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The Level of Luvisols Biochemical Activity in Midfield Shelterbelt and Winter Triticale (x*Triticosecale* Wittm. ex A. Camus) Cultivation

Renata Jaskulska

Institute for Agricultural and Forest Environment, Polish Academy of Science, 60-809 Poznań, Poland; reniajas@tlen.pl

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Abstract: This study aimed to evaluate the usage of Luvisols under the midfield shelterbelt and in the cultivation of winter triticale, together with the influence of physicochemical properties on enzymatic activity. Soil samples were collected in spring, summer, and autumn from the depth of 0–15 cm along the following measurement transect: midfield shelterbelt (S), the border between the shelterbelt and the triticale field (B), cultivated field at a distance of 50 m from point B (F_{50}), and cultivated field at a distance of 100 m from point B (F_{100}). The activities of dehydrogenase (DHA), acid phosphatase (PAC), and alkaline phosphatase (PAL) were determined, and a water retention curve (pF) was established. The pH, soil organic carbon (SOC) content, and total nitrogen (N_{total}) were also measured. The analysis of the activity results for DHA, PAC, and PAL showed that the triticale soil had a higher level of enzyme activity than the midfield shelterbelt soil during the entire growing season. The soil under the triticale cultivation was slightly acidic, and the shelterbelt soil was very acidic. It was observed that the timing of soil sampling had an impact on the activity of the studied enzymes. The highest levels of DHA, PAC, and PAL activity were found in summer. The midfield shelterbelt demonstrated greater water retention than the winter triticale. The SOC content and N_{total} were higher in the shelterbelt than in the winter triticale field.

Keywords: agricultural land; enzymatic activity; soil physicochemical properties

1. Introduction

Midfield shelterbelts are important from the point of view of the landscape's natural balance [1]. Vegetation from natural succession forms the so-called pioneering phytocoenoses in the shelterbelts, which provide protection against the erosive effects of weather conditions, including intense rainfall and winds. The soil under shelterbelts has very good water absorption, which reduces surface runoffs [2,3]. Additionally, it is not ploughed, and its lumpy structure remains intact. Tree root systems extend much deeper than those of cultivated plants [4]. In periods of moisture deficiency, trees use capillary water from the soil profile, and shelterbelts enrich the air with water vapor as a result of transpiration [5]. The decomposition of organic compounds increases several or even a dozen times due to higher soil moisture and intense microbiological processes [6,7]. In the shelterbelts, the total inflow of organic matter to the soil consists of the fall from trees and shrubs, undergrowth, and dying roots and parts of plants [8]. Dead roots and plant debris enrich the soil with organic matter, growth substances, and growth inhibitors [9,10]. For arable plants, midfield shelterbelts create favorable microclimatic conditions, improve the functioning of agricultural physiocoenoses, and protect soil and water resources [11,12].

The function of each agricultural soil is to produce an appropriate amount of organic matter of high quality, i.e., with high content of nutrients, for cultivated plants [13,14].

In Poland, the cultivation of triticale (x*Triticosecale* Wittm. Ex A. Camus) is very popular [15]. Triticale grain is primarily a raw material intended for animal feed. It is grown in two forms: winter and spring. The winter form is of dominant economic importance. Individual cultivars show different hardiness, but in general, triticale is a species quite resistant to frost [16]. Triticale is a grain that combines the features of wheat and rye. It also has lower soil requirements than wheat and barley and shows greater tolerance to low soil pH. Additionally, triticale is more resistant to diseases, so it requires less intensive protection. One of the advantages of triticale is its very high yield potential [17]. The most important factors shaping the yield efficiency from the unit area is the application of an appropriate nitrogen dose and the influence of weather conditions during the growing season. The appropriate amount of rainfall in the first phase of plant growth results in the optimal use of supplied nutrients and the correct form of grain [18]. The excess of water in the period of grain formation and maturation aids the development of fungal diseases and fungal growth. Good weather and soil conditions as well as excellent agrotechnics, including fertilization, ensure high yield. However, each agrotechnical operation and the use of fertilizers may bring about changes in the soil environment. In cultivated fields, the main substrate of soil humus is harvest residues as well as aboveground and underground parts of plants that die during the growing season [4]. They enrich the soil with minerals and substances that bind soil aggregates. They are also a source of easily available carbon for microorganisms and contribute to the increase in organic matter content in the soil, which has an impact on the increase in water capacity, thus providing good conditions for the development of plants, soil microorganisms, and the activity of soil enzymes [19].

The interaction between microorganisms and higher plants leads to the creation of a certain kind of balance in biocenotic systems in the soil environment [20,21]. The activity of microorganisms and the enzymatic activity of the soil result in the transformation of mineral compounds. It is related to the periodic accumulation of reactants produced by enzymes in the soil, which are of great importance for green plants [22,23]. During the vegetation season, the course of enzymatic processes in the soil is difficult to explain due to the influence of various factors shaping the properties of the soil environment. These factors include, among others, the periodically changing temperature and soil moisture, which affect the life activity of microorganisms [24,25]. When it comes to soil enzymes, dehydrogenases (DHA) and phosphatases play a large role. Their proteinaceous nature makes them susceptible to environmental factors, both natural and anthropogenic. Soil enzymes are involved in metabolism and catalyze processes related to the transformation of matter and energy that take place in the soil [26]. Dehydrogenases (EC 1.1.1) are enzymes that accelerate the processes of biochemical oxidation of organic components [27]. They constitute a large group of oxidoreductases located inside living cells, so their activity depends on the entire population of soil-inhabiting microorganisms. The activity of dehydrogenases is considered to be a general, indirect indicator of the abundance and activity of soil microorganisms. Active dehydrogenase can transfer electrons to oxygen, carbon, and other bonds. Dehydrogenases are more sensitive to the activity of toxic compounds than extracellular enzymes. Therefore, the activity of dehydrogenases is a sensitive test in the assessment of the level of soil environment pollution [28].

