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Easily degradable carbon – an indicator of microbial hotspots and soil degradation**

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Abstract. The effect of arable soil was quantified against non-cultivated soil on easily degradable carbon and other selected microbiological factors, *i.e.* soil microbial biomass, respiration activity, and dehydrogenase activity. The intent was to ascertain whether easily degradable carbo can be useful as a sensitive indicator of both soil biological degradation and microbial hotspots indication. As a result, it was found that soil respiration activity was significantly higher ($p < 0.0001$) in all controls, ranging between 30-60 *vs.* 11.5-23.7 μmol CO_2 kg d.m.$^{-1}$ h^{-1} for the arable soils. Dehydrogenase activity was significantly lower in the arable soil (down to 35-40% of the control values, $p < 0.001$) varying depending on the soil type. The microbial biomass was also significantly higher at the non-cultivated soil (512-2807 *vs*. 416-1429 μg g^{-1} d.m., $p < 0.001$), while easily degradable carbon ranged between 620-1209 mg kg^{-1} non-cultivated soil and 497-877 mg kg^{-1} arable soil ($p < 0.0001$). It was demonstrated that agricultural practices affected soil properties by significantly reducing the levels of the studied parameters in relation to the control soils. The significant correlations of easily degradable carbon-respiration activity (ρ = 0.77*), easily degradable carbon-dehydrogenase activity (ρ = 0.42*), and easily degradable carbon-microbial biomass (ρ = 0.53*) reveal that easily degradable carbon is a novel, suitable factor indicative of soil biological degradation. It, therefore, could be used for evaluating the degree of soil degradation and for choosing a proper management procedure.

Keywords: agriculture, carbon, degradation, microbial activity, soil

INTRODUCTION

Soils are the main pool of organic carbon on Earth, storing above 1500 Pg of C in the top one metre layer. This is greater than the amount of carbon accumulated by vegetation present in the atmosphere (Zhao *et al.*, 2014). The soil organic matter pool is a balance between the addition and decomposition rate of plant and animal remains. It is suggested that up to two-thirds of deposited residues are returned to the atmosphere as CO_2 in a single season, whilst subsequent decomposition is significantly slower and results in the accumulation of more stable organic and less decomposed carbon forms in the soil (Li and Fang, 2002). The organic carbon cycle is a dynamic process, depending on physical and chemical conditions. Soil organic carbon (SOC) plays many functions, as it is a main determining factor of soil fertility, influencing its biological, chemical and physical properties, and protecting it against soil erosion. The SOC pool affects soil's capability to sustain plant growth as it is a source and sink of nutrients and water absorption, promotes soil aggregation and density, determines ion exchange, protects against soil reaction (pH) due to application of agricultural chemicals, and regulates soil temperature and the vegetation period (Lal, 2004). SOC also supports the activity of bacteria, fungi, earthworms, plant roots and other soil inhabitants. Therefore, it is considered a soil degradation indicator and a factor enhancing the breakdown of soil contaminants (Šarapatka and Bednář, 2014). Organic matter consists of a wide range of C compounds that vary in terms of susceptibility to biological degradation. According to a more recent classification (Breulmann *et al.,* 2014; Strosser, 2010) based on the carbon function in soil, three groups of C compounds are distinguished: labile, stable and inert. Labile organic carbon is quickly reactive and susceptible to biodegradation, providing nutrients for both soil microorganisms and plants (Breulmann *et al.,* 2014). This fraction of soil organic

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matter is rapidly transformed during the year. The stable carbon fraction is a reservoir of less decomposable organic matter responsible for soil cation exchange capacity (Strosser, 2010). These forms are usually bound in organic-mineral aggregates (Breulmann *et al.* 2014). Its half-life is estimated as being even up to decades (Strosser, 2010). The most non-reactive organic matter, the inert fraction, determines the physical properties of the soil, protecting it against decomposition. It has a half-life between decades and centuries (Strosser, 2010). According to this classification, the labile carbon forms are substantially important factors supporting soil microbiological activity. As these are easily degradable, in the study they are referred to as easily degradable carbon (EDC). However, as the methods of its measurement are based on carbon oxidation using potassium permanganate (Weil *et al.*, 2003), EDC is often called permanganate oxidisable carbon (POXC) or active C. Culman *et al.* (2012) indicate that POXC is a relatively new method that can quantify labile carbon both rapidly and inexpensively. Of note, studies devoted to POXC measurements and correlations with other important biological factors (*i.e.* respiration and enzyme activity) in soil environment are still little recognised.

