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The use of enzyme assays to assess soil biodiversity of diverse land use systems integrating trees – Preliminary research

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Abstract: Most studies of agroforestry system biodiversity focus on assessing visible, aboveground biodiversity, largely ignoring soil biodiversity. To fill this gap, a preliminary assessment of soil biodiversity in an agroforestry system was undertaken based on changes in soil enzyme activity. The study was conducted in the village of Maziarnia, Lubelskie Voivodeship, Poland, Europe. Arable fields with spring wheat, mid-field trees and perennial mixed forest were selected for the study. Soil material for physicochemical analyses (pH_{H_2O}, pH_{KCl}, sorption properties, total carbon and total nitrogen) and biochemical analyses (activity of acid phosphatase, alkaline phosphatase, urease and dehydrogenases) was collected in the spring and autumn of 2022. The present study showed that the biochemical properties of the soils of the selected study sites varied depending on the type of ecosystem determining habitat conditions. Each ecosystem that makes up the agroforestry system studied is characterised by a distinctive microbiome composition and its own level of enzymatic activity. The obtained results support the thesis that agroforestry systems significantly increase the functional diversity and overall biodiversity of agricultural landscapes. However, a full, objective characterisation of the processes taking place in agroforestry systems requires long-term monitoring.

Keywords: activity of soil enzymes; agroforestry systems; arable fields; mid-field trees; perennial mixed forest

Biodiversity is the foundation of life. It is essential for humans as well as for environmental and climate protection. Understanding and maintaining biodiversity is becoming an increasingly important area of research as well as a resource management objective (Caldwell 2005). Biodiversity loss – one of the most visible forms of contemporary environmental change – is largely driven by the loss of terrestrial habitats and, in particular, the spread, intensification and monoculturization of agriculture (Ortiz et al. 2021).

According to Mupepele et al. (2021) agroforestry is a "collective name for diverse land-use systems integrating tree husbandry with livestock or arable cultivation". Agroforestry areas include arable fields, mid-field woodlots and forests. Agroforestry practices are often a part of strategies to improve natural resource management (Ong & Kho 2015). The literature increasingly emphasises the valuable contribution of trees to more ecological forms of agricultural intensification (Tscharntke et al. 2012). The perennial nature of trees in agroforestry systems has a profound impact on soil microclimate and properties. By positively influencing the abundance, diversity and activity of soil biota, trees in agroforestry systems contribute to soil health and functional resilience (Barrios et al. 2012).

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Agroforestry has been proposed as a sustainable agricultural system compared to conventional agriculture and forestry, protecting biodiversity and improving the provision of ecosystem services, without compromising productivity. It is the practice of deliberately integrating woody vegetation (trees or shrubs) into crop and/or livestock production systems to benefit from the resulting ecological and economic interactions (Mosquera-Losada et al. 2009). Agroforestry can provide many benefits in terms of biodiversity conservation and associated ecological threats (Udawatta et al. 2019). Agroforestry is a potentially transformative solution for sustainable agriculture as it restores the structure and function of natural ecosystems (Jose 2009).

Given the complexity of the potential benefits of agroforestry systems, soil quality indicators are used to show how these systems contribute to soil conservation (Lima et al. 2010; Iwata et al. 2012). In scientific research, biodiversity indicators can be used as measurable environmental factors. Since the biodiversity of even a small area is too complex to be comprehensively measured and quantified, suitable indicators need to be found. Determination of soil enzyme activity (SEA) is among many biochemical methods of measuring biodiversity. Soil traits such as enzyme activity are used as potential indicators of soil quality (Burns et al. 2013). SEA is influenced by soil characteristics related to nutrient availability, soil microbial activity and land use management processes that modify potential substrate catalysis via soil enzymes (Meena & Rao 2021).

Most studies of agroforestry system biodiversity focus on assessing visible, aboveground biodiversity (Rösch et. al. 2013, 2019; Boinot et. al. 2019a, b; Mupepele et. al 2021), largely ignoring soil biodiversity (Barrios et al. 2018). To fill this gap, a preliminary assessment of soil biodiversity in an agroforestry system was undertaken based on changes in soil enzyme activity. For this purpose, acid phosphatase (APhac), alkaline phosphatase (APhal), urease (AUr) and dehydrogenase (ADeh) activities were assayed as an indicator of overall microbial activity. The utilitarian aim of the study was to use enzyme assays to rapidly assess both the potential activity and functional diversity of the soil microbiome.

