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Depth-Related Changes in Soil P-Acquiring Enzyme Activities and Microbial Biomass—The Effect of Agricultural Land Use/Plant Cover and Pedogenic Processes

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Abstract: Although the phosphatase enzymes regulate phosphorus (P) turnover throughout the soil profile, at present, they are rarely studied and are less well understood in the deeper soil layers than in the surface horizons. Hence the changes in P-associated soil properties were assessed throughout five Phaeozem profiles for different agricultural land uses including alfalfa, winter wheat, grapevine, apple trees and hops. The acid phosphatase (AcP), alkaline phosphatase (AlP) and phosphodiesterase (PDE) activity was assayed, as were the microbial biomass carbon (MBC) and phosphorus (MBP) contents and also other properties (e.g., available phosphorus, total organic carbon, total nitrogen). We have also determined the mass, length and surface area of the plant roots. In general, the activities of the studied enzymes were highest in the topsoil in four out of the five profiles studied, which corresponded to the highest level of root mass. The older the plant, the greater the root mass and increased enzymatic activity in the deeper horizons of apple trees and hop profiles in comparison to the surface layers. The greatest enzymatic activity, MBC and MBP contents were found in the horizons with a TOC content >0.5% and decreased down the soil profiles similarly to the changes in TOC and TN contents. While the studied properties were determined to varying degrees by means of the organic C content and availability in all of the genetic horizons, the influence of the prevailing conditions and the factors related to soil depth and pedogenic processes were less pronounced. The clay content was related to a significant extent to all of the studied enzyme activities, but only in horizons with a TOC content <0.5%. Significantly higher phosphatase activity under aerobic as opposed to anaerobic conditions were determined in this study, while the opposite trend was found for the content of MBC and MBP as well as the ratio of MBC/MBP. Overall, we pointed out the complex effect of the soil depth, soil forming-processes and cultivated plants on soil P-associated enzyme activities and other properties throughout the soil profiles. This knowledge will allow better understanding of the state of enzymes and their contribution to the biogeochemical cycle of soil P, especially in subsoils, where the enzyme activities follow different patterns than those in the surface horizons.

Keywords: cultivated plants; land management; microbial biomass; phosphatase activity; soil profile; soil-forming processes

Citation: Długosz, J.; Piotrowska-Długosz, A.; Siwik-Ziomek, A.; Figas, A. Depth-Related Changes in Soil P-Acquiring Enzyme Activities and Microbial Biomass—The Effect of Agricultural Land Use/Plant Cover and Pedogenic Processes. *Agriculture* **2022**, *12*, 2079. https://doi.org/10.3390/agriculture12122079

Academic Editor: Barbara Futa

Received: 29 October 2022
Accepted: 1 December 2022
Published: 3 December 2022

1. Introduction

Organic phosphorus (Po) forms may contain between 30 and 60% of soil total P and are grouped into some classes of compounds [1]. Orthophosphate esters are classified according to the number of ester bonds (monoesters such as sugar phosphates, mononucleotides and inositol phosphates as well as diesters, e.g., phospholipids, RNA and DNA). Orthophosphate anhydrides, that include both organic and inorganic compounds, consist

of linear chains of orthophosphates [2]. Phosphonates differ from other soil organic P compounds since they consist of two hydroxyl groups as wells as P=O bonds and stable covalent C-P bonds [3]. Although phosphonates account for less than 3% of the entire soil P, they are an important source of P for microorganisms in P-limited soils since they are the components of extracytoplasmic macromolecules such as exopolysaccharides, phosphonolipids and glycoproteins [4]. Phosphonates occurring in soil originate form biogenic processes or came from anthropogenic input (e.g., herbicides). Phosphonates in soil are strongly adsorbed on the soil mineral surface, increasing their ability to degrade by soil microorganisms via the enzymatic degradation (biodegradation) as well as chemical and/or oxidative degradation [5]. The main path for microbial breakdown and utilization of phosphonate to get nutrients for growth and development is across the C–P lyase enzymatic hydrolysis [6].

Phosphorus is a crucial element that controls microbial and plant growth and is often more limited in terrestrial ecosystems than nitrogen [7]. However, mechanisms causing P limitation as well as their relationships with N status in soils, are less known [8]. The N limitation is often found in young soils (when symbiotic N fixation is restricted and when P input from rock weathering is high comparative to biological N fixation), while P limitation prevails rather in geologically old sites (due to the weathering exhaustion) [9]. As regards profile studies, the microbial P limitation was found to increase with depth of soil profiles, parallelly to the decrease in soil P availability in deep soil horizons [10]. In the P limited sites, where the availability of inorganic P is low, soil microbial communities may preferentially allocate resources to synthetize P-acquiring enzymes to prevent limitation by increasing the enzymatic hydrolysis of organic phosphorus [11]. The availability and limitation of soil nutrients have been recently assessed by the enzymatic stoichiometry, which allows determination of the microbial C, N and P acquisition and nutrient limitations [12]. Herold et al. [13] elaborated three ratios of enzyme activities to assess changes in resource allocation considering various soil horizons, e.g., ratio of β-glucosidase activity to phosphatase activity, which was considered as an indicator of potential C/P acquisition activity.

Among the phosphatases present in the soil, phosphomonoesterases (PMEs) have the main role in the mineralization of organic phosphorus. This group of enzymes catalyses the hydrolysis of anhydrides and esters of orthophosphoric acid into inorganic phosphates, which are directly available to soil microorganisms and plants [14–16]. The difference between acid and alkaline phosphatases is based upon their main sources, substrate specificity and the differences in optimum pH at which these enzymes are active. And thus, acid phosphomonoesterase (EC 3.1.3.1) which originates mainly from fungi and plant roots, displays low substrate specificity and is one of the most important group of enzymes involved in mineralization of organic P in soils with a pH below 7.0 [17]. On the other hand, the alkaline phosphomonoesterase (EC 3.1.3.2), which is mainly produced by soil bacteria and fauna, is active in accessing phosphorus bound in organic compounds in alkaline conditions [18]. According to some authors [19], acid phosphatase is more widespread than alkaline phosphatase at soil pH values that are the most characteristic for natural soils. In addition to phosphomonoesterases (PMEs), phosphodiesterase activity (PDE) (EC 3.1.4.1) has also been detected in soils. These enzymes hydrolyse phosphate diesters, such as nucleic acids and phospholipids to phosphate monoesters, which are further hydrolysed by the PMEs to free ortophosphates [20]. Although it is increasingly clear that phosphate diesters constitute a great proportion of the fresh organic P inputs into the soil [21], phosphodiesterases are studied far less in the soil than PMEs [22]. Since both phosphomonoesterases and phosphodiesterases are necessary for the release of inorganic P in the soil, as they catalyse a distinct step of the Po mineralization pathway, they are therefore considered to be enzymes of great agricultural and economic importance [23,24].

Soil microbes play a crucial role in phosphorus transformation in different ways. On the one hand, microorganisms secrete protons, carboxylates and enzymes, which mobilize organic and inorganic P [25,26]. On the other hand, they can immobilize soil P into the

microbial biomass P [27]. Microbial phosphorus (MBP) is considered to be an important component of the total soil P since it is equivalent to, or exceeds, the P content of the plant biomass [28]. The MBP in bulk soil is generally estimated to account for 2–10% of the total soil P, although in the surface soil, the P content can reach as high as 40–50% [29]. Biomass P is believed to be released directly from the cells when microorganisms die; they decompose and it becomes readily available to plants. Some authors consider MBP to be a better indicator of soil P availability than other P forms [30].

Many researchers have only measured the extracellular enzyme activities (EEAs) in the surface soils despite the large quantities of organic C and P as well as other nutrients found in subsoils. It was found that microbial biomass and the EEAs as well as their substrates usually decline with profile depth, but they are all the time substantially active in deep soil layers [31–33]. Concerning the conditions mentioned above, the restrictions of research regarding surface horizons not only limits the knowledge concerning the functional diversity of soil microorganisms and changes in the activity of soil enzymes, but also restricts our understanding of organic matter transformation. Although some attempts have been made to determine the variations in microbial biomass, enzymatic activity and nutrient cycling in different soil profiles, the importance of the factors that affect the extent of these parameters within a particular profile under different land use has not been explained in full, to date [34]. The enzyme activities at a certain depth may reveal different patterns than those in the surface layers, given that enzymes at that depth are less prone to environmental disturbance [35], subsoils have a greater spatial heterogeneity of organic substrates than topsoils [36], and the microbial communities at depth are dominated by oligotrophic microorganisms [37].

One of the key biotic factors affecting the enzymatic activity is the type of plant cultivated, which may differ in root biomass and structure along the soil profile [38]. As a general rule, all plant roots may exude extracellular enzymatic proteins and enhance the activity of soil microbial communities [39], nevertheless, differences in root structure, mainly with regard to the amount of small roots and their metabolic activity, seem to be pivotal in shaping the soil microbial content and diversity, but also in changes in enzymatic activity [40]. Because plant species may have a positive influence over microbial biomass and activity along the soil profile, there is a demand to determine the effect of plant species differentiation, e.g., plants that have a contrasting morphology with regard to their root systems, on EEAs with depth [31,41].

A number of investigators [33,42] have stated that the soil pedogenic processes across the soil profiles have a substantial effect on various soil physico-chemical and microbiological features. In turn, the nature of the relationship between soil-forming processes and various soil properties occurring in deeper soil layers is less recognized. The status of the soil biological properties in surface horizons is closely associated with the content and quality of soil organic matter (SOM) [42,43]. The rate of SOM decomposition in the surface horizons is often much higher than in the deeper, saturated and anaerobic soil layers [44]. In the gleyic horizons, the enzymatic and microbial properties can be directly affected by changes in the air–water conditions, but mainly by water saturation, which leads to a depletion of oxygen and, as a consequence, to changes in the key soil properties. The facultative and obligate anaerobic microorganisms are the main decomposers of SOM in the saturated and anaerobic soil layers and decomposition occurs at a much slower rate than in the surface horizons [44]. The lessivage process may affect the vertical distribution of soil P as well as the activity of P-related enzymes within the agricultural and horticultural soil [33,45]. Lessivage is understood to bring about a substantial vertical transfer of particles of less than 2 μm (clay, iron oxides, humus compounds) from the surface horizons to the deeper layers [46]. These particles, mainly those of clay minerals due to their specific features such as a large specific surface area which is often predominantly negatively charged, retain nutrients against leaching and react with hydrogen and aluminium ions, while buffering the soil against extreme pH changes [47]. They can also affect the microbial communities of the soil, which is its main source of enzymes as well as determining the

EEAs themselves [48]. The enzymatic activity could be increased, or at least preserved, due to adsorption on clay minerals, which maintain their structure, which permits enzymes to keep their catalytic abilities [49].

