-oriented vitality (daylight) that is reflected into space. In the case of mineral stores in a mine load, low albedo implies that these surfaces assimilate more sun-powered vitality and are less reflective.

Temperature Increment: Low albedo surfaces, which retain more daylight, tend to warm up more rapidly. In a mine load, this may result in raised temperatures on and around the mineral stores. Raised temperatures can influence biomass deterioration.

Microbial Action: Higher temperatures can increase microbial movement. Organisms play a significant part in breaking down natural matter within the deterioration process. In this manner, raised temperatures seem to possibly speed up biomass deterioration by providing a more favorable environment for microorganisms.

Chemical Responses: Raised temperatures can also speed up chemical responses involved in biomass deterioration. These responses can be incorporated in the breakdown of complex natural particles into easier degradable ones. Expanded temperatures seem to encourage these responses, possibly driving quicker deterioration.

Dampness Levels: Low albedo surfaces may also influence dampness levels. If the surface warms up quickly due to low albedo, dissipation of dampness from the encompassing environment can result. Lower dampness levels can impact biomass deterioration.

Dampness Accessibility: Biomass deterioration regularly requires a certain level of dampness. Deficient dampness can decrease decay rates as many decomposers, such as fungi and microbes, require water to operate viably. Low albedo surfaces that contribute to dampness deficits may possibly destroy decay processes in parched or semi-arid situations.

Plant Development: The conditions on a mine pile, including albedo, can impact plant growth. Low albedo surfaces may be debilitating to plant development due to higher temperatures and decreased dampness. Plants contribute to biomass through their natural matter, which can, in turn, influence decay rates.

Plant Inputs: More plant biomass implies a more noteworthy supply of organic fabric for deterioration. As low albedo conditions may restrain plant development, there may be less natural fabric accessible for decomposition, potentially reducing decomposition processes.

Long-Term Impacts: It is critical to consider the long-term impacts. Over time, the particular environmental conditions, including low albedo, can lead to changes in soil properties, microbial communities, and the composition of the biomass itself. These changes can have complex and possibly cascading impacts on decay forms. In outline, the low albedo of mineral stores in a mine load can have a few potential impacts on biomass decay. These impacts incorporate raised temperatures, changes in dampness levels, and changes in plant development designs, all of which can impact the rate and proficiency of deterioration processes in these novel environments. However, the real affect would depend on different components and the particular characteristics of the mine pile and the encompassing environment.

4.7. The Understanding of the Biomass Relationships to the Biotic Conditions of Novel Ecosystems on Coal Mine Heaps

The extraordinary poor, mineral, oligotrophic habitat conditions of novel ecosystems on coal mine heaps cannot be returned to the historic state, i.e., the previous pre-mining state [52]. The relationships between plant species assemblages and the soil, or abiotic soil substrate (mineral stone and gravel sites), condition can change during succession development, particularly the spontaneous succession development taking place on a very special type of post-mineral excavation site. In the early stages of succession, soil (or soil substrate) organisms may depend on a specific combination of plant species biochemical characteristics [20,119]. Post-mineral excavation sites allow the study of the relationships between the dominant plant species (vegetation type), vegetation cover, biomass, soil enzyme activity, soil fauna, and soil respiration parameters.

Apart from the complexity of primary producer plant combinations, the microbial assemblages, the specific abiotic conditions, and the limited understanding of these relationships in natural and semi-natural ecosystems, the human industrial activity brings a completely new set of challenges [120]. The mineral resource extraction results in significant transformation of the landscape [121]. The deposition of mineral material from deep black coal mining, as well as the open cast mining of different ores, changes the landscape, including the removal of the existing vegetation and the modification the soil hydrological conditions and the soil structure and composition [20,39,51]. Specific physical and chemical parameters of the mineral material substrates create harsh abiotic conditions such as the specific texture, poor nutrient concentrations, low water-holding capacity, extreme pH, and high temperatures [49,122]. The spontaneous non-analogous vascular plant species composition, mosses and lichens, has been studied in the novel ecosystems on coal mine heaps [50]. The recorded biotic diversity has identified the existence of a wide variety of habitat conditions of coal mine heaps regarding moisture, granulometry, and salinity, in addition to slope inclination, height, and aspect differences. Moreover, the habitat diversity within one coal mine heap is frequently much higher than that between two or more heaps. The great mosaic of microhabitats (e.g., moisture, granulometry, salinity) is reflected by the diverse vegetation patchiness. The wide microhabitat variety is crucial in the single coal mine heap scale [123,124]. The available studies on non-analogous species composition developed on coal mine heaps have revealed that the novel ecosystems are significantly different from the surrounding non-industrial areas. The differences include flora, fauna, bryophytes, lichens, and associated saprophytic organisms, such as bacteria, fungi, mites (Mesostigmata group), saprophytic protists, etc. These have become established as a result of the natural processes of colonization and recruitment in the novel habitats. The non-analogous species composition of the novel ecosystems presents a self-sustaining system that develops without human intervention [52]. It also creates unusual opportunities to study the processes of primary succession in specific combinations of habitat conditions, with special attention to substrates with low initial biological activity [50,124–128].

5. Conclusions

The ecosystems development, based on the spontaneous vegetation of the non-analogous species composition, might be significantly different in terms of ecosystem functioning. The relationships between the biomass amount and biotic parameters are distinctive from those recorded from the natural and semi-natural vegetation types and the subordinate environments.

Opposite to our hypothesis, the diversity indexes of vegetation species composition measured by means of different indexes does not present a common trend in terms of the relationship between the biomass amount and the examined vegetation diversity indicators. The biomass of the dominant species is related to the Evenness (E) diversity indicator, while the biomass of the non-dominant plant species is related to the species richness and H' Shannon-Weiner diversity indexes.

Contrary to our expectations, the biomass of the dominant species in the novel ecosystems on post-coal mining sites is negatively and statistically significantly related to the soil organic matter (SOM) in the heap soil substrate, while the biomass of the non-dominant species has no impact on the biomass amount in the studied vegetation types.

Opposite to our expectations, the activity of soil enzymes such as dehydrogenase, acid phosphatase, and alkaline phosphatase does not increase significantly with the amount of biomass. None of the enzyme activity is related to the biomass of the studied vegetation types developing spontaneously on the post-coal mine heaps and mineral soil substrate.

Unlike our expectations, the number of *Nematodes* and *Enchytraeidae* is not related either to the amount of biomass of the dominant species and the biomass of the non-dominant species or to the overall biomass amount of the studied vegetation types that developed on the mineral soil substratum of the novel ecosystems on the coal mine heaps.

Our results revealed that, along with our expectations, the vegetation types with higher plant biomass amounts had a positive relationship with the soil substrate respiration parameters in the post-coal mining novel ecosystems. Further study related to the total organic carbon (TOC) should consider the carbon (C) stored in soil organic matter (SOM). Organic carbon (OC) enters the soil through the decomposition of plant and animal residues, root exudates, living and dead microorganisms, and soil biota. SOM is the organic fraction of soil exclusive of non-decomposed plant and animal residues. Nevertheless, most analytical methods do not distinguish between decomposed and non-decomposed residues. SOM is a heterogeneous, dynamic substance that varies in particle size, C content, decomposition rate, and turnover time.

Soil organic carbon (SOC) is the main source of energy for soil microorganisms. The ease and speed with which SOC becomes available is related to the SOM fraction in which it resides. The future study of the SOM present on hard coal mining heaps has to be focused on the participation of C in TOC and SOC organic matter forms.

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