Geoderma 416 (2022) 115779

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Enzymatic activity and functional diversity of soil microorganisms along the soil profile – A matter of soil depth and soil-forming processes

Anna Piotrowska-Długosz [a,*], Jacek Długosz [a], Magdalena Frąc [b], Agata Gryta [b], Barbara Breza-Boruta [c]

[a] Department of Biogeochemistry and Soil Science, Laboratory of Soil Science and Biochemistry, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Bernardyńska 6 St., 85-029 Bydgoszcz, Poland
[b] Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4 St., 20-290 Lublin, Poland
[c] Department of Microbiology and Food Technology, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Bernardyńska 6 St., 85-029 Bydgoszcz, Poland

ARTICLE INFO

Handling Editor: Naoise Nunan

Keywords:
Enzyme activity
Functional diversity
Microbial biomass
Pedogenic processes
Soil profile

ABSTRACT

Most studies concerning soil microorganisms and enzymes are focused on the upper horizons of the soil profile even though they transform soil organic matter at every depth of the soil profile. The aim of this study was to determine how the soil microbial functional diversity and enzymatic activity changed as a function of depth across the soil profiles which developed on the same parent material (glacial till) but differed in their soil-forming processes thereby resulting in the formation of different reference soil groups (RSG) (Luvisols, Gleysols and Stagnosols). Four soil profiles were excavated at different sites that are located in two mesoregions of the South Baltic Lake District (central Poland) in fields with alfalfa (*Medicago sativa* L.) in the fourth year of its cultivation. Using the soil samples collected in June 2019, we assessed the oxidoreductase activity (DHA – dehydrogenases, POX – phenol oxidase, PER – peroxidase), the fluorescein diacetate hydrolysis (FDAH) rate, the microbial biomass carbon and nitrogen (MBC, MBN) content, the number of microbial groups and the related physico-chemical properties. The community-level physiological profiles of the soil microorganisms were evaluated using the Biolog EcoPlate™ method. In general, the highest level of enzymatic activity per soil mass unit was found in the Ap horizons, whereas the dehydrogenases activity and FDAH decreased gradually with depth, while the phenol oxidase and peroxidase activity revealed no consistent trends between the sub-surface horizons. The specific enzymatic activity, expressed per unit of soil TOC and MBC, was more variable in response to profile depth than the enzymatic activity, which was expressed per soil mass, and this did not allow for clear trends to be determined. Also, the specific activity was different with regard to the intracellular and extracellular enzymes. The DHA/MBC ratio was markedly higher in the Ap horizons (all profiles) and generally decreased with depth. The phenol oxidase and peroxidase activities, expressed in the terms of the MBC content, did not exhibit any clear trends in the studied profiles, however, they reached their highest levels in the deepest layers in all of them. The abundance of all of the microbial groups was generally highest in the Ap horizon and decreased sharply with depth. A greater abundance of microbial groups was found in the gleyic horizons of the Gleysols and Stagnosols as compared to the upper and lower horizons. The highest average well color development (AWCD) under aerobic conditions was observed in the Ap genetic horizons (with the exception of the Haplic Luvisol profile), while the highest values of AWCD in anaerobic conditions were found in the illuvial and gleyic horizons. In contrast to the AWCD, the Shannon diversity index (H) and Shannon evenness index (S) (equal usage of

Abbreviations: HL, Haplic Luvisol; AES, Albic Eutric Stagnosol; MSG, Mollic Stagnic Gleysol; DHA, dehydrogenases; FDAH, fluorescein diacetate hydrolysis; POX, phenol oxidase; PER, peroxidase; TOC, total organic carbon; TN, total nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; Btot, the number of aerobic heterotrophic bacteria; Bfan, facultative anaerobic bacteria; Act, actinomycetes; Fun, filamentous fungi; AWCDa, the average well color development under aerobic condition; AWCDan, the average well color development under anaerobic condition; Ha, Shannon diversity index under aerobic condition; Han, Shannon diversity index under anaerobic condition; Ea, Shannon evenness index under aerobic condition; Ean, Shannon evenness index under anaerobic condition; Hh, hydrolytic acidity; Fed, free iron oxides; CEC, cation exchange capacity; BD, bulk density.

* Corresponding author at: Bydgoszcz University of Science and Technology, Bernardyńska 6 St., 85-029 Bydgoszcz, Poland.
E-mail addresses: apiotr@pbs.edu.pl (A. Piotrowska-Długosz), jacekd@pbs.edu.pl (J. Długosz), m.frac@ipan.lublin.pl (M. Frąc), a.gryta@ipan.lublin.pl (A. Gryta), breza@pbs.edu.pl (B. Breza-Boruta).

https://doi.org/10.1016/j.geoderma.2022.115779
Received 13 March 2021; Received in revised form 31 January 2022; Accepted 17 February 2022
Available online 9 March 2022

compounds) indices were more similar in the compared horizons. Our findings suggest that the studied microbial properties in the Ap horizons were primarily determined by the TOC content, while their activity in the subsurface layers was also affected by factors associated with soil-forming processes.

1. Introduction

Microbial communities and the enzymes they produce play an essential role in the functioning of the soil environment. They participate in the transformation of soil organic matter (SOM), thereby making nutrients available for plants and degrading any contaminants (Kramer et al., 2013; Ko et al., 2017). Soil dehydrogenases (EC 1.1.), which are the major representatives of oxidoreductases, have often been used as an indicator of the overall soil microbiological activity. They are known to oxidize soil organic matter by transferring protons and electrons from the substrates to the acceptors (Wolińska and Bennicelli, 2010). However, soil oxidase activity such as phenol oxidase and peroxidase are associated with the turnover of 'recalcitrant' polyphenolic compounds (Sinsabaugh, 2010). Phenol oxidase (EC. 1.10.3.2), which is characterized by a low substrate specificity, is responsible for the degradation and mineralization of lignin and other polyphenolic molecules, while peroxidases (EC1.11.1.7) are known for their role in the depolymerization of lignin (Sinsabaugh, 2010). In turn, soil hydrolases preferentially catalyze the nutrient-rich 'labile' soil organic matter, which is directly related to meeting the C, N and P nutrient requirements of soil microorganisms and plants. The overall level of soil hydrolytic activity is often determined by the hydrolysis of fluorescein diacetate sodium salt, which is performed by proteases, lipases and esterases (Adam and Duncan, 2001). Additionally, information concerning microbial biomass content and changes in soil microbial functional diversity (metabolic potential) is essential for understanding the role of microbial communities in the intensity and direction of organic matter transformation and nutrient cycling (Garland, 1996; Gryta et al., 2020).

To date, most studies concerning microbial and enzymatic properties in agricultural soil have been restricted to the upper 30 cm of the soil, despite the fact that soil microbes and their related enzymes influence biogeochemical processes throughout the entire soil profile (Buss et al., 2005; Ko et al., 2017). Fierer et al. (2003) and van Leeuwen et al. (2017) reported that approximately 30–35% of the microbial biomass in the soil profile was found below the 25 cm depth. The subsurface soil microbial communities play an important role in soil C sequestration since large amounts of organic C (about 60%) are stored in the subsurface horizons (below 30 cm) (Rumpel and Kögel-Knabner, 2011; Schnecker et al., 2015). Generally, the carbon in the deep soil horizons has a long turnover time compared to the surface horizons, and its availability to soil microbes and enzymes may be affected by depth-specific, unfavorable factors such as oxygen limitation, low temperature and reduction in the level of nutrients and energy.

It is commonly known that the microbial biomass, the functional diversity of soil microorganisms and the activity of hydrolytic enzymes generally decline with increasing soil depth (Goberna et al., 2005; Herold et al., 2014; Eilers et al., 2012; Stone et al., 2014). The decrease in these properties with depth is mainly associated with changes in the edaphic factors such as pH, the air–water conditions as well as in the quantity and quality of soil organic matter, mainly in the amount of labile, easily degradable substrates (Ko et al., 2017). Goberna et al. (2005) observed different patterns in the utilization of C substrates throughout the soil profiles and a decrease in the values of the biodiversity indices (Shannon and evenness) down the soil profiles. In turn, some authors have found a relatively high oxidase activity in the deeper soil layers as compared to the surface ones, which may be associated with the number of irregular polymers or the amount of toxins, which are both independent of the SOM content (Eilers et al., 2012; Schnecker et al., 2015).

Relatively few studies have been conducted to associate the changes in soil properties with the various soil-forming processes (Marinari and Vittori Antisari, 2010). Generally, differences in soil properties throughout the soil profile are expected to be associated with the differences in their genesis, the degree of weathering and the mineral composition of the parent material (Wilson et al., 2017; Kögel-Knabner and Amelung, 2021). The rate of organic matter decomposition in the surface horizons is often much higher than in the deeper, saturated and anaerobic soils layers. In the gleyic horizons, the microbiological properties can be directly affected by changes in the air–water properties that are associated with water saturation, which leads to a depletion of oxygen in the soil and results in changes in the soil features. In such conditions, the facultative and obligate anaerobic microorganisms primarily decompose organic matter, wherein the decomposition can occur at a much slower rate than in the surface horizons (Jackson et al., 2014).

In turn, in the process of lessivage the movement of materials, e.g. clay fraction < 2.0 μm, free iron oxides, humus substances, from the upper to the deeper horizons of the soil profile occur. Because of their properties such as a large specific surface and negative charge these materials, especially the clay minerals, play a key role in an ecosystem by affecting the soil hydrology and fertility as well as the weathering and redox reactions by retaining nutrients against leaching (Świtoniak et al., 2016). Additionally, they can also modify a wide range of physico-chemical properties, which in turn affects the microbial communities, the main source of enzymes in soil as well as the extracellular enzymes themselves. Any significant modification of the microorganisms and enzymes absorbed onto clay minerals or organo-mineral complexes with depth can influence both the potential enzyme activity and the enzyme turnover rate (Alison and Jastrow, 2006; Burns et al., 2013; Wei et al., 2014).

The aim of the research was to estimate the response of the microbial and enzymatic properties of the soil to soil-forming processes that result in the formation of different reference soil groups that had been subjected to the same agriculture use. In addition, we selected the fields where the same plant had been cultivated in the previous four years in order to avoid the influence of different tillage practices and plants. For the purposes of the study, four soil profiles that had formed on the same parent material but differed in their pedogenic processes, and as a consequence, had different physico-chemical properties, were compared. An index-based approach was used to determine the soil enzymatic activity. Thus, the most frequently used indicators of overall microbial activity (dehydrogenases), and an indicator of soil hydrolytic activity (FDAH) were determined. The oxidative enzymes (phenol oxidase, peroxidase) were assessed in order to measure the organic matter recalcitrance. Additionally, the abundance of the main microbial group and microbial functional diversity were measured with depth in the compared horizons that were situated at the same/similar depth. We hypothesized that (1) changes of the studied enzymatic activity as well as in the abundance of the main microbial groups and microbial functional diversity down the soil profiles would depend on the soil properties and horizons/soil-forming processes. In the surface horizons these properties would be mainly determined by the soil organic matter content (C availability), while in sub-surface horizons they would be also affected by properties associated with soil-forming processes, (2) differences in the soil hydrolases and oxidases, which are expressed as the activity per unit dry soil mass (absolute enzyme activities) and per unit microbial biomass and TOC (specific enzyme activities) in the soil horizons would be expected due to their various roles in SOM transformation. These are associated with the amount of the available substrate, and any changes in the relative abundance of the enzyme producers and the activity of the extracellular enzymes that are

Table 1
Morphology of the soil profiles.

