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Responses of N-Cycling Enzyme Activities and Functional Diversity of Soil Microorganisms to Soil Depth, Pedogenic Processes and Cultivated Plants

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Abstract: The rates of N cycling and soil enzyme activities involved in the transformation of soil N-related nutrients are rarely measured in soils below a 30 cm depth, even though substantial amounts of nitrogen are also stored in deep soils. The aim of this study was to determine how soil microbial and enzymatic properties changed as a function of depth across soil profiles that were developed on the same parent material but differed in terms of soil-forming processes. Two soil profiles were excavated in fields with lucerne and two under winter wheat. We assessed the N-cycling enzymes, the microbial utilization of the N-substrates, the microbial biomass carbon and nitrogen (MBC, MBN) content, and the related physicochemical properties. The most beneficial enzymatic (on a soil mass) and microbial properties, as well as nitrogen substrate utilization, were found in the Ap horizons and decreased with depth to varying degrees. The specific enzymatic activity (per unit of soil TOC and MBC), was more variable in response to the depth of the profile, but did not exhibit clear trends. The potential enzyme activities in the subsurface layers were also affected by factors that are associated with the pedogenic processes (e.g., the lessivage process, clay content). Only nitrate reductase activity was significantly higher in the horizons with potential reducing conditions compared to oxidative horizons, while the opposite trend was found for N-acetyl-β-D-glucosaminidase (NAG) activity. The cultivated plants had a significant impact on the degree of enzymatic activity and N-substrate utilization. The lessivage process significantly reduced microbial biomass and enzymatic activity (except for NAG activity). In general, nitrogen substrate utilization decreased with increasing soil depth and was greater in lucerne than the winter wheat profiles. Mollic Stagnic Gleysols (MSG) and Cambic Stagnic Phaeozems (CSP) horizons also have higher nitrogen substrate utilization than Luvisol profiles.

Keywords: functional diversity; metabolic capacity; microbial biomass; N-cycling enzymes; soil-forming processes; soil profile

Citation: Piotrowska-Długosz, A.; Długosz, J.; Gryta, A.; Frąc, M. Responses of N-Cycling Enzyme Activities and Functional Diversity of Soil Microorganisms to Soil Depth, Pedogenic Processes and Cultivated Plants. *Agronomy* **2022**, *12*, 264. https://doi.org/10.3390/agronomy12020264

Academic Editors: Jorge Paz-Ferreiro, Gabriel Gascó Guerrero and Ana Méndez

Received: 13 December 2021
Accepted: 18 January 2022
Published: 20 January 2022

1. Introduction

Nitrogen (N) is the primary limiting nutrient in many terrestrial ecosystems, and, as a consequence, the content and transformations of soil N compounds have been widely studied [1]. A global dataset indicates that over 60% of soil organic N occurs at a depth from 20 to 100 cm in the soil profile, but substantial amounts of nitrogen are also stored below a depth of 1 m [2]. As a consequence, especially when the substantial thicknesses of subsurface profiles are taken into consideration, even low rates of microbial activity per unit soil mass at depth could contribute meaningfully to ecosystem-scale soil N cycling [3,4]. Despite this fact, most of the studies concerning soil N content and transformation involving different land uses have been focused on carbon-rich surface mineral layers (usually

occurring to a depth of 30 cm), where plant root density and soil organic matter (SOM) concentrations typically reach the highest values [5]. As a consequence, the rates of N cycling and soil extracellular enzyme activities (EEAs) involved in the decomposition of complex organic matter and in the transformation of soil N-related nutrients are rarely measured in deeper soils. Furthermore, the assumed relationships between N mineralization rates and the activities of various decomposing EEAs are poorly characterized. That is why some studies have suggested that nitrogen occurring in deeper soil layers should be included when estimating the total soil N pool [6,7]. An assessment of the N concentration and its transformations in deeper soil horizons are also important because the available N from soil, fertilizers, and manure sources, when inefficiently used in crop production systems, can migrate from agricultural fields and contaminate surface and groundwater resources [8,9]. In Europe, where N fertilizers are used most frequently, leaching and denitrification are the main processes responsible for N losses to the environment, wherein ammonium ions, despite the fact that they are not susceptible to leaching, like nitrate ions, may contribute directly to soil acidification [10]. Similarly, in Chinese orchards, approximately 67% of the total N surplus for the last 28 years has accumulated in the soil profile in the form of nitrate [11].

Few studies have reported a direct correspondence between enzyme activities and the concentration and transformation of N compounds [1,6]. The mineralization of inorganic N is assumed to correspond directly with the activity of the N-acquiring enzymes and microbial functional diversity related to N-source transformations. Among the N transforming extracellular enzymes, urease (EC 3.5.1.5) and nitrate-reductase (EC 1.7.99.4) activities are more frequently determined. The enzyme urease catalyzes the hydrolysis of urea to CO_2 and NH_3 with a concomitant rise in soil pH, which results in a rapid N loss to the atmosphere through NH_3 volatilization [12]. Nitrate reductase activity (NR) is important in the process of denitrification, which catalyzes the reduction of NO_2^- to N_2O under anaerobic conditions. Nitrate reductase is an adaptive enzyme and is only synthesized in the presence of NO_3^- ions, and that is why its activity is commonly used as an indicator of the ability of plants to utilize NO_3^- from the soil [13]. A large proportion of the nitrogen (N) in the soil is in the form of proteinaceous material. Proteins in the soil are readily decomposed by proteases and peptidases into smaller, membrane-permeable peptides and amino acids, which are further metabolized with the release of NH_4^+ [14]. Proteolysis is an important process in reference to N-cycling in many ecosystems, as it is considered to be a rate-limiting step during N mineralization in soil [15]. It is usually assumed that soil proteases originate from free-living microbes and those associated with roots [16]. The enzyme, N-acetyl-glucosaminidase (NAG, EC 3.2.1.30), is in turn involved in releasing N-acetylglucosamine from aminopolysaccharides, such as chitin and peptidoglycan, and is considered to be one of the N-acquiring enzymes [17]. Living organisms, such as fungi, bacteria, plants, and animals, are the main sources of NAG activity in soils [18].

Existing studies concerning microbial properties throughout the soil profile have found that microbial biomass, the functional diversity of soil microorganisms, the activity of the hydrolytic enzymes and their substrates generally decline with increasing soil depth but they are still detectable in deeper soils [17,19,20]. With reference to the above, the limitations of research concerning topsoil not only restricts the identification of microbial functional diversity and changes in soil enzymatic activity but also limits our level of understanding with regard to the common processes of organic matter transformation. As soil microbes are involved in soil nitrogen cycling and, thus, can affect the global climate change [21], it is necessary to study the poorly understand microbial functional diversity changes under various crops cultivation, including leguminous crops and cereals (plants with contrasting morphology of root systems). In general, all plant roots may secrete extracellular enzymes and stimulate the activity of soil microorganisms [22]; however, differences in root morphology, mainly the number of plant's small roots and their metabolic activity seems to have a key impact on soil microbial content and diversity, as well as enzymatic activity [23].

The vertical decrease in enzymatic activity is mainly affected by edaphic factors, such as soil pH, soil texture, air–water conditions, as well as soil nutrient content and the quantity and chemical composition of soil organic matter, particularly the content of labile, easily degradable substrates [24]. In the study by Yao et al. [25], organic carbon content explained approximately 50% of the variations in enzyme activities. While amounts of carbon, nitrogen, and other resources are often greater at the soil surface (because of inputs from plant litter and root exudates), deeper layers receive limited plant inputs; mainly dissolved organic matter that moves downward through mass flow or diffusion [26]. Although the content of soil organic C is considered to be a major factor influencing the level and distribution of enzymatic activity throughout the profile, this relationship could be modified within particular horizons by the soil type and various soil-forming processes [27]. Since, to the best of our knowledge, only a few papers have focused on the effects of pedogenic processes on soil microbial properties and processes [28,29], a combined pedological, chemical, and microbial approach has been undertaken in this study to increase the level of knowledge concerning soil microbial diversity and the activity of N cycling enzymes along the soil profiles. For this purpose, we have selected four soil profiles that differ in their pedogenic processes, thereby resulting in the formation of different reference soil groups (Luvisols, Gleysols and Phaeozems). The differences in the soil properties throughout the soil profiles are expected to be associated with the differences in their genesis, the degree of weathering and the mineral composition of the parent material [30]. The rate of organic matter decomposition in the surface horizons is often much higher than in the deeper, saturated, and anaerobic soils layers [31]. In gleyic horizons, microbiological properties can be directly affected by changes in the air–water properties that are associated with water saturation, which leads to a depletion in the level of oxygen in the soil and results in changes in soil features. In such conditions, mainly the facultative and obligate anaerobic microorganisms decompose organic matter, wherein the decomposition occurs at a much slower rate in the saturated and anaerobic soil layers [31]. Lessivage has been described as a major or secondary pedogenetic process for many soil types [32,33]. This process consists of a substantial vertical transfer of fine particles, ranging in size from less than 2 µm to 10 µm (clay fraction < 2.0 µm, free iron oxides, humus substances), from the eluvial E-horizon to the illuvial B-horizon [34,35]. These materials, especially the clay minerals, because of their properties, such as large specific surface and negative charge, play a key role in an ecosystem and affect soil hydrology, fertility, weathering, and redox reactions by retaining nutrients against leaching and reaction with hydrogen and aluminum ions, thus buffering the soil against extreme soil reactions [35].

In this study, we aimed to estimate the response of soil N-related properties (some N forms, nitrogen compound utilization and N-acquiring enzymes) to different soil types and soil depths, as well as to different soil-forming processes and cultivated plants (winter wheat, lucerne). For the purpose of the study, we compared four soil profiles formed from the same parent material, but that differed in their soil-forming processes, and consequently had different physicochemical properties. Due to the differences in the TOC content in the compared horizons, we calculated the specific enzymatic activity (expressed per unit of TOC and MBC biomass), which are considered to be the appropriate indicators of any changes in soil quality parameters. Additionally, the utilization of the main nitrogen substrates by microbes was determined according to depth in the compared horizons that were situated at the same/similar depth. We hypothesized that (1) the potential enzyme activities of N cycling per unit of soil mass would decrease with depth, because the expected decreases in SOM concentrations provide less organic substrate for decomposition; (2) In addition to the soil organic matter content (C availability), the soil properties associated with pedogenic processes are also important drivers of the differences in enzymatic and microbial activity; (3) Cultivated plants (winter wheat and lucerne) would change the values of the studied properties, mainly in the surface horizons. (4) Differences in the studied N cycle enzymes, both the absolute activities (expressed in terms of the soil mass) and specific activity (expressed per unit of soil TOC and MBC) in the soil horizons, are

expected due to their various roles in N transformation and are associated with the amount of available substrate, any changes in the relative abundance of the enzyme producers, and the activity of the extracellular enzymes.

2. Materials and Methods

2.1. Study Area and Soil Sampling

The study was conducted on four soil profiles that were from two mesoregions of the South Baltic Lake District, Kuiavian-Pomerania Province (Central Poland) that have a long history of agricultural use. Although the soils were formed from the same glacial till (deposited during the Vistulian glaciations), they differ in their pedogenic processes, which results in the formation of different reference soil groups (RSGs) (Luvisols, Gleysols and Phaeozems). We studied the following profiles: Haplic Luvisol (HL, profile 1), Mollic Stagnic Gleysol (MSG, profile 2), Haplic Luvisol (HL, profile 3), and Cambic Stagnic Phaeozem (CSP, profile 4). Profiles 1 and 2 were excavated from Salno (53°8′59.6″ N, 17°26′1.1″ E and 53°9′9.8″ N, 15°25′56.6″ E, respectively), which is located in the Chełmno Lakeland (part of the Chełmno-Dobrzyń Lakeland), while profile 3 was excavated from Samostrzel (53°29′5.8″ N18°55′50.5″ E and 53°28′58.1″ N, 18°55′18.9″ E, respectively), which is located in the South-Krajna Lakeland (part of the South-Pomeranian Lakeland).

Profile 4 was excavated from Chełmce near Kruszwica (53°37′36.3″ N18°25′34.5″ E), which is located in the Inowrocław Plain. Descriptions of the soil profiles are given in Table 1.

Table 1. Morphology of the soil profiles.

