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How Important Are the Relations between Vegetation Diversity and Bacterial Functional Diversity for the Functioning of Novel Ecosystems?

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Abstract: Understanding ecosystem development of post-mining areas requires observing the development of the plant and microbial communities. It is widely known that mutual interaction is important for both of these groups, and both benefit significantly. The aim of this study was to broaden the knowledge about the relation between the vegetation and functional diversity of bacterial communities in novel ecosystems of post-mining areas and to discuss the potential applicability of methods of studies of bacterial functional diversity in these ecosystems with special attention paid to the BIOLOG method. The functional diversity of microbial communities of five types of microhabitats of post-coal mining heap (Upper Silesia, Poland) was studied using the BIOLOG method. Four of them were covered by spontaneously developed vegetation (two dominated by grasses *Calamagrostis epigejos* and *Poa compressa* and two others by dicotyledonous species *Daucus carota* and *Tussilago farfara*). The results obtained for vegetated microhabitats were compared with the diversity of microbial communities from non-vegetated types of microhabitat. Our study confirmed that microbial functional diversity measured by the summed area under the curve for all substrates, the richness index, the Shannon-Wiener index and the evenness index mirrors aboveground vegetation diversity. All of these measures differ, especially between non-vegetated patches and grassland patches dominated by *C. epigejos* and *P. compressa*.

Keywords: primary succession; non-analogous species composition; mineral resources exploitation; land reclamation; BIOLOG method

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1. Introduction

Natural and semi-natural ecosystems are self-sustained, fundamental units in ecology, based on the cycles of matter and energy flow. The preliminary feature of each ecosystem is the species composition and functional diversity of primary producers. Plant species richness and functional identity significantly influence the above- and belowground ecosystem processes, particularly the decomposition of organic matter in all types of natural and semi-natural ecosystems [1–5]. Biodiversity in the taxonomic aspect and its influence on the whole ecosystem's functioning are still of interest for current research and future challenges [2,6]. Species and functional diversity affect the use of resources, which forms the basis for the partitioning selection and complementarity concept in explaining the mechanisms and maintenance of biodiversity in ecosystems [7]. Studies of the global

biodiversity scenarios are still developing and they prove that functional diversity affects decomposition processes in natural, semi-natural or experimental ecosystems [8–11].

For a long time, the study of biodiversity in various natural and semi-natural ecosystems on Earth focused on aboveground plant diversity. Fewer studies attempted to unravel and understand the importance of belowground biodiversity [12–17]. The above- and belowground elements of wetland and terrestrial ecosystems are linked via microorganisms colonizing the plant rhizosphere [18–21], endosphere and phyllosphere [22]. The autotrophic organisms, mostly plants, deposit litter and root exudates in soils providing a source of carbon and other nutrients for assemblages of decomposers. It is widely known that soil microbiota (with bacteria as its significant part), and their activity in decomposition are dependent on biochemical characteristics of soil organic matter (mostly plant remnants). They also play an essential role in the cycling of elements and providing inorganic nutrients for plant growth [6,23,24].

In the last decades the processes taking place during the Anthropocene revealed new aspects of natural phenomena. To a large extent, the growing area of habitats completely transformed by man with plant communities showing totally new species compositions (e.g., novel ecosystems established, with non-analogous species composition) not occurring in natural and semi-natural systems, generates many new research questions (Figure 1). Novel ecosystems develop in habitats that have been transformed so deeply (i.e., those where the crossing of the ecological threshold took place) that a return to the earlier state is no longer possible. In completely new habitat conditions, the assembly rules expressed by natural processes lead to the establishment of new, previously unknown, species assemblages and network of interactions [25–27].

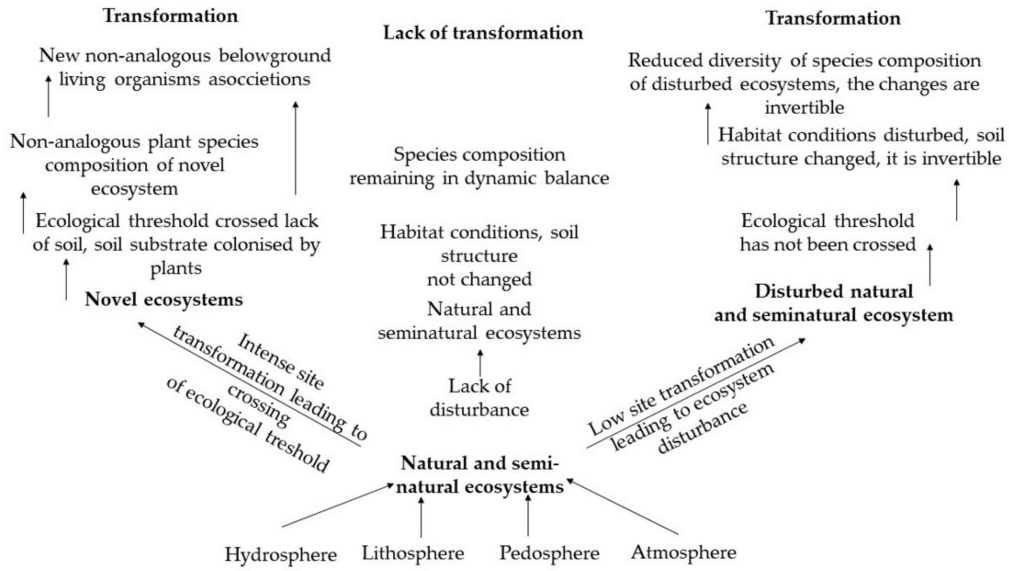

Figure 1. Effects of the transformation of ecosystems.

Understanding the nature of novel ecosystems requires a study of their functional character. Apart from the identification of the non-analogous species composition of vascular plants, the biological analyses of the substrate (i.e., the activity of soil enzymes, functional diversity of microorganisms) as well as studies of species functional traits (morphological, biochemical or physiological) revealed that the adaptations that enable them to colonize harsh habitats are necessary. The harsh complex system of habitat conditions are caused by the lack of initial soils in the sites where the novel ecosystems develop. In many sites, the mineral material substrate without organic matter of granulometric structure (referred to as the soil substrate further in the text) is colonized by vascular plant species. It can be hypothesized that along with non-analogous plant species composition, the associated

bacteria, fungi and animals will appear in unexpected arrangements, [28,29] leading to the establishment of a novel ecosystem. This is the reason for undertaking this complex study.

Apart from the complexity of microbial, plant combinations and abiotic conditions, and the limited understanding of these relations in natural and semi-natural ecosystems, human industrial activity brings completely new challenges. The extraction of mineral resources causes strong transformation of the landscape. The open cast mining and deposition of mineral material from deep mining change the land relief, remove the existing vegetation, and modify the soil structure and composition as well as hydrological conditions [30–33]. Specific chemical and physical properties of the mineral substrates are responsible for creating harsh conditions such as low water-holding capacity, poor nutrient concentrations, extreme pH and high temperatures [34,35]. In some sites, the changes of conditions and availability of resources are so fundamental that the given bio-geo-chemical habitat threshold is crossed, and then returning to the initial species composition is impossible. The habitats with vegetation appearing de novo are examples of novel ecosystems *sensu* (Hobbs et al. [27,36]). The spontaneous non-analogous plant species composition has been studied in coal mine heaps of novel ecosystems [15–17]. Studies have proven the existence of a wide variety of habitat conditions of coal mine heaps regarding moisture, granulometry and salinity, not to mention 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 [14,21,33]. The wide microhabitat variety is crucial in the single coal mine heap scale. The available studies of non-analogous species composition developed on coal mine heaps revealed that the novel ecosystems were significantly different from the surrounding non-industrial areas. The differences includes flora, fauna and associated saprophytic organisms, such as bacteria, fungi, mites (Mesostigmata group), saprophytic protists, etc., established as a result of the natural processes of colonization and recruitment in the novel habitats [25]. The non-analogous species composition of the novel ecosystems presents a self-sustaining system that develops without human intervention [31,36–43]. 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 [44].