Phosphatases are also an indicator of the biological activity of the soil environment. They hydrolyze organic phosphorus compounds into inorganic phosphates, which are directly available to plants and soil microorganisms [29]. The main sources of soil phosphatases are microorganisms, plant roots, and soil fauna. The most frequently studied activity is the activity of acid phosphatase (PAC) (EC 3.1.3.2) and alkaline phosphatase (PAL) (EC 3.1.3.1). They differ in a soil pH range that is optimal for their activity. The acidic pH of the soil (pH 4–6) is optimal for acid phosphatase, while alkaline (pH 8–10) is optimal for alkaline phosphatase [30].

Measuring the level of soil enzymes provides us with information about the quality and biochemical activity of the soil. Enzymes are believed to be good and sensitive indicators that respond quickly to changes in the soil caused by natural and anthropogenic factors. Moreover, their activity is easy to measure and influences main microbial reactions involving soil nutrient cycles. The studies of many authors have also shown that agrotechnical treatments affect enzymatic activity more than other biochemical parameters [7,24].

The research hypothesis assumes that the distance from the shelterbelt and the weather conditions prevailing during the growing season have an impact on the level of soil enzyme activity. It has also been hypothesized that the physicochemical properties of Luvisols influence the results of enzyme activity.

This study aimed to demonstrate the variability of the activity level of dehydrogenases and acid and alkaline phosphatases combined with the physicochemical properties of the soil.

2. Materials and Methods

2.1. Description of the Study Site

The research was carried out in 2018 in the Gen. D. Chłapowski Landscape Park in Rogaczewo Wielkie, Greater Poland. Geomorphologically, the research area is part of the basal moraine of the Baltic glaciation, Leszno stadial, which ended about 10,000 years ago [12]. The glacial tills occurring there were washed and stratified to various degrees. The clays are highly sanded and exhibit the graining of loamy sands and sandy loam. According to FAO—WRB [31] classification, the studied soil is a Luvisol formed from parent materials with favorable water infiltration properties (Table 1). The topography consists mainly of postglacial plains with slight height differences and slopes [32]. The agricultural landscape of the park consists of a mosaic of arable fields intersected by various types of trees, enclaves of meadows, and a network of water reservoirs. The characteristic features of this landscape are streak, linear, compact, clump, or wedge-shaped midfield shelterbelts. The arable fields, meadows and wetlands, and forests and shelterbelts make up 70%, 12%, and 16% of the area, respectively [33].

Table 1. Physicochemical properties of the topsoil (0–15 cm).

Variable	Clay	Silt	Sand	Texture	pH_{KCl}	CEC
		%				Cmol kg^{-1}
Shelterbelt (S)	2	24	74	loamy sand	3.51	6.97
Border (B)	5	22	73	sandy loam	4.50	9.29
Field (F_{50})	3	17	79	loamy sand	6.24	7.60
Field (F_{100})	2	20	77	loamy sand	6.15	7.22

CEC, cation exchange capacity.

The study subject was the old, 200-year-old midfield shelterbelt together with the neighboring cultivated field. The GPS coordinates are as follows: midfield shelterbelt (Poland 52°04′19″ N and 16°82′19″ E) and the neighboring cultivated field (Poland 52°04′20″ N and 16°82′67″ E). In this area, four soil sampling points were designated along the measurement transect S, B, F_{50}, and F_{100}. Point S was marked in the middle of the shelterbelt, while point B was marked about 20 m in a straight line from point S and was located on the boundary between the shelterbelt and the cultivated field. The next two points were located on the cultivated field at a distance of about 50 m (point F_{50}) and 100 m (point F_{100}) from point B.

The midfield shelterbelt is an old, 200-year-old stand that is 2 km long and 36 m wide. The tree layer formed by forest species is dominated by an alien species, *Robinia pseudoacacia* L. (82.9%), naturalized in the flora of Poland, accompanied by the native tree species *Quercus petraea* (Matt.) Liebl. and *Q. robur* L. (7.4%), *Acer platanoides* (6.7%), *Acer pseudoplatanus* L. (1.5%), and *Alnus glutinosa* L. (1.5%). The shrub layer consists of forest species *Euonymus europaea*, *Rubus idaeus*, and *Sambucus nigra* and shrub species *Crataegus monogyna*, *Humulus lupulus*, and *Rubus plicatus*. The herbaceous layer consists of forest, segetal, ruderal, scrub, grassland, and meadow species (e.g., *Alliaria petiolata*, *Allium vineale*, *Anthriscus sylvestris*, *Chelidonium majus*, *Elymus repens*, *Festuca rubra* *Galeopsis ladanum*, *Geraniuma robertianum*, *Geum urbaninervia*, *Moehringa robertianum*, *Geum urbaninervia*, *Moehringa robertianum*, *Geum urbaninervia*, *nemoralis*, *Stellaria media*, and *Veronica hederifolia*). The geographical position of the midfield shelterbelt is on a north–south axis.

The species grown in the cultivated field was winter triticale (x*Triticosecale* Wittm. ex A. Camus), Rotundo variety. It is a short-straw variety with good stiffness. It is characterized by very good frost resistance and a high, stable level of yield throughout the area of Poland. It has a beautiful, large wheat-type grain with a high protein content.

In this field, the forecrop for winter triticale was maize. The treated (Funaben Plus 02 WS) certified seeds were sown in the last week of September 2017 at a dose of 150 kg ha^{-1}. Agrotechnical treatments were carried out in accordance with the principles of good agricultural practice for winter triticale. Herbicide (Legato Plus 600 SC, 1.3 L ha^{-1}) and fungicide (Delaro 325 SC, 1.0 L ha^{-1}) were applied for chemical protection of crops from weeds and diseases. In autumn, presowing fertilization was applied using the multicomponent POLIFOSKA 4 fertilizer (N: 4%, P: 12%, and K: 32%) at a dose of 230 kg ha^{-1}. Nitrogen fertilization in the dose of 110 kg ha^{-1} was applied on three different dates. The first dose of nitrogen in ammonium form (Zaksan®, 50 kg ha^{-1}) was sown in spring, shortly before the resumption of vegetation, and it primarily increased the number of productive stalks. The second dose was applied from the end of tillering to the stem formation phase (which positively influences the final number of caryopses in the ear), and the third dose of nitrogen was applied in the earing stage (which positively influences grain formation and protein content).