Genetic Horizon	Depth	Munsell Color		Texture *	Structure #	Bulk Density
		Dry	Moist			[g cm^3]
Profile 1. Haplic Luvisol (Cutanic)						
Ap	0–30	5/3 2.5Y	3/3 2.5Y	L	AB, ME, 2	1.64
E1	30–55	6/3 10YR	4/4 10YR	SL	AB, FI, 1	1.62
E2	55–82	6/3 10YR	4/4 10YR	SL	AB, FI, 1	1.72
Bt	82–144	6/4 10YR	4/6 10YR	SiL	SB, CO, 3	1.77
BC	144–150	5/6 10YR	4/6 10YR	L	SB, CO, 3	1.83
Profile 2. Mollic Stagnic Gleyosol						
Ap	0–30	4/1 10YR	2/1 10YR	L	AB, ME, 2	1.67
A2	30–53	6/2 5Y	3/2 5Y	L	AB, VC, 3	1.69
2ACgg	53–70	5/2 5Y	4/2 5Y	L	AB, CO, 3	1.77
3G1	70–110	5/2 5Y	4/3 5Y	L	AB, CO, 3	1.78
3G2	110–150	4/1 10YR	2/1 10YR	L	AB, ME, 2	1.79
Profile 3. Haplic Luvisol (Cutanic)						
Ap	0–32	5/3 10YR	3/3 10YR	SL	AB CO 2	1.55
E	32–46	6/4 10YR	4/4 10YR	L	AB ME 1	1.63
EB	46–58	6/4 10YR	4/6 10YR	L	SB ME 2	1.71
Bt	58–135	5/4 10YR	3/6 10YR	L	SB CO 3	1.73
Ck	135–150	6/4 10YR	4/6 10YR	L	AB CO 2	1.73
Profile 4. Cambic Stagnic Phaeozem						
Ap	0–35	3/2 10YR	2/1 10YR	L	AB ME 2	1.46
BCkg	35–67	7/2 2.5Y	6/3 2.5Y	L	SB VC 3	1.61
Ck	67–90	7/2 2.5Y	6/3 2.5Y	L	AB CO2	1.71
2Ck1	90–123	7/1 2.5Y	6/2 2.5Y	FSL	AB ME 2	1.72
2Ck2	123–150	7/2 2.5Y	5/3 2.5Y	FS	AB FI 1	1.75

* L—loam; SL—sandy loam; SiL—silty loam; FSL—fine sandy loam, FS—fine sand; # type: AB—angular blocky; SB—subangular blocky. Size classes: ME—medium; FI—fine/thin; VC—very coarse/thick; CO—coarse/thick. Grades: 1—weak; 2—moderate; 3—strong.

The climate in the study regions is temperate with well-below-zero (°C) temperatures in the winter, an average annual temperature of 7 °C and an average precipitation of 550 mm $year^{-1}$. Profiles 1 and 2 were excavated from a field with lucerne (*Medicago sativa* L.) in the fourth year of its cultivation, while profiles 3 and 4 were located in a field with winter wheat (*Triticum aestivum* L.).

The winter wheat was fertilized with mineral phosphorus (P) and potassium (K) (Lubofoska P-12%, K-24%) in the autumn at rates of 50 kg ha^{-1} and 80 kg ha^{-1} (pure ingredients), respectively. Nitrogen fertilization, NH_4NO_3 (N-34%) and urea (N-46%), was applied at 3 rates (given as pure ingredient): 40 kg ha^{-1} was applied before the sowing of winter wheat (together with K and P fertilization), 60 kg ha^{-1} was applied in as top dressing at tillering, and the last application was made (40 kg ha^{-1}) during the shooting of winter wheat. Lucerne was fertilized with 50 kg of phosphorus (P) and 100 kg of potassium (K) (Lubofoska, P-12%, K-24% and Korn-Kali, K-40%) every year. The fertilizers were applied at two rates; after the first and second cuts of lucerne.

In each genetic horizon, 5 blocks with dimensions dependent on horizon thickness were selected. Three soil samples were collected from the middle portion of each block with a gouge auger for stepwise sampling and thoroughly mixed to make one pooled sample. The five pooled samples (from each block) were analyzed separately for selected properties and the mean value for the five blocks was presented for each horizon. The surface soil samples were placed in plastic containers (which permitted gas exchange) and chilled to 4 °C in order to minimize any changes in the microbial populations. The samples that were taken from the deeper layers of the soil were placed in sealed containers that generated an atmosphere with a reduced oxygen content and were chilled to 4 °C. The microbial and enzymatic activities were determined based on fresh soil samples within two weeks. The soil samples used to determine the physicochemical properties were air-dried and sieved (2 mm). In order to determine root biomass and morphology, soil samples were taken from each horizon with a core sampler (diameter 10 cm, 250 cm^3). Three corings per horizon were performed and root biomass and morphology were determined separately for each coring. The mean value for the three corings is presented.

2.2. Physicochemical Properties

The physicochemical properties were determined according to the standard methods used in soil sciences and each sample was analyzed in triplicate. The particle size was defined using the Casagrande method, as modified by Prószyński, and the sand fraction content was determined using the sieving method [36]; the pH in a solution of 0.01 M $CaCl_2$ was measured using the potentiometric method [37]; the contents of total organic carbon (TOC) and total nitrogen (TN) were determined using a dry combustion CN analyzer (Vario Max CN). The bulk density was determined using the method of applying volume cylinders (100 cm^3). The cation exchange capacity (CEC) was calculated as the sum of the basic saturation and hydrolytic acidity (data not presented). The contents of N-NO_3^- and N-NH_4^+ were extracted from field-moist soil with KCl and K_2SO_4, respectively. The nitrate nitrogen content was determined using the phenoldisulfonic acid method and the ammonium nitrogen concentration was assayed using the indophenol blue method [38].

2.3. Potential Enzyme Activities

All assays of enzyme activities were performed on fresh, moist, sieved (<2 mm) soils and the activity values were calculated based on the oven-dried (105 °C) weight of soil. Soil urease activity (UR) was assayed, as described by Kandeler and Gerber [39]. In brief, 1 g of soil was incubated with borate buffer (pH 10.0) and urea solution in reaction flasks for 2 h at 37 °C. After the incubation, 1 M KCl was added to all of the flasks for 30 min. In order to assess the ammonium content, the filtrate was mixed with water, Na salicylate/NaOH and sodium dichloroisocyanide and allowed to stand at room temperature for 30 min prior to measuring the optical density at 690 nm. The UR activity was expressed in terms of mg N-NH_4^+ kg^{-1} h^{-1}. We determined the nitrate reductase activity (NR) according

to the method of Kandeler [40]. Soil samples were incubated for 24 h at 25 °C with a 2,4 DNP (dinitrophenol) and the substrate (25 mM KNO_3). After incubation, a 4 M KCl solution was added to all samples; the contents of the test tubes were mixed briefly and filtered immediately. For the spectrophotometric analysis, filtrate, ammonium chloride buffer (pH 8.5) and a color reagent were added to the test tubes and mixed. The extinction was measured at 520 nm. The NR activity was expressed in terms of mg $N\text{-}NO_2^-$ kg^{-1} 24 h^{-1}. We used the method of Ladd and Butler [41] to determine the casein-protease (PRO) activity method [28]. The soil samples (1 g) were incubated with Tris-buffer (0.2 M, pH 8.0) and a Na-caseinate solution at 40 °C for 2 h. After incubation, the remaining casein was precipitated with cold 10% trichloroacetic acid. The suspension was then filtered and a three-fold diluted Folin-Ciocalteu reagent was added to the filtrate and mixed. The tyrosine concentration was measured photometrically at 680 nm and was expressed in terms of mg TYR kg^{-1} h^{-1}. N-acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30) was assayed using a method based on determining the *p*-nitrophenol released after the soil was incubated with *p*-nitrophenyl-N-acetyl-β-D-glucosaminide using acetic buffer (pH 5.5) for one hour at 37 °C. The intensity of the yellow color of the filtrate was measured at 400 nm and the activity was expressed in terms of mmol *p*NP kg^{-1} h^{-1} [42].

2.4. Microbial Biomass C and N Content

The microbial biomass C and N content was determined using the chloroform fumigation-extraction method [43,44]. Moist soil samples (50% WHC, 25 g) were fumigated with ethanol-free $CHCl_3$ at 25 °C for 24 h. After incubation, the chloroform was removed by repeated evacuation. Both fumigated and unfumigated soils (controls) were extracted with 0.5 M of K_2SO_4 for 30 min and analyzed for soluble C, as proposed by Vance et al. [44]. The total N from both fumigated and unfumigated soil samples was determined according to Bremner and Mulvaney [45]. To account for any incomplete recovery of microbial C and N, the microbial biomass was calculated by dividing the difference between the fumigated and unfumigated samples using a correction factor of 0.38 (kEC) [44] and 0.54 (=k_{EN}) [46], respectively. The MBC/C_{ORG} (%) and MBN/N_{TOT} (%) ratios were also calculated [47].

2.5. Nitrogen Substrate Use Capacity Expressed as Microbial Metabolic Potential Diversity

The capacity of microbial communities to mineralize different substrates characterizes their functional diversity [48]. In this study, the utilization of selected substrates present in the Biolog EcoPlate (Biolog Inc., Hayward, CA, USA) containing nitrogen in the structure was analyzed in aerobic and anaerobic (85% N_2, 10% CO_2, 5% H_2) conditions, using an incubator (BD240, Binder, Germany) and Anoxomat (MART Microbiology B.V., Norwood, MA, USA), respectively. We tested the eleven nitrogen sources from the group of amino acids (AA), amines, and amides (AM), N-included organic acids (OA) and N-included carbohydrate compounds (CC). The analysis included the following N-substrates: L-Arginine, L-Asparagine, L-Phenylalanine, L-Serine, L-Threonine, Glycyl-L-Glutamic Acid (AA), Phenylethylamine, Putrescine (AM), ɣ-Amino Butyric Acid, D-Glucosaminic Acid (OA) and N-Acetyl-D-Glucosamine (CC). The substrate utilization was determined according to Frąc et al. [49] with the modifications of Gryta et al. [50] and Oszust et al. [51] in 100 mL of soil suspension containing 1 g of soil and 99 mL of saline peptone water (Biomaxima, Lublin, Poland). Color development was observed when microorganisms from the soil suspension were inoculated into wells (120 µL) and the utilization of N-substrates was measured at 590 nm using a Biolog Microstation (Biolog Inc., Hayward, CA, USA) every 24 h for 8 days. The microbial response with regard to N-substrate utilization was expressed in terms of absorbance values after the deduction of water control values. To perform the necessary calculations, the average value of the measurements for all of the incubation hours was used.

2.6. Evaluation of Root Biomass and Morphology

After manual separation from the soil cores, the roots were placed on a sieve and the soil was removed by gentle rinsing with water. After washing the roots, their fresh weight was determined. Measurements of the morphological parameters of the roots (root length, root surface area) were performed using an EPSON EXPRESSION 10,000 XL root scanner with WinRhizo software [52]. After scanning, the roots were dried at 70 °C until a constant weight was achieved.

2.7. Data Analyses

The studied properties did not show a normal distribution, and therefore, were transformed accordingly (Gaussian anamorphosis). Since the transformation improved the normality of the properties, further analyses were performed with the corrected data. First, a one-way analysis of variance was performed to assess the effect of soil depth (five horizons of up to 150 cm). Additionally, we performed a one-way analysis of variance to assess the significance of the influence of cultivated plants (lucerne and winter wheat) on the soil being studied. Finally, two one-way analyses of variance were used to determine the influence of the soil forming processes/genetic horizons across the soil profiles on the studied enzymatic properties. Firstly, an analysis of variance was carried out to determine changes in enzymatic properties under the influence of the gleyic process (reductive and oxidative conditions), and to accomplish this a comparison was made between three of the deepest horizons situated at the same/similar depths in HP profiles (considered together) and in MSG and CSP profiles (considered together). Secondly, the influence of the lessivage process was assessed with regard to soil enzymatic activity by comparing the E1 and E horizons (HP profiles) with the A2 and BCKg horizons in the MSG and CSP profiles. Any significant differences between the means were determined using Tukey's post hoc test with a 95% confidence interval. Differences between the soil samples were analyzed using a principal component analysis (PCA) based on the mean data values of all of the studied properties. The first two principal components (PC1 and PC2) were selected for the ordination of the cases. Additionally, we performed an analysis of correlation to investigate the relationship between the studied properties. A cluster analysis was performed to determine the groups in the dataset based on the dendrograms prepared separately for N-substrate utilization in aerobic and anaerobic conditions. Moreover, on the basis of the heatmaps obtained, the most and least intensively utilized N-substrates were identified for the tested plants, soil types, and soil depths. All of the statistical analyses were conducted using Statistica 8.1 for Windows software.