The Relation between the Diversity of Plant Species Composition of a Vegetation Cover and Bacterial Functional Diversity—Theory and Methods

The microbiota assemblages are significant drivers of soil organic matter decomposition processes [45,46]. The decomposition of dead organic matter depends on the metabolic activity of microorganisms and the biochemical parameters of the biomass [47,48]. The characteristics of the deposited biomass are controlled by factors such as humidity and temperature [48,49], soil properties [48,50] and vegetation type [51,52]. Due to the low homeostasis and high surface-to-volume ratio, the microorganisms respond quickly to changes in the environment [52]. Apart from the methods based on molecular assays such as the analysis of phospholipid fatty acids (PLFA) [53,54] or denaturing/temperature gradient gel electrophoresis (DGGE/TGGE) [55,56], the measurement of the physiological activity of microorganisms represents an important approach. The physiological activity of microorganisms allows for the study of functional characteristics of microbial communities [57]. The BIOLOG method is frequently used to compare the metabolic activity of heterotrophic microbial communities from the rhizosphere [58]. The intensity of the utilization of carbon substrates varies in different groups of microorganisms, which results in a given "metabolic fingerprint" of a microbial community [59–65].

The physiological profiles analyzed by means of multidimensional statistical methods allows for the comparison and assessment of similarity between microbial communities from different ecosystems and habitats. This method can be used for estimating changes in soil microbial communities that result from a long-term or a short exposure of the soil to the influence of extreme factors. The differences in metabolic response enable the evaluation of

adverse changes in microbial communities between communities from transformed and reference areas. The parameters characterized by the BIOLOG method provide information about the changes in microbial community abundance. The community-level physiological profile (CLPP) allows for the disclosing of the effects on microbial functional diversity.

The aim of this study was to (1) broaden the knowledge about the relationship between vegetation and bacterial functional diversity in novel ecosystems of post-mining areas; and (2) discuss the potential applicability of methods of studies of bacteria functional diversity in novel ecosystems with special attention paid to the BIOLOG method.

2. Materials and Methods

2.1. Study Site Characteristics

The study site is situated in the central part of the Silesian Upland (southern Poland). This is a temperate climate area, with the highest mean temperature of 14–16 °C in July and an annual rainfall of 600–800 mm. The western winds are the most frequent in the region. The days with mist range from 30 to over 100, while the share of days with cloud cover is approximately 60–80% [66]. The fieldwork was conducted and the samples were collected from the "Makoszowy" coal mine heap (Zabrze, Sośnica; 50°16′22″ N, 18°44′43″ E); "Kostuchna" in Katowice (50°11′4″ N, 19°0′33″ E); and "Wesoła" in Mysłowice (50°10′28″ N, 19°5′44″ E). These coal mine heaps cover an area of approximately 170 hectares, and are located at an altitude of 310–339 m above the sea level. They are still active [66]. They are formed mainly from carboniferous rocks extracted from a depth of about 0.5 to 1 km below the surface and deposited at the spoil site [66]. New material is still transported to the top of the heap by a railway connecting the mine and the heap. The studied heaps are generally irregular and built of carboniferous gangue with unfavorable soil texture (mainly claystone and siltstone, sandstone, conglomerate, and coal shale) with admixtures of coal. Organisms colonizing the post-mineral excavation sites (Figures 2–5), including the deep coal-mine heaps, are often subjected to extreme abiotic factors and conditions such as low water availability and retention, low nutrient availability, fast drying of the surface layer of substrate, low availability of organic matter, high temperature (reaching endogenous thermal activity) and high/variable salinity levels (crystallized salt can sometimes be observed). The substrate of coal mine heaps does not contain high concentrations of heavy metals and other dioxins in comparison to other post-industrial sites (e.g., lead and zinc spoil heaps) of anthropogenic origin [28,31,33–35,41,42]. Non-analogous species composition in these habitats has been formed in the process of primary succession.

2.2. Vegetation and Soil Sampling Methods

The studied vegetation patches represented a non-vegetated microhabitat and four vegetation types. Two groups of patches were chosen for detailed investigation: (i) those dominated by herbaceous species forb species (dicotyledons) (*Daucus carota*, *Tussilago farfara*); and (ii) those dominated by grass species (monocotyledons) (*Calamagrostis epigejos*, *Poa compressa*). Ten sample plots (circle, diameter 6 m) for each microhabitat type with plant cover and for non-vegetated patches were established to study the vegetation and functional diversity of the bacterial communities. The plots were separated at least 10 m from each other. All of the study plots were established on flat terrain (on the tops of spoil heaps) to unify the conditions between the plot exposure to sunlight and the susceptibility to erosion.

Figure 2. The organisms colonizing the post-mineral excavation sites subjected to extreme abiotic factors and conditions ("Wesoła" coal mine heap in Mysłowice; phot. G. Woźniak).

Figure 3. The spontaneous occurrence of *Phragmites australis* on coal mine novel habitats ("Kostuchna" coal mine heap in Katowice; phot. G. Woźniak).

Figure 4. The range of hydrological conditions reflecting the micro-habitat diversity ("Makoszowy" coal mine heap in Zabrze; phot. G. Woźniak).

Figure 5. The frequently spontaneously established patch with the domination of *Tussilago farfara* ("Wesoła" coal mine heap in Mysłowice; phot. G. Woźniak).

In the studied plots, the cover of vascular plants (estimated as cover for each species) was evaluated according to the scale as follows: 1, 2, 5, 10%, and the next at 10% intervals up to 100%.

To obtain material for analysis of bacterial functional diversity at each plot, the rhizosphere mineral material (substrate) was sampled from three points at a depth of 10 cm and mixed. The bulk samples were immediately transported to the laboratory and sieved with a 2 mm mesh sieve. The sieved material was put in polyurethane bags at 4 °C before the analysis was conducted.

Before the start of the procedure, both the needed equipment and the 0.85% saline solution were sterilized. A total of 10 g of dry soil was suspended in 90 mL of saline solution (dilution 10^{-1}), incubated for 60 min on a shaker (120 rpm), and left to settle for 30 min. Next, 2 mL of the prepared soil suspension was transferred to 18 mL of fresh sterile saline solution (dilution 10^{-2}). A total of 120 µL of solution was used to inoculate each of the 96 wells of the BIOLOG microplates. Immediately after inoculation (and every 24 h afterwards), the absorbance of soil suspension in the wells was measured at 590 nm using the BIOLOG MicroStation. The microplates were incubated at 22 °C for 5 days.

To measure and characterize the bacteria's functional diversity, the study used the bacteria's ability to diversify the intensity of digesting different sources of carbon used in the plates [67–69]. The following measurements were calculated: the summed area under the curve for all substrates (ΣAUC), the richness index (Rs), the Shannon-Wiener index (H'), and the evenness index (Eh) [70].

A statistical analysis was performed with Statistica 12 software. Assumptions for parametric tests were checked by the following methods: a normal probability plot followed by the Shapiro-Wilk test for normality of distribution and Levene's test for the equity of variances. A non-parametric test (Kruskal-Wallis test with post-hoc test) was applied when it appeared that the assumptions were not fulfilled. In other cases, statistical significance of differences between mean values was tested with a one way ANOVA test along with the post-hoc Tukey's test with the ω^2 factor calculated additionally [71,72].