MATERIAL AND METHODS

The study was conducted in the village of Maziarnia (50°98'15"N; 23°64'51"E), Lubelskie Voivodeship, Poland. Arable fields with spring wheat (A), strip of mid-field trees (T) and perennial mixed forest (F) were selected for the study (Figure 1). This forest (F)

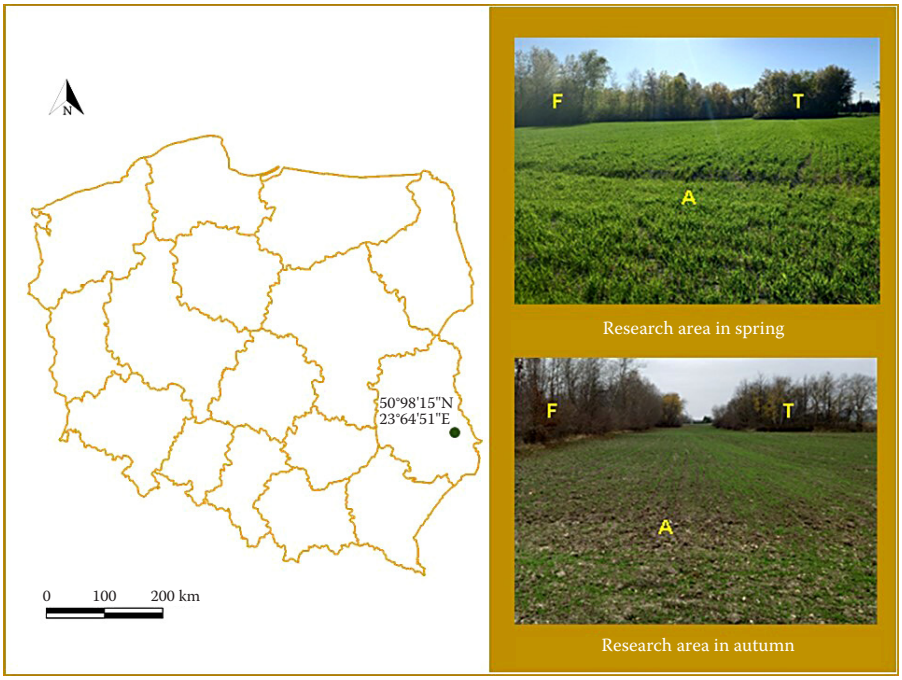

Figure 1. Location of the research area against the background of Poland and in the agricultural landscape in different seasons
A – arable fields; T – mid-field trees; F – perennial mixed forest

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was planted in the 1960 s and the tree strips (T) in the 1990 s. The experiment was established on an Albic Luvisol made from silt loam (IUSS Working Group WRB 2015).

Soil material for physicochemical and biochemical analyses was collected in the spring and autumn of 2022. Soil material for laboratory tests was collected under stable weather conditions from the 0–30 cm layer. Soil samples for biochemical analyses were collected, sieved through a 2 mm sieve and stored at 4 °C, in accordance with ISO 18400 (2018). Soil samples for physicochemical analyses were dried at room temperature.

The pH was measured by the potentiometric method in H_2O (1 : 2.5 ratio) and in KCl solution at the concentration of 1 mol/dm^3 (1 : 2 : 5 ratio), (ISO 10390:2005). The hydrolytic acidity (HA) and exchangeable base cations (EBC) were determined with Kappen's method (Soil Survey Investigation Report 1996). The cation exchange capacity (CEC) of the soil was calculated according to the following formula:

$$CEC = HA + EBC \quad (1)$$

Total organic carbon (TOC) was determined using a TOC-VCSH apparatus (PN-EN 15936:2013-2) with an SSM-5000A module. The total nitrogen (N_{tot}) content was determined by the modified Kjeldahl method using a Kjeltech TM 8100 distillation unit (ISO 13878:1998). The C/N ratio was calculated from the ratio of TOC and N_{tot}.

Determination of APhac and APhal was performed according to Tabatabai and Bremner (1969). AUr was determined by the method of Zantua and Bremner (1975). The activity of ADeh was determined by Thalmann's method (1968). APhac, APhal, AUr, and ADeh determined colourimetrically using a spectrophotometer CECIL CE 2011 (Cecil Instrumentation Ltd., UK) at the following wavelengths: λ = 410 nm for both groups of phosphatases and urease and λ = 485 nm for dehydrogenases.

