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# Changes in soil quality associated with tillage system applied**

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A b s t r a c t. The aim of this research was to evaluate changes in soil quality associated with the tillage system applied with chosen parameters of soil biological properties. The long-term field experiments were located at a private farm in Rogów (Zamość region, E Poland) on a silt soil and at the Experimental Station in Laskowice (Wrocław region, S-W Poland) on a sandy loam soil. Soil samples were collected from 0-15 and 15-30 cm layers. Winter wheat was grown under traditional, reduced and no-tillage systems. The analyses included estimations of microbial biomass C and N content, microbial respiration rate, activity of dehydrogenase and arylsulfatase, and fluorescein diacetate hydrolysis. After eight years the effects of tillage on both soils were clearly noticed. In general, the less disturbing tillage systems enhanced the increase of soil biological activity by 15-40%, on average, than conventional tillage system. The significant correlations between microbial biomass, and/or enzyme activities with total organic C content indicate that concentration of organic C in soil environment plays an extremely important role in enhancing the stabilization and activity of soil microorganisms, and protection of an extracellular enzymes. The studied parameters of soil biological activity showed their sensitivity to tillage applied and may be considered as an useful indicators of soil quality in monitoring all conditions alter soil environment.

K e y w o r d s: tillage system, microbial biomass C and N, soil enzyme activities, FDA hydrolysis

## INTRODUCTION

Soil quality can be defined as its capacity to work properly within ecosystem boundaries maintaining biological productivity, environment quality and also to promote plant and animal health (Doran and Safely, 1997; Madejón *et al*., 2009). Physical and chemical properties have been extensively used to measure soil quality. However, these properties of soil change very slowly usually, and therefore significant changes may occur only over many years (Puglisi *et al*., 2006; Pupin *et al*., 2009). By contrast, soil biological and biochemical properties as activity of soil microorganisms and/or activity of enzymes are sensitive to sudden environmental changes, and providing sensitive information on changes in soil quality (Frąc *et al*., 2012; Green *et al*., 2007; Melero *et al*., 2010). Microbial biomass has been shown to be a sensitive indicator of qualitative and quantitative changes in the soil organic matter because of variations in applied soil and crop management practices. As an active component of soil organic matter, microbial biomass is involved in the transformations and accumulation of nutrients in soil. It is a good measure of organic matter turnover and biological activity in natural habitats and agricultural ecosystems (Green *et al*., 2007; Madejón *et al*., 2009). Microbial enzymes have essential functions in the soil and have been used to measure the soil quality and the influence of soil management (Bielińska *et al*., 2008; Mohammadi, 2011; Pupin *et al*., 2009). Therefore, soil microbial biomass and enzymes activity have been suggested as potential indicators of soil quality because of their relationship to soil biology, and rapid response to changes originated by management and environmental factors (Frąc and Jezierska-Tys, 2011; Gajda, 2008, 2010; Madejón *et al*., 2009; Mohammadi, 2011).

The objective of presented studies was to evaluate the changes in soil quality associated with the different tillage system applied measured with chosen parameters of soil biological properties as microbial biomass C and N, and soil enzyme activities.

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## MATERIALS AND METHODS

The studies were conducted on the long-term field experiment started in 2002 at a private farm in Rogów (Eastern part of Poland) on a fine textured soil type – silt soil, and at the IUNG-PIB Experimental Station in Laskowice (South-Western part of Poland) on a coarse textured soil type – sandy loam soil. Characteristics of soils are presented in Table 1. At both experimental sites winter wheat was grown under three soil tillage systems: traditional tillage (TT) use of a moldboard plough and traditional soil tillage equipment, and conservation tillage (CST), reduced tillage (RT), non-inversion tillage (NT) based on soil crushing-loosening equipment and a rigid-tine cultivator, and no-tillage system. The working depth of the moldboard plow was about 20 cm, while the depth for the rigid-tine cultivator was about 10 cm. The each tillage system has been continuously applied to the same fields (about 1 ha each) for several years (since 2002). For the purpose of these studies, representative soil samples were collected in a random manner 2 times during each vegetation season (2007-2010) from in-row planting area at a two depths 0-15 and 15-30 cm. Immediately following sampling composite soil samples (about 1 500 g each) were placed in a plastic bags to prevent the water loss, homogenized and stored in a coolers (inside temperature ranged between 3-8°C) for transportation time from field to laboratory. All field moist soil samples were sieved through a 2 mm mesh sieve and divided into two parts: one part was stored at 4°C in a refrigerator in loosely tied plastic bags to ensure sufficient aeration for the microbiological and biochemical analysis and the other part was air dried and kept at room temperature for chemical analysis. Soils were analyzed for water content, texture, pH and electrical conductivity (EC) (Smith and Doran, 1996), total organic carbon ($C_{org}$) and total nitrogen ($N_{tot}$). Based on pH and lack of reactivity to acid, all C contained in both soils assumed to be in the organic form.

