Original Research Article

Biological degradation of agricultural soils from Lublin region (SE Poland)

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A B S T R A C T

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The purpose of the study was to evidence the phenomenon of soil biological degradation being a result of an intensive soil agricultural practices. This aim is strictly consistent with the recommendations of The European Environmental Agency and Biodiversity Strategy to 2020 strongly promoting monitoring of soil microbiological degradation state in arable soils. For our study, 16 soil units agriculturally exploited and the same number of control soils were selected. Arable soils were collected form the surface layer (0-20 cm) during Spring season (April 2014) from non ploughed places. Control samples were taken from non agriculturally cultivated and non forested sites, located in close neighboring to basic soils and belonging to the same soil type. Under laboratory conditions the following analyses were performed: pH, total carbon content (TC), forms of nitrogen and phosphorus, number of copiotrophic and oligotropohic bacteria, microbial biomass (MB) and respiration activity (RA). Spearman's rho correlation coefficient was used to assess relationships between chemical and biological soil properties. Our study clearly demonstrated that cultivated soils are biologically degraded, what was evidenced by lower values of all biological parameters (MB, RA, abundance of copio- and oligotrophic bacteria) in relation to control sites. Statistical analyses proved that pH is the most important factor determining soil biological activity, what was confirmed by positive correlations with: TC (0.55***), $N-NO_2$ (0.32**), MB (0.77***), RA (0.59***) and abundance of copio- (0.30**) and oligotrophic bacteria (0.38***). Among investigated N forms in relation to soil biological aspect the most important was nitrite nitrogen ($N-NO_2$), whereas RA was the most sensitive indicator of soil agricultural management.

Introduction

It is known form the world-wide literature (Torsvik, et al. 2002; Tscharntke, et al. 2012), that agricultural types of soil contain much smaller number of microorganisms

(about 140-150 species per g), in comparison with natural soils (thousand of species per g). Soil biodiversity degradation has been estimated to affect 16-40% of terrestrial area (Chappell and LaValle, 2011) and even for Europe, meaningful soil losses causing reduced yields are predicted for the coming century (Banwart, 2011).Van Eekeren et al. (2008) observed the microbial biomass-reduction phenomenon in the agricultural soils, which was expressed as a 34% drop of bacterial biomass in relation to grasslands. Differences were noted also in the abundance of bacterivores, herbivores and nematodes population (decline in arable soils by 20, 59 and 47%, respectively). Due to this fact, agricultural soils may not contain the stable microbial communities, but have greater abundance of groups living according to strategy *r* (copiotrophs) and adapting to maximizing their intrinsic rate of growth when resources are abundant (Fierer, et al. 2007). Copiotrophs preferentially consume labile soil organic C pools and have high nutritional requirements, so their metabolic activity is quickly reduced and consequently their number in the soil decreases. In contrast, oligotrophs (strategy *K*) exhibit slower growth rates and are likely to outcompete copiotrophs in conditions of low nutrient availability due to their higher substrate affinities (Fierer, et al. 2007, 2012). Soils with large amounts of available organic C seem to favor copiotrophs while oligotrophs tend to predominate in soils where organic C quality and/or quantity is low (Campbell, et al. 2013). Consequently, not only does oligotrophs/copiotrophs ratio provide a proxy for soil-nutrient supply but also predominantly about the microbial-community degradation as a consequence of agricultural practices. The reduction in the number of bacterial phylogenetic groups as affected by human agricultural activities is a reason for small taxonomic group content in arable soils. Finally, the lack of biodiversity

is another reason for agricultural soils not being able to become naturally regenerated which may lead to their inability of regaining the satisfactory level of fertility. Winding et al. (2005) described a wide range of possible microbial indicators, providing different information on soil quality, which are included in soil monitoring programs implemented e.g. in Germany, The Netherlands, Switzerland, Czech Republic, The United Kingdom, Austria and New Zealand. It is recommended that as many microbial indicators as possible should be included to gain experience (Winding, et al. 2005). At a minimum, measures of microbial biomass, respiration activity and microorganisms abundance should be studied.

In the current study we hypothesized that:
- soil microbiological and chemical features are different in the area under cultivation than in natural soils (non-cultivated),
- human agricultural activity and soil physico-chemical parameters are important conducive factors for bacterial selection in arable soils,
- soils agriculturally exploited are biologically degraded.

