

Soil biological activity as affected by tillage intensity**

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A b s t r a c t. The effect of tillage intensity on changes of microbiological activity and content of particulate organic matter in soil under winter wheat during 3 years was studied. Microbial response related to the tillage-induced changes in soil determined on the content of biomass C and N, the rate of CO₂ evolution, B/F ratio, the activity of dehydrogenases, acid and alkaline phosphatases, soil C/N ratio and microbial biomass C/N ratio confirmed the high sensitivity of soil microbial populations to the tillage system applied. After three year studies, the direct sowing system enhanced the increase of labile fraction of organic matter content in soil. There were no significant changes in the labile fraction quantity observed in soil under conventional tillage. Similar response related to the tillage intensity was observed in particulate organic matter quantities expressed as a percentage of total organic matter in soil. A high correlation coefficients calculated between contents of soil microbial biomass C and N, particulate organic matter and potentially mineralizable N, and the obtained yields of winter wheat grown on experimental fields indicated on a high importance of biological quality of status of soil for agricultural crop production.

Key words: soil tillage system, microbial biomass C and N, dehydrogenase activity, potentially mineralizable N, particulate organic matter

INTRODUCTION

Soil organic carbon (SOC) and total N (TN) contents play a crucial role in sustaining soil quality, crop production, and environmental quality. Soil quality is a necessary indicator of sustainability land management (Doran and Parkin, 1996). The quality of soil depends on a large number of physical, chemical and biological soil properties. Changes in soil chemical and physical conditions influence microbial activity and population structure. Microbial communities

are integral parts of soil and their activity is very important to the functioning of soil. The role of microorganisms in mediating soil processes, and their relatively high rate of turnover, logically suggests that microbiological and biochemical soil properties could be a sensitive indicator and early predictor of changing soil organic matter processes (Acosta-Martinez *et al.*, 2008; Balashov *et al.*, 2010; Gajda, 2008; Marinari *et al.*, 2006). It has been well established that the more dynamic characteristics such as microbial biomass, soil enzyme activity and soil respiration respond more quickly to changes in crop management practices than do characteristics such as total soil organic matter content (Doran *et al.*, 1996; Mastro *et al.*, 2006). It has also been observed that the labile fraction of soil organic matter showed high sensitivity to changes in soil environment caused by an applied management system (Cambardella *et al.*, 2001; Marriott and Wander, 2006; Wander and Yang, 2000). Wardle *et al.* (1999) pointed out that long-term investigations are necessary for microbial studies in order to discern the effects of different intensive agricultural managements on soil microbial properties. The presented results obtained in 2007-2009 are the part of the studies conducted on a long-term experimental fields under different management systems, established in 2002 in four regions of Poland differ in soil and climatic conditions.

The aim of presented studies was to evaluate the effect of tillage intensity on soil on response of several parameters of biological activity as: microbial biomass C (MBC) and N (MBN) contents, rate of CO₂ evolution, dehydrogenase system and phosphatases activity, potentially mineralizable nitrogen (PMN), and particulate organic matter (POM) content.

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MATERIALS AND METHODS

The research was conducted in 2007-2009 on long-term field experiment established in 2002 in Experimental Station in Baborówko (Poznań region, Poland) of the Institute of Soil Science and Plant Cultivation, National Research Institute in Puławy on a light loamy sand soil (18% silt and clay fraction; 1.38% organic matter content). Winter wheat was grown on non-replicated 9 experimental fields (1 ha each) under conventional (CT) and alternative tillage systems: reduced (RT) and direct sowing (DS). Soil samples were collected randomly two times during each vegetation season from the in-row planting area at a depth of 0-25 cm across each trial. The samples were taken from the experimental fields with a soil corer (internal diameter 29 mm) at the end of June and/or first decade of July, and after harvest. A representative sample of soil taken from each experimental field weighted about 1 500 g. Immediately after sampling, soil was placed in double plastic bags, homogenized by hands and stored in the coolers for transportation time from fields to laboratory. After when samples reached laboratory were thoroughly mixed and 10-15 g subsamples were dried at 105°C for 24 h to determine field soil water content. The fresh soil samples were sieved through 2 mm mesh sieve and dry matter content and pH in soil was determined. The following microbiological and biochemical analyses were performed using standard methods:

- microbial biomass C (MBC) content using fumigation-incubation (F-I) method,
- microbial biomass N (MBN) using fumigation-extraction method (F-E),
- rate of CO₂ evolution with titration procedure,
- activity of dehydrogenase,
- activity of phosphatases,
- potentially mineralizable nitrogen (PMN) in soil.