The soil water content was determined for each depth with a standard method drying the soil at 105°C to constant weight. The changes in soil biological activity associated with tillage system used were measured using the microbial biomass C and N content, microbial respiration rate, dehydrogenase system activity, arylsulfatase activity, and fluorescein diacetate hydrolysis. All microbiological and biochemical analyses were made in a three replicates for each representative soil sample within two weeks.

The microbial biomass C (MBC) content was determined using the Jenkinson and Powlson (1976a) fumigation-incubaction (F-I) modified method (Gajda, 2010). The moist soil samples (55% of water holding capacity, WHC) were placed into a glass beakers and fumigated in a vacuum desiccator in vapours of ethanol-free chloroform for 24 h at 25°C. Control samples (unfumigated) as well as fumigated were placed into airtight jars (0.9 l) and incubated for 10 days at 25°C together with vials containing 0.5M NaOH to trap respired $CO_2$. MBC was calculated from the difference between amount of $CO_2$ released from the fumigated and unfumigated soil during 10 days of incubation, and divided by conversion factor $Kc = 0.45$ for F-I procedure.

The rate of microbial respiration (MR) was calculated from the cumulative $CO_2$-C evolved from the control soil during 10 days of incubation.

The microbial biomass N (MBN) content in soil was estimated using the Jenkinson and Powlson (1976b) fumigation-extraction (F-E) method modified by Keeney and Nelson (1982). The conversion factor $K_N = 0.68$ for F-E procedure was used for calculation MBN content.

The dehydrogenases activity (DH) was estimated according to Casida *et al*. (1964) standard method, using TTC (2,3,5-triphenyltetrazolium chloride) as a substrate.

The activity of arylsulfatase (arylsufate sulfohydrolase, EC 3.1.6.1) (AS) was determined after soil incubation with p-nitrophenylsulphate as a substrate and measuring the absorbance at 400 nm of p-nitrophenol released according to procedure described by Alef and Nannipieri (1995).

**T a b l e 1.** Some physical and chemical characteristics of soils

| Type of soil | Tillage | Soil fraction content (%) | | | pH $H_2O$ | EC (dS $m^{-1}$) | $C_{org}$ (%) | $N_{tot}$ (%) | C:N |
|---|---|---|---|---|---|---|---|---|---|
| | | sand | silt | clay | | | | | |
| | | 1.0-0.1 | 0.1-0.02 | ≤0.02 | | | | | |
| | | (mm) | | | | | | | |
| Silt soil | TT | 4 | 54 | 42 | 6.1 | 1.4 | 0.88 | 0.06 | 14.7 |
| | RT | | | | 6.7 | 1.3 | 0.90 | 0.06 | 15.0 |
| | NT | | | | 6.8 | 1.2 | 0.92 | 0.07 | 13.1 |
| Sandy loam soil | TT | 56 | 20 | 24 | 5.7 | 1.8 | 0.72 | 0.04 | 18.0 |
| | RT | | | | 6.0 | 1.6 | 0.74 | 0.04 | 18.5 |
| | NT | | | | 5.9 | 1.5 | 0.74 | 0.05 | 14.8 |

The fluorescein diacetate hydrolysing (3,6-diacetylfluorescein, FDA) (EC 3.2.1.21) activity of the soil was determined by measuring the concentration of fluorescein released after soil incubation at 490 nm using the method described by Dick *et al*. (1996). All laboratory analysis were performed in a three replicates in the Department of Agricultural Microbiology at IUNG-PIB in Puławy, Poland.

Data discussed in this paper were obtained within 3 year research 2007-2010.

The obtained data were statistically analyzed by analysis of variance ANOVA.

Pearson correlation coefficients were determined among several soil properties using all replicates of the studied soils (all tillage systems, depths, and/or sampling positions) separately for each experimental site.