Consequently, the aim of the work was to demonstrate the effect of soil agricultural usage on selected chemical properties as: pH, TC, biogenic forms of N and P and on biological soils features as: abundance of copio- and oligotrophic bacteria, microbial biomass and respiration activity levels, and finally to determine correlations between mentioned parameters. This aim is strictly consistent with the recommendations of The European Environmental Agency (EEA) and The Commission of the European Communities (EC), European Union (EU), Biodiversity Strategy to 2020 and European project ENVASSO, strongly promoting the

protection strategy of microbial community, especially in the arable soil with description of soil microbiological degradation state.

Materials and Methods

Study site

16 soil units agriculturally used (representatives for the Lublin region), and the same number of control soils not agriculturally used were studied. Soil materials have been selected on the basis of earlier work for the typological soil recognition within the framework of the Bank of Soil Samples (BSS) belonging to the Institute of Agrophysics PAS in Lublin. BSS was organized inspired by Gliński, Ostrowski, Stępniewska and Stępniewski – scientists from IA PAS in Lublin and from the Institute of Land Reclamation and Grassland Farming in Falenty (Bieganowski, et al. 2013). Precise localization of the samples catalogued in the BSS created a possibility of the precise return to the sampling place (Gliński, et al. 1991).

Locations of soils selected for the current study with crop type from 1991 and present (2014) are shown in Table 1. Limitation of the tested site to one region resulted from the fact that the Lublin district is represented by a great diversity of soil types and it is one of the largest and the most important agricultural areas in Poland.

Soil sampling

Extractions of soils were performed using Egner's bow from the surface layer (0-20 cm) agriculturally subjected (three replicates of 2 kg, consisting of about 50 samples taken from a 100 m^2 area). Arable soils were collected during spring season (24-26 April 2014) from non ploughed places in order to avoid artefacts from ploughing perturbations. Control samples were taken (analogically like described above) from non agriculturally cultivated and non forested sites (covered at least 1 hectare area), located in close neighboring to basic soils and belonging to the same soil type (i.e. non cultivated from years fallow lands or grasslands).

Eutric Cambisols and *Orthic Podzols* are dominating soil types in Poland, acting by 82% of the country, thus their share in the study material was significant (9 representatives among 16 soil units). 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 (2-3 days).

pH measurement

The pH were determined from a 2:1 soil suspension in distilled water using a multifunctional potential meter pIONneer 65 (Radiometer Analytical S.A., France), equipped with a glass electrode (Cartrode pH E16M340). The measurements were taken in triplicate after stabilisation of the readings.

TC determination

Total carbon (TC) were determined using an automatic carbon analyzer TOC-V$_{CSH}$ SSM 5000A (Shimadzu, Japan). Soil samples (150 mg) were pulverized, dried prior to analysis and then combusted at 900°C, in a column containing platinum and cobalt oxide catalyst. Under mentioned conditions all carbon compounds were converted into carbon dioxide form, and detected by an infrared detector. Each TC recordings were realized in triplicate.

Forms of N and P

The concentrations of soluble phosphorus, nitrate, nitrite and ammonium were

determined colorimetrically using Auto Analyser 3 System (Bran+Luebbe, Germany). Prior to analysis examined nutrients were extracted (Banach, et al. 2009) using deionised water (35 g fresh soil and 100 ml water). PO_4-P was analysed according to Henriksen (1965) with ammonium molybdate. NH_4-N and NO_3-N were measured using, respectively, hydrazine sulphate (Kamphake, et al. 1967) and salicylate (Grasshoff and Johannsen, 1972) as a colour marker. NO_2-N analysis were based on the latter method excluding hydrazine sulphate. Obtained results have been corrected for the amount of soil sample and expressed as μg per g of fresh soil.

Abundance of copio- and oligotrophic bacteria

Method of Koch platelet was applied (Hattori and Hattori, 1980). Abundance of copiotrophs on nutritive agar (BTL) were performed, whilst the number of oligotrophs on agar with broth 1000-fold diluted (BTL) were estimated. From each of soil samples three portion (10 g) were weighed and placed in 95 ml of sterile saline solution (SSS). The suspension was shaken (20 min.) at a room temperature. After that, 10 ml of suspension was sterilely collected and added to further 90 ml SSS and shaken (5 min.). This way all soil's dilutions in the range of $10^{-1} - 10^{-5}$ were prepared and plated on three parallel a Petri dishes. Cultures were incubated at 28ºC for 7 and 21 days, for copio- and oligotrophic bacteria, respectively (Hattori and Hattori, 1980). The number of tested microbial groups as a colony form unit (CFU) per gram of dry soil were expressed (CFU·g^{-1} d.m. soil).