3. Results

3.1. Physical and Chemical Properties of the Soil Profiles

The soil bulk density was found to be the lowest in the Ap horizons and increased with depth in all profiles (between 1.46 and 1.83 g cm^{-3}) (Table 1). The soil pH in 1 M KCl ranged from slightly acid to alkaline (5.71–8.36). There were no significant differences in pH across the HL (profile 1) and CSP (profile 4). A significantly higher pH in KCl was found in the surface and in the deepest horizons of HL (profile 2) and MSG (profile 2) when compared to the other horizons of these profiles. All of the soil horizons of the CSP (profile 4) had markedly higher pH values than comparable horizons in the other profiles (Table 2). There were no consistent trends in the clay content in the studied profiles. In general, the clay content significantly increased with depth in profiles 1 and 3, while in profiles 2 and 4 its content was the highest in the middle horizons (2ACgg and Gk) and significantly decreased, both upwards and downwards.

Table 2. Depth distribution of some physico-chemical properties; mean (±SE), $n = 5$.

Profile	Genetic Horizon	pH KCl	Clay	TOC	TN	TOC/TN	N-NO_3^-	N-NH_4^+	CEC
			%	g kg^{-1}			mg kg^{-1}		mmol kg^{-1}
Haplic Luvisol (Cutanic)	Ap	5.71 ± 0.18 A	8 ± 0.3 C	10.8 ± 1.23 A	1.32 ± 0.32 A	10.8 ± 0.30 A	1.31 ± 0.01 A	2.46 ± 0.04 A	46 ± 1.7 B
	E1	5.94 ± 0.14 A	7 ± 0.2 C	2.70 ± 0.56 B	0.31 ± 0.09 B	8.7 ± 0.20 B	0.40 ± 0.01 D	0.62 ± 0.01 B	36 ± 1.0 B
	E2	6.03 ± 0.11 A	6 ± 0.1 C	1.59 ± 0.24 C	0.26 ± 0.07 C	6.1 ± 0.14 C	0.58 ± 0.01 C	0.27 ± 0.01 C	28 ± 0.7 C
	Bt	5.96 ± 0.08 A	16 ± 0.6 A	1.34 ± 0.45 C	0.35 ± 0.10 B	3.8 ± 0.15 D	0.91 ± 0.02 B	0.07 ± 0.01 D	108 ± 4.0 A
	BC	5.58 ± 0.11 A	13 ± 0.4 B	1.22 ± 0.31 C	0.35 ± 0.13 B	3.5 ± 0.10 D	0.34 ± 0.01 E	0.26 ± 0.01 C	97 ± 3.1 A
Molic Eutric Stagnosol	Ap	6.91 ± 0.09 A	10 ± 0.3 C	17.7 ± 1.54 A	1.96 ± 0.34 A	9.0 ± 0.35 B	1.40 ± 0.04 A	2.23 ± 0.05 A	142 ± 4.9 A
	A2	6.49 ± 0.11 C	12 ± 0.2 C	9.66 ± 0.87 B	0.90 ± 0.11 B	10.7 ± 0.22 A	0.36 ± 0.01 B	0.54 ± 0.01 B	126 ± 2.7 A
	2ACgg	6.46 ± 0.05 C	25 ± 0.7 A	4.02 ± 0.35 C	0.42 ± 0.09 C	9.6 ± 0.25 AB	0.21 ± 0.01 C	0.34 ± 0.01 C	139 ± 4.0 A
	3G1	6.67 ± 0.05 B	14 ± 0.3 B	2.34 ± 25 D	0.37 ± 0.09 C	6.3 ± 0.09 C	0.13 ± 0.01 C	0.21 ± 0.01 D	96 ± 2.3 B
	3G2	6.74 ± 0.03 B	14 ± 0.5 B	2.13 ± 0.33 D	0.29 ± 0.05 D	7.3 ± 0.31 C	0.07 ± 0.01 D	0.22 ± 0.01 D	92 ± 3.5 B
Haplic Luvisol (Cutanic)	Ap	6.86 ± 0.15 A	9 ± 0.2 C	10.1 ± 0.41 A	1.25 ± 0.02 A	8.0 ± 0.18 A	9.80 ± 0.40 B	2.40 ± 0.04 A	70 ± 1.0 B
	E	5.81 ± 0.19 B	8 ± 0.2 C	3.07 ± 0.10 B	0.41 ± 0.01 B	7.3 ± 0.19 A	7.85 ± 0.09 C	0.49 ± 0.01 B	57± 1.5 C
	EB	5.72 ± 0.20 B	14 ± 0.4 B	2.17 ± 0.02 C	0.37 ± 0.01 B	6.2 ± 0.22 B	23.5 ± 0.69 A	0.26 ± 0.01 C	64 ± 2.6 BC
	Bt	5.63 ± 0.21 B	18 ± 0.4 A	1.60 ± 0.04 C	0.36 ± 0.01 B	4.4 ± 0.17 C	8.13 ± 0.18 B	0.25 ± 0.01 C	109 ± 2.3 B
	Ck	7.46 ± 0.30 A	15 ± 0.4 B	1.72 ± 0.03 C	0.30 ± 0.01 C	5.9 ± 0.24 B	2.19 ± 0.05 D	0.20 ± 0.01 C	132 ± 4.3 A
Cambic Stagnic Phaeozem	Ap	7.41 ± 0.36 A	9 ± 0.2 C	19.1 ± 0.21 A	2.43 ± 0.06 A	7.9 ± 0.38 A	3.36 ± 0.14 D	2.78 ± 0.05 A	287 ± 10.7 C
	BCkg	7.82 ± 0.08 A	21 ± 0.5 B	2.97 ± 0.03 B	0.36 ± 0.01 B	8.3 ± 0.08 A	34.4 ± 0.56 C	0.48 ± 0.01 B	499 ±14.4 B
	Gk	7.71 ± 0.27 A	24 ± 0.4 A	2.28 ± 0.05 B	0.36 ± 0.01 B	6.3 ± 0.22 B	77.3 ± 0.77 A	0.52 ± 0.01 B	576 ±13.5 A
	2Gk1	8.36 ± 0.14 A	7 ± 0.2 C	0.68 ± 0.02 C	0.08 ± 0.00 C	8.9 ± 0.15 A	41.8 ± 1.25 B	0.17 ± 0.01 C	156 ± 2.9 D
	2Gk2	8.19 ± 0.48 A	2 ± 0.10 D	0.32 ± 0.01 C	0.08 ± 0.00 C	4.2 ± 0.25 C	26.6 ± 0.92 C	0.04 ± 0.01 D	60 ± 1.9 E

TOC—total organic carbon, TN—total nitrogen, N-NO_3^-—nitrate nitrogen form, N-NH_4^+—ammonium; form, CEC—cation exchange capacity The uppercase letters indicate significant differences; ($p < 0.05$) between genetic horizons within the same soil profile.

The highest concentration of TOC and TN was generally observed in the top layers and decreased gradually in successively lower sections of the soil profiles. In general, the MSG and CSP profiles had higher TOC and TN contents as compared to the Haplic Luvisols (profiles 1 and 3), especially in the Ap horizons. Both HL profiles showed a similar content and profile distribution of TOC and TN, while the MSG profile showed definitively higher values for these properties in subsurface horizons as compared to the CSP profile. A relatively high content of TOC and TN was found in the subsurface layer (A2) of the MSG profile (9.66 g kg^{-1} and 0.90 g kg^{-1}, respectively). No clear trends were found for the TOC/TN ratio with regard to the changes throughout the soil profiles, with the exception of profile 1, where the above-mentioned ratio significantly decreased with depth. Clearly, a higher N-NO_3^- content was determined in the soil profiles collected under winter wheat as compared to lucerne cultivation. With regard to the N-NH_4^+ content, all of the studied profiles showed a similar content of this N form with a gradual and significant decrease down the soil profiles.

No clear trends were found; however, for the N-NO_3^- values throughout the soil profiles, except for the MSG profile (profile 2), where the content of this N-form decreased significantly with depth. For both profiles (3 and 4) excavated in the field under winter wheat cultivation, the highest content of N-NO_3^- was determined in the middle horizons (EB and Gk, respectively) with lower values above and below these horizons (Table 2).

3.2. Soil Microbial Biomass C and N

Microbial biomass content, expressed as carbon (MBC) and nitrogen (MBN), was higher in both profiles with a mollic horizon (profile 2 and 4) as compared to both of the Haplic Luvisol profiles (Table 3).

Both the MBC and MBN contents were the highest in the Ap horizon (in all of the profiles) and decreased sharply with depth. They were 50–65% lower in the subsurface horizons (E1, E, BCkg) than in the surface layers (Ap) (except for the MSG profile). The MBC/MBN ratio ranged from 4.01 to 6.24, but no clear trends were found for this ratio with regard to changes throughout the soil profiles. The contribution of MBC to TOC was generally lower than 2.0% in profiles 1–3, and the highest values were observed in the middle horizons of the profiles. In turn, in the CSP profile the MBC/TOC values ranged from 1.51 to 13.8 and increased with depth. The contribution of MBN to TN in profiles 1–3 ranged between 0.70 and 3.40 and was highest in the subsurface horizons (E1, A2, and E). A significantly higher MBN/TN ratio was found in the CSP profile, with the highest values being found in the two deepest horizons (16.0 and 12.8).

Table 3. Depth distribution of microbial biomass C and N; mean (±SE), n = 5.

Genetic Horizon	Depth	MBC	MBN	MBC/MBN	MBC/TOC	MBN/TN
		(mg kg^{-1})			(%)	
Profile 1. Haplic Luvisol						
Ap	0–30	123.3 ± 6.67 A	25.6 ± 2.97 A	4.81 ± 0.38 C	1.15 ± 0.09 B	1.94 ± 0.12 C
E1	30–55	47.1 ± 3.98 B	10.5 ± 1.22 B	4.47 ± 0.29 CD	1.74 ± 0.15 A	3.40 ± 0.29 A
E2	55–82	27.2 ± 1.89 C	6.56 ± 0.41 C	4.01 ± 0.34 D	1.71 ± 0.16 A	2.52 ± 0.19 B
Bt	82–144	18.5 ± 1.75 D	3.42 ± 0.37 D	5.41 ± 0.28 B	1.38 ± 0.16 B	0.98 ± 0.18 D
BC	>144	15.3 ± 1.44 D	2.45 ± 0.32 D	6.24 ± 0.23 A	1.25 ± 0.14 B	0.70 ± 0.13 D
Profile 2. Mollic Stagnic Gleyosol						
Ap	0–30	145.6 ± 5.9 A	30.2 ± 1.82 A	4.83 ± 0.69 C	0.82 ± 0.09 C	1.54 ± 0.09 B
A2	30–53	113.6 ± 5.4 B	25.5 ± 0.93 A	4.46 ± 0.77 C	1.18 ± 0.15 A	2.83 ± 0.12 A
2ACgg	53–70	53.1 ± 4.38 C	10.5 ± 0.41 B	5.06 ± 0.82 B	1.32 ± 0.14 A	2.50 ± 0.18 A
3G1	70–110	25.6 ± 3.37 D	4.12 ± 0.67 C	6.21 ± 0.89 A	1.09 ± 0.12 B	1.11 ± 0.13 C
3G2	110–150	16.5 ± 1.02 E	2.56 ±0.35 D	4.44 ± 0.50 C	0.78 ± 0.14 C	0.88 ± 0.11 D
Profile 3. Haplic Luvisol						
Ap	0–32	113.9 ± 1.12 A	23.2 ± 0.34 A	4.93 ± 0.05 A	1.12 ± 0.02 B	1.83 ± 0.02 B
E	32–46	57.1 ± 0.31 B	12.1 ± 0.32 B	4.72 ± 0.17 A	1.86 ± 0.05 A	2.84 ± 0.10 A
EB	46–58	39.0 ± 1.57 C	9.1 ± 0.37 C	4.32 ± 0.23 B	1.78 ± 0.08 A	2.54 ± 0.12 A
Bt	58–135	26.6 ± 0.77 D	6.1 ± 0.13 D	4.42 ± 0.13 B	1.64 ± 0.12 A	1.64 ± 0.08 B
Ck	135–150	10.0 ± 0.43 E	2.1 ± 0.11 E	4.76 ± 0.21 A	0.58 ± 0.09 C	0.71 ± 0.06 C
Profile 4. Cambic Stagnic Phaeozem						
Ap	0–35	302.1 ± 9.57 A	61.8 ± 1.07 A	4.63 ± 0.09 B	1.51 ± 0.03 D	2.61 ± 0.05 E
BCkg	35–67	106.1 ± 5.99 B	22.9 ± 0.34 B	4.71 ± 0.12 B	3.65 ± 0.04 C	6.36 ± 0.32 C
Gk	67–90	88.8 ± 3.40 C	13.8 ± 0.34 C	6.04 ± 0.18 A	3.74 ± 0.13 C	4.01 ± 0.08 D
2Gk1	90–123	74 2 ± 2.03 D	12.0 ± 0.23 CD	5.96 ± 0.16 A	10.8 ± 0.25 B	16.0 ± 0.41 A
2Gk2	123–150	46.7 ± 0.55 E	9.8 ± 0.15 D	4.66 ± 0.12 B	13.8 ± 0.19 A	12.8 ± 0.31 B

MBC—microbial biomass carbon; MBN—microbial biomass nitrogen, TOC—total organic carbon, TN—total nitrogen. The uppercase letters indicate significant differences ($p < 0.05$) between genetic horizons within the same soil profile.