The aim of this study was to investigate the effect of different cultivated plants and soil-forming processes/soil depths on soil P-related properties (P-acquiring enzymes, available and microbial biomass P content) across the studied soil profiles. For the purpose of the study, five Phaeozem profiles which were developed from the same raw material (glacial till), but differed due to soil management and plants cultivated (alfalfa, winter wheat, grapevine, apple trees, hop) and consequently had different physico-chemical properties, were compared. We hypothesized that (1) the potential activity of the P-transforming enzymes would be highest in the surface horizons because of the accumulation of organic matter and that the enzymatic activity would decrease down the soil profiles, because the parallel decline in SOM content gives lower quantity of organic substrate for enzymatic transformation, (2) apart from the SOM content (C availability), other soil properties, such as clay content, soil pH and bulk density as well as oxidative and reducing conditions are also important drivers of the differences in enzymatic and microbial properties across the soil profiles, (3) although the effects of vegetation cover on EEAs is expected to be most predominant in the topsoil layers, a plant species with a contrasting root biomass and structure would also change the properties found along the soil profile; we therefore hypothesize the effects of plant species on EEAs throughout the depth profile.

2. Materials and Methods

2.1. Study Area and Soil Collection

The sampling sites were located in selected mesoregions of the South Baltic Lake District (Central Poland). The studied soils were developed on the same glacial till (deposited during the Vistulian glaciations) but they vary in a history of agricultural use, namely in long-term soil management and cultivated plants. The climate is temperate with temperatures below zero in the winter and a mean annual temperature of 7 °C. The mean annual precipitation is 550 mm year^{-1}.

Profile 1 was excavated in Salno (53°9′9.8″ N, 15°25′56.6″ E), which is located in the Chełmno Lakeland (located at Chełmno-Dobrzyń Lakeland), while profile 2 was excavated from Chełmce near Kruszwica (53°37′36.3″ N 18°25′34.5″ E), which is the part of the Inowrocław Plain. Profile 3 was located in Malice, near Kcynia (52°59′30″ N 17°29′17″ E), situated in the Kujawy-Pomerania Province. Samples for profile 4 were dug in Wtelno (53°14′24.2″ N, 17°54′14.7″ E), which is in part of the mesoregion, Krajna Lake District (Kujawy-Pomerania Province). Profile 5 was excavated from Malice (52°59′48.7″ N, 17°31′20.2″ E) which is located in the Chodzież Lake District (Kujawy-Pomerania Province). Profile 1 was dug in a field with alfalfa (AA) (*Medicago sativa* L.) after 4 years of its cultivation, profile 2 was sourced from a field experiment concerning agricultural practices in 12-year-long winter wheat monoculture (after the harvest in August) (W), profile 3 came from a vineyard (GV), profile 4 came from an apple trees orchard (AT), while profile 5 was located in a hop plantation (H). The studied profiles differ in the morphology of their genetic horizons. The Mollic Stagnic Gleyosol (MSG) (profile 1) has mollic horizons with a high thickness value and lithological discontinuity in the 2ACgg layer, in which an accumulation of the clay fraction of lithological origin was found. The Cambic Stagnic Phaeozem (profile 2) is a bipartite soil. The upper horizons (up to 90 cm) were formed from the glacial till, while in the deeper layers fluvioglacial sands occur. The increased clay fraction in the BCkg and Gk horizons is not of illuvial origin because no traces of the lessivage process were found. Carbonates in the BCkg and Gk horizons originate from both the parent material (original) and from the process of precipitation from groundwater (secondary). The BCkg and Gk horizons are characterized by an increased Fea content, which may originate from the weathering of primary minerals (braunification) or it may be the effect of precipitation during the redox processes which take place on the border of the oxidation and reduction zones during the fluctuation of groundwater levels. The Eutric

Loamic Regosol (profile 3) is a soil in the early stages of development which is formed from the carbonate glacial till (carbonates occur across the soil profile). Humification was the only soil-forming process found in this profile. In the Stagnic Luvic Phaeozem (profile 4) the argic horizon was found at a depth of 40–82 cm, this was confirmed by the increased amount of the clay fraction. This profile is characterized by the thickness of the mollic horizon. In addition, the Cambic Phaeozem (profile 5) revealed the high thickness value of the mollic horizon (66 cm). The cambic diagnostic horizon was characterized by the increased content of iron oxides and clay fraction which were found below the humic horizons. No traces of the illuvial process were found in this profile.

Alfalfa (*Medicago sativa* L.) was fertilized each year with 50 kg of phosphorus (P) and 100 kg of potassium (K) in the form of Lubofoska, (P-12%, K-24%) and Korn-Kali (K-40%). The fertilizers were used at two rates; after the first and second harvests of alfalfa. The mineral fertilization of winter wheat included nitrogen as NH_4NO_3 (N-34%) and urea (N-46%), and was divided into three doses (pure ingredient): 40 kg ha^{-1} was applied before the sowing of winter wheat, 60 kg ha^{-1} was used as a top dressing at tillering, and the last application was made (40 kg ha^{-1}) during the shooting of winter wheat. Mineral phosphorus (P) and potassium (K) (in the form of Lubofoska) were applied in the autumn at doses of 50 kg ha^{-1} and 80 kg ha^{-1} (pure ingredients), respectively. The W profile was excavated from the plot after a single ploughing where the seeder–cultivator unit was used and straw was removed. The cultivation system in the apple orchard was traditional, with mowed green grass between the rows and with herbicide-treated fallow in the tree rows. The orchard was fertilized with 120 kg ha^{-1} of mineral N (as NH_4NO_3) at the beginning of the vegetation period (mainly occurring at the end of March) and at a dose of 70 kg ha^{-1} after flowering. Additionally, fertilization with 150 kg ha^{-1} of KCl and 100 kg ha^{-1} of $Ca(H_2PO_4)_2$ was applied in the spring. The hops plantation was fertilized with mineral P (60 kg ha^{-1}) and K (180 kg ha^{-1}) in the autumn as well as with N (as NH_4NO_3) at a dose of 150 kg ha^{-1} (50% of the dose was applied in the spring and 50% in the summer). The cultivation system in the hop plantation had a mechanical fallow (in plant rows and between them) to prevent an excess of water evaporation and for the maintenance of adequate soil aeration.

Soil samples in the studied profiles were taken according to their genetic horizons and in each horizon, five blocks were selected with dimensions that were dependent on the horizon thickness. We have collected three soil samples from the middle part of each block (to avoid the edge effect) and mixed them to make one bulk sample. The five composited samples were analysed individually for soil properties and the mean value for each block was statistically analysed. The surface and subsurface samples were treated differently. The soil samples collected from the surface layers were placed in plastic boxes, which allowed for gas exchange, and chilled to 4 °C in order to minimize any possible changes in their microbial populations. The samples taken from the deeper soil layers were put into sealed containers which generated an atmosphere with a reduced oxygen content and were also chilled to 4 °C. The samples were sieved through a sieve with a mesh of 2 mm and divided into two parts. First part was used for physicochemical analyses after air-drying, and the second part was stored in field-moist conditions in the fridge and used within two weeks for determination of microbial and enzymatic properties.

The plant root mass and morphology (length, surface), were determined in soil samples that were collected from each genetic horizon using a core sampler (diameter 10 cm, 250 cm^3). Three corings were performed per one horizon and the root biomass and morphological parameters were assessed individually for each coring. Finally, the mean values for each of the three corings were presented.

2.2. Laboratory Assays

2.2.1. Soil Physicochemical Properties Assessment

The set of physicochemical properties was assayed using the standard methods and three replications of each sample were performed. The soil water content (SWC) was

determined by drying at 105 °C for 2 days. The particle size was defined according to the method of Casagrande, in the modification of Prószyński, and the content of sand fraction was determined according to a sieving method [50]. The soil reaction was measured in a solution of 0.01 M $CaCl_2$ using the potentiometric method [51], whereas the hydrolytic acidity (Hh) was determined in 1M CH_3COONa. The contents of total organic carbon (TOC) and total nitrogen (TN) were assessed using a dry combustion CNS analyser (Vario Max CNS). Dissolved organic carbon (DOC) concentration was measured after extraction in 0.004 M $CaCl_2$ using a soil-to-extractant ratio of 1:10 (w/v) over 1 h. The DOC content was determined with a Multi N/C 3100 Analityk Jena analyser (Jena, Germany), and was expressed in mg C kg^{-1} of the dry weight of a soil sample and as a percentage of TOC content. The method of applying volume cylinders (100 cm^3) was used to assess the bulk density. We have calculated the cation exchange capacity (CEC) as the sum of the hydrolytic acidity and basic saturation. The concentration of plant available P (Pavail) was determined using spectrophotometry after colour development with ammonium molybdate and $SnCl_2$ after extraction in 0.1 M ammonium lactate (pH = 3.7) [52]. The concentration of $CaCO_3$ was determined by the dissolution of the carbonate in an excess of 1N HCl. The amount of remaining acid was measured by titrating it with 1N NaOH. After extraction, the content of amorphous iron oxides (Fea) was extracted with ammonium oxalate and determined according to the method proposed by Tamm [53] by applying the ASA technique by using a Philips 9100PU apparatus (Amsterdam, The Nethedlands) [54].

2.2.2. Determination of Enzyme Activities and Microbial Biomass

Moist soil samples were taken to assess the enzyme activities. The activity of both phosphomonoesterases (AcP, AlP, respectively) and phosphodiesterase (PDE) was assayed according to Tabatabai and Bremner [55] and Browman and Tabatabai [56], respectively. The amount of 1 g of soil samples (d.w.) was incubated in 4 mL of modified universal buffer (MUB, pH = 6.5 for AcP, pH = 11 for AlP, pH = 8 for PDE), and 1 mL of 15 mM *p*-nitrophenyl phosphate disodium for both AcP and AlP and 5 mM bisnitrophenyl-*p*-nitrophenyl phosphate for PDE, at 37 °C for 1 h. The reaction was stopped by the addition of 0.5 M NaOH (4 mL) and 0.5 M $CaCl_2$ (1 mL), the suspension was mixed and filtered. Finally, the filtrate was analysed using UV spectrophotometry (410 nm). The activity of the studied enzymes was expressed as mM *p*-nitrophenol kg^{-1} soil h^{-1} (mM *p*NP kg^{-1} h^{-1}). The content of microbial biomass C (MBC) and P (MBP) was quantified according to the chloroform fumigation–extraction protocol [57,58]. Fresh samples of soil were fumigated with ethanol-free chloroform at 25 °C for 1 day. Subsequently, the $CHCl_3$ was removed. In order to determine MBC both fumigated and controls were extracted with 0.5 M of K_2SO_4 and assessed for soluble C, according to the protocol of Vance et al. [58]. The correction factor of 0.38 (kEC) was used to account for any incomplete recovery of MBC. [58]. Additionally, we have calculated the MBC/TOC (%) and the MBC/DOC (%). In order to determine the phosphorus of microbial biomass (MBP) both the fumigated and control samples were extracted with 0.5 M $NaHCO_3$ (pH 8.5) within 30 min at a solution:soil ratio of 4:1. Then, the suspensions were filtered and P concentration in the filtrate was determined colorimetrically [59]. A correction for the incomplete extraction of Pi released by chloroform was made by determining the percentage of the recovery of a known quantity of Pi spiked (50 μg P g^{-1} soil) in the $NaHCO_3$ solution followed by the extraction of a control (non-fumigated) soil. The microbial biomass P was calculated using the following formula: MBP = Ep/Kp, where Ep is the increase in extractable P in fumigated soil over that in the control and Kp is the fraction of MBP that is extracted after fumigation. A Kp factor of 0.4 was applied as was proposed by Brookes et al. [57]. Additionally, the MBC/MBP ratio was also calculated.