Respiration activity

Soil subsamples (5 g with roots removed, sieved, 3 replicates) were placed in dark, sterile bottles (60 ml) tightly closed and incubated in 20°C. At the beginning of the experiment and after 7 days the level of accumulated CO_2 was analyzed in the headspace of soil samples by means of a GC (Varian CP-3800, USA), equipped with a thermal conductivity detector – TCD (120°C) and two types of columns: Poraplot Q (25 m) and a molecular sieve 5A (30 m) connected together and at 40°C (Szafranek-Nakonieczna and Stępniewska, 2014). Based on the differences between concentration of CO_2 at start and at the end of experiment respiration activity (RA) 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}).

Microbial biomass

Microbial biomass (MB) was determined using the chloroform fumigation technique (Franzluebbers, et al. 1999 with small modification) by measurement of total extractable organic biomass material from freshly killed microorganisms. Triplicate subsamples from the composite soil sample of each type (5 g fresh-weight) were placed inside 60 ml glass bottle. Two treatments, control and fumigated, were used. $CHCl_3$ was placed in a 2 ml glass vial into fumigated treatments. Both combinations were incubated for 5 days (room temperature). Then, vial with $CHCl_3$ was removed and both samples were vented and transferred for next 7 days into new 60 ml glass bottles with a rubber membrane. Concentration of headspace CO_2 released by microorganisms which survived incubation with $CHCl_3$ was measured by a GC (Varian CP-3800, equipped with a TCD detector). The amount of MB was calculated as (Kandeler, 2007):

$$biomass\ C - \frac{G_{ch} - G_{ch} \times 0.029 - G_0 \times 0.24}{0.41}$$

where:
biomass C − the carbon trapped to the microbial biomass,

G_{ch} − the amount of CO_2 produced by fumigated soils (mg),

G_0 − the amount of CO_2 produced by not fumigated soils (mg).

Results were expressed as milligrams of biomass C on gram of dry soils (mg g d.m.$^{-1}$).

Statistical analysis

Data were tested by using standard variance analysis (ANOVA) for the randomized complete block. Mean separations were made for significant effects with LSD and Tukey's test at the probability of $p<0.05$ (Swędrzyńska, et al. 2013). Spearman's rho correlation coefficient between chemical and biological soils properties was also determined. All statistical analyses were carried out using Statistica 9.0 (Statsoft Ltd., UK) software.

Results and Discussion

Chemical properties of cultivated and control soils

Differences in soil chemical factors (pH, TC, forms of N and P) between cultivated and control sites are presented in Table 2. It was found that agricultural treatments strongly modified soil chemical parameters. pH in the cultivated soils ranged between 4.18 − 6.98, however generally oscillated in a range of 4.6 − 5.6 in the most of investigated soil types. It is worth to emphasize, that in each of the agricultural exploited soil, a decline of pH towards acidic condition was registered compared to control sites, where pH amounted c.a. 6 − 7. It should be remembered, that soil acidification modifies population numbers of soil microorganisms and availability of

nutrients. Similarly, depletion of TC level in cultivated soils was observed and ranged between 0.83 to 2.69%, whilst in control soils TC values were significantly higher and amounted between 1.23 to 5.8% ($p<0.01$). These observations are comparable with the study of Swędrzyńska et al. (2013).

Dominated form of N was the nitrate nitrogen (NO_3-N), which in cultivated soils was extremely higher than in controls and amounted between 2.99 to 77.17 µg/g, whereas in controls reached the level of 1.68 − 13.82 µg/g. Mentioned differences are the result of systematically fertilizing of agricultural soils. Similar observations were performed by Li and Lang (2014).

The second form of N in terms of quantity was ammonium nitrogen (N-NH_4). Its content was in the range of 0.01 − 0.43 µg/g in relation to cultivated soils and 0.02 − 4.94 µg/g in controls.

