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Can the Biological Activity of Abandoned Soils Be Changed by the Growth of *Paulownia elongata* × *Paulownia fortunei*?—Preliminary Study on a Young Tree Plantation

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Abstract: Bioenergy crops play an ecologically and economically fundamental role as an alternative to agri-food productions and as renewable energy sources. Thus far, less attention has been given to assessing microbiological indicators of soil quality in bioenergy crops on abandoned land. The current study assessed microbial and biochemical properties of two soils with different textures in agroforestry plantations of *Paulownia elongata* × *Paulownia fortunei*, with regard to the analysis of potential for the reclamation and redevelopment of abandoned lands. The soil samples were characterised by measuring microbial biomass C and N, key enzyme activities, and determining the community-level physiological profiles (CLPP) using Biolog EcoPlates. Soil texture, sampling time (June and October), and distance of sampling (0.1 m and 1 m from a tree) had significant effects on microbiological properties. Moreover, dehydrogenases and acid phosphatase activities as well as microbial biomass C and N decreased with distance from the trees, and were significantly higher in the October than in the June. The community-level physiological profiles (CLPP) and diversity indices showed a similar trend to other parameters of biological activity. The results showed that there were significant differences in the AWCD (average well-colour development) of all carbon sources among the *Paulownia* microbial communities ($p < 0.05$). In summary, already after one year of tree planting, a statistically significant increase in microbial activity was found, regardless of soil texture, when evaluated by various methods. This proves the value of the *Paulownia* as fast-growing plant for recultivation and improvement of soil quality on abandoned land.

Keywords: *Paulownia*; abandoned land; metabolic diversity; microbiological activity; soil quality

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1. Introduction

Land use and land management are some of the most important factors that influence soil key properties and the environment [1–3]. The recent years have seen dynamic changes in the use of agricultural land. They mainly concern transformation of farmland for abandonment land [4,5]. The definition of term "agricultural land abandonment" refers to land that has not been used for a minimum of two to five years, which means total cessation of agricultural activities [6,7]. Agricultural land abandonment is a common process in parts of North America and Europe [8]. This process can be triggered a combination of socio-economic, political factors, farm structure, agricultural viability, and constraints to the natural environment [5,9]. The European Commission denote an alarming trend concerning that, in the period 2015–2030, about 11% (more than 20 million ha) of agricultural land in the EU can be at potential risk of abandonment, bringing the total abandoned land to 5.6 million ha by 2030, the equivalent of 3% of the total agricultural land [10].

Bell (2020) [11] suggested strategies that can be applied in the process of abandoned land management. These strategies underline, i.e., the importance of the multi-functionality of agriculture and renewable energy sources, including bio-energy crops. The multifunctionality of agriculture is an essential condition for sustainable development. In addition, the multifunctionality of agriculture is understood as the phenomenon that, in addition to the basic function of agriculture, which is the production of food products and other organic raw materials, generates additional income through the production of goods other than those traditionally associated with agricultural production [12–14]. The attractive strategy for farmers is recultivating abandoned agricultural lands, especially where biophysical conditions are favourable, and transforming them into sustainable and multifunctional bioenergy crops, assuring climate change mitigation, energy security, and increased economic opportunities [8,15]. In this regard, the cultivation of bioenergy trees/short rotation forestry have received great interest as a potential alternative to agri-food production, guiding the world towards a sustainable and circular bioeconomy [16–18]. However, soil quality indicators, such as microbial and metabolic parameters, have been less studied in the assessment of soil response to reclamation of land abandonment.

Short rotation forestry (SRF) does not compete with traditional crops for the most productive agricultural land because they are usually cultivated on lower-class agricultural land, reclaimed land, previously uncultivated land, or land that had been abandoned [19]. The genus *Paulownia* contains fast-growing varieties of deciduous trees, which belong to the Paulowniaceae family. *Paulownia* spp. grows on different types of soil, as well as degraded soils [20,21]. However, the most appropriate soils are those that are loose and permeable, with a pH ranging from 5 to 8 [21,22].

The microbial activity is important for plant growth and development in natural ecosystems [23,24]. In forest ecosystems, trees influence the metabolism and structure of soil microorganisms through root secretions and leaf litter fall [25,26]; however, they also directly affect the chemical and physical properties of soil [27]. Microbiological activity is a significant element of soil quality. All disturbances and changes in environmental ecosystems are reflected by changes in the diversity and metabolic activity of microorganisms, with changes in both their distribution and nutrients intake [28]. Microbiological parameters that are widely used in the analysis of the soil environment status are represented by community level physiological profiles (CLPP) [29,30] and enzymatic activity [30,31], as well as microbial biomass C and N [31–33]. The EcoPlate test contains 31 of the most useful carbon sources for microbial community analysis [34], enabling for community-level physiological profiling (CLPP) of microorganisms. This method for the analysis of environmental samples was proposed by Garland and Mils [35]. In addition, Lehman et al. [36] confirmed that the selected set of compounds is appropriate for the characterisation of soil in terms of their metabolism. The population of microorganisms gives a characteristic response pattern called a metabolic fingerprint [29]. However, Ros et al. (2008) [37] argue that the Biolog test does not reflect the functional capacities of the all microorganisms in the sample, but only reflects the capacities of a limited subset of the microbial community. The use of single biological parameters when assessing soil condition is burdened with many limitations. It is therefore recommended that the results of analysis are developed based on the group of parameters, for example, the Biolog EcoPlates technique with the assessment of enzymatic activity and microbial biomass [38].

Land-use transition from agricultural to bioenergy crops has become increasingly common in Europe. However, we have a poor understanding of the effect of these land conversions on soil quality attributes on abandoned land. There were previously such studies, but they were based on different plant species and performed on arable land. Most ecological studies have focused on regions of America and tree taxa, i.e., poplar and willow [39–41]. The information about microbiological properties of soil in *Paulownia* plantations is limited. Only a few studies on this subject have been done in Europe, especially concerning the long-term research of the microbial properties of soil in *Paulownia* plantations [42–44]. Analysing the biological soil quality, it becomes important to under-

stand the diversity, activity, and functioning of microbial communities, as well the need to protect edaphic species of microorganisms [43,45]. Thus far, less attention has been given to assessing microbiological indicators of soil quality in bioenergy crops on abandoned land.

The aim of the study was to assess the potential of *Paulownia* bioenergy crops, which is applicable in multifunctional agriculture for the reclamation and redevelopment of abandoned lands. Therefore, this research explored the responses and changes of the activity of three soil enzymes (acid and alkaline phosphatase, dehydrogenases), microbial biomass, and functional diversity in two soils under plantations of *Paulownia elongata* × *Paulownia fortunei* in a temperate climate in Poland established on abandoned lands, taking into account sampling date and distance from the tree, in order to identify the actual status and the future prospects for this type of land transformation.

2. Materials and Methods

2.1. Study Sites and Soil Sampling

The soil samples were collected from two plantations of *P. elongata* × *P. fortunei* located in Poland, established on abandoned land. This land had not been used in the last 5 years. The *P. elongata* × *P. fortunei* plantations were located in Podkampinos (52°14′ N, 20°27′ E) and Otrębusy (52°07′ N, 20°45′ E), near Warsaw, Mazowieckie Voivodeship, Poland. The area was characterised by a warm summer humid continental (Dfb) climate type, according to the Köppen classification system, with mean annual temperatures of 15 °C and an annual precipitation of 927.6 mm during the study year. The soil samples were collected once in the summer (in the June) and once in the autumn (in the October) of 2017 at both areas, at a distance of 0.1 and 1 m from the nearest tree and at a depth 0–20 cm. The samples were collected in the first year of plantation. All the trees were the same age, in the juvenile phase. The canopy diameter ranged from 40 to 50 cm.