2.2.3. Determination of Root Biomass and Morphology

The roots were separated from the soil using a combination of hand-sorting and wet-sieving. Once the roots were washed, their fresh weight was assessed immediately. Deter-

mination of the morphological structure of the roots (length and surface area) were carried out using a root automated scanner EPSON EXPRESSION (Suwa, Japan) 10,000 XL fitted with WinRhizo software package (Regent Instruments Inc., Quebec City, QC, Canada) [60]. After scanning, the roots were placed in the dryer and left at 70 °C until they obtained constant weight.

2.2.4. Data Analyses

The dataset was not normally distributed, which is why it was transformed via Gaussian anamorphosis (GA). The applied transformation enhanced the normality of the results and that is why the further analyses were carried out with the transformed results. A one-way analysis of variance was performed to estimate the changes in soil properties down the soil profiles. The second one-way analysis of variance was carried out to compare the values of the studied properties in the genetic horizon occurring at the same/similar depth in order to compare the effect of, e.g., vegetation covers (alfalfa, winter wheat, grapevine, apple trees, hop), oxidation–reduction conditions as well as to show the connection between the studied properties. An analysis of variance was performed for randomized block design where the profiles were treated as blocks. We have used the Tukey test ($p < 0.05$) to assess significant differences between the means. Soil depths, genetic horizons were independent variables, as were plants, while the studied properties (e.g., enzymatic activity, MBC content) were the dependent variables. The Pearson's correlation analysis was performed to exhibit the connections between the studied properties and results are displayed as a heatmap. The differences between the soil samples were analysed using principal component analysis (PCA) based on the raw dataset. The first two principal components (PC1 and PC2) were selected for the ordination of the cases. We have performed all statistical analyses using Statistica 13.1 for Windows software (TIBCO Statistica Inc., Tulsa, OK, USA) [61].

3. Results

3.1. Physico-Chemical Properties throughout the Soil Profiles

The content of clay ranged widely between 2% and 25%, while the highest content was noted in the middle layers (2ACgg, CK, Ck1, Btg, AB) and also in the deepest horizon of the grapevine profile (Table 1). The silt content, which ranged from 20 to 52%, was highest in the subsurface layers of profiles 1 and 2 and the deepest horizons of profiles 3 and 5. The soil pH in $CaCl_2$ ranged from moderately acidic to moderately alkaline (6.29 to 8.36) and increased significantly with depth in the grapevine, apple trees and hop profiles. No significant changes in pH were found in the alfalfa and winter wheat profiles. No clear trends in depth-related changes were found for Hh. In general, the highest Hh levels were found in the surface and subsurface horizons (Ap, A2, A) of the studied profiles, with the exception of the grapevine profile. In this profile, the Hh value was highest in the deepest layer as compared to the four horizons closer to the surface. The CEC values ranged broadly between 60.7 and 576.0 mmol kg^{-1} in the studied profiles and no clear trends in depth-related changes were found for this property. The $CaCO_3$ was present in each horizon for the winter wheat and vineyard profiles, while in the orchard and hop profile, it was only determined in the deepest layers. No $CaCO_3$ was found throughout the alfalfa profile. The highest Fea content was noted in the surface horizons of profiles 1, 2 and 4 and the level decreased with depth in these profiles. In turn, in profiles 3 and 5, the highest Fea content to a significant extent was found in the Ck1-Ck3 horizons (profile 3) and in the A2 and AB horizons (profile 5).

Table 1. Depth distribution of selected physico-chemical properties.

Plant	Genetic Horizon	Depth (cm)	Clay	Silt	pH	Hh	CEC	$CaCO_3$	Fea
			(%)			(mmol kg^{-1})		(%)	(g kg^{-1})
Profile 1. Mollic Stagnic Gleyosol (MSG)									
Alfalfa (AA)	Ap	0–30	10 c *	45 a	6.87 a	3.72 a	141.3 a	0.0	1.58 a
	A2	30–53	12 bc	45 a	6.76 a	3.66 a	127.8 b	0.0	0.69 b
	2ACgg	53–70	25 a	41 b	7.01 a	2.60 b	139.3 a	0.0	0.54 c
	3G1	70–110	14 b	33 d	7.16 a	1.09 c	97.6 c	0.0	0.30 d
	3G2	110–150	14 b	38 c	7.31 a	3.43 a	91.9 c	0.0	0.27 d
Profile 2. Cambic Stagnic Phaeozem (CSP)									
Winer wheat (W)	Akp	0–35	9 c	33 c	7.41 a	2.23 a	286.5 c	5.3 c	1.01 a
	BCkg	35–67	21 b	39 a	7.82 a	0.79 b	498.7 b	20.9 a	0.79 b
	Ck	67–90	24 a	36 b	7.71 a	0.20 c	576.0 a	23.7 a	0.58 c
	2Ck1	90–123	7 d	33 c	8.36 a	0.09 d	156.4 d	9.2 b	0.21 d
	2Ck2	123–150	2 e	20 d	8.19 a	0.10 d	60.7 e	4.0 d	0.20 d
Profile 3. Eutric Loamic Regosol (ELR)									
Grapevine (GV)	Ak	0–18	14 b	46 bc	7.19 b	0.75 b	138.3 c	8.6 b	0.92 c
	ACk	18–40	13 b	43 c	7.26 b	0.74 b	137.2 c	8.9 b	0.97 c
	Ck	40–82	17 a	42 c	7.63 a	0.74 b	206.3 a	12.3 a	1.53 a
	Ck2	82–120	14 b	49 ab	7.66 a	0.76 b	159.8 b	11.1 a	1.29 b
	Ck3	120–150	18 a	52 a	7.62 a	1.12 a	121.4 d	8.0 b	1.64 a
Profile 4. Stagnic Luvic Phaeozem (SLP)									
Apple trees (AT)	A	0–18	14 b	49 a	6.67 c	4.00 a	225.4 a	0.0	1.82 a
	A2	18–40	15 b	47 b	7.04 b	1.00 c	130.8 b	0.0	1.91 a
	Btg	40–82	25 a	44 c	7.35 a	1.00 c	91.5 d	0.0	0.97 b
	Ckgg	82–120	15 b	50 a	7.64 a	1.00 c	139.9 b	16.1 a	0.51 c
	Gk	120–150	13.b	46 b	7.66 a	2.00 b	111.9 c	14.5 b	0.50 c
Profile 5. Cambic Phaeozem (CP)									
Hop (H)	A	0–34	7 e	36 d	6.29 c	8.34 a	117.1 d	0.0	0.94 d
	A2	34–66	12 c	42 c	6.57 c	7.78 a	353.3 a	0.0	2.17 a
	AB	66–88	16 a	44 bc	6.87 b	4.77 b	254.0 bc	0.0	1.84 b
	Bw	88–114	15.b	47 b	6.70 b	4.49 b	237.9 c	0.0	1.18 c
	Ck	114–150	10.d	51 a	7.83 a	1.10 c	288.4 b	11.7	0.51 e

*—Various letters designate significant differences ($p < 0.05$) between genetic horizons throughout the same soil profile. Clay—clay content, silt—silt content, pH—soil reaction in $CaCl_2$, Hh—hydrolytic acidity; CEC—cation exchange capacity; $CaCO_3$—calcium carbonate; Fea—amorphous iron oxides. A—surface humic horizon, B—enriched horizon, C—parent material horizon, G—gleyic horizon, AB, AC—transitional horizons, t—illuvial accumulation of silicate clay, k—accumulation of pedogenic carbonates, g—stagnic conditions, gg—strong gleying, Prefixes, e.g., 2 and 3—lithological discontinuity, suffixes, e.g., 2, 3 sub-horizon number

3.2. Soil Phosphorus, Carbon and Nitrogen Forms along the Soil Profiles

The available P content was found to be the highest in the surface horizons (A, Ap, Ak) in profiles four and five (Table 2). In the orchard profile, the highest Pavail content was noted in the subsurface horizon (A2). No clear trends in depth-related changes were found for this property with regard to the deeper soil horizons. Only in the hop profile was the Pavail concentration found to decrease gradually with depth and in the vineyard profile, all horizons below the A layer revealed an insignificant P content. According to the recommendations of Polish Norm (PN-R-04023), the concentration of Pavail in the surface horizons was in a very high class (134.8–231.8 mg kg^{-1}) in the alfalfa, apple trees and hop profiles, while there was a high level (65.8 mg kg^{-1}) in the winter wheat profile and a very low one (13.5 mg kg^{-1}) in the vineyard profile (Table 2). In all of the profiles studied, the content of MBP and MBC was the highest in the surface horizons and decreased gradually with depth with just two exceptions. The MBP content in the apple trees profile was markedly higher in the Ckgg horizon than in the horizon above (Btg). The same result was

found for the MBC content in the A2 and AB horizons of the hop profile. The TOC, TN and DOC contents were generally highest in the topsoils and declined gradually with depth with one exception (Table 2). In the Cambic Phaeozem (profile 5), the TOC and TN contents were significantly higher in the A2 horizon than in the A layer, while DOC content was not statistically differentiated between these horizons.

Table 2. Depth distribution of soil phosphorus, carbon and nitrogen, mean (SE), n = 5.