Nitrite nitrogen (N-NO_2), remaining on the level of 0.04 − 0.14 µg/g and 0.09 − 0.87 µg/g in cultivated and control soils, respectively, was the least representative form of N in the soils investigated. Based on N forms levels and their relations, it seems that nitrification was a dominant processes in cultivated soils whereas in controls denitrifiaction prevailed. In work of Bissett et al. (2014) soil ammonium concentrations were up to 6.7 µg/g in non cultivated open grassy woodland whilst in cultivated soils N-NH_4 ranged between 1.4 and 6.6 µg/g with the exception to additional N fertilization treatments where levels of ammonium were higher than in control. Similar findings were presented by Zhang et al. (2013) for woodland and agricultural soils. In the cultivated soils higher content of phosphate phosphorus (P-PO_4) were found (1.04 − 19.6 µg/g) in comparison with control sites (0.6 − 7.52 µg/g).

Stępniewska and Stępniewski (2009) indicated that vegetation type, carbon and nutrients availabilities may influence microbial community composition at local scales, whereas soil pH was a better predictor of a community structure at the continental scale (Fierer and Jackson, 2006).

Copiotrophic and oligotrophic bacteria number

Differentiation in abundance of copio- and oligotrophic bacteria in agricultural and control soils are shown in Table 3.

In the current study copiotrophs counts were significantly lower in comparison with oligotrophic bacteria and ranged between 29 to 286 (cfu·10^4·g^{-1} d.m.) and 22 − 300 (cfu·10^4·g^{-1} d.m.) for cultivated and control soils, respectively ($p<0.0001$). Generally their counts were higher in controls rather than in agricultural exploited sites, what suggests microbiological degradation of arable soils. Domination of oligotrophic over copiotrophic bacteria due to economic processing of the energy substrate by oligotrophs is of significant importance from the point of view of maintenance OM in soil (Swędrzyńska, et al. 2013). Weyman-Kaczmarkowa (1996) indicated that above mentioned domination is essential for maintaining constant level of OM and soil biological balance.

Microbial biomass

The term of microbial biomass (MB) is commonly used for description of the total mass of microorganisms present in soil (Broos, et al. 2007; Wolińska, et al. 2012). Effect of soil cultivation on microbial biomass is presented on Fig. 1.

MB reached higher level in control (non-cultivated) soils, what indicate on negative influence of farming treatments on soil biological parameters. Maximal MB amounted 11.73 and 10.39 mg/g d.m of soil were found in the case of *Haplic Phaeozem* (No 10) and *Rendzina Leptosol* (No 16) control samples, respectively. Relatively high MB level ranging c.a. 8.0 mg/g d.m of soil were noted in the case of *Eutric Cambisols* control samples (No 4, 7 and 8). MBs estimated in cultivated soils were generally lower from controls c.a. by 30-80% and amounted between 0.82 − 6.93 mg/g d.m of soil. The highest decrease of MB (by 84.3%) as an effect of soil agricultural usage was noted in *Orthic Podzol* (Nr 2) and in *Haplic Phaeozem* (No 10) by 55.4%. Majority of soils were characterized by MB decline in the range 32 − 42%.

However, in the case of *Eutric Cambisols* (No 3, 5 and 9), *Eutric Histosol* (No 14) and *Rendzina Leptosol* (No 15) not significant differences in MB level were observed, regardless of the way of soil usage. Balota, et al. (2003) and Jia, et al. (2010) observed similar trend of MB like presented in the current study. They noted significantly higher MB level in the natural than in agricultural soils. Broos, et al. (2007) considered that anthropogenic activities and soil management in particular, are mostly responsible for disturbing the chemical, physical and biological equilibrium of soil. A particularly serious problem is the decrease in the OM content of agricultural soils, which may endanger soil fertility and enhance erosion (Wolińska, et al. 2012).

Respiration activity

Differentation in soil RA between cultivated and control soils is shown in Fig. 2. It is considered that soil RA (defined as the process of CO_2 release by microorganisms and plant roots) is a factor that provide one

of the most important characteristics of the soil biological activity (Cerhanova, et. al. 2006; Wolińska, et al. 2011) and is a sensitive indicator of variability's in soil carbon cycling which may derive from anthropogenic environmental changes (Baronti, et al. 2008). It was decidedly stated that intensive agricultural land management resulted in significant ($p<0.01$) decrease of soil RA in relation to non-agricultural exploited areas (controls).

Each of 16 arable soil units evidenced biological degradation expressed by RA inhibition. The values of RA determined in control soils were approximately 2-3 times higher than in cultivated soils. The highest RA differentiation between cultivated and control sites in the case of *Rendzina Leptosol* (No 16), and *Eutric Cambisol* (No 8) were stated.