The average ratio of bacteria to fungi B/F in soil was calculated for each tillage system separately.

For organic C, total N and POM analyses air dried soil was used. Organic carbon and total nitrogen concentrations in soil were determined once a year using Dumas method – FP-528-LECO. The quantity of POM was measured using the Cambardella *et al.* (2001) modified method in which POM was estimated according to loss-on-ignition (LOI) procedure.

All laboratory analyses were performed in 3 replicates of each soil sample. Statistical analysis were made using the ANOVA method. Obtained differences at $P < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Determination of microbial biomass is an essential baseline parameter in monitoring changes of soil biological properties. Balashov and Buchkina (2011), Masto *et al.* (2006), and Winding *et al.* (2005) reported that soil manage-

ment strongly affect the size of microbial biomass pool. In the presented studies, the highest measurements of the content of MBC were obtained in the soil under winter wheat grown in RT system. In comparison to soil managed conventionally, contents of MBC in soil under RT and DS were 22 and 18% higher, on average, respectively. During three year studies, statistically significant changes of the size of MBC pool in soil under RT were not observed between studied years, similarly to the soil under DS with an exception of the year 2008. In 2008 MBC content in soil under studied management systems reached relatively low values, as compared to the obtained values of MBC content in 2007 and 2009. It was probably the effect of very high temperatures of the air measured in the end of June, what resulted not sufficient enough level of water content in soil. The slight decrease of size of MBC pool was noted in soil managed conventionally (Fig. 1). Similar results were published by Acosta-Martinez *et al.* (2008), Gajda (2010) and Winding *et al.* (2005).

The oxidation of organic compounds to CO₂ by aerobic heterotrophic microorganisms in soil is a key process in carbon cycle of all terrestrial ecosystems. The measurement of the intensity of CO₂ evolution is helpful in estimation of physiological status and activity level of soil native microbial population (Winding *et al.*, 2005). In this study, significantly higher amounts of CO₂ evolved were obtained in soil under RT and DS system, and averaged 27 and 40%, respectively, as compared to the amounts of CO₂ evolved from CT soil (Fig. 2). Also, Gajda (2008) and Marinari *et al.* (2006), and reported that the decrease of major disturbance in soil, such is tilling, increased the size of soil organic carbon pool, the main source of easy available nutrients and energy, which supports bigger size and diversity of microbial populations, and its higher activity.

The MBN pool behaved in the similar manner as MBC, showing strong relation with tillage system. During three year studies, the greatest pool of MBN was measured in RT soil in all experimental years, which ranged from 30.6 to 38.5 µg N g⁻¹ d.m. soil. In soil under DS tillage, the size of MBN pool was 17% lower, on average, than the MBN content measured in RT soil. In comparison to soil managed conventionally, contents of MBN in soil under less disturbed tillage systems (RT and DS) were 30% higher, on average (Fig. 3). Similar responses of soil MBN to the different intensity of tillage disturbances were reported by *eg* Doran and Parkin (1996) and Govaerts *et al.* (2007).

Differences in MBC and MBN content, and the rate of CO₂ evolution as an effect of applied soil tillage systems were considered as statistically significant at $P < 0.05$.

At the end of three years of tillage practice, the ratio of soil organic carbon and nitrogen varied with respect to the studied tillage system, but obtained differences were not significant. The calculated C-to-N ratios of the organic matter in soil under DS, RT and CT system reached values 17.8; 18.4, and 20.7, respectively (Table 1). Also, Al-Kaisi *et al.* (2005) reported that after 7 years tillage experiment no

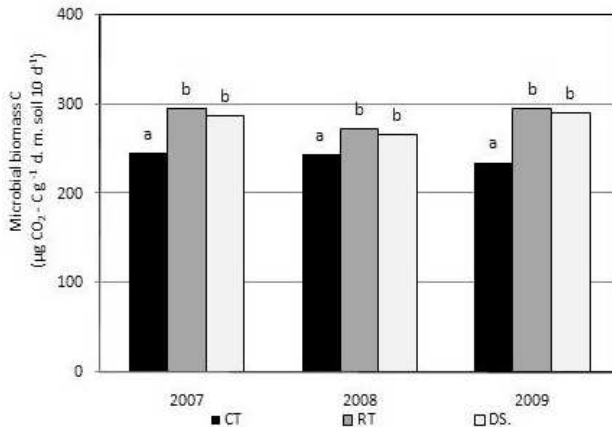


Fig. 1. Microbial biomass C content in soil during experiments; a, b, c – values marked with different letters are significantly different at $P < 0.05$.