Discriminant analysis, after checking all the assumptions, was performed to verify if bacterial functional diversity follows the vegetation characteristics of studied novel ecosystems patches; and Statistica 12 software was again used [71,72].

3. Results

The characteristics of the vegetation are given in Table 1. The most abundant vegetation cover was founded in microhabitats dominated by *C. epigejos*, although the plant diversity there, measured by the mean number of species, was comparable to the diversity of the other vegetation types.

Table 1. Characteristics of plant cover in four studied vegetation types.

Dominant Species	Mean Cover	Abundance of Dominant Species	Species Composition (Mean Number of Species; the Most Abundant Species)
Calamagrostis epigejos *	73%	10–80%	12.8; *Picris hieracioides*, *Oenothera* sp., *Senecio viscosus*, *Solidago gigantea*, *Poa compressa*
Poa compressa *	70%	20–40%	13.9; *Leontodon autumnalis*, *Lotus corniculatus*, *Daucus carota*, *Plantago lanceolata*, *Achillea millefolium*, *Medicago lupulina*, *Echium vulgare*, *Picris hieracioides*, *Calamagrostis epigejos*
Daucus carota **	54%	5–30%	12.8; *Calamagrostis epigejos*, *Lotus corniculatus*, *Hieracium piloselloides*, *Picris hieracioides*, *Matricaria maritima* subs. *inodora*
Tussilago farfara **	46%	20–40%	8.3; *Calamagrostis epigejos*, *Daucus carota*, *Chamaenerion palustre*, *Hieracium piloselloides*

Monocotyledons (grasses *) and dicotyledons (forbs **).

The BIOLOG-CLPP analysis revealed significant effects of plant species composition on the physiological diversity of microbial communities in the studied novel ecosystems. The highest values of total catabolic activity (∑AUC) were observed for the communities associated with *C. epigejos*. ∑AUC of microbial communities associated with *T. farfara* and non-vegetated patches were about 10 times lower than those of the microbial communities associated with the other plant species and differed significantly from the other vegetation types (Figure 6). The H' index and the numbers of oxidized substrates (Rs) were the highest for the microbial communities associated with *C. epigejos* and *P. compressa*, but the observed differences were not so evident.

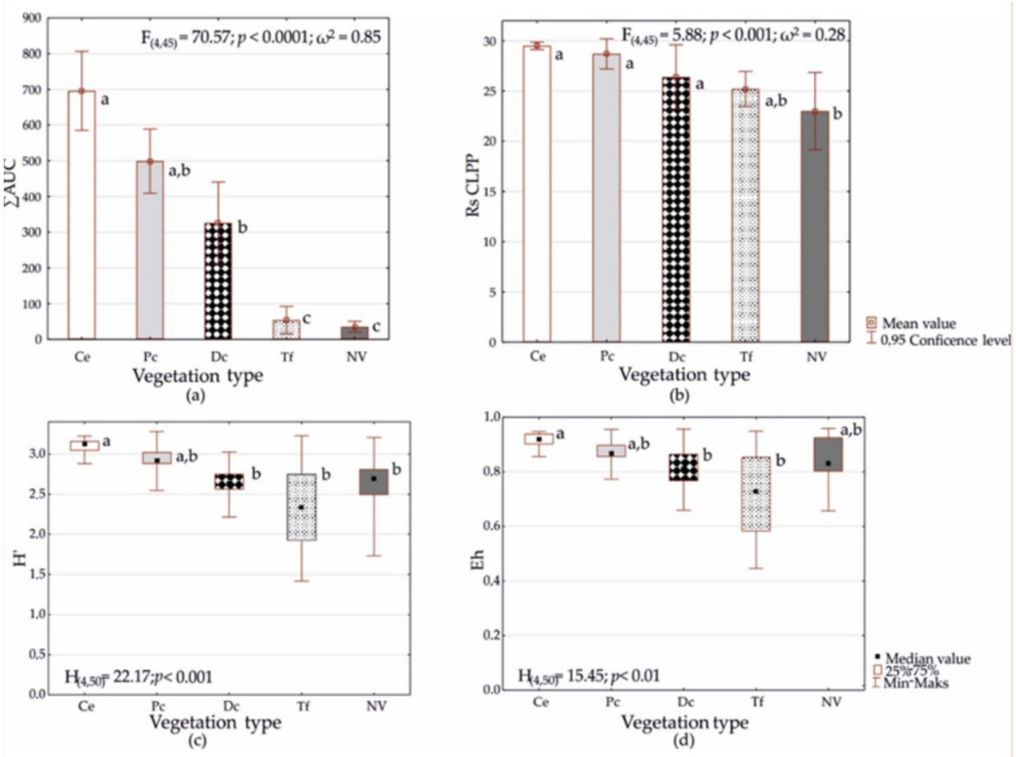

Figure 6. Results of the one-way ANOVA (**a**,**b**) and Kruskal-Wallis test (**c**,**d**) of measures characterizing bacteria functional diversity. (**a**) ΣAUC—summed area under the curve for all substrates; (**b**) Rs—richness index; (**c**) H′—Shannon-Wiener index; (**d**) Eh—evenness index; $N = 10$, post-hoc tests were performed (Tukey's HSD test or U Mann-Whitney test; different letters indicate significantly different values, with $p < 0.05$. Vegetation type: Ce—*C. epigejos* dominated, Pc—*P. compressa*, Dc—*Daucus carota*, Tf—*Tussilago farfara*, NV—non vegetated patches.

Discriminant analyses performed to check if the pattern of microbiome characteristics follows the differences in vegetation cover showed high levels of co-occurrence (Figure 7). Discrimination performed on the basis of microbiome characteristics covered the division of patches made on the basis of vegetation structure in 60% (90% in the case of non-vegetated patches, 70% in *C. epigejos*, 60% in *D. carota* and 40% in the case of *P. compressa* and *T. farfara* patches) and additionally showed high levels of similarity of microbiome characteristics between *T. farfara* and non-vegetated patches as well as in the case of grass-dominated vegetation types.