Generally RA level in controls oscillated between $27 - 76$ µM CO_2 kg d.m.$^{-1}$ h^{-1}, whilst in agriculturally exploited soils ranged from 11.5 to 35 µM CO_2 kg d.m.$^{-1}$ h^{-1}.

Decrease of soil RA in cultivated soils were also observed by Steenwerth, et al. (2005) and Araujo, et al. (2009). Current study clearly demonstrated that RA can be more sensitive to the way of land use than MB, what is a reason that RA is strongly recommended for measuring in soil monitoring programs realized by many countries.

Correlations between investigated factors

The obtained results allowed for the determination of numerous relationships between the soil chemical and biological parameters (Table 4). From all analyzed parameters, pH seemed to be one of the most important factor determining soil biological activity, what was confirmed by positive correlations and high values of ρ coefficients with majority of factors: TC, N-NO_2, MB, RA and abundance of copio- and oligotrophic bacteria. The most sensitive on pH was MB (0.77, $p<0.0001$) and RA (0.59, $p<0.0001$).

The strong correlation between soil pH and MB could be a result of soil pH integrating a number of other individual soil and site variables. However, soil pH is also considered to be an independent driver of soil bacterial diversity, because the intracellular pH of most microorganisms is usually within 1 pH unit of neutral (Fierer and Jackson, 2006). Furthermore, biological factors (MB, RA, Copio and Oligo) demonstrated a significant positive correlations with TC and (excluding abundance of oligotrophic bacteria number) with N-NO_2.

These results are in agreement with the findings of study performed by Jia, et al. (2010), who suggested that soil OM is fundamental to the build-up and development of microorganisms. Greater TC and N forms may favor the growth of microbial populations (Balota, et al. 2003) and consequently result in greater MB and RA. Anyway, RA exhibited negative correlation with N-NO_3. We found that among investigated nitrogen forms in relation to soil biological aspect the most important is nitrite nitrogen, displaying significant relationship with MB, RA and copiotrophic bacteria number.

Table.1 Location of agricultural soils and description of control sites (Lublin region)

Soil number	Type of soil (FAO)	Crop type (1991)	Crop type (2014)	Village	Geographic coordinates	Control sites
1	*Orthic Podzol*	Potatoes	Oat	Dęba	22º10'17,7'' 51º26'24,6"	30 year old meadow planted with fruit trees
2		Barley	Triticale	Pryszczowa Góra	22º27'10,3'' 51º24'3,8"	20 year old woodlots with birches
3		Herbs	Wheat	Niemce	22º36'51,8'' 51º21'27,0"	50 year old meadow (mowed once a year)
4	*Eutric Cambisol*	Wheat	Triticale	Klementowice	22º06'54,2'' 51º21'52,2"	Unmoved meadow, wasteland
5		Potatoes	Oat	Łany	22º15'19,0'' 51º23'0,9"	20 year old field-woodlots
6		Potatoes	Oat	Markuszów	22º15'55,5'' 51º23'1,9"	20 year old field-woodlots
7		Thyme	Field prepared for seeding	Rogalin	24º04'0,3'' 50º51'15,81	Meadow (mowed once a year)
8		Barley	Triticale	Sady	23º22'52,4'' 50º51'14,8"	Unmoved meadow, wasteland
9		Potatoes	Strawberries	Chrząchówek	22º07'29,9'' 51º25'5,5"	Unmoved meadow, wasteland
10	*Haplic Phaezoem*	Bean	Triticale	Hostynne	50º44'48,3" 23º42'56,6''	Meadow (mowed once a year)
11	*Mollic Gleysol*	Oat	Colza	Pożóg Nowy	22º06'18,8'' 51º22'48,0"	30 year old pine woodlots
12		Field prepared for seeding	Wheat	Bałtów	22º01'25,5'' 51º29'15,3"	70 year old meadow (mowed once a year)
13	*Eutric Fluvisol*	Barley	Oat	Kośmin	21º59'10,1'' 51º33'47,7"	15 year old meadow (mowed once a year)
14	*Eutric Histosol*	Oat	Oat	Wólka Kątna	22º16'38,9'' 51º25'27,3"	20 year ld meadow (mowed once a year)
15	*Rendzina Leptosol*	Beets	Celeries	Siedliszcze	23º10'58,3'' 51º12'22,3"	40 year old meadow (mowed once a year)
16		Oat	Oat	Brzeziny	23º11'43,9'' 51º12'10,8"	Meadow (mowed once a year)