Soil microbial activity is limited by EDC (Schimel and Weintraub, 2003). However, by removing this restriction (the input of labile C), the activity of microorganisms significantly increases and produces microbial hotspots (Kuzyakov and Blagodatskaya, 2015). Kuzyakov (2010) defined such hotspots as small soil volumes with much faster microbiological processes than that in average soil conditions. Kuzyakov and Blagodatskaya (2015) divided hotspots into four groups, taking into account their biotic (rhizosphere, detritusphere, biopores) and abiotic (aggregate surface) origin. Three potential mechanisms for the transport of labile carbon to microorganisms have also been distinguished (Kuzyakov and Blagodatskaya, 2015):

– direct release of organics,
– advection system – transport of organics dissolved in water, and
– transport by diffusion.

The key pathway in soil carbon accumulation is sequestration from plant biomass; hence, the rate of carbon accumulation and storage can be determined by the type of vegetation or amount of plant remains. These processes can also be influenced by agriculture management systems and practices. Tillage practices affect aeration in the surface level of soil profiles and thus it is expected that the rate of organic matter decomposition will be greater due to quicker carbon mineralisation via being in an aerobic state than being anaerobic. This hypothesis was confirmed by Moussa-Machraoui *et al.* (2010) and Moussadek *et al.* (2014) for Vertisols and Cambisols while there were no statistical differences between tillage and no-tillage soils (Thomas *et al.*, 2007; Moussadek *et al.*, 2014) for Luvisols. In these experiments, the total organic carbon pool was considered, but the labile forms, as discussed above, seem to play the crucial role for microbial activity and plant cultivation. Still, studies related to EDC pools on cultivated soils *versus* non-cultivated and correlations with soil biological factors (especially respiration activity and dehydrogenase activity) are limited.

Thus, in the presented laboratory study, we aimed to quantify the effect of soil cultivation in comparison to non-cultivated soil on a labile (bioavailable) carbon stock in seven soil types: Albic Luvisol, Brunic Arenosol, Haplic Phaezoem, Mollic Gleysol, Eutric Fluvisol, Eutric Histosol, and Rendzina Leptosol, which are characteristic for the Lublin region of SE Poland. A hypothesis was formulated that arable soils might be biologically degraded in terms of their biodiversity and microbial activity as a result of intensive agricultural practices. Bioavailable carbon analyses (or easily degradable carbon, EDC) were applied as a tool to determine how labile carbon forms affected soil microbial biomass (MB) carbon, respiration activity (RA) and dehydrogenase activity (DHA), *i.e.* factors reflecting microbial activity in soils. The aim was to ascertain whether EDC can be used as a sensitive indicator of the biological degradation phenomenon and a factor in the generation of microbial hotspots in the soil environment.

MATERIALS AND METHODS

The study was conducted in the south-east part of Poland, in the Lublin region (51°13′N 22°54′E), a vast and important agricultural area. For the current study, 16 sites were selected (Fig. 1) based on database created by Bieganowski *et al.* (2013), allowing a precise return to the sampling locality (Gliński *et al.*, 1991). Each site was described in the database of the Bank of Polish Soil Samples (BPSS) established in 1991 by the Institute of Agrophysics, the Polish Academy of Sciences in Lublin

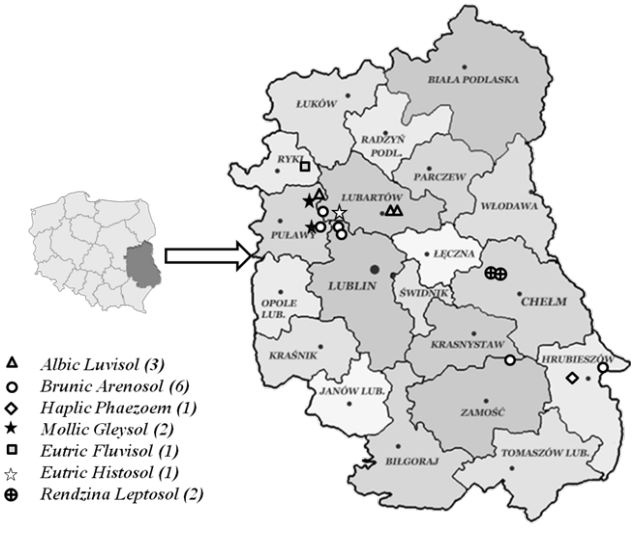

Fig. 1. Location of sampling sites in the Lublin region (SE Poland). Different symbols represent soil types and the number of sampled sites for each soil type (own image).