The statistical analysis of the results was performed using Microsoft Office Excel 2019 and Statistica PL (Ver. 13.3) (TIBCO Software Inc., USA). One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to compare groups across variants. Linear correlation analysis (r) and principal component analysis (PCA) were performed to illustrate the relationships between the parameters studied. Ward's cluster analysis was carried out to determine the similarity of the soils of the study sites and the results were presented in the form of dendrograms.

RESULTS

The physicochemical and biochemical properties of the soils of the selected study sites varied according to ecosystem type and soil sampling date. The concentrations of active hydrogen ions (active acidity – pH_{H2O}) and the concentrations of exchangeable hydrogen ions determined per 1 mol KCl/dm^3 (exchangeable acidity – pH_{KCl}) in the solutions of the tested soils were significantly different (Table 1). Compared to the spring period, the values of pH_{H2O} and pH_{KCl} were lower in the autumn period, with the differences not being statistically significant. The pH of the investigated soils, determined on the basis of pH_{KCl}, varied from strongly acidic (F), through slightly acidic (T) to neutral (A).

The parameters, i.e. HA, EBC and CEC, describe the sorption properties of the investigated soils (Table 2). The highest, statistically significant HA value was recorded in the soil F. In contrast, the highest BC and CEC values were found in soil A. Ecosystem type was a significantly differentiating factor between the values of exchangeable base cations and cation exchange capacity in soils. The values of the parameters describing the sorption properties of the soils (HA, EBC, CEC) were lower in the autumn term compared to the spring term, but the differences were not statistically significant. The sorption properties of soils are closely related to their pH, as confirmed

Table 1. pH values in H_2O and 1 mol KCl/dm^3 in tested soils

Ecosystem type	pH_{H2O}		pH_{KCl}	
	spring	autumn	spring	autumn
Arable fields (A)	7.46 ± 0.10^a	7.24 ± 0.05^a	7.18 ± 0.05^a	7.01 ± 0.02^a
Mid-field trees (T)	6.33 ± 0.6^b	5.98 ± 0.10^b	6.11 ± 0.02^b	5.74 ± 0.01^b
Perennial mixed forest (F)	4.13 ± 0.09^c	3.95 ± 0.03^c	3.85 ± 0.03^c	3.52 ± 0.01^c

$^{a–c}$different small letters indicate significant difference at $P \leq 0.05$ for land use

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Table 2. Values of hydrolytic acidity, exchangeable base cations and cation exchange capacity in the tested soils

Ecosystem type	HA		EBC		CEC	
	(cmol(+)/kg)					
	spring	autumn	spring	autumn	spring	autumn
Arable fields (A)	1.23 ± 0.06^{a}	1.17 ± 0.06^{a}	46.17 ± 0.15^{a}	36.10 ± 0.10^{a}	47.40 ± 0.20^{a}	37.27 ± 0.15^{a}
Mid-field trees (T)	1.30 ± 0.10^{a}	1.27 ± 0.06^{a}	34.90 ± 0.70^{b}	26.03 ± 0.49^{b}	36.20 ± 0.70^{b}	27.30 ± 0.51^{b}
Perennial mixed forest (F)	2.43 ± 0.06^{b}	2.03 ± 0.06^{b}	12.12 ± 0.20^{c}	5.50 ± 0.10^{c}	14.63 ± 0.25^{c}	7.53 ± 0.06^{c}

HA – hydrolytic acidity; EBC – exchangeable base cations; CEC – cation exchange capacity; $^{a–c}$different small letters indicate significant difference at $P \leq 0.05$ for land use

by a simple correlation analysis (Table 6). It was shown that HA was negatively correlated with pH_{H2O} ($r = -0.91$), pH_{KCl} ($r = -0.90$), EBC ($r = -0.85$) and CEC ($r = -0.84$). EBC and CEC indices correlated significantly positively with concentrations of active (pH_{H2O}) and exchangeable (pH_{KCl}) hydrogen ions ($r = 0.94–0.95$), (Table 6).

A Ward cluster analysis was performed on the basis of pH_{H2O}, pH_{KCl} and the parameters describing the sorption properties (HA, EBC, CEC) of the soils. Sites A and T were found to be the most similar in terms of these parameters.

The different habitat conditions generated by the type of ecosystem constituted the factors significantly differentiating the TOC and N_{tot} in the studied soils (Table 3). The presence of woody communities within the T and F sites had a beneficial impact on the content of TOC and N_{tot} in soils. This effect was most evident in the site T, where the content of these components in the soil was the highest. When comparing the study dates, it was observed that there was generally an increase in soil TOC and N_{tot} content in the autumn period. Compared to the spring period, a lower N_{tot} content was found in the soil of the arable field in the autumn. It should be noted that statistically significant differences were recorded only for the amount of TOC in the soils of sites with woody vegetation (T and F), (Table 3).