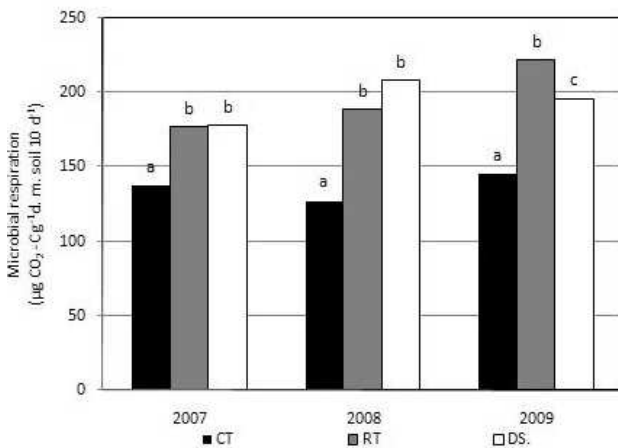


Fig. 2. Microbial respiration content in soil during experiments. Explanations as in Fig. 1.

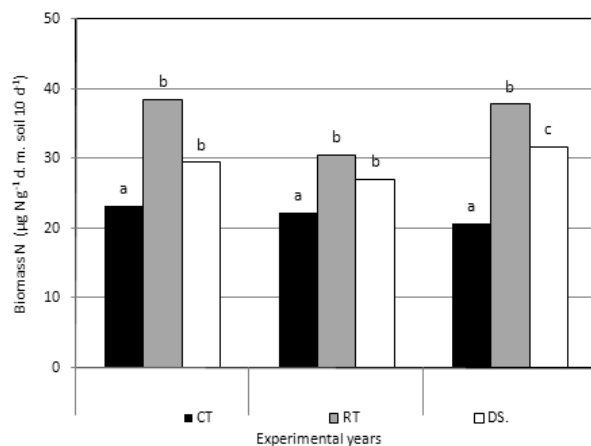


Fig. 3. Microbial biomass N content in soil during experiments. Explanations as in Fig. 1.

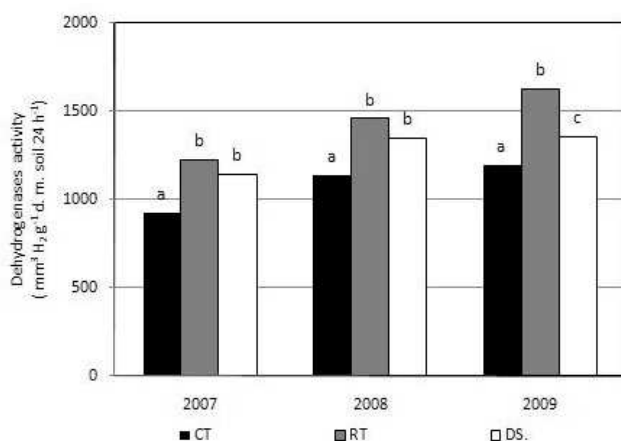
significant difference in C:N ratio was observed between no-tillage and chisel plowing soil. In our studies, the influence of the tillage system on soil environment was reflected stronger in the C-to-N ratio of the microbial biomass. The significant differences in the C_{mic} -to- N_{mic} ratio were obtained between RT and CT soil. The calculated values of C_{mic} -to- N_{mic} ratio in DS soil and CT soil ranged from 7.7 to 8.6 and 10.6 to 11.4, respectively. According to Fließbach *et al.* (2000) the C_{mic} -to- N_{mic} ratio of arable soils vary only in a small range. A small range of C_{mic} -to- N_{mic} ratio may be due to the fact that only a minor part (about 10%) of microbial biomass is actively taking part in the metabolic processes in soil. The obtained differences between the tillage systems at the similar intensity in the ratio of C:N were little, but visibly tended towards lower values in the soil under less intensive tillage system, as compared to the ratio of C:N in conventionally managed soil (Table 1).

