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QUANTIFIED CHARACTERIZATION OF SOIL BIOLOGICAL ACTIVITY UNDER CROP CULTIVATION

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ABSTRACT

Soil biological activity is extremely important for sustaining high soil fertility, crop yields, and therefore can effect economy. Poland is particularly relevant example where approx. 60% of land is used for crop cultivation. It is expected that agricultural soils contain much smaller number of microorganisms relative to non-cultivated soils. We hypothesise that agricultural soils are biologically degraded and cannot naturally regenerate which in turn may lead to relative infertility. The aim of the study was to evaluate which crop cultivation, oat or triticale, is more favourable for sustaining soil microbiological activity. Eight different cultivated soils and the same number of non-cultivated (control) soils were selected for the study. Soil samples were extracted from the surface layer, and taken to laboratory for determination of the: pH, total carbon (TC), permanganate oxidizable carbon (POXC), phosphate phosphorus (PO_4-P) and total nitrogen (TN) content, microbial biomass (MB), soil respiration (SR), dehydrogenase activity (DHA) and DNA content. Spearman's rho correlation coefficient was used to assess multicolinearity between the above physico-chemical and biological soil properties. Our data suggest that cultivated soils are biologically degraded as reflected by lower values of all microbiological parameters with relation to non-cultivated soils. In terms of cultivated crop type, we show that triticale is more favourable for sustaining soil microbiological activity than oat. Positive correlations suggest that pH, TC, POXC, P, N content are the critical factors determining soil microbiological activities.

Indexing terms/Keywords

Crop type; Oat; Triticale; Biological activity; Microbial biomass; Soil Respiration

Academic Discipline And Sub-Disciplines

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SUBJECT CLASSIFICATION

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1. INTRODUCTION

Soil organisms are major components of all soil types and they are often described as a “biological engine of the earth” [1]. Soil microorganisms are responsible for a large part of biological activity (60-80%) which is associated with processes regulating nutrient cycles and decomposition of organic matter [2,3]. Doran and Parkin [4] defined soil quality as the capacity of soil to function effectively at present and in the future or, more precisely, as the capacity of soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health. In this context, soil quality plays an important role for conservation, health, good agricultural practices and agro-ecosystems sustainability [5,6]. Consequently, high soil quality should be defined as balance between high productivity and high biodiversity without soil microbiological degradation [7].

Studying soil biology is based on the interpretation and combined evaluation of various parameters, which can be incorporated in a European Union Monitoring Programme established in various countries, including Germany, United Kingdom, Switzerland, France, New Zealand [8]. However, in Poland until now no such monitoring programme was implemented. Our study is the first step in introducing the monitoring programme with relation to Polish agricultural soils. Changes in soil quality can be measured using different indicators which include physical, chemical and biological processes and characteristics to determine soil quality [6]. It appears that microbial biomass (MB), soil respiration (SR), total nitrogen (N) and phosphate phosphorus (PO_4-P) content are commonly regarded as part of a minimum data set to describe the microbial part of the soil organisms [3,8].

It is known that soil management strongly influences soil biodiversity, for example, in agricultural ecosystems [2,3]. Different practices cause shifts in habitat quality and in substrate availability, resulting in changes in abundance of individual species [6,9]. The management practices used in many agro-ecosystems (e.g. monocultures, extensive use of tillage, chemical inputs) degrade the fragile web of community interactions between pests and their natural enemies and lead to increased pest and disease problems [3]. It was estimated that with up to 40% of the world’s agricultural land are seriously degraded [9].

A question arises about what management practices adversely impact the functioning of soil ecosystems. We hypothesise that agricultural soils are most of all biologically degraded and unable to become naturally regenerated, possibly resulting in their inability of regaining the satisfactory level of fertility. Consequently, the purpose of the study was to indicate which crop type (oat or triticale) is more favourable for maintaining biological activity in the systematically cultivated soils for a sustainable balance among productivity, environmental quality and profitability. According to OECD recommendations soil biological activities were determined by measurement of MB, SR, and dehydrogenase activity (DHA). We also recommend measurement of soil DNA content as a sensitive indicator linked topsoil ecosystem processes and function. Moreover, differentiation in chemical parameters: pH, total carbon (TC), permanganate oxidizable carbon (POXC), and total nitrogen (TN) with regards to crop type is presented. Finally, statistical data analysis was done to find relationships between investigated factors and point at those parameters which more strongly determine biological properties of soils.