The C : N ratio values in the studied soils varied depending on the land development method (Table 3). In spring, the soils of arable field and mid-field trees had a significantly narrower C : N ratio than forest soil. This could have been related to the different habitat conditions, e.g. the acid reaction of the forest soil (Table 1). In contrast, a significant variation in this parameter was found in autumn depending on the land development method. Comparing the test dates, it was found that in the autumn period, the tested soils generally had a significantly wider C : N ratio. Statistically significant differences were not found in the forest soil (Table 3).

Ward's analysis based on TOC, N_{tot} and C : N indicates significant similarity of sites with woody plant communities (T and F), (Figure 3).

Land cover and habitat conditions had a significant effect on the activity levels of enzymes from the phosphatase group (Table 4). The highest APhac was found in forest soil, while in arable soil the highest APhal. When analysing the effect of test date on the enzymatic activity of phosphatases, it was found

Table 3. Contents of total carbon and total nitrogen and ratio of total organic carbon to total nitrogen

Ecosystem type	TOC		N_{tot}		C : N	
	(g/kg)					
	spring	autumn	spring	autumn	spring	autumn
Arable fields (A)	15.93 ± 0.19^{aA}	16.55 ± 0.19^{aA}	1.52 ± 0.02^{a}	1.12 ± 0.02^{a}	10.51 ± 0.14^{aA}	14.83 ± 0.24^{aB}
Mid-field trees (T)	24.62 ± 0.24^{bA}	34.37 ± 0.18^{bB}	2.38 ± 0.01^{b}	2.54 ± 0.03^{b}	10.33 ± 0.15^{aA}	13.51 ± 0.15^{bB}
Perennial mixed forest (F)	21.61 ± 0.09^{cA}	26.58 ± 0.20^{cB}	1.85 ± 0.02^{c}	2.52 ± 0.03^{c}	11.66 ± 0.06^{bA}	11.95 ± 0.11^{cA}

TOC – total organic carbon; N_{tot} – total nitrogen; C:N – ratio of total organic carbon to total nitrogen; $^{a–c}$different small letters indicate significant difference at $P \leq 0.05$ for land use (in the column); $^{A–B}$different capital letters indicate significant difference at $P \leq 0.05$ for date of sampling (in a row)

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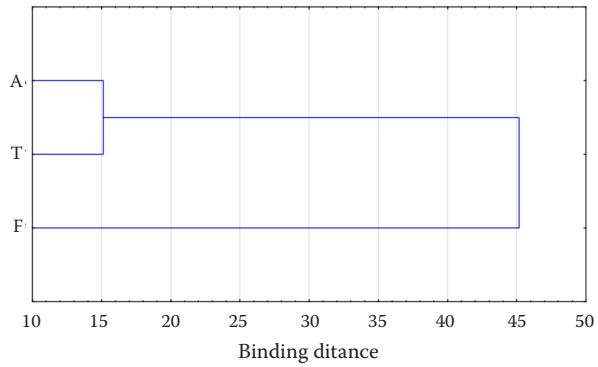

Figure 2. Tree diagram – Ward's dendrogram for pH_{H_2O}, pH_{KCl} and sorption properties
A – arable fields; T – mid-field trees; F – perennial mixed forest

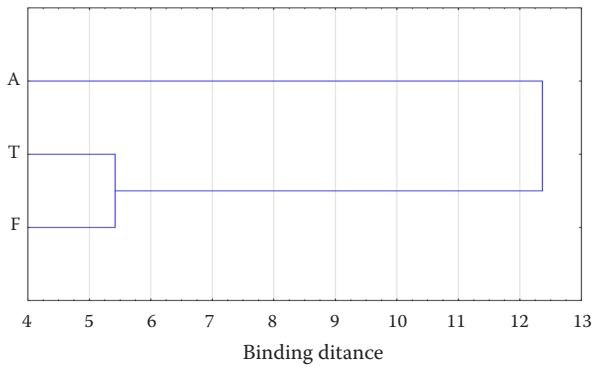

Figure 3. Tree diagram – Ward's dendrogram for total organic carbon, total nitrogen and C : N
A – arable fields; T – mid-field trees; F – perennial mixed forest

that APhac was higher in the spring period than in autumn. With statistically significant differences recorded in arable soil and forest soil. In the case of APhal, there was a significant increase in the activity of this enzyme during the autumn period in the soil of the site with mid-field tree stands, (Table 4). A simple correlation analysis showed that APhac correlated negatively with pH_{H_2O} (r = –0.73), pH_{KCl} (r = –0.73), EBC (r = –0.64) and CEC (r = –0.65) and positively with HA (r = 0.64), (Table 6). For alkaline phosphatase, opposite correlations were noted. APhal was positively correlated with pH_{H_2O} (r = 0.84), pH_{KCl} (r = 0.83), EBC (r = 0.87) and CEC (r = 0.86). Furthermore, there was a negative correlation of APhal with HA (r = 0.86) and APhac (r = 0.51), (Table 6).