The enzymes activity in soil is considered to be a major contributor of overall soil microbial activity and soil quality. Because they may rapidly respond to changes in soil caused by both natural and anthropogenic factors and are relatively easy to measure, thus being suggested as suitable early indicators of soil alteration in both natural and agro-ecosystems (Masto *et al.*, 2006; Puglisi *et al.*, 2006). In the presented studies the activity of soil enzymes (dehydrogenases and phosphatases) displayed different activity patterns as, probably, a result of applied tillage. The activity of dehydrogenase is considered an indicator of the oxidative metabolisms in soils and thus of the microbiological activity, because it is exclusively intracellular and, theoretically, can function only within viable cells (Masto *et al.*, 2006; Truu *et al.*, 2008). During three year studies the highest activity of dehydrogenases was observed in soil taken from fields under RT system. In comparison to the activity of dehydrogenase system measured in soil managed conventionally, the activity of dehydrogenases in RT soil was higher and ranged from 18 to 28%, on average. Similar trends in the activity of dehydrogenase system were observed in soil under DS system. The obtained results were 13-17% higher, as compared to soil under conventional tillage (Fig. 4). Also, Burns and Dick (2002), and Marinari *et al.* (2006) reported that decreasing of plough use enhanced the activity of dehydrogenases in soil. The dehydrogenase activity appeared quite sensitive to the tillage systems here studied. However, as it was mentioned by Mijangos *et al.* (2006) the earlier studies did not always showed a consistent correlation between dehydrogenase activity and activity of population of soil microorganisms. In this respect Howard (1972) hypothesized that extracellular phenol oxidizes, which are known to exist in soil, might also catalase the dehydrogenase reaction, thus reducing the potential for dehydrogenase activity to serve as an index of viable microbial activity (Mijangos *et al.*, 2006).

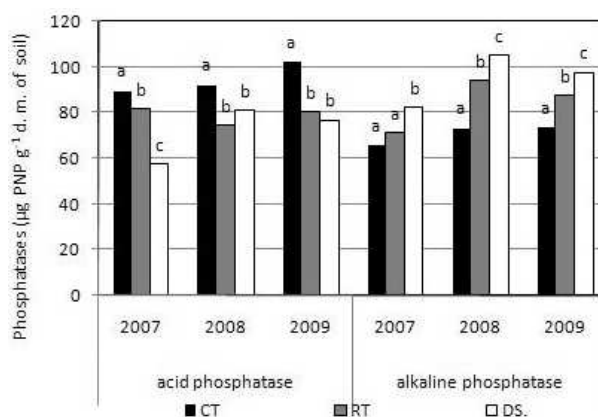
Table 1. Some characteristics of studied soil

Tillage system	Soil C:N ratio	Experimental year	Microbial biomass		
			C _{mic} (mg C-CO ₂ kg ⁻¹ soil)	N _{mic} (mg N kg ⁻¹ soil)	C _{mic} : N _{mic} ratio
CT	20.7 a	2007	246.3 a	23.3 a	10.6 a
		2008	244.9 a	22.2 a	11.0 a
		2009	235.3 a	20.7 a	11.4 a
RT	18.4 a	2007	296.3 b	38.5 b	7.7 b
		2008	262.6 a	30.6 b	8.6 b
		2009	295.8 b	37.8 b	7.8 b
DS	17.8 a	2007	287.5 b	32.5 b	8.8 a
		2008	267.2 a	28.1 b	9.5 a
		2009	290.8 b	33.7 b	8.6 a

a, b, c, – values marked with different letters show significant differences between tillage systems at $P \leq 0.05$.

**Fig. 4.** The dehydrogenase system activity in soil during experiments. Explanations as in Fig. 1

Phosphatases are important soil enzymes, because they provide P for plant uptake by releasing PO₄ from immobile organic P. In the present studies, the activity of acid phosphatase was 20 and 26% higher, on average, in soil tilled in conventional manner than in soil under RT or DS system, respectively. The activity of alkaline phosphatase reached the highest values in RT and/or DS soil. In comparison to CT soil, the activity of alkaline phosphatase measured in RT soil and in DS soil was 18 and 30% higher, on average. Least significance difference (LSD) at $P < 0.05$ were considered as the significant differences between treatment means (Fig. 5). The higher activity of acid phosphatase in conventionally managed soil than in RT and/or DS soil may be due to pH of soil, which was influenced probably by application

**Fig. 5.** The phosphatases activity in soil during experiments. Explanations as in Fig. 1.

of mineral nitrogen fertilizers (according to Polish agro-technics requirements) into soil under conventional tillage. Similar effects of tillage system on phosphatases activity were earlier reported by Masto *et al.* (2006) and Winding *et al.* (2005).