(Gliński *et al.*, 1991). At each site, both agriculturally exploited and uncultured plots were selected. The arable soils were sampled (coded A – arable) during the spring season (April 2014) from non-ploughed sites in order to avoid artefacts from ploughing perturbations (Wolińska *et al.*, 2014). At the same time, the control samples (coded NC – controls, non-cultivated soils) were taken from non-agriculturally cultivated and non-forested sites (covering at least a 1 ha area) close to the basic soils and belonging to the same soil type (*i.e.* fallow lands non-cultivated for years or grasslands). A description of the fields differing in the crop type and of the control site is presented in Table 1.

Table 1. Location of agricultural soils and description of the control sites used in the study

Soil No.	Type of soil (FAO)	Crop type	Village	Geographic coordinates	Control sites
1	Albic Luvisol (AL)	Oat	Dęba	22º10’17.7’’ 51º26’24.6’’	30 year old meadow planted with fruit trees
2		Triticale	Pryszczowa Góra	22º27’10.3’’ 51º24’30.8’’	20 year old woodlots with birches
3		Wheat	Niemce	22º36’51.8’’ 51º21’27.0’’	50 year old meadow (mowed once a year)
4	Brunic Arenosol (BA)	Triticale	Klementowice	22º06’54.2’’ 51º21’52.2’’	Unmoved meadow, wasteland
5		Oat	Łany	22º15’19.0’’ 51º23’00.9’’	20 year old field-woodlots
6		Oat	Markuszów	22º15’55.5’’ 51º23’10.9’’	20 year old field-woodlots
7		Field prepared for seeding	Rogalin	24º04’00.3’’ 50º51’15.8’’	Meadow (mowed once a year)
8		Triticale	Sady	23º22’52.4’’ 50º51’14.8’’	Unmoved meadow, wasteland
9		Strawberries	Chrząchówek	22º07’29.9’’ 51º25’50.5’’	Unmoved meadow, wasteland
10	Haplic Phaezoem (HP)	Triticale	Hostynne	50º44’48.3’’ 23º42’56.6’’	Meadow (mowed once a year)
11	Mollic Gleysol (MG)	Colza	Pożóg Nowy	22º06’18.8’’ 51º22’48.0’’	30 year old pine woodlots
12		Wheat	Bałtów	22º01’25.5’’ 51º29’15.3’’	70 year old meadow (mowed once a year)
13	Eutric Fluvisol (EF)	Oat	Kośmin	21º59’10.1’’ 51º33’47.7’’	15 year old meadow (mowed once a year)
14	Eutric Histosol (EH)	Oat	Wólka Kątna	22º16’38.9’’ 51º25’27.3’’	20 year old meadow (mowed once a year)
15	Rendzina Leptosol (RL)	Celeries	Siedliszcze	23º10’58.3’’ 51º12’22.3’’	40 year old meadow (mowed once a year)
16		Oat	Brzeziny	23º11’43.9’’ 51º12’10.8’’	Meadow (mowed once a year)

Soil samples were collected during 24-26 April 2014. 10 × 10 m squares were chosen from the areas of the sample sites characterised by homogeneity of the vegetation cover. Generally, 16 sites represented agricultural areas and the same number of sites was taken for the controls (non-cultivated) (Fig. 1). Within each square, *ca*. 50 random soil samples were taken from the top layer (0-20 cm), using a 2.5 cm diameter auger. Randomised samples were drawn (due to the considerable heterogeneity of soils) in order to get the most representative soil material. In total, 3 replicates of 2 kg were sampled. The collected material represented 7 soil types: Brunic Arenosol (BA), Albic Luvisol (AL) (dominating soil types in Poland, representing 82% of the country soils, thus their share in the studied material was significant – 6 and 3 soil samples, respectively), Mollic Gleysol (MG), Rendzina Leptosol (RL) (2 representatives), Haplic Phaezoem (HP), Eutric Fluvisol (EF), and Eutric Histosol (EH) (all from 1 site, Table 1, Fig. 1). Under laboratory conditions, each sample was passed through a 2.0 mm sieve to remove large pieces of rocks and plant material was stored at 4ºC till to the analysis.