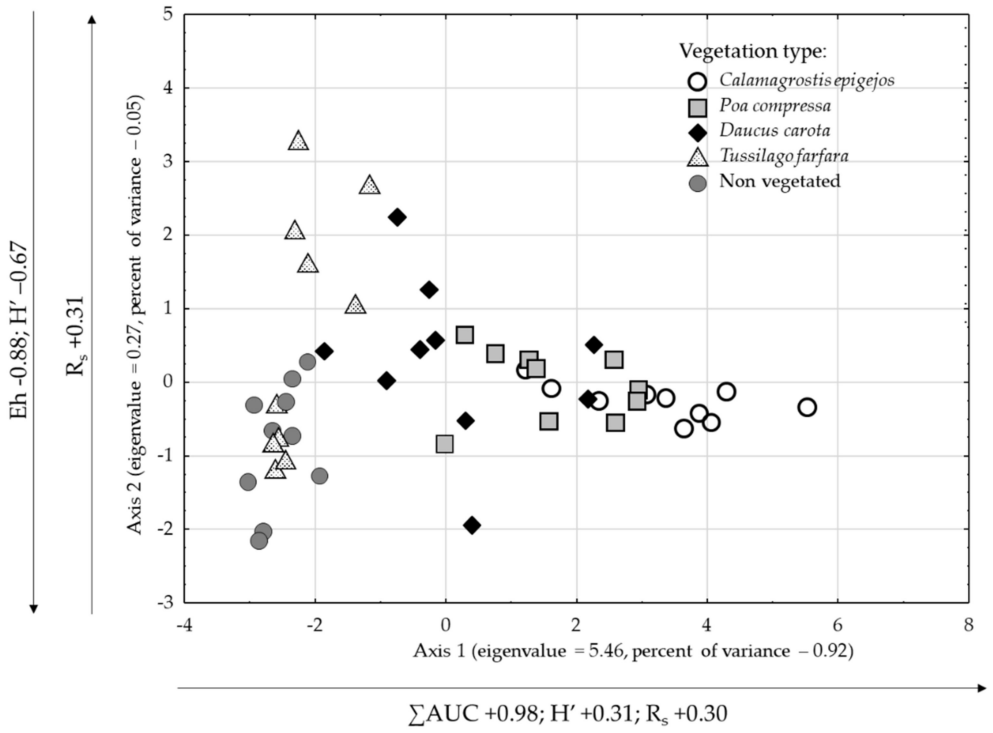

Figure 7. Results of discrimination analysis made on four characteristics of microbiome (∑AUC, H', Eh and Rs). Extra axes explain the influence of four given characteristics of the microbiome with the discriminative axes and values show the strength of the influence (range from −1.0 to 1.0). Axis 1 discriminates *T. farfara* and non-vegetated patches from the other vegetation types and the most important discriminating factor is ∑AUC. Axis 2 discriminates non-vegetated and *C. epigejos* patches from the other vegetation types and the main discriminating factor is the evenness index.

4. Discussion

The awareness that the development of stable ecosystems on degraded lands depends on complicated interactions among organisms, with the essential role played by plants and microbial assemblages, is steadily growing among scientists and practitioners. Understanding ecosystem development on post-mining areas requires following the development of both plant and microbial communities [73,74]. Mutual interaction is important for both groups, and both benefit significantly from it. Studies of the effect of the presence of *T. farfara*, an early colonizer of spoil heaps [75], on the development of the soil microbial community showed that plant roots increased microbial diversity and that biomass and roots influenced by the microbial community, including nitrogen fixators and arbuscular mycorrhizal fungi, were bigger with higher metabolic potential than the control [76–79]. In our study, measures characterizing bacterial functional diversity of non-vegetated patches and patches dominated by *T. farfara* did not differ significantly, and they were difficult to distinguish on the basis of the used data. The summed area under the curve for all substrates (∑AUC) and the richness index (Rs) were only slightly higher for *T. farfara* patches, but mean values did not differ significantly. More evident differences were noted between measurements calculated for the above mentioned two vegetation types and grassland patches (with *C. epigejos* and *P. compressa*) with significantly higher values. It is worth mentioning that the development of denser and more diverse grass-dominated vegetation types lasts longer than the development of sparse vegetation (the mean cover about 30%) dominated mostly by *T. farfara,* and that the time factor can significantly influence the development of the bacterial community. Studies across chronosequence plots spanning 54 years located in a brown coal mine spoil deposit area in the Czech Republic demonstrated an important role of bacteria in the initial stage of succession (soil development stage). Later bacterial communities changed less dynamically and followed changes in soil

parameters rather than vegetation changes. The transformation of plant assemblages was mirrored by soil fungal communities to a higher extent [74]. The functional diversity of bacteria in the soil is significantly related to SOM in the soil. It affects the energy provision for microbial growth and enzyme production [54]. Most studies revealed positive correlations between microorganism activities and SOC and TN in human-disrupted areas [55–57,80]. Urbanová et al. [44] detected that during spontaneous succession on heaps after open-cast brown coal extraction, the presence of SOC and TN in the soil layer had a crucial influence on the activities of microorganisms. Wang et al. [81] revealed that the management of vegetation restoration impacted different carbon sources that influenced the functional diversity of the microbial community in sandy soils. For vegetated and unvegetated plots, it is possible that most of the carbon in the mineral material was related to organic matter of recent or geogenic origin [60,61]. Geogenic coal is not available to microorganisms, and therefore, despite the high content of organic carbon (10–18%), the substrate in the studied plots had low available carbon sources for microorganisms [62]. The lack of correlation between SOC and the activity of the microorganisms may be related to the small quantity of available carbon in the total pool of SOC in some studies [9–11].

Stefanowicz at al.'s [73] studies of microbial communities conducted using the BIOLOG method on 20 spoiled heaps in two age categories (5–10 and 15–25 years from the end of heaping) covered with three different vegetation types (*T. farfara*, *C. epigejos*, and *Chamaenerion palustre* as dominant species) also confirmed the importance of time and vegetation type for microbial community activity, bacterial functional richness and basal respiration [73]. All of these measurements were significantly higher for older heaps overgrown by all vegetation types in comparison to the control ones (bare ground in all heaps age categories).

The Constraints of Methods for Determining Functional Diversity

The BIOLOG tool allows for the assessment of the relationships between microbial communities of varied vegetation types and the land use type [63,82,83] under environmental stresses such as high salinity and high soil pH [84], pollution with hydrocarbons or heavy metals, or high temperatures [85–90]. The BIOLOG method enables the detection of even small variations in microbial functional communities, such as the responses of rhizosphere microorganisms to the plant's aging [91]. Biodiversity decrease (e.g., bacterial diversity) is often caused by the extinction of species with narrow ecological requirements that are unable to face new environmental constraints [91–93].

The BIOLOG method enables one to obtain data about the microorganismal community based on the characteristic pattern of substrate utilization, since the microbial growth rate and substrate utilization reflect the microbial community function. In this respect, the results of the BIOLOG method application provide information about both the structure and function of the studied microbial communities [94]. The results display the potential and not the actual catabolic activity of a microorganismal community [55,62]. Different microorganisms have diverse abilities to utilize the substrates in the plates. The variety of the carbon substrates provided in the plates does not always reflect the substrates that are available to the microorganisms in the soil or the soil substratum environment. On the other hand, some bacterial species are unable to grow on plates because the crucial substrates are not present [95]. In some studies, complicated interactions between species, different nutrient requirements, and/or high microbial species diversity might cause ambiguities and problems. It can be very difficult to precisely assess which microorganisms used the substrates on the plate. It is possible that the fast-growing bacteria contribute the most to the reduction of the tetrazolium dye on bacterial plates, as they are adapted to high substrate concentrations [96,97]. The slow-growing bacteria, incapable of growing in conditions of high substrate concentrations, can sometimes remain undetected in the analysis [97]. Some bacteria are unable to reduce tetrazolium or are able to reduce it only in cases of high substrate concentrations [98,99]. Several studies confirmed that the physiological profile obtained on a plate reflects only a part of the microbial community diversity,

and the conclusions about the function and structure of a whole bacteria assemblage must consider this. Taking the above into account, the results obtained by using the BIOLOG method provide data about the metabolic potential and functional diversity of the studied community which is able to metabolize actively and grow in plate conditions [90,100].

Physiological profiles of the microbial community can also be studied by the catabolic responses of the bacteria community to the addition of carbon substrates to separate soil samples. The measurement of CO_2 efflux along with the direct measurements of CO_2 production or O_2 consumption reflects the catabolic response and assessment of respiration of the microbial community in the studied vegetation type. Sometimes the utilization of a particular substrate demands the presence of other organic compounds [101,102]. In this method, the soil extraction is not necessary, so inefficient extraction and inoculum density does not cause a problem. The microbial community activity is generally measured after 0–6 h and, different from the BIOLOG plates, relies on indigenous microbial activity rather than on microbial growth [101,103]. In the BIOLOG method, each substrate is in a separate well on the plate. The catabolic profiling approach enables one to avoid some problems documented in the BIOLOG plates method. High substrate concentrations, which can inhibit some species or cause substrate-accelerated death, are crucial [103,104].