2. MATERIAL AND METHODS

2.1. Site description

This study was conducted as a part of an experiment (established since 1991) connected with organization of database of Polish arable mineral soil, being result of the collaborative project of the Institute of Agrophysics, Polish Academy of Sciences in Lublin and the Institute of Land Reclamation and Grassland Farming in Falenty [10]. Bank of Soil Samples (BSS) was established to serve a depository of catalogued soil samples stored dried and ready for use for soil research [11]. Precise locations of soils selected for the current study with an indication to crop type are shown in Table 1. Lublin district is a representative region characterized by a great diversity of soil types and it is one of the largest and the most important agricultural areas in Poland [12].
Selected soils have the same crop history and both in 2013 and 2014 were under oat (*Avena sativa*) or triticale (x *Triticosecale*) cropping system. Cereal production is one of the main directions for agricultural production in Poland. The area sown to basic cereals (wheat, rye, triticale, barley and oats) amounted to 6.3 million ha and equalled nearly 80% of the cereal sown area in general.

2.2. Soil sampling

Eight cultivable areas of 100 m^2 were selected (Table 1), from each of them three replicates of 2 kg of soils consisting of about 50 single samples were extracted and then mixed into one sample.

Agricultural soils were collected during spring season (24–26 April 2014) from non ploughed sites in order to avoid artifacts from ploughing perturbations [12]. Control samples were taken from non-agriculturally cultivated and non-forested sites (covering at least 1 hectare area), located in close vicinity to basic soils and belonging to the same soil type (Table 1). As a control uncultivated for a long time sites, like old meadows or filed-woodlots were selected.

For the study 5 type of soils were chosen: *Albic Luvisols*, *Haplic Luvisols, Brunic Arenosols*, *Eutric Fluvisol* and *Haplic Phaeozem*. Under laboratory conditions each sample was passed through a 2.0-mm sieve, to remove large pieces of rocks and plant material and were stored at 4ºC prior analysis [12].

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Table 1. Location of agricultural soils (SE Poland) and crop type

Soil number	Type of soil (FAO)	Crop type	Village	Geographic coordinates	Control sites
1	*Albic Luvisols*	Oat	Dęba	22º10'17,7'' 51º26'24,6"	30 year old meadow (mowed once a year)
2	*Brunic Arenosols*	Oat	Łany	22º15'19,0'' 51º23'0,9"	20 year old field-woodlots
3	*Brunic Arenosols*	Oat	Markuszów	22º15'55,5'' 51º23'1,9"	20 year old field-woodlots
4	*Eutric Fluvisol*	Oat	Kośmin	21º59'10,1'' 51º33'47,7"	15 year old meadow (mowed once a year)
5	*Albic Luvisols*	Triticale	Pryszczowa Góra	22º27'10,3'' 51º24'3,8"	20 year old woodlots with birches
6	*Haplic Luvisol*	Triticale	Sady	23º22'52,4'' 50º51'14,8"	Unmoved meadow, wasteland
7	*Haplic Luvisol*	Triticale	Klementowice	22º06'54,2'' 51º21'52,2"	Unmoved meadow, wasteland
8	*Haplic Phaezoem*	Triticale	Hostynne	50º44'48,3" 23º42'56,6''	Meadow (mowed once a year)

The terrain investigations realized in the frame of BSS comprised characteristics of the terrain where the profile is located and description of the soil samples with preliminary assessment of soil properties [11]. General data of soils characteristics used in the current study with description of territory of sampling are shown in Table 2.

Table 2. Characteristics of the individual soil outline, according BSS data sets [11]

Soil number	Terrain location	Exposure	Landscape	Bedrock	Erosion type	Severity of erosion	Complex of agricultural usability
1	flat with good outflow	south-east	plain	less	absent	absent	wheat
2	flat with good outflow	eastern	plain	sand	absent	absent	rye very weak
3	flat with good outflow	eastern	plain	sand	absent	absent	rye very weak
4	flat with weak outflow	southern	river valley	silt	absent	absent	wheat and pasture
5	flat with good outflow	south-east	plain	clay	surface	weak	wheat-rye
6	average slop	southern	hilly	less	surface	medium	wheat good
7	flat with good outflow	south-east	plain	less	surface	weak	wheat good
8	flat with good outflow	southern	plain	less	surface	low	very good wheat

2.3. Soil 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 of 1.33 for water as the dispersing phase. During the measurement the pump speed was set at 1750 rpm, while the stirrer speed was at 700 rpm [10]. The *soils were dispersed* using *ultrasound* at 35W for 4 min without removing the organic matter [13]. The measurements were carried out in 3 replications.