3.3. Absolute and Specific Enzymatic Activity

The enzymatic activity, which was expressed on a soil mass basis, was generally the highest in the surface horizons in all of the profiles and decreased significantly with depth. We found however, a different pattern in response to soil depth, which was both profile and enzyme specific (Table 4).

The activity of urease (UR) in the Ap horizons was much higher in both profiles with a mollic horizon (profile 2 and 4) than in the Haplic Luvisol profiles. In the deepest horizons (30–150 cm) the UR activity was significantly lower and the values were closer to each other (the mean value for all subsurface horizons of all profiles was between 1.22 and 4.57 mg N-NH_4^+ kg^{-1} h^{-1}). The soil nitrate-reductase activity (NR) was mainly located in the Ap horizons and decreased sharply with depth in all of the profiles, being close to zero in the subsurface horizons. With regard to the Ap horizons, the NR activity was higher in the HL profiles (mean value 3.10 mg N-NO_2^- kg^{-1} 24 h^{-1}) than in the MSG and CSP profiles (mean value 2.61 mg N-NO_2^- kg^{-1} 24 h^{-1}). We found that the activity of PRO in the surface horizons (Ap) was more than twice as high for both profiles with a mollic horizon (mean value 45.6 mg TYR kg^{-1} h^{-1}) as compared to the HL profiles (mean value 20.8 mg TYR kg^{-1} h^{-1}). There were no consistent trends in this activity between the subsurface horizons of the studied profiles. In the Haplic Luvisols (profile 1), the PRO activity decreased significantly with depth, while for the other profiles, the activity was significantly higher in some subsurface layers than in the higher horizons (e.g., 2ACgg in profile 2, EB horizon in profile 3 and 2Ck2 horizon in profile 4). It should be emphasized that a substantial activity of PRO was found in the deepest horizons, and thus 5.4% of the activity determined in the Ap horizon of HP (profile 1) was found in the deepest layer

(BC). For the other profiles, we found the following relationship: 9.3% in profile 2, 6.8% in profile 3 and 5.9% in profile 4. A higher NAG activity in the Ap horizon was found for profiles 3 and 4 (winter wheat cultivation) as compared to the activity in this layer that was assessed in profiles 1 and 2 (lucerne cultivation). The subsurface (all layers below the Ap) NAG activity in the MSG and CSP profiles was significantly lower (the mean for both profiles was 0.030 mmol *p*NP kg^{-1} h^{-1}) than that found for both HL profiles (the mean for both profiles was 0.081 mmol *p*NP kg^{-1} h^{-1}). In the CSP profile the NAG activity in the subsurface horizons was found to be close to zero.

Table 4. Depth distribution of the soil absolute enzyme activities; mean (±SE), n = 5.

Genetic Horizon	Depth	UR activity	NR Activity	PRO Activity	NAG Activity
Profile 1. Haplic Luvisol (Cutanic)					
Ap	0–30	6.32 ± 0.325 A	0.33 ± 0.002 A	13.7 ± 0.344 A	0.26 ± 0.004 A
E1	30–55	2.09 ± 0.072 C	0.05 ± 0.002 B	11.2 ± 0.342 B	0.17 ± 0.001 B
E2	55–82	3.31 ± 0.120 B	0.03 ± 0.001 C	11.1 ± 0.338 B	0.07 ± 0.002 C
Bt	82–144	2.61 ± 0.125 BC	0.02 ± 0.001 C	6.15 ± 0.339 C	0.02 ± 0.001 D
BC	144–150	1.88 ± 0.064 C	0.02 ± 0.001 C	2.54 ± 0.127 D	0.01 ± 0.000 D
Profile 2. Mollic Stagnic Gleyosol					
Ap	0–30	42.1 ± 0.127 A	1.38 ± 0.043 A	29.9 ± 1.487 A	0.20 ± 0.032 A
A2	30–53	12.4 ± 0.628 B	0.05 ± 0.005 C	22.2 ± 1.021 B	0.08 ± 0.010 B
2ACgg	53–70	3.82 ± 0.315 C	0.19 ± 0.090 B	26.0 ± 0.714 AB	0.03 ± 0.001 C
3G1	70–110	1.73 ± 0.050 D	0.02 ± 0.001 C	3.34 ± 0.084 C	0.02 ± 0.000 C
3G2	110–150	0.77 ± 0.063 D	0.01 ± 0.000 C	3.22 ± 0.108 C	0.01 ± 0.000 C
Profile 3. Haplic Luvisol (Cutanic)					
Ap	0–32	4.37 ± 0.176 A	5.87 ± 0.002 A	27.8 ± 1.140 A	0.54 ± 0.010 A
E	32–46	1.11 ± 0.058 C	0.03 ± 0.001 B	9.85 ± 1.207 C	0.14 ± 0.001 B
EB	46–58	3.04 ± 0.060 B	0.01 ± 0.001 B	18.3 ± 0.003 B	0.10 ± 0.001 C
Bt	58–135	0.42 ± 0.008 D	0.01 ± 0.001 B	11.1 ± 0.378 C	0.10 ± 0.001 C
Ck	135–150	0.33 ± 0.063 D	0.01 ± 0.001 B	4.10 ± 0.005 D	0.04 ± 0.001 D
Profile 4. Cambic Stagnic Phaeozem					
Ap	0–35	65.6 ± 15.80 A	3.83 ± 0.030 A	61.2 ± 0.388 A	0.47 ± 0.006 A
BCkg	35–67	3.20 ± 0.900 B	0.11 ± 0.003 B	25.3 ± 0.395 B	0.04 ± 0.022 B
Ck	67–90	3.19 ± 0.262 B	0.08 ± 0.003 B	7.01 ± 0.810 D	0.03 ± 0.001 B
2Ck1	90–123	1.25 ± 0.069 C	0.04 ± 0.002 C	7.18 ± 0.003 D	0.01 ± 0.000 B
2Ck2	123–150	0.91 ± 0.101 C	0.01 ± 0.001 C	10.4 ± 0.861 C	0.02 ± 0.001 B

UR urease activity (mg N–NH_4^+ kg^{-1} h^{-1}), NR nitrate reductase (mg N-NO_2^- kg^{-1} 24 h^{-1}), PRO proteases (mg TYR kg^{-1} h^{-1}), NAG N-acetyl-glucosaminidase (mmol pNP kg^{-1} h^{-1}). The uppercase letters indicate significant differences ($p < 0.05$) between genetic horizons within the same soil profile.

In order to assess the influence of reducing conditions (water saturation and low O_2 concentration) on the studied enzymes, three of the deepest horizons of the MSG and CSP profiles were compared with the corresponding horizons of both of the Haplic Luvisols (Figure 1). The UR and PRO activity was not significantly differentiated in comparison with the air–water conditions. In turn, the NR activity was significantly higher in the horizons with reducing conditions, as compared to more highly oxygenated conditions, while in the case of the NAG activity the tendency was the opposite.

Since lessivage is the main soil forming process in the studied profiles of the Haplic Luvisols, we compared the enzymatic activity in the E1 and E horizons (profile 1 and 2) with the activity in the humic horizons situated at the same depth (A2 and BCKg) in profiles 3 and 4. The UR, NR and PRO activity was significantly higher in the humic horizons as compared with their activity in the corresponding eluvial horizons (E1 and E) (Figure 2). In the case of NAG activity, the tendency was the opposite.

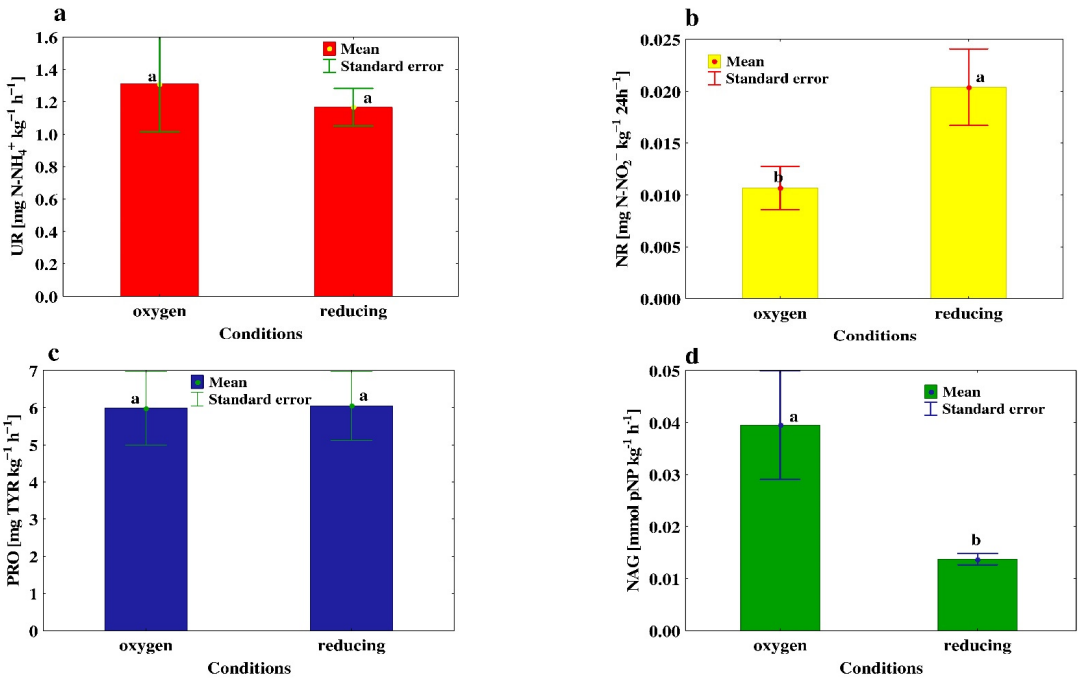

Figure 1. Effect of reducing conditions on the potential UR (**a**), NR (**b**), PRO (**c**), NAG activity (**d**), whereas oxygen conditions refer to three the deepest horizons in the profiles 1 and 3, while reducing conditions refer to three the deepest horizons in the profiles 2 and 4 (mean ± SE). Values in compared horizons followed by different small letters are statistically different at $p < 0.05$.

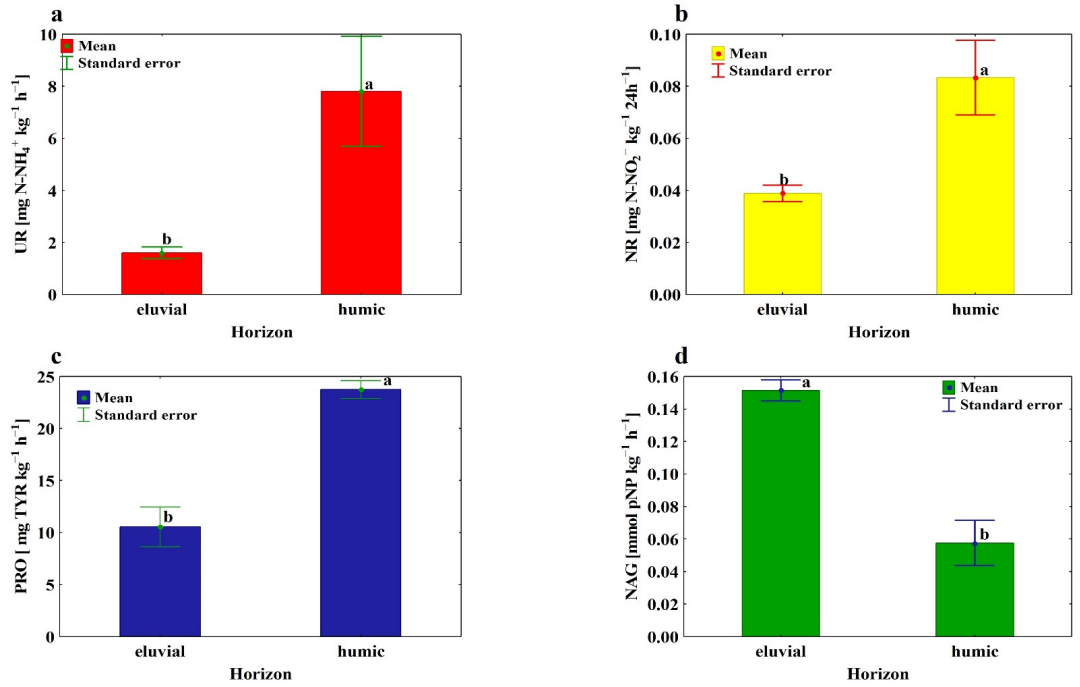

Figure 2. Effect of the lessivage process on the potential UR (**a**), NR (**b**), PRO (**c**), NAG activity (**d**); whereas eluvial horizons refer to the E1 and E horizons in the profiles 1 and 3, while humic horizons refer to the A2 and BCkg horizons in the profiles 2 and 4 (mean ± SE). Values in compared horizons followed by different small letters are statistically different at $p < 0.05$.