Table.2 Chemical characteristics of cultivated and control soils (±SD)

| Soil No.	Type of soil (FAO)	Land use	pH (H$_2$O)	TC (%)	N-NH$_4$ (µg/g)	N-NO$_3$ (µg/g)	N-NO$_2$ (µg/g)	P-PO$_4$ (µg/g)
1		**CULTIVATED**	**5.23±0.06**	**0.98±0.002**	**0.01±0.006**	**9.34 ±0.8**	**0.11±0.003**	**2.56±0.04**
		CONTROL	6.27±0.005	1.76±0.12	0.09±0.006	1.68±0.014	0.17±0.001	1.77±0.03
2	*Orthic Podzol*	**CULTIVATED**	**4.66 ±0.02**	**1.23±0.04**	**0.02±0.001**	**7.37±0.05**	**0.08±0.001**	**1.51±0.01**
		CONTROL	5.02±0.02	1.40±0.05	0.04±0.014	5.84±0.03	0.10±0.001	1.01±0.01
3		**CULTIVATED**	**4.78±0.02**	**1.24±0.04**	**0.01±0.001**	**53.32±0.52**	**0.05±0.005**	**19.6±0.98**
		CONTROL	6.22±0.09	1.79±0.14	0.06±0.006	3.58±0.09	0.42±0.005	1.16±0.05
4		**CULTIVATED**	**6.98±0.02**	**1.96±0.05**	**0.43±0.006**	**18.25±0.06**	**0.10±0.004**	**12.9±0.04**
		CONTROL	7.08±0.06	2.52±0.14	0.48±0.008	7.57±0.32	0.53±0.003	5.9±0.03
5		**CULTIVATED**	**5.45±0.04**	**1.01±0.04**	**0.07±0.006**	**25.53±0.18**	**0.12±0.001**	**6.88±0.01**
		CONTROL	5.58±0.04	2.06±0.19	0.69±0.009	10.18±0.14	0.21±0.002	3.52±0.09
6		**CULTIVATED**	**4.78±0.006**	**0.83±0.09**	**0.01±0.007**	**20.26±0.07**	**0.09±0.004**	**4.01±0.01**
		CONTROL	5.58±0.04	2.06±0.19	0.69±0.009	10.18±0.14	0.21±0.002	3.52±0.09
7	*Eutric Cambisol*	**CULTIVATED**	**6.93±0.006**	**0.97±0.06**	**0.05±0.001**	**14.48±0.04**	**0.04±0.005**	**4.61±0.01**
		CONTROL	6.99±0.03	3.49±0.11	0.41±0.008	5.41±0.14	0.87±0.003	3.85±0.03
8		**CULTIVATED**	**5.96±0.12**	**0.96±0.11**	**0.36±0.02**	**17.35±0.03**	**0.12±0.002**	**6.81±0.02**
		CONTROL	6.06±0.009	2.68±0.07	2.61±0.04	11.07±0.05	0.24±0.02	2.94±0.03