Plant	Genetic Horizon	Depth (cm)	Pavail	MBP	TOC	TN	DOC	MBC
			(mg kg^{-1})		(g kg^{-1})		(mg kg^{-1})	
Profile 1. Mollic Stagnic Gleyosol (MSG)								
Alfalfa (AA)	A	0–30	231.7 ± 2.2 a*	13.82 ± 0.17 a	17.7 ± 1.54 a	1.96 ± 0.34 a	124.6 ± 5.5 a	145.6 ± 5.9 a
	A2	30–53	47.1 ± 0.6 d	10.19 ± 0.04 b	9.66 ± 0.87 b	0.90 ± 0.11 b	86.5 ± 2.1 b	113.6 ± 5.4 b
	2ACgg	53–70	32.9 ± 0.45 e	3.49 ± 0.21 c	4.02 ± 0.35 c	0.42 ± 0.09 c	78.4 ± 2.5 bc	53.1 ± 4.4 c
	3G1	70–110	86.2 ± 0.61c	3.11 ± 0.02 cd	2.34 ± 0.25 d	0.37 ± 0.09 c	63.7 ± 1.5 cd	25.6 ± 3.4 d
	3G2	110–150	110.8 ± 0.2 b	2.63 ± 0.08 d	2.13 ± 0.33 d	0.29 ± 0.05 d	54.5 ± 2.6 d	16.5 ± 1.0 b
Profile 2 Cambic Stagnic Phaeozem (CSP)								
Winter wheat (W)	Akp	0–35	65.8 ± 0.2 a	40.47 ± 0.44 a	19.1 ± 0.21 a	2.43 ± 0.06 a	142.7 ± 1.6 a	302.1 ± 9.6 a
	BkCg	35–67	9.9 ± 0.2 c	11.51 ± 0.04 b	2.97 ± 0.03 b	0.36 ± 0.01 b	45.2 ± 1.7 b	106.1 ± 6.0 b
	Gk	67–90	9.7 ± 0.1 c	12.53 ± 0.09 b	2.28 ± 0.05 b	0.36 ± 0.02 b	43.3 ± 1.4 b	88.8 ± 3.4 c
	2Gk1	90–123	9.7 ± 0.3 c	7.13 ± 0.07 c	0.68 ± 0.02 c	0.08 ± 0.01 c	29.2 ± 1.0 c	74.3 ± 2.0 c
	2Gk2	123–150	17.7 ± 0.4 b	5.69 ± 0.08 c	0.32 ± 0.05 c	0.08 ± 0.01 c	29.2 ± 0.2 c	46.7 ± 1.6 e
Profile 3. Eutric Loamic Regosol (ELR)								
Grapevine (GV)	Ak	0–18	13.5 ± 0.3 a	38.09 ± 1.06 a	12.11 ± 0.14 a	1.31 ± 0.02 a	118.3 ± 2.0 a	288.8 ± 4.2 a
	ACk	18–40	12.3 ± 0.1 b	12.51 ± 0.12 b	6.42 ± 0.25 b	0.76 ± 0.03 b	94.7 ± 2.5 b	119.9 ± 4.6 b
	Ck	40–82	11.6 ± 0.3 b	6.79 ± 0.04 c	2.14 ± 0.07 c	0.25 ± 0.01 c	57.4 ± 1.3 c	64.2 ± 2.1c
	Ck2	82–120	12.1 ± 0.1 b	3.20 ± 0.04 d	1.59 ± 0.06 d	0.19 ± 0.01 c	38.2 ± 1.1 d	35.9 ± 1.2 d
	Ck3	120–150	12.4 ± 0.4 b	2.41 ± 0.01 d	1.27 ± 0.04 d	0.21 ± 0.01 c	41.1 ±1.0 d	17.9 ± 0.6 e
Profile 4. Stagnic Luvic Phaeozem (SLP)								
Apple trees (AT)	A	0–18	134.8 ± 1.6 b	20.38 ± 0.22 a	27.64 ± 0.44 a	2.41 ± 0.03 a	115.1 ± 2.7 a	233.5 ± 4.0 a
	A2	18–40	178.4 ± 3.8 a	13.14 ± 0.08 b	15.20 ± 0.23 b	1.64 ± 0.06 b	88.9 ± 3.0 b	145.7 ± 3.8 b
	Btg	40–82	14.3 ± 0.1 c	4.81 ± 0.07 d	3.80 ± 0.07 c	0.54 ± 0.02 c	60.9 ± 2.5 c	51.2 ± 1.2 c
	Ckgg	82–120	12.3 ± 0.1 c	5.33 ± 0.04 c	3.71 ± 0.05 c	0.27 ± 0.01 d	41.0 ± 0.9 d	62.1 ± 1.7 c
	Gk	120–150	13.7 ± 0.3 c	4.04 ± 0.05 e	2.51 ± 0.09 c	0.16 ± 0.01 d	47.9 ± 1.2 d	36.0 ± 0.8 d
Profile 5. Cambic Phaeozem								
Hop (H)	A	0–34	146.4 ± 0.6 a	7.09 ± 0.02 a	8.87 ± 0.13 b	0.85 ± 0.03 b	80.8 ± 1.2 a	43.58 ± 2.77 a
	A2	34–66	109.8 ± 0.7 b	6.10 ± 0.17 a	10.40 ± 0.31 a	0.93 ± 0.03 a	76.9 ± 2.3 a	18.07 ± 0.52 c
	AB	66–88	72.0 ± 1.1 c	3.01 ± 0.01 b	4.35 ± 0.10 c	0.41 ± 0.01 c	51.9 ± 1.2 b	36.02 ± 1.62 b
	Bw	88–114	55.2 ± 0.9 d	2.18 ± 0.04 b	2.18 ± 0.06 d	0.24 ± 0.01 d	53.8 ± 1.6 b	18.13 ± 0.36 c
	Ck	114–150	24.7 ± 0.1 e	1.99 ± 0.01 b	0.09 ± 0.01 e	0.04 ± 0.01 e	5.8 ± 0.09 c	7.09 ± 0.23 d

* —Various letters inicate significant differences ($p < 0.05$) between genetic horizons within the same profile. Pavail—available phosphorus, MBP—microbial biomass phosphorus, TOC—total organic carbon, TN—total nitrogen, DOC—dissolved organic carbon, MBC—microbial biomass carbon. Abbreviations of soil horizon names are explained under Table 1.

The amount of TOC, and TN in the hop profile was significantly higher in the A2 horizon in comparison with the surface horizon. The TOC/TN ratio was highest in the surface horizons (A, A2, Ak, AB) and decreased gradually with depth in the alfalfa, grapevine and hop profiles (Table 3). In the apple trees profile, the highest TOC/TN ratio was found in the deepest horizon, while in the winter wheat profile, this occurred in the second from the bottom horizons (2Gk1). The lowest contribution of DOC in TOC was indicated in the surface horizons and increased significantly with depth.

Table 3. Ratios of the studied properties.

Plant	Genetic Horizon	Depth (cm)	TOC/TN	DOC/TOC (%)	MBC/TOC (%)	MBC/DOC	MBC/MBP
Profile 1. Mollic Stagnic Gleyosol (MSG)							
Alfalfa (AA)	A	0–30	9.0 ± 0.4 a*	0.74 ± 0.04 c	0.82 ± 0.09 c	1.17 ± 0.08 a	10.5 ± 0.38 b
	A2	30–53	10.7 ± 0.2 a	0.93 ± 0.03 c	1.18 ± 0.15 ab	1.30 ± 0.04 a	11.0 ± 0.22 b
	2ACgg	53–70	9.6 ± 0.2 ab	2.01 ± 0.08 b	1.32 ± 0.14 a	0.68 ± 0.03 b	15.4 ± 0.82 a
	3G1	70–110	6.3 ± 0.1 c	2.79 ± 0.09 a	1.09 ± 0.12 b	0.40 ± 0.01 c	8.25 ± 0.10 c
	3G2	110–150	7.3 ± 0.3 c	2.67 ± 0.17 a	0.78 ± 0.14 c	0.30 ± 0.02 c	6.32 ± 0.24 c
Profile 2. Cambic Stagnic Phaeozem (CSP)							
Winter wheat (W)	Ap	0–35	7.9 ± 0.4 b	0.75 ± 0.03 d	1.58 ± 0.03 d	2.11 ± 0.09 b	7.74 ± 0.28 c
	BCkg	35–67	8.3 ± 0.1 ab	1.52 ± 0.05 c	3.57 ± 0.18 c	2.34 ± 0.04 a	9.22 ± 0.51 ab
	Gk	67–90	6.3 ± 0.2 c	1.91 ± 0.10 c	3.90 ± 0.07 c	2.06 ± 0.14 b	7.09 ± 0.30 c
	2Gk1	90–123	8.9 ± 0.3 a	4.21 ± 0.02 b	10.8 ± 0.61 b	2.55 ± 0.15 a	10.4 ± 0.38 a
	2Gk2	123–150	4.2 ± 0.3 d	9.14 ± 0.60 a	14.6 ± 0.37 a	1.60 ± 0.06 c	8.22 ± 0.21 b
Profile 3. Eutric Loamic Regosol (ELR)							
Grapevine (GV)	Ak	0–18	9.2 ± 0.3a	0.98 ± 0.01 e	2.38 ± 0.01 b	2.44 ± 0.01 a	7.59 ± 0.17 c
	ACk	18–40	8.4 ± 0.1 b	1.48 ± 0.02 d	1.87 ± 0.02 d	1.26 ± 0.02 b	9.58 ± 0.34 b
	Ck	40–82	8.7 ± 0.1 ab	2.68 ± 0.03 c	3.00 ± 0.01 a	1.12 ± 0.01 c	9.47 ± 0.31 b
	Ck2	82–120	8.3 ± 0.1 b	2.41 ± 0.04 b	2.26 ± 0.01 c	0.93 ± 0.02 d	11.2 ± 0.31 a
	Ck3	120–150	6.1 ± 0.1 c	3.24 ± 0.04 a	1.40 ± 0.01 e	0.43 ± 0.5 e	7.41 ± 0.27 c
Profile 4. Stagnic Luvic Phaeozem (SLP)							
Apple trees (AT)	A	0–18	11.9 ± 0.1 bc	0.40 ± 0.00 e	0.82 ± 0.01 d	2.03 ± 0.02 a	11.5 ± 0.17 a
	A2	18–40	9.4 ± 0.1 c	0.58 ± 0.00 d	0.95 ± 0.01 c	1.64 ± 0.02 b	11.1 ± 0.27 a
	Btg	40–82	6.9 ± 0.1 d	1.62 ± 0.00 c	1.36 ± 0.02 b	0.84 ± 0.01 d	10.7 ± 0.22 a
	Ckgg	82–120	13.9 ± 0.1 b	1.10 ± 0.01 b	1.66 ± 0.02 a	1.52 ± 0.01 c	11.7 ± 0.32 a
	Gk	120–150	16.3 ± 0.1 a	1.86 ± 0.01 a	1.40 ± 0.01 b	0.75 ± 0.00 e	8.92 ± 0.20 b
Profile 5. Cambic Phaeozem (CP)							
Hop (H)	A	0–34	10.4 ± 0.2 ab	0.91 ± 0.02 d	0.49 ± 0.02 c	0.54 ± 0.00 v	6.18 ± 0.12 c
	A2	34–66	11.2 ± 0.3 a	0.74 ± 0.03 d	0.17 ± 0.01 d	0.23 ± 0.01 d	3.21 ± 0.66 d
	AB	66–88	10.6 ± 0.3 a	1.19 ± 0.01 c	0.83 ± 0.01 b	0.69 ± 0.00 b	12.0 ± 0.22 a
	Bw	88–114	9.1 ± 0.3 b	2.47 ± 0.03 a	0.83 ± 0.02 b	0.23 ± 0.01 d	8.32 ± 0.12 b
	Ck	114–150	6.2 ± 2.8 c	1.79 ± 0.35 b	8.72 ± 1.65 a	4.90 ± 0.24 a	3.57 ± 0.07 d

*—Various letters indicate significant differences ($p < 0.05$) between genetic horizons within the same soil profile. TOC—total organic carbon, TN—total nitrogen, DOC—dissolved organic carbon, MBC—microbial biomass carbon, MBP—microbial biomass phosphorus. Abbreviations of soil horizon names are explained under Table 1.

The participation of MBC in TOC was in the broad range of (0.78–14.6%) but no explicit pattern was noted for the MBC/TOC proportion with regard to changes down the soil profiles, except for the winter wheat profile, where the ratio above increased systematically with depth. In the profiles 1, 3 and 4, the highest MBC/DOC ratio was found in the topsoils and decreased gradually with depth, while no clear trends in depth-related changes were found for this property with regard to horizons 2 and 5. The MBC/MBP ratio behaves differently with depth in subsequent profiles and no clear trends were found.