Chemical soil characteristics were performed by measuring the following soil factors: pH, TC, POXC, total N and PO_4-P content.

The pH values were determined from a 2:1 soil suspension in distilled water using a multifunctional potential meter (Hach Lange). The measurements were taken in triplicate after stabilisation of the readings [12].

TC were determined using an automatic carbon analyzer TOC-V_{CSH} SSM 5000A (Shimadzu, Japan), as was earlier described by Wolińska et al. [12].

The principle of the method used for POXC determination was described by Weil et al. [14] and based on the carbon oxidation by permanganate resulting in bleaching of purple solution. POXC is an equivalent of easily degradable carbon or is considered as biologically active carbon, easily available for microorganisms. The air-dried soil samples (2.5 g) were placed in graduated polypropylene conical centrifuge tubes (50 ml). 2 ml of 0.2 $KMnO_4$ in 1 M $CaCl_2$ (pH 7.2) was added followed by the addition of distilled water up to 20 ml mark. The tubes were capped and shaken for 2 minutes at 120 rpm. After that they were placed in a rack for 5-10 min in dark to allow soil settle. Prior the analysis standard curve was prepared using 0.2 $KMnO_4$ in 1 M $CaCl_2$ (pH 7.2) stock solution. Absorbance was measured spectrophotometrically (Shimadzu U-1800, Japan) at 550 nm using distilled water as a background. For the calculation the POXC content in soil sample the following formula was used [14]:

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

where:
0.02 mol/l is the initial solution concentration, *a* and *b* are the intercept and the slope of the standard curve, respectively, 9000 is amount of C in mg oxidized by 1 mole of MnO_4, 0.02 l is the volume of $KMnO_4$ solution reacted, and 0.0025 is the amount of soil sample in kg.

The concentrations of TN and PO_4-P were determined colorimetrically using Auto Analyser 3 System (Bran+Luebbe, Germany), as described by Wolińska et al. [12] and according to Henriksen [15] method. Obtained results have been corrected for the amount of soil sample and expressed as mg per g of fresh soil [12].

MB was determined using the chloroform fumigation technique as described by Zou et al. [16], by measurement of total extractable organic biomass material from freshly killed microorganisms [12]. Triplicate subsamples of each soil type (5 g fresh-weight) were placed inside 60 ml glass bottle [12]. Two treatments, control and fumigated, were applied. More details are presented in our earlier study [12]. Concentration of CO_2 released by microorganisms which survived incubation with $CHCl_3$ was measured by a GC (Varian CP-3800). Results were expressed as grams of biomass C on kilogram of dry soils (g kg d.m.$^{-1}$).

SR was determined also by means of a GC (Varian CP-3800, USA), equipped with the two types of columns: Poraplot Q (25 m) and a molecular sieve 5A (30 m) connected together and at 40°C [17]. Soil subsamples (5 g, 3 replicates) were placed in dark, sterile bottles (60 ml) tightly closed and incubated (7 days) in 20°C [12]. Based on the differences between concentration of CO_2 at start and at the end of experiment SR was calculated and expressed as a mass of produced carbon dioxide per mass of dry peat used in the experiment and per unit of time (μM CO_2 kg d.m.$^{-1}$ h^{-1}).

Soil DHA was estimated by reducing 2,3,5- triphenyltetrazolium chloride (TTC), according to the procedure of Casida et al. [18]. Product of the reaction - triphenylformazan (TPF) was extracted with ethanol and absorption was measured at 485 nm with a spectrophotometer (UV-1800, Shimadzu). DHA was quantified and expressed in μg TPF g^{-1} min^{-2} [19].

Total DNA was isolated according to the modified procedure for soil samples as described by Tomczyk-Żak et al. [20]. Briefly, soil samples (6 g) were mixed with several glass beads (0.4-0.6 mm, Sartorius) in 50 ml Falcon tubes filled with extraction buffer (100 mM Tris-HCl [pH 8.0], 100 mM sodium EDTA [pH 8.0], 100 mM sodium phosphate [pH 8.0], 1.5 M NaCl) and shaken carefully for short time. Then, 30 μl of lysozyme (100 mg ml^{-1}) was added and the samples were incubated at 37°C for 30 min. After that, 60 μl of proteinase K solution (20 mg ml^{-1}) was added, followed by a further incubation at 37°C for 30 min, after which 1.8 ml of 20% SDS was introduced and the samples were incubated at 65°C for 2 h, and mixed by inversion every 15 min. Subsequently, the samples were centrifuged (10 min., 7000 rpm) at room temperature, supernatant guarded and sediment extracted with the same procedure but in half buffer's volumes. The two supernatants were combined, extracted with equal volume of chloroform:isoamyl solution and precipitated with 0.6 volume of isopropanol. DNA recovered by 20 min centrifugation at 9000 rpm was suspended in 500 μl of water. In order to reduce the presence of humic substances and other soil impurities an additional purification step was performed on the DNA sample. The crude total DNA was further purified by CsCl gradient centrifugation (16h, 70 000 rpm, 20°C; Sorvall WX Ultra ThermoScietific). Concentrations of the isolated DNA were assessed with NanoDrop spectrophotometer (ThermoScientific) after 10 times dilution. Each of described laboratory analysis were performed in triplicate.