In the spring period, AUr was not statistically significantly different. In contrast, during the autumn period, the highest AUr was found in the cultivated soil and the lowest in the forest soil. The differences observed were statistically significant (Table 5). This supports the view that urease activity is dependent on the availability of the substrate urea. Having analysed the effect of the timing of the study, we concluded that there was a significant increase in AUr

Table 4. Acid phosphatase and alkaline phosphatase activities in the tested soils

Ecosystem type	APhac		APhal	
	(mmol PNP/kg/h)			
	spring	autumn	spring	autumn
Arable fields (A)	109.93 ± 1.14^{aA}	97.42 ± 0.75^{aB}	182.73 ± 1.31^{aA}	199.31 ± 0.90^{aA}
Mid-field trees (T)	136.00 ± 0.63^{bA}	127.37 ± 0.16^{bA}	171.35 ± 1.36^{bA}	210.95 ± 0.70^{bB}
Perennial mixed forest (F)	146.55 ± 0.62^{cA}	130.83 ± 0.10^{cB}	137.25 ± 0.37^{cA}	123.22 ± 0.24^{cA}

APhac – acid phosphatase, APhal – alkaline phosphatase, $^{a–c}$different small letters indicate significant difference at $P \leq 0.05$ for land use (in the column); $^{A–B}$different capital letters indicate significant difference at $P \leq 0.05$ for date of sampling (in a row)

Table 5. Activity of urease and dehydrogenases in the tested soils

Ecosystem type	AUr		ADeh	
	(mg $N\text{-}NH_4^+$/kg/h)		(mg TPF/kg/24 h)	
	spring	autumn	spring	autumn
Arable fields (A)	42.43 ± 0.08^{aA}	55.25 ± 0.12^{aB}	19.01 ± 0.09^{aA}	21.11 ± 0.10^{aA}
Mid-field trees (T)	46.23 ± 0.12^{aA}	31.07 ± 0.16^{bB}	25.20 ± 0.06^{bA}	28.02 ± 0.14^{bB}
Perennial mixed forest (F)	44.80 ± 0.09^{aA}	21.08 ± 0.05^{cB}	21.21 ± 0.08^{aA}	24.63 ± 0.12^{aB}

AUr – activity of urease; ADeh – activity of dehydrogenases; $^{a–c}$different small letters indicate significant difference at $P \leq 0.05$ for land use (in the column); $^{A–B}$different capital letters indicate significant difference at $P \leq 0.05$ for date of sampling (in a row)

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Table 6. Significant correlation coefficients between analysed properties of soils

	pH_{H_2O}	pH_{KCl}	HA	EBC	CEC	TOC	N_{tot}	APhac	APhal	AUr	ADeh
pH_{H_2O}		0.99	–0.91	0.95	0.95	–0.40	–0.44	–0.73	0.84	0.59	–0.24
pH_{KCl}	*		–0.90	0.94	0.95	–0.42	–0.45	–0.73	0.83	0.61	–0.24
HA	***	***		–0.85	–0.84	0.08	0.16	0.65	–0.85	–0.33	–0.08
EBC	***	***	***		0.99	–0.30	–0.38	–0.65	0.87	0.47	–0.35
CEC	***	***	***	***		–0.31	–0.39	–0.65	0.87	0.47	–0.36
TOC	ns	ns	ns	ns	ns		0.90	0.59	0.06	–0.67	0.69
N_{tot}	ns	ns	ns	ns	ns	***		0.76	–0.15	–0.66	0.80
APhac	**	**	**	**	**	**	***		–0.51	–0.41	0.50
APhal	***	***	***	***	***	ns	ns	*		0.40	–0.06
AUr	**	**	ns	*	*	**	**	ns	ns		–0.31
ADeh	ns	ns	ns	ns	ns	**	***	*	ns	ns	