Apart from the wide range of applications, the BIOLOG method generates some problems that need to be overcome. An example of such a problem is that the commonly used phosphate buffer for the extraction of the microorganisms is not an appropriate solution for the extraction of microorganismal communities from a soil with a high zinc content. Phosphate reacts with zinc and influences absorbance measurements, leading to unprecise readings [60,105,106]. Another problem that has to be taken into consideration is that physiological profiles of bacterial communities can provide different results depending on the used buffer type. A source of variation might be the fact that the phosphate buffer is also a source of nutrients for microorganisms [106]. The extraction method is essential when comparing physiological profiles. Special attention should be paid to the extraction of microbes from the soil. The extraction has to be effective enough to allow the preparation of a representative inoculum of the studied bacterial community [103,104].

An additional issue is the impact of the inoculum density standardization depending on the study aim. Inoculum density standardization is crucial when the study aims to estimate the dynamics of the microbial community composition [107]. It affects the average well colour development (AWCD) [89]. The suitable dilution of the soil solution fixes the number of microorganisms to a certain level. The standardized inoculum is inoculated into plate wells [108]. Unfortunately, soil solution dilution can lead to the loss of rare species in the studied community [89].

The analysis of average well colour development, AWCD, is the least sensitive to the impact of inoculum density [89]. Greater than ten-fold dilutions affect classification with AWCD to a significant extent. Additionally, the dependence of colour development on the number of bacterial cells in the inoculum is not apparent because of the complex nature of the metabolic response. The metabolic response is influenced by the competition and cooperation between the microorganisms [108].

5. Conclusions

The microorganism assemblages, along with plants, play a significant role in the development of sustainable ecosystems in post-excavated, transformed areas. Their composition depends on two main factors. The first one is the complex system of habitat conditions and the second is vegetation composition, both of which change significantly over time. Our study confirmed that microbial functional diversity measured by the summed area under the curve for all substrates, the richness index, the Shannon-Wiener index and the evenness index mirrors aboveground vegetation changes. All of these measures differ, especially between non-vegetated patches and grassland patches dominated by *C. epigejos* or *P. compressa*.

The data obtained by using the method allows us to evaluate the functional diversity of microbial communities developed in habitats overgrown by non-analogous plant species composition, e.g., in post mineral exploitation novel ecosystems, and enables us to compare it with data from non-transformed ecosystems. The results obtained by using this method can be used to perform an environmental risk assessment.

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References

1. Hooper, D.U.; Bignell, D.E.; Brown, V.K.; Brussard, J.; Dangerfield, M.; Wall, D.H.; Wardle, D.A.; Coleman, D.C.; Giller, K.E.; Lavelle, P.; et al. Interactions between Aboveground and Belowground Biodiversity in Terrestrial Ecosystems: Patterns, Mechanisms, and Feedbacks: We assess the evidence for correlation between aboveground and belowground diversity and conclude that a variety of mechanisms could lead to positive, negative, or no relationship—Depending on the strength and type of interactions among species. *BioScience* **2000**, *50*, 1049–1061.
2. Lavelle, P.; Spain, A.V. *Soil Ecology*; Kluwer Academic Publishers: New York, NY, USA; Boston, MA, USA; Dordrecht, The Netherlands; London, UK; Moscow, Russia, 2001; pp. 1–654.
3. Loreau, M.; Naeem, S.; Inchausti, P.; Bengtsson, J.; Grime, J.P.; Hector, A.; Hooper, D.U.; Huston, M.A.; Raffaelli, D.; Schmid, B.; et al. Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science* **2001**, *294*, 804–808. [CrossRef] [PubMed]
4. Naeem, S.; Loreau, M.; Inchausti, P. Biodiversity and ecosystem functioning: The emergence of a synthetic ecological framework. In *Biodiversity and Ecosystem Functioning—Synthesis and Perspectives*; Loreau, M., Naeem, S., Inchausti, P., Eds.; Oxford University Press: Oxford, UK, 2002; pp. 3–11.
5. Van der Putten, W.H.; Macel, M.; Visser, M.E. Predicting species distribution and abundance responses to climate change: Why it is essential to include biotic interactions across trophic levels. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2010**, *365*, 2025–2034. [CrossRef] [PubMed]
6. Scherer-Lorenzen, M. Functional diversity affects decomposition processes in experimental grasslands. *Funct. Ecol.* **2008**, *22*, 547–555. [CrossRef]
7. Loreau, M.; Hector, A. Partitioning selection and complementarity in biodiversity experiments. *Nature* **2001**, *412*, 72–76. [CrossRef] [PubMed]
8. Whittaker, R.H. Dominance and diversity in land plant communities: Numerical relations of species express the importance of competition in community function and evolution. *Science* **1965**, *147*, 250–260. [CrossRef]