2.4. Statistical analysis

The data were subjected to one-way ANOVA using Statistica 9.0 (Statsoft Ltd., UK) software. Means were calculated for three replicate values. Mean separations were made for significant effects with LSD and Tukey's test at the probability of $p<0.05$. Spearman's rho correlation coefficient between chemical and biological soils properties was also determined [12].

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3. RESULTS AND DISCUSSION

3.1. Soils classification

Particle size distribution (PSD) in the soils investigated, taking into account both the World Reference Base for soil resources (WRB) and Polish Society of Soil Science (PSSS) classifications is shown in Table 3.

Table 3. Clay, silt and sand fractions (in volume percentage) obtained by Hydro G unit of laser diffractometer Mastersizer 2000

Sample No.	Clay	Silt	Sand	Particle size group	
	(mm)				
	<0.002	0.002-0.05	0.05-2.0	WRB[1]	PSSS[2]
1	4.76	37.66	57.58	sandy loam	sandy loam
2	2.06	22.96	74.98	loamy sand	loamy sand
3	3.64	30.88	65.47	sandy loam	sandy loam
4	2.35	34.50	63.15	sandy loam	sandy loam
5	1.25	17.28	81.47	loamy sand	loamy sand
6	5.26	74.37	20.37	silt loam	loamy silt
7	5.60	50.80	43.59	silt loam	loamy silt
8	5.26	77.14	17.60	silt loam	loamy silt

[1]World Reference Base for soil resources, [2] Polish Society of Soil Science

A comparison of the contents of the particular fractions allowed for classification of tested soils into three groups: sandy loam, loamy sand and silt loam, due to domination of coarser fractions (silt, and sand). PSD is one of the most important soil parameter often used in geological, and geomorphologic laboratories. However, the knowledge of PSD is needed to determine not only the physicochemical processes occurring in the soil [20] but also for microbiological activity. It should be emphasized that soil texture plays a key role in carbon storage and also influences nutrient availability [21], and ithus is crucial factor for soil microorganisms activity. Hamarashid et al. [21] indicated that the capacity of soils to preserve soil carbon and nitrogen in clay and silt sized particles is greater than sandy one.

3.2. Crop type effect on chemical soils properties

Differentiation in soil chemical factors level (pH, TC, POXC,TN, PO_4-P) in relation to crop type (oat, triticale) in cultivated and control sites are presented in Figure 1. It was observed, that crop type strongly influenced soil chemical properties. Triticale variant was the reason of higher values of pH, TC, TN and PO_4-P content in cultivated soils.

Soil pH values in arable soils ranged from 4.9±0.5 to 6.1±0.91 for oat and triticale crop system, respectively, whereas in controls reached higher values from 5.87±0.25 to 6.23±0.93. This indicates that systematic agricultural treatments contribute to a significant decrease of pH values towards acidification which is one of the causes of soil degradation. Shrestha et al. [9] also noted decrease of pH values in cultivated soils with a pH range of 5.6 – 7.1. It was reported that although soil acidity is a natural process, it is greatly accelerated by more intensive and productive farming systems [22,23]. Strong divergence among pH (p<0.001) noted in cultivated soils proved that crop system is a significant determinant of soil chemical properties and indicated that triticale cropping prevents the pH declines. It should be also emphasised, that soil acidification reduces population numbers of soil microorganisms and availability of some macro- and microelements [12].