The planting density for *Paulownia* is 5 × 5 m. Three study areas (1.5 m × 1.5 m area) were selected in each plantation. Three permanent study areas were selected in the indicated plantations and sampled in both June and October. Two study areas were located at the edge of the field, approximately 10 m from the boundary, and one area was in the middle of the field. The designated study areas were biological replicates. In each area, 10 random pits at a distance of 0.1 m and 15 random pits at a distance of 1 m were made to collect soil samples (subsamples). The subsamples from each distance (separately for 0.1 m and 1 m) were pooled into one sample representing one biological replicate. In summary, 3 samples (consisting of 10 subsamples) were taken from each area in the field at a distance of 0.1 m, and 3 samples (consisting of 15 subsamples) were taken at a distance of 1 m from the tree (Figure 1). The sampling scheme is shown below.

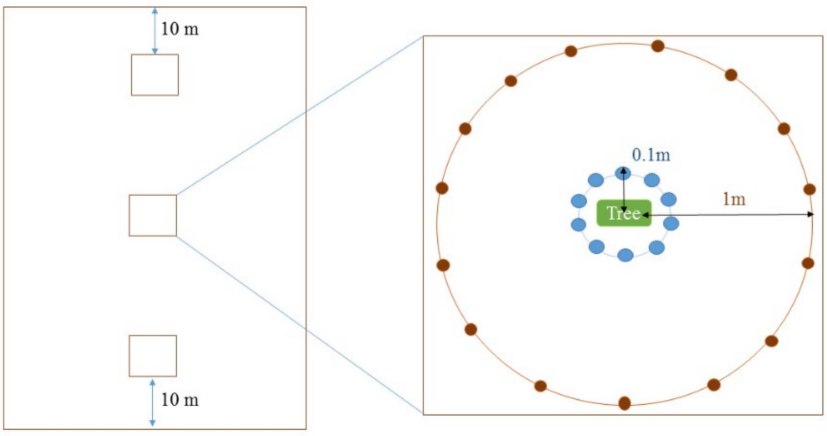

Figure 1. The sampling scheme for *Paulownia* tree plantations.

A summary of the collected samples is shown in Table 1. The samples were brought to the laboratory in plastic bags and refrigerated at 4 °C until analysed. All samples were

passed through a 2-mm mesh sieve. Except for the case of the Biolog EcoPlates, the results were based on dry soil analysed at 105 °C.

Table 1. Soil samples included in the study, with soil texture, site of collection, season, and distance of nearest tree.

Sites	Soil Texture	June		October	
Podkampinos	sandy loam	0.1 m	1 m	0.1 m	1 m
Otrębusy	loamy sand	0.1 m	1 m	0.1 m	1 m

Podkampinos: degraded Cambisol developed from sandy and silty glacial deposits; Otrębusy: Podzol developed from coarse glacial deposits, according to the soil-agricultural map of Poland 1:25,000, managed by Institute of Soil Science and Plant Cultivation-State Research Institute.

Physical and chemical properties of the soils are presented in Table 2.

Table 2. Physical and chemical properties of the soil at two *P. elongata* × *P. fortunei* plantation sites.

Soil Properties	Site	
	Podkampinos	Otrębusy
Sand %	62.35	80.60
Silt %	34.66	17.73
Clay %	2.99	1.66
Texture [1*]	sandy loam	loamy sand
$N\text{-}NH_4$ (mg kg^{-1}) [2*]	0.75	0.28
$N\text{-}NO_2$ (mg kg^{-1}) [2*]	0.03	0.09
$N\text{-}NO_3$ (mg kg^{-1}) [2*]	66.70	108.45
Total nitrogen (TN %) [3*]	0.10	0.18
Soil organic carbon % [3*]	0.81	2.07
pH in H_2O [4*]	5.82	6.39
Available phosphorus (mg P_2O_5 100 g^{-1}) [5*]	17.90	18.00
Available potasium (mg K_2O 100 g^{-1}) [6*]	26.10	27.90
Exhangeable Ca (cmol kg^{-1}) [7*]	2.02	5.49
Exhangeable Mg (cmol kg^{-1}) [7*]	0.44	1.14
Exhangeable K (cmol kg^{-1}) [7*]	0.49	0.44
Exhangeable Na (cmol kg^{-1}) [7*]	0.13	0.13

[1*] According to the United States Department of Agriculture (USDA) soil texture classification using a Mastersizer 2000 laser diffraction particle size analyzer (Malvern Instruments, Worcestershire, UK); [2*] by flow spectrometry after extraction with 1 M K_2SO_4 using a QuAAtro39 analyzer (Seal Analytical, Norderstedt, Germany); [3*] by combustion with a vario Macro 8 cube CN analyzer (Elementar, Langenselbold, Germany); [4*] by the potentiometric method, with 10 g soil in 40 mL solution, using an AAnalyst 800 atomic absorption spectrometer (AAS) (Perkin Elmer, Waltham, MA, USA); [5*] by the Egner-Riehm colourimetric method, using extraction with 0.02 M calcium lactate in 0.01 M HCl, followed by colourimetric measurement in a Lambda 45 Spectrometer (Perkin Elmer, Waltham, MA, USA), based on the reaction with ammonium molybdate [46]; [6*] by the Egner-Riehm method [47], with K measurement using an AAnalyst 800 AAS; [7*] using an AAnalyst 800 atomic absorption spectrometer (AAS) (Perkin Elmer, Waltham, MA, USA) [48]; Abbreviations: $N\text{-}NH_4$—nitrogen as ammonium compounds; $N\text{-}NO_2$—nitrogen as nitrites; $N\text{-}NO_3$—nitrogen as nitrates.

2.2. Determination of Soil Microbial Biomass and Enzyme Activity

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined by the chloroform fumigation–extraction method (PN ISO 14240-2. 2001) [49]. Soil samples were fumigated with ethanol-free chloroform for 24 h, with non-fumigated samples being retained. MBC and MBN were then extracted from samples with 0.5 mol L^{-1} K_2SO_4, stirring at 180 rpm for 30 min. The samples were analysed using a C/N analyser (Analytik Jena AG, Jena, Germany). The MBC was calculated by the difference between carbon (C) in the fumigated and non-fumigated samples, with a correction factor of 0.45 [50]. Similarly, MBN was analysed, but with a factor of 0.54 [51]. The amount of microbial biomass C was calculated as:

$$\text{Microbial biomass C} = (Fc - NFc)/kEC \quad (1)$$

where: Fc—microbial carbon extracted from fumigated soil; NFc—microbial carbon extracted from non-fumigated soil; kEC—extraction efficiency (0.45).

The activities of acid (AcP) and alkaline phosphatase (AlP) were analysed using 1 g of soil incubated for 1 h (37 °C) with p-nitrophenyl phosphate at their optimum pHs of 6.5 and 11, respectively, using the spectrophotometric method described by Tabatabai and Bremner [52]. Soil dehydrogenases activity (DHA) was determined in 6 g soil (natural moisture) by colourimetric measurement of the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) solution to triphenylformazan (TPF) after incubation at 37 °C for 24 h, according to the method of Casida et al. [53]. The enzyme activity was measured spectrophotometrically at 485 nm. Then, all microbial analyses were carried out in three replicates.

2.3. Bacterial Community-Level Physiological Profiling (CLPP)

Metabolic profiling (phenotypic) of all soil samples was carried out using the Biolog EcoPlates containing 31 different carbon sources divided into five compound groups: amines and amides, amino acids, carboxylic acids, carbohydrates, and polymers—plus a blank well as a control (Biolog System Inc., Hayward, CA, USA) [30]. For the EcoPlates, 1 g soil was suspended in 99 mL sterile water, shaken for 20 min at 20 °C, and incubated at 4 °C for 30 min [54]. The soil extracts were then passed through 60 µm cellulose filters to reduce interference during optical density (OD) measurements. Next, each well of the Biolog EcoPlates was inoculated with 120 µL of the prepared suspension and was incubated at 25 °C. Absorbance at 590 nm was measured on a Biolog Microstation after 24, 48, 72, 96, and 120 h of incubation. The analysis was carried out in three replicates [29].