9. Grubb, P.J. The maintenance of species-richness in plant communities: The importance of the regeneration niche. *Biol. Rev.* **1977**, *52*, 107–145. [CrossRef]
10. Benton, M.J. The Red Queen and the Court Jester: Species diversity and the role of biotic and abiotic factors through time. *Science* **2009**, *323*, 728–732. [CrossRef]
11. Prach, K.; Walker, L.R. *Comparative Plant Succession among Terrestrial Biomes of the World*; Cambridge University Press: Cambridge, UK, 2020; pp. 1–300.
12. Faliński, J.B. Long-term studies on vegetation dynamics: Some notes on concepts, fundamentals and conditions. *Community Ecol.* **2003**, *4*, 107–113. [CrossRef]
13. Wardle, D.A.; Hörnberg, G.; Zackrisson, O.; Kalela-Brundin, M.; Coomes, D.A. Long-term effects of wildfire on ecosystem properties across an island area gradient. *Science* **2003**, *300*, 972–975. [CrossRef]
14. Chmura, D.; Molenda, T.; Błońska, A.; Woźniak, G. Sites of Leachate Inflows on Coalmine Heaps as Refuges of Rare Mountainous Species. *Pol. J. Environ. Stud.* **2011**, *20*, 551–557.
15. Piekarska-Stachowiak, A.; Szary, M.; Ziemer, B.; Besenyei, L.; Woźniak, G. An application of the plant functional group concept to restoration practice on coal mine spoil heaps. *Ecol. Res.* **2014**, *29*, 843–853. [CrossRef]
16. Błonska, A.; Kompała-Bąba, A.; Sierka, E.; Bierza, W.; Magurno, F.; Besenyei, L.; Rys, K.; Woźniak, G. Diversity of vegetation dominated by selected grass species on coal-mine spoil heaps in terms of reclamation of post-industrial areas. *J. Ecol. Eng.* **2019**, *20*, 209–217. [CrossRef]
17. Woźniak, G.; Jagodziński, A.M. Post-mineral Excavation Sites as Novel Ecosystems and Examples of Socio-environmental Resilience. In *Green Scenarios: Mining Industry Responses to Environmental Challenges of the Anthropocene Epoch—International Mining Forum 2021*; Dyczko, A., Jagodziński, A.M., Woźniak, G., Eds.; CRC Press Balkema, Taylor & Francis Group: Boca Raton, FL, USA, 2022; pp. 57–67.
18. Grime, J.P. Benefits of plant diversity to ecosystems: Immediate, filter and founder effects. *J. Ecol.* **1998**, *86*, 902–910. [CrossRef]
19. Bradshaw, A.D. The use of natural processes in reclamation—Advantages and difficulties. *Landsc. Urban Plan.* **2000**, *51*, 89–100. [CrossRef]
20. Bardgett, R.D.; Bowman, W.D.; Kaufmann, R.; Schmidt, S.K. A temporal approach to linking aboveground and belowground ecology. *Trends Ecol Evol.* **2005**, *20*, 634–641. [CrossRef]
21. Woźniak, G.; Markowicz, A.; Borymski, S.; Piotrowska-Seget, Z.; Chmura, D.; Besenyei, L. The relationship between successional vascular plant assemblages and associated microbial communities on coal mine spoil heaps. *Community Ecol.* **2015**, *16*, 23–32. [CrossRef]
22. Thijs, S.; Sillen, W.; Rineau, F.; Weyens, N.; Vangronsveld, J. Towards an enhanced understanding of plant-microbiome interactions to improve phytoremediation: Engineering the metaorganism. *Front. Microbiol.* **2016**, *124*, 341. [CrossRef]
23. Witkamp, M.; Ausmus, B.S. Processes in decomposition and nutrient transfer in forest systems. In *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes*; Anderson, J.M., Macfadyen, A., Eds.; Blackwell Scientific Publications: Hoboken, NJ, USA, 1976; pp. 375–396.
24. Porazinska, D.L.; Bardgett, R.D.; Blaauw, M.B.; Hunt, W.H.; Parsons, A.N.; Seastedt, T.R.; Wall, D.H. Relationships at the aboveground–belowground interface: Plants, soil biota, and soil processes. *Ecol. Monogr.* **2003**, *73*, 377–395. [CrossRef]
25. Keith, S.A.; Newton, A.S.; Herbert, R.J.H.; Morecroft, M.D.; Bealey, C.E. Non-analogous community formation in response to climate change. *J. Nat. Conserv.* **2009**, *17*, 228–235. [CrossRef]
26. Morse, N.B.; Pellissier, P.A.; Cianciola, E.N.; Brereton, R.L.; Sullivan, M.M.; Shonka, N.K.; Wheeler, T.B.; McDowell, W.H. Novel ecosystems in the Anthropocene: A revision of the novel ecosystem concept for pragmatic applications. *Ecol. Soc.* **2014**, *19*, 12. [CrossRef]
27. Rotherham, I.D. *Recombinant Ecology—A Hybrid Future?* Springer: Cham, Switzerland, 2017; pp. 1–85.
28. Kałucka, I.L.; Jagodziński, A.M. Successional traits of ectomycorrhizal fungi in forest reclamation after surface mining and agricultural disturbances: A review. *Dendrobiology* **2016**, *76*, 91–104. [CrossRef]
29. Błaszkowski, J.; Niezgoda, N.; Piątek, M.; Magurno, F.; Malicka, M.; Zubek, S.; Mleczko, P.; Yorou, N.S.; Jobim, K.; Vista, X.M.; et al. *Rhizoglomus dalpeae*, *R. maiae*, and *R. silesianum*, new species. *Mycologia* **2019**, *111*, 965–980. [CrossRef] [PubMed]
30. Cardinale, B.J.; Duffy, E.; Gonzalez, A.; Hooper, D.U.; Perrings, C.; Venail, P.; Narwani, A.; Mace, G.M.; Tilman, D.; Wardle, D.A.; et al. Biodiversity loss and its impact on humanity. *Nature* **2012**, *486*, 59–67. [CrossRef] [PubMed]
31. Frouz, J. (Ed.) *Soil Biota and Ecosystem Development in Post Mining Sites*; CRC Press, Taylor & Francis Group: Boca Raton, FL, USA, 2014; pp. 1–316.
32. Jagodziński, A.M.; Wierzcholska, S.; Dyderski, M.K.; Horodecki, P.; Rusińska, A.; Gdula, A.K.; Kasprowicz, M. Tree species effects on bryophyte guilds on a reclaimed post-mining site. *Ecol. Eng.* **2018**, *110*, 117–127. [CrossRef]
33. Kompała-Bąba, A.; Sierka, E.; Dyderski, M.K.; Bierza, W.; Magurno, F.; Besenyei, L.; Błońska, A.; Ryś, K.; Jagodziński, A.M.; Woźniak, G. Do the dominant plant species impact the substrate and vegetation composition of post-coal mining spoil heaps? *Ecol. Eng.* **2020**, *143*, 105685. [CrossRef]
34. Novák, J.; Prach, K. Vegetation succession in basalt quarries: Pattern on a landscape scale. *Appl. Veg. Sci.* **2003**, *6*, 111–116. [CrossRef]

35. Řehounková, K.; Čížek, L.; Řehounek, J.; Šebelíková, L.; Tropek, R.; Lencová, K.; Bogusch, P.; Marhoul, P.; Máca, J. Additional disturbances as a beneficial tool for restoration of post-mining sites: A multi-taxa approach. *Environ. Sci. Pollut. R.* **2016**, *23*, 13745–13753. [CrossRef]
36. Hobbs, R.J.; Higgs, E.S.; Hall, C.M. *Novel Ecosystems: Intervening in the New Ecological World Order*; John Wiley & Sons: Chichester, UK, 2013; pp. 1–384.
37. Naeem, S.; Hahn, D.R.; Schuurman, G. Producer—Decomposer co-dependency influences biodiversity effects. *Nature* **2000**, *403*, 762–764. [CrossRef]
38. Hobbs, R.J.; Arico, S.; Aronson, J.; Baron, J.S.; Bridgewater, P.; Cramer, V.A.; Epstein, P.R.; Ewel, J.J.; Klink, C.A.; Lugo, A.E.; et al. Novel ecosystems: Theoretical and management aspects of the new ecological world order. *Glob. Ecol. Biogeogr.* **2006**, *15*, 1–7. [CrossRef]
39. Frouz, J.; Prach, K.; Pižl, V.; Háněl, L.; Starý, J.; Tajovský, K.; Materna, J.; Balík, V.; Kalčík, J.; Řehounková, K. Interactions between soil development, vegetation and soil fauna during spontaneous succession in post mining sites. *Eur. J. Soil Biol.* **2008**, *44*, 109–121. [CrossRef]
40. Loreau, M. Linking biodiversity and ecosystems: Towards a unifying ecological theory. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2010**, *365*, 49–60. [CrossRef] [PubMed]
41. Tropek, R.; Kadlec, T.; Karesova, P.; Spitzer, L.; Kocarek, P.; Malenovsky, I.; Banar, P.; Tuf, I.H.; Hejda, M.; Konvicka, M. Spontaneous succession in limestone quarries as an effective restoration tool for endangered arthropods and plants. *J. Appl. Ecol.* **2010**, *47*, 139–147. [CrossRef]
42. Tropek, R.; Kadlec, T.; Hejda, M.; Kocarek, P.; Skuhrovec, J.; Malenovsky, I.; Vodka, S.; Spitzer, L.; Banar, P.; Konvicka, M. Technical reclamations are wasting the conservation potential of post-mining sites. A case study of black coal spoil dumps. *Ecol. Eng.* **2012**, *43*, 13–18. [CrossRef]
43. Mudráka, O.; Dolezală, J.; Frouz, J. Initial species composition predicts the progress in the spontaneous succession on post-mining sites. *Ecol. Eng.* **2016**, *95*, 665–670. [CrossRef]
44. Urbanová, M.; Kopecký, J.; Valásková, V.; Ságová-Marecková, M.; Elhottová, D.; Kyselková, M.; Moënne-Loccoz, Y.; Baldrian, P. Development of bacterial community during spontaneous succession on spoil heaps after brown coal mining. *FEMS Microbiol. Ecol.* **2011**, *78*, 59–69. [CrossRef]
45. Chapin, F., III; Zavaleta, E.S.; Eviner, V.T.; Naylor, R.L.; Vitousek, P.M.; Reynolds, H.L.; Hooper, D.U.; Lavorel, S.; Sala, O.E.; Hobbie, S.E.; et al. Consequences of changing biodiversity. *Nature* **2000**, *405*, 234–242. [CrossRef]
46. Wardle, D.A.; Bardgett, R.D.; Klironomos, J.N.; Setälä, H.; van der Putten, W.H.; Wall, D.H. Ecological linkages between aboveground and belowground biota. *Science* **2004**, *304*, 1629–1633. [CrossRef]
47. Couteaux, M.M.; Bottner, P.; Berg, B. Litter decomposition, climate and litter quality. *Trends Ecol. Evol.* **1995**, *10*, 63–66. [CrossRef]
48. Babur, E.; Dindaroğlu, T.; Solaiman, Z.M.; Battaglia, M.L. Microbial respiration, microbial biomass and activity are highly sensitive to forest tree species and seasonal patterns in the Eastern Mediterranean Karst Ecosystems. *Sci. Total Environ.* **2021**, *775*, 145868. [CrossRef]
49. Aerts, R. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. *Oikos* **1997**, *79*, 439. [CrossRef]
50. Prescott, C.E. Influence of forest floor type on rates of litter decomposition in microcosms. *Soil Biol. Biochem.* **1996**, *28*, 1319. [CrossRef]
51. Wardle, D.A.; Nilsson, M.C.; Zackrisson, O.; Gallet, C. Determinants of litter mixing effects in a Swedish boreal forest. *Soil. Biol. Biochem.* **2000**, *35*, 827. [CrossRef]
52. Boivin, M.E.Y.; Breure, A.M.; Posthuma, L.; Rutgers, M. Determination of field effects of contaminants—significance of pollution-induced community tolerance. *Hum. Ecol. Risk Assess.* **2002**, *8*, 10–35. [CrossRef]
53. Dickens, H.E.; Anderson, J.M. Manipulation of soil microbial community structure in bog and forest soils using chloroform fumigation. *Soil Biol. Biochem.* **1999**, *31*, 2049–2058. [CrossRef]
54. Enami, Y.; Okano, S.; Yada, H.; Nakamura, Y. Influence of earthworm activity and rice straw application on the soil microbial community structure analyzed by PLFA pattern. *Eur. J. Soil Biol.* **2001**, *37*, 269–272. [CrossRef]
55. Smalla, K.; Wachtendorf, U.; Heuer, H.; Liu, W.T.; Forney, L. Analysis of BIOLOG GN substrate utilization patterns by microbial communities. *Appl. Environ. Microbiol.* **1998**, *64*, 1220–1225. [CrossRef]
56. Yrjälä, K.; Katainen, R.; Jurgens, G.; Saarela, U.; Saano, A.; Romantschuk, M.; Fritze, H. Wood ash fertilization alters the forest humus Archaea community. *Soil Biol. Biochem.* **2004**, *36*, 199–201. [CrossRef]
57. Stefanowicz, A. The Biolog plates technique as a tool in ecological studies of microbial communities. *Pol. J. Environ. Stud.* **2006**, *15*, 669–676.
58. Garland, J.L.; Mills, A.L. Classification and characterization of heterotrophic microbial communities on the basis or patterns of community-level sole-carbon-source utilization. *Appl. Environ. Microbiol.* **1991**, *57*, 2351–2359. [CrossRef]
59. Choi, K.-H.; Dobbs, F.C. Comparison of two kinds of Biolog microplates (GN and Eco) in their ability to distinguish among aquatic microbial communities. *J. Microbiol. Methods* **1999**, *36*, 203. [CrossRef] [PubMed]
60. Baudoin, E.; Benziri, E.; Guckert, A. Metabolic fingerprint of microbial communities from distinct maize rhizosphere compartments. *Eur. J. Soil Biol.* **2001**, *37*, 85–93. [CrossRef]