pH_{H_2O} – active acidity; pH_{KCl} – exchangeable acidity; HA – hydrolytic acidity; EBC – exchangeable base cations; CEC – cation exchange capacity of the soil; TOC – total organic carbon, N_{tot} – total nitrogen; APhac – acid phosphatase; APhal – alkaline phosphatase; Aur – urease, ADeh – dehydrogenases; ***$P \leq 0.001$; **$P \leq 0.01$; *$P < 0.05$; ns – not statistically significant

activity in the cultivated soil in autumn compared to spring. There was a significant inhibition of this enzyme at the other sites during the autumn period (Table 5). Correlation analysis showed that AUr correlates closely, positively with pH_{H_2O} (r = 0.59), pH_{KCl} (r = 0.61), EBC (r = 0.47) and CEC (r = 0.47). In addition, there was a negative correlation of AUr with TOC (r = –0.67) and N_{tot} (r = –0.66) (Table 6).

The activity of dehydrogenases (ADeh), intracellular enzymes of the oxidoreductase class, in the tested soils varied depending on the type of vegetation cover and the season of the year. The highest, statistically significant, activity of this enzyme group was found in the soil with mid-field trees and the lowest in the farmland soil. Compared to the spring period, Deh activity was found to increase in the autumn period, with significant differences at sites T and F. Dehydrogenase activity was closely correlated with TOC (r = 0.69), N_{tot} (r = 0.80) and APhac (r = 0.50), (Table 6).

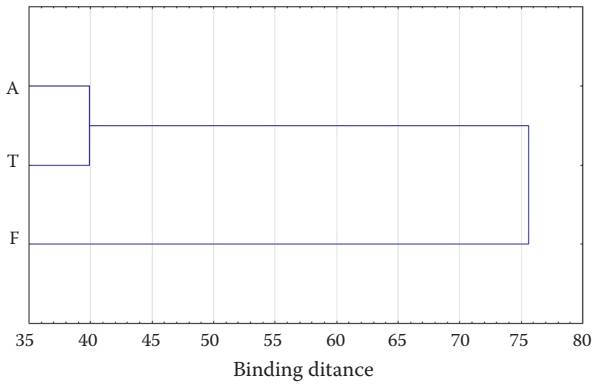

Figure 4. Tree diagram – Ward's dendrogram for enzymatic activity

A – arable fields; T – mid-field trees; F – perennial mixed forest

The results of the cluster analysis by Ward's method performed based on soil enzyme activities, i.e. APhac, APhal, AUr and ADeh, are shown in Figure 4. After analysing the resulting dendrogram, it was found that sites A and T were most similar in terms of overall enzyme activity. A similar grouping of sites is shown in Ward's dendrogram for pH_{H_2O}, pH_{KCl} and sorption properties (Figure 2).

Figure 5 shows the results of the principal component analysis (PCA). Factors 1 and 2, extracted during the analysis, together explain 100% of the variance of the analysed properties of the studied soils. Factor 1 explains 74.66% of the variation in the analysed properties (total variance), factor 2 explains 25.34% of the variation in the analysed properties. Based on PCA, soil parameters pH_{H_2O}, pH_{KCl}, EBC, CEC, N_{tot} and TOC were found to be strongly, positively correlated with each other. Based on PCA, soil parameters pH_{H_2O}, pH_{KCl}, EBC, CEC, N_{tot} and TOC were found to be strongly, positively correlated with each other. If the content of one indicator increases, the other one also increases. However, HA is negatively correlated with APhal, EBC, CEC, pH_{H_2O} and pH_{KCl}, so as HA increases, the content of the remaining indicators decreases. The multiyear mixed forest differed significantly in terms of individual indicators from the cultivated field. Perennial mixed forest stood out from other types of ecosystems with the