With regard to the Ap horizons, a significantly higher enzymatic activity was found in the winter wheat profiles as compared to the lucerne profiles (Figure 3a–d). In the subsurface horizons in turn, the UR and NAG activity was significantly higher under the lucerne as opposed to the winter wheat, while in the case of NR and PRO activity the difference was not significant. The UR and NR activity in the three deepest horizons was significantly higher in the lucerne profiles than in the winter wheat profiles. In the case of PRO and NAG activity in the two deepest horizons, the tendency was the opposite.

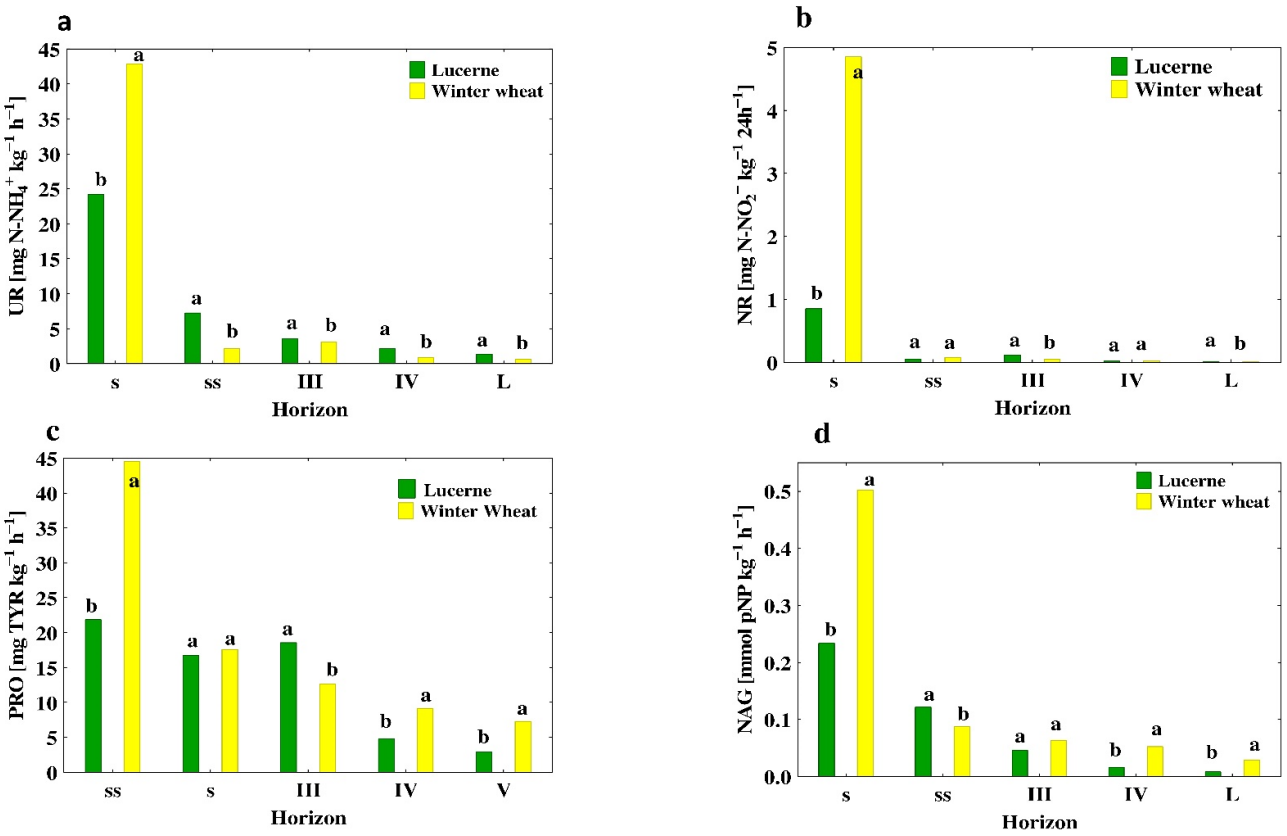

Figure 3. Effect of the cultivated plants on the potential UR (**a**), NR (**b**), PRO (**c**), NAG activity (**d**); s the surface (Ap) horizons, ss the sub-surface horizons, III the middle (thirds) horizons, IV the second from the bottom horizons, L the deepest horizons. Values in compared horizons followed by different small letters are statistically different at $p < 0.05$.

We found that the specific enzyme activity (expressed per unit of soil TOC and MBC) was more variable in response to the depth of a profile when compared to the enzymatic activity which was expressed per soil unit, and this prevented clear trends from being determined (Tables S1 and S2). The UR activity which was expressed per unit of soil TOC and MBC reached its highest levels in the Ap horizons of the MSG and CSP profiles and significantly decreased with depth. In turn, in both Haplic Luvisols the highest specific UR activity (per TOC and MBC units) was found in the middle and lower horizons as compared to the surface layers. Both NR/TOC and NR/MBC ratios were the highest in the Ap horizons of all of the profiles and were significantly lower in the deeper layers. It is worth noting that the changes in both ratios with profiles depth were similar. With regard to the specific activity of PRO and NAG, there were no consistent trends in their changes between the horizons and those changes were both enzyme and profile specific. The same point was only the highest value of PRO activity (both per the TOC and MBC units) in the middle horizons of 1–3 profiles, as well as the highest value of NAG activity (also per TOC and MBC) in surface or subsurface layers of the studied profiles.

3.4. Potential Microbial Metabolic Diversity Expressed in Terms of N-Substrate Utilization

On the basis of cluster analysis for N-substrate utilization, for the assumed Sneath criteria of 66% and 33%, two and four main groups in the dataset were identified, respectively (Figure 4). The effect of the cultivated plants was more pronounced when substrate utilization by aerobes was considered (Figure 4a), while the influence of soil type was more visible for substrate degradation in anaerobic conditions (Figure 4b). In aerobic incubation, the first group included soil samples collected from lucerne cultivation mainly from deeper soil layers (IA). The second cluster (IB) included all soil samples from winter wheat cultivation. The third (IIA) and fourth (IIB) groups contained only samples collected from lucerne profiles. However, for cluster IIA, samples from the top layer were recognized and for the IIB samples from the subsurface layers, mainly L2 and L3, were placed. From the 11 different nitrogen sources, the highest rates of substrate utilization were recorded for the samples grouped in clusters IIA and IIB and then IA collected from the lucerne profiles, thereby indicating that soil microbial communities had a higher potential to utilize N-compounds in comparison with microbes colonizing the soil under wheat cultivation of.

Lucerne increased the microbial utilization of all the tested N-sources as compared to winter wheat cultivation, especially L-asparagine, ɣ-amino butyric acid, L-serine, L-arginine in all soil layers, and L-phenylalanine, L-threonine, glycyl-L-glutamic acid, phenylethylamine, putrescine, D-glucosaminic acid and N-acetyl-D-glucosamine in the upper soil horizons (Figure 5a). For winter wheat cultivation all of the tested N substrates were utilized at a very low level, particularly L-arginine and phenylethylamine, which was characterized by almost no utilization (Figure 5a).

In anaerobic conditions (Figures 4b and 5b), it was found that almost all soil samples collected from HL profiles could be placed in the first cluster regardless of the cultivated plant (IA). The second (IB), third (IIA) and fourth (IIB) clusters mainly included soil samples from the MSG and CSP profiles. However, in the IIB cluster only samples from the top layer were identified, in IIa they were from layers L1 and L3, while in Ib most of the samples were identified as being collected from deeper layers (Figure 4b). The use of almost all of the tested N-sources was very low in the HL profiles (Figure 5b).

When relating N-source utilization with specific substrate use in the MSG and CSP profiles, the relationship with N-source degradation was affected by the soil layer. In the Ap horizon of MSG profile only L-serine and N-acetyl-D-glucosamine were utilized, while the CSP profile was characterized by a relatively high utilization level of the majority of tested nitrogen substrates in the Ap and subsurface layers (Figure 5b).

In summary, due to a combination of the effects of the plant cultivated, soil types and soil horizons, significant differences were observed (Figures S1 and S2). Lucerne significantly increased the soil microbial aerobic utilization of L-arginine, L-asparagine, L-serine, ɣ-amino butyric acid and phenylethylamine while decreasing the levels of L-phenylalanine and D-glucosaminic acid as compared to winter wheat cultivation. There were no significant differences in the utilization of the other tested N-sources between cultivated plants.

Under anaerobic conditions only the utilization of L-serine and ɣ-amino butyric acid increased and decreased, respectively in soil under lucerne cultivation. In general, the level of L-phenylalanine and N-acetyl-D-glucosamine was higher and that of ɣ-amino butyric acid and D-glucosaminic acid was lower in the MSG and CSP profiles than in the HL profiles under aerobic conditions of soil incubation. However, in anaerobic incubations, glycyl-L-glutamic acid, N-acetyl-D-glucosamine and ɣ-amino butyric acid were utilized more intensively in the MSG and CSP profiles than in the HL profiles. A significant increase in the utilization of N-acetyl-D-glucosamine was noted in the Ap horizons as compared to the deeper soil layers for all of the tested soils incubated in aerobic and anaerobic conditions. The level of L-phenylalanine was significantly higher in the Ap horizon and the level of L-serine was significantly lower in the deepest layers in comparison with the other soil depths, but only after soil incubation in aerobic conditions. In general, N substrate

utilization decreased with increasing soil depth, and was greater in the lucerne than in the winter wheat profiles, and also in the MSG and CSP profiles than in the HL profiles.