## RESULTS AND DISCUSSION

The differences found in size of microbial biomass C and N pools were significantly influenced by tillage system ($p<0.05$) applied continuously on the same experimental fields at both sites and the most marked effects of tillage were shown at the upper layer of soils 0-15 cm (Figs 1-4). In relation to 2007, the increase of MBC content determined in 2010 in silt soil under conservation tillage systems CST – RT and NT was about 28 and 17% bigger (on average), respectively, but in traditionally tilled soil reached only 11% (Fig. 1A). Similar trends were observed in the sandy loam soil, but the values of obtained measurements were about 40% lower, on average, of those obtained in the silt soil (Fig. 1B). The increase of size of MBC pools determined in 2010 was about 13 and 17% bigger, on average, in RT and NT soils, respectively, as compared to 2007. In traditionally tilled soil about 10% increase of size of MBC pool was noticed only (Fig. 2).

The evaluation of the effects of tillage system on size of MBN pools showed similar trends to the changes of the size of MBC pools observed in studied soils (Figs 3, 4). The biggest differences between effects of tillage systems used on MBN content significant at $p<0.05$ were obtained in TT and CST soils up to 15 cm depth at both experimental sites (Fig. 3A, B). The increase of MBN content determined in 2010 in a silt soil under conservation tillage systems – RT and NT was 22% bigger, on average, in relation to MBN content in 2007, but in traditionally tilled soil the increase of MBN reached only 8% (Fig. 3A). The MBN measurement values obtained in sandy loam soil showed similar trends, but accounted for only 10-20%, on average, of the values obtained in the silt soil (Fig. 3B). After 3 year studies, the increase of size of MBN pools at the layer of 0-15 cm of the silt soil under RT and NT system was 32 and 37% bigger, on average, respectively in relation to TT system. Also, in the sandy loam soil the increase of size of MBN pools in RT and

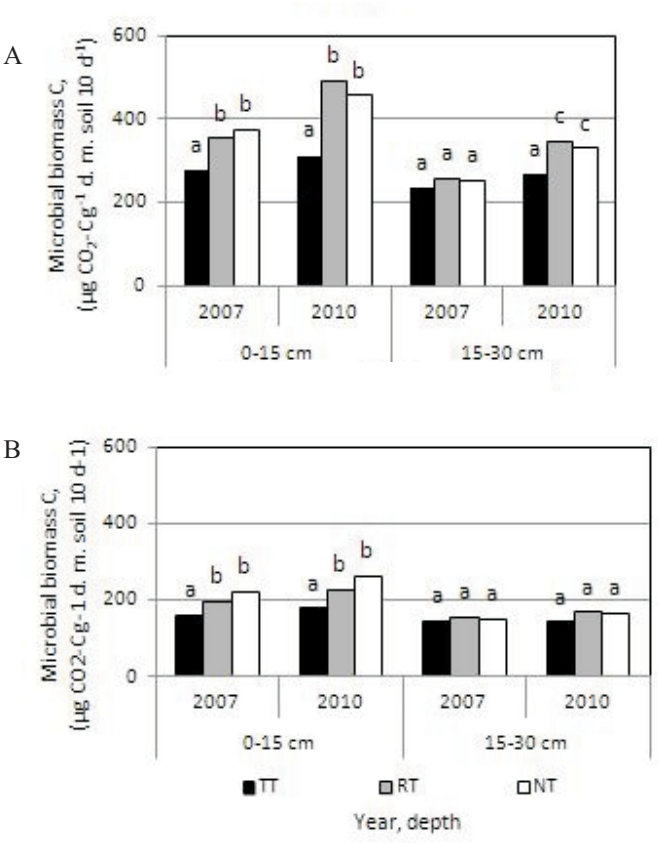


**Fig. 1.** Microbial biomass C content in soils under different tillage: A – silt, B – sandy loamy soil. a, b, c – values marked with different letter are significant at $p<0.05$.

NT system calculated in relation to TT system was bigger but not exceed 20 and 22%, on average, respectively (Fig. 4). Similarly to the results discussed by Melero *et al*. (2010) and Balota *et al*. (2004, 2011), the fact that size of MBC and MBN pools was considerably bigger in both soils under conservation tillage systems RT and NT, as compared to TT, demonstrated the differences in soil organic matter (OM) quality induced by tillage system disturbances and an amount of plant residues incorporated into studied soils.