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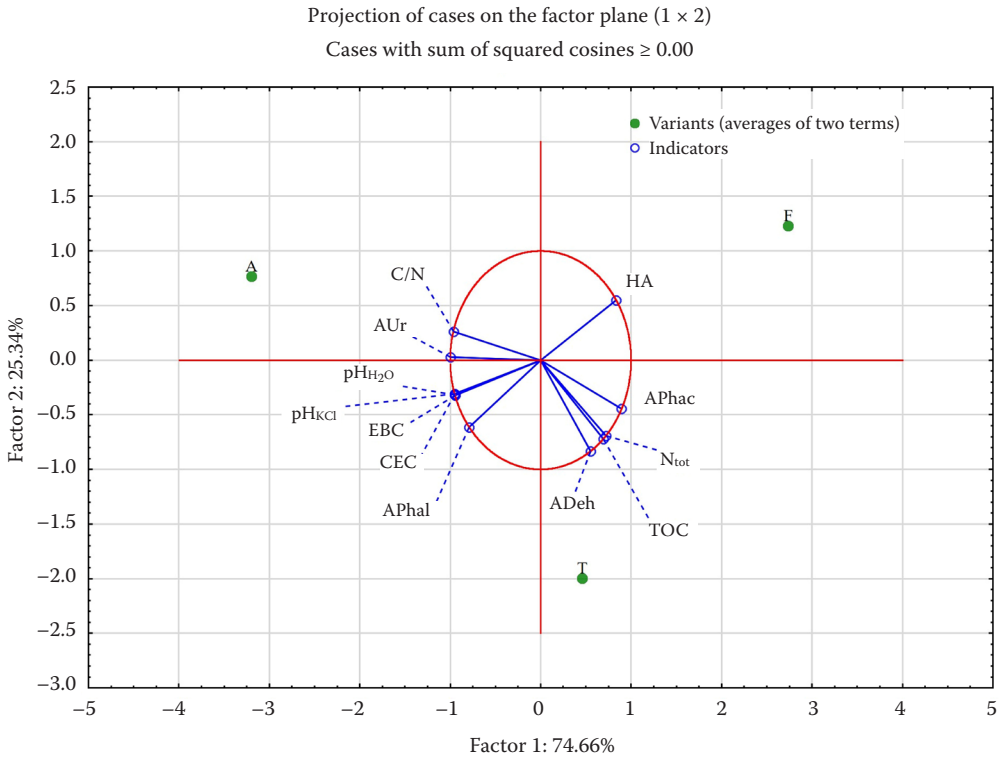

Figure 5. Biplot (combination of a 2D factorial plot for cases (A, T, F) with a 2D factorial plot for variables (pH$_{H2O}$, pH$_{KCl}$, HA, EBC, CEC, TOC, N$_{tot}$, C/N, APhac, APhal, Aur, ADeh)

A – arable fields; T – mid-field trees; F – perennial mixed forest; pH$_{H2O}$ – active acidity; pH$_{KCl}$ – exchangeable acidity; HA – hydrolytic acidity; EBC – exchangeable base cations; CEC – cation exchange capacity of the soil; TOC – total organic carbon; Ntot – total nitrogen; C : N – ratio of total organic carbon to total nitrogen; APhac – acid phosphatase; APhal – alkaline phosphatase; Aur – urease, ADeh – dehydrogenases

highest HA content, mid-field plantings with the highest N$_{tot}$, TOC, ADeh content, and arable fields with the highest EBC, CEC content and pH$_{H2O}$, pH$_{KCl}$.

DISCUSSION

Soil biodiversity is insufficiently studied despite being responsible for soil quality and health (Barrios et al. 2018). The present study showed that the land use systems integrating trees significantly differentiated the enzymatic activity of the soils, indicating the differential biodiversity of this system (Tables 4–5, Figures 3–4). The physicochemical properties of the soils, related to the habitat, determined the development and activity of the soil microbiome which is the main source of many enzymes (Nannipieri et al. 2018).

The activity of APhac and APhal shown to be significantly (positively or negatively) dependent on environmental conditions, including pH and sorption properties of soils, as confirmed by simple correlation (Table 6) and PCA analyses (Figure 5). Similar ones were obtained by Bueis et al. (2018) and Wesołowska et al. (2022), among others. Soil pH is important for the synthesis of the microbiome and soil enzymes (Nannipieri et al. 2018). Higher APhal than APhac was found in the cultivated soil, which was related to the neutral reaction of the soil tested. In contrast, the forest soil was characterised by a higher APhac and a strongly acidic reaction. Enzymes of the phosphatase group are enzymes that are very sensitive to changes in soil pH (Lemanowicz 2013).

Our own research showed that AUr in the soils of the study sites varied significantly only in autumn. The highest AUr was found in the arable soil. This may indicate a strong relationship between AUr and substrate availability – urea. Ammonia and carbonic acid, products of the decomposition catalysed by urease, are involved in the nitrogen and carbon cycles in the soil and also influence the regulation of pH (Wyszkowska & Wyszkowski 2010). Thus, AUr con-

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verts soil nutrients into the appropriate forms required by plants and suitability of the living environment for soil microbes (Kuscu 2019; Cao et al. 2023). Turner et al. (2014) showed that AUr correlates positively with the amount of N_{tot} in soil. Cao et al. (2023), also observed an increase in AUr as soil organic matter content increased. Our own research did not confirm this relationship (Table 3 and 5), which may be the result of regular fertilisation of arable fields with urea fertiliser (Meena & Rao 2021).