The nitrogen mineralization capacity refers to the capability of soils to transform organic nitrogen compounds into ammonium/nitrate under optimum moisture and temperature conditions over a given period time (Gil-Sotres *et al.*, 2005). In presented studies an influence of tillage intensity on the content of mineralized N in soil was noticed. The highest amounts of potentially mineralized N (PMN) were estimated in 2009 in soil under alternative tillage systems, RT and DS – 4.4 and 4.0 µg N-NH₄ g⁻¹ d.m. soil, respectively.

During three year research period, in CT soil the measured contents of mineralized N were 13-27% smaller, on average, as compared to the contents of mineralized N measured in RT and DS soil (Fig. 6). This shows that the activity of soil microorganisms involved in the processes of N mineralization under less disturbed conditions was markedly higher, as compared to the soil managed in conventional manner. Similar observations were reported by Marzaioli *et al.* (2010) and Mijangos *et al.* (2006). According to Deenik (2006) soils with high N mineralization potential tend to be inherently fertile, while soils with low N mineralization potential tend to be less fertile and require greater agricultural inputs. Considering the obtained findings, the potential of nitrogen mineralization is a suitable measure of soil quality.

The ratio of bacteria to fungi (B/F) in soil was calculated, also. The B/F ratio is used as a one of good parameters of soil biological activity for characterization of microbial community in soil. The highest values of B/F ratio were calculated for soil under DS and RT – 30.5 and 27.5, respectively, but the lowest values were typical for soil under conventional tillage – 17.2, on average (Fig. 7). Wielgosz and Szember (2006) reported that the higher values of B/F ratio inform about weaker development of fungi population in soil, but the lower values suggest stronger development of fungi, as compared to bacteria population in soil.

The intensity of tillage caused the changes in quantity of particulate organic matter fraction in soil. In 2007, the measured quantities of POM in RT and DS soil were 12% higher, on average, than the quantity of POM measured in CT soil. The POM contents in RT soil and DS soil assessed in 2009 were 28 and 34% higher, respectively, as compared to the POM quantity in soil under CT tillage. After three year studies, the DS system enhanced the increase of POM content in soil (20%, on average), as compared to the POM concentration in DS soil measured in 2007. There were no significant changes in POM quantity observed in soil under conventional tillage (Fig. 8). It is interesting to point out that in comparison to CT soil, the content of POM fraction expressed as the percentage of total soil organic matter (SOM) was the highest in RT and DS soil, and amounted 29.8, 29.7%, respectively, in 2007, and 32.0, 33.6%, respectively, in 2009. The lowest share of POM, reaching 24.6 and 20.9% of SOM in 2007 and 2009, respectively, was in soil under intensive tillage system with plough use (Fig. 9). Our three year studies showed that the less disturbed tillage systems (RT and DS) enhanced the accumulation processes of POM fraction in soil expressed as a percentage of SOM (2.2 and 3.9%, respectively), whereas the soil under CT tillage with the practice of soil inversion enhanced a decreasing trends in the quantity of POM fraction in the total SOM (3.7%, on average) (Fig. 10). Similar trends in obtained results have been reported earlier by Cambardella *et al.* (2001), Gajda (2010), and Gajda *et al.* (2001).

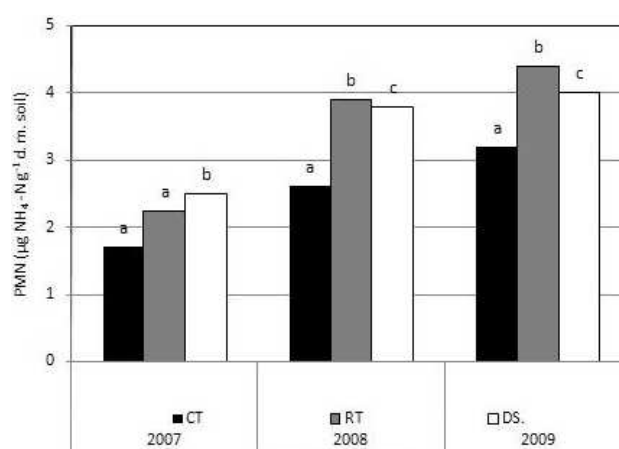


Fig. 6. Potentially mineralizable N content in soil during experiments. Explanations as in Fig. 1.