Particle size distribution was measured using laser diffractometer (Mastersizer 2000 (Malvern, UK) with Hydro G dispersion units). The intensity of laser light registered on the particular detectors of the measurement system can be converted to particle size distribution according to the Mie theory, assuming the following values of the indices: refraction index 1.52 and absorption index 0.1 for the dispersed phase, and refraction index 1.33 for water as the dispersing phase. During the measurement, the pump speed was set at 1750 r.p.m., while the stirrer speed was at 700 r.p.m. (Bieganowski *et al.*, 2013). The soils were dispersed using ultrasound at 35 W for 4 min without removing the organic matter (Lamorski *et al.*, 2014). The measurements were carried out in 3 replications.

For the current study, several biochemical and chemical analyses were performed. Respiration activity (RA), expressed as µmol of the produced carbon dioxide per mass of dry soil in unit of time (µmol CO_2 kg^{-1} d.m. h^{-1}) was determined during the soil incubation experiment (dark, tightly closed vessels, at 25°C, for 10 days). The levels of accumulated CO_2 were subsequently analysed by means of a gas chromatograph (Varian CP-3800, USA, equipped with a thermal conductivity detector – TCD (120°C): Poraplot Q (25 m) and a molecular sieve 5A (30 m) connected together and at 40°C) at the start and the end of the incubation, always in triplicate (Szafranek-Nakonieczna and Stępniewska, 2014). Microbial biomass (MB) carbon was determined using the fumigation incubation method and calculated from the difference between CO_2 evolved from fumigated (at 50% WHC, in a vacuum desiccator, 24 h, at 25°C in vapours of ethanol-free chloroform) and unfumigated soils after 10 days of incubation, and divided by conversion factor Kc=0.45 (fraction of biomass C mineralised to CO_2) (Gajda and Martyniuk, 2005). The amount of the released CO_2 was measured by the GC method as described above. Results were expressed as µg of biomass C per gram of dry soils.

Dehydrogenase Activity (DHA) was measured spectrophotometrically (λ = 485 nm, UV-1800, Shimadzu, Japan), using tetrazolium salt: 2.3.5-TTC as a substrate (Casida *et al.*, 1964). Easily Degradable Carbon (EDC), known also as permanganate oxidisable carbon (POXC) and synonymous with 'active carbon', was measured as follows: a 2.5 g soil sample was mixed with 2 ml of 0.2 $KMnO_4$ in 1 M $CaCl_2$ (pH 7.2) and diluted to 20 ml, using distilled water. After 2 min of shaking (100 r.p.m.), the sample was left for 10 min to allow the soil to settle while being protected against light. A 0.5 ml of a clear liquid from the upper 1 cm of the soil-$KMnO_4$ suspension was then diluted 10 times, and the obtained solution was used for spectrophotometric analysis (λ = 550 nm, UV-1800, Shimadzu, Japan). The calibration curve was produced using standards 0.005, 0.01 and 0.02M $KMnO_4$, in 0.1M $CaCl_2$. EDC was calculated using the following formula:

$$\text{Active C (mg kg}^{-1}\text{)} = [0.02 \text{ mol/l} - (a + b \text{ absorbance})] \\ (9000 \text{ mg C/mol}) (0.02 \text{ l solution}/0.0025 \text{ kg soil}),$$

where: 0.02 mol/l is the initial solution concentration, a is the intercept and b is the slope of the standard curve, 9000 is mg C (0.75 mol) oxidised by 1 mol of MnO_4 changing from Mn^{7+} to Mn^{2+}, while 0.02 l is the volume of $KMnO_4$ solution reacted, and 0.0025 is kg of soil used (Weil *et al.*, 2003; Wolińska *et al.*, 2016).

All collected data were statistically processed by means of Statistica 9 PL (StatSoft, USA). The assumptions of parametric tests were checked with Shapiro-Wilk W statistics, and, if the assumptions were not met, ln(x+1) transformation was applied. The effect of soil use and its type on the studied variables were tested using a MANOVA test, with Tukey post-hoc, followed by the analysis of regression (Pearson's *r* or Spearman's *rho* depending on data normality). The significance was accepted at $p < 0.05$. Data are presented as non-transformed values with either standard deviation (SD) or standard error of mean (SEM) (n = 3).

RESULTS AND DISCUSSION

The particle size analysis (PSD) of the studied topsoil samples revealed mainly silt loam soil type, with only the BA and EH soils being described as sandy loam types (World Reference Base for soil resources). This difference was attributed mainly to the presence of higher contents of coarser fractions, silt and sand, ranging from 17.5-63.2 and 35.1-76.6%, respectively.