9		**CULTIVATED**	**5.13±0.006**	**0.88±0.06**	**0.19±0.009**	**4.96±0.06**	**0.14±0.001**	**13.9±0.24**
		CONTROL	5.40±0.006	1.42±0.11	0.18±0.001	1.76±0.06	0.80±0.002	7.52±0.02
10	*Haplic Phaezoem*	**CULTIVATED**	**6.61±0.05**	**1.64±0.03**	**0.02±0.001**	**27.43±0.08**	**0.09±0.003**	**1.36±0.05**
		CONTROL	7.22±0.02	5.43±0.14	0.02±0.002	8.23±0.02	0.44±0.006	1.35±0.02
11	*Mollic Gleysol*	**CULTIVATED**	**6.73±0.006**	**1.18±0.02**	**0.41±0.04**	**10.11±0.07**	**0.13±0.004**	**5.77±0.13**
		CONTROL	6.76±0.01	3.15±0.29	0.78±0.01	10.06±0.09	0.15±0.001	1.09±0.02
12		**CULTIVATED**	**4.74±0.02**	**0.91±0.05**	**0.03±0.004**	**21.90±0.02**	**0.09±0.001**	**2.04±0.03**
		CONTROL	6.25±0.03	1.80±0.13	4.94±0.08	6.75±0.05	0.10±0.001	1.68±0.008
13	*Eutric Fluvisol*	**CULTIVATED**	**4.18±0.05**	**0.98±0.07**	**0.14±0.04**	**2.99±0.03**	**0.09±0.001**	**2.64±0.09**
		CONTROL	5.64±0.06	1.23±0.08	0.27±0.03	2.20±0.05	0.13±0.002	1.33±0.008
14	*Eutric Histosol*	**CULTIVATED**	**4.85±0.03**	**2.69±0.19**	**0.01±0.001**	**10.22±0.12**	**0.08±0.002**	**3.09±0.10**
		CONTROL	5.27±0.01	3.63±0.14	0.02±0.002	9.05±0.03	0.09±0.001	1.74±0.38
15	*Rendzina*	**CULTIVATED**	**5.58±0.06**	**0.97±0.06**	**0.05±0.01**	**77.17±0.14**	**0.08±0.007**	**6.83±0.19**
		CONTROL	5.76±0.01	1.59±0.12	3.39±0.06	10.12±0.07	0.09±0.004	0.60±0.007
16	*Leptosol*	**CULTIVATED**	**5.58±0.11**	**1.25±0.05**	**0.22±0.01**	**32.98±.27**	**0.09±0.001**	**1.04±0.02**
		CONTROL	7.39±0.02	5.80±0.43	0.28±0.02	13.82±0.5	0.13±0.004	1.01±0.02
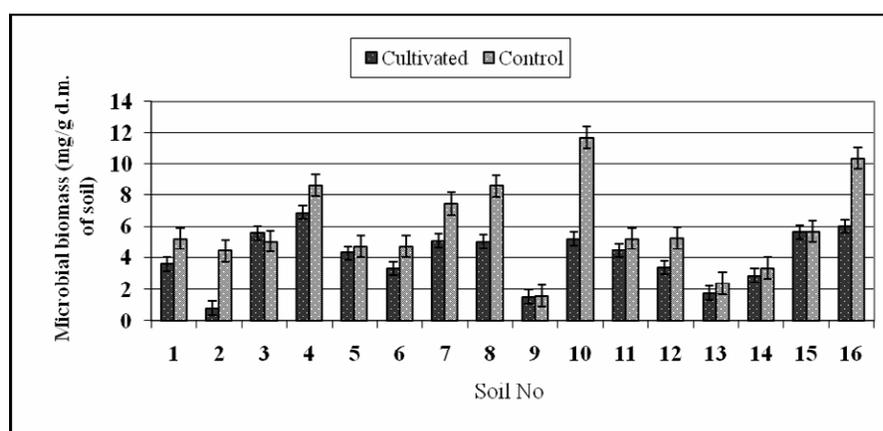
Fig.1 Differences in microbial biomass level between cultivated and control soils. Bars present means of three replicates with SE