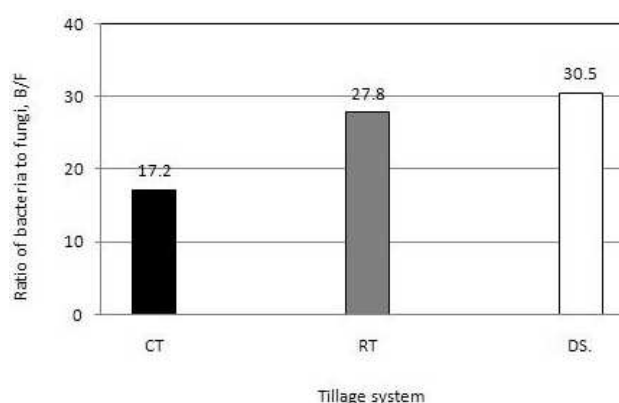


Fig. 7. The ratio of bacteria to fungi B/F calculated for soil during experiments. Explanations as in Fig. 1.

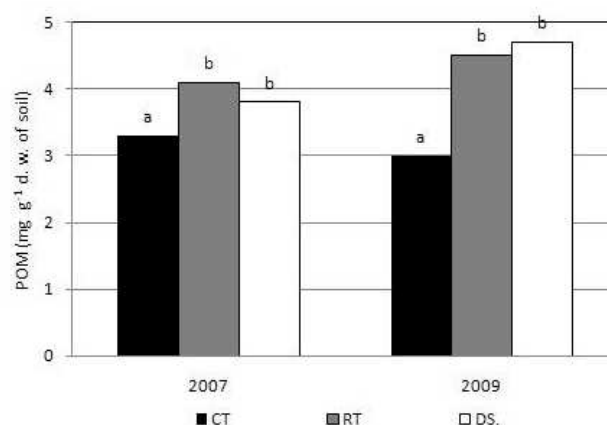


Fig. 8. The POM fraction content in soil during experiments. Explanations as in Fig. 1.

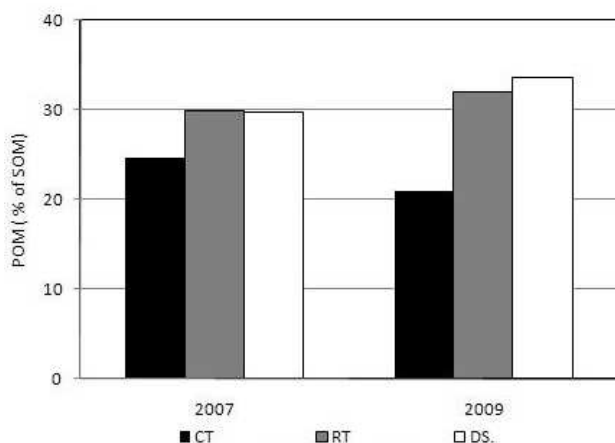


Fig. 9. The POM content expressed as the % of SOM in soil during experiments. Explanations as in Fig. 1.

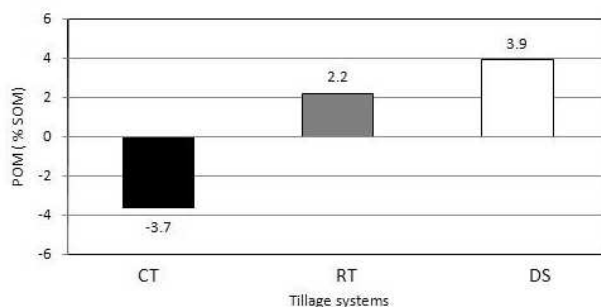


Fig. 10. The increase and/or decrease of POM quantity (expressed as % SOM) in soil estimated in 2009 in relation to 2007. Explanations as in Fig. 1.

The obtained high correlation coefficients between studied parameters of soil biological activity confirmed earlier findings that chosen parameters well characterize the effects of tillage intensity on soil (Fig. 11A, B, C, D). It is commonly known, that soil organic matter is a key factor of the quality and productivity of soil, especially the ongoing transformation processes of SOM in soil. In mineralization processes of SOM are involved microorganisms, which play an extremely important role in creation of microbiological and biochemical properties of soil. A high correlation coefficients obtained between content of soil organic C and microbial biomass C and N, POM and activity of dehydrogenase system ($r = 0.785; 0.723; 0.553; 0.588$, respectively) confirmed the strong relationship between development and activity of soil microorganisms and the content of SOM, which serves as a main source of nutrients and energy for soil microorganisms (Fig. 11E, F, G, H). The close relationships were found among almost all biochemical properties studied and soil organic C and total N concentrations by

Alvear *et al.* (2005) and Jiménez *et al.* (2002), also. Increases of soil organic matter and microbial biomass are associated with improved soil quality, usually (Mijangos *et al.*, 2006).