Genetic horizon	Depth	Munsell colour		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
Eet1	30–55	6/3 10YR	4/4 10YR	SL	AB, FI, 1	1.62
Eet2	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	5/6 10YR	4/6 10YR	L	SB, CO, 3	1.83
Profile 2. Albic Eutric Stagnosol						
Ap	0–30	5/3 10YR	3/3 10YR	SL	AB, ME, 2	1.56
AC	30–58	5/3 10YR	3/3 10YR	SL	AB, ME, 1	1.60
Cg1	58–106	7/2 2.5Y	5/3 2.5Y	SL	AB, ME, 1	1.52
Cg2	106–145	5/4 2.5Y	4/4 2.5Y	FSL	AB, ME, 2	1.68
Cg3	>145	6/4 2.5Y	5/4 2.5Y	FSL	AB, ME, 1	1.65
Profile 3. 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 4. Cutanic Luvisol						
Ap	0–28	5/4 10YR	4/6 10YR	L	AB ME 2	1.74
Bt1	28–78	5/6 10YR	3/6 10YR	L	SB VC 3	1.75
Bt2	78–130	6/4 10YR	4/6 10YR	L	SB CO 3	1.78
Ck	130–150	4/3 10YR	3/2 10YR	L	AB CO2	1.75

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

absorbed and protected on soil clay minerals. That is why fluorescein diacetate hydrolysis, which reflects the enzymes that target the easily decomposable compounds is limited to the surface soil layers, while phenol oxidase and peroxidases are significantly active in the deeper horizons due to the stabilization of their residual activity on clay minerals and because of the greater amount of substrates in the deeper soils.

2. Materials and method

2.1. Study area and soil sampling

The study was conducted on four soil profiles which are situated in two mesoregions of the South Baltic Lake District, Cuiavia-Pomerania Province (Central Poland) that have a long history of agricultural use. Although the soils were formed from the same glacial till, they differed in their soil-forming processes, which result in the formation of different reference soil groups (RSGs) (Luvisols, Gleysols and Stagnosols). The following profiles were studied: Haplic Luvisol (HL, profile 1), Albic Eutric Stagnosol (AES, profile 2), Mollic Stagnic Gleysol (MSG, profile 3) and Cutanic Luvisol (CL, profile 4). Profiles 1 and 2 were excavated in 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 profiles 3 and 4 were from Samostrzel (53° 29′ 5.8″ N, 18° 55′ 50.5″ E and 53° 28′ 58.1″ N, 18° 55′ 18.9″ E, respectively), which is located in the South-Krajna Lakeland. Descriptions of the soil profiles are provided in Table 1. The climate in the study area 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}. Soil samples were collected from fields with alfalfa (*Medicago sativa* L.) in the fourth year of its cultivation in June 2019 in order to avoid any significant effect of previous land use or the homogenization of the soil by tillage/plowing. Five soil samples were collected from the middle portion of each horizon with a gouge auger for stepwise sampling, pooled, thoroughly mixed and analyzed separately for the selected properties. The mean values of five repetitions that were performed for each horizon are presented. The soil samples from the 0–30 cm layers were placed in plastic containers (which permitted gas exchange) and chilled to 4 °C in order to minimize any changes in the populations of microorganisms. 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 chilled to 4 °C. The soil sampling tools were sterilized between sample collections, and the soil samples were placed in sterile bags to prevent cross-contamination. The microbial and enzymatic activity was determined within two weeks based on fresh soil samples. The soil samples used to determine the physicochemical properties of the soil were air-dried and sieved (2 mm).

2.2. Physico-chemical properties

The physico-chemical properties of the soil were determined according to the standard methods that are 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 (PN-ISO 11277, 2005), the pH in a solution of 0.01 M $CaCl_2$ was measured using the potentiometric method (PN-ISO 10390, 1997), the content of total organic carbon (TOC) and total nitrogen (TN) was determined using a dry combustion CN analyzer (Vario Max CN). The bulk density was determined with using the method of applying volume cylinders (100 cm^3). The content of free iron oxides (Fed) was determined in a sodium citrate and dithionite extract (Mehra and Jackson, 1960). The cation exchange capacity (CEC) was calculated as the sum of the basic saturation and hydrolytic acidity.

2.3. Potential enzyme activities

The enzyme activity was determined in fresh, moist and sieved (<2 mm) soil and calculated based on the oven-dry (105 °C) weight of the soil. Soil dehydrogenases (DHA, EC 1.1.) activity was determined using a 0.2% INT solution (2-(4-iodophenyl)-3-(4-nitrophenyl(-5-phenyl-2H-tetrazolium chloride) as the substrate (von Mersi and Schinner, 1991). The controls were prepared in the same way but using autoclaved soil. The samples were incubated for two hours at 37 °C and after incubation, 10 ml of the extraction solution was added. The soil suspension was filtered in the dark after one hour and the optical density of the product

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

Genetic horizon	Depth	pH $CaCl_2$	Clay	TOC	TN	TOC/TN	DOC/TOC	Fed	CEC
			%	g kg^{-1}			%	gkg^{-1}	mmol(+) kg^{-1}
Profile 1. Haplic Luvisol (Cutanic) (HP)									
Ap	0–30	5.41(±0.34)Cb	8(±1)Cb	10.8(±1.23)Ac	1.32(±0.32)Ab	10.8	1.09(0.06)Ca	1.99(±0.33)Dd	45.9(±8.6)Cc
Eet1	30–55	5.89(±0.25)Bb	7(±1) Cc	2.70(±0.56)Bb	0.31(±0.09)Bb	8.71	2.78(0.10)Ba	2.14(±0.45)Db	36.2(±4.3)Dd
Eet2	55–82	6.15(±0.44)Ab	6(±1)Cc	1.59(±0.24)Cc	0.26(±0.07)Cb	6.11	3.71(0.20)Aa	2.63(±0.41)Cb	28.4(±3.8)Ec
Bt	82–144	6.38(±0.40)Ab	16(±3)Aa	1.34(±0.45)Cb	0.35(±0.1)Ba	3.82	3.99(0.24)Aa	7.67(±1.12)Aa	106.9(±14.2)Aa
BC	>144	6.18(±0.76)Ab	13(±3)Ba	1.22(±0.31)Cb	0.35(±0.1)Ba	3.48	3.98(0.17)Ca	5.71(1.05±)Ba	97.4(±10.1)Ba
Profile 2. Albic Eutric Stagnosol (AES)									
Ap	0–30	6.64(±0.66)Ca	11(±3)Aa	12.7(±1.43)Abc	1.68(±0.22)Aab	7.56	1.01(0.03)Ca	2.97(±0.36)Ac	81.7(±8.88)Ab
AC	30–58	6.95(±0.47)Ba	9(±2)Ac	8.80(±1.03)Ba	1.05(±0.13)Ba	8.38	1.19(0.04)Cb	1.44(±0.22)Cc	77.6(±5.24)Bc
Cg1	58–106	7.27(±0.98A)a	7(±2)Bc	2.43(±0.35)Cb	0.24(±0.06)Db	10.1	2.94(0.14)Ba	2.09(±0.33)Bc	25.3(±4.12)Dc
Cg2	106–145	7.26(±1.23)Aa	10(±3A)b	1.07(±0.12)Dc	0.22(±0.05)Db	4.86	4.79(0.09)Aa	2.54(±0.43)Bc	73.1(±5.78)Bb
Cg3	>145	7.34(±0.86)Aa	7(±2)Bb	1.09(±0.25)Db	0.30(±0.06)Ca	3.63	4.63(0.15)Aa	2.49(±0.53)Bc	64.8(±7.56)Cb
Profile 3. Mollic Stagnic Gleyosol (MSG)									
Ap	0–30	6.87(±0.66)Ca	10(±3)Ca	17.7(±1.54)Da	1.96(±0.34)Aa	9.03	0.73(0.04)Cb	4.10(±0.78)Ab	141.3(±23.1)Aa
A2	30–53	6.76(±0.87)Ca	12(±2)Cb	9.66(±0.87)Ba	0.90(±0.11)Ba	10.7	0.93(0.03)Cb	2.14(±0.34)Bb	127.8(±19.6)Ba
2ACgg	53–70	7.01(±0.83)Ba	25(±4)Aa	4.02(±0.35)Ca	0.42(±0.09C)a	9.57	2.01(0.08)Bb	1.50(±0.23)Cc	139.3(±21.4)Aa
3G1	70–110	7.16(±0.57)Ba	14(±3)Ba	2.34(±0.25)Da	0.37(±0.09)Ca	6.32	2.79(0.08)Ab	1.01(±0.15)Dc	97.6(±17.9)Ca
.3G2	110–150	7.31(±0.84)Aa	14(±3)Ba	2.13(±0.33)Dab	0.29(±0.05)Da	7.34	2.67(0.17)Ab	1.41(±0.23)Cd	91.9(±18.3)Ca
Profile 4. Cutanic Luvisol (CL)									
Ap	0–28	6.91(±0.67)Ba	12(±2)Ba	13.6(±1.77)Ab	1.65(±0.21)Aab	8.24	1.07(0.04)Ca	5.25(±0.78)Ba	138.6(±12.7)Aa
Bt1	28–78	7.18(±0.58)Aa	16(±4)Ab	2.12(±0.41)Cb	0.35(±0.07)Bb	6.06	3.31(0.22)Aa	6.04(±0.56)Aa	108.6(±7.91)Bb
Bt2	78–130	7.13(±0.47)Aa	13(±2)Ba	1.58(±0.22)Db	0.35(±0.08)Ba	4.51	3.47(0.11)Aa	4.77(±0.45)Cb	102.1(±12.5)Ba
Ck	130–150	7.34(±0.79)Aa	11(±2)Ba	2.63(±0.54)Ba	0.33(±0.08)Ba	7.97	1.78(0.07)Bc	4.09(±0.76)Db	93.9(±9.99)Ca

TOC – total organic carbon; TN – total nitrogen; DOC – dissolved organic carbon; Fed – free iron oxides; CEC – cation exchange capacity. Following horizons were compared: a) all of the Ap horizons; b) Eet1, AC, A2 and Bt1; c) Eet2, Cg1, 2ACgg, Bt1; d) Bt, Cg2, 3G1, Bt2; e) BC, Cg3, 3G2, Ck. Values in compared horizons followed by different small letters are statistically different at $P < 0.05$. Different uppercase letters indicate differences ($P < 0.05$) between genetic horizons within the same soil profiles.

was measured at 464 nm. The overall soil hydrolytic activity was evaluated by measuring the activity of fluorescein diacetate sodium salt hydrolysis. The soil was treated with a phosphate buffer (60 mM, pH 7.6) with fluorescein diacetate as the substrate and the mixture was incubated for one hour at 37 °C. After incubation, the reaction was stopped by adding a mixture of methyl alcohol and chloroform (1:2). After that, the soil suspension was centrifuged, and the optical density of the clear supernatant was measured at 490 nm (Adam and Duncan, 2001). Phenol oxidase (EC 1.10.3.2) activity was measured using 3,4-dihydroxy-L-phenylalanine (L-DOPA) as the substrate (Bach et al., 2013). Three mL of an acetate buffer was added to 10-ml centrifuge tubes containing one gram of soil. After 10 min of mixing, 2 ml of 10 mM L-DOPA was added to three replicates and 2 ml of acetate buffer was added to the control sample. All of the tubes were shaken in a water bath (100 rev min^{-1}; 25 °C) for 30 min. After incubation, the contents of the tubes were centrifuged to stop the reaction and the optical density of the product was measured at 475 nm. The peroxidase (EC 1.11.1.7) activity was determined in the same way as the phenol oxidase, but eight replicates were prepared, four to determine the phenoloxidase activity (as above) and four with the addition of 0.3% H_2O_2, as a second substrate to determine the total oxidative activity (Sinsabaugh et al., 2008). The enzymatic activity was expressed on both a soil mass basis (absolute enzyme activities) and per unit of soil TOC and MBC (specific enzyme activities).