Soil respiration gives an overall potential of microbial activity and is considered as a bioindicator of soil quality

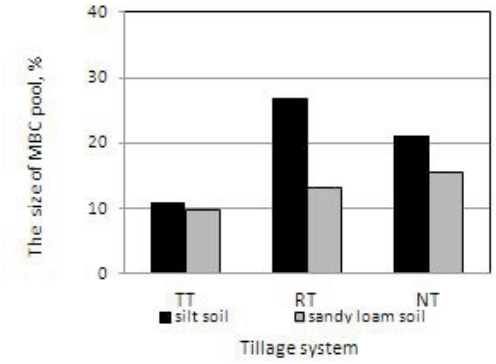


**Fig. 2.** Effect of different tillage on size of MBC pool in soil (0-15 cm).

(Puglisi *et al*., 2006; Dutta *et al*., 2010). In general, in presented studies the trends in variations in the rate of microbial respiration between tillage systems in both soils have been found to be similar to the trends in changes of MBC pool. In a silt soil, at the layer of 0-15 cm, 21 and 25% higher (on average) respiration rate was measured in 2007 and 2010, respectively, as compared to TT soil (Fig. 5A). Although, differences in respiration rate between tillage systems in a silt soil were relatively bigger (about 2 times, on average) than these observed in a sandy loam, the trends in respiration intensity between tillage systems were similar (Fig. 5B). Similar results in the $CO_2$ evolution rate as a consequence of microbial respiration influenced by tillage system were discussed earlier by Gajda (2008), Marinari *et al*. (2006) and Sánchez-Monedero *et al*. (2008). It is commonly known that soil respiration is related to carbon availability in biomass and usually the greater amount of $CO_2$-C is generated at the upper layer of NT soil than plowed soil because of greater population and activity of soil microorganisms (Gajda 2010; Gajda and Przewłoka, 2012; Stark *et al*., 2007). In our studies, the intensity of mineralization of total soil carbon ($C_{tot}$) (ratio of $CO_2$-C respired and $C_{tot}$) varied with the tillage system and soil depth. In soil taken from 0-15 cm under conservation tillage systems CST, the proportion of total mineralized C was 22% greater (on average) than under TT and significant at $p<0.01$ at both soil types. In the deeper

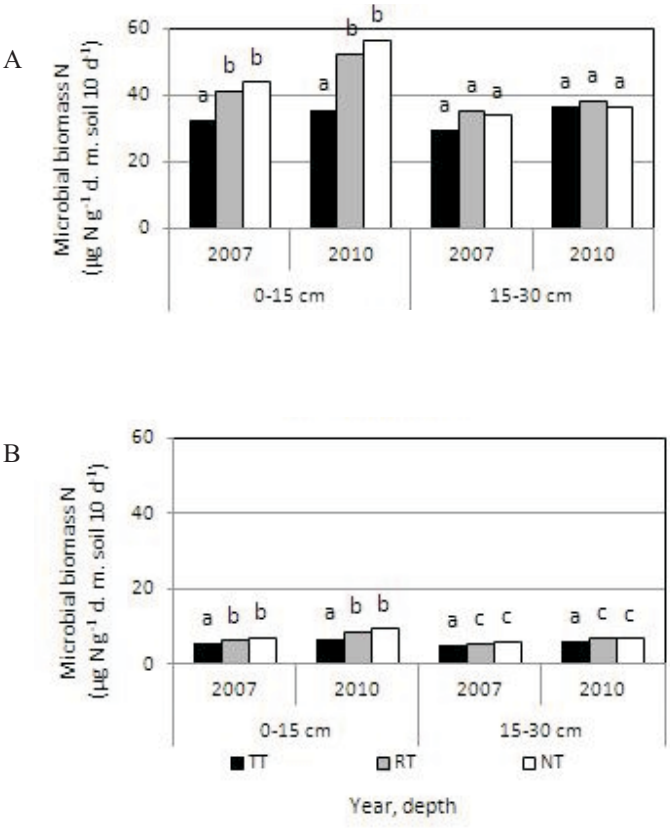


**Fig. 3.** Microbial biomass N content in soils under different tillage: A – silt, B – sandy loamy soil. Explanations as in Fig. 1.

layer (15-30 cm) the intensity of mineralization of $C_{tot}$ significantly decreased at both soils. The proportion of mineralized $C_{tot}$ was 1.6-2.0 times smaller in RT and NT soils than in soils coming from TT. The smallest differences in the mineralization intensity of total C between soil depth were noticed in soils under plow tillage at both experimental sites (Fig. 6). Also, similar results were published by Green *et al*. (2007).