Table.3 The number of oligotrophic and copiotrophic microorganisms (cfu·10^4·g^{-1} d.m. of soil) in agricultural and control soils (±SD)

| Soil No.	Soil type (FAO)	Land use	Oligotrophic bacteria	Copiotrophic bacteria
1		**CULTIVATED**	**254.19±31.53**	**76.81±17.07**
		CONTROL	290.64±30.78	98.54±12.70
2	*Orthic Podzol*	**CULTIVATED**	**89.55±11.92**	**63.91±20.71**
		CONTROL	142.76±18.96	59.42±11.75
3		**CULTIVATED**	**129.99±19.69**	**64.28±12.78**
		CONTROL	251.57±20.04	167.72±30.73
4		**CULTIVATED**	**366.73±28.90**	**159.66±42.24**
		CONTROL	449.38±6.48	284.00±28.30
5	*Eutric Cambisol*	**CULTIVATED**	**199.54±27.73**	**246.27±7.61**
		CONTROL	276.42±28.82	270.72±22.62
6		**CULTIVATED**	**250.85±27.69**	**58.41±6.83**
		CONTROL	276.42±28.82	270.72±22.62
7		**CULTIVATED**	**124.51±5.72**	**189.07±4.23**
		CONTROL	87.36±13.18	114.00±25.35
8		**CULTIVATED**	**424.02±29.28**	**286.17±54.68**
		CONTROL	473.29±14.73	299.29±9.79
9		**CULTIVATED**	**270.78±13.24**	**141.29±30.58**
		CONTROL	339.67±44.44	300.33±26.08
10	*Haplic Phaeozem*	**CULTIVATED**	**595.14±43.93**	**117.48±33.54**
		CONTROL	744.14±58.07	188.07±41.92
11		**CULTIVATED**	**129.86±16.09**	**40.61±4.35**
	Mollic Gleysol	CONTROL	209.38±22.25	97.11±30.57
12		**CULTIVATED**	**213.73±7.61**	**94.32±30.17**
		CONTROL	321.94±40.85	73.62±4.36
13	*Eutric Fluvisol*	**CULTIVATED**	**32.04±10.91**	**29.69±6.20**
		CONTROL	101.04±4.65	142.58±32.00
14	*Eutric Histosol*	**CULTIVATED**	**153.98±3.54**	**100.53±22.81**
		CONTROL	291.78±5.49	170.13±7.71
15		**CULTIVATED**	**246.19±26.72**	**193.07±5.09**
	Rendzina	CONTROL	319.91±38.62	218.8±26.32
16	*Leptosol*	**CULTIVATED**	**74.57±9.68**	**42.67±1.92**
		CONTROL	82.94±2.60	52.74±8.92
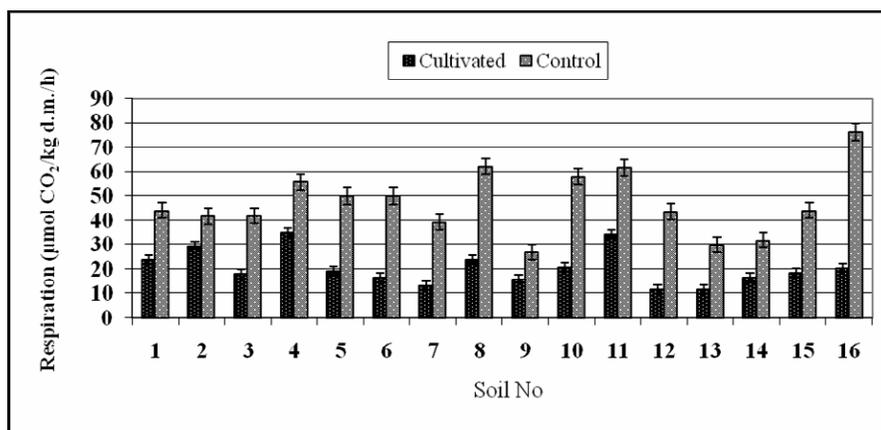
Fig.2 Differences in respiration activity between cultivated and control soils. Bars present means of three replicates with SE

Table.4 Interrelations between analyzed parameters
(n=96, Spearman's rho correlation coefficient)

	pH	TC	N-NH$_4$	N-NO$_3$	N-NO$_2$	P-PO$_4$	Oligo	Copio	MB	RA
pH	-	0.55***	-0.13 ns	-0.12 ns	0.32**	0.11 ns	0.38***	0.30**	0.77***	0.59***
TC		-	-0.11 ns	-0.22*	0.27**	-0.07 ns	0.27**	0.32**	0.71***	0.72***
N-NH$_4$			-	0.41***	0.29**	0.08 ns	0.04 ns	0.09 ns	0.08 ns	-0.06 ns
N-NO$_3$				-	0.26*	-0.10 ns	-0.04 ns	-0.06 ns	0.12 ns	-0.34***
N-NO$_2$					-	0.24*	0.14 ns	0.27**	0.39***	0.22*
P-PO$_4$						-	0.13 ns	0.35***	-0.05 ns	0.13 ns
Oligo							-	0.54***	0.42**	0.29**
Copio								-	0.32**	0.49***
MB									-	0.70***
RA										-

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

Analogical trend was stated in the case of P-PO$_4$. Several studies also reported stimulating effect of phosphorus fertilization on microbial abundance (Raesi and Ghollarata, 2006), what was in agreement with our results in regards to copiotrophs abundance (0.35***, *p*<0.0001). However, Thirukkumaran and Parkinson (2000) observed that P additions had an inhibitory impact on RA, what was compatible with our findings (Table 4).

Summarising, our study confirmed assumptions of wagered hypotheses that soils with systematically, intensive agricultural exploitation are biologically degraded as evidenced by lower values of all biological parameters (MB, RA, abundance of copio- and oligotrophic bacteria) in cultivated areas in relation to control sites. We also proved that soil micro- biological and chemical features are different in the area under cultivation than in non-cultivated soils – mentioned differences were the best illustrated by decrease of pH into acidic conditions and drop of TC in cultivated soils. Additionally, pH seemed to be the most important chemical factor determining soil biological activity. And last but not least we demonstrated that among investigated biological factors RA was the most sensitive indicator of soil agricultural management.

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