Our previous studies (Wolińska *et al.*, 2014, 2016) showed that the examined soils differed in their properties depending on their type and management (Table 2). In general, the control soils had higher moisture, pH, total carbon content, nitrite-nitrogen and ammonium concentrations, whilst nitrate-nitrogen and phosphates were higher

Table 2. Selected soil properties (means ± SD) depending on their type and management

Type	Usage	Moisture (%)		pH		Eh (mV)		TC (%)	
AL	NC	9.97	0.89	5.85	0.60 bcde	472.16	42.87 a	1.65	0.21 ab
	A	9.13	0.95	4.89	0.26 ab	519.94	32.27 a	1.15	0.14 a
BA	NC	11.03	5.06	5.99	0.74 cde	418.10	32.89 a	2.37	0.67 bcd
	A	11.13	4.28	5.93	0.84 cde	457.63	28.12 a	1.11	0.41 a
HP	NC	30.90	5.48	7.23	0.02 e	529.27	0.23 a	5.43	0.13 e
	A	24.50	3.73	6.62	0.05 cde	561.30	0.36 a	1.64	0.03 abc
MG	NC	12.15	2.03	6.49	0.29 de	540.43	3.29 a	2.47	0.77 bcd
	A	9.25	3.89	5.73	1.09 bcde	474.40	205.08 a	1.05	0.15 a
EF	NC	8.80	0.25	5.64	0.03 abcd	545.20	0.40 a	1.23	0.09 ab
	A	5.10	0.47	4.18	0.05 a	551.30	0.30 a	0.98	0.07 a
EH	NC	9.10	0.85	5.27	0.01 abcd	519.97	0.25 a	3.63	0.14 de
	A	6.50	0.79	4.85	0.03 abc	523.43	0.23 a	2.69	0.19 bcd
RL	NC	15.80	3.72	6.58	0.90 de	469.98	26.09 a	3.70	2.32 cde
	A	11.75	1.04	5.57	0.09 bcd	496.05	8.60 a	1.11	0.16 a
		$N\text{-}NO_3$ (mg kg^{-1})		$N\text{-}NO_2$ (mg kg^{-1})		$N\text{-}NH_4$ (mg kg^{-1})		$P\text{-}PO_4$ (mg kg^{-1})	
AL	NC	4.21	2.51 a	0.23	0.15 abc	0.07	0.02 ab	1.32	0.35 ab
	A	22.83	22.92 cd	0.08	0.03 a	0.02	0.00 a	7.91	8.83 c
BA	NC	7.69	3.37 abc	0.36	0.27 bc	0.82	0.86 bc	4.29	1.92 c
	A	16.81	6.46 d	0.10	0.03 a	0.18	0.17 ab	10.33	3.50 d
HP	NC	8.23	0.02 abcd	0.44	0.01 bc	0.02	0.00 ab	1.35	0.02 abc
	A	27.43	0.08 de	0.10	0.00 abc	0.02	0.00 ab	1.36	0.05 abc
MG	NC	8.40	1.82 abcd	0.12	0.02 ab	0.41	0.41 abc	1.39	0.33 ab
	A	16.00	6.46 cd	0.12	0.04 abc	2.67	2.48 d	3.91	2.04 bc
EF	NC	2.20	0.05 a	0.13	0.00 abc	0.14	0.04 abc	1.33	0.01 abc
	A	2.99	0.03 ab	0.11	0.00 abc	0.28	0.03 abc	2.65	0.09 abc
EH	NC	9.05	0.03 abcd	0.08	0.00 abc	0.02	0.00 ab	1.74	0.38 abc
	A	10.22	0.12 abcd	0.09	0.00 abc	0.02	0.00 ab	3.10	0.10 abc
RL	NC	11.97	2.05 bcd	0.12	0.02 ab	0.16	0.13 ab	0.81	0.22 a
	A	55.07	24.21 e	0.47	0.43 c	1.81	1.74 cd	3.93	3.17 abc

Numbers indicated by the same letter do not differ at $p < 0.05$ (MANOVA, comparison between soil usages and soil types). AL – Albic Luvisol, BA – Brunic Arenosol, HP – Haplic Phaezoem, MG – Mollic Gleysol, EF – Eutric Fluvisol, EH – Eutric Histosol, RL – Rendzina Leptosol, Eh – redox potential, TC – total carbon, NC – non-cultivated soil, A – arable soil.

in the arable soils (all at least significant at $p < 0.05$). These factors could be driving forces for the changes observed in the microbial diversity and activity.