2.4. Microbial biomass C and N content

The microbial biomass C and N content was determined using a chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). 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 was proposed by Vance et al. (1987). The total N from both fumigated and unfumigated soil samples was determined according to Bremner and Mulvaney (1982). 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 by a correction factor of 0.38 (kEC) (Vance et al., 1987) and 0.54 ($=k_{EN}$) (Joergensen and Mueller, 1996), respectively. The MBC/TOC (%) and MBN/TN (%) ratios were also calculated (Anderson and Domsch, 1990).

2.5. Community-level physiological profiling (CLPP) analysis

The CLPP of soil microorganisms was assessed based on a metabolic fingerprinting technique using Biolog ECO™ plates (Garland, 1996). The Biolog ECO™ plate system is a useful method to describe the shifts and metabolic diversity of soil microbial communities. One gram of the sieved soil was suspended in 99 ml of sterile saline peptone water and shaken for 20 min at 20 °C, after which the sample was incubated at 4 °C for 30 min. Next, the ECO plates were inoculated with 120 μL of a prepared suspension of the soil microorganisms. The inoculated plates were incubated under aerobic and anaerobic conditions. The anaerobic conditions were created by placing the plates in special jars with a gas mixture containing 5% H_2, 10% CO_2 and 85% N_2; the gas was injected using an Anoxomat apparatus (MART Microbiology B.V., USA). The metabolic profile was determined based on measurements of absorbance (590 nm) over 192 incubation hours at 24 h intervals using Biolog microstation (BIOLOG®, Hayward, CA, USA). The metabolic activity of the soil microbiome was also calculated including the average well color development (AWCD) index. Additionally, biodiversity indices such as Shannon-Weaver (H) and evenness (E) were determined.

2.6. Microbial analyses

The number of aerobic heterotrophic bacteria (total bacteria) (Btot), facultative anaerobic bacteria (Bfan), actinomycetes (Act) and filamentous fungi (Fun) were determined. Ten grams of soil sample was added to 90 ml of Ringer's solution. After homogenization for 30 min, ten-fold serial dilutions were performed (10^{-1} to 10^{-7}). The pour plate technique was used to isolate the bacteria, 1 ml of each dilution was transferred into sterile Petri dishes. The aerobic heterotrophic bacteria were isolated on a yeast extract-peptone-soil extract medium (YPS) and

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 (Cutanic)						
Ap	0–30	123.3 (±6.67)Ad	25.6 (±2.97)Ab	4.81 (±0.38)Ab	1.15 (±0.09)Cbc	1.94 (±0.12)Cb
Eet1	30–55	47.1 (±3.98)Bc	10.5 (±1.22)Bb	4.47 (±0.29)Bb	1.74 (±0.15)Ab	3.40 (±0.29)Ab
Eet2	55–82	27.2 (±1.89)Cc	6.56 (±0.41)Cc	4.01 (±0.34)Cb	1.71 (±0.16)Ab	2.52 (±0.19)Bc
Bt	82–144	18.5 (±1.75)Dc	3.42 (±0.37)Dc	4.41 (±0.28)Bb	1.38 (±0.16)Bc	0.98 (±0.18)Dc
BC	> 144	15.3 (±1.44)Db	2.45 (±0.32)Db	4.44(±0.23)Bc	1.25 (±0.14)Db	0.70 (±0.13)Db
Profile 2. Albic Eutric Stagnosol						
Ap	0–30	201.5 (±4.9)Ab	44.6 (±1.65)Aa	4.52 (±0.16)Bb	1.58 (±0.09)Bb	2.65 (±0.16)Bab
AC	30–58	54.4 (±2.42)Bc	12.3 (±0.32)Bb	4.44 (±0.32)Bb	0.62 (±0.05)Dd	1.17 (±0.13)Dd
Cg1	58–106	35.5 (±3.45)Cc	8.56 (±1.35)Cc	4.14 (±0.27)Bb	1.46 (±0.07)Bbc	3.57 (±0.27)Ab
Cg2	106–145	25.3 (±2.55)Db	5.00 (±0.40)Db	5.06 (±0.15)Ab	2.36 (±0.28)Ab	2.27 (±0.11)Cb
Cg3	> 145	10.1 (±0.43)Ec	1.85 (±0.17)Ec	5.46 (±0.25)Ab	0.93 (±0.09)Cc	0.62 (±0.08)Eb
Profile 3. Mollic Stagnic Gleyosol						
Ap	0–30	145.6 (±5.9)Ac	30.2 (±1.82)Ab	4.83 (±0.69)Cb	0.82 (±0.09)Dc	1.54 (±0.09)Cc
A2	30–53	113.6 (±5.4)Bb	25.5 (±0.93)Ba	4.46 (±0.77)Db	1.18 (±0.15)Bc	2.83 (±0.12)Ac
2ACgg	53–70	53.1 (±4.38)Cb	10.5 (±0.41)Cb	5.06 (±0.82)Ba	1.32 (±0.14)Ac	2.50 (±0.18)Bc
3G1	70–110	25.6 (±3.37)Db	4.12 (±0.67)Dbc	6.21 (±0.89)Aa	1.09 (±0.12)Cc	1.11 (±0.13)Dc
3G2	110–150	16.5 (±1.02)Db	2.56 (±0.35)Db	4.44 (±0.50)Dc	0.78 (±0.14)Dd	0.88 (±0.11)Eb
Profile 4. Cutanic Luvisol						
Ap	0–28	289.6 (±7.5)Aa	49.6 (±1.69)Aa	5.84 (±0.25)Ca	2.12 (±0.17)Ca	3.00 (±0.09)Ca
Bt1	28–78	146.5 (±6.4)Ba	27.0 (±1.57)Ba	5.43 (±0.23)Ca	6.91 (±0.32)Aa	7.70 (±0.35)Aa
Bt2	78–130	78.5 (±3.36)Ca	12.5 (±0.78)Ca	6.28 (±0.18)Ba	4.97 (±0.22)Ba	3.57 (±0.12)Ba
Ck	130–150	52.5 (±2.22)Da	7.31 (±0.24)Da	7.19 (±0.12)Aa	2.08 (±0.15)Ca	2.21 (±0.08)Da

MBC – microbial biomass carbon; MBN – microbial biomass nitrogen; TOC – total organic carbon; TN – total nitrogen. Following horizons were compared: a) all of the Ap horizons; b) Eet1, AC, A2 and Bt1; c) Eet2, Cg1, 2ACgg, Bt1; d) Bt, Cg2, 3G1, Bt2; e) BC, Cg3, 3G2, Ck. Values in compared horizons followed by different small letters are statistically different at $P < 0.05$. Different uppercase letters indicate differences ($P < 0.05$) between genetic horizons within the same soil profiles.

the filamentous fungi were isolated on Rose-Bengal agar containing 30 μg ml^{-1} streptomycin (Atlas, 2010). Modified yeast extract-glucose medium (YGA) was used to determine the number of Actinobacteria (Crawford et al., 1993). The facultative anaerobic bacteria were cultivated on a sodium thioglycolate medium base (FTG) (Sheikh et al., 2015). The duration of incubation for the Btot, Fun and Act was four, five and ten days at 25–28 °C, while the facultative anaerobic bacteria were incubated in anaerobic conditions for three to five days at 30 °C. The number of microorganisms was determined in four replicates and is given per 1 g of soil dry matter (CFU g^{-1} d.m. of soil).

2.7. Data analyses

A one way analysis of variance was performed to assess the changes in soil properties down the soil profiles. Moreover, five one-way analyses of variance (ANOVA) were performed to determine the influence of genetic horizons across the soil profiles on the studied enzymatic, microbial as well as physical and chemical properties. Firstly, we compared all of the Ap horizons which were the only horizons common to all profiles. All of the other analyzes of variance were carried out to determine how soil properties changed in the various genetic horizons laying at the same/similar depths. An analysis of variance was performed for randomized block design where profiles were treated as blocks. Significant differences between means were determined using the Tukey test at a 95% confidence level. Before conducting the ANOVA, we tested whether the normality and homogeneity of the variances of the residuals were fulfilled; if they were not, the data were log transformed. The Multiple regressions were performed to show the relationships among the studied properties. Differences between the soil samples were analyzed using 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. 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 increased with depth in the Haplic Luvisol, Albic Eutric Stagnosol and Mollic Stagnic Gleysol profiles (between 1.56 and 1.83 g cm^{-3}), while in the Cutanic Luvisol, it was similar throughout the entire profile (1.74–1.78 g cm^{-3}) (Table 1). The soil pH in $CaCl_2$ ranged from acid to neutral (5.41–7.34) and significantly increased with depth in all of the studied profiles (Table 2). In the surface horizons (Ap), the clay content was significantly lower in the Haplic Luvisol than in the other three profiles, while it was significantly higher in the sub-surface and the middle horizons of the Mollic Stagnic Gleysol and Cutanic Luvisol profiles than it was in the Haplic Luvisol and Albic Eutric Stagnosol profiles. The highest concentration of TOC, TN and TOC/TN ratio was generally observed in the top layers and significantly decreased down the soil profiles. It was only significantly higher in the Ck horizons in the Cutanic Luvisol than in the upper layers (Bt1, Bt2). A relatively high content of TOC was found in the sub-surface layers of the Albic Eutric Stagnosol and Mollic Stagnic Gleysol (8.8 and 9.7 g kg^{-1}, respectively). In contrast to TOC/TN ratio, the DOC/TOC ratio tended to gradually increase with depth. No clear trends were found for the Fed and CEC values down the soil profiles. The highest CEC values were found in the first three horizons of the Mollic Stagnic Gleysol and Cutanic Luvisol profiles as well as in the two deepest horizons of the Haplic Luvisol. In the surface and sub-surface horizons, the highest Fed content was found in the Cutanic Luvisol and was significantly lower in the other profiles studied. The highest Fed content in the deepest layers was found in the illuvial horizons (Bt in the Haplic Luvisol and Bt1 in the Cutanic Luvisol).