2.2. Weather Conditions

The climatic conditions are shaped mainly by the masses of polar sea air (about 60% of days a year) flowing in from the North Atlantic, characterized by a high water vapor content. There are also polar continental air masses (less than 30% of days a year) flowing from the east. All this results in a relatively mild climate in this region [34].

The average annual air temperature in 2018 was +10.8 °C. The coldest month was February at −2.3 °C, while the warmest was August at +21.3 °C. The period with the average daily temperature above +5 °C lasted about 245 days, which is very favorable for agriculture.

The wind directions in the ground layer of the atmosphere depend on the directions of the influx of air masses. Western winds prevailed almost all year.

Precipitation varies greatly over time compared to other weather elements. There are large differences between monthly and annual rainfall totals [34]. In 2018, the amount of rainfall was about 425.9 mm, and in the growing season, it was 282.1 mm. The rainfall was unevenly distributed, and the droughts were particularly unfavorable for plants in April, May, June, and August.

2.3. Soil Sampling for Physicochemical Analyses

The research material was soil samples with disturbed structure collected on three different dates (spring, summer, and autumn) taken from surface level (0–15 cm) from the designated measurement points (S, B, F_{50}, and F_{100}). Soil samples were collected from five sites, three times for each of the four measurement points. This way, we obtained 12 soil samples in each analysis period, each sample weighing 1 kg. The soil samples were dried at room temperature and then sieved through a sieve with a mesh diameter of 2 mm. After sifting, the soil was stored in plastic containers. The samples prepared this way were the starting material for the analyses. The analyses were performed three times. Determination of moisture, activity of dehydrogenases, and acid and alkaline phosphatases were performed in fresh soil samples. and the remaining measurements were made in air-dry material.

Soil samples were analyzed according to the methodological procedures described by Mocek et al. [35] and Van Reeuwijk [36].

The particle size distribution was determined with the Cassagrande aerometric method modified by Prószyński. Contents of sand (2.0–0.05), silt (0.05–0.002 mm), and clay (<0.002 mm) [36] were determined according to the standard PN-ISO 11,277 [37]. Soil pH was determined in a soil 1M KCl suspension (soil: 1M KCl ratio of 1:2.5) and measured with a pH meter (CP-501 Elmetron, Zabrze, Poland).

The exchangeable acidity was determined potentiometrically in the 1M KCl solution. The soil organic carbon (SOC) was measured by catalytic burning to CO_2 at 900 °C in a 5000A total organic carbon (TOC) analyzer (Shimadzu, Kyoto, Japan). The total nitrogen (N_{total}) content was determined using the modified Kjeldahl method in the Kjeltec-Tecator (Gerhardt, Wertheim, Germany) analyzer. The cation exchange capacity (CEC) was assessed using a modified Mehlich method with $BaCl_2$ at pH = 8.2.

2.4. Soil Sampling to Determine the Water Retention Curve

Soil samples with intact structure were taken at each of the measurement points from S to F_{100} into cylinders with a standard volume of 100 cm^3, three times from the level (5–10 cm). A total of 12 soil samples were collected and transported to the research laboratory. They were then saturated to full water capacity. Two standard measurement methods were used to create the retention curve, i.e., the method of displacing water from a sample put on porous material (dust) and pressure chamber method [38]. In case of the porous material, the moisture of soil samples (θ_v) was determined at predefined suction pressures, i.e., pF = 0.4, pF = 1.0, pF = 1.7, and pF = 2.0. After determining the moisture at the field water capacity (pF = 2.0), the soil samples were moved to the pressure chamber, where the moisture was determined under steady-state conditions at suction pressures pF = 2.7, pF = 3.0, pF = 3.4, and pF = 4.2. After the measurements, the samples were dried at a temperature of 105 °C [35] to determine their dry weight and gravimetric moisture content.

2.5. Soil Enzymatic Activity

The analyses of soil enzymatic activity were based on the colorimetric method. To measure the dehydrogenase activity (EC 1.1.1), 1% triphenyltetrazolium chloride (TTC), which turns red when reduced to formazan, was used as an acceptor of hydrogen ions. The formazan formed in the soil was extracted with ethanol after the 24 h incubation at 30 °C. The color intensity of the ethanol extract was determined at a wavelength of 485 nm on a Novospec spectrophotometer and expressed in μmol of triphenylformazan TPF 24 h^{-1} g^{-1} dm of soil [39].

Soil biochemical analyses also included the determination of acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) activities using the method developed by Tabatabai and Bremner [40]. Activities were determined with 0.2% disodium p-nitrophenyl phosphate (PNP) used as substrate after 1 h incubation at 37 °C. As a result of hydrolysis, p-nitrophenol was released, and its amount was determined based on yellow color at a wavelength of 400 nm on a Novospec spectrophotometer. The results were converted into μmol (p-nitrophenol) PNP h^{-1} g^{-1} dm of soil.

2.6. Statistics

The results were statistically processed using the Statistica 7.0 software (Statsoft, Cracow, Poland). The correlations between the variables were assessed using Pearson's correlation coefficients at the significance level of $p \leq 0.05$. Data were analyzed using ANOVA.

3. Results

3.1. Physical and Chemical Properties of Soils

Significant differences in physicochemical and biochemical properties were observed in soil samples from four measurement points located along the measurement transect: S, B, F_{50}, and F_{100}.

The surface soil formations at the analyzed measurement points S, F_{50}, and F_{100} showed the grain size distribution of loamy sand (LS), while point B showed sandy loam (SL) (Table 1).

Table 2 summarizes the averaged characteristic moisture conditions of the water retention curve for the analyzed soil formations from S to F_{100}. Based on the presented data, it can be concluded that the soil under the shelterbelt (S) and the soil on the boundary between the shelterbelt and the cultivated field (B) demonstrated higher moisture values ($\theta_{pF=0.4}$) than the soil forms under the cultivated field. Increased soil moisture in conditions close to full saturation in the forest and on its border proved a significant share of organic matter in the soil matrix. Soils in the cultivated field, regardless of the location (F_{50} and F_{100}), were characterized by very similar moisture values at the suction pressure pF = 0.4 and oscillated between 40.3 and about 46.7%; the values were lower than in the soils in B and S.

Table 2. Results of measurements of water retention curves.