In the cultivated sites, TC ranged from 9.5±0.09 to 16.9±0.15 g kg^{-1} for oat and triticale options, respectively, whereas reached significant higher level in non-cultivated soils, where its amount achieved to 24±1.27 g kg^{-1}. Significantly higher (by 84%) TC content has been found under triticale crop type rather than oat. By comparing variations of TC between cultivated and non-cultivated soil it was noted that TC in controls demonstrated an increasing trend, even by 71 and 162% for triticale and oat combinations, respectively. Despite the fact that investigated soils included low TC content and were classified as minerals soils, the amount of easily available carbon for microorganisms were even lower and oscillated 0.55±0.05 and 0.64±0.11 g kg^{-1} for oat and triticale, respectively. In controls reached significantly higher values till to 0.83±0.19 g kg^{-1}. Thereby, pool of POXC accounted for only 3.5–5.8% of TC. However, Zou et al. [16] indicated that microbial carbon contributes usually 1-3% of TC. Our observations are comparable with the study of Valpassos et al. [3] and Villarino et al. [24] who reported that no-tillage (controls) system showed the highest carbon content. Gajic et al. [25] assumed that continuous cropping and soils cultivation, has resulted in a decrease in TC content.

TN in arable soils oscillated between 14.74±9.17 mg kg^{-1} in oat combination and 17.53±8.03 mg kg^{-1} in triticale crop. However, this range has insignificant character (p=0.4363). Distinctly lower levels of TN was noted in reference to control soils, amounting to 6.65±4.63 mg $kg^{-1}$and 9.69±2.60 mg kg^{-1} under oat and triticale crop, respectively. Undoubtedly, higher N richness in cultivated soils comes from their continuous nitrogen fertilization. This is consistent with the results of Li and Lang [26], indicating similar trend with regards to uncultivated and cultivated black soil. Also, it was pointed that high level of N fertilization can drive soil acidification [22].

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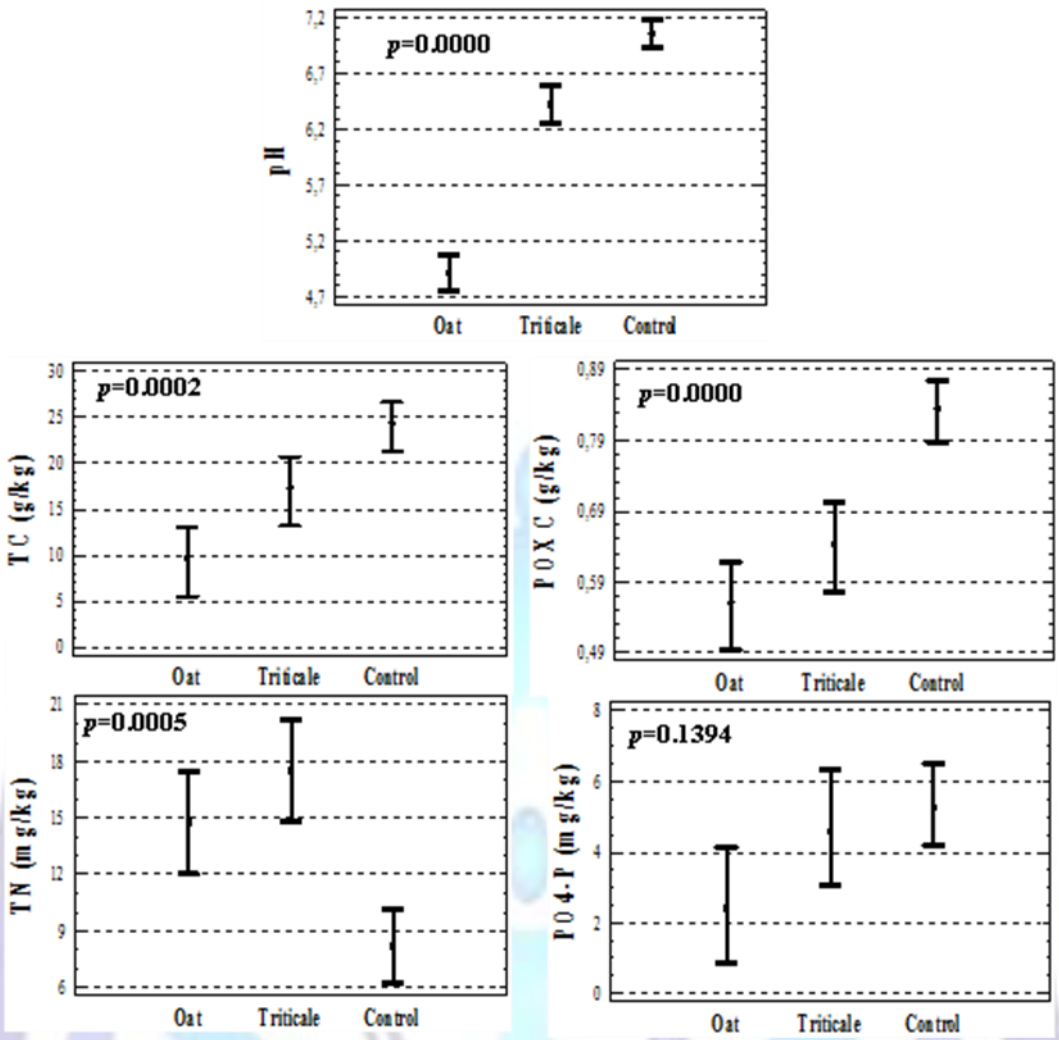