The results obtained indicate that the site T had the highest ADeh and also TOC and N_{tot} compared to other ecosystems (A and F). ADeh was significantly correlated with the pool of TOC and N_{tot} in the soil, as confirmed by statistical analyses (Table 6 and Figure 5). In the present study, the relationship of ADeh with pH and sorption properties was not demonstrated. According to Lasota et al. (2021), the negative effect of pH on ADeh and other enzymes may be masked by organic matter, which is a more influential factor in SEA.

Numerous authors claim that the activity of soil enzymes is determined primarily by the TOC content (Błońska et al. 2016; Piotrowska-Długosz et al. 2022). In contrast, our own research shows that the enzymatic activity of soils of agroforestry systems was also influenced by other factors related to the properties of the studied ecosystems. Our analysis of the obtained dendrograms shows that, in terms of the overall enzymatic activity, the sites A and T were most similar (Figure 4). The same grouping of sites is shown by Ward's dendrogram for pH_{H2O}, pH_{KCl} and sorption properties (Figure 2). Cluster analysis for TOC, N_{tot} and C : N shows significant similarity of sites with woody plant communities (T and F), (Figure 3). TOC and N_{tot} are the two basic biogenic elements in the ecosystems and originate mainly from the decomposition of organic matter. They are important in maintaining ecosystem structure, function and stability (Lu et al. 2023). Furthermore, they regulate microbiome composition and ecosystem productivity (Wolna-Maruwka et al. 2023). The afforestation can increase the pool of plant biomass and SOM content, modifies the quality and quantity of litter and microclimatic conditions, improve microbiome structure and SEA (Myszura et al. 2021; Bierza et al. 2023).

Błonska et al. (2017) and Wolna-Maruwka et al. (2023) indicate changes in enzymatic activity depending on the species composition of the plant cover. Root secretions have a quantitatively variable chemical composition depending on the species and type of plant. Nevertheless, they contain significant amounts of organic acids, carbohydrates, amino acids, and inorganic compounds (Galloway et al. 2020). According to Ouahmane et al. (2007), organic acids are among the main factors influencing soil microbial succession and activity. On the other hand, Galloway et al. (2020) consider that wheat root secretions may contain large amounts of polysaccharides, which are a readily available carbon source for microorganisms. By intensifying the activity of the microbiome, saccharides promote the secretion of various enzymes and thus stimulate the mineralisation of soil organic matter. Root secretions during the growing season, as well as post-harvest residues in the arable field (A) and dead organic matter in woody communities (T and F) with different C : N ratios, determine the directions of changes in the composition and size of the soil microbial population, as well as its enzymatic activity.

Seasonal changes in enzymatic activity varied according to the type of enzyme and the type of ecosystem determining habitat conditions. SEA changes may be related to meteorological conditions, i.e. changes in environmental temperature and humidity and seasonal dynamics of soil nutrient content (Koch et al. 2007; Silvestro et al. 2017). Water deficiency causes strong disturbances in the development and activity of soil microflora (Bielinska et al. 2017).

Landscaping, plant biodiversity, humus content and litter appear to significantly influence soil enzyme activities in land use systems integrating trees (Daunoras et al. 2024). According to Gianfreda (2015), higher SEA means greater functional diversity of the microbiome. It should be remembered that the decrease in the microbiological diversity of the soil environment poses a significant threat to the balance of the ecosystem. Currently, land use intensification is the main anthropogenic factor that has led to significant changes in local biodiversity and has had a significant impact on ecosystem processes (Daunoras et al. 2024). Agroforestry systems seem to be a good solution to protect the biodiversity of the soil and the entire ecosystem.

In summary, the biochemical properties of the soils of the selected study sites varied depending on the type of ecosystem determining habitat conditions. Each ecosystem that makes up the agroforestry system studied is characterised by a distinctive microbiome composition and its own level of enzymatic activity. The testing of the activity of a range of soil enzymes (APhac, APhal, AUr and ADeh) provided a potential

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integrative assessment of the biochemical status of soils and the potential activity, as well as functional diversity, of soil microbial communities. The obtained results support the thesis that land-use systems integrating trees significantly increase the functional diversity and overall biodiversity of agricultural landscapes. However, a full, objective characterisation of the processes taking place in agroforestry systems requires long-term monitoring.

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