A high correlation coefficients calculated between contents of soil microbial biomass C and N, POM and PMN, and the obtained yields of winter wheat grown on experimental fields ($r = 0.777; 0.768; 0.898; 0.678$, respectively) indicated on a high importance of biological quality status of soil for agricultural crop production. The discussed here results were well agreed with published earlier by Gajda *et al.* (2009) (Fig. 12).

Due to sensitivity, rapid response, and capacity to provide information that integrates many environmental factors, the biological parameters are useful tools for farmers and land use managers to assess the effects of farming practices on the capacity of the land to remain productive and on soil quality (Doran *et al.*, 1996; Gajda, 2010; Mijangos *et al.*, 2006).

CONCLUSIONS

1. The less intensive tillage systems, as compared to the conventional tillage, caused 20% increase, on average, of the content of labile fractions of soil organic matter as microbial biomass C and N, and particulate organic matter.

2. Microbial response related to the tillage-induced changes in soil determined on the rate of CO_2 evolution, B/F ratio, the activity of dehydrogenases along with acid and alkaline phosphatases confirmed the high sensitivity of soil microbial populations to the tillage system applied. The less intensive tillage systems caused 20-33% higher values of measured parameters of microbial activity, on average, than the respective ones obtained in the conventionally tilled soil.

3. The soil C/N ratio and microbial biomass C/N ratio showed higher values for soil under conventional tillage, as compared to the alternative, less intensive tillage systems (RT and DS), indicate on a greater requirements of microorganisms for N per unit of C biomass in soil managed conventionally.

4. There was a much higher content of potentially mineralizable nitrogen in soil under less intensive tillage systems (RT and DS), as compared to conventional tillage. That indicated on higher activity of soil microorganisms involved in the processes of N mineralization under less disturbed conditions, as compared to the soil under conventional tillage.

5. The presented studies showed that the less disturbed tillage systems (RT and DS) enhanced the process of accumulation of POM fraction in soil expressed as a percentage of SOM (2.2 and 3.9%, respectively), whereas the soil under conventional tillage with the practice of soil inversion enhanced a decreasing trends in the quantity of POM fraction as a percentage of SOM (3.7%, on average).