2.4. Statistical Analysis

Statistical analyses were performed using Statistica 13.1 software (StatSoft, Inc., Tulsa, OK, USA). Data were analysed by three-way analysis of variance (ANOVA) for the comparison of means. Post-hoc analysis used Tukey's honestly significant difference (HSD) test at a significance level of $p < 0.05$. The functional diversity of microbial communities was evaluated by the Shannon diversity index (H'), Simpson diversity index (1-D), Shannon evenness index (E), and average well-colour development (AWCD), which was calculated as described by Garland and Mills [35]. The variables included in the analysis are the main effects: Soil, Date, and Distance, as well as interactions effects: Soil*Date, Soil*Distance, Date*Distance, and Soil*Date*Distance. In addition, multivariate statistical assessment used principal component analysis (PCA) to summarize the variability of the samples, and to determine the association among the measured properties for the two different soils, sandy loam and loamy sand. Correlations between the properties of each soil texture were tested by Spearman's rank correlation coefficient, since most of the data were non-parametric. Cluster analysis of the soil samples, using Ward's method with bond distance, was conducted based on all the measured microbiological soil parameters. For Spearman's rank correlation, PCA, and heatmap analysis, all result values were standardised so that each score contributed equally to the analysis.

3. Results

3.1. The Soil Microbial Biomass and Enzyme Activity

In this current study, MBC showed similar patterns in both the sandy loam and loamy sand soils, with sampling dates variability at the two distances (Figure 2a). Soil MBC decreased with an increasing distance from the nearest tree, independently of when the soil was sampled. Overall, a higher MBC was recorded in the loamy sand than the sandy loam soil, both relative to the date and distance of sampling. MBC of samples from loamy sand soil showed significant differences among samples at the two distances, 0.1 m and 1 m, in both June and October ($p < 0.05$). Samples from sandy loam soil also exhibited significant differences in MBC in the October for samples taken at the distances 0.1 m and 1 m; however, distance of sampling in the June had no significant impact on MBC. MBN showed a similar trend to MBC (Figure 2b). The highest N assimilation was recorded in

loamy sand, specifically in the October. Generally, MBN concentrations of all samples were significantly different in loamy sand ($p < 0.05$). No significant differences were observed for MBN in samples from sandy loam soils in the June ($p > 0.05$; Figure 2b).

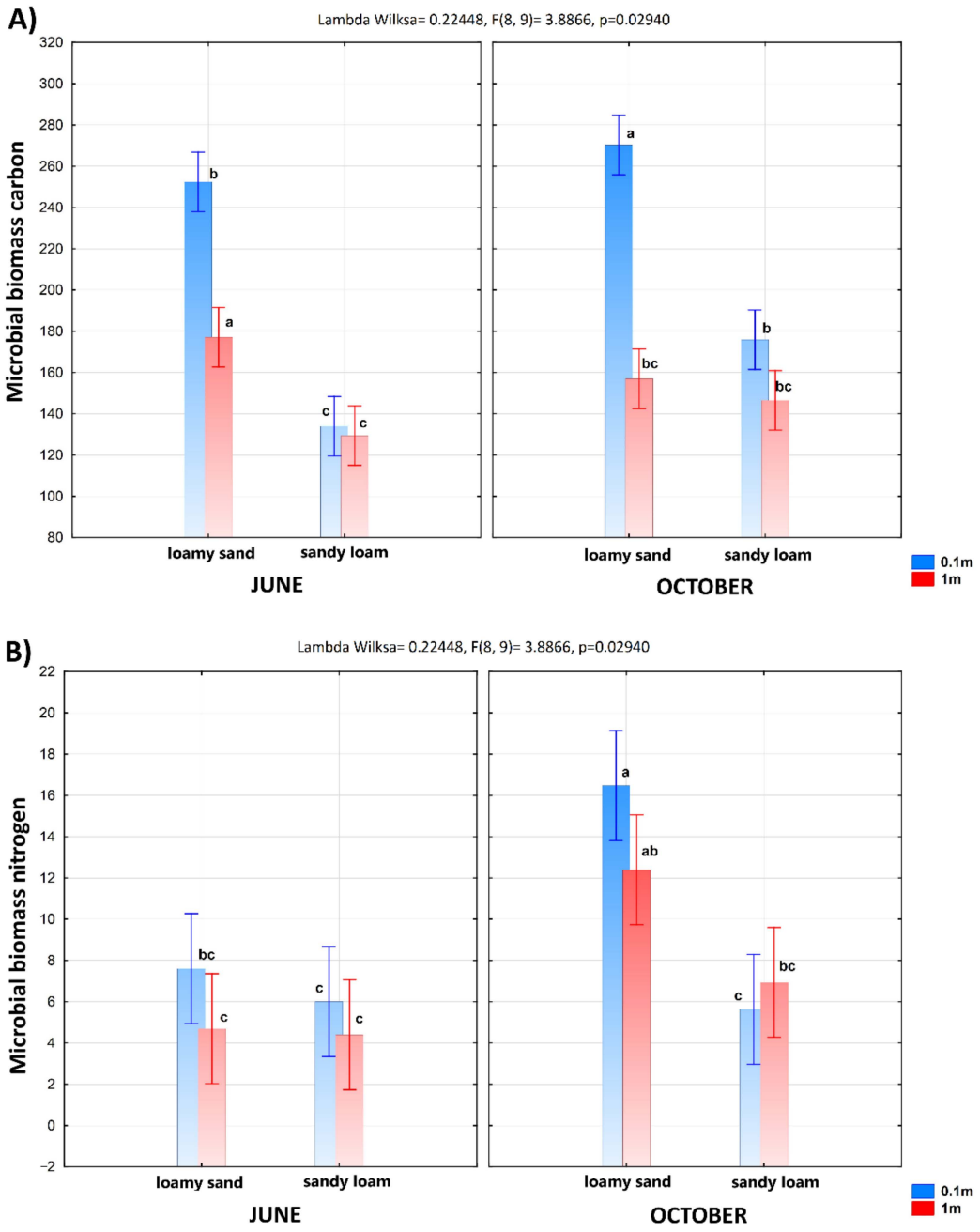

Figure 2. (**a**) Microbial biomass carbon (MBC; μg C g^{-1} of soil) and (**b**) microbial biomass nitrogen (MBN; μg N g^{-1} of soil) three-way analysis of variance (ANOVA). Treatment means separated by different letters are significantly different (Tukey's mean separation test $p < 0.05$; $n = 3$; biological replication).

DHA was found to be higher in soil near trees, similar to the values of microbial biomass. Moreover, it changed with sampling time at all sites and was lower in June, indicating a sampling dates differentiation. The trend toward higher biological activity in loamy sand soil for these parameters was also recorded. Three-way ANOVA showed that the DHA in loamy sand and sandy loam soils was significantly affected by the date of sampling and distance from trees (Figure 3).

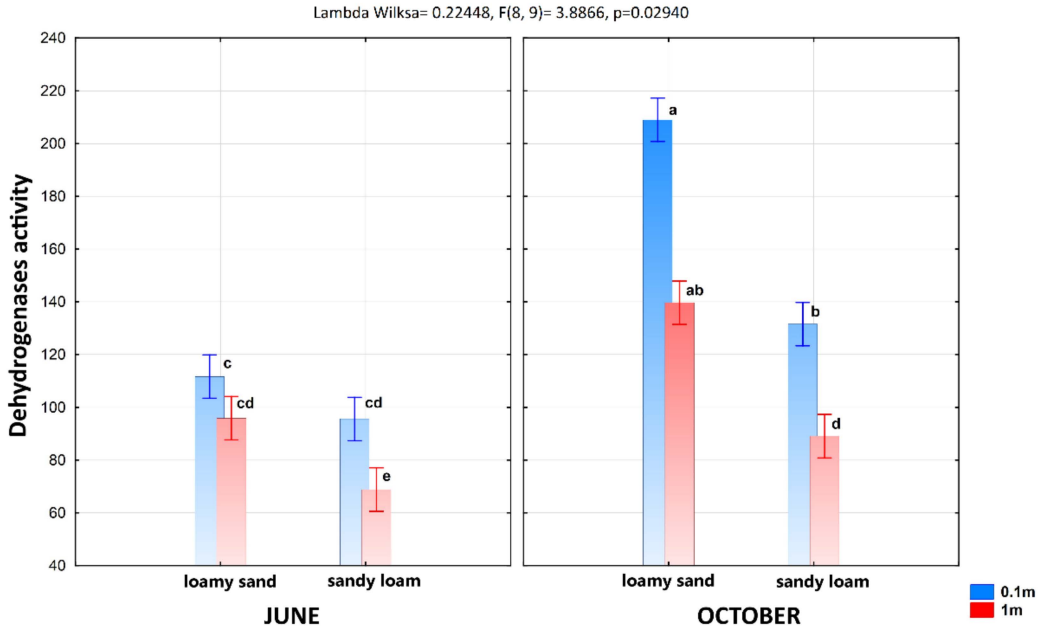

Figure 3. Dehydrogenases activity (DHA; μg TPF g^{-1} dry matter of soil 24 h^{-1}) three-way analysis of variance (ANOVA). Treatment means separated by different letters are significantly different (Tukey's mean separation test $p < 0.05$; $n = 3$; biological replication).