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

Genetic horizon	Depth	DHA activity	POX activity	PER activity	FDA hydrolysis
Profile 1. Haplic Luvisol (Cutanic)					
Ap	0–30	0.874 (±0.038)Ab	4.02 (±0.57)Aa	15.1 (±2.61)Aa	18.5 (±1.30)Ab
Eet1	30–55	0.112 (±0.013)Bc	1.98 (±0.62)Ba	8.84 (±1.15)Ba	6.67 (±0.94)Ba
Eet2	55–82	0.081 (±0.013)Cb	1.95 (±0.18)Ba	8.64 (±1.06)Ca	3.98 (±0.83)Ca
Bt	82–144	0.027 (±0.009)Dd	0.92 (±0.14)Cb	4.28 (±0.55)Da	1.72 (±0.17)Db
BC	> 144	0.016 (±0.008)Dc	1.86 (±0.17)Bb	6.56 (±0.68)Ea	1.16 (±0.13)Dc
Profile 2. Albic Eutric Stagnosol					
Ap	0–30	1.529 (±0.086)Aa	1.76 (±0.045)Bc	8.84 (±0.21)Ac	26.1 (±0.68)Aa
AC	30–58	0.402 (±0.032)Ba	2.33 (±0.068)Aa	4.15 (±0.15)Bb	6.93 (±0.38)Ba
Cg1	58–106	0.055 (±0.012)Cc	0.46 (±0.018)Dc	0.97 (±0.11)Ec	3.84 (±0.26)Ca
Cg2	106–145	0.042 (±0.009)Cc	0.63 (±0.021)Cc	2.34 (±0.13)Cb	0.96 (±0.13)Dc
Cg3	> 145	0.021 (±0.005)Cc	0.74 (±0.024)Cc	1.46 (±0.25)Dc	1.00 (±0.13)Dc
Profile 3. Mollic Stagnic Gleyosol					
Ap	0–30	0.678 (±0.029)Ac	4.38 (±0.204)Aa	5.66 (±0.33)Ad	16.7 (±0.67)Ab
A2	30–53	0.359 (±0.018)Ba	1.10 (±0.088)Bb	2.17 (±0.14)Bc	5.82 (±0.12)Bb
2ACgg	53–70	0.183 (±0.016)Ca	0.84 (±0.064)Cab	2.12 (±0.10)Bc	3.90 (±0.10)Ca
3G1	70–110	0.171 (±0.014)Ca	1.31 (±0.097)Ba	2.08 (±0.13)Bb	3.26 (±0.05)Ca
3G2	110–150	0.065 (±0.013)Db	0.56 (±0.036)Dc	2.04 (±0.11)Bc	1.93 (±0.08)Da
Profile 4. Cutanic Luvisol					
Ap	0–28	0.744 (±0.022)Abc	3.54 (±0.083)Ab	10.7 (±0.63)Ab	24.3 (±1.23)Aa
Bt1	28–78	0.152 (±0.017)Bb	1.05 (±0.052)Bb	4.51 (±0.22)Bb	2.15 (±0.09)Bc
Bt2	78–130	0.094 (±0.012)Db	1.31 (±0.023)Da	4.32 (±0.33)Ba	1.29 (±0.02)Cb
Ck	130–150	0.261 (±0.027)Ca	2.75 (±0.065)Ca	5.50 (±0.26)Cb	1.46 (±0.05)Cb

DHA – dehydrogenases (μg INTF $g^{-1}h^{-1}$); POX – phenol oxidase (μM DOPA-chrome $g^{-1}h^{-1}$); PER – peroxidase (μM DOPA-chrome $g^{-1}h^{-1}$); FDAH - fluorescein diacetate hydrolysis (μg F $g^{-1}h^{-1}$). Following horizons were compared: a) all of the Ap horizons; b) Eet1, AC, A2 and Bt1; c) Eet2, Cg1, 2ACgg, Bt1; d) Bt, Cg2, 3G1, Bt2; e) BC, Cg3, 3G2, Ck. Values in compared horizons followed by different small letters are statistically different at $P < 0.05$. Different uppercase letters indicate differences ($P < 0.05$) between genetic horizons within the same soil profiles.

3.2. Soil microbial biomass and enzymatic activity

Microbial biomass content, expressed in terms of carbon (MBC) and nitrogen (MBN), was the highest in the Ap horizon (in all of the profiles) and decreased sharply with depth (Table 3). The content of MBC and MBN was 50–70% lower in the subsurface horizons (Eet1, AC, Bt1) than in the surface layers (Ap). In the Haplic Luvisol and Albic Eutric Stagnosol profiles, the MBC/MBN ratio was lower than 5.0, while it was higher than 5.0 in the Cutanic Luvisol. The contribution of MBC to the TOC was generally lower than 2.0% in the Haplic Luvisol, Albic Eutric Stagnosol and Mollic Stagnic Gleysol profiles, while it was higher than 2.0% (up to 7.19%) in the Cutanic Luvisol profile. A broader range of results (0.62–7.70) was found for the MBN/TN ratio. No clear trends were found for the MBC/MBN, MBC/TOC and MBN/TN ratios down the soil profiles. In general, when the horizons at a similar depth in all of the profiles were compared, the highest contribution of MBC and MBN to TOC and TN, respectively, was found in the Cutanic Luvisol profile and the lowest in the Haplic Luvisol profile.

The enzymatic activity, which was expressed on a soil mass basis, was the highest in the surface horizons of all of the profiles and generally decreased significantly with depth (Table 4). The only exception to this rule was the activity of the enzymes in the Cutanic Luvisol, which was significantly higher in the Ck horizon than in the upper horizons (Bt1 and Bt2) of this profile. For the Ap horizon, the highest DHA activity was determined in the Albic Eutric Stagnosol profile, while in the sub-surface horizons, the most active DHA was in the Haplic Luvisol and Albic Eutric Stagnosol profiles. In the deepest horizons, the highest DHA activity was found in the Cutanic Luvisol profile (0.261 μg INTF $g^{-1}h^{-1}$) as compared to the other profiles. The highest peroxidase activity in all of the horizons was found in the Haplic Luvisol profile, while the lowest was found in the Mollic Stagnic Gleysol profile. When the PER activity and PER/TOC ratio in the two deepest horizons (in all of the profiles) were compared, we found less activity in the profiles 2 and 3 than in the Haplic Luvisol and Mollic Stagnic Gleysol profiles (Fig. 1). There was an inversely proportional relationship between the POX and PER enzyme activity and the clay content throughout the Haplic Luvisol profile (Fig. 1S a, b) and between the Eet1 and Bt1horizons (Fig. 2 a, b). The results of one-way ANOVA analyses for POX and PER activity are shown in Table 3S and 4S.

The specific enzyme activity (expressed per unit of soil TOC and MBC) was more variable in response to the depth of a profile than the enzymatic activity that was expressed on a soil mass, and this did not enable clear trends to be determined (Table 1S and 2S). Therefore, in general, the DHA/TOC ratio decreased significantly with the profile depths in the Haplic Luvisol and Albic Eutric Stagnosol profiles, while in the Cutanic Luvisol profile, the trends seemed to be the opposite. The tendency for a specific activity (per TOC) to increase throughout the soil profile was also found for the PER activity and FDAH in the Haplic Luvisol profile, the POX activity in the Albic Eutric Stagnosol profile, the PER activity in the Mollic Stagnic Gleysol profile and the activities of POX and PER and also FDAH in the Cutanic Luvisol profile. The results of one-way ANOVA analyses for POX/TOC and PER/TOC are presented in Table 3S. The DHA/MBC ratio was markedly higher in the Ap horizons (for all of the profiles) and sharply decreased with depth, with the exception of the Albic Eutric Stagnosol profile, in which the ratio was higher in the deepest (Cg2 and Cg3) than in the upper horizons (AC and Cg1).

3.3. Community-level physiological profiling analysis

The metabolic activity (AWCD) was definitely higher (1.7–23.0-fold) for the microbial community under aerobic rather than anaerobic conditions for each of the tested soil profiles and horizons (Table 5). The highest metabolic activity under aerobic conditions was observed in the Ap genetic horizon of each of the tested soil profiles, except for the Haplic Luvisol profile, in which the highest AWCD under anaerobic conditions was observed in the Eet1 horizon (0.611). In turn, there were no clear trends in the AWCD changes in the sub-surface horizons. In anaerobic conditions, the highest values of AWCD were found in the illuvial and gleyic horizons (e.g., Bt and Bt2 in the Cutanic Luvisol, Cg2, Cg3 in Albic Eutric Stagnosol, 2ACgg in the Mollic Stagnic Gleysol). Moreover, in aerobic conditions, the AWCD was higher in some of the deeper horizons than in upper horizons. The surface horizons of the considered profiles had a lower functional diversity in the anaerobic