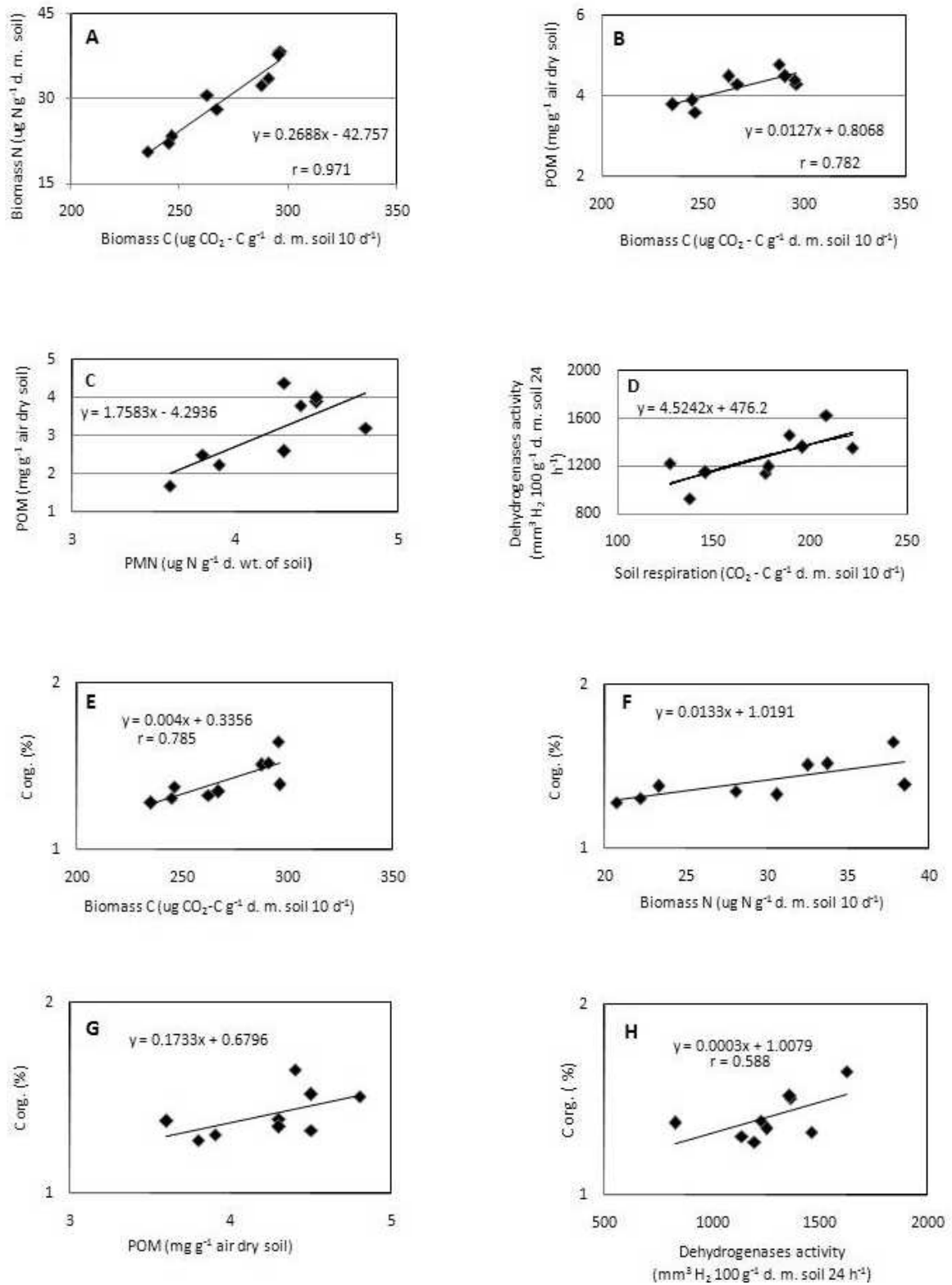


Fig. 11. The relationship between some parameters of biochemical properties (A, B, C, D, E, F, G, H) of soil during experiments (significance level $P < 0.05$). Explanations as in Fig. 1.

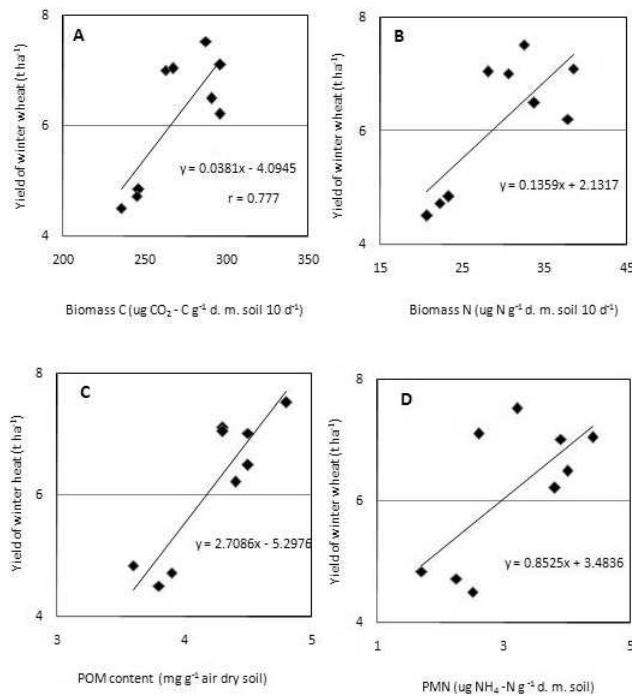


Fig. 12. Relationship between yield of winter wheat and some parameters of biochemical properties (A, B, C, D) of soil during experiments (significance level $P < 0.05$). Explanations as in Fig. 1.

6. Calculated high correlation coefficients among studied parameters of soil biological activity and soil organic carbon, and obtained yields of winter wheat confirmed the strong relationship between development and activity of soil microorganisms and the content of SOM, which serves as a main source of nutrients and energy for soil microorganisms and indicated on a high importance of biological quality status of soil for agricultural crop production.

7. Continuous application of reduced tillage and/or no-tillage systems affected beneficially soil environment what reflected the changes of studied parameters of soil biological activity. The results pointed clearly out the importance and the need of continuation of investigations on the changes in soil biological properties induced by tillage practices for better understanding the reaction of soil and for application of the more appropriate tillage systems for increasing soil sustainability or productivity.

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