The measurement of the soil microbiological activity by dehydrogenase systems (DH) is very attractive because these systems are an integral part of microorganisms and their apparent role in oxidation of soil organic matter (Madejón *et al*., 2009; Melero *et al*., 2010). The significant influence of tillage on DH activity was observed in both studied soils. In a silt soil values of DH activity were 20, 28% higher in 2007, and 30, 40% in 2010 under RT and NT (on average), respectively than under TT, especially at the upper layer (0-15 cm). At the lower layer (15-30 cm) the activity of DH dropped down and was 2-3 times lower as compared to the upper soil (Fig. 7A). In the upper layer of sandy loam soil values of measurements of DH activity were 10, 12% higher in 2007, and 32, 22% in 2010 hihger (on average) under RT and NT, respectively than under TT. The increase in soil depth caused the significant decrease of the activity of DH (about 2 times, on average) in relation to the measurements obtained for 0-15 cm layer, particularly under RT and NT systems (Fig. 7B). Also, Gajda (2010), Gajda and Przewłoka (2012), Marinari *et al*. (2006), Melero *et al*. (2010) and Stark *et al*. (2007), reported higher activity of dehydrogenases in soil under conservation tillage system in relation to ploughed soil under conventional tillage. According to Stark *et al*. (2007) the difference in DH activity between NT and/or RT soil and TT soil could be related either to a positive effect of organic fertilizers applied mostly into soil under conservation tillage systems or a negative effect of mineral fertilization applied into soil under conventional tillage. The appliation of mineral fertilizers mainly might influence DH activity without affecting the size of microbial community by inhibiting certain metabolic

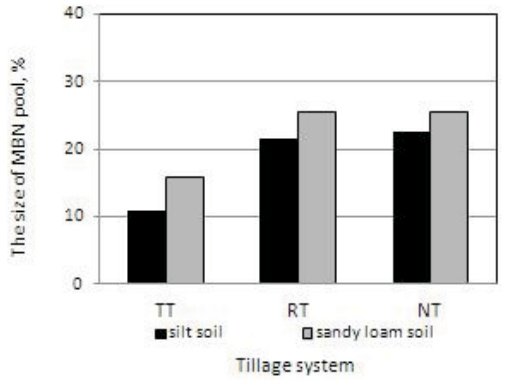


**Fig. 4.** Effect of different tillage on size of MBN pool in soil (0-15 cm).

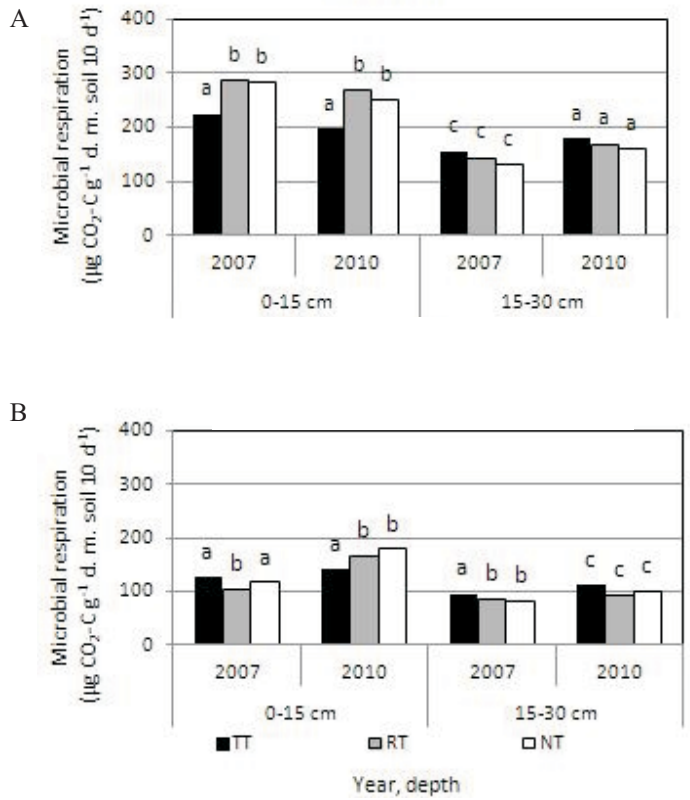


**Fig. 5.** Microbial respiration rate in soils under different tillage: A – silt, B – sandy loamy soil. Explanations as in Fig. 1.

processes or microbial groups. There were some research done earlier on soil microbial population groups involved in DH activity. For example, Kumar and Tarafdar (2003) reported that DH activity in soil is mainly carried out by populations of bacteria and actinomycetes while the participation of fungi is usually limited.