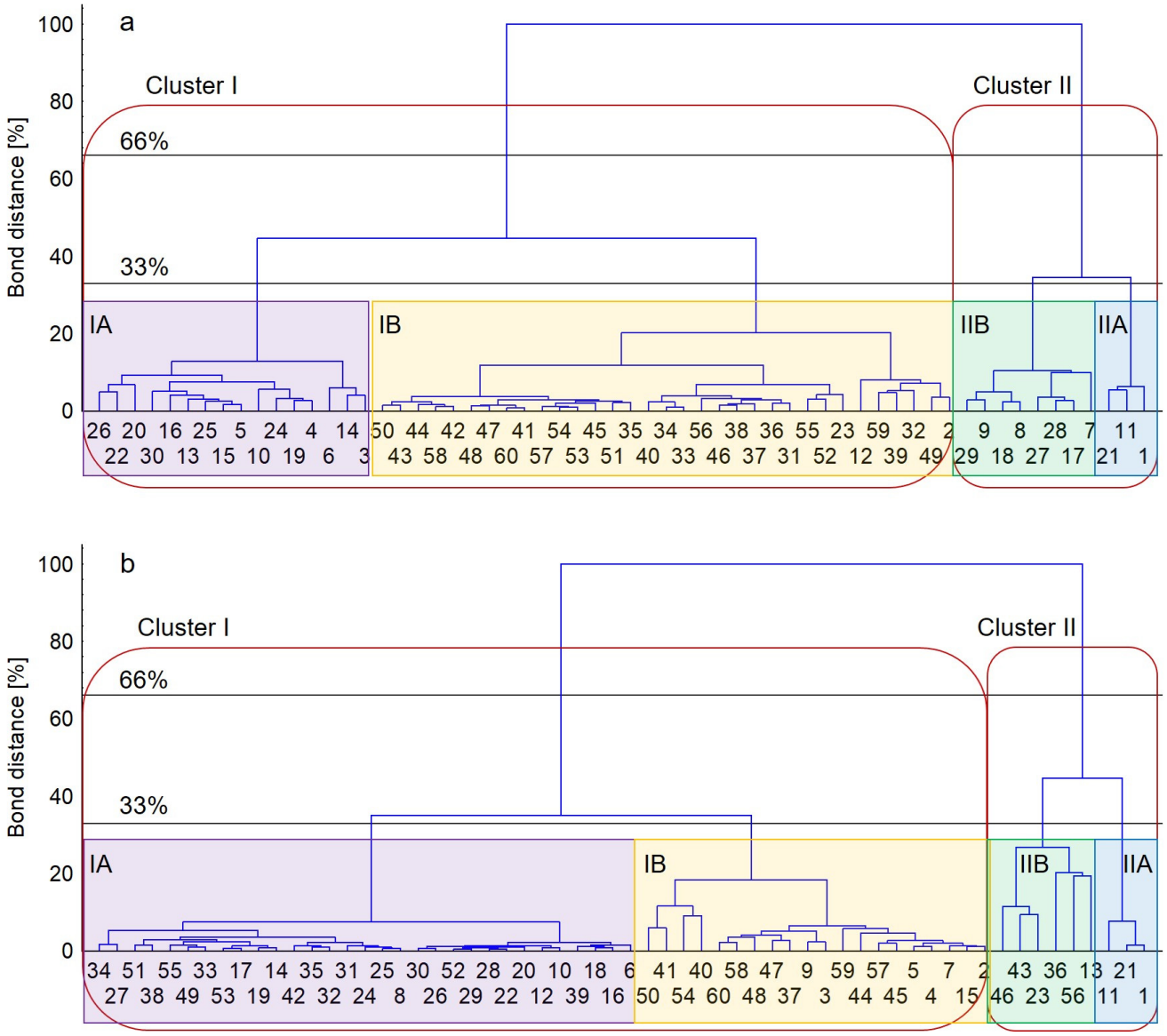

Figure 4. Dendrograms showing the distribution of soil samples with different microbial metabolic capacity under aerobic (**a**) and anaerobic (**b**) conditions. Explanations: 1,11,2 1 lucerne, Mollic Staginic Gleysol (MSG), Ap, 2, 12, 22 lucerne, MSG, A2, 3, 13, 23 lucerne, MSG, 2ACgg, 4, 14, 24 lucerne, MSG, 3G1, 5, 15, 25 lucerne, MSG, 3G2, 6, 16, 26 lucerne, Haplic Luvisol (HL), Ap, 7, 17, 27 lucerne, HL, E1, 8, 18, 28 lucerne, HL, E2, 9, 19, 29 lucerne HL, Bt, 10, 20, 30 lucerne, HL, BC, 31, 41, 51 winter wheat, Haplic Luvisol (HP), Ap, 32, 42, 52 winter wheat, HL, E, 33, 43, 53 winter wheat, HL, EB, 34, 44, 54 winter wheat, HL Bt, 35, 45, 55 winter wheat, HL, Ck, 36, 46, 56 winter wheat, Cambic Stagnic Phaeozem (CSP), Ap, 37, 47, 57 winter wheat, CSP, BCkg, 38, 48, 58 winter wheat, CSP, Ck, 39, 49, 59 winter wheat, CSP, 2Ck1, 40, 50, 60 winter wheat, CSP, 2Ck2.

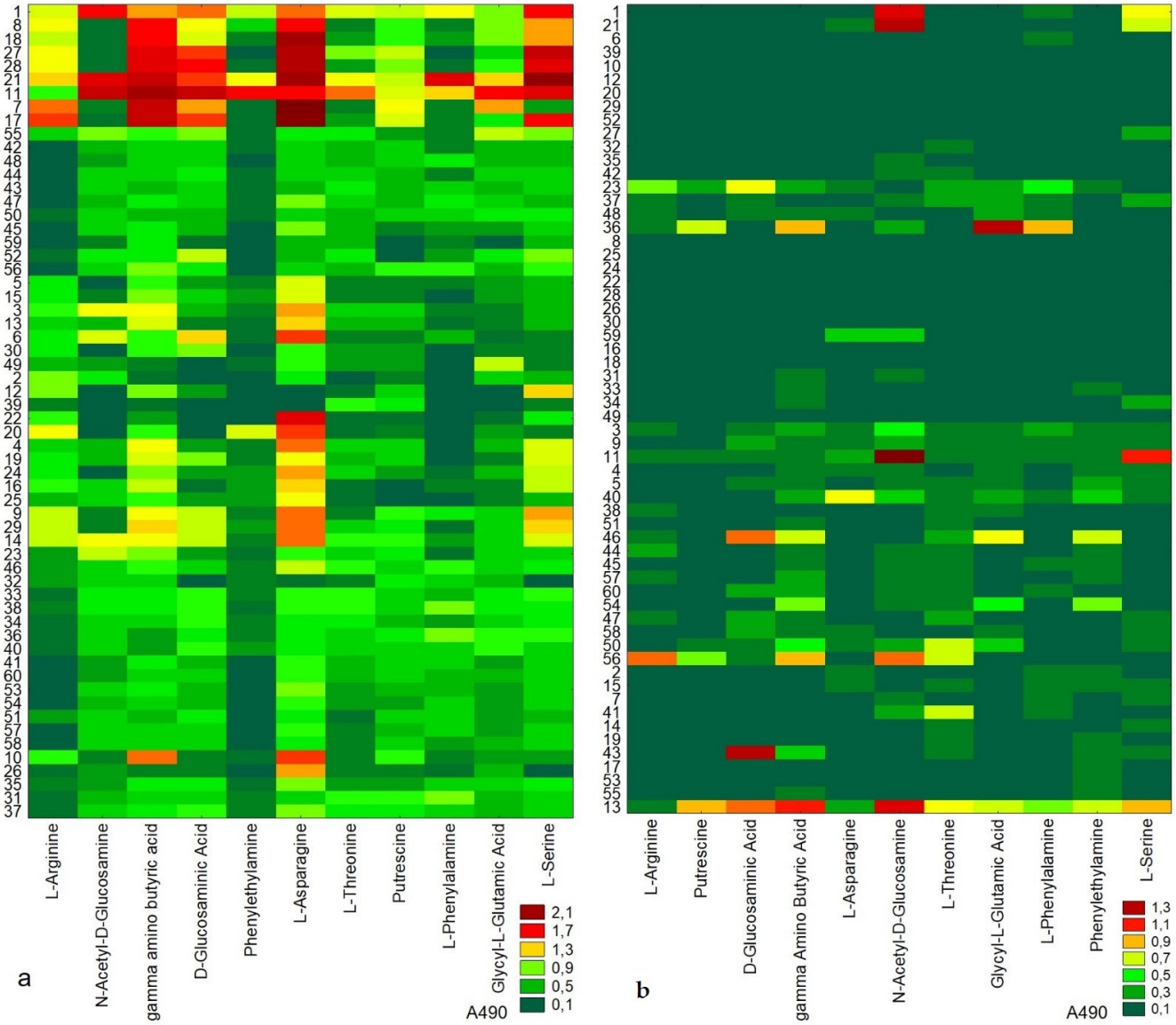

Figure 5. Heat maps showing the substrates utilization by soil microbial communities under aerobic (**a**) and anaerobic (**b**) conditions. Explanations: 1,11,21 lucerne, Mollic Staginic Gleysol (MSG), Ap, 2, 12, 22 lucerne MSG, A2, 3, 13, 23 lucerne, MSG, 2ACgg, 4, 14, 24 lucerne, MSG, 3G1, 5, 15, 25 lucerne, MSG, 3G2, 6, 16, 26 lucerne, Haplic Luvisol (HL), Ap, 7, 17, 27 lucerne, HL, E1, 8, 18, 28 lucerne, HL, E2, 9, 19, 29 lucerne, HL, Bt, 10, 20, 30 lucerne, HL, BC, 31, 41, 51 winter wheat, Haplic Luvisol (HL), Ap, 32, 42, 52 winter wheat, HL, E, 33, 43, 53 winter wheat, HL, EB, 34, 44, 54 winter wheat, HL, Bt, 35, 45, 55 winter wheat, HL, Ck, 36, 46, 56 winter wheat, Cambic Stagnic Phaeozem (CSP), Ap, 37, 47, 57 winter wheat, CSP, BCkg, 38, 48, 58 winter wheat, CSP, Ck, 39, 49, 59 winter wheat, CSP, 2Ck1, 40, 50, 60 winter wheat, CSP, 2Ck2.

3.5. Root Biomass and Morphology

In general, the root biomass decreased with increasing soil depth and was greater in the lucerne profiles than in the winter wheat profiles, especially in the surface and subsurface soil layers (Table S3). Almost no roots were found in the deepest horizons of the lucerne profiles as compared to the deepest layers of the winter wheat profiles, where the root biomass in the deepest layers was over 20% of the mass found in the Ap horizons. Irrespective of the plant being cultivated, the length and surface area of the roots were greatest in the Ap horizons of the soil profiles, while no clear changes in these morphological features were found with depth (Figure 6a–d, Table S3). Only in profile 4

did both the root length and surface area decrease progressively down the soil profile. In the lucerne profiles, the average root length in all subsurface horizons was about 6% of the length determined in the Ap horizons, while in the winter wheat profiles this value was 47% (mean for both profiles). For the winter wheat profiles, irrespective of the soil horizon, roots with a diameter below 0.5 mm made up over 80% of the total root system length, while almost no roots with a surface area of between 2.5 and 5.0 mm were found (Figure 6a,b). In the lucerne profiles in turn, the greater contribution of roots with a larger diameter was found, including those with a diameter ranging from 2.5–5.0 mm (Figure 6c,d).

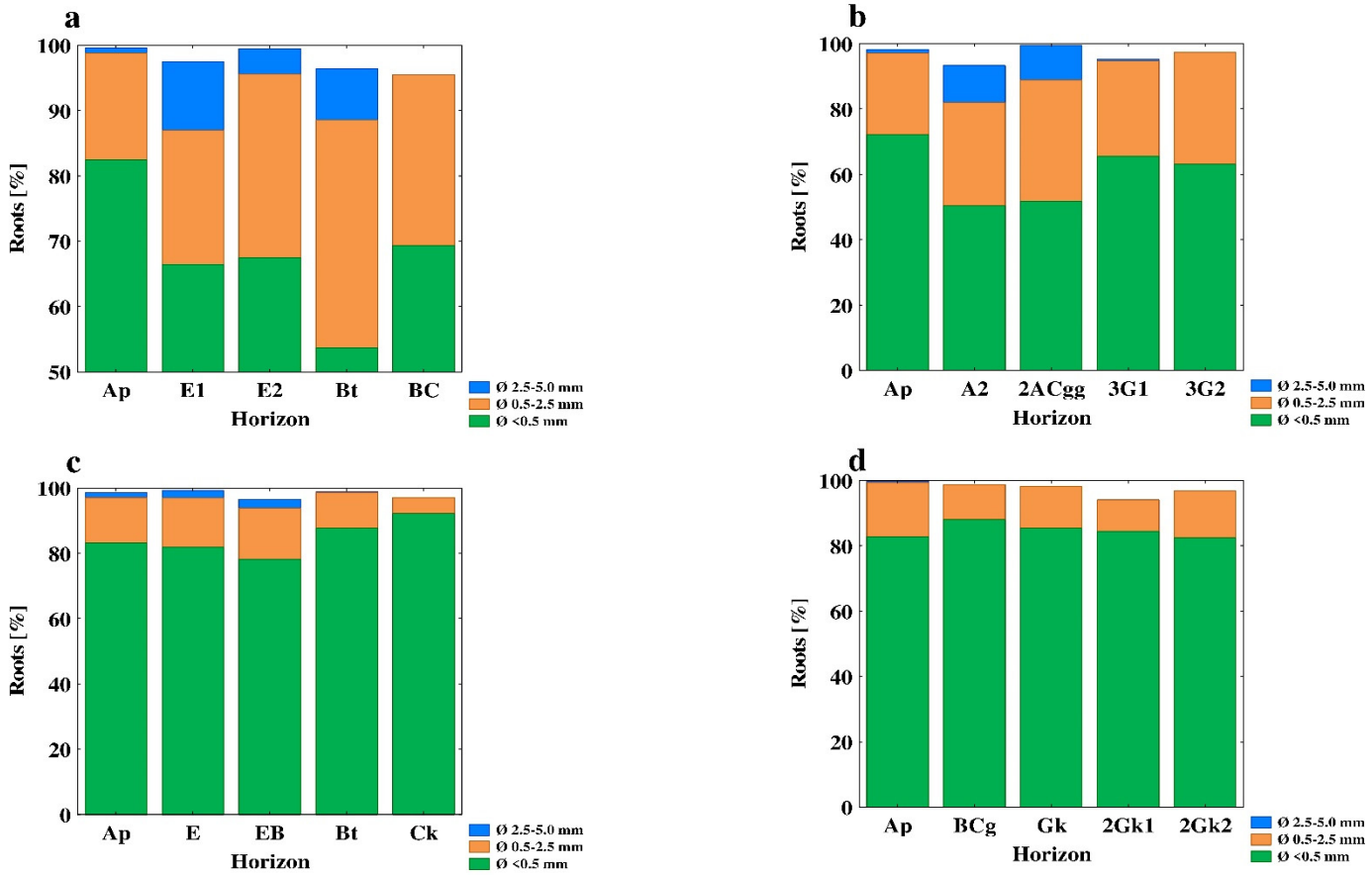

Figure 6. Contribution of different root diameters (mm) in the total length of the root system; winter wheat profiles (**a**,**b**) and lucerne profiles (**c**,**d**).