3.3. Depth-Related Changes in the Activities of P-Acquiring Enzymes

As a general rule, the activity of all of the studied enzymes was highest in the surface horizons (Ap, A) in all of the profiles and decreased significantly with depth with the exception of the AcP and AlP activities in the hop profile (Table 4).

Table 4. Depth-related changes in the activities of P transforming enzymes; mean (±SE), n = 5.

Plant	Genetic Horizon	Depth (cm)	AcP	AlP	PDE	AlP/AcP
Profile 1. Mollic Stagnic Gleyosol (MSG)						
Alfalfa (AA)	A	0–30	3.74 ± 0.016 a*	7.30 ± 0.078 a	1.15 ± 0.011 a	1.95 ± 0.023 b
	A2	30–53	0.97 ± 0.003 b	2.35 ± 0.109 b	0.30 ± 0.004 b	2.42 ± 0.014 a
	2ACgg	53–70	0.86 ± 0.026 b	1.91 ± 0.043 c	0.26 ± 0.001 b	1.79 ± 0.011 b
	3G1	70–110	0.79 ± 0.004 c	1.19 ± 0 012 d	0.18 ± 0.001c	1.51 ± 0.015 c
	3G2	110–150	0.69 ± 0.005 d	0.84 ± 0.007 e	0.19 ± 0.002 c	1.22 ± 0.013 d
Profile 2. Cambic Stagnic Phaeozem (CSP)						
Winter wheat (W)	Akp	0–35	4.99 ± 0.022 a	12.70 ± 0.095 a	1.734 ± 0.031 a	2.55 ± 0.013 a
	BCkg	35–67	1.02 ± 0.006 b	2.01 ± 0.023 b	0.55 ± 0.002 b	1.98 ± 0.013 b
	Gk	67–90	0.87 ± 0.012 c	1.34 ± 0.014 c	0.55 ± 0.006 b	1.56 ± 0.012 c
	2Gk1	90–123	0.16 ± 0.006 d	0.18 ± 0.004 d	0.06 ± 0.002 c	1.13 ± 0.015 d
	2Gk2	123–150	0.13 ± 0.002 d	0.10 ± 0.003 d	0.03 ± 0.001 c	0.77 ± 0.026 e
Profile 3. Eutric Loamic Regosol (ELR)						
Grapevine (GV)	Ak	0–18	3.00 ± 0.010 a	9.94 ± 0.031 a	1.69 ± 0.009 a	3.31 ± 0.033 a
	ACk	18–40	1.50 ± 0.009 b	4.51 ± 0.012 b	0.76 ± 0.002 b	3.01 ± 0.029 a
	Ck	40–82	0.49 ± 0.020 c	0.90 ± 0.010 c	0.17 ± 0.010 c	1.84 ± 0.015 b
	Ck2	82–120	0.41 ± 0.011 d	0.55 ± 0.014 d	0.09 ± 0.006 d	1.34 ± 0.019 bc
	Ck3	120–150	0.41 ± 0.007 d	0.45 ± 0.001 e	0.08 ± 0.010 d	1.10 ± 0.012 c
Profile 4. Stagnic Luvic Phaeozem (SLP)						
Apple trees (AT)	A	0–18	8.44 ± 0.219 a	8.69 ± 0.225 a	1.85 ± 0.026 a	1.03 ± 0.013 a
	A2	18–40	5.46 ± 0.008 b	6.46 ± 0.039 b	1.48 ± 0.009 b	1.18 ± 0.016 a
	Btg	40–82	2.45 ± 0.055 c	1.99 ± 0.035 c	0.53 ± 0.012 c	0.81 ± 0.018 b
	Ckgg	82–120	1.23 ± 0.008 d	1.10 ± 0.009 d	0.32 ± 0.002 d	0.89 ± 0.013 b
	Gk	120–150	0.90 ± 0.012 d	0.50 ± 0.001 e	0.17 ± 0.008 e	0.56 ± 0.011 c
Profile 5. Cambic Phaeozem (CP)						
Hop (H)	A	0–18	2.44 ± 0.829 b	2.13 ± 0.004 d	0.48 ± 0.006 b	0.87 ± 0.012 b
	A2	18–40	3.27 ± 0.014 a	4.75 ± 0.023 a	0.68 ± 0.030 a	1.45 ± 0.011 a
	AB	40–82	2.15 ± 0.043 b	3.07 ± 0.020 c	0.51 ± 0.020 b	1.43 ± 0.020 a
	Bw	82–120	2.36 ± 0.048 b	3.39 ± 0.025 b	0.50 ± 0.016 b	1.44 ± 0.015 c
	Ck	120–150	0.75 ± 0.003 c	0.83 ± 0.018 e	0.06 ± 0.001 c	1.11 ± 0.007 d

*—Different letters indicate differences ($p < 0.05$) between genetic horizons within the same soil profile. AcP—acid phosphatase activity; AlP—alkaline phosphatase activity; PDE—phosphodiesterase activity; Enzyme activities were expressed as mM *p*NP kg^{-1} h^{-1}; AlP/AcP—the ratio of alkaline phosphatase activity to acid phosphatase activity. Abbreviations of soil horizon names are explained under Table 1.

In this profile, the enzymatic activity was the greatest in the A2 horizon, and it was significantly lower in the upper and lower horizons. As a general rule, AcP activity dominated over the activity of AlP and PDE. The exception was the activity of both phosphomonoesterases in selected horizons (Btg, Ckgg, Gk) of the apple trees profile. The ratio of the AlP/AcP ranged between 0.56 and 3.31 across the studied profiles. The highest ratio of AlP/AcP was calculated for the surface and/or subsurface horizons and it decreased significantly with depth.

In order to better explain the changes in the studied properties as well as the relationships between the profiles studied, we have compared those properties in the genetic horizon which lie at the same/similar depth (Figures 1–3). In the surface horizons of the AA, W and GV profiles, the AlP activity was definitely higher than the AcP activity, while no significant differences between these enzymes were found in the upper layers of the AT and H profiles (Figure 1a).

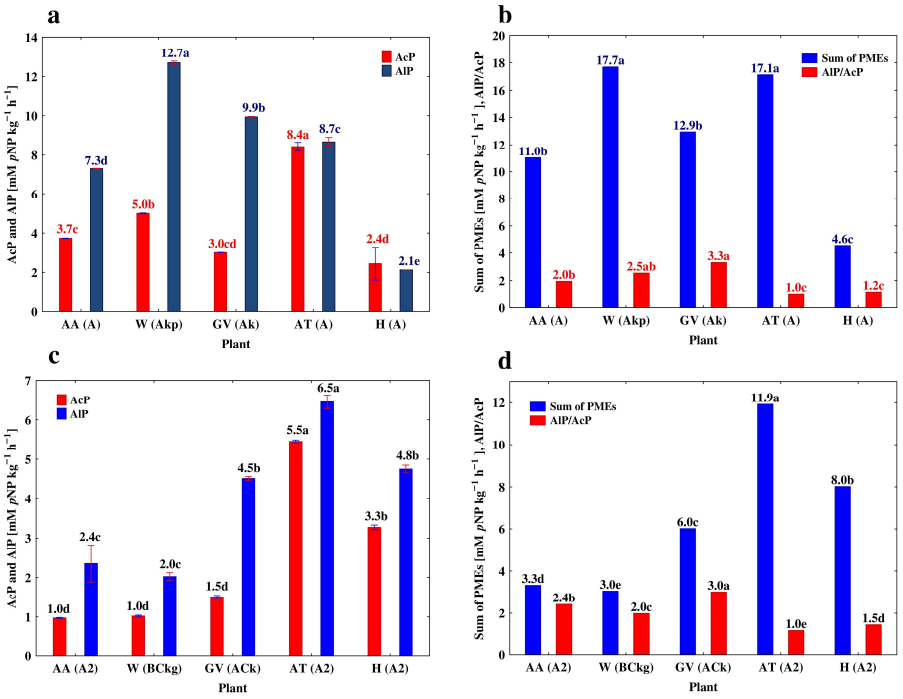

Figure 1. Effect of cultivated plants on the acid and alkaline phosphatase activity (AcP, AlP) and sum of PMEs activities as well as the AcP/AlP ratio in surface horizons (Ap, A, Akp, Ak) ((**a**,**b**), respectively) and in the sub-surface horizons (A2, BCkg, ACk) ((**c**,**d**), respectively). PMEs—the sum of phosphomonoesterases (AcP and AlP). AA—alfalfa, W—winter wheat, GV—grapevine, AT—apple trees, H—hop. Values for each property followed by different letters are statistically different at $p < 0.05$. Abbreviations of soil horizon names are explained under Table 1.

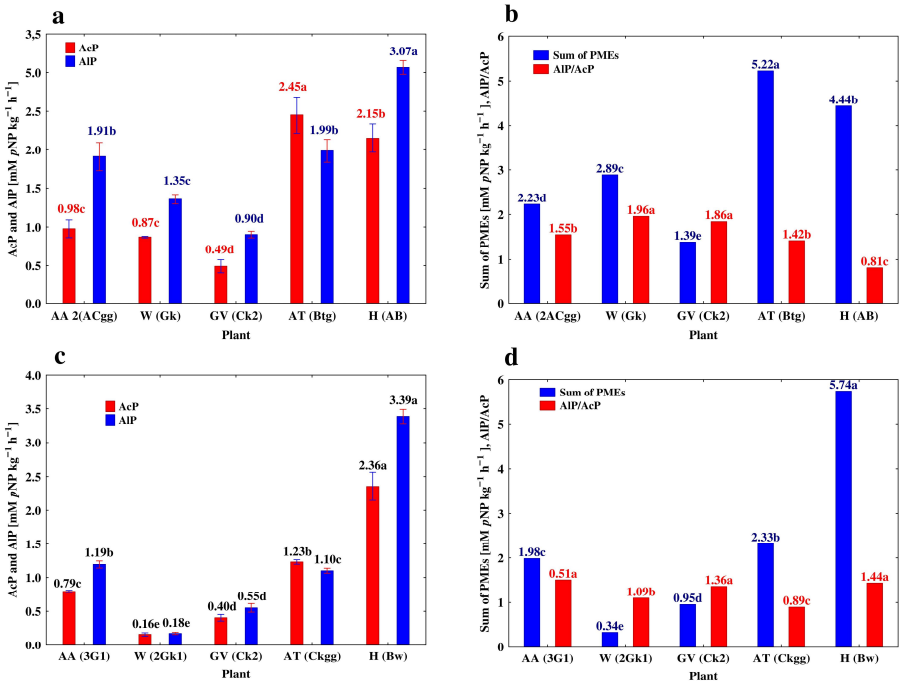

Figure 2. Effect of cultivated plants on the activity of acid and alkaline phosphatase (AcP, AlP) and sum of PMEs activities as well as the AcP/AlP ratio in third horizons (ACgg, Gk, Ck2, Btg, AB) ((**a**,**b**), respectively) and in the fourth horizons (3G1, 2Gk1, Ck2, Ckgg, Bw) (**c**,**d**), respectively). AA—alfalfa, W—winter wheat, GV—grapevine, AT—apple trees, H—hop. PMEs—the sum of phosphomonoesterases (AcP and AlP). Values for each property marked by different small letters are statistically different at $p < 0.05$. Abbreviations of soil horizon names are explained under Table 1.