Variable	Soil Moisture (cm^3 cm^{-3}) at Suction Pressure (pF)							
	0.4	**1.0**	**1.7**	**2.0**	**2.7**	**3.0**	**3.4**	**4.2**
S	0.5500	0.5400	0.5130	0.4430	0.3670	0.2967	0.2324	0.1679
B	0.5689	0.5103	0.4779	0.4072	0.3577	0.2992	0.1905	0.1117
F_{50}	0.4674	0.4262	0.4053	0.3455	0.2218	0.1740	0.0943	0.0681
F_{100}	0.4027	0.3901	0.3621	0.3254	0.2371	0.1697	0.1140	0.0819

average values (n = 3).

The reaction of the soils along the measurement transect (S, B, F_{50}, and F_{100}) varied from very acidic to slightly acidic. The soil under the shelterbelt (S) was very acidic (pH < 4.5), while that on the border of the shelterbelt and the field with winter triticale (B) was acidic (pH < 4.7). In contrast, the soil in the triticale field (F_{50} and F_{100}) was slightly acidic (pH < 6.5) (Table 1).

The sorption capacity (CEC) did not show any significant differences between the soil under the shelterbelt (S, 6.97 Cmol kg^{-1}) and the soil under the triticale (F_{50}, 7.60 Cmol kg^{-1} and F_{100}, 7.22 Cmol kg^{-1}). Higher values were observed in the soil on the border between the shelterbelt and the triticale field (B, 9.29 Cmol kg^{-1}). This soil demonstrated a particle size distribution of sandy loam (Table 1).

When it came to the soil moisture level, the shelterbelt (S) showed higher values throughout the entire growing season compared to the measurement points B, F_{50}, and F_{100}. No differences were observed between B, F_{50}, and F_{100}. However, significant differences in the soil moisture were found at the measuring points depending on the season. All studied soils showed high moisture in spring (11.13–25.24%) and autumn (4.16–10.17%) and low moisture in summer (2.85–11.60%) (Figure 1).

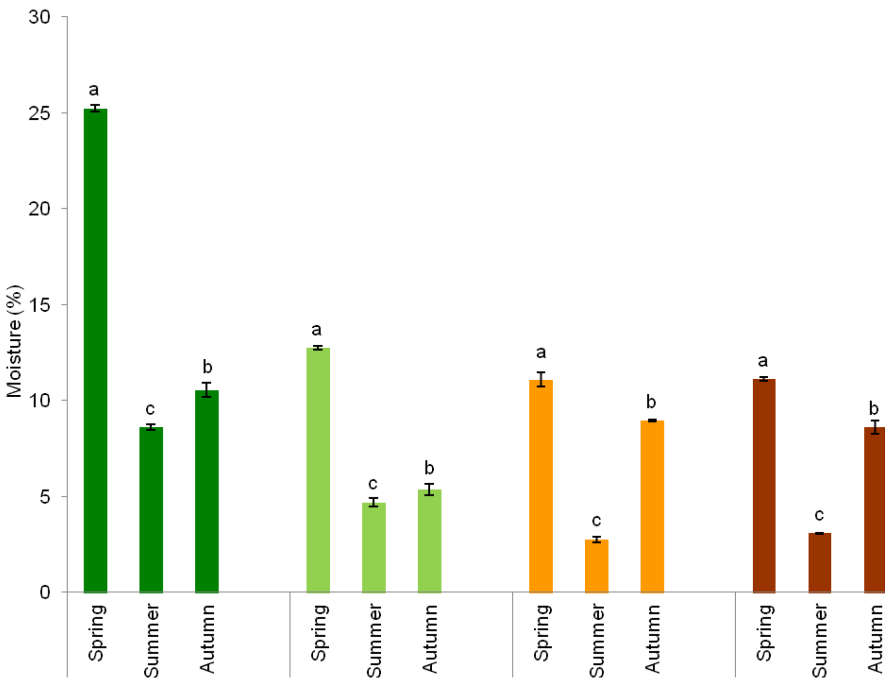


Figure 1. Luvisol moisture in midfield shelterbelt (S), the border between the shelterbelt and the triticale field (B), cultivated field at a distance of 50 m from point B (F_{50}), and cultivated field at a distance of 100 m from point B (F_{100}) determined on three different dates (average values ± standard deviation; n = 3). Different letters indicate significant differences; ANOVA carried out separately for measuring point and sampling time, p < 0.05.

The SOC content decreased along the measurement transect. The highest SOC values were recorded in S (4.83%) and the lowest in the soil under the triticale (F_{50}, 0.98% and F_{100}, 0.92%). In soil B, the SOC content was 2.74%. The SOC content in the soil was significantly higher in S than in soil B, F_{50}, and F_{100}. No significant differences were found in the SOC content between the soil in points F_{50} and F_{100} in the field with winter triticale cultivation (Figure 2).

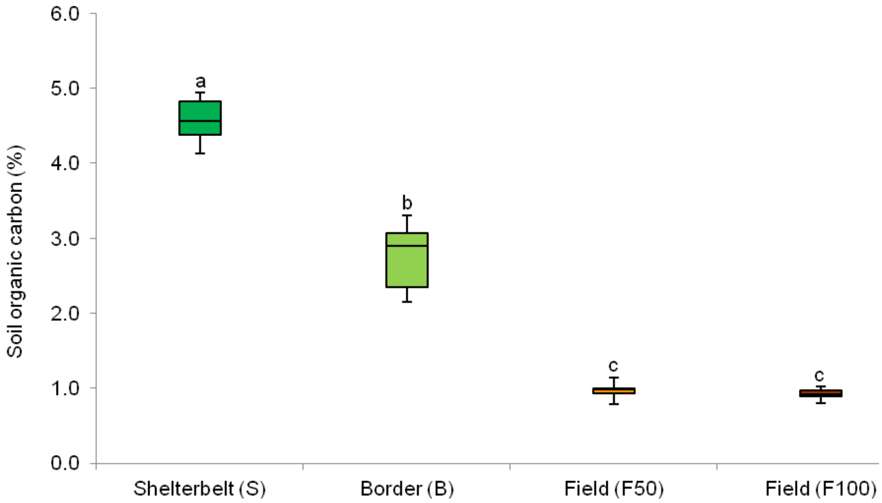


Figure 2. Box plots and results of one-way ANOVA showing the effect of soil use on soil organic carbon (SOC) content along the measurement transect. The top hanging bar represents the high edge, and the lower hanging bar is the lower edge; – median; □ quartile 25–75%; significant difference at $p < 0.05$; $n = 9$; different letters indicate significant differences.