Arylsulfatases are one of many types of sulfatases involved in the mineralization of ester sulfate in soil. Most arylsulfatases are not constitutive enzymes, and their synthesis may be controlled by the C and S content. Thus, arylsulfatase (AS) activity is dependent on sulfate content and nutrient content in soil. AS activity is usually the enzyme with the lowest levels in soils (Balota, 2011). The obtained in 2007 and 2010 measurements of the activity level of AS in both soils are within the ranges obtained by researchers in many regions of the world and reported in literature (1.4 -113.0 μg PNP $g^{-1}$ of soil $h^{-1}$) (Acosta-Martinez *et al*., 2010). The levels of activity of *AS* varied from 7.7 to 26.7, and 5.5 to 17.3 μg PNP $g^{-1}$ of soil $h^{-1}$ in a silt soil and sandy loam soil, respectively (Fig. 8). The significantly higher activity of AS (2-3 times) were observed in the upper layer of both soils (0-15 cm) under conservation tillage system, especially in NT, than that observed in TT soil. As was mentioned above, the much higher levels of AS activity measured in soils under RT and NT tillage than the ones under tillage with plough used were probably related to a larger input of organic C in RT and /or NT soil *via* plant residues, which are a basic reservoir of sulfate esters, the main substrate for this group of enzymes (Balota *et al*., 2011). In general, higher AS activity was measured in the silt soil with a higher clay particles content and fertility level, as compared to the sandy loam soil (Fig. 8).

The fluorescein diacetate (FDA) hydrolysis a sensitive and nonspecific test able to depict the hydrolytic activity of soil microorganisms in a wide range of soils was commonly accepted as a simple measure of total microbial activity

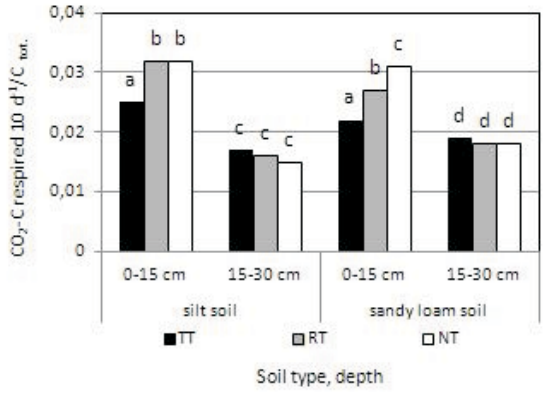


**Fig. 6.** Effect of tillage system on intensity of total C mineralization in soil. Explanations as in Fig. 1.

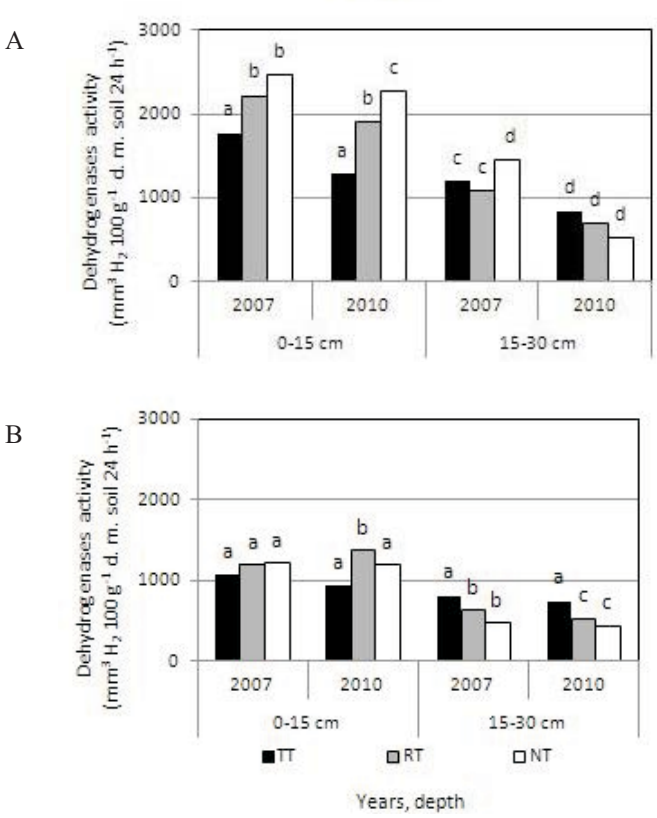


**Fig. 7.** Dehydrogenase system activity in soils under different tillage: A – silt, B – sandy loamy soil. Explanations as in Fig. 1.