Figure 1: Differences in soil chemical factors in relation to crop type in cultivated and control sites (n=48). Bars present means of three replicates with SE

Content of PO_4-P was very low, below the critical level of 10 mg kg^{-1} of soil, according to ratings given by Okalebo et al. [27]. In the cultivated soils it ranged from 2.49±0.71 mg kg^{-1} to 4.67±1.99 mg kg^{-1} for oat and triticale, respectively (p=0.1467). Differentiation in control soils was also insignificant (p=0.0782), even though PO_4-P reached higher level (6.91±5.20 mg kg^{-1}) under oat crop type, rather than under triticale system (3.77±2.73 mg kg^{-1}). Phosphorus is found in soil in two forms: organic or inorganic. The last (PO_4-P) is the form in which P is used by plants, but its availability strongly depends on soil pH [28]. It is known that soils with inherent pH values between 6 and 7.5 are ideal for P availability, while pH values below 5.5 and between 7.5 and 8.5 limits P-availability to plants due to fixation by aluminum, iron, or calcium [28]. In our study, the best conditions for PO_4-P availability taking into account pH were found in relation to triticale crop type. Also both in cultivated and in control soils pH above 6.0 were noted. In contrast, under oat crop type pH was below 5.5 which strongly limited P availability. Furthermore, a study by Valpassos et al. [3] demonstrated that no-tillage system usually provides a vertical deposition of crop residues, once it is not periodically revolved and it manifests high microbial activities. Typically small cycled reservoirs of phosphate are dissolved in living and dead organic matter [3].

3.3. Crop type effect on biological soils properties

Differentiation in soil biological factors (MB, SR, DHA, DNA) in relation to crop type (oat, triticale) and control sites are shown in Figure 2. Analogically to chemical factors, also biological parameters seemed to be strongly affected by crop type. Triticale variant was the reason for higher values of MB, SR, DHA and DNA content in cultivated soils. The same pattern was noted in control sites, just with one exception when DNA content, achieving insignificantly (p=0.2506) higher value under oat crop variant (Figure 2). In addition, in control soils each of measured biological factors achieved higher levels, which confirms the hypothesis that soil biological degradation is a result of human agricultural practices. It was assumed that the soil MB quickly responds to changes in agricultural practices [3,29]. Balota et al. [2] indicated that cultivating winter cover crop is a beneficial practice enhancing soil microbial biomass and also soil organic C stocks.

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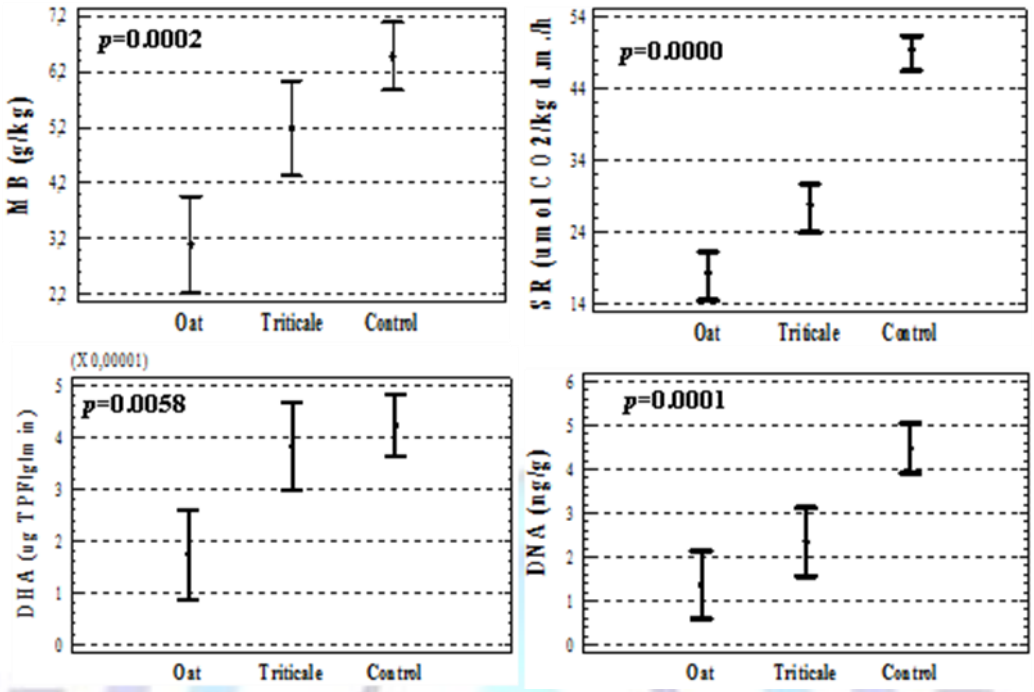