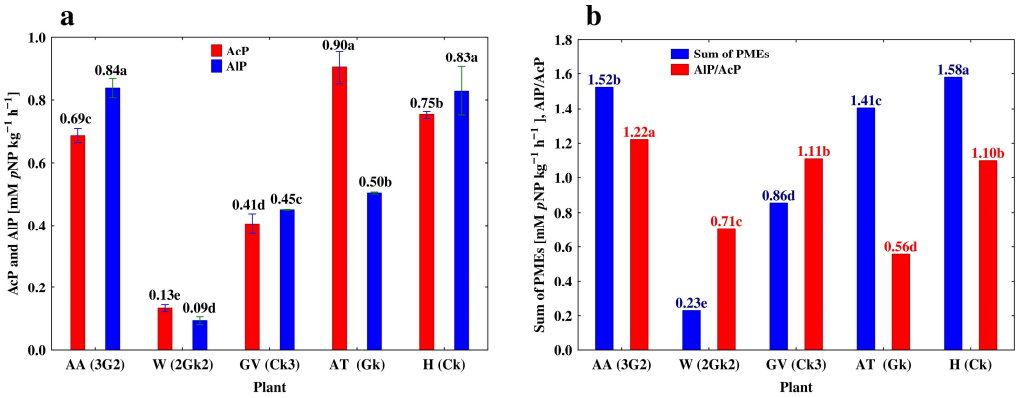

Figure 3. Effect of cultivated plants on the acid and alkaline phosphatase activity (AcP, AlP) (**a**), and sum of PMEs activities as well as the AcP/AlP ratio (**b**), in the deepest horizons (3G2, 2Gk2, Ck3, Gk, Ck). PMEs—the sum of phosphomonoesterases (AcP and AlP). AA—alfalfa, W—winter wheat, GV—grapevine, AT—apple trees, H—hop. Values for each property marked with different letters are statistically different at $p < 0.05$. Abbreviations of soil horizon names are explained under Table 1.

The sum of both PMEs was found to be highest (and close to each other) in the soil sampled under winter wheat and apple trees and the lowest level was found under the hop plantation (Figure 1b). The AlP/AcP ratio calculated for enzyme activities determined in the soil under AA, W and GV cultivation (mean 2.6) was definitely higher when compared to the values of this ratio obtained in the soil collected under the AT and H plantations (mean 1.1) (Figure 1b). In the subsurface horizons, the trend in PMEs activities was different when compared to the upper layer, and thus the highest PMEs activity was noted in the AT and H profiles and the lowest in the other three profiles (Figure 1c). In all profiles the AlP was more active than the AcP. Variations in the ratio of AlP/AcP were similar to those calculated in the upper layer, while a different trend was found for the sum of PMEs. This was highest in the AT profile (11.9 mM *p*NP kg^{-1} h^{-1}) and lowest in the A and W profiles (mean 3.15 mM *p*NP kg^{-1} h^{-1}) (Figure 1d). In the three subsequent horizons (third, fourth and fifth), the direction of the changes in PMEs activity were similar, and both enzymes had higher levels in the AT, H and AA profiles compared to the W and GV profiles (Figures 2 and 3). Only in the AT profile was the AcP activity higher than that of AlP. The very low activity level of both PMEs in the two deepest horizons of the W profile should be emphasized as compared to the other profiles. No clear tendency was indicated in these horizons regarding the AlP/AcP ratio and the sum of the PMEs activity.

We compared the layers which were expected to have reducing conditions with the oxidative horizons, lying at a similar depth (three of the deepest horizons in profile 1, 2 and 4 were compared with the three deepest layers in profiles 3 and 5, respectively) (Figure 4). The AlP activity, the sum of both PMEs and the PMEs/PDE ratio were significantly higher in the horizons with oxidative conditions as compared to the layers with reducing conditions. In turn, the content of microbial biomass C and P, but also the ratio of MBC/MBP, were significantly higher in reducing conditions in comparison with the oxidative ones.

3.4. Root Biomass and Morphology

In considering all of the profile horizons together, the root biomass was found to be greater in the GV, AT and H profiles than in the W and AA profiles (Table 5). The biomass of plant roots decreased with depth in the AA and W profiles, while in the remaining profiles it was significantly greater in the middle layers than in the surface and the deeper ones. In the AA, W and GV profiles, the root length and surface area were higher in the surface horizons (Ap, Akp, Ak), and decreased gradually with depth (except of AA profile). In turn, ambiguous variations in these root parameters with depth were indicated across the AT and H profiles. The exception was the longest root length and surface area in the A2 horizons in the H profile.

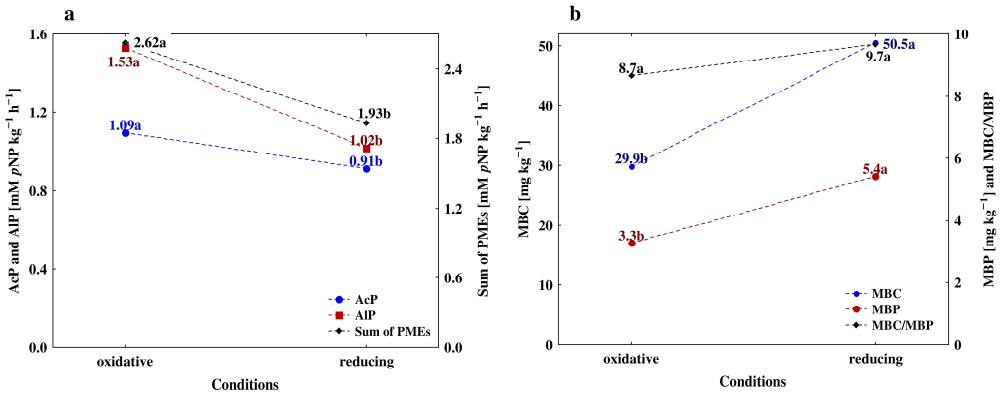

Figure 4. Effect of oxidative and reducing conditions on the AcP, AlP activities and the sum of PMEs (**a**), and on the microbial biomass C and P content (MBC, MBP) and the ratio of MBC/MBP (**b**). Horizons with the expected reducing conditions were compared with the oxidative horizons, lying at a nearing depth (three the deepest horizons in profile no 1, 2 and 4 with three of the deepest layers in profiles no 3 and 5, respectively). Values for each property marked with different letters are statistically different at $p < 0.05$.

Table 5. Root mass, length and surface area in the considered profiles.

Plant	Horizons	Depth (cm)	Root Mass (g dm^{-3})	Root Length (mm dm^{-3})		Root Surface (mm^{-2} dm^{-3})	
				All	^ Ø < 0.5 mm	All	Ø < 0.5 mm
Profile 1. Mollic Stagnic Gleyosol (MSG)							
Alfalfa (AA)	Ap	0–30	14.2 a*	3138 a	2265 a	688a	164 a
	A2	30–53	1.20 b	181 d	91.0 d	78.6 b	7.8 c
	2ACgg	53–70	0.74 c	215 c	112.4 c	64.5 b	10.8 b
	3G1	70–110	0.20 d	249 b	164 b	42.4 c	12.7 b
	3G2	110–150	0.04 d	83.1 e	52.1 e	12.2 d	5.1 d
Profile 2. Cambic Stagnic Phaeozem (CSP)							
Winter wheat (W)	Akp	0–35	1.91 a	2680 a	2221 a	292 a	143 a
	BCkg	35–67	1.01 b	1750 b	1540 b	175 b	115 b
	Gk	67–90	1.02 b	1278 c	1092 c	141.1 c	84.5 c
	2Gk1	90–123	0.41 c	603 d	363 d	36.5 d	23.4 d
	2Gk2	123–150	0.32 c	207 e	172 e	22.6 e	12.2 e
Profile 3. Eutric Loamic Regosol (ELR)							
Grapevine (GV)	Ak	0–18	10.0 c	1530 a	820 a	513 a	57.9 a
	ACk	18–40	12.9 b	686 b	325 b	277 b	24.0 b
	Ck	40–82	11.9 b	738 b	352 b	285 b	26.0 b
	Ck2	82–120	14.5 a	385 c	161 c	257 c	10.9 c
	Ck3	120–150	2.97 d	237 c	116 d	58.6 d	9.91 c
Profile 4. Stagnic Luvic Phaeozem (SLP)							
Apple trees (AT)	A	0–18	4.40 b	1634 a	955 a	329 b	75.8 a
	A2	18–40	16.8a	1771 a	741 b	691 a	58.7 b
	Btg	40–82	1.91 c	482 c	214 d	143 c	19.8 d
	Ckgg	82–120	1.40 d	643 b	276 c	158 c	25.3 c
	Gk	120–150	0.85 e	174 d	44.6 e	58.8 d	3.71 e
Profile 5. Cambic Phaeozem (CP)							
Hop (H)	A	0–34	3.98 c	1438 b	395 c	765 b	35.6 c
	A2	34–66	11.7 a	2119 a	765 a	979 a	71.2 a
	AB	66–88	9.69 b	1046 c	470 b	348 c	34.6 d
	Bw	88–114	9.86 b	1104 c	762 a	161 d	55.7 b
	Ck	114–150	2.11 d	215 d	104 d	54.9 e	8.02 e

*—Different letters show significant differences ($p < 0.05$) between genetic horizons within the same soil profile; ^ roots diameter. Abbreviations of soil horizon names are explained under Table 1.

For the winter wheat profile, roots with a diameter below 0.5 mm made up over 80% of the total root system length in all horizons, with the exception of the 2Gk1 horizon where the contribution was 60%. The root length with a diameter below 0.5 mm in the surface layer of the AA profile formed 72% of the total root system length, while in the deeper layers it was between 50 and 65%.

Similarly, in the surface layers of the GV and AT profiles, the shortest roots accounted for 53% and 58% of the total root system length. Below the surface layer, the relationship above was between 42–49%. Only in the H profile did roots with a diameter below 0.5 mm make up 27% of the total root system length in the surface horizon and 36–69% in deeper layers. Ambiguous tendency was noted concerning the root surface with a diameter below 0.5 mm across the studied profiles. In the topsoil and in the deepest layer of the AA profile (Ap, 3G2) a root surface < than 0.5 mm constituted 42% of the total root system surface, while in the layers the relationship above was between 10–30%. Similar trends were found in the W and GV profiles, while in the AT profile the highest contribution of the root surface < than 0.5 mm in the total root system surface was found in surface layer (AA) and decreased with depth.

3.5. Relationship between the Studied Properties—Analysis of Correlation and PCA

An analysis of correlation was performed for all horizons together, as well as separately, for horizons with a TOC content > 0.5% (higher TOC) and horizons with a TOC content < 0.5% (lower TOC) (Figure 5).