AcP activity in loamy sand was significantly higher at a distance of 0.1 m than at 1 m for both of the sampling dates. In addition, significant differences in AcP activity were observed between these same distances over two dates, with higher values in October (Figure 4a). Such relations of AcP activity were presented only for the June sampling in the case of sandy loam soil. Sampling distance significantly affected AcP activity in the June, but not in the October (Figure 4a). The tendency of AlP activity was not similar to the other enzymes (DHA and AcP), as well as microbial biomass C and N. In the current study, AlP activity was lower than AcP activity. AlP activity only showed a relationship with research sites, having higher activity in loamy sand soil (Figure 4b). In the loamy sand samples in the June, significantly lower AlP activity was observed at a distance of 1 m from the tree, while, in the October, there were significantly lower values of AlP activity at 0.1 m from the tree than at a distance of 1 m. By contrast, all samples from sandy loam soil represented one homogenous group. No significant differences in AlP activity were observed among all the samples in relation to distance and season ($p > 0.05$; Figure 4b).

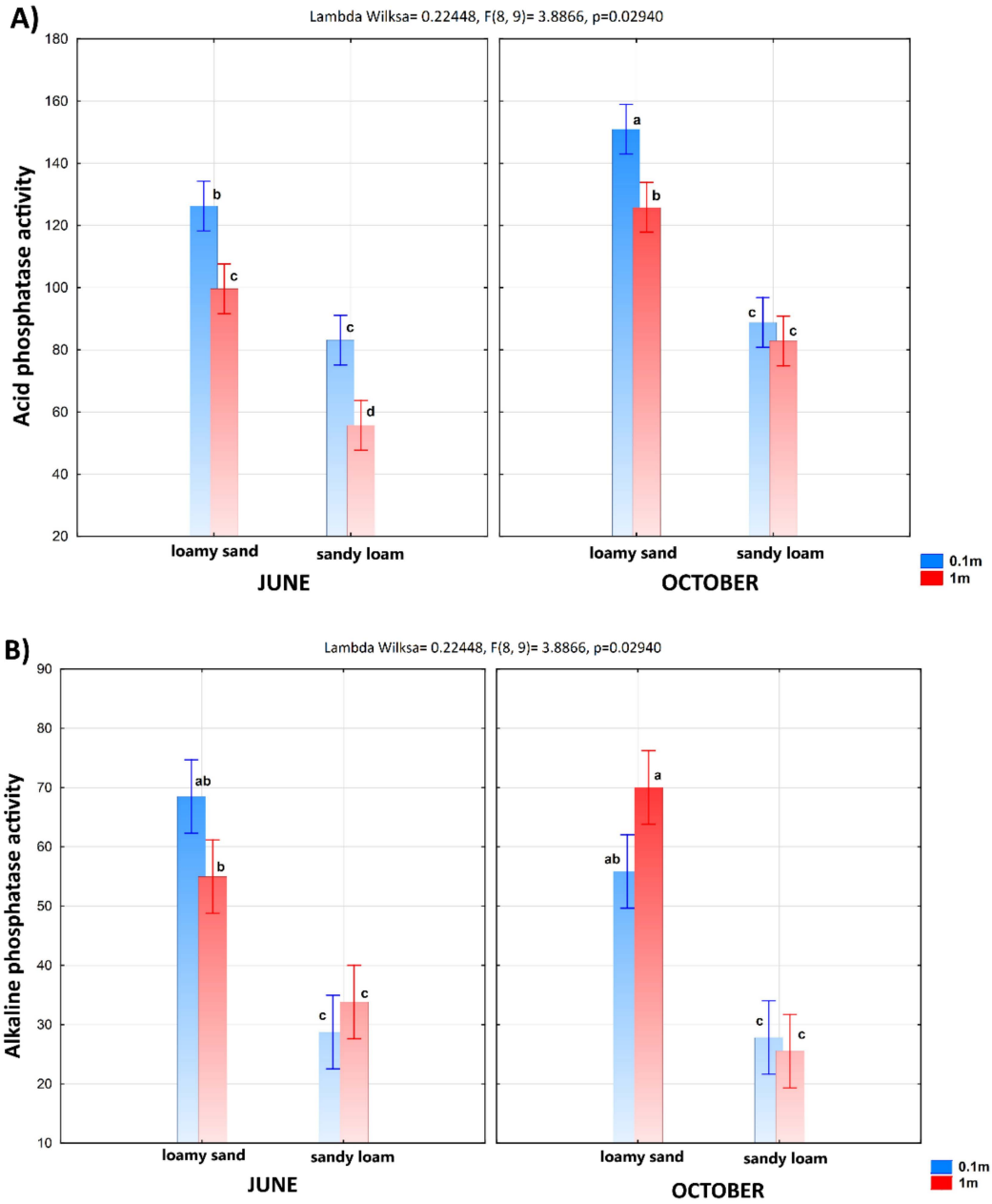

Figure 4. (**a**) Acid phosphatase activity (AcP) and (**b**) alkaline phosphatase activity (AlP; µg p-nitrophenol g^{-1} h^{-1}) three-way analysis of variance (ANOVA). Treatment means separated by different letters are significantly different (Tukey's mean separation test $p < 0.05$; $n = 3$; biological replication).

3.2. Bacterial Community-Level Physiological Profiling (CLPP)

Based on the utilisation patterns of 31 different carbon sources, microbial functional diversity in the soil of *P. elongata* × *P. fortunei* plantations in relation to soil texture, date, and distance of sampling was evaluated. The time selected for analysis using Biolog EcoPlates was 120 h of incubation. This was the optimal time to analyse the samples, since the AWCD achieved maximum values. Microbial communities from loamy sand soil collected in October at distance 0.1 m ($AWCD_{590nm}$ 2.231) ($AWCD_{590nm}$ is an indicator of general microbial activity and was assessed as the average optical density at 590 nm of all wells per plate) had a stronger metabolic activity in utilising carbon substrates than sandy loam. In samples from sandy loam soil, the highest utilisation of all carbon sources was shown by the soil sample collected in October at distance 0.1 m ($AWCD_{590nm}$ 2.017). For

comparison, H' values were 3.29–3.33 in samples of sandy loam and 3.27–3.36 in loamy sand, with higher values in October than in June. The range for all functional diversity indices between the samples was rather small. The highest values of H' for loamy sand occurred in the soil sample collected in October at distance 0.1 m, and for sandy loam in the soil sample collected in October at distance 0.1 m, indicating that the microorganisms exhibited a high metabolic potential and a high metabolic biodiversity in these samples. By contrast, soil samples collected in June at a distance 1 m in two types of soils had the lowest microbial diversity in relation to both indices. Notably, 1-D slightly decreased together with the increasing distance from the nearest tree, independent of soil texture and date of sampling (Table 3). Three-way ANOVA was performed for testing the interaction between the three independent variables, soil, date of sampling and distance of sampling. The dependent variables were parameters of the biodiversity indices AWCD, H', E, and 1-D. Table 4 summarises the three-way ANOVA. The biodiversity indices measured from the Biolog EcoPlates revealed differences between soil texture, date, and distances of sampling. In the loamy sand soil, higher biodiversity indices were observed than in the sandy loam soil. Moreover, statistically lower values were recorded for these indices at a distance of 1 m from the nearest tree in both types of soils. In October, higher values of the AWCD and H' indices were noted in all tested samples. There were no significant differences between the values of H' in the samples collected in October, either between distances 0.1 m and 1 m or between loamy sand and sandy loam soils. Soil texture and distance of sampling significantly affected AWCD, H', and 1-D. Moreover, a greatly significant impact of the variable date of sampling on AWCD, H' and E was observed ($p < 0.01$). The effect of interactions between the variables soil and date was significant only for the 1-D index, while soil and distance of sampling was significant for AWCD and E. Interactions among the three independent variables were significant for parameters E and 1-D (Table 4).