The contents of N_{total} in the analyzed soils were similar. Furthermore, the content of N_{total} was significantly higher in the shelterbelt (S) soil and contained more N_{total} (0.42%) compared to the soil under the triticale (F_{50}, 0.17% and F_{100}, 0.16%). In the soil on the border between the shelterbelt and triticale (B), the content of N_{total} was 0.28% and differed significantly from F_{50} and F_{100} (Figure 3).

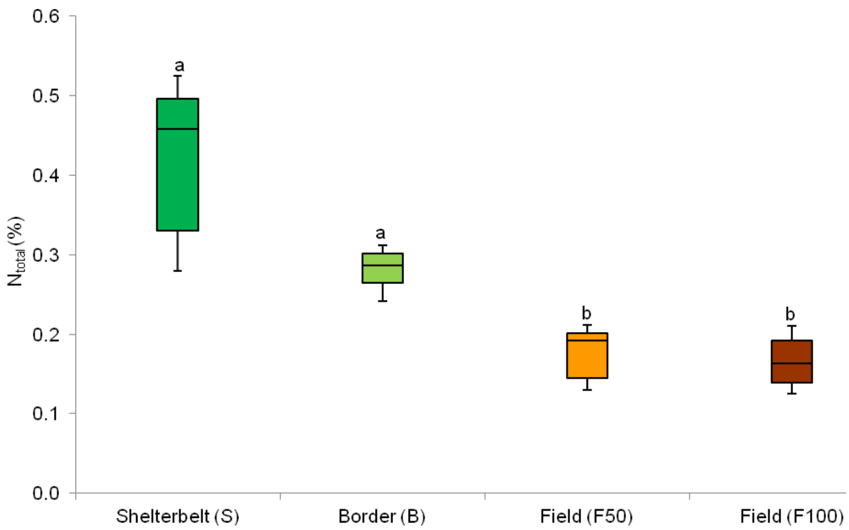


Figure 3. Box plots and results of one-way ANOVA showing the effect of soil use on total nitrogen (N_{total}) content along the measurement transect. The top hanging bar represents the high edge, and the lower hanging bar is the lower edge; – median; □ quartile 25–75%; significant difference at $p < 0.05$; $n = 9$; different letters indicate significant differences.

3.2. Soil Enzymatic Activity

The presented physical and chemical properties of soils influenced the diversification of the level of enzymatic activity along the measurement transect. Some changes were observed in dehydrogenase activity depending on the place and date of soil sampling. The highest level of DHA activity in the soil took place in summer under the winter triticale field (F_{50}, 0.101 µmol TPF 24 h^{-1} g^{-1} dm of soil and F_{100}, 0.052 µmol TPF 24 h^{-1} g^{-1} dm of soil). Significantly lower activity of this enzyme was observed in spring and autumn in the field with winter triticale cultivation along the distance of 50 and 100 m from the border between the shelterbelt and the field. In the shelterbelt (S), the level of DHA activity was the lowest compared to the other research points (B, F_{50}, and F_{100}). There were no significant differences in DHA activity between the dates of soil sampling in the shelterbelt (S). On the border of the shelterbelt and the field (B), DHA activity was significantly higher in autumn compared to spring and summer (Figure 4).

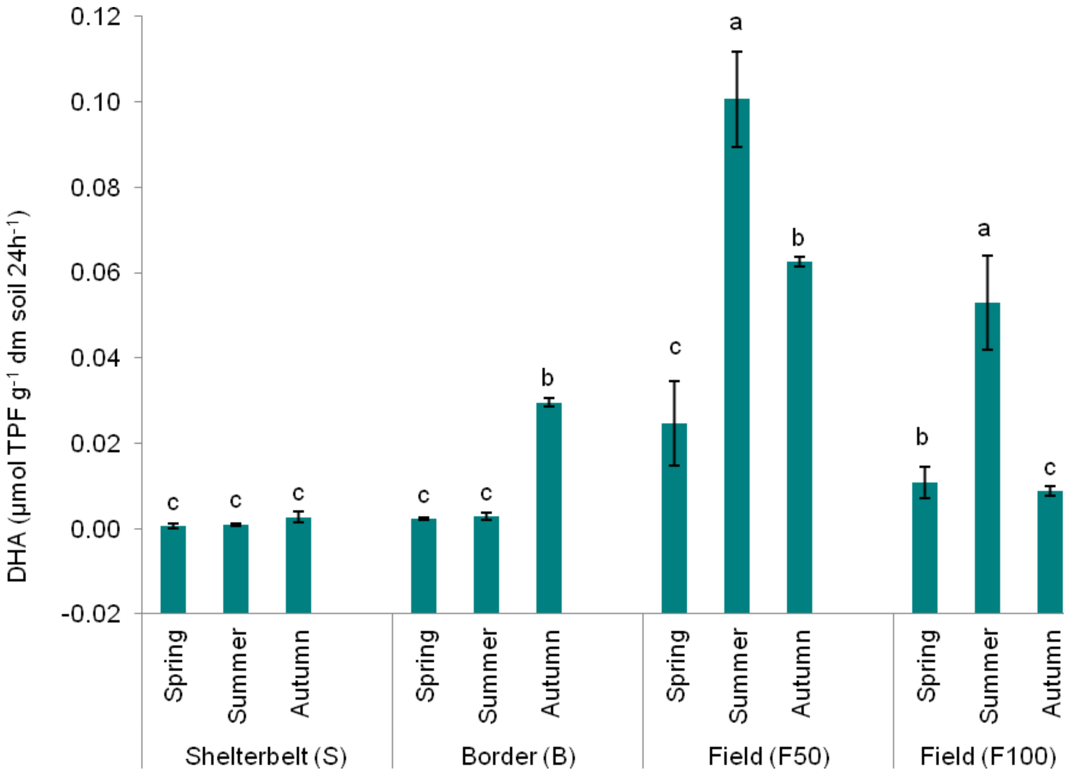


Figure 4. The enzymatic activity of dehydrogenase (DHA) in Luvisol on three different research dates (average values ± standard deviation; $n = 3$). Different letters indicate significant differences; ANOVA carried out separately for measuring point and sampling time, $p < 0.05$.