The studied soils differed substantially in microbial biomass, respiration activity and DHA in a function of the type of land use (Fig. 2). Soil RA was significantly higher ($p < 0.0001$) in all the controls, ranging between 30 and 60 μmol CO_2 kg^{-1} d.m. h^{-1}, in comparison to 11.5-23.7 μmol CO_2 kg^{-1} d.m. h^{-1} for the A soils. We found the strongest differences for Rendzina Leptosol, Haplic Phaezoem, Eutric Fluvisol, and Brunic Arenosol (Fig. 2a) – the reduction of RA amounted to 30-40% of the control values. In general, DHA in the studied soils was significantly lower in the A sites ($p < 0.001$). However, a considerable variation in

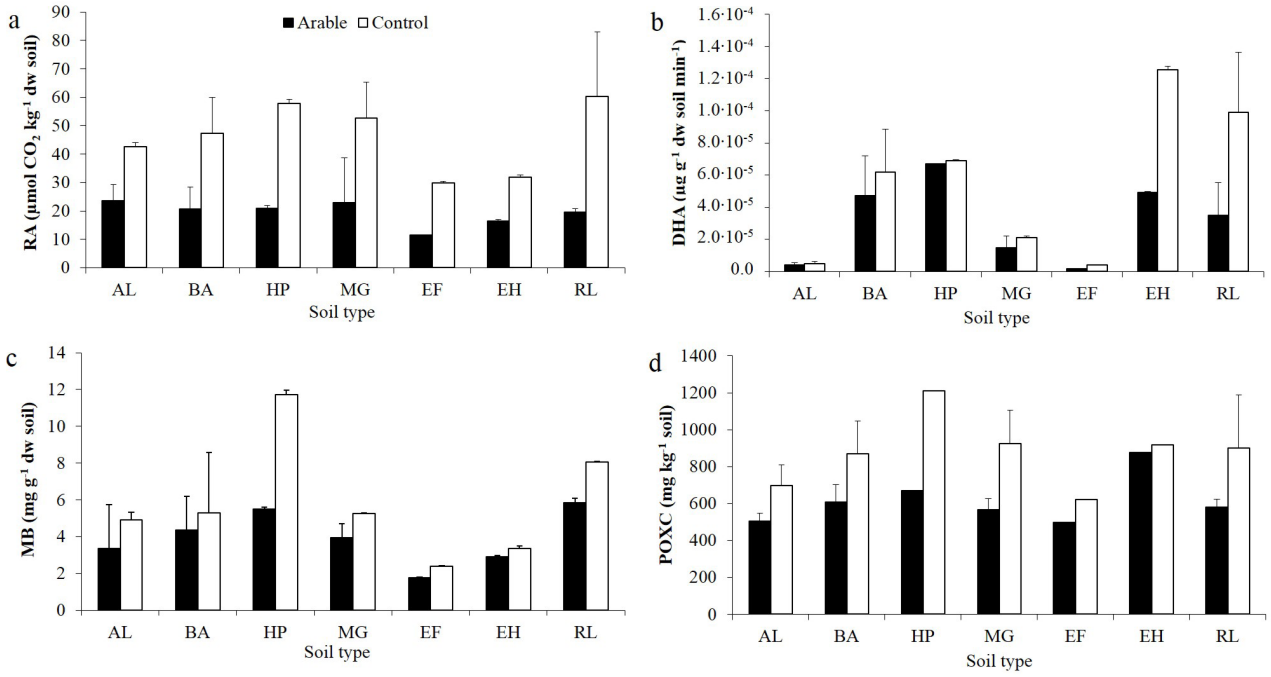

Fig. 2. Average (±SEM) values of: a – respiration activity, b – dehydrogenase activity, c – microbial biomass, and d – easily degradable carbon for both arable and the control soils.

this parameter between the soil types was found. In Eutric Histosol and RL, the highest DHA was recorded: 1.25 10^{-4} and 9.92 10^{-5} µg g^{-1} d.m. soil min^{-1}, respectively. In addition, these two sites revealed the highest reduction in DHA - down to 35-40% of the control values. BA and HP were characterised by high activity irrespective of the type of land use: 4.7-6.9 10^{-5} µg g^{-1} d.m. soil min^{-1}. Mollic Gleysol, Albic Luvisol, and Eutric Fluvisol had the lowest DHA and the lowest DHA reduction level (Fig. 2b). In general, the microbial biomass was significantly higher in the NC soils ($p < 0.001$); there were, however, differences depending on the soil type. The HP soil had the highest biomass of 2807.145 µg g^{-1} d.m. in the control, while most of the soils had a biomass lower by half. In the case of the other soils, the MB was in the range of 1 093-1 855 µg g^{-1} d.m. (NC soils) and 478-1 429 (A soils), with the exception of EF, where it was lower than 520 (NC soils) and 420 (A) µg g^{-1} d.m. (Fig. 2c).