61. O'Connel, S.P.; Garland, J.L. Dissimilar response of microbial communities in Biolog GN and GN2 plates. *Soil Biol. Biochem.* **2002**, *34*, 413–416. [CrossRef]
62. Preston-Mafham, J.; Boddy, L.; Randerson, P.F. Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles—A critique. *FEMS Microbiol. Ecol.* **2002**, *42*, 1–14.
63. Gomez, E.; Garland, J.; Conti, M. Reproducibility in the response of soil bacterial community-level physiological profiles from a land use intensification gradient. *Appl. Soil Ecol.* **2004**, *26*, 21–30. [CrossRef]
64. Grayston, S.J.; Campbell, C.D.; Bardgett, R.D.; Mawdsley, J.L.; Clegg, C.D.; Ritz, K.; Griffiths, B.S.; Rodwell, J.S.; Edwards, S.J.; Davies, W.J.; et al. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. *Appl. Soil Ecol.* **2004**, *25*, 63–84. [CrossRef]
65. Bhattacharjee, A.; Datta, R.; Gratton, E.; Hochbaum, A.I. Metabolic fingerprinting of bacteria by fluorescence lifetime imaging microscopy. *Sci. Rep.* **2017**, *7*, 3743. [CrossRef]
66. Kompała-Bąba, A.; Bierza, W.; Błońska, A.; Sierka, E.; Magurno, F.; Chmura, D.; Besenyei, L.; Radosz, Ł.; Woźniak, G. Vegetation diversity on coal mine spoil heaps—How important is the texture of soil substrate? *Biologia* **2019**, *74*, 419–436. [CrossRef]
67. Di Giovanni, G.D.; Watrud, L.S.; Seidler, R.J.; Widmer, F. Comparison of parental and transgenic alfalfa rhizosphere bacterial communities using Biolog GN metabolic fingerprinting and entero bacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR). *Microb. Ecol.* **1999**, *37*, 129–139. [CrossRef]
68. Insam, H.; Goberna, M. Use of Biolog® for Community Level Physiological Proffiling (CLPP) of Environmental Samples. In *Molecular Microbial Ecology Manual*; Akkermans, A.D.L., Elsas, J.D., Bruijn, F.J., Eds.; Kluwer Academic: Dordrecht, The Netherlands, 2004; pp. 853–860.
69. Rutgers, M.; Wouterse, M.; Drost, S.M.; Breure, A.M.; Mulder, C.; Stone, D.; Creamer, R.E.; Winding, A.; Bloem, J. Monitoring soil bacteria with community-level physiological profiles using Biolog™ ECO-plates in the Netherlands and Europe. *Appl. Soil Ecol.* **2016**, *97*, 23–35. [CrossRef]
70. Magurran, A.E. *Measuring Biological Diversity*; Blackwell Science: Oxford, UK, 2004; pp. 1–256.
71. Stanisz, A. *Przystępny Kurs Statystyki z Zastosowaniem STATISTICA PL na Przykładach z Medycyny*; StatSoft Polska: Kraków, Poland, 2006; pp. 1–532.
72. StatSoft, Inc. STATISTICA (Data Analysis Software System), Version 12. Available online: www.statsoft.com (accessed on 17 July 2022).
73. Stefanowicz, A.M.; Kapusta, P.; Błońska, A.; Kompała-Bąba, A.; Woźniak, G. Effects of *Calamagrostis epigeios*, *Chamaenerion palustre* and *Tussilago farfara* on nutrient availability and microbial activity in the surface layer of spoil heaps after hard coal mining. *Ecol. Eng.* **2015**, *83*, 328–337. [CrossRef]
74. Harantová, L.; Mudrák, O.; Kohout, P.; Elhottová, D.; Frouz, J.; Baldrian, P. Development of microbial community during primary succession in areas degraded by mining activities. *Land Degrad. Develop.* **2017**, *28*, 2574–2584. [CrossRef]
75. Woźniak, G. *Zróżnicowanie Roślinności na Zwałach Pogórniczych Górnego Śląska*; Instytut Botaniki im. W. Szafera PAN: Kraków, Poland, 2010; pp. 1–320.
76. Laczkó, E.; Rudaz, A.; Aragno, M. Diversity of antropogenically influenced or disturbed soil microbial communities. In *Microbial Communities*; Insam, H., Rangger, A., Eds.; Springer: Berlin/Heidelberg, Germany, 1997; pp. 57–67.
77. Elhottová, D.; Krišůfek, V.; Malý, S.; Frouz, J. Rhizosphere Effect of Colonizer Plant Species on the Development of Soil Microbial Community during Primary Succession on Postmining Sites. *Commun. Soil Sci. Plant Anal.* **2009**, *40*, 758–770. [CrossRef]
78. Söderberg, K.H.; Probanza, A.; Jumpponen, A.; Bååth, E. The microbial community in the rhizosphere determined by community-level physiological profiles (CLPP) and direct soil- and cfu-PLFA techniques. *Appl. Soil Ecol.* **2004**, *25*, 135–145. [CrossRef]
79. Graystong, S.J.; Wang, S.; Campbell, C.D.; Edwards, A.C. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol. Biochem.* **1998**, *30*, 369–378. [CrossRef]
80. Chesson, P. Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* **2000**, *31*, 343–366. [CrossRef]
81. Wang, S.; Mori, T.; Zou, S.; Zheng, H.; Heděnec, P.; Zhu, Y.; Wang, W.; Li, A.; Liu, N.; Jian, S.; et al. Changes in vegetation types affect soil microbial communities in tropical islands of southern China. *Glob. Ecol. Conserv.* **2022**, *37*, e02162. [CrossRef]
82. Banerjee, M.R.; Burton, D.L.; Depoe, S. Impact of sewage sludge application on soil biological characteristics. *Agric. Ecosyst. Environ.* **1997**, *66*, 241. [CrossRef]
83. Mondini, C.; Insam, H. Community level physiological profiling as a tool to evaluate compost maturity: A kinetic approach. *Eur. J. Soil Biol.* **2003**, *39*, 141. [CrossRef]
84. Pankhurst, C.E.; Yu, S.; Hawke, B.G.; Harch, B.D. Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. *Biol. Fertil. Soils* **2001**, *33*, 204. [CrossRef]
85. Pietikäinen, J.; Hiukka, R.; Fritze, H. Does short term heating of forest humus change its properties as a substrate for microbes? *Soil Biol. Biochem.* **2000**, *32*, 277. [CrossRef]
86. Ellis, R.J.; Neish, B.; Trett, M.W.; Best, J.G.; Weightman, A.J.; Morgan, P.; Fry, J.C. Comparison of microbial and meiofaunal community analyses for determining impact of heavy metal contamination. *J. Microbiol. Meth.* **2001**, *45*, 171. [CrossRef] [PubMed]
87. Bundy, J.G.; Paton, G.I.; Campbell, C.D. Combined microbial community level and single species biosensor responses to monitor recovery of oil polluted soil. *Soil Biol. Biochem.* **2004**, *36*, 1149. [CrossRef]