Table 3. Biodiversity indices of soil based on substrate utilisation patterns for Biolog EcoPlates containing 31 different carbon sources at 120 h. Three-way analysis of variance (ANOVA) was performed. Treatment means separated by different letters are significantly different (Tukey's mean separation test $p < 0.05$; $n = 3$; biological replication).

Soil	Date	Distance	Biodiversity Indices			
			AWCD	*H'*	E	1-D
sandy loam	June	0.1 m	1.75 ^{cd}	3.29 ^{bc}	0.97 ^{ab}	0.98 ^{bc}
		1 m	1.48 ^{e}	3.27 ^{c}	0.98 ^{ab}	0.98 ^{a}
sandy loam	October	0.1 m	2.01 ^{b}	3.33 ^{abc}	0.98 ^{ab}	0.98 ^{bc}
		1 m	1.79 ^{cd}	3.33 ^{abc}	0.98 ^{a}	0.98 ^{b}
loamy sand	June	0.1 m	2.14 ^{ab}	3.35 ^{ab}	0.98 ^{a}	0.98 ^{c}
		1 m	1.66 ^{de}	3.27 ^{c}	0.96 ^{b}	0.98 ^{bc}
loamy sand	October	0.1 m	2.23 ^{a}	3.36 ^{a}	0.98 ^{a}	0.98 ^{c}
		1 m	1.94 ^{bc}	3.36 ^{a}	0.99 ^{a}	0.98 ^{ab}

AWCD—average well colour development; *H'*—Shannon diversity index; E—Shannon evenness index; 1-D—Simpson diversity index.

Metabolic profiles of the microbial populations of each soil sample based on the utilisation of a range of carbon sources were compared (Figure 5). Scoring in this analysis was derived from differences in consumption of the same carbon sources by individual microbial communities. Carbon substrate utilisation differed between soil textures. Overall, carbon substrates were also more intensively utilised in the October than in the June. For loamy sand soil—the sample collected in October at distance 0.1 m—the most intensively used substrates were Phenylethylamine, N-Acetyl-D-Glucosamine, Tween 40, and D-Glucosaminic Acid. By contrast, the lowest utilised carbon sources were beta-Methyl-D-Glucoside and D-Galactonic Acid gamma-Lactone. For sandy loam soil—sample collected in October at distance 0.1 m—the highest utilised substrates were Tween 80, D-Cellobiose,

D-Galactonic Acid-gamma-Lactone, and Glycogen, while the lowest utilised substrates were i-Erythritol, alfa-Keto-Butyric Acid, Phenylethylamine, and L-Serine.

Table 4. Summary of three-way analysis of variance (ANOVA) results testing the effects of soil (loamy sand and sandy loam), date of sampling (June and October), and distance of sampling (0.1 m and 1 m) on basal biodiversity indices. Data shown represent *F*-value and significance levels for each factor and interaction.

	Variables	AWCD		*H′*		E		1-D	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Main effects	Soil	62.2	0.00	8	0.01	0.2	0.69	15	0.00
	Date	62.4	0.00	25.1	0.00	13.7	0.00	1	0.25
	Distance	110.9	0.00	6.4	0.02	0.6	0.46	56	0.00
Interaction effects	Soil * Date	3.4	0.08	0.30	0.59	2.1	0.16	10	0.01
	Soil * Distance	5.3	0.03	3.9	0.07	11.7	0.00	4	0.08
	Date * Distance	4.1	0.06	6.9	0.02	4.5	0.05	0	0.98
	Soil * Date * Distance	1.3	0.27	4.1	0.06	4.8	0.04	8	0.01

AWCD—average well colour development; *H′*—Shannon diversity index; E—Shannon evenness index; 1-D—Simpson diversity index.

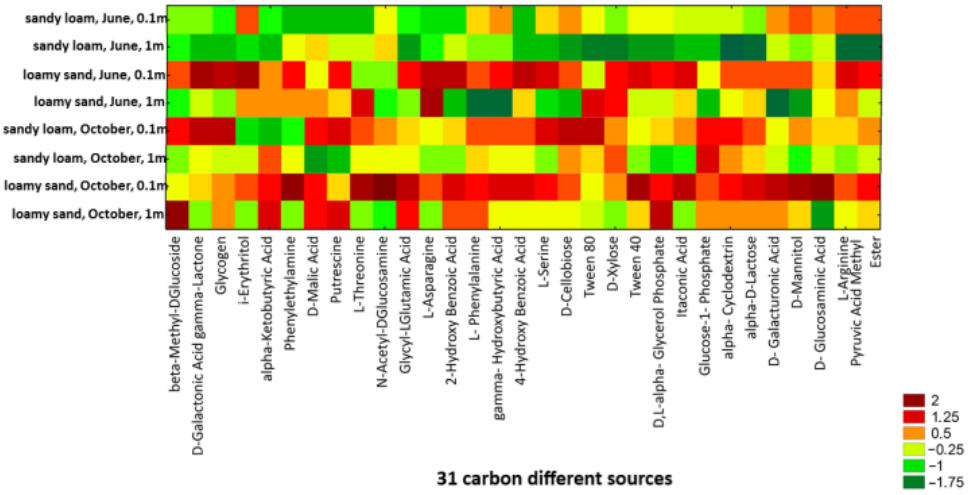

Figure 5. Heatmap based on the analysis of 31 carbon sources from Biolog EcoPlates. The results illustrate the difference in microbial communities in each sample based on the utilisation of the same substrate. The scale indicates that the highest utilisation of substrate has been marked by the red colour, while the lowest utilisation has been represented by the green. Moreover, the values on the scale (from −1.75 to 2) are the standardised OD values at 590 nm. The data for this analysis were standardised. The highlighted samples exhibit the highest activity in the metabolising substrates found in the Biolog EcoPlates in each texture soil (n = 3 biological replication).

The bacterial communities from the *P. elongata* × *P. fortunei* plantations used all 31 different carbon sources on the Biolog EcoPlates. To investigate the utilisation of these substrates, they were categorised in five classes: amines and amides, amino acids, carboxylic acids, carbohydrates, and polymers. Figure 6 shows the total percentage of each carbon group utilised by each sample. Generally, regardless of the soil texture, carbohydrates were the best utilised carbon substrates, while polymers, amines, and amides showed the lowest utilisation level (Figure 6). The utilisation patterns of carbohydrates, amines and amides, and amino acids did not differ significantly between the tested soil samples (Figure 6). Among the polymers, Tween 40 was the best utilised by bacteria in all samples, while among the amines and amides, phenylethylamine was the most used (Figure 6). In the June, for the two soil textures, significant differences in the utilisation of carboxylic acids were observed by microbial communities. Microorganisms from sandy loam soil at a distance of

1 m showed a distinctly higher percent utilization of carboxylic acids than those collected at a distance of 0.1 m in the same soil. By contrast, there was more utilisation of this group of carbon sources at 0.1 m than at 1 m in loamy sand. Significant differences were also noted in the use of polymers by microorganisms from sandy loam samples collected in the summer.