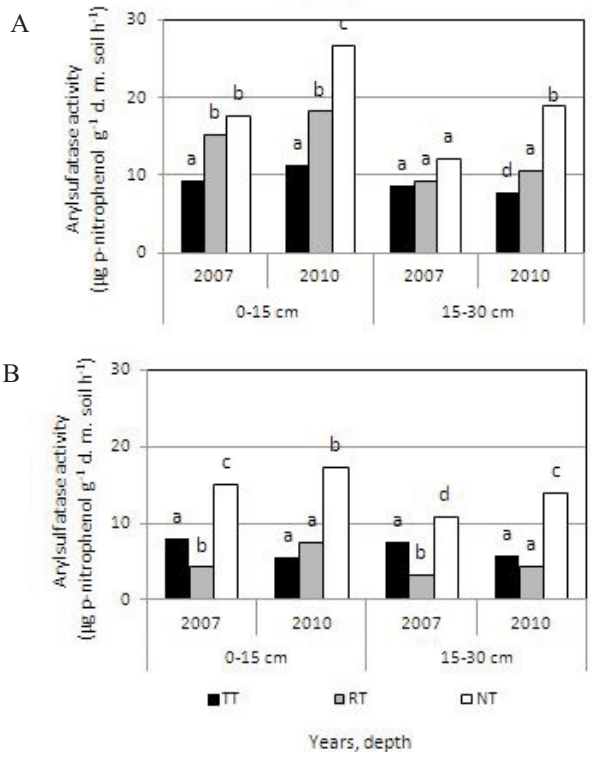


**Fig. 8.** Arylsulfatase activity in soils under different tillage: A – silt, B – sandy loamy soil. Explanations as in Fig. 1 .

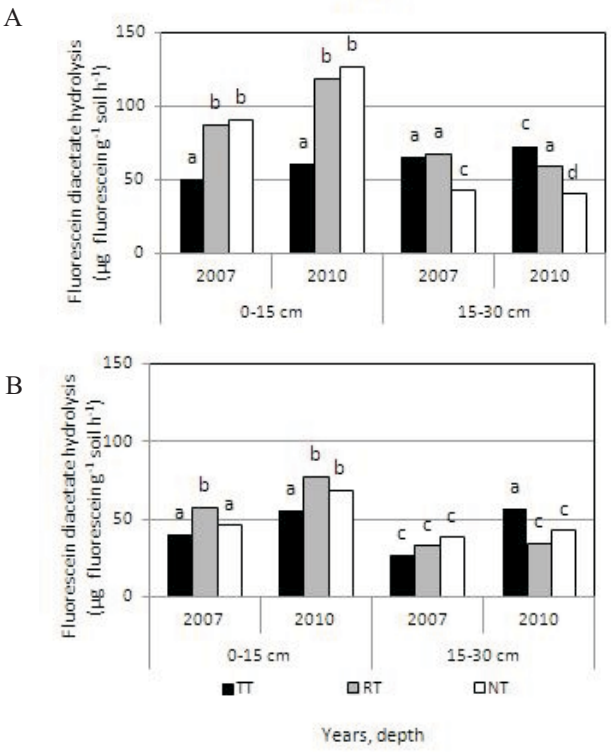


**Fig. 9.** Fluoresce in diacetate hydrolysis in soils under different tillage: A – silt, B – sandy loamy soil. Explanations as in Fig. 1.

**T a b l e 2.** Pearson correlation coefficients among some properties of silt and sandy loam soils across all treatments and sampling positions