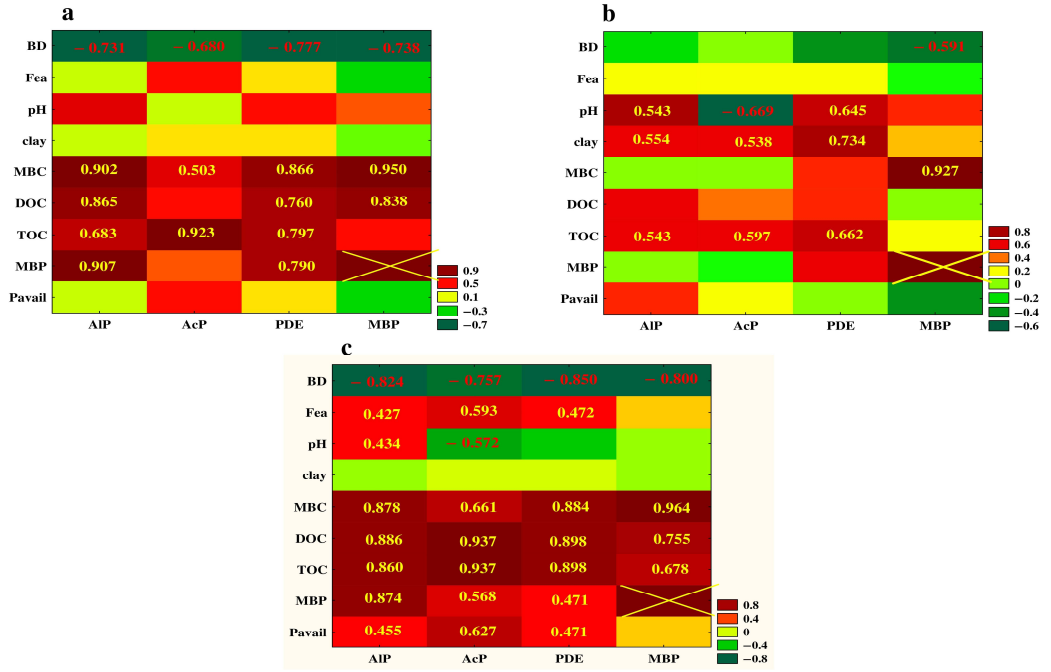

Figure 5. Heatmaps of correlations between studied properties (all profiles are considered together), horizons with TOC >0.5% (**a**), horizons with TOC,0.5% (**b**), all horizons (**c**) Only significant values are presented ($p < 0.05$), AlP—alkaline phosphatase, AcP—acid phosphatase, PDE—phosphodiesterase, MBP—microbial biomass phosphorus, Pavail—available phosphorus, TOC—total organic carbon, DOC—dissolved organic carbon, MBC—microbial biomass carbon, Fea—amorphous iron oxides, BD—bulk density, pH—soil reaction in $CaCl_2$, clay—clay content. Abbreviations of properties are explained under previous tables and figures.

In all genetic horizons considered together, the enzyme activities and MBP contents related to a significant extent with the physicochemical properties, wherein the relationship with BD was negative. The soil reaction (pH in $CaCl_2$) was positively correlated with the AlP and PDE activities, while it correlated negatively with AcP activity. A different pattern in the relationship among the studied properties was found when horizons with

a TOC content > 0.5% and < 0.5% were analysed separately. The MBP content was only significantly and positively associated with the AlP and PDE activities in the layer with TOC > 0.5%. As expected, the studied enzyme and MBP contents were highly and positively correlated with the TOC, DOC and MBC content, while they were negatively correlated with BD, mainly in horizons with a TOC content > 0.5%. By contrast, the clay content was only significantly and positively correlated with the soil enzyme activity in horizons with a TOC content < 0.5%. A negative relationship was found between the Pavail and $CaCO_3$ content in horizons 2 and 3 (Figure 6). The PCA analysis recognized three components which generated 75% of the total existing variance. Most of this variance was explained by two main components, namely PC1 and PC2 (Figure 7). PC1, which described 51.4% of the variance, was correlated with AcP, AlP and PDE activity, MBC and MBP, TOC and DOC, since all of these properties had high negative loading scores for this component (Table 6). In turn, the Pavail, BD and pH in $CaCl_2$ was significantly but positively related to PC1. PC2, which distinguished 16.3% of the total variance was correlated with some chemical properties, both positively (pH in $CaCl_2$) and negatively (Pavail, Fea). The PCA analysis revealed a negative association of the C-rich surface and subsurface layers of all of the considered profiles with PC1 as well as both positively and negatively with PC2, wherein the strength of the dependence varied widely (Figure 7).

Table 6. Loading scores of the properties for the principal component analysis.

Property	PC1	PC2	Property	PC1	PC2
AcP	−0.906	−0.113	clay	0.176	0.124
AlP	−0.945	0.201	silt	−0.092	−0.214
PDE	−0.951	0.217	BD	0.903	−0.152
Pavail	−0.612	−0.580	CEC	−0.077	0.197
MBP	−0.777	0.578	Hh	−0.378	−0.797
MBC	−0.854	0.545	pH	0.630	0.667
TOC	−0.959	−0.042	Fea	−0.535	−0.430
DOC	−0.914	0.047			

AcP—acid phosphatase, AlP—alkaline phosphatase, PDE—phosphodiesterase, Pavail—available phosphorus, MBP—microbial biomass phosphorus, MBC—microbial biomass carbon; TOC—total organic carbon, clay—clay content, silt—silt content BD—bulk density, CEC—cation exchange capacity, pH—soil reaction measured in $CaCl_2$, Hh—hydrolytic acidity, Fea—amorphous iron oxides, PC1—first principial component, PC2—second principial component.

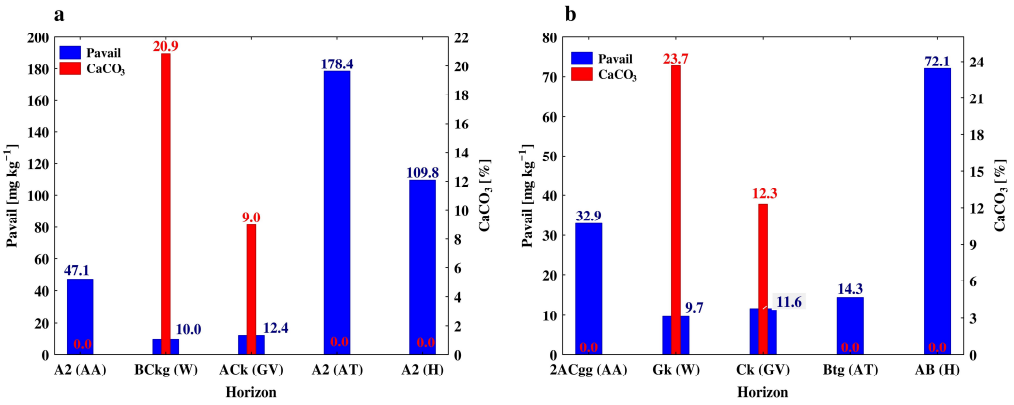

Figure 6. Effect of cultivated plants on the available phosphorus (Pavail) and $CaCO_3$ content in the sub-surface horizons (**a**) and third horizons (**b**) of the studied profiles. AA—alfalfa, W—winter wheat, GV—grapevine, AT—apple trees, H—hop. Abbreviations of soil horizon names are explained under Table 1.

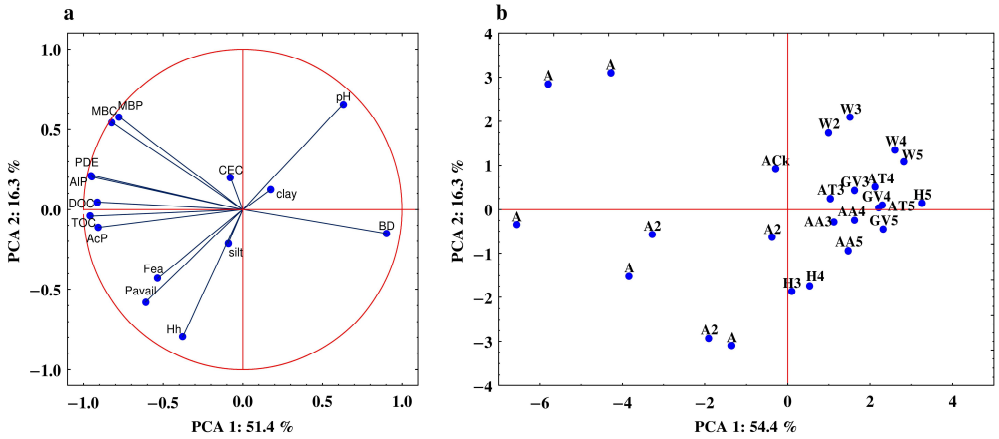

Figure 7. PCA analysis obtained from the studied soil variables: (**a**) plot of the PC1 and PC2 for the determined soil properties: Pavail—available phosphorus content, AcP—acid phosphatase activity, AlP—alkaline phosphatase activity, PDE—phosphodiesterase activity, MBC—microbial biomass carbon, TOC—total organic carbon, TN—total nitrogen content, DOC—dissolved organic carbon, Fea—amorphous iron oxides, BD—bulk density, Hh—hydrolytic acidity, CEC—cation exchange capacity, pH—pH in $CaCl_2$; (**b**) PCA analysis of the properties in the genetic horizons in all 5 considered profiles: A—all the surface horizons. A2 and Ack—sub-surface horizons (with TOC content > 0.5%) (except that of winter wheat profile), AA3–AA5—three of the deepest horizons in alfalfa profile, W2–W5—horizons of winter wheat profile (except the surface layer), GV3–GV5—three of the deepest horizons in the grapevine profile, AT3–AT5—three of the deepest horizons in the apple trees profile, H3–H5—three of the deepest horizons in the hop profile.

4. Discussion

4.1. Vertical Differentiation in Soil Enzyme Activities—The Effect of Different Agricultural Land Use

It is well established that different plants influence the soil enzymatic activity in two ways; directly when the roots secrete enzymes themselves and indirectly when the plant specifically stimulates the growth of various microbial communities in the rhizosphere via root exudation of different compounds including sugars, amino acids, organic acids, hormones and vitamins [62]. No clear link has been recorded between the enzymatic properties tested in this study and their dependence on the plants being cultivated and also the relationship was strictly dependent on the depth in soil profile. The differences in the activity of PMEs may be related to their source and thus it is possible to derive AcP mainly from plants and fungi, while AlP and PDE activity is largely derived from bacteria [63]. Since no suitable method exists to distinguish between the activity of phosphatases from various sources, it is difficult to interpret the data of soil PMEs activity which is obtained using the existing methods [15].