The research showed that the level of dehydrogenase activity in the studied soils of the shelterbelt (S) was the lowest compared to the other research points located along the measurement transect (B, F_{50}, and F_{100}). Within the shelterbelt (S), the activity of DHA was significantly lower compared to the field with winter triticale (F_{50} and F_{100}) (Figure 5).

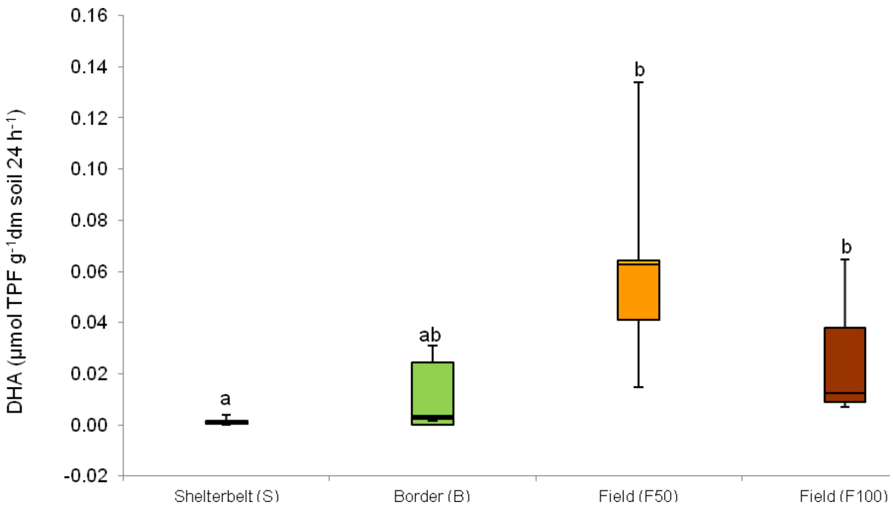


Figure 5. Box plots and results of one-way ANOVA showing the effect of soil use on DHA activity level along the measurement transect. The top hanging bar represents the high edge, and the lower hanging bar is the lower edge; − median; ◻ quartile 25–75%; significant difference at $p < 0.05$; $n = 9$; different letters indicate significant differences.

In the case of hydrolase enzymes, the activity of acid and alkaline phosphatases in the shelterbelt (S) did not show any significant differences depending on the timing of soil sampling in the growing season. A significantly higher level of PAC activity compared to PAL was found in the soil under the shelterbelt (S). It was found that the level of PAC activity was higher in soil S, B, and F_{50} compared to the activity level of PAL. On the other hand, the activity of acid and alkaline phosphatases in the studied soil of the winter triticale cultivation showed high variability during the growing season. A large increase in the PAC level was observed in summer in the soil of winter triticale (F_{50}, 1.68 µmol PNP g^{-1} d.m. h^{-1} and F_{100}, 1.85 µmol PNP g^{-1} d.m. h^{-1}). In spring and autumn, PAL activity was higher than PAC activity in F_{100} (Figure 6).