Figure 2: Differences in soil biologicall factors in relation to crop type in cultivated and control sites (n=48). Bars present means of three replicates with SE

The current paper clearly demonstrated that in the cultivated soils MB ranged from 3.09±1.01 g kg^{-1} to 5.18±1.25 g kg^{-1} for as follows, oat and triticale crop system, whilst in the control sites were even by 109% higher (in compare to oat treatment), achieving concentration of 6.48±2.71 g kg^{-1}. These values are consistent with other studies. Balota et al. [2], Valpassos et al. [3], Kara and Bolat [29] reported that permanent crops such as grassland generally have enhanced levels of MB, while arable crops and soil cultivation promote the biomass decline. Van Eekeren et al. [30] observed the MB-reduction phenomenon in the agricultural soils, which was expressed as a 34% drop of bacterial biomass and a 21% decrease in fungi in relation to grasslands. Wolińska et al. [12] noted that MB reached higher level in non-cultivated soils, which points to the negative influence of farming treatments on soil biological parameters.

Soil respiration is one of the most frequently used parameter for quantifying microbial activities in soils. SR is an integrated signal of the complete biotic and abiotic processes that occur in soil, and is therefore a sensitive indicator of alternations in soil carbon cycling that may result from human-caused environmental change [31]. In the presented experiment SR in arable soils oscillated between 17.75±5.82 and 27.25±5.99 µmol CO_2 kg^{-1} d. m. h^{-1}, respectively for oat and triticale crop type. However, significantly higher SR values were reached in control soils amounting from 43.54±8.77 to 54.37±8.53 µmol CO_2 kg^{-1} d. m. h^{-1}, which was by 2-2.5 times more than in cultivated soils. Besides, stimulatory effect of triticale crop type rather than oat cultivation on SR should be emphasized, as there is lack of similar reports in literature database. Nonetheless, decreasing trend of SR in agricultural soils demonstrated in current study was earlier reported [2,9,12].

Dehydrogenases (EC1.1.1) are very important among various soil enzymes as their activity levels is considered to be an indicator of overall microbial activity, due to their intracellular presence in all living microbial cells [19,32]. DHA in agricultural soils varied between 1.74±0.06 to 3.83±0.08×10^{-5} µg TPF g^{-1} min^{-2} for oat and triticale crop type (p=0.0279), whereas in control sites reached much higher values of 2.06±0.02-4.81±0.03×10^{-5} µg TPF g^{-1} min^{-2} (p=0.0070). These also confirm degradation of soil biological activity from agricultural practices as DHA is a sensitive indicator of soil disturbances. Triticale crop type has proven to be the preferable option for maintaining soil DHA, rather than oat, where enzyme activity achieved c.a. 55% lower level. Pascual et al. [33] noted almost 2-fold higher DHA in natural rather than in agricultural soils. Consequently, our results are consistent with the findings of other studies which reported a decline of enzymatic activities in cultivated soils relative to uncultivated [32, 34].

Also, nucleic acids are ubiquitous compounds of soil environment. Most of the information about soil microbial ecology and diversity is lodged in this genetic material [19], so we suggest the measurement of soil DNA content as a sensitive indicator linked with soil ecosystem processes and function. Comparison between cultivated and control soils proved that agricultural management strongly affected the total DNA content (Figure 2). In agricultural soils DNA ranged from 1.36±0.76 to 2.34±1.58 ng g^{-1} (p=0.0647) and was dependent from crop type, achieving higher level under triticale than oat system. Significantly higher DNA content was extracted from control soils and amounted 4.47±2.37 ng g^{-1} (p=0.0001). Decrease of DNA in agricultural soils was noted also by Miller et al. [35]. However, to our knowledge in the literature there is lack of any information about DNA variability depending on crop type (i.e. triticale, oat). This study is therefore the first work that clearly demonstrates sensitivity of soil DNA to different crop type.