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

An analysis of correlation took all of the data into account (all of the profiles and horizons considered together) (Table S4). All of the studied enzyme activities were significantly and positively correlated with TOC, TN, MBC and MBN. A significant but negative relationship was calculated between the clay content and the NAG activity, mainly in the Ap horizons. The PCA analysis identified three components which accounted for 85% of the total variance, most of which were explained by PC1 and PC2 (Figure 7a).

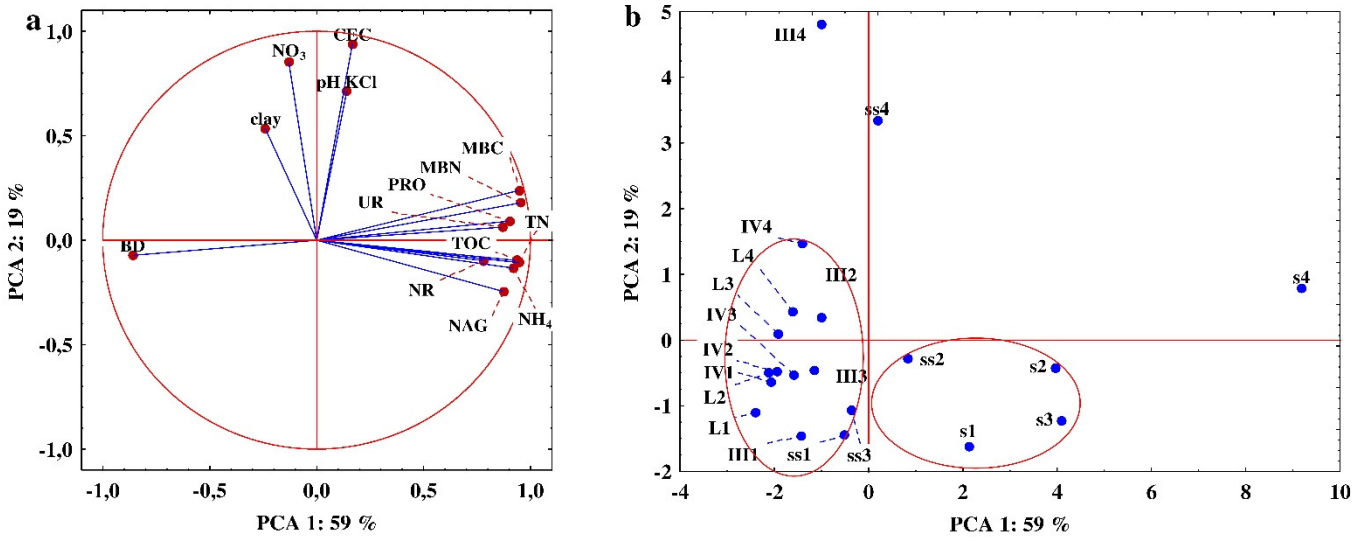

Figure 7. Principal component analysis derived from the studied soil properties. (**a**) Plot of the first two principal components (PC) for the measured soil properties, UR urease, NR nitrate reductase, PRO proteases, NAG N-acetyl-glucosaminidase, TOC total organic carbon, TN total nitrogen, MBC—microbial biomass carbon, MBN microbial biomass nitrogen, DB bulk density, N-NO_3^- nitrate nitrogen, N-NH_4^+ ammonium nitrogen, CEC cation exchange capacity; (**b**) principal component analysis of the variables in the soil horizons in all 4 profiles: s1–s4 the surface (Ap) horizons, ss1 ss4 the sub-surface horizons, III1–III4 the middle (thirds) horizons, IV1–IV3 the second from the bottom horizons, L1–L4 the deepest horizons.

PC1, which accounted for 59% of the variance, was related to enzymatic activity, microbial biomass C and N, TOC and TN because all of them had high positive loading scores for this component (Table 5). The above-mentioned properties were most positively correlated with PC1 as well as with each other. PC2 correlated with the chemical properties such as pH in KCl, clay content, NO_3^- and CEC. A PCA analysis showed a strong and positive correlation of the surface horizons of all of the studied profiles, while all horizons below the Ap layer in all profiles were negatively correlated with this component (Figure 7b).

Table 5. Loading scores of the variable for PCA.

Variable	PCA1	PCA2	Variable	PCA1	PCA2
UR	0.870	0.006	clay	−0.242	0.535
NR	0.782	−0.100	TOC	0.936	−0.097
PRO	0.905	0.091	TN	0.951	−0.108
NAG	0.875	−0.247	CEC	0.166	0.939
MBC	0.947	0.238	BD	−0859	−0.072
MBN	0.955	0.179	N-NO_3^-	−0.130	0.852
pH in KCl	0.140	0.711	N-NH_4^+	0.920	−0.134

UR urease, NR nitrate reductase, PRO proteases, NAG N-acetyl-glucosaminidase, MBC microbial biomass carbon; MBN microbial biomass nitrogen, TOC total organic carbon, TN total nitrogen, CEC cation exchange capacity, BD bulk density, N-NO_3^- nitrate nitrogen, N-NH_4^+ ammonium nitrogen.

4. Discussion

4.1. Changes in the Soil Properties along the Soil Profiles

In accordance with previous studies [53,54] our data have shown a significant decline in soil microbial properties and N-source utilization as a function of soil depth. In general, such a decrease can be explained by the fact that both microbial biomass and functional diversity expressed in terms of substrate use capacity as well as enzymatic activity, especially those originating from hydrolases, are often directly associated with SOM content and they mirrored the expected availability of their respective substrates [48,54]. Since the

SOM content and the number of regular substrates that can be hydrolyzed decrease with depth [55], the activity of the hydrolytic enzymes can also be expected to decrease [20]. In the study by Darby et al. [1], the correspondence with soil C was strongest for the N-acquire enzyme (NAG) (R^2 = 0.83) and lower for C-acquire enzymes. In fact, similar to previous studies [1,56], our results showed that the potential enzyme activities of the soil and metabolic microbial potential in the upper horizons were primarily determined by the content of organic carbon (TOC) and total nitrogen (TN) and generally decreased with soil depth. A clear dependence on TOC was found for PRO activity in the A2 and 2ACgg horizons (the MSG profile) as well as in the Ap horizon of the CSP profile. The NAG activity was observed to decrease with increase in N availability, indicating that this enzyme reflects the extent to which N limits the growth of microorganisms [57]. The relationship between the NAG activity and mineral N forms in this study (relatively high and positive correlation, Table S4) did not confirm the above observations. In turn, the NAG activity in our study was strongly correlated with fungal CFUs (data not presented), both of which were the highest in top layers and decreased significantly with depth. In fact, NAG activity was proposed earlier as a specific indicator of the presence of fungi biomass in soil [58]. Additionally, the patterns of enzymatic activity and substrate use capacity with depth and could also be affected by a range of factors, such as soil texture, moisture, oxygen levels, and plant residues [59].

Soil enzymes are mainly produced by microorganisms, and the microbial biomass of C and N have been shown to decrease with increasing soil depth as a result of the decreasing availability of the nutrient concentration in the deeper soil layers [60]. A decreased MBC and MBN content with depth was closely associated with decreasing enzymatic activity, and this relationship was confirmed by the high values of the correlation coefficients between all of the studied enzymes and the MBC content (Table S4). A decrease in the microbial biomass of C and N with depth may be associated with the fact that more of the microbial biomass could have been less active as well as not active or dead due to the extreme deep-soil conditions, such as a higher soil density, lower oxygen concentration and less available carbon and nutrients than in the surface horizons [61]. This was confirmed by changes in the MBC/MBN ratio throughout the soil profiles. A high values for the ratio in the deeper soil horizons indicate long-term decreases in the availability of nutrients to the soil microorganisms [62]. It is commonly known that the substrate quality is lower in the subsoil than in the topsoil, which suggests that the SOM is less degradable at deeper depths [27]. Based on the literature [61], we can also assume that due to the low degree of competition between microbial communities, certain microbial taxa are consistently more abundant in deep soils and are preferentially adapted to low-nutrient conditions due to their ability to synthesize and store specific enzymes [63].

However, while potential enzyme activity (expressed in terms of soil mass units) usually decreases with soil depth [19,20], the specific enzyme activity (expressed in terms of organic C or microbial biomass C content) had either similar values throughout the soil profile or increased with depth [64,65]. In the study by Dove et al. [17], enzyme activities per MBC or SOC units were substantially higher at depth (soils below 20 cm accounted for 80% of whole-profile enzymatic activity), suggesting an accumulation of enzymes in subsoil horizons on mineral and organic surfaces. The enzyme activity on a soil mass basis provides an estimate of the rate at which the products of enzymatic activity are being made available to microorganisms and plants; as such, it is a quantitative measure. By contrast, the expression of enzymatic activity per TOC or MBC units provides an estimation of how eligible organic matter is to being degraded by those enzymes, and that is why it is considered to be an organic matter quality index [27]. We expected that specific enzymatic activities would increase with depth, thereby reflecting a greater degree of microbial potential to produce enzymes in response to the decreased availability of carbon and nutrients [66]. The relatively high specific enzyme activity per unit of TOC in the deeper horizons of the soil profiles studied when compared to the upper layers may suggest the presence of substrates and favorable conditions for substrate mineralization (i.e., more

degraded and humified soil organic matter) with the contribution of these enzymes. Stone et al. [20] suggested that constant rates of specific enzyme activity throughout the soil profile could be due in part to constitutive enzyme activity. Alternatively, soil microorganisms may expend more energy to produce enzymes in deep soils because microbes continuously secrete a low level of enzymes in order to maintain the capacity to rapidly respond to changes in substrate availability even in a nutrient-poor environment [66].

An accumulation of NO_3^- was observed in the deeper layers of winter wheat profiles, which was probably related to the N fertilization applied for the cultivation of this plant. In general, under the reducing conditions in deep soil, NO_3^- is rapidly reduced to N_2 by denitrifying bacteria. A low-oxygen environment contributes to the favorable conditions for denitrification [67]; however, the process of denitrification, apart from NO_3^-, also requires a sufficient quantity of organic matter to serve as an electron donor. In the deep reductive soils, organic matter could not decompose completely and unavailable organic matter substrates for denitrifiers (such as humic-like substances) were included in the dissolved organic matter parameter [67]. Several studies have shown that an increase in soil organic matter increases the soil denitrification rate [68]. However, in the winter wheat profiles, soil organic matter as well as DOC content (data not presented) in the deeper layer was rather low which probably contributed to the low denitrifying activity and the accumulation of nitrate nitrogen. In fact, the scarcities of organic matter, microbial populations and water content in subsoils produce aerobic conditions, promote NO_3^- stability and inhibit denitrification [69]. In addition, nitrate N accumulation in deep soil profiles of vegetable systems (>100 cm) was positively correlated with N and water input rates, and was negatively correlated with soil organic carbon, C/N ratio, and the clay content [70]. Similarly, in the study by Huang et al. [71], the available N increased with depth after N fertilization because of NO_3^- leaching. It is expected that the majority of NO_3^- in excess of the biological immobilization rates will be transported through the soil into ground or surface waters [72].

Nitrate reductase is an adaptive enzyme and is synthesized only in the presence of NO_3^- ions, while in the soil solution it is repressed by NH_4^+ ions [73]. This result is not entirely consistent with our study, whereas NR activity was positively correlated with both NO_3^- and NH_4^+ content when they are considered separately for the surface and all subsurface horizons (but for all profiles).