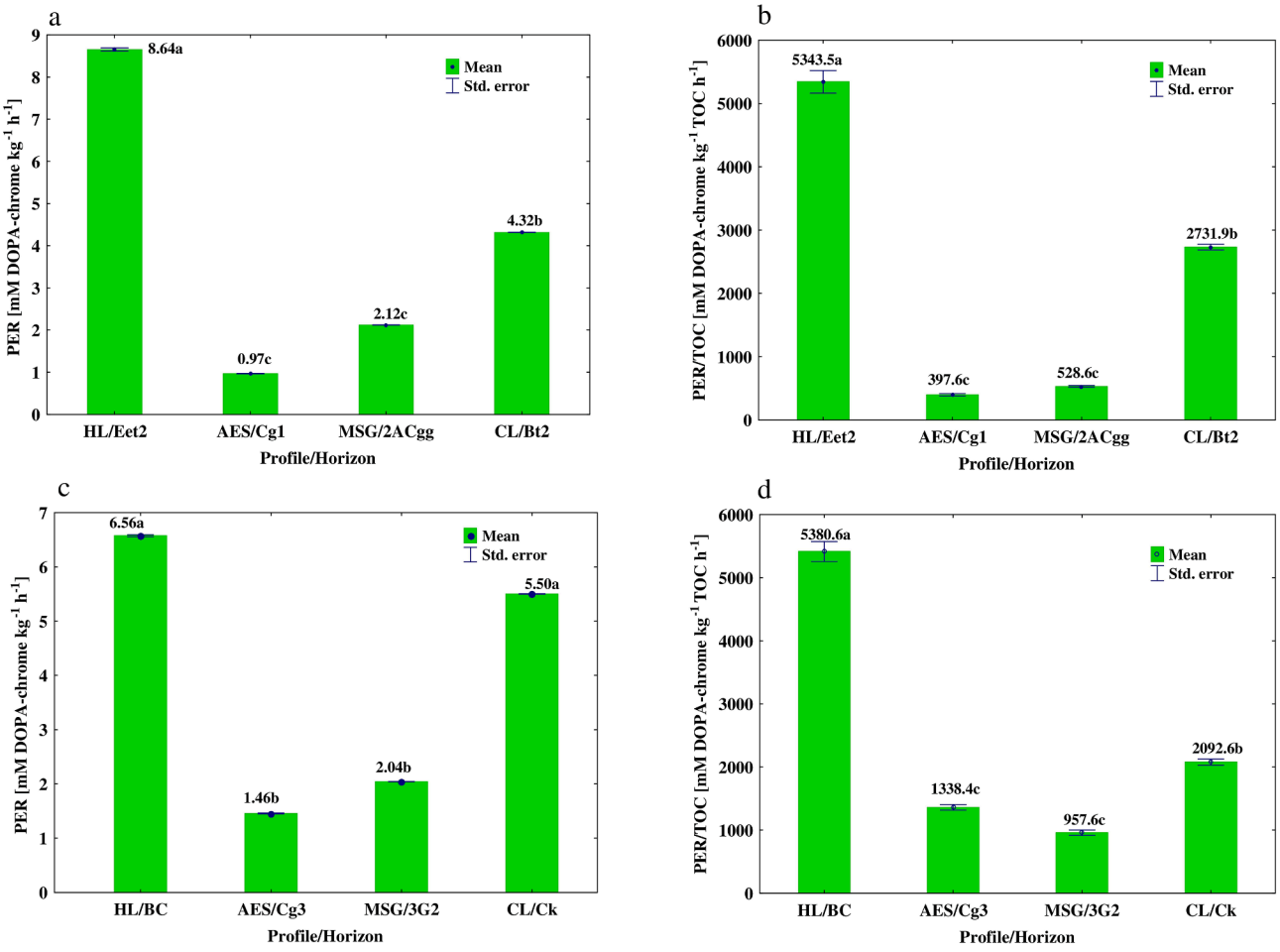

Fig. 1. Effect of gleic processes on the potential peroxidase (PER) activity (a, c) and the PER/TOC ratio (b, d); whereas a and b – third horizon in all profiles and c and d - the deepest horizons in the all profiles. HL - Haplic Luvisol, AES - Albic Eutric Stagnosol, MSG - Mollic Stagnic Gleyosol, CL - Cutanic Luvisol. The oxidative conditions (in Eet2 and Bt2 as well as BC and Ck horizons) were compared with the reducing conditions (in Cg1 and 3G1 as well as Cg3 and 3G2 horizons). Values in compared profiles/horizons followed by different small letters are statistically different at $P < 0.05$.

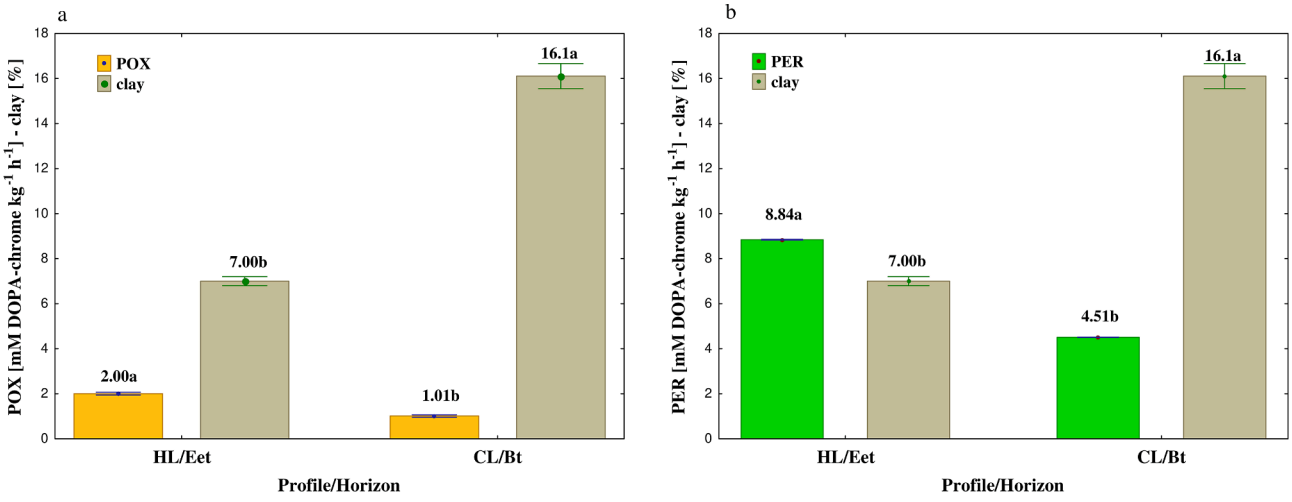

Fig. 2. Effect of the clay content on the phenol oxidase, POX (a) and peroxidase, PER (b) activity in Eet1 horizon (Haplic Luvisol, HL) and Bt1 horizon (Cutanic Luvisol, CL) (mean ± SE). Values in compared horizons followed by different small letters are statistically different at $P < 0.05$.

Table 5
Microbial catabolic diversity indexes calculated for the data obtained from the EcoPlate™.

Genetic horizon	AWCD anaerobic	AWCD aerobic	H anaerobic	H aerobic	E anaerobic	E aerobic
Profile 1. Haplic Luvisol (Cutanic) (HP)						
Ap	0.024 (±0.001)Dc	0.363 (±0.014)Cd	2.364 (±0.041)Ca	2.853 (±0.107)Ba	0.865 (±0.032)Bb	0.904 (±0.034)Aa
Eet1	0.044 (±0.001)Ba	0.611 (±0.013)Ab	2.276 (±0.034)Ca	2.778 (±0.080)Ba	0.833 (±0.017)Ba	0.873 (±0.025)Ba
Eet2	0.031 (±0.003)Cb	0.544 (±0.018)Bc	2.582 (±0.063B)a	2.749 (±0.065)Ba	0.849 (±0.028)Ba	0.886 (±0.021)Ba
Bt	0.082 (±0.002)Ab	0.573 (±0.023)Ba	2.785 (±0.054)Aa	3.028 (±0.114)Aa	0.911 (±0.037)Aa	0.926 (±0.035)Aa
BC	0.020 (±0.002)Ec	0.300 (±0.009)Db	2.156 (±0.034)Db	2.552 (±0.081)Cb	0.872 (±0.025)ABa	0.850 (±0.027)Ca
Profile 2. Albic Eutric Stagnosol (AES)						
Ap	0.098 (±0.003)Ab	0.917 (±0.013)Ab	1.992 (±0.030)Cb	3.028 (±0.071)Aa	0.829 (±0.012)Bb	0.914 (±0.021)Aa
AC	0.028 (±0.001)Db	0.488 (±0.027)Cc	2.675 (±0.038)Aa	2.762 (±0.085)Ba	0.877 (±0.027)ABa	0.922 (±0.028)Aa
Cg1	0.041 (±0.004)Cb	0.674 (±0.032)Ba	2.785 (±0.054)Aa	2.988 (±0.140)Aa	0.910 (±0.043)Aa	0.898 (±0.042)Aa
Cg2	0.050 (±0.001)Bc	0.400 (±0.013)Db	2.610 (±0.036)Ba	2.851 (±0.106)Ba	0.887 (±0.029)ABa	0.895 (±0.033)Aa
Cg3	0.052 (±0.003)Bb	0.288 (±0.029)Eb	2.740 (±0.041)Aa	2.699 (±0.118)Bb	0.916 (±0.037)Aa	0.868 (±0.033)Aa
Profile 3. Mollic Stagnic Gleyosol (MSG)						
Ap	0.213 (±0.005)Ba	1.132 (±0.046)Aa	2.222 (±0.048)Da	3.144 (±0.109)Aa	0.958 (±0.039)Aa	0.953 (±0.033)Aa
A2	0.046 (±0.002)Ea	0.278 (±0.007)Cd	2.609 (±0.045)Ca	2.637 (±0.055)Ca	0.898 (±0.019)Aa	0.863 (±0.018)Da
2ACgg	0.239 (±0.004)Aa	0.416 (±0.011)Bb	2.184 (±0.034)Da	2.825 (±0.082)Ba	0.796 (±0.021)Ba	0.902 (±0.026)Ca
3G1	0.062 (±0.003)Dc	0.422 (±0.006)Bb	2.837 (±0.042)Ba	2.743 (±0.064)Ba	0.926 (±0.013)Aa	0.896 (±0.021)Ca
3G2	0.073 (±0.002)Ca	0.398 (±0.017)Ba	3.013 (±0.065)Aa	3.104 (±0.116)Aa	0.942 (±0.041)Aa	0.922 (±0.035)Ba
Profile 4. Cutanic Luvisol (CL)						
Ap	0.218 (±0.004)Aa	0.773 (±0.037)Ac	2.150 (±0.037)Ca	2.998 (±0.078)Aa	0.839 (±0.022)Bb	0.933 (±0.024)Aa
Bt1	0.030 (±0.003)Db	0.703 (±0.031)Ba	2.598 (±0.063)Aa	2.967 (±0.103)Aa	0.915 (±0.040)Aa	0.918 (±0.032)Aa
Bt2	0.104 (±0.004)Ba	0.336 (±0.006)Cc	2.485 (±0.037)Ba	2.462 (±0.058)Ca	0.899 (±0.016)Aa	0.861 (±0.020)Ba
Ck	0.046 (±0.002)Cb	0.173 (±0.005)Dc	2.655 (±0.046)Aa	2.652 (±0.062)Bb	0.887 (±0.021)Aa	0.855 (±0.020)Ba

AWCD - the average well color development; H - Shannon diversity index; E - Shannon evenness index; mean (±SE).
Following horizons were compared: a) all of the Ap horizons; b) Eet1, AC, A2 and Bt1; c) Eet2, Cg1, 2ACgg, Bt1; d) Bt, Cg2, 3G1, Bt2; e) BC, Cg3, 3G2, Ck. Values in compared horizons followed by different small letters are statistically different at $P < 0.05$.
Different uppercase letters indicate differences ($P < 0.05$) between genetic horizons within the same soil profiles.

communities (Shannon's diversity index, H) than the sub-surface horizons, while in the case of the aerobic H index, the tendency was the opposite (except for the Haplic Luvisol). The values of the evenness index (E) in aerobic conditions were higher in the Ap and AC horizons, while in anaerobic conditions this index was higher in the gleyic horizons. In contrast to AWCD, the E and H indices were more similar in the compared horizons. There were no statistically significant differences in the values of the H biodiversity index for the aerobic communities in horizons 1–3 of Haplic Luvisol profile compared to the other profiles. In the deepest horizon, the highest H values for anaerobic and aerobic conditions were found in the Mollic Stagnic Gleysol profile.

3.4. The number of microorganisms

In general, the abundance of all of the microbial groups was the highest in the Ap horizon (in all of the profiles) and decreased sharply with depth, wherein the lowest values were found in the deepest horizons (except for the Cutanic Luvisol profile) (Table 6). The abundance of some of the microbial groups decreased by>99% from 0 to 150 cm in depth, e.g., almost no fungal CFUs were measured at two the deepest horizons of the Haplic and Cutanic Luvisol profiles. A higher amount of total bacteria, facultative anaerobic bacteria (Bfan) and filamentous fungi (Fun) abundance was found in the gleyic horizons as compared to the upper and lower horizons. When the sub-surface horizons (Eet1, AC, A2, Bt1) were analyzed, the highest total bacteria count, actinomycetes and filamentous fungi growth was observed in the AC horizon, while the highest abundance of facultative anaerobic bacteria was found in the A2 horizon. We found a significantly higher number of BFan and BFan/TOC ratio in the Cg1 and 3G1 horizons as compared to the Eet2 and Bt2 horizons (Fig. 3), wherein these horizons had a similar TOC content and were at a similar depth in the profiles. The results of the one-way ANOVA for facultative anaerobic bacteria and BFan/TOC ratio are given in Table 4S.