| Parameter | Biomass C | Biomass N | Dehydrogenases activity | Arylsulfatase activity | FDA | Microbial respiration | Total organic C |
|---|---|---|---|---|---|---|---|
| | | | Silt | | | | |
| Biomass C | 1 | 0.96** | 0.42 | 0.82** | 0.96** | 0.72* | 0.82** |
| Biomass N | | 1 | 0.52 | 0.95** | 0.99** | 0.58* | 0.90** |
| Dehydrogenases activity | | | 1 | 0.63* | 0.58* | 0.87** | 0.74** |
| Arylsulfatase activity | | | | 1 | 0.93** | 0.44 | 0.92** |
| FDA | | | | | 1 | 0.58* | 0.92** |
| Microbial respiration | | | | | | 1 | 0.70* |
| Total organic C | | | | | | | 1 |
| | | | Sandy loam | | | | |
| Biomass C | 1 | 0.88** | 0.46 | 0.87** | 0.78** | 0.90** | 0.85** |
| Biomass N | | 1 | 0.62* | 0.99** | 0.60* | 0.66* | 0.88** |
| Dehydrogenases activity | | | 1 | 0.55 | 0.58* | 0.84** | 0.84** |
| Arylsulfatase activity | | | | 1 | 0.59* | 0.62* | 0.91** |
| FDA | | | | | 1 | 0.42 | 0.85** |
| Microbial respiration | | | | | | 1 | 0.68* |
| Total organic C | | | | | | | 1 |

*, **significant at $p<0.05$ and $p<0.01$, respectively.

(Adam and Duncan, 2001; Dutta *et al*., 2010). FDA is hydrolyzed by esterases, proteases and lipases, all enzymes involved in the microbial decomposition of organic matter in soil. In our studies, the FDA activity in both soils was affected significantly at $p<0.05$ by variations in tillage systems applied and ranged from 50.5 to 126.7 and 40.2 to 77.2 μg fluorescein $g^{-1}$ of soil $h^{-1}$ in a silt soil and sandy loam, respectively. In relation to TT soil, 42-50 and 20-30% higher activity of FDA (on average) was observed in the upper layer of less disturbed RT and/or NT silt soil and sandy loam soil, respectively (Fig. 9).

The enzyme activities were significantly intercorrelated, as observed in previous studies of *eg* Green *et al*. (2007), which suggest that tillage and crop rotations systems have similar effects on the activities of those enzymes involved in C, N, P and S cycling in soils. The obtained high correlations between analyzed enzymes and total soil organic C indicate likely that organic C provides a better environment for stabilizing and protecting extracellular enzymes through the association with organic and inorganic colloids, and thus supporting a greater microbial biomass and its activity (Balota *et al*., 2011). Also, arylsulfatase (silt soil-0.82**, 0.95**; sandy loam soil-0.87**, 0.99**) and FDA (silt soil-0.96**, 0.99**; sandy loam soil-0.78**, 0.60*) showed strong correlations with microbial biomass C and N at both studied soils (Tables 1, 2). This indicates that enzyme activities were associated with active microorganisms in soil which are the major source of soil enzymes (Balota *et al*., 2011). Similar results were obtained earlier by Gajda *et al*. (2012).

It should be noted that received values of analyzed parameters of biological activity were higher for a silt soil than the ones for a sandy loam soil. In addition, the conventional tillage influenced on much lower differentiation of measured parameters of soil biological activity as observed in alternative systems (NT and RT tillage) at both experimental sites.

Summarizing, under conservation tillage systems, the reduction and/or absence of soil disturbances produces a modification of surface soil conditions, which reduces soil organic matter decomposition, favours the formation and stabilization of microagregates, which provide the habitats for microorganisms and increases the microbial activity, thus in consequences resulted in a higher values of measured parameters of biochemical and microbiological properties of both soil types.

## CONCLUSIONS

1. In general, the tillage systems examined, no- and reduced tillage maintained greater biological activity than conventional tillage, what was reflected in 15-40% higher (on average) values of measurements of microbial biomass C and N content and soil enzyme activities, especially in the layer of 0-15 cm of both studied soil types.

2. In all tillage systems soil microbial biomass C and N content and soil enzyme activities were related to depth, especially in soil under no- and reduced tillage system. In the upper layer of soil 0-15 cm, much higher microbial biomass C and N content and enzyme activities were measured as compared to the ones in the lower 15-30 cm layer. The conventional tillage influenced much lower differentiation of measured parameters of soil biological activity as observed in an alternative systems (no- and reduced tillage) at both experimental sites.

3. Significant correlations between microbial biomass C and N, analyzed enzymes and total soil organic C indicate likely that organic C supporting a greater pool of microbial biomass and its activity maintaining a better environment for stabilizing and protecting extracellular enzymes. Also, the high correlations confirmed high sensitivity of studied parameters to changes in soil biological properties and might be considered as an useful indicators in monitoring of soil quality changes due to tillage system applied.

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