The relationship between the soil enzymes, nitrogen-substrate utilization and the cultivated plants was also considered, since the roots are a significant source of some soil enzymes and organic compounds [74] or contribute to favorable conditions for the microbial synthesis [75]. Based on this statement, we may suppose that the extent of enzymatic activity and the degradation of nitrogen compounds throughout the soil profiles (at least in the upper layers) may be due to the nature of the plant root system. With regard to the surface soil horizon, the occurrence of enzyme activities in the rhizosphere soils is of great importance [27]. The stronger correlation between most enzymes and root biomass in the topsoil than in the subsoil (e.g., for NAG activity $R^2 = 0.54$ in topsoil and $R^2 = 0.12$ in subsoil) indicates intensified production of extracellular enzymes by plants to acquire soil nutrients [18]. The findings of Greenfield et al. [76] suggest that cereal roots contribute one-fifth of rhizosphere protease activity. The higher level of enzyme activity of the rhizosphere as compared to the bulk soil depends not only on the stimulation of root-associated microbial activity by rhizodeposition but also on the release of enzymes and root exudates that can solubilize some mineral-associated organic matter and can also vigorously stimulate the activities of some enzymes [77]. For this reason, a comparison has been made between the shallow-rooted, bunched annual crop system (winter wheat) with the deeply-rooted, 4 year old plantation of lucerne in this study. In general, the enzyme activities were the highest in the topsoil in all of the studied profiles which corresponded to the highest level of root biomass. No clear difference was recorded however for the tested enzymatic properties in the deeper soil layers, which depended on the plants being cultivated. Although the highest level of biomass as well as root length and surface area

(Table S3) was found in the Ap horizons of the lucerne profiles, the studied enzymatic activity level was significantly higher in the winter wheat surface horizons. In the 4 year old plantations of lucerne used in this study, the roots of other plants, such as grasses and weeds, may participate in the overall root system. Considering that the Ap horizons of the winter wheat profiles had one and half times more MBC and MBN than the lucerne profiles (with a comparable TOC content) we may assume that the enzymatic activity was mainly of microbial rather than of plant origin. In addition, a greater microbial biomass of C and N as well as the activity of enzymes in the Ap layer of winter wheat profiles may be affected by applied mineral N fertilization. In fact, a strong non-linear negative relationship between mineral N content and root biomass of spring wheat was found in the topsoil, whereas a linear decrease was observed for the subsoil [18]. That is why data related to the effect of roots on soil enzymatic properties should be considered with caution, since other factors may also contribute to this.

4.2. Changes Associated with Soil Type, Genetic Horizons and Soil-Forming Processes

While the soil organic carbon content and the oxygen conditions that result from certain agricultural practices were the major factors affecting the state of enzymatic activity in the surface, humic horizons of agricultural soils [78], in the deeper horizons, other factors such as soil reaction, air-water conditions and soil texture, could also explain the shift in microbial and enzymatic properties. Such conditions may affect both the nature and extent of extracellular enzymatic activity and also the activity of microbial communities taking part in organic compound transformation and utilization. The significant effect of soil type/soil formation processes on the enzyme activities and on the N-substrate utilization profile was revealed, this may be attributed to the different physicochemical properties of the studied soils. The main features of Mollic Stagnic Gleysol (MSG) and Cambic Stagnic Phaeozem (CSP) is the mollic diagnostic horizon and the gleyic process, while Haplic Luvisols have ochric horizons and the lessivage process in common [34]. We assumed that, because of these differences, the studied profiles would reveal significant differences in the examined soil microbial and enzymatic properties.

Reducing conditions (water saturation and a low O_2 concentration) are common in the subsoil horizons of the studied MSG and CSP profiles, as expected, both of these soils were waterlogged by groundwater. In order to evaluate the effect of reducing conditions on the studied enzymes, we compared the enzymatic activity in the 2ACgg-3G2 horizons (in the MSG profile) and the Gk-2Gk2 horizons (in the CSP profile), which are expected to have reducing conditions, to the oxidative layers in the Haplic Luvisol profiles, occurring at the same/similar depth. Although hydrolytic enzyme activities do not directly require the presence of O_2, experiments and theory suggest that reducing conditions may decrease the activity of hydrolases indirectly via at least two mechanisms (1) oxygen limitation may inhibit hydrolytic enzyme activity by promoting the accumulation of phenolic substances, a ubiquitous components of organic matter that interferes with enzymes catalysis [79]. Polyphenolic compounds accumulate in anaerobic wetland soils because their decomposition presumably requires O_2-dependent phenol oxidative enzymes [79]; (2) the adenosine triphosphate (ATP) yield of C mineralization declines in the absence of oxygen, corresponding with decreased microbial biomass and a relative decline in enzyme synthesis under anaerobic condition [79,80]. Our results did not indicate the significant influence of reducing conditions on UR and PRO activity, while the NAG activity was significantly reduced in such conditions. In turn, in the study of Hall et al. [80] the NAG activity increased as soil O_2 declined across all the studied sites and both reducing conditions and phenolic compounds did not appear to constrain the NAG activity. According to Hall et al. [80] the NAG activity can be resilient to periodic anaerobiosis and may actually stimulate O_2 consumption at the microsite scale. As stated earlier [81], the UR activity was apparently modified in soil horizons with gleyic properties. In gley podzolic soil, there was no correlation between urease activity and SOC. Since mottling in this soil was indicative of waterlogging, this factor may influence the rapid destruction of urease or the

production of inhibitory substances. Similarly, the absence of urease activity in the presence of organic carbon in mottled BG-horizon of the gley podzolic soil suggests either a lack of ureolytic microorganisms or the rapid destruction or inhibition of urease [81]. As expected, NR activity was significantly higher in the horizons with potential reducing conditions as compared to the oxidative horizons. In fact, dissimilatory nitrate reductase in soils is the enzyme that catalyzes the reduction of NO_3^- to NO_2^- under anaerobic conditions [13]. In addition, the nitrate content in this study was more than four times higher in horizons that are expected to have reducing condition, as compared to the oxidative layers. Since the compared oxidative and gleyic horizons had a similar TOC content (2.25 and 1.97 g kg^{-1}, respectively) and thickness, these changes were caused by the gleyic properties in the MSG and CSP horizons.

The process of lessivage (Haplic Luvisol profiles) involves the translocation of fine particles of clay from the upper to the deeper horizons of the soil profile. In successive stages, the partially soluble salts, free iron, and aluminum migrate, and all of these have a significant impact on soil microbial properties [82]. In general, clay minerals inhibit the activity of enzymes, which is followed by their stabilization and the protection of their residual activity against thermal and proteolytic deactivation [33,83]. The study of Tzanakakis et al. [84] documented the inhibitory effect of zeolite on urease activity in soil treated either with manure or urea, while the NAG activity remained unaffected. Previous studies have reported that the clay content affects enzyme kinetics through a decrease in substrate turnover and an increase in the half-life of the enzyme, and therefore, the impact of clay on the soil enzymes is not consistent [83]. It should be noted that we may have underestimate enzyme activities in soils with high clay content because the sorption of an enzyme on a clay surface can increase its optimum pH by one or two pH units [17]. In general, when all of the data were considered together, an analysis of correlation showed that the UR, NR and PRO activity did not depend on the clay content, while NAG activity was inversely dependent, suggesting an inhibition effect on activity. A more detailed analysis has shown however that in profile 3, a higher PRO activity was found in the EB and Bt layers, as compared to the neighboring horizons. A relatively high clay content was also found in these layers. Additionally, in order to show the effect of the lessivage process on soil properties, the eluvial horizons (E1 and E) were compared with the A2 and BCKg horizons situated at the same depth in the MSG and CSP profiles. Because the E1 and E horizons had more than half the TOC and clay content and a 1.75 times lower TN content than the A2 and BCKg horizons, this suggests that the UR, NR and PRO activity was rather dependent on soil organic matter content, however, there is no explanation for NAG activity. This may be due to the fact that it was inhibited by the higher clay content in the A2 and BCKg horizons (mean value 12%) in contrast to the eluvial horizons (mean value 7.5%).

4.3. Changes of Microbial Functional Diversity under Different Plants Cultivation and Various Soil Depth

In this study we aimed to understand the impact of various plants (lucerne and winter wheat) on microbial functional diversity including nitrogen-based substrates utilization. Legume plants, including lucerne, are capable of N-cycling participation, due to the capacity to fix of atmospheric nitrogen (N_2) through interactions with rhizobia bacteria [85]. They are beneficial to increase microbial biomass and change the structure and functional diversity of soil microbial communities [86]. In addition, the leguminous crops improve soil fertility by increasing soil organic carbon content and availability of nutrients such as N and P as well as improve soil structure [86,87]. Thus, it is crucial to understand the ecological importance of legumes to soil properties and microbial communities in agroecosystems under different crops cultivation. The results of our study clearly indicated that lucerne increased the microbial utilization of all the tested N-sources as compared to winter wheat cultivation. In addition, Fahey et al. [88] indicated that soil microbiomes are strongly affected by different vegetation plants due to root exudates. Moreover Zuo et al. [89], who tested various

factors affecting soil microbial activity and diversity, showed differences in microbial shifts under different plant cover. However, there is still a gap in understanding which pathways and substrates can be changed in the soil under various crop systems. Therefore, our data indicated higher utilization of N-based compounds, such as L-asparagine, ɣ-amino butyric acid, L-serine, and L-arginine in all soil layers, and L-phenylalanine, L-threonine, glycyl-L-glutamic acid, phenylethylamine, putrescine, D-glucosaminic acid and N-acetyl-D-glucosamine in the upper soil horizons under lucerne cultivation as compared to winter wheat. However, this effect decreased significantly with the soil depth, which was confirmed by Kang et al. [90]. A higher level of nitrogen substrate utilization (mainly amino acids) in the soil under lucerne cultivation was observed as compared to winter wheat, which may be explained by the levels of various amino acids of these plants [91, 92]. The high content of particular amino acids in the lucerne biomass, e.g., arginine and asparagine [92], may cause the adaptation of soil microbes to these compounds and therefore their greater degree of utilization in metabolic potential diversity tests.

5. Conclusions

We found that the studied soil profiles demonstrated the highest potential enzymatic activity and nitrogen substrate utilization in surface soil horizons, which decreased with depth. The NR and NAG activity decreased systematically with soil depth, while in the case of UR and PRO activity there were no clear changes in the distribution of the enzymatic activity with depth. We found that the decreasing availability of organic carbon and total nitrogen and also available substrates are likely to be the principal drivers of the decreases in microbial abundance and enzymatic activity with depth. The specific enzymatic activity was more variable in response to the depth of the profiles than the enzymatic activity expressed on a soil mass basis, and this did not allow for clear trends to be determined.

Although the significant impact of the plants cultivated on the soil enzymatic properties was revealed, the data should be considered with caution because other factors (organic matter content, mineral N fertilization) could also contribute to this.

We found that the potential enzyme activity in the subsurface layers was also affected by factors associated with soil-forming processes (e.g., the lessivage process, clay content, gleyic conditions). As expected, only NR activity was significantly higher in the horizons with potential reducing conditions as compared to the oxidative horizons, while the NAG activity behaved opposite. The lessivage process significantly reduced the microbial biomass and enzymatic activities (except of NAG activity). In general, nitrogen substrate utilization decreased with increasing soil depth and was greater in the lucerne than in the winter wheat profiles, as well as in soil with a mollic horizon rather than in Luvisol profiles. In order to better understand the relationship between the soil microbial properties and soil fractions, these properties should be determined separately in individual soil fractions (e.g., clay, silt).

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12020264/s1, Figure S1. Capacity of the soil microbial community to use different N-substrates in aerobic and anaerobic conditions under lucerne and wheat cultivation; Figure S2. Capacity of the soil microbial community to use different N-substrates in aerobic and anaerobic conditions in soils with mollic layers—Mollic Staginc Gleysol and Cambic Stagnic Phaeozem (MOL) and Haplic Luvisol (LUV); Table S1. Depth distribution of soil enzyme activities expressed per TOC unit; Table S2. Depth distribution of soil enzyme activities expressed per MBC unit; Table S3. Root length and surface in the studied profiles (the average values and selected intervals); Table S4. Correlation matrix between the studied properties.

Author Contributions: Conceptualization, A.P.-D., J.D., M.F. and A.G.; methodology, A.P.-D., J.D., M.F. and A.G.; software, A.P.-D., J.D., M.F. and A.G.; investigation, A.P.-D., J.D., M.F. and A.G.; resources, A.P.-D., M.F.; writing—original draft preparation, A.P.-D. and M.F.; writing—review and editing, A.P.-D., M.F. and A.G.; visualization, A.P.-D. and M.F.; 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.

Informed Consent Statement: Not applicable.

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

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

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