3.5. Relationship between the studied properties – PCA analysis and regression

The PCA analysis identified four components that accounted for 81.7% of the total variance, most of which were explained by PC1 and PC2 (Fig. 4a, b). PC1, which accounted for 48.2% of the variance, was related to enzymatic and microbiological properties, because all of them had high positive loading scores concerning this component (Table 7). Properties such as the level of dehydrogenases, hydrolysis of fluorescein diacetate sodium salt, phenol oxidase, microbial biomass C and N, total organic carbon, total nitrogen, total bacteria, facultative anaerobic bacteria, actinomycetes and filamentous fungi were most correlated with PC1 as well as with each other. PCA2 was correlated with chemical properties such as pH, Hh and CEC, while PC3 was correlated with the bulk density and clay content. The PCA analysis showed a strong and positive correlation of the surface horizons of three profiles (Albic Eutric Stagnosol, Mollic Stagnic Gleysol, Cutanic Luvisol), a low and negative correlation of the sub-surface horizons of these profiles as well as a strong and negative correlation of the deepest horizons of these profiles with PC1 (Fig. 4b).

According to the multiple linear regression analysis, the soil enzymes in the surface and sub-surface horizons were significantly correlated with some chemical properties, wherein the clearest example was the relationship with the TOC content (Table 8). The activity of the soil enzymes was also significantly related to the clay content, wherein for the DHA and FDAH rate in surface horizons, the relationships were mostly positive, while negative correlations were found for the phenol oxidase and peroxidase activity in both the surface and sub-surface layers.

4. Discussion

4.1. Changes in the soil properties along the soil profiles

The results of our study confirmed the fact that the soil type and depth in a soil profile are the key factors that significantly affect the soil microbial properties (Tripathi et al., 2018; Hao et al., 2020). As with previous studies (Stone et al., 2014; Parvin et al., 2018), our results showed that the enzymatic activity and functional diversity in the upper horizons were primarily determined by the amount and quality of organic substances and generally decreased with soil depth in parallel to the microbial biomass and organic carbon content. The clearest

Table 6
The number of aerobic heterotrophic bacteria (total bacteria) (Btot), facultative anaerobic bacteria (Bfan), actinomycetes (Act) and filamentous fungi (Fun) (CFU g^{-1} d. m. of soil); mean (±SE).

Genetic horizon	Aerobic heterotrophic bacteria	Facultative anaerobic bacteria	Actinomycetes	Filamentous fungi
Profile 1. Haplic Luvisol (HL)				
Ap	9.60 10^6 (±3.60 10^5)Ac	2.75 10^5 (±4.75 10^3)Ac	1.48 10^6 (±1.31 10^3)Aa	8.97 10^4 (±3.05 10^3)Ac
Eet1	6.34 10^5 (±1.31 10^4)Bc	2.81 10^4 (±4.18 10^2)Bc	5.65 10^5 (±2.42 10^4)Ba	9.94 10^3 (±3.03 10^2)Bc
Eet2	3.11 10^5 (±1.01 10^4)Bc	4.41 10^3 (±1.08 10^2)Cb	3.47 10^5 (±3.89 10^4)Cb	1.30 10^4 (±2.98 10^2)Ba
Bt	6.35 10^4 (±2.56 10^3)Cd	7.39 10^2 (±1.42 10^1)Dc	1.03 10^4 (±3.15 10^2)Dc	8.67 10^1 (±3.33 10^0)Cc
BC	2.90 10^4 (±8.37 10^2)Cd	4.67 10^2 (±7.36 10^0)Dc	1.11 10^4 (±6.38 10^2)Db	1.00 10^2 (±5.77 10^0)Cb
Profile 2. Albic Eutric Stagnosol (AES)				
Ap	1.76 10^7 (±2.55 10^5)Ab	2.86 10^6 (±4.25 10^4)Aa	2.13 10^6 (±1.84 10^5)Ab	2.23 10^5 (±6.14 10^3)Ab
AC	3.02 10^6 (±7.89 10^4)Ba	1.44 10^5 (±3.23 10^3)Bb	6.16 10^5 (±1.21 10^4)Bc	1.62 10^4 (±2.71 10^3)Ba
Cg1	6.73 10^5 (±2.72 10^4)Cb	4.13 10^4 (±5.38 10^2)Cb	1.89 10^5 (±2.66 10^4)Cd	7.15 10^3 (±1.03 10^2)Cb
Cg2	3.64 10^5 (±7.82 10^3)Cb	2.32 10^2 (±3.20 10^0)Dc	5.50 10^4 (±9.27 10^2)Db	1.55 10^3 (±1.53 10^2)Db
Cg3	5.44 10^4 (±1.76 10^3)Dc	1.61 10^2 (±2.41 10^0)Dc	2.68 10^4 (±3.09 10^3)Db	6.37 10^2 (±6.06 10^1)Da
Profile 3. Mollic Stagnic Gleyosol (MSG)				
Ap	2.59 10^7 (±1.05 10^6)Aa	2.01 10^6 (±4.36 10^4)Ab	3.36 10^6 (±3.18 10^4)Ac	1.54 10^5 (±1.27 10^4)Ab
A2	1.51 10^6 (±3.18 10^4)Bb	2.79 10^5 (±4.81 10^3)Ba	4.52 10^5 (±9.44 10^3)Bb	1.09 10^4 (±1.26 10^3)Bb
2ACgg	2.09 10^6 (±5.41 10^4)Ba	2.71 10^4 (±4.26 10^2)Ca	3.50 10^5 (±1.26 10^4)Cc	1.80 10^4 (±3.15 10^2)Ba
3G1	1.29 10^6 (±1.87 10^4)Ba	5.66 10^4 (±8.43 10^2)Ca	3.25 10^5 (±5.61 10^3)Ca	2.71 10^3 (±1.88 10^2)Ca
3G2	6.89 10^5 (±2.97 10^4)Ba	8.70 10^3 (±1.89 10^2)Ca	1.65 10^5 (±2.54 10^4)Db	5.40 10^2 (±6.35 10^1)Ca
Profile 4. Cutanic Luvisol (CL)				
Ap	1.74 10^7 (±4.54 10^5)Ab	9.50 10^5 (±1.62 10^4)Ac	6.65 10^6 (±4.06 10^5)Ab	2.70 10^5 (±1.25 10^4)Aa
Bt1	2.25 10^6 (±9.79 10^4)Ba	2.64 10^4 (±6.43 10^2)Bc	2.43 10^5 (±1.27 10^4)Ba	3.74 10^3 (±6.35 10^1)Bc
Bt2	1.75 10^5 (±3.02 10^3)Cc	4.44 10^3 (±6.63 10^1)Cb	7.32 10^4 (±3.25 10^3)Cd	1.10 10^2 (±2.20 10^0)Cc
Ck	1.70 10^5 (±4.00 10^3)Cb	1.50 10^3 (±2.56 10^1)Cb	4.53 10^4 (±1.77 10^3)Ca	1.67 10^2 (±3.18 10^1)Cb

Following horizons were compared: a) all of the Ap horizons; b) Eet1, AC, A2 and Bt1; c) Eet2, Cg1, 2ACgg, Bt1; d) Bt, Cg2, 3G1, Bt2; e) BC, Cg3, 3G2, Ck. Values in compared horizons followed by different small letters are statistically different at $P < 0.05$. Different uppercase letters indicate differences ($P < 0.05$) between genetic horizons within the same soil profiles.

dependence on TOC was found for the dehydrogenases, whose activity was markedly higher in the AC and A2 horizons (the Albic Eutric Stagnosol and Mollic Stagnic Gleysol profiles) than in the E1 and Bt1 horizons (the Haplic and Cutanic Luvisols). It is generally accepted that the soil C in the topsoil appears in a labile, easy-to-mineralize form, below 30 cm in the soil profile, the soil C that is absorbed by clay or other minerals is more protected against mineralization and is stabilized as a C sink (Hsiao et al., 2018). The organic matter in the subsoil layers is older and more humified than the organic matter in the topsoil layers. (Fang et al., 2005; Callesen et al., 2007). As decomposition progresses, carbon is lost (e.g., as CO_2), while nitrogen is immobilized in the microbial biomass and remains in the system, which frequently results in a lower TOC/TN ratio of the subsoil than the topsoil organic matter (Rumpel and Kögel-Knabner, 2011). This, however, was not fully confirmed in our study because the TOC/TN ratio in some of the deeper horizons (AC, Cg1, A2, 2ACgg, Ck) was higher or similar to the ratio obtained for the surface layers, thus suggesting a significant contribution of C sources, e. g., from the plant roots of deeply-rooted, four-year-old plantation of alfalfa.

Changes in the TOC along the soil profiles are closely associated with the size and activity of the soil microbial population (Sun et al., 2020). On the one hand, soil microbial biomass is part of labile organic C and influences the decomposition of the old organic matter (Liang et al., 2011). On the other hand, it was stated that MBC is precursor of the organic substances that form stable SOC (Kallenbach et al., 2016; Liang et al., 2017). In fact, Liang et al. (2011) and Liang et al. (2019) found that the dead microbial biomass and the by-products of various microbially mediated soil processes account for as much as 80% of soil organic C and thus significantly contribute to the soil C sequestration. These statements were confirmed in our study by the MBC/TOC ratio, which was significantly higher in some of the deeper horizons compared to the surface layers of the studied profiles. Given the significant role of MBC in regulating the vertical change in TOC, the MBC/TOC ratio could be considered to be a potential regulator of the TOC dynamics (Sun et al., 2020). Based on the above, the changes of the ratio in the studied soil profiles could be explained in two ways. Firstly, enough available organic substrates (per TOC content) were available to support microbial content and activity. In fact, in this study the contribution of dissolved organic carbon to TOC increased throughout the soil profiles, which confirmed that soils with more labile C (per TOC content) tend to have a higher microbial biomass (per TOC content) (Marinari and Vittori Antisari, 2010). Secondly, the reason for the higher MBC/TOC in some of the deeper horizons, despite the lower enzymatic activity, 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 nutrients than in the surface horizons (Brewer et al., 2019). The statement above was partially confirmed by the DHA/MBC ratio. Because the dehydrogenases activity (DHA) reflects the population of living and proliferating microorganisms, this ratio indicated that there were worse conditions for microbial life in the deeper horizons of the profiles.

We did find differences in the depth distribution between the hydrolytic and extracellular oxidative enzyme activity. The hydrolytic enzyme activity (expressed as FDAH), systematically decreased with depth, while the oxidative enzymes had a different depth distribution in their activity in the deeper horizons that was sometimes even higher than in the upper horizons (e.g., the Cutanic Luvisol profile). That increase may be explained by the fact that the activity of the hydrolytic enzymes is often directly associated with the SOM content (Sinsabaugh et al., 2008; Schnecker et al., 2015). Since the SOM content and the number of regular substrates that can be hydrolyzed decrease with depth (Rumpel and Kögel-Knabner, 2011), the activity of the hydrolytic enzymes can also be expected to decrease. The relatively high FDAH/MBC ratio, which was even higher in the deepest than in the surface horizons (e.g., the Mollic Stagnic Gleysol) indicates that the enzymatic activity was stabilized by the clay minerals and therefore protected from degradation. Our interpretation is based on the assumption that all of the hydrolytic enzymes are mainly affected by the changing soil abiotic conditions and that the vertical translocation of the enzymatic protein is minimal. The enzymes that are immobilized on the clay minerals are present and active in a soil in which no microbial proliferation occurs (Hsiao et al., 2018). Additionally, soil 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 (Chacon et al., 2006).