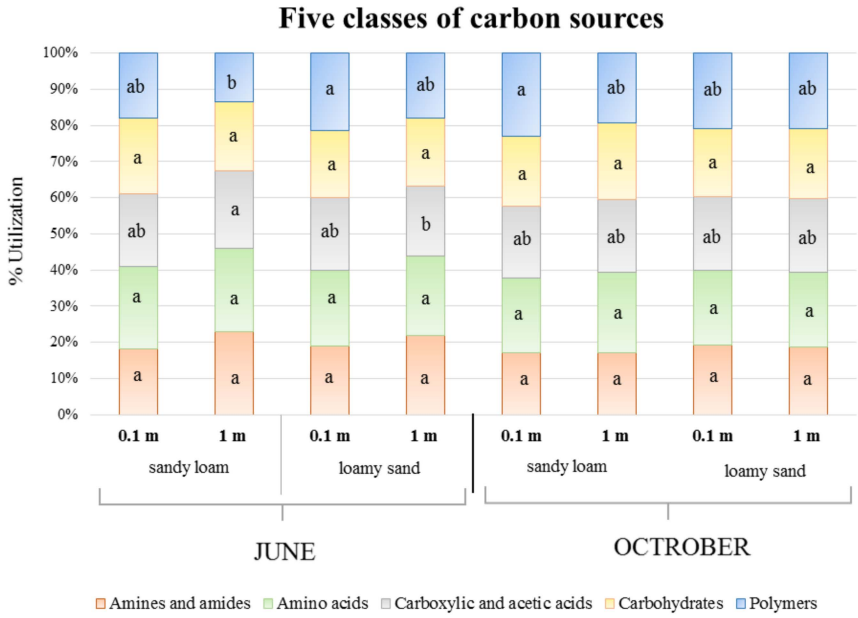

Figure 6. Utilisation of the all five categorised guilds of carbon substrates by microbial communities from two soil textures. Three-way analysis of variance (ANOVA) was used. Treatment means separated by different letters are significantly different (Tukey's mean separation test $p < 0.05$; $n = 3$; biological replication).

3.3. Statistical Analysis

3.3.1. Hierarchical Cluster Analysis

The results of cluster analysis of all samples from sandy loam and loamy sand soils are shown in Figure 7. The analysis was performed using all measured microbiological properties of the soils. A clear separation of samples representing loamy sand soil from those of sandy loam soil was observed. The biological activity of the soil allowed the distinction of two groups (zones), almost coinciding with the soil texture. The first group included all samples from sandy loam soil and one sample from loamy sand soil collected in the June at distance 1 m, while the second group included the other samples from loamy sand soil. The classification of the loamy sand soil sample collected in the June at distance 1 m to the first cluster of soil samples was likely caused by low values for its microbiological activity.

3.3.2. Principal Component Analysis

The correlation among all the samples and soil microbial activity for the two different soils were assessed by PCA. To reduce the dimensionality of the data set, PCA was used to compare the relationships among enzymatic activity, MBC, MBN, *H'*, and AWCD. The values from the PCA indicated that the first two principal components (PCs) accounted for 62.42% and 17.85% of the total variance of samples from sandy loam and loamy sand soils. The PCA plot of the first two PCs showed that all samples differed relative to distance and soil texture (Figure 8). All microbiological properties were positively related to samples of loamy sand collected in the October. By contrast, samples of sandy loam collected in the June were negatively related to dehydrogenases and phosphatase activities, AWCD, MBC, and MBN. The superimposed circles in Figure 8 illustrate that the samples typically clustered based on soil texture and date of sampling.

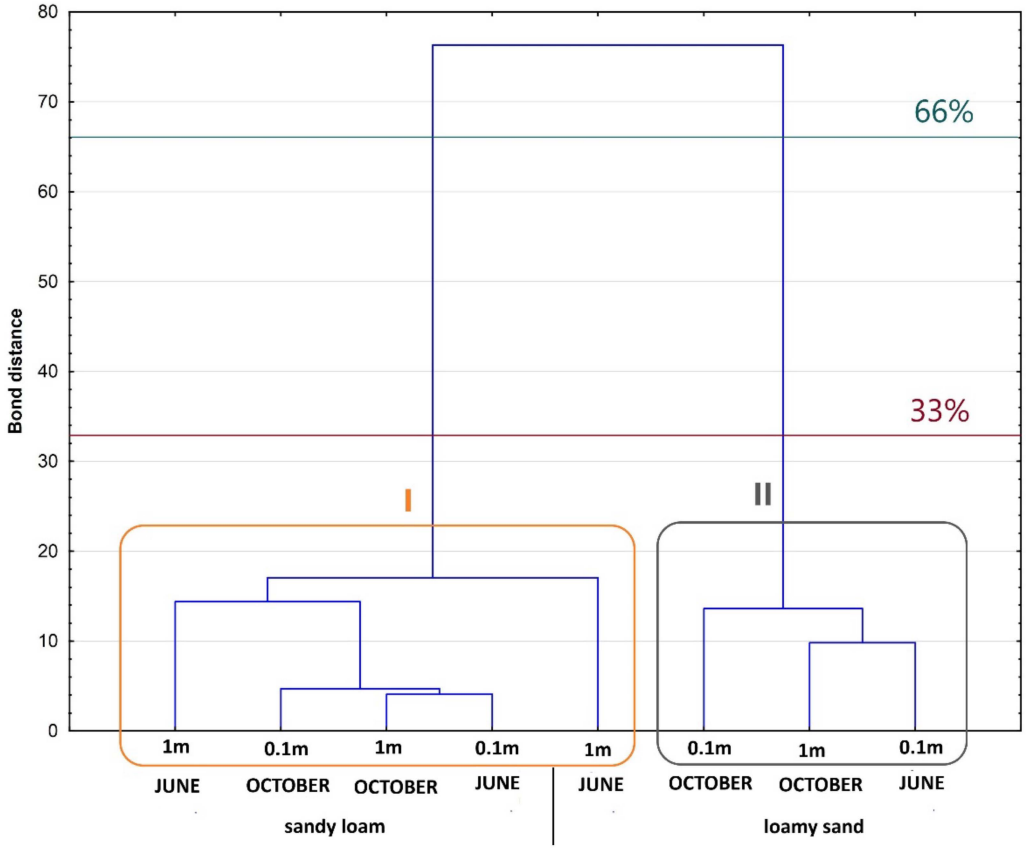

Figure 7. Hierarchical dendrogram of samples from two soil textures, loamy sand and sandy loam, using Ward's clustering method with bond distances. Boundaries of Sneath's criteria, restrictive (33%) and less restrictive (66%), are marked on the dendrogram.

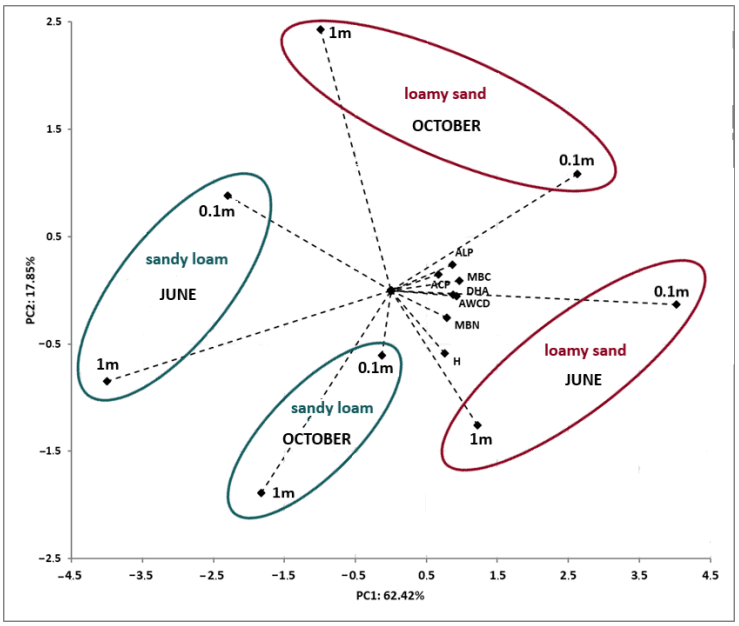

Figure 8. Bi-plot generated from principal component analysis (PCA) of microbial parameters, such as microbial biomass C and N, phosphatase and dehydrogenases activities, Shannon index (*H'*), and the average well-colour development (AWCD) of loamy sand and sandy loam soils. Mean values from three replicates were used for PCA analysis. The data for this analysis were standardised.