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3.4. Correlations between analyzed factors

Noted in the current study numerous relationships between the soil chemical and biological parameters are summarized in Table 4.

Table 4. Correlation matrix between analyzed parameters (n=48, Spearman's rho correlation coefficient)

	TC	POXC	TN	PO_4-P	MB	SR	DHA	DNA
pH	0.76***	0.62***	0.66**	0.75***	0.92***	0.56**	0.62**	0.39**
TC		0.91***	0.29 ns	0.45*	0.58**	0.61**	0.59**	0.26 ns
POXC			0.15 ns	0.36*	0.88***	0.81***	0.68***	0.39**
TN				0.15 ns	0.72***	0.41*	0.81***	-0.20 ns
PO_4-P					0.69***	0.57**	0.93***	0.77***
MB						0.39*	0.60**	0.58**
SR							0.41*	0.65***
DHA								0.12 ns

*, **, *** - indicate significance at the 5, 1 and 0.1% level, respectively,
ns – not significant differences

One of the most important factor in maintaining soil biological activities turned out to be pH, displaying a strong positive correlations with MB, SR, DHA, DNA and further with TN, TC, POXC and PO_4-P content, what was confirmed by high values of rho Spearman's coefficient (Table 4). Correlations between pH, MB and TN supposing resulted from the fact that high N concentration increase bacterial concentrations. However, in general bacterial diversity is the highest in the soils with neutral pH. Similar observations were reported by Fierer and Jackson [36] who estimated bacterial richness for two tropical forest soils to be 26% higher at pH approx. 5.5 than in the more acidic soil (pH 4.1). Strong correlations pH-MB (r=0.77***) and pH-SR (r=0.59***) for soils under different management practices was demonstrated by Wolińska et al. [12].

Positive correlations were noted also between both TC as its available for microorganisms forms (POXC) and MB, SR, DHA, PO_4-P. The higher level of MB in control soils was probably due to its close relation with TC (r=0.58**) and POXC (0.88***). Also Balota et al. [2] demonstrated good correlation of microbial parameters among themselves and with TC. Valpassos et al. [3] reported that in no-tillage system, biological activity was high due to the high amount of TC content in the soil. In the same way, the lowest amount of MB was detected on that treatment which also presented the lowest TC and POXC content [3]. The same, SR was related to carbon availability (r=0.81***) in the biomass, and thus was generally higher because of greater biological activity [3]. Additionally, TC and POXC have important effects on soil enzymes activities (r=0.59** and r=0.68***), the higher pool of TC and POXC can provide enough substrate to support higher microbial biomass, hence result in higher enzyme production [2, 37].

TN content demonstrated significant positive effect on almost all investigated biological factors (MB, SR, DHA). Only DNA appeared to be invulnerable to any TN fluctuations ($p>0.05$). The most significant nitrogen influence was noted in relation to MB and DHA, which was confirmed by the highest rho values: 0.72*** and 0.81***, respectively. Wolińska et al. [12] also reported positive correlation TN-MB (0.39***) in agricultural Polish soils, while Kara and Bolat [29] showed the same relationship (0.58**) in soils from Bartin Province (Turkey).

Total extracted DNA has been used as an index of soil microbial biomass, but Leckie et al. [38] found no correlation between DNA extracted and MB. Opposing, in our study positive relationships were noted between DNA-MB (0.58**), -SR (0.65***), –PO_4-P (0.77***) and POXC (0.39**). Presented findings well consistent with those reported by Blagodatskaya et al. [39], although studies describing correlations between extracted DNA and soil biological parameters are still limited.

4. CONCLUSIONS

Results from the present study clearly indicate that human management practices and certain types of crop (oat, triticale) can exert a profound influence on soil biological factors. We demonstrated that cultivated soils are biologically degraded which was evidenced by lower values of all microbiological parameters in relation to control sites. In terms of crop type comparison, we showed that triticale is more favourable for sustaining soil microbiological activity than oat. Our recommendation for adding DNA parameter to sensitive indicators linked with soil ecosystem processes and function proves to be worthwhile, as we noted significant differences in DNA content under oat and triticale crop type. This demonstrates a high sensitivity of DNA to human agricultural practices and type of crop. Positive correlations highlight the following chemical factors: pH, TC, POXC, PO_4-P and TN content are the most important in regulating soil microbiological activities in the agricultural soils.

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