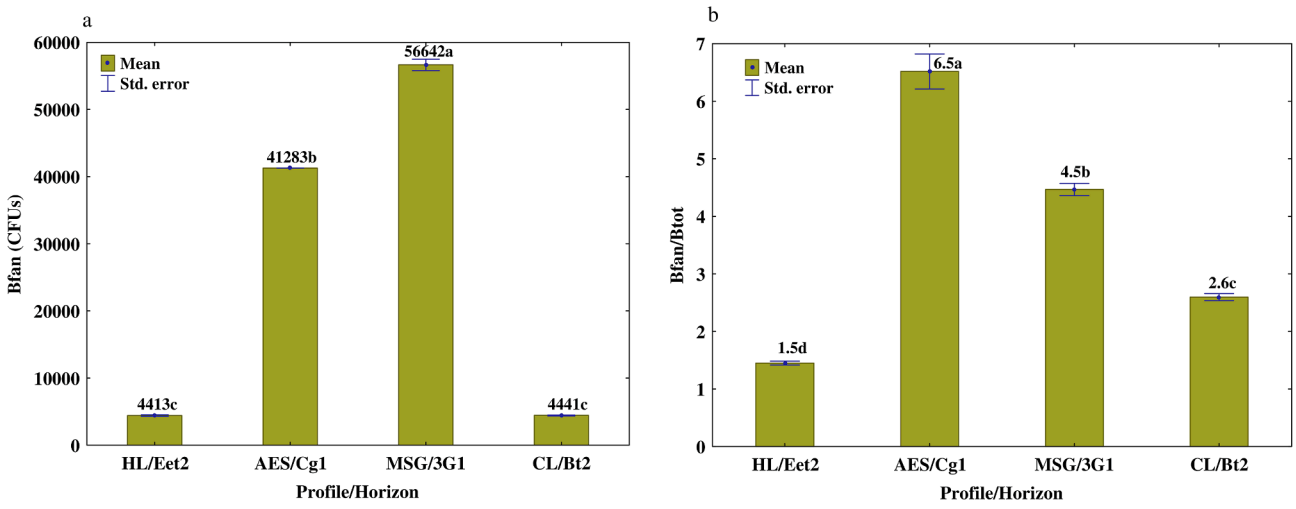

Fig. 3. Effect of gleic processes on the facultative anaerobic bacteria, BFan (a) and BFan/Btot ratio (b) in the third horizon in all profiles. HL - Haplic Luvisol; AES - Albic Eutric Stagnosol; MSG - Mollic Stagnic Gleyosol; CL - Cutanic Luvisol. The oxidative conditions (in Eet2 and Bt2 horizons) were compared with the reducing conditions (in Cg1 and 3G1 horizons). Values in compared profiles/horizons followed by different small letters are statistically different at $P < 0.05$.

Oxidoreductases, by contrast, are unspecific enzymes and are often not produced to directly acquire nutrients, but rather to degrade the humic complexes or toxic substances such as phenols (Talbot et al., 2008; Sinsabaugh, 2010). Although it is commonly accepted that the lignin content decreases with depth, an increase in the lignin content of the SOM with depth has been observed in some cases, which could be attributed to its vertical transport and protection of the lignin in the deeper soil layers (Mason et al., 2009). This may explain the relatively high POX and PER activities in the deeper horizons in our study where they contributed to the lignin degradation, humification, C mineralization and dissolved organic C export (Sinsabaugh, 2010).

Both the phenol oxidase and peroxidase, which were expressed in terms of the microbial biomass content (MBC) content were significantly active in the deeper horizons of the studied profiles, and were the highest in the deepest horizons (BC, Cg3, 3G2 and Ck). This may be due to stabilization of the residual enzymatic activity on the clay minerals and also because of the greater number of substrates in deep soils. The substrates that are translocated into the deeper layers could either be immediately assimilated by microorganisms or become more closely adsorbed onto mineral particles than in the surface layers (Kramer et al., 2013).

4.2. *Changes associated with genetic horizons and soil-forming processes*

In the deeper soil horizons, factors other than organic carbon content may explain the shift in the microbial and enzymatic properties. The process of lessivage (in the Haplic Luvisol and Cutanic Luvisol) 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

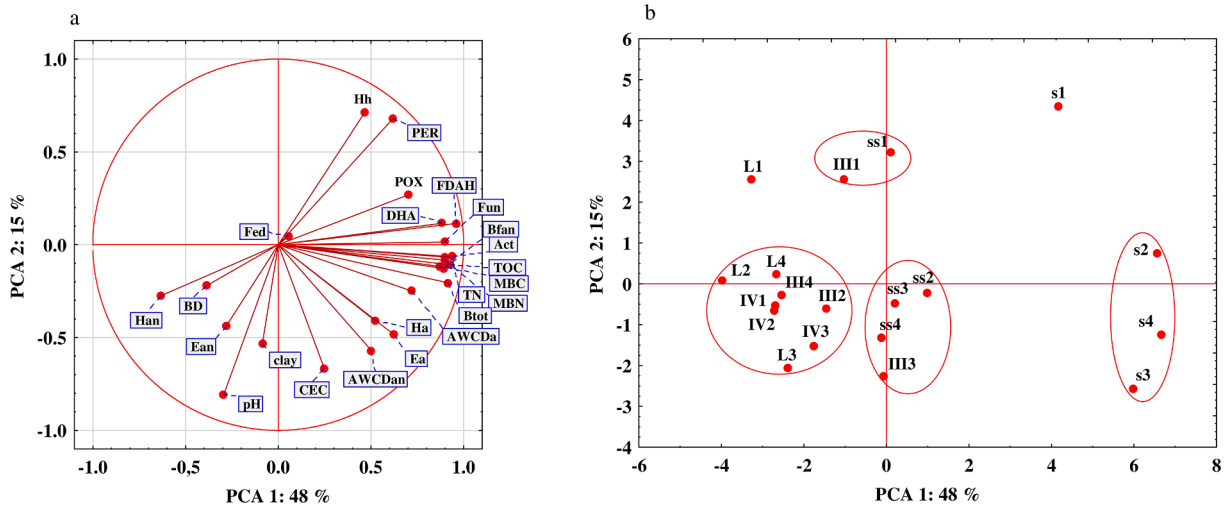

Fig. 4. Principal component analysis derived from the studied soil properties' plot of the first two principal components (PC) for the measured soil properties, DHA – dehydrogenases; FDAH - fluorescein diacetate hydrolysis; POX – phenol oxidase; PER – peroxidase; TOC – total organic carbon; TN – total nitrogen; MBC – microbial biomass carbon; MBN – microbial biomass nitrogen; Btot - the number of aerobic heterotrophic bacteria; Bfan - facultative anaerobic bacteria; Act – actinomycetes; Fun - filamentous fungi; AWCDa - the average well color development under aerobic condition; Ha - Shannon diversity index under aerobic condition; Ea - Shannon evenness index under aerobic condition; AWCDan - the average well color development under anaerobic condition; Han - Shannon diversity index under anaerobic condition; Ean - Shannon evenness index under anaerobic condition; Hh– hydrolytic acidity; Fed – free iron oxides; CEC – cation exchange capacity; BD – bulk density, principal component analysis of the variables in soil horizons/depths, horizons in all 4 profiles: s1-s4 – the surface (Ap) horizons, ss1-ss – the sub-surface horizons, III1-III4 – the middle (thirds) horizons, IV1-IV3 – the second from the bottom horizons, L1-L4 - the deepest horizons.

Table 7
Loading scores of the variable for PCA.

Variable*	PCA1	PCA2
DHA	0.882	0.116
FDAH	0.960	0.115
POX	0.703	0.269
PER	0.619	0.679
TOC	0.927	−0.109
TN	0.936	−0.064
MBC	0.868	−0.116
MBN	0.896	−0.082
Btot	0.916	−0.205
Bfan	0.892	−0.127
Act	0.900	−0.067
Fun	0.897	0.014
AWCDa	0.719	−0.242
Ha	0.522	−0.409
Ea	0.622	−0.482
AWCDan	0.502	−0.569
Han	−0.634	−0.275
Ean	−0.282	−0.439
pH in $CaCl_2$	−0.295	−0.809
Hh	0.469	0.715
CEC	0.248	−0.666
Fed	0.054	0.045
Clay	−0.086	−0.534
BD	−0.387	−0.220

*Abbreviations are explain under Tables 1-6 and Figs. 1-4.

impact on the soil microbial properties (Błońska and Lasota, 2013). In general, clay minerals inhibit the activity of the enzymes, which is followed by their stabilization and protection of the residual activity against thermal and proteolytic deactivation (Burns et al., 2013). Previous studies have reported that the clay content affects the enzyme kinetics through a decrease in the 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 (Burns et al., 2013; An et al., 2015). In our study, the activity of phenol oxidase and peroxidase in the Eet1 and Bt1 horizons of the Haplic Luvisol and Cutanic Luvisol profiles was inversely dependent on the clay content, this was also confirmed by the analysis of regression. Because the compared horizons had a similar TOC content, this suggests that the enzymatic activity is inhibited by the clay content. Our data are not in agreement with Allison and Jastrow (2006) who found the highest level of peroxidase in the clay-size fraction, which indicates that this enzyme activity pattern down the soil profiles may be explained by the increasing adherence of enzymatic proteins to the mineral surfaces within the soil profiles. Additionally, according to Hsiao et al. (2018), the peroxidase activity within the deeper layers of claypan soil was correlated to the clay content, thereby suggesting a greater potential activity in the clay-enzyme complex. The observed increase might occur because the clay minerals protect the extracellular enzymes from degradation. The clay content of the soil also modifies the microbial community structure by favoring bacteria over fungi (Wei et al., 2014). This statement was confirmed in our study in which there was a very low fungi CFUu in the horizons with the highest clay content (Bt and BC in the Haplic Luvisol or Bt1-Ck in the Cutanic Luvisol) as compared to the similar horizons/depths in the other profiles.