3.3.3. Pearson's Correlation Analysis

The data obtained by PCA were extended by correlation analysis (Tables 5 and 6), in which all results obtained for the two soils in the June and October were included. Spearman's rank correlation coefficient was used to test the correlations between the microbial activity and functional diversity of the soils. Spearman's correlation matrix demonstrated that a large number of soil variables were significantly correlated with each other, both in sandy loam and loamy sand soil (Tables 5 and 6). In sandy loam soil, diverse correlations were found between soil variables, with values of 1-D having a significantly negative correlation with soil pH, as well as DHA and AcP activity. A strong positive correlation was observed between DHA and pH, as well as AWCD. This enzyme also showed a positive correlation with AcP activity, MBC, *H*′, and utilisation of polymers (Table 5). In loamy sand, a significantly negative correlation was observed between 1-D and soil pH, as well as MBC. DHA was positively and significantly correlated with AcP activity, MBN, AWCD, *H*′, and E. In addition, AcP activity, MBC, MBN, and soil pH were positively correlated with AWCD, though in some cases, the coefficients were not very high (Table 6).

Table 5. Matrix correlation (correlation coefficient values) between the different variables of sandy loam.

Parameters	DHA	AlP	AcP	MBC	MBN	pH	AWCD	*H*′	E	1-D	AandA	A	CandAA	C
DHA														
AlP	−0.36													
AcP	**0.79** *	−0.38												
MBC	**0.64** *	−0.01	0.49											
MBN	0.06	−0.39	0.17	−0.07										
pH	**0.88** *	−0.35	**0.68** *	**0.65** *	0.14									
AWCD	**0.87** *	−0.44	**0.75** *	**0.78** *	0.22	**0.86** *								
H′	**0.64** *	−0.53	**0.64** *	**0.71** *	0.18	0.49	**0.80** *							
E	−0.19	−0.20	−0.04	0.17	−0.33	−0.35	−0.01	0.46						
1-D	**−0.74** *	0.34	**−0.67** *	−0.42	−0.43	**−0.87** *	**−0.71** *	−0.25	**0.622** *					
AandA	−0.36	**0.66** *	−0.28	−0.28	0.04	−0.45	−0.30	−0.36	−0.03	0.30				
A	−0.37	0.03	−0.02	−0.50	−0.24	−0.45	−0.54	−0.41	0.09	0.24	0.00			
CandAA	−0.22	0.01	−0.30	**−0.62** *	−0.16	−0.27	−0.49	−0.38	−0.04	0.32	−0.10	0.14		
C	−0.01	−0.51	−0.11	−0.19	0.13	0.02	−0.20	−0.15	−0.22	−0.11	**−0.73** *	0.25	0.22	
P	**0.76** *	−0.31	**0.64** *	**0.64** *	−0.15	**0.81** *	**0.70** *	0.54	−0.06	−0.57	**−0.68** *	−0.24	0.00	0.10

Significant correlations ($p < 0.05$) are marked with an asterisk and are in bold. The data for this analysis were standardised. DHA—dehydrogenases activity, AlP—alkaline phosphatase activity, AcP—acid phosphatase activity, MBC—microbial biomass C, MBN—microbial biomass N, AWCD—average well colour development, *H*′—Shannon diversity index, E—Shannon evenness index, 1-D—Simpson's diversity index, AandA—amines and amides, A—amino acids, CandAA—carboxylic acids, C—carbohydrates, and P—polymers.

Table 6. Matrix correlation (correlation coefficient values) between the different variables of loamy sand.

Parameters	DHA	AlP	AcP	MBC	MBN	pH	AWCD	*H*′	E	1− D	AandA	A	CandAA	C
DHA														
AlP	0.09													
AcP	**0.87** *	0.04												
MBC	0.36	−0.25	**0.61** *											
MBN	**0.87** *	0.07	**0.70** *	0.21										
pH	0.19	−0.18	0.53	**0.81** *	0.06									
AWCD	**0.78** *	0.10	**0.87** *	**0.71** *	**0.65** *	**0.69** *								
H′	**0.80** *	0.45	0.56	0.03	**0.73** *	−0.10	0.55							
E	**0.80** *	0.45	0.56	0.03	**0.73** *	−0.10	0.55	1.00						
1−D	−0.17	0.39	−0.35	**−0.68** *	0.04	**−0.71** *	−0.57	0.22	0.22					
AandA	−0.53	−0.45	−0.57	−0.32	−0.54	−0.23	−0.52	−0.57	−0.57	−0.08				
A	−0.39	−0.38	−0.27	0.00	−0.33	−0.19	−0.46	−0.36	−0.36	0.11	0.11			
CandAA	0.30	−0.44	0.25	−0.05	0.43	0.10	0.22	0.08	0.08	−0.29	−0.13	0.15		
C	−0.12	0.01	−0.20	−0.13	0.06	−0.48	−0.23	−0.06	−0.06	0.09	−0.11	0.52	0.15	
P	0.12	0.57	0.15	0.17	0.11	0.15	0.25	0.25	0.25	0.20	−0.53	**−0.59** *	−0.56	−0.20

Significant correlations ($p < 0.05$) are marked with an asterisk and are in bold. The data for this analysis were standardised. DHA—dehydrogenases activity, AlP—alkaline phosphatase activity, AcP—acid phosphatase activity, MBC—microbial biomass C, MBN—microbial biomass N, AWCD—average well colour development, *H*′—Shannon diversity index, E—Shannon evenness index, 1-D—Simpson's diversity index, AandA—amines and amides, A—amino acids, CandAA—carboxylic acids, C—carbohydrates, and P—polymers.

4. Discussion

Abandoned and marginal lands are considered a promising strategy for producing biomass (Short rotation plantations) and avoiding conflicts with food production. Abandoned land is a land lower in agricultural value and mostly less profitable for agricultural production. Thus, fast growing plants (including *Paulownia* sp.) on unused plots constitute an economically interesting option for farmers. In Eastern Europe, land abandonment is common process [55,56]. Therefore, this study focused on the region in central Poland (a country facing worrying abandoned land trends), which, due to the occurrence of *Paulownia* plantations (bioenergy crops), was appropriate for our research [10].

Land use change can transform key soil properties, influencing the capacity of soil to maintain ecological functions. Providing an evidence base of the impacts of *Paulownia* sp. on microbiological activity of soils is an important element in understanding the sustainability of these bioenergy crops and their potential for reclaiming abandoned land.

The microbiological properties of soil in SRF are important, particularly in the context of soil biodiversity and protection against the loss of its quality. SRF is expected to have a positive effect on biodiversity [57,58], as well as on soil [59]. Microorganisms are the major factors of biological and biochemical processes in the soil. They are much more sensitive than physiochemical factors to changes in the soil ecosystem, and therefore, they are excellent indicators of agricultural sustainability [60]. Porta et al. [41] found little variation in physicochemical properties because the parental rock material, climatic conditions and geomorphology were very similar in the studied area. Therefore, in this study, we analysed several microbiological and biochemical properties. An evaluation of soil microbiological properties under *P. elongata* × *P. fortunei* trees was important to understand the role of microbes and its biodiversity in soil.

The soils of *P. P. elongata* × *P. fortunei* plantations from Podkampinos and Otrębusy differed in their chemical properties and textures. The soil from Podkampinos was a sandy loam, whereas that from Otrębusy was loamy sand. The selected plots are characterised by favourable conditions for plant growth. However, for economic and social reasons, these plots were abandoned. On these soils of relatively high fertility, the *Paulownia* tree may produce more green biomass.