88. Rutgers, M.; Breure, A.M. Risk assessment, microbial communities, and pollution-induced community tolerance. *Human Ecol. Risk Assess.* **1999**, *5*, 661. [CrossRef]
89. Garland, J.L.; Mills, A.L.; Young, J.S. Relative effectiveness of kinetic analysis vs single point readings for classifying environmental samples based on community-level physiological profiles (CLPP). *Soil Biol. Biochem.* **2001**, *33*, 1059. [CrossRef]
90. Zak, J.C.; Willig, M.R.; Moorhead, D.L.; Wildman, H.G. Functional diversity of microbial communities: A quantitative approach. *Soil Biol. Biochem.* **1994**, *26*, 1101. [CrossRef]
91. Garland, J.L. Patterns of potential carbon source utilization by rhizosphere communities. *Soil Biol. Biochem.* **1996**, *28*, 223. [CrossRef]
92. Kandeler, E.; Kampichler, C.; Horak, O. Influence of heavy metals on the functional diversity of soil microbial communities. *Biol. Fertil. Soils.* **1996**, *23*, 299. [CrossRef]
93. Rutgers, M.; Van't Verlaat, I.M.; Wind, B.; Posthuma, L.; Breure, A.M. Rapid method for assessing pollution-induced community tolerance in contaminated soil. *Environ. Toxicol. Chem.* **1998**, *17*, 2210. [CrossRef]
94. Garland, J.L.; Mills, A.L. A community-level physiological approach for studying microbial communities. In *Beyond the Biomass*; A Wiley-Sayce Publication; Ritz, K., Dighton, J., Giller, K.E., Eds.; British Society of Soil Science (BSSS): Chichester, UK, 1994; pp. 77–83.
95. Glimm, E.; Heuer, H.; Engelen, B.; Smalla, K.; Backhaus, H. Statistical comparisons of community catabolic profiles. *J. Microbiol. Meth.* **1997**, *30*, 71. [CrossRef]
96. Verschuere, L.; Fievez, V.; Van Vooren, L.; Verstraete, W. The contribution of indyvidual populations to the Biolog pattern of model microbial communities. *FEMS Microbiol. Ecol.* **1997**, *24*, 353. [CrossRef]
97. Winding, A.; Hendriksen, N.B. Biolog substrate utilisation assay for metabolic fingerprints of soil bacteria: Incubation effects. In *Microbial Communities (Functional versus Structural Approaches)*; Insam, H., Rangger, A., Eds.; Springer: Berlin/Heidelberg, Germany, 1997; pp. 195–205.
98. Winding, A.; Binnerup, S.J.; Sørensen, J. Viability of indigenous soil bacteria assayed by respiratory activity and growth. *Appl. Environ. Microbiol.* **1994**, *60*, 2869. [CrossRef] [PubMed]
99. Heuer, H.; Smalla, K. Evaluation of community-level catabolic profiling using Biolog GN microplates to study microbial community changes in potato phyllosphere. *J. Microbiol. Meth.* **1997**, *30*, 49. [CrossRef]
100. Schutter, M.; Dick, R. Shifts in substrate utilization potential and structure of soil microbial communities in response to carbon substrates. *Soil Biol. Biochem.* **2001**, *33*, 1481. [CrossRef]
101. Degens, B.P.; Harris, J.A. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biol. Biochem.* **1997**, *29*, 1309. [CrossRef]
102. Garland, J.L.; Roberts, M.S.; Levine, L.H.; Mills, A.L. Community-level physiological profiling performed with an oxygen-sensitive fluorophore in a microtiter plate. *Appl. Environ. Microbiol.* **2003**, *69*, 2994. [CrossRef]
103. Campbell, C.D.; Chapman, S.J.; Cameron, C.M.; Davidson, M.S.; Potts, J.M. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Appl. Environ. Microbiol.* **2003**, *69*, 3593. [CrossRef]
104. Konopka, A.; Oliver, L.; Turco, R.F., Jr. The use of carbon substrate utilization patterns in environmental and ecological microbiology. *Microb. Ecol.* **1998**, *35*, 103. [CrossRef]
105. Hitzl, W.; Rangger, A.; Sharma, S.; Insam, H. Separation power of 95 substrates of the Biolog system determined in various soils. *FEMS Microbiol. Ecol.* **1997**, *22*, 167. [CrossRef]
106. Kelly, J.J.; Tate, R.L. Use of Biolog for the analysis of microbial communities from zinc-contaminated soils. *J. Environ. Qual.* **1998**, *27*, 600. [CrossRef]
107. Garland, J.L. Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiol. Ecol.* **1997**, *24*, 289–300. [CrossRef]
108. Haack, S.K.; Garchow, H.; Klug, M.J.; Forney, L.J. Analysis of factors affecting the accuracy, reproducibility and interpretation of microbial community carbon source utilization patterns. *Appl. Environ. Microb.* **1995**, *61*, 1458–1468. [CrossRef] [PubMed]