Water saturation and a low O_2 concentration (reducing conditions) are common in the subsoil horizons of Gleysol and Stagnosol, although these two soils may differ in their source of waterlogging (groundwater or atmospheric water) and in their manifestations in the soil profile. In order to assess the effect of the reducing conditions on the studied enzymes, we compared the enzymatic activity in the Cg1 and Cg3 horizons (Albic Eutric Stagnosol) and the 3G1 and 3G2 horizons (Mollic Stagnic Gleysol), which are expected to have reducing conditions, as compared to the oxidative layers, lying at a similar depth. Since the compared horizons had a similar TOC content (1.58–2.43 g kg^{-1}) and thickness, the possible changes across the soil profiles can be assigned to the gleyic properties or other soil features. The reducing conditions did not affect most of the studied enzyme activities. Only the peroxidase activity was significantly lower in the reducing horizons of the studied profiles compared to the oxidative layers. In contrast to the expected results, the dehydrogenases did not have the highest activity in the horizons with potential reducing conditions compared to the oxidative horizons. Furthermore, no significant relationship was found between the dehydrogenases and the number of facultative anaerobic bacteria or the anaerobic AWCD, H and E indices, although we found that these properties were higher in the reducing horizons compared to the oxidative layers (considered as the average of four horizons). It was found earlier that most of the dehydrogenases are produced to a large degree by anaerobic microorganisms (Wolińska and Bennicelli, 2010). It was shown in many studies that DHA is significantly affected by the water content and decreases with a decrease in the soil humidity. For example, Gu et al. (2009) observed a higher DHA level (by as much as 90%) in flooded soil than in non-flooded conditions. Similar results were also found by Zhao et al. (2010) and Weaver et al. (2012). The lack of a significant effect of the reducing conditions on the studied oxidoreductases may be explained by the fact that the fluctuating oxidative and reducing conditions might lead to the abiotic oxidation of organic matter in the presence of iron and manganese and might mimic the oxidative enzyme activities (Bach et al., 2013; Hall and Silver, 2013; Jones et al., 2020). In fact, some of recent studies have suggested that a significant amount of oxidative decomposition is due to a nonenzymatic or abiotic reaction (Blankinship et al., 2014; Kéraval et al., 2018). One possible explanation is the presence of reactive metal intermediates such as the Mn and Fe forms, which mediate the oxidation of macromolecules by molecular oxygen, and have been shown to enhance oxidative decomposition in a soil system (Hall and Silver, 2013; Kranabetter, 2019). The difference in the POX and PER activities as affected by the reducing conditions in the compared genetic horizons could be associated with the different contribution of the Mn- and Fe-mediated oxidative decomposition (Jones et al., 2020). As regards the changes in the POX activity (without the addition of H_2O_2), which better reflects field conditions, Mn best explained soil oxidative activity. In the case of the PER activity (when H_2O_2 was added to the assay), Fe became the better predictor of oxidative activity by far (Jones et al., 2020). This

Table 8
Regression summary for dependent variables (y) calculated for all considered profiles.

Dependent variable (y)	Horizon	Regression	*p*	R^2
DHA	*Surface	y = 0.160TOC - 0.031Hh - 0.023CEC + 0.320clay - 1.895 ± 0.090	<0.0001	0.960
	**Sub-surface	y = 0.103TOC + 0.003CEC - 0.023clay - 0.091 ± 0.034	<0.0001	0.785
FDAH	Surface	y = 2.984TOC - 0.290CEC + 4.424clay - 37.867 ± 4.223	0.0001	0.774
	Sub-surface	y = 1.644TOC + 0.229Hh - 0.030CEC + 1.007 ± 0.678	<0.0001	0.845
POX	Surface	y = 0.0237Hh + 0.035CEC - 0.862clay + 0.0004Fed + 5.259 ± 0.432	<0.0001	0.906
	Sub-surface	y = 0.828TOC + 0.165Hh + 0.042CEC - 0.347clay - 0.145 ± 0.356	<0.0001	0.738
PER	Surface	y = -0.590TOC + 1.080Hh - 0.932clay + 0.003Fed + 8.279 ± 0.322	<0.0001	0.996
	Sub-surface	y = 1.687TOC + 0.905Hh + 0.074CEC - 0.732clay + 0.619 ± 1.156	<0.0001	0.819

*Ap horizons of all profiles, ** all sub-surface horizons of all profiles considered together, DHA – dehydrogenases, POX – phenol, PER – peroxidase, FDAH - fluorescein diacetate hydrolysis TOC – total organic carbon, Hh -hydrolytic acidity, CEC – cation exchange capacity, Fed – free iron oxides.

observation is in agreement with the well-known stimulation of the Fenton chemical reactions by H_2O_2 as well as the H_2O_2-mediated inhibition of Mn (II) oxidation and the reduction of reactive Mn (III) forms (Learman et al., 2013). Because we did not determine any Mn forms only Fed forms, these explanations should be considered with caution since other factors might also contribute to this phenomenon.

The hydrolytic enzyme activities (expressed as FDAH), which were similar in the reducing and oxidative horizons of the studied profiles, do not directly require the presence of O_2 and can be resilient to periodic anaerobiosis (Hall et al., 2014). Additionally, Alves et al. (2020) found that some hydrolytic enzymes isolated from microorganisms living in mangroves (anaerobic/aerobic sediments) were able to fluctuate in oxic/anoxic conditions. It is also possible that a variation in the frequency and extent of the reducing conditions in the soils that accompany weather change might be insufficient to affect the dynamics of the hydrolytic enzymes (Liptzin et al., 2011). In addition, as was found by Hall et al. (2014), field-relevant periods of anaerobiosis might not inhibit enzymes in laboratory incubations.

4.3. Community-level physiological profiling (CLPP) and the number of microorganisms

The culture-dependent methods such as the CLPP and the viable plant count have often been used to characterize the metabolic potential and functional diversity of microbial communities and the microbial numbers in the bulk and rhizosphere soils of different environments (Mącik et al., 2020; Lladó et al., 2017; Lee et al., 2021). Previous studies found many limitations of the culturing approaches (culturing and Biolog®), including the requirements to unify the equivalence of the inoculum sample size, the incubation duration, the data analysis and the interpretation of the results (Preston-Mafham et al., 2002). What is more, the carbon substrates used in the Biolog method do not always correspond to the substrates that are present in the studied environmental sample (Stefanis et al., 2013). It is necessary to stress that culturing-based methods such as CLPP and plate counts do not represent the functional potential of the entire microbial community, but only of the fast-growing microorganism fraction (Grządziel et al., 2019; Lladó et al., 2017). When microbial CFUs are analyzed, it must be kept in mind that usually <1% of all soil microorganisms can be determined by direct counts of the colonies that form on Petri dishes (Davis et al., 2005). Other disadvantages of plate counts are that this method does not distinguish between vegetative and spore cells or the shift toward fungal species that produce large quantities of spores. The advantages of the culturing approaches over the molecular-based techniques are the simplicity of the protocol and the low analytical costs as well as possibilities to assess different biochemical processes in the soil environment, thereby providing many results of the microorganism's metabolism that correlate with other soil quality indicators (Gałązka et al., 2017). However, as was mentioned above, these methods are still being used in environmental microbiology research. Therefore, we decided to use these methods to compare the microbial numbers and the activity of the aerobic and anaerobic microorganisms in different soil profiles because this issue is poorly understood and should be investigated.

The significantly higher metabolic activity for a microbial community under aerobic rather than 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 (Reith et al., 2002); moreover, the aerobic processes are more effective than the anaerobic processes. However, anaerobic soil processes are also quite relevant to the functioning of the soil ecosystem (Keiluweit et al., 2017). In anaerobic processes, chemicals are metabolized in other metabolic pathways, and therefore, different sets of substrates may be utilized by the anaerobic populations.

The order of the aerobic AWCD values was associated with the properties of the soil types and is as follows: Mollic Stagnic Gleysol > Albic Eutric Stagnosol > Cutanic Luvisol > Haplic Luvisol. According to the previous studies (Grządziel and Gałązka, 2019), the Mollic Stagnic Gleysol profile is rich in organic matter, while the Haplic Luvisol profile is a rather poor and acidic soil. This was also confirmed by the results of our study, which showed the lowest content of TOC, TN, Fed, CEC, clay and a low pH in the surface horizon of this profile (Table 2). Moreover, the highest values of the H and E indices in the Mollic Stagnic Gleysol profile indicate that it had the most stable substrates utilization value (H) and the most equal use of substrates by the microbial communities as compared to the other soil profiles.

The results of the E index indicated an increasing trend in the utilization of carbon sources by a microbial community in the following order: Haplic Luvisol < Albic Eutric Stagnosol < Mollic Stagnic Gleysol < Cutanic Luvisol, which shows that in the poorer profiles, the utilization of the substrates was not equal but became more equal with increasing soil fertility. The lowest values of E in the Haplic Luvisol profile (E1 horizon) were probably due to this soil playing a filtering role (Keesstra et al., 2012), and therefore it contains various carbon sources that cause an unequal utilization of substrates.

In the studied profiles, the highest number of microbial CFUs were found in the surface soil and decreased with an increase in depth. We found that the lower availability of organic matter in the deeper soil horizons can be considered to be the main factor that controls the structure and activity of microbial communities. This is in agreement with Braun et al. (2006) and Bhattarai et al. (2015), who also found that a decrease in the abundance of microbial groups with depth might be attributed to changes in the moisture and a decreasing aeration. In contrast to this, other authors found that the soil particle size and soil pH had the greatest impact on microbial diversity and community structure (Fierer et al., 2003). This, however, does not reflect our results since no statistically significant relationships were found between the microbial numbers and soil texture or soil pH, although the raw data did indicate that while the abundance of the microbial groups decreased with depth, soil pH increased.

Despite the shortcomings of the culture-dependent methods, combining them with determining the soil enzymatic activities was considered to be a good approach for acquiring better information on the characteristics of soil microorganisms. As was stated by Lee et al. (2021), no single method for undertaking an absolute microbial count and diversity using culture-dependent methods or even culture-independent methods exists.

5. Conclusions and future research

Our results have shown that the hydrolytic enzyme activity (FDAH) systematically decreased with increasing soil depth, while the oxidative enzymes had a different depth distribution which occasionally produced even higher activity in the deeper horizons than in the upper layers. The data indicated that the decrease in the potential activity of dehydrogenases and FDAH was parallel to the decrease in the microbial biomass and organic carbon content. On the other hand, the different depth gradients observed may be associated with the different degrees of availability of the substrates for phenol oxidase and peroxidase and also for the hydrolytic enzymes in specific profile horizons. It was found that the potential enzyme activity in the surface horizons was mainly determined by organic matter content and composition, while their activity in the sub-surface layers was also affected by certain factors that are associated with the soil-forming processes (e.g., the lessivage process, clay content, gleyic conditions). The impact of clay positively affected the dehydrogenases in the surface layers, but, contrary to expectations, it negatively affected the phenol oxidase and peroxidase activity. Additional research is required to identify the relationship between the soil microbial properties and soil fractions. To examine the soil microbial properties separately in individual soil fractions could be an appropriate way to explain these relationships.

Since the specific enzymatic activity (expressed as the TOC and MBC content) was clearly different for the intracellular and extracellular enzymes, it may be considered as a good indicator of changes in the size of the microbial population and activity with depth.

The pattern of substrate utilization by the microbial communities was different in aerobic and anaerobic conditions and was unequal in the poorer profiles but was more equal in the more fertile profiles (with a higher organic carbon content). The differences in substrate utilization patterns with depth possibly reflect changes in the dominance of those microorganisms that can maintain their metabolism under various substrate contents and quality conditions. The highest values of the AWCD in anaerobic conditions, which were noted in the Ap horizons of the Cutanic Luvisol and Mollic Stagnic Gleysol profiles, corresponded with the highest content of TOC and TN in those profiles, thus confirming that these profiles are rich in humus, which can stimulate microbial activity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by the National Science Centre, Poland (project no 2018/29/B/NZ9/00982). Much gratitude is due to Michele Simmons for proof reading the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2022.115779.

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