Acosta-Martınez et al. [61] reported that soil physicochemical properties, such as pH, organic matter, and texture, could influence soil enzyme activities, which provides information on biological activity and the maintenance of soil fertility. The overall microbial properties were closely related to the soil physiochemical properties, which showed better values in loamy sand soil. These changes could be attributed to the content of nutrient elements. The loamy sand soil had higher values of parameters such as nitrogen as nitrates ($N\text{-}NO_3$), TON, TOC, exchangeable Ca, and the level of humus than soil from Podkampinos. However, it had lower values for nitrogen as ammonium compounds ($N\text{-}NH_4$) and exchangeable K (Table 2). Das et al. [62] showed that the amount of soil microorganisms exhibited positive correlation with the available K^+, as well as the exchangeable Ca^{2+} and Mg^{2+} of soil, which is confirmed by our research. The better microbiological activity, assessed by various methods, was shown in the loamy sand texture soil with a higher content of exchangeable Ca^{2+} and Mg^{2+}. Consistent to our finding, Liu et al. [63] found that the soil microbes in agroforestry systems are shaped by soil physiochemical properties. Pang's [64] findings suggest that soil microbial functional diversity, evaluated on Biolog EcoPlates, is governed by soil physicochemical properties in subtropical forests.

Moreover, among soil attributes, pH is considered the main variable of soil. It influences microorganisms, thereby determining plant growth and biomass yield [65]. The values of the soil pH recorded for both soil textures (sandy loam and loamy sand) were slightly acidic. Hinsinger et al. [66] and Hagen-Thorn et al. [67] showed that the roots of trees acidified the soil through the release of acidic compounds and microbial respiration. Moreover, Khan et al. [68] found that several physicochemical properties of the soil in plantations of *Robinia pseudoacacia*, e.g., pH, were modified through the decomposition of leaves. The slightly lower pH was noted for the sandy loam soil, which may be reflected in

the different microbial activity. Xu et al. [69] emphasised that pH and soil texture exerted a significant impact on microbial community structure. Similarly, Rousk et al. [70] investigated the influence of pH on the microorganisms in soil, and this study showed that higher values of pH favoured microorganism growth.

It has been reported that growth of tree species affects the physicochemical and biological properties of soils [25–27,71]. Thus, the structure, diversity, and metabolic activity of soil microorganisms are dependent on the tree species [72]. In general, a relationship between parameters (MBC, MBN, DHA, AcP activities, and the metabolic diversity of microorganisms) was observed. Soil enzyme activities and parameters used to describe microbial functional diversity were generally higher at the tree row than at the middle of the alley. The increased activity abundance of soil microorganisms in the nearest tree of the agroforestry systems corroborated previous findings of trends of the amount of bacteria in the Canadian agroforestry systems [73]. Similarly, Mungai et al. [74] studies indicated that microbiological activity expressed as AWCD, substrate richness, and the Shannon diversity index are positively linked with distance from the analysed plants, whereas these parameters are higher in the tree row. The observed trend may indicate the existence of metabolically and functionally different microorganisms at the two distances of 0.1 m and 1 m. These changes could be attributed to reserves of nutrients in the soil that come from leaves incorporated into the soil. In close proximity to the tree stem, there is more leaf residue and root secretions, and hence, there is higher microbiological activity. The higher biological activities in the soil near the tree can be due to an increased supply of carbon and nutrients from litter, dead root cells, and rhizo- depositions [75,76]. Yadav et al. [76] have also recorded higher dehydrogenase activity under trees. Similarly, Wan and Chen [77] observed higher microbial activities under trees, probably resulting from the increased content of carbon, nutrients, leaf residues, and root secretions in the soil. Decomposition processes of leaf residue and root secretions are the result of the most active microorganisms, whose main goal is to obtain nutrients. The high content of organic compounds in the substrate contributes to a significant increase in the number of microorganisms and changes in their structure, thus improving the soil microbial activity [78–80]. Furthermore, these microorganisms can have a beneficial effects on plant productivity and also agroecosystem stability [80].

In the current study, much higher microbiological activity and metabolic diversity were observed in the October (autumn period) in both soil textures, which could be associated with the large amount of abscised leaves and the climatic conditions, such as high humidity. Notably, leaves of *Paulownia* spp. contain many nutrients, such as nitrogen, phosphorus, and potassium, and, in China, they are used as a green fertilizer [77,81]. Wang and Shogren [82] confirmed that leaves of *Paulownia* spp. could be a valuable source of organic matter and nutrients. In addition, they emphasised the potential of autumn leaves. Plant phenology and photosynthetic activities affect seasonal dynamics of microorganisms because plants influence C and N availability for soil microorganisms as a result of the exudation of labile C through the roots and the substrate input by litter fall [83]. Plant root exudates provide labile carbon in summer, while winter microorganisms use dead plant material [84–86] and microbial cellular products [87]. At the end of summer, when the plants join the aging phase, microorganisms have access to easily available plant litter and show less competition for nutrients and minerals [84,86,88]. The results of our study are consistent with the results of Mungai et al. [74] from a 21-year-old pecan (*Carya illinoinensis*) and a 12-year-old silver maple (*Acer saccharinum*), and Koranda et al. [89] from a *Fagus sylvatica* as well as Bini et al. [90] from mixed plantations of *Eucalyptus grandis* and *Acacia mangiu.* In the autumn period, higher MCB values were observed for Mixed Middle—Aged, Deciduous Young, and Deciduous Mature forests than in the summer period. However, dehydrogenase activity was higher in the autumn for Coniferous Young and Mixed Mature. The seasonal dynamics of the metabolic activity of microorganisms and soil biological activity are a frequently observed phenomenon [91–93]. Understanding the seasonal dynamics (intra-annual) of soil microbial communities and their activity is

very important for improving the ecosystem management policy and can help us clarify the drivers of community stability and ecosystem functioning [91,94].

Chauhan et al. [95] emphasised that SRF has an enormous potential in maintaining physical, chemical, and biological properties of the soil, e.g., enhanced productivity, nutrient recycling, erosion control, and reclamation of problematic/degraded land. In the research of Swamy et al. [96], Singh and Sharma [97], and Gupta et al. [98], they revealed significant improvements in available nitrogen, phosphorus, potassium, and organic carbon content in soil under agroforestry plantations. Turley et al. [99] indicated that the restoration of abandoned land increased bacterial diversity by 13.8% and fungal diversity by 60.1%. Xu et al.'s [100] results indicate that recovery after cropland abandonment causes an increase in microbial activity in the soil and depends on plant characteristics and soil physicochemical parameters. Therefore, it is so important to choose a suitable plant species. Our study presents the status of microbial activity in soil under *Paulownia* plantations, considering variability of date and distance of sampling. This research emphasised the importance of fast-growing *Paulownia* as a plant with great potential to reclaim the abandoned land due to the improvement of the soil enzyme activities, microbial biomass, and metabolic diversity of microorganisms. Overall, *Paulownia* crops can be ecologically and economically profitable as an alternative to agri-food productions and as renewable energy sources.

5. Conclusions

In this study, soil texture, date, and distance variability were definitely the primary factors affecting the soil biological properties and determining the diversity of microorganisms. After only one year of observation, it can be concluded that some soil microbial parameters (microbial biomass C and N, dehydrogenase and acid phosphatase activity, and catabolic activity based on Biolog EcoPlates) decreased with an increase in distance from the nearest tree, which is related to the decreasing content of nutrients derived from root secretions and leaf residues. The temporal fluctuation is the key factor affecting soil biological properties considering the intra-annual trend of each of the analysed parameters. This provides evidence that microorganisms have the capability to modulate their activity in response to modified nutritional conditions during a year in individual seasons. The combination of studies on the activity and diversity of microorganisms from soil allow for a better understanding of the interactions between bioenergy crops and microorganisms and their role in abandoned land. Learning the dynamics of soil microbial communities is very important for improving ecosystem management policy and can help us evaluate the potential of SRF for recultivation. There are several soil ecological advantages of short rotation plantations of annual crops on abandoned land. However, further study-based knowledge is necessary, especially on the fundamentals of the long-term effects and on the sustainability of the effects. Based on the conducted microbiological analyses and their results, the main conclusion can be drawn. Already after one year of tree planting, a statistically significant increase in microbial activity was recorded. This stresses the value of the *Paulownia* as fast-growing plant for recultivation, biomass sequestration, and improvement of soil quality.

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Conflicts of Interest: The authors declare no conflict of interest.

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