Marschner *et al.* (2012) reported that hotspots are characterised by higher microbial abundance, resulting in 2-3 times greater microbial biomass in respect to bulk soil. This fact was also confirmed in our study, as we noted *c.a.* 2-times higher MB in NC soils than in the A variant. Gajda and Martyniuk (2005) observed similar MB level in agricultural soils under winter wheat as the values noted in our study. However, it should be emphasised that, usually, total MB reflects dormant microorganisms, whereas active microorganisms perform the majority of biochemical processes (Kuzyakov and Blagodatskaya, 2015). Furthermore, the active fraction is expressed by such biological parameters as RA and DHA (Gajda and Martyniuk, 2005). We demonstrated that RA reached *c.a.* 3-times higher level in NC soils, analogically as DHA, with 40% higher values in NC rather than in A soils. In this way, we confirmed the fact that more hotspots are created in non-cultivated soils, whereas long-term and systematic soil cultivation limits hotspots development.

Easily degradable carbon, termed as biologically active and available carbon showed a similar trend as the other studied biological factors. In general, its values were significantly higher in the controls than in the agriculturally exploited soils ($p < 0.0001$). It ranged between 620 (EF) and 1209 mg kg^{-1} (HP) in the NC, and between 497 (EF) and 877 mg kg^{-1} (EH) in the A soils. The strongest reduction of EDC was noted in HP, MG, and RL (down to 55-65% of the control values, Fig. 2d).

Other studies have demonstrated that both organic carbon and microbial biomass are sensitive factors that quickly react to changes in management such as reduced tillage, cover cropping and land use practice (Grandy and Robertson, 2007; Wander and Bidart, 2000). This sensitivity has led to a wide adoption of these parameters in soil science as indicators of change in the soil ecosystem (Gil-Sotres *et al.*, 2005; Kaschuk *et al.*, 2010).

The correlative study allowed us to verify if EDC could be used as a tool for determining the degree of soil degradation due to agricultural practices. We have found that soil microbial and biochemical activities are strongly dependent on the type of land use, which is linked with the physico-chemical parameters (Wolińska *et al.*, 2014, 2016). The