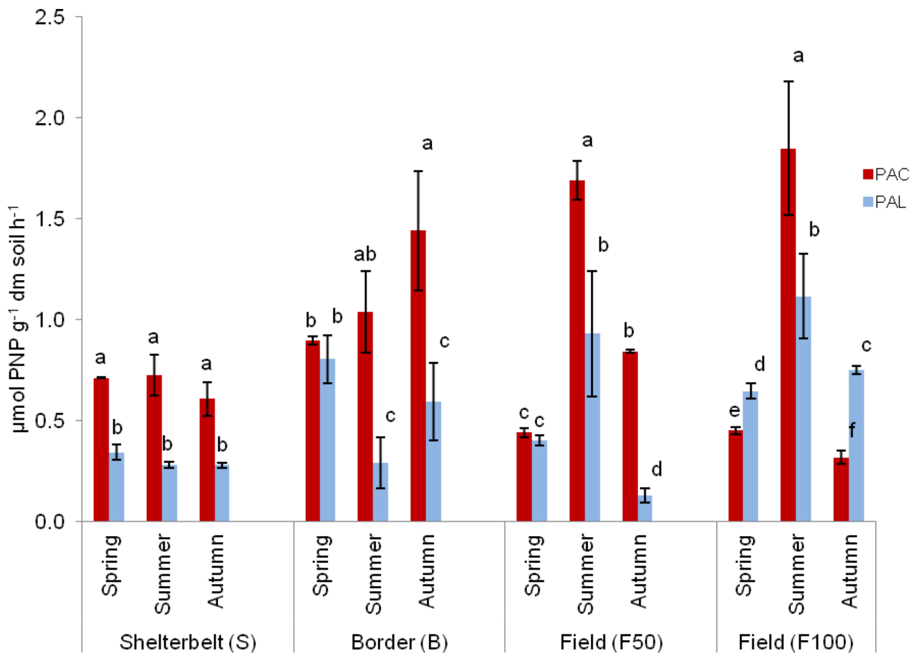
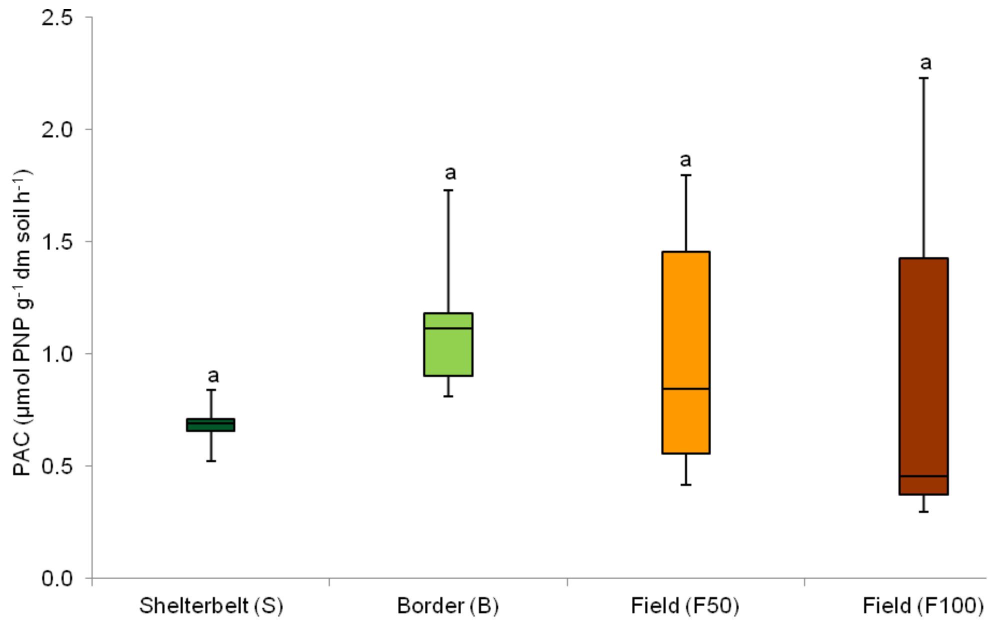
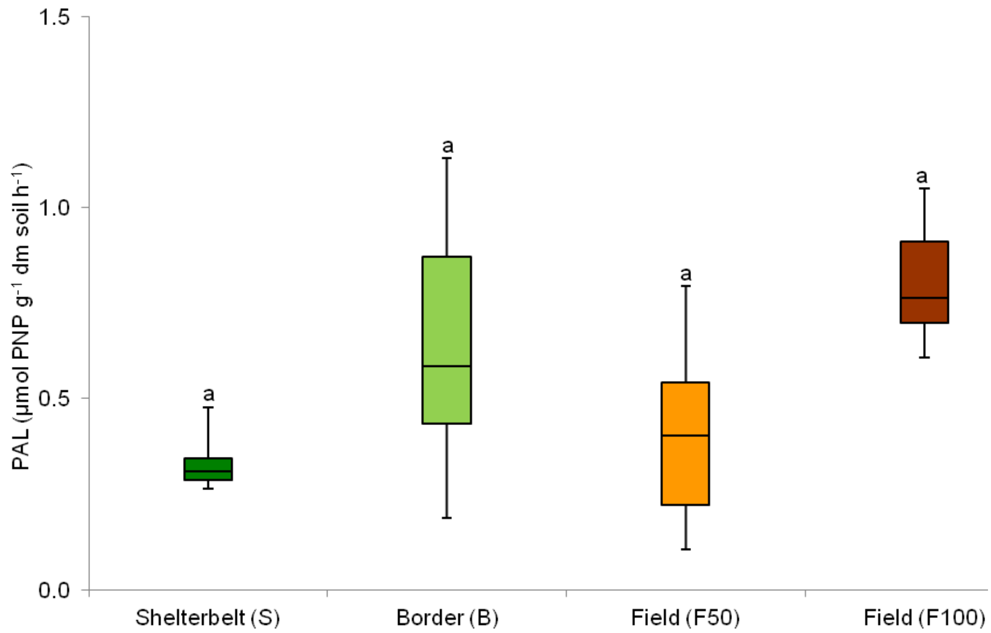


Figure 6. Phosphatase acid (PAC) and alkaline phosphatase (PAL) activity in Luvisols on three research dates (average values ± standard deviation; $n = 3$). Different letters indicate significant differences; ANOVA carried out separately for measuring point and sampling time, $p < 0.05$.

Analyses of the acid and alkaline phosphatase activity showed no significant differences in the studied soils along the measurement transect from S to F_{100}. The research revealed that the level of acid and alkaline phosphatase activity was the lowest in the shelterbelt (S). On the other hand, the soil of the field with winter triticale cultivation at 100 m (F_{100}) from the border with the shelterbelt (B) demonstrated high activity of both acid and alkaline phosphatases (Figure 7a,b).



(**a**)



(**b**)

Figure 7. Box plots and results of one-way ANOVA showing the effect of soil use on PAC (**a**) and PAL (**b**) activity level along the measurement transect. The top hanging bar represents the high edge, and the lower hanging bar is the lower edge; – median; □ quartile 25–75%; significant difference at $p < 0.05$; $n = 9$; the same letters indicate no statistical differences.

To determine the relationship between the studied variables, a linear Pearson's correlation analysis was performed at the significance levels $p \leq 0.01$ and $p \leq 0.05$. Our research showed that the level of activity of DHA was positively correlated with soil pH ($r = 0.61$ $p \leq 0.05$). On the other hand, it was negatively correlated with moisture ($r = -0.56$ $p \leq 0.05$), SOC content ($r = -0.57$ $p \leq 0.05$), and N_{total} ($r = -0.48$ $p \leq 0.01$). PAC activity was negatively correlated with moisture ($r = 0.58$ $p \leq 0.05$), while the level of PAL activity was positively correlated with pH_{KCl} ($r = 0.53$ $p \leq 0.05$) and negatively correlated with the SOC content ($r = 0.47$ $p \leq 0.01$) (Table 3).

Table 3. Pearson's linear correlation results for independent variables.

Variable	pH_{KCl}	Moisture	CEC	SOC	N_{total}
DHA	0.61 **	−0.56 **	n.s.	−0.57 **	−0.48 *
PAC	n.s.	−0.58 **	n.s.	n.s.	n.s.
PAL	0.53 **	n.s.	n.s.	−0.47 *	n.s.

* significance level $p \leq 0.01$; ** significance level $p \leq 0.05$; n.s., not significant.

4. Discussion

The lowest values of pH_{KCl} were recorded in the shelterbelt (S) soil. pH was very acidic, which is unfavorable for most microorganisms [41]. According to Piotrowska et al. [42], the increase in soil acidification is closely related to the content of organic matter and biological activity. Organic matter undergoes the processes of mineralization and humification, thus providing acidic products that penetrate the soil with rainfall [43].

The low pH of the shelterbelt (S) soil may be caused by the sorption of significant amounts of H^+ and Al^{3+} by the soil sorption complex [44]. Earlier studies carried out in the same area showed that the shelterbelt (S) soil demonstrated high exchangeable acidity [12]. Our research revealed that the pH increased along the measuring transect from B to F_{100}, showing a slightly acidic pH under triticale. This proves the increasing anthropogenic pressure on arable land. Our own research showed a high proportion of basic cations in the soil of the cultivated field. The dominant cation was calcium [14]. Kobierski et al. [45] presented similar relationships in arable soils.