It is commonly known that plants are an important source of AcP activity in the soil and AcP activity increased near the rhizoplane of some plants; as such, this increase depended on both the plant species and their age [17]. During the process of plant development, the available inorganic P decreases as it becomes increasingly incorporated into organic forms. Brook et al. [64] found lower soil phosphatase activity at younger and canopy closure stands, and this activity was negatively related to phosphate contents across all stands, but the described relationship was related exclusively to the upper soil layers. Our study fits this model mainly in the lower horizons concerning the AcP activity, while the relationship mentioned above in the surface horizons was probably disturbed by mineral fertilization (mainly fertilization with P and N). Some other authors [15,19,65] who determined the phosphatase activity throughout the soil profiles, have also reported that the range of phosphatase activity and the phosphorus mineralization across the soil profiles may be due to the mass and structure of the root system. We have considered all roots (not only those of the cultivated plants) that were found in the collected samples. Normally, the

enzymatic activity were found to be greatest in the surface layers in four out of five of the considered profiles which coincided with the highest level of root mass (except for profile 5). We observed that the older the plant, the greater the mass of roots in the deep horizons as compared to the upper layers (apple trees and hop). The increase in enzyme activity with plant age was probably caused by the progressive formation of rhizosphere microflora and also by the excretion of plant PMEs [15]. In spite of the fact that the highest level of root biomass, length and surface area was assessed in the upper layers of the A profile, the level of enzymatic activity was markedly higher in the topsoil of winter wheat profiles. In the alfalfa profile considered in our study, the roots of other plants, such as grasses and weeds, may share in the total root system, we have determined. The greater microbial biomass of carbon and phosphorus and also the enzymatic activity in the Ap horizon of the W profiles may be influenced by the implemented mineral N fertilization. Actually, a close non-linear negative relationship between the mineral nitrogen content and the biomass of spring wheat roots was noted in the surface horizon [66]. In addition, Fageria and Moreira [67] suggested that mineral fertilizers inhibit the development of a root system.

The correlation analysis showed a significant link between the activity of all of the studied phosphatases and the biomass, length and surface area of the small roots (with Ø < 0.5 mm), which was closer in the surface layers than in the deeper soils (results not presented). The strength of the relationship reached its highest point, however, for acid phosphatase activity (r = 0.778–0.886), this indicates an intensified production of this enzyme by plants in order to acquire soil phosphorus, and their significant role in the production of acid phosphatase. In fact, some studies indicated that soil AcP activity is derived to a high degree from plant roots, and thus dissimilarities in root structure, are mostly due to the amount of small roots and their metabolic activity which appear to be very important for the soil enzymatic activity in response to the variation in P availability [68]. Furthermore, as was found by Cabugao et al. [69], the level of soil acid phosphatase increased with fine-root mass density and organic P content, which together can explain more than 50% of the differences in soil phosphatase activity.

Although the activity of AlP and PDE was also correlated with root parameters, the obtained results could be masked by other phenomena. Based on the literature, the activity of these enzymes, in contrast to acid phosphatase, were rather related with organic matter content (there were significant correlations with TOC, DOC and MBC content). The closer dependence of AlP and PDE activities on TOC and microbial biomass C and P than on the root biomass and structure suggests the importance of the microbial rather than the plant origin of these enzymes.

The alkaline and acid phosphatase activity was used to calculate the ratio of AlP/AcP which was proposed by Dick et al. [70] and is known as an enzyme pH indicator, that may be used to check the optimum conditions for plant growth and development in the surface of intensively used agricultural soils. The above ratio lower than 0.50 in the surface soil designates that the soil is acid and should be limed [70,71]. The value of the AlP/AcP ratio across the studied profiles was 0.87–3.31 and that is why no liming was required. The relatively high AlP/AcP ratio in the deeper layers was connected with increasing soil pH.

4.2. The Effect of Soil Depth and Pedogenic Processes on the Studeod Properties

Although the biogeochemical cycling of soil P is affected by the pedogenic processes and soil factors, less is known about their influence on the soil phosphatase activities, which are considered to be the key agents of soil organic P transformation [24]. As was found previously [41,72] in our study, the enzyme activities and the content of microbial biomass in the surface (Ap, Ak, A) and subsurface (A2, ACk) layers were mainly assessed by the accumulation of organic matter content and generally decreased with soil depth similarly to the changes in TOC and TN concentration. Such a decrease may be elucidated by the fact that both enzymatic activity of hydrolases and microbial biomass content are frequently directly associated with soil organic matter content and they reflected the

expected availability of their specific substrates [73]. Since the content of SOM and the amount of respective substrates that can be hydrolysed decreases down the soil profile, the activity of the specific hydrolytic enzymes would also be expected to decline [41].

Similar to previous studies [74] a relatively high activity of PMEs was found in the subsurface parts of some of the considered profiles (orchard and hop plantation) as compared to the surface layers, and this phenomenon may be explained in two ways. Firstly, if soil P availability declines with depth in parallel with the carbon and nitrogen levels, as was found in this study in the orchard and hop profiles, the soil microorganisms will invest resources in a P-acquiring enzyme and, because of that, a relatively high PMEs activity was observed in the deeper soil layers. From a resource allocation perspective, a high microbial investment in P-acquiring enzymes indicates microbial P-limitation [75]. In turn, in other profiles in our study soil, Pavail did not decline with depth similarly to TOC and TN but it even increased with depth. In this case, a pattern of decreasing activity in the studied phosphatases was observed. Secondly, the relatively high PMEs activity in the deeper horizons (with a simultaneously decreasing microbial biomass) may be associated with the possible transport of extracellular phosphatases across the soil profile. In a column transport experiment, Guber et al. [76] provided evidence which linked enzyme transport to soil colloids. Between 52–88% of the total enzyme activity in the effluents was associated with the clay fraction, while the remaining part of the enzyme activity was attributed to enzymes that were connected with organic colloids and were free in the soil solution [76]. Some studies indicated that the enzymatic activity was adsorbed on solid surfaces, e.g., on clay minerals, which stabilized and protected the enzymatic protein from degradation [77]. The enzymatic proteins that are immobilized on the clay fraction are active in the soil, in which no microbial proliferation takes place [77]. Furthermore, soil microbes constantly excrete a low level of enzymes to keep the ability to quickly respond to changes in substrate accessibility even in sites with low nutrients concentration [78].

The complex dependency between the Pavail content and phosphatases activity was also affirmed by a correlation analysis (Figure 5). In this study, no significant interaction between phosphatase activity and available P was found in horizons where a TOC content over and below 0.5% were considered together, while significant but low correlation coefficients were found for all horizons taken together. As already mentioned, the relationship between the available P concentration and phosphatase activity may be very complex and either a positive, a negative or no relationship between these two properties was found [16]. When no relationship is observed between AcP activity and available P content, P may not limit the system but some other factors may affect enzyme secretion and their activity, for example, N availability [79] or the interaction between the enzyme and humus or clay [80].

In deeper horizons, the enzymatic activity was still associated with SOM but was also related to soil-forming processes and associated factors such as soil texture (mainly the clay content), oxidative/reductive conditions or soil reaction [41,74]. Water saturation and a low oxygen concentration, which is responsible for the reducing conditions, were common in three of the deeper horizons of the alfalfa, winter wheat and apple tree profiles, which were waterlogged by groundwater (especially the two deepest horizons). To assess the influence of reducing conditions on the determined enzymes, we compared their activity in these horizons to the oxidative layers in the grape vine and hop profiles, which occurred at the same/similar depth. The significantly higher phosphatase activity under aerobic as opposed to anaerobic conditions that was obtained in this study may be connected with the principle that the crucial roles in the soil biochemical processes (the decomposition of organic matter, availability of nutrients for plants and the cycling of elements) are dependent on the availability of oxygen and the aerobic activity of microorganisms [81]. What is more, the aerobic processes are more rapid compared to the processes occurring in anaerobic conditions.

The increased content of the clay fraction (up to 25%), e.g., as a result of the lessivage process was found in some of the profiles studied. In general, the activity and stability of enzymes may be promoted, inhibited or maintained after immobilization on clay

minerals [82]. Previous studies reported the inconsistent effects of the clay content on enzymatic kinetics by the reduction in the substrate turnover and an increase in the enzyme half-life [82,83]. Generally, the activity of the inhibited enzymes are lower, but the residual activity is stabilized and protected against thermal and proteolytic deactivation [15]. In this study, low correlation coefficients were noted between enzyme activities and clay content only in horizons with TOC content <0.5%. This suggests that in surface horizons, the effect of clay minerals on enzyme activities could be interfered with by the content of organic matter.

It is well established that the soil reaction (pH) reveals a remarkable effect on the activity of soil phosphatases since it acts on (1) conformational and chemical changes in amino acids that are substantial for binding the substrate and its catalysis, (2) its adsorption on solid surfaces, (3) the ionization and solubility of substrates and cofactors [84], and (4) the structure of microbial diversity and community composition [85]. Soil pH is particularly important for phosphatases, since it determines the magnitude of their activities and their types [86]. The lack of a significant relationship between soil pH and phosphatase activities in horizons with a TOC content > 0.5% may be masked by associations of enzymes with soil carbon content. Since the pH in the deeper parts of the studied profiles was over the optimum level for AcP (6.5), and below the optimum for AlP and PDE (11.0 and 8.0, respectively), we have found both negative (for AcP) and positive (AlP and PDE) correlations between enzyme activities and the pH in $CaCl_2$.

5. Conclusions

Although the effects of plant cover on enzymatic activity were the most predominant in the topsoil layers, a plant species with a contrasting root biomass and structure also changed the properties studied along the soil profile. Among the enzymes studied, only the AcP activity was closely related to the root biomass and morphological parameters (also in the deeper layers). In addition, the amount of small roots and their metabolic activity is believed to have a crucial effect on soil enzymatic activity in response to a variation in P availability.

As expected, the activity of P-transforming enzymes was highest in surface horizons, as was the organic C concentration and accessibility (except for hop profile) and decreased with depth simultaneously with the TOC and TN content. A close relationship between enzymatic activity and the C and N properties was also found in the deeper layers of the studied profiles. The impact of the soil-forming processes as well as soil properties (e.g., vertical texture) in deeper horizons was, in turn, less noticeable, with the exception of oxidative/reductive conditions. A significantly higher activity for all phosphatases were found under aerobic as compared to the anaerobic conditions.

As a general rule, the enzyme activities were found to be highest in the surface horizons of the studied profiles (except for the hop profile), which corresponded to the highest level of root mass in topsoils. The older the plant, the greater the root mass and increased enzymatic activity in the deeper horizons of AT and H profiles, as compared with the surface horizons. Even though we have found some clear relationships between the cultivated plants and soil phosphatase activities, the obtained results need to be considered with care since such dependences could be impacted by other factors, such as air–water conditions, soil tillage and/or applied fertilization. Additional study should be considered, however, to obtain more reliable conclusions concerning the status of PMEs activity in the deeper parts of soil profiles. For example, to better explain the association between soil enzymatic activity and soil texture, the appropriate properties ought to be assessed independently in separate soil fractions, such as sand, silt and clay.

Author Contributions: Conceptualization, J.D. and A.P.-D.; methodology, A.P.-D., J.D., A.S.-Z. and A.F.; software, A.P.-D., J.D., A.S.-Z. and A.F.; investigation, A.P.-D., J.D., A.S.-Z. and A.F.; resources, A.P.-D. and J.D.; writing—original draft preparation, A.P.-D. and J.D.; writing—review and editing, A.P.-D., J.D., A.S.-Z. and A.F.; visualization, A.P.-D. and J.D.; project administration, A.P.-D.; funding acquisition, A.P.-D. and J.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Centre, Poland, grant number 2018/29/B/NZ9/00982.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data used to support the findings of this study are available from the authors upon request.

Acknowledgments: Much gratitude is due to David Scott for proof reading the article.

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

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