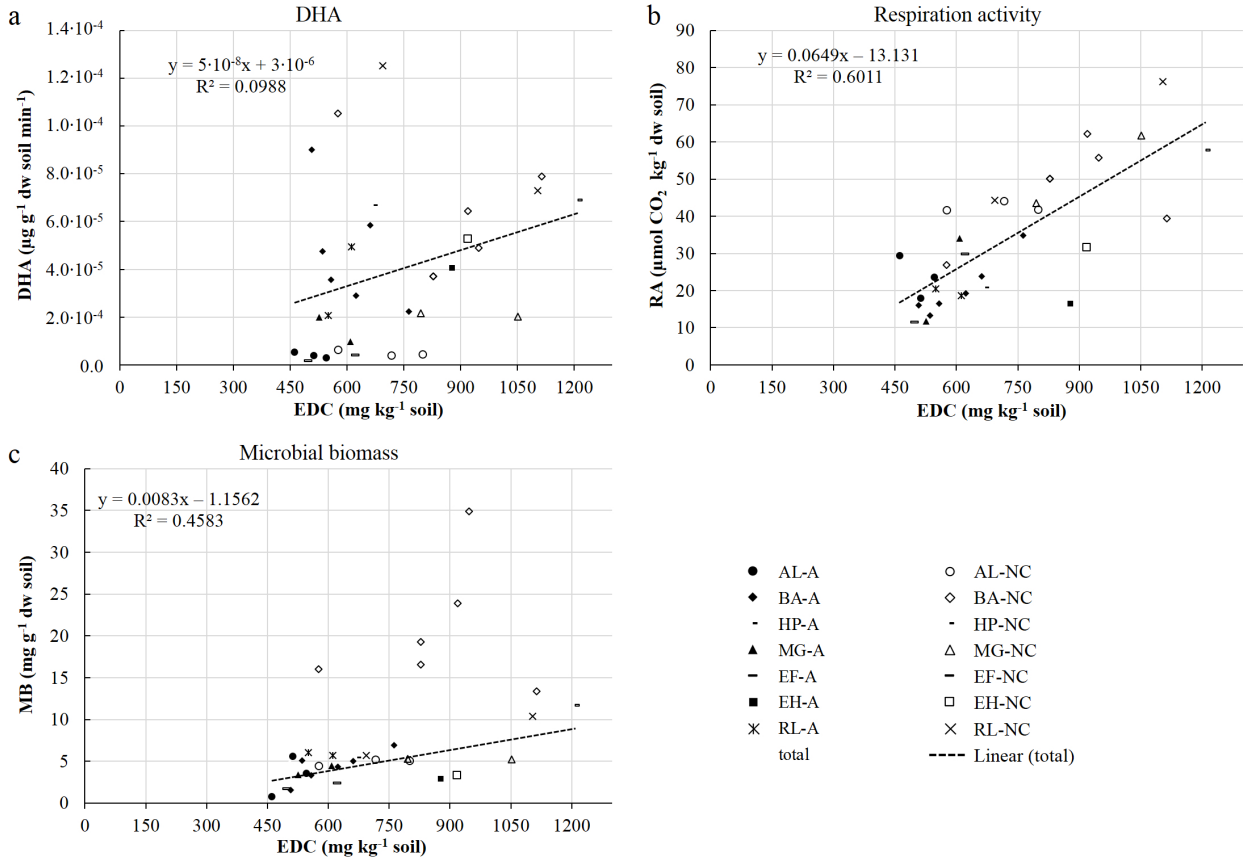

Fig. 3. Relationships between EDC and: a – dehydrogenase activity (DHA), b – respiration activity (RA), and c – microbial biomass (MB) for both arable and the control soils.

availability of easily degradable carbon also changed due to management practices. We revealed that this parameter was strongly reduced in all the agricultural soils (Fig. 2d). This pool of carbon is important to soil organisms and can affect their activity. It can be seen in Fig. 2 that the tested parameters were much lower in the arable soils, which confirms this statement. Also, by noting a decreasing trend in EDC content in A soils, the direct connection of EDC with hotspots creation in the non-cultivated soils was confirmed. Agricultural activities consume nutrients (including EDC), affecting their pools, which could explain their lower contents in A soils affecting soil health (Culman *et al.*, 2012; Panettieri *et al.*, 2015).

In order to ascertain if EDC may be a measure of soil degradation, the values of RA, DHA, and MB were plotted against the levels of EDC in the studied soils, and Spearman's rho was calculated for correlations. Very strong positive correlations were found for RA (ρ = 0.77*) and moderate correlations for DHA (ρ = 0.42*) and MB (ρ = 0.53*). The graphical representation of the observed correlations is presented in Fig. 3. The higher values of EDC indicate stronger microbial activity in the studied soils in terms of the selected parameters. This relationship is represented by linear equations for MB and RA, whilst the best equation for DHA is a power series. For the first two parameters, these equations explain 46 and 60% their variability. For DHA, it is less sound – only 19% variability in DHA may be explained by EDC.

Since both MB and EDC are based on chemical extractions of labile soil A, the agreement between these two methods is logical (Culmen *et al.*, 2012). Weil *et al.* (2003) found high a positive correlation (R^2 = 0.72) between MB and EDC, whilst Culmen *et al.* (2012) determined the same relationship (R^2 = 0.44) analogically to the authors study (Fig. 3). However, studies describing the relationship between EDC and such important soil factors as RA and DHA are limited. In that context, the determination of close correlations between EDC, RA and DHA is a novel element which fills the gap of knowledge about EDC with regard to soil environment.

CONCLUSIONS

1. In the study, it was demonstrated that agricultural practices significantly affect the biological and chemical properties of soil by reducing the levels of microbial biomass, respiration activity, dehydrogenase activity and easily degradable carbon, in relation to the control soils.

2. Consequently, the hypotheses of progressive microbiological degradation caused by soil agricultural management, and a direct connection between the level of easily degradable carbon and microbial hotspots in soils was confirmed.

3. It was demonstrated that decidedly more hotspots (taking into account easily degradable carbon input and the value of biological factors) are created in non-cultivated soils, whilst soil cultivation, except for the impact on the biological properties (decrease effect) reduces the possibility for hotspots creation.

4. Finally, by determination of Spearman rho correlation coefficient, it was revealed that easily degradable carbon can be considered a suitable factor for assessing soil biological degradation, expressed as soil respiration activity, microbial biomass and dehydrogenase activity).

5. The demonstrated sensitivity of easily degradable carbon to other biological factors and the relative ease of its measurement suggest that easily degradable carbon is a useful indicator of both the degradation phenomenon in agricultural soils and microbial hotspots indication.

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