The content of SOC and N_{total} in the shelterbelt (S) soil were much higher than in the winter triticale field. This can be attributed to the fact that the total plant fall remains in the shelterbelts. The lack of ploughing might have also contributed to this fact. In addition, the accumulation of organic matter is larger in the shelterbelts than in cultivated fields [46,47].

Low SOC and N_{total} values in the soil of winter triticale are caused by conventional agrotechnical practices related to the soil tillage system, crop rotation, or fertilization [48]. Crop residue is a very important component of the stability of cultivated fields. It enriches the soil with minerals and substances that bind soil aggregates and is a source of easily available carbon for microorganisms. Crop residue also contributes to the increase in the content of organic matter in the soil, which improves the water capacity and thus provides good conditions for the development of plants and microorganisms [49].

The moisture conditions in the soil essentially determine its hydrophysical and microbiological properties. Soil moisture is one of the most important physical parameters in agriculture, and it has a decisive influence on the quality and quantity of crops [33]. Midfield shelterbelts change the microclimatic conditions, especially the wind speed over the fields, and thus affect the soil moisture [5]. The water content in soil is described by the pF curve, which provides information about the water retention capacity of soil pores [50]. The water content potentially available to plants is defined as the amount of water maintained in the soil in the range from pF = 2.0 to pF = 4.2. Research has shown that the best retention properties are found in soils with higher organic matter content [51].

Some authors have proven that crop residue entering the soil is the main factor of variability in soil enzymatic activity [52].

According to Bielińska et al. [53], the classic field cultivation affects both the activity of enzymes and the number of microorganisms by changing the chemical composition, the content of organic matter, and air and water conditions in the soil environment.

Dehydrogenases play a significant role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors [54]. Active dehydrogenases are present only in living cells [55,56]; therefore, the activity of dehydrogenases is considered to be an indicator of the number of microorganisms in the soil [26].

Soil studies carried out along the measurement transect from S to F_{100} show that dehydrogenases are enzymes that demonstrate strong fluctuations in activity depending on the season. It is related to the dynamics of the activity of soil microorganisms. The temperature change in spring is strongly related to the increase in the activity of microorganisms, which indirectly influences the activity of DHA and contributes to its slight increase. Our own research showed high activity of DHA in winter triticale soil in summer. Considering that active dehydrogenases are present only in living cells of microorganisms, its activity was highest at 20–30 °C, which is close to the optimal temperature for the growth and activity of soil microorganisms [57].

Interestingly, it is not only the amount of organic matter that is important but also its quality. Organic matter affects the supply of energy for the growth of microorganisms and, at the same time, the production of enzymes, including DHA. Our research showed that there was low level of DHA activity in the shelterbelt (S) soil. This soil demonstrated high content of SOC, but it had a low, very acidic, pH, which influenced the level of DHA. It was probably also related to the low number of soil microorganisms, as demonstrated by previous studies [58]. According to the authors of [59], the weakening of the enzymatic activity in the soil along with the increase in soil acidity is the result of the destruction of ionic and hydrogen bonds in the enzyme active site. Studies by Brzezińska et al. [56] have also shown that the best conditions for DHA exist when the pH ranges between 6.6 and 7.2. Our studies carried out in the field with winter triticale (F_{50} and F_{100}) showed that DHA also reached high levels at lower pH values between 6.15 and 6.24.

Phosphatases belong to the group of enzymes catalyzing the hydrolysis of organic phosphorus bonds. Acid and alkaline phosphatase activity changed depending on the distance of the studied measurement points (B, F_{50}, and F_{100}) from the shelterbelt (S). According to Lemanowicz et al. [60], the higher activity of acid phosphatase results from the fact that phosphatases are the enzymes that are most sensitive to changes in soil pH. Ciereszko et al. [61] suggest that root systems of plants with phosphorus deficiency demonstrate increased secretion of acid phosphatase into the soil. Furthermore, Nannipieri et al. [29] found that the deficiency of this macronutrient stimulated the secretion of acid phosphatase by plants. On the other hand, the activity of alkaline phosphatase significantly increased in the cultivated field within 100 m (F_{100}) from the shelterbelt (S). This could be caused by the increased activity of soil microorganisms stimulated by organic phosphorus compounds released into the soil by triticale. Waldrip et al. [62] noticed a similar dependence in their research, i.e., the content of organic phosphorus forms was correlated with the activity of alkaline phosphatase in soil. Phosphatases also show the fastest reaction to an increase in the content of heavy metals in soil [63]. The research by Mocek-Płóciniak [64] also reveals that low concentrations of metals may stimulate the activity of phosphatases, while their large amounts reduce the number of microorganisms secreting this enzyme.

5. Conclusions

Environmental factors that have a positive effect on enzymatic activity include pH, moisture, SOC content, N_{total}, season, temperature, and fertilization. The enzymatic activities of DHA, PAC, and PAL in the analyzed soils were clearly differentiated depending on the distance from the shelterbelt. They were characterized by variability during the growing season. The highest activity levels of DHA, PAC, and PAL were found in the soil under the winter triticale in summer, which then decreased in autumn. Such a trend in the variability of the enzymatic activity might have been caused by both human activity and the variability of weather conditions.

Research in this area should be continued as it will allow to relatively determine the influence of various environmental factors on DHA, PAC, and PAL in soil. To conclude, it can be stated that DHA is a sensitive bioindicator that reacts quickly to changes in the soil environment.

This research also shows that shelterbelts adjacent to cultivated fields are good examples of sustainable management of agricultural land. As is well known, on the one hand, they reduce surface runoff from agricultural fields, and on the other hand, they reduce evaporation from fields in the part sheltered by trees by changing the wind speed in the fields adjacent to them. The influence of trees on groundwater chemistry is of particular importance for protective measures aimed at increasing landscape resistance to degradation and limiting the spread of area pollution generated by agriculture.

Agriculture is crucial for ensuring food security, and therefore it is important to establish and maintain shelterbelts in agricultural areas.

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