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The Impact of Tillaging Spring Barley on Selected Chemical, Microbiological, and Enzymatic Soil Properties

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Abstract

The aim of this study was to determine the long-term effects of different tillage systems on some chemical and biological soil properties. The research was carried out in 2011-14 based on a static field experiment set up in 1999 at Brody Research Station of Poznań University of Life Sciences, Poland, in a temperate climate, on Albic Luvisols. Soil samples were collected from under spring barley from two layers: 0-10 cm and 10-20 cm. The results of this experiment showed that reduced tillage (RT) and no-tillage (NT) increased total carbon, total nitrogen, the number of microorganisms (heterotrophic bacteria, oligotrophic and copiotrophic bacteria, actinobacteria and fungi) and enzymatic activity (dehydrogenases, acid and alkaline phosphatase, urease, protease, and catalase) compared with conventional tillage (CT) – especially in a 0-10 cm layer of soil. This suggests that tillage has a significant impact on soil C cycling through its effects on soil microbial activity.

Keywords: enzymatic activity of soil, soil microorganisms, tillage systems, soil fertility, spring barley

Introduction

Agricultural intensification from intensive tillage-based production systems generally has had a negative effect on the quality of many of the essential natural resources such as soil, water, biodiversity, and the associated ecosystem services provided by nature. This degradation of the land resource base has caused crop yields and factor productivities to decline and has

forced farmers and scientists to search for an alternative paradigm of sustainable production intensification. The new paradigm of sustainable production intensification needs productive and remunerative agriculture, which at the same time conserves and enhances the natural resource base and environment [1]. Under ploughless tillage agriculture, the soil is not inverted and this seems to profoundly impact many soil properties, particularly in the upper soil layer [2-4]. Soil under no-till and reduced tillage have greater storage of diverse plant biomass on the surface, which results in moist soil and low temperature with efficient microbial activity, and considerable improvement in organic carbon content, which positively

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affects soil structure and increases the C/N ratio compared to conventional tillage [5-8]. Soil organic matter is considered the indicator of soil quality because of its contribution to influencing soil biological, chemical, and physical properties and crop yields.

Some have suggested that soil quality should be assessed using a wide variety of biological, physical, and chemical indicators [9]. Integrating multiple indicators into an index of soil quality provides a way to comprehensively monitor changes in soil quality as a result of land management over time. The particulate organic matter, organic C, total N, microbial biomass, biological activities, enzymes, bulk density, and soil aggregation are important indicators of dynamic soil quality because of their quick response to management practices [2, 9]. Soil biochemical properties, such as population of the microbial and enzymatic activities, are directly involved in soil organic matter dynamics and thus highly correlated with soil organic C content [5, 10]. Increasing soil microbial diversity and the interactions among the soil biota have a great effect on the incidence of diseases, which affects production levels and the beneficial organisms, e.g., cycle nutrients. This is because the availability of water, nutrients, and certain microorganisms at the root surface is mediated by such interactions [11-12].

Soil quality is of great importance in determining the sustainability of land management systems. Therefore, comprehensive analysis of the advantages and disadvantages of different tillage systems requires a thorough knowledge of this effect on the soil ecosystem. Biological indicators, especially those related to soil microbial communities, are becoming increasingly used due to their quick response, high sensitivity, ecological relevance, and capacity to provide information that integrates many environmental factors. Biological indicators may serve as early indicators of soil quality improvement or degradation in agroecosystems [9]. The objective of the present study was to determine the long-term effects of different tillage systems on some chemical and biological soil properties. We hypothesized that populations of different groups of soil microorganisms and enzymatic activity are higher in reduced tillage (RT) and no-tillage (NT) than in conventional tillage (CT).

Materials and Methods

The research was carried out in 2011-14 based on a static field experiment set up in 1999 at the Brody Agricultural Experimental Station (52°26'N; 16°17'E) at Poznan University of Life Sciences, Poland. The soil was included in Albic Luvisols and, according to Soil Taxonomy in Typic Hapludalfs, regarding granulation in loamy sand underlined by loam [13]. The soil of the experimental field was classified in the IIIB-IVa bonitation class. Prior to the start of this experiment, only ploughing tillage was applied for crops (mainly cereals), and straw of cereals was removed.

Spring barley was grown in a four-year rotation of pea, winter wheat, spring barley, and winter triticale. Three tillage systems were arranged in a randomized complete block design in four replications, resulting in a total of 12 plots. The size of each tillage plot was 30 m length and 5 m width. The plots were separated by 0.3 m-wide buffer strips and a 6 m-gap between blocks for the tractor. The straw of previous crop (winter wheat) was removed from all plots in all years.

The following tillage systems were applied in continuation: 1) conventional tillage (CT), 2) reduced tillage (RT), and 3) no-tillage (NT). CT consisted of tilling with a disk harrow (2.5 m wide) to a depth of 8 cm after harvesting the previous crop, ploughing to a depth of 25 cm with a three-furrow reversible plough (the first week of October) and pre-sowing tillage for seedbed preparation with a field cultivator followed by harrowing and rolling to 8 cm deep one week before sowing. RT occurred in the autumn (the first week of October) only with a stubble cultivator (2.5 m wide). NT involved sowing directly into the stubble of the previous crop. The CT plots were drilled with a Suffolk coulters grain drill and the RT and NT plots with a double-disk drill (Great Plains Solid Stand 10').

Spring barley *cv.* Nadek was sown at a density of 400 seeds·m⁻² for all tillage sown in late March (between 20 and 29), except in 2013, in which sowing was carried out on 16 April. In autumn, mineral fertilization was applied at the following rate per 1 ha: P – 35 kg and K – 66 kg, while in spring 90 kg N·ha⁻¹ were used. The herbicide program for tillage systems used pre-plant and post-emergence applications. Before planting, 3 l ha⁻¹ of glyphosate herbicide was applied on all plots with NT and RT to control perennial weed and volunteers. For weed control during the growing season post-emergence, Lintur 70 WG (dikamba 65,9%) + Chwastox Extra 300 SL (MCPA) herbicides were applied at the rate of 150 g·ha⁻¹ and 1.0 dm³·ha⁻¹, respectively. The seeds were dressed with Raxil Extra 060 FS fungicide (0.06 dm³ per 100 kg seeds) containing thiuram and tebuconazole and for disease control Falcon 460 EC (tebuconazole + spiroxamine + triadimenol) fungicide at the rate 0.6 dm³·ha⁻¹.

Soil samples for chemical analyses were collected before the start of the experiment in 2011. The replication plot was represented by a mean sample consisting of 10 individual samples collected using an Egner sampler from the 0-10 cm and 10-20 cm layer. After drying, the soil was crushed by hand and sieved through a 2-mm sieve. Organic carbon was determined by the Tiurin method (K dichromate oxidation), total N by Kjeldahl method, and pH in 1 mol KCl · dm³ [14].

Soil samples for biological analyses and pH were collected three times during the growing season of spring barley: I term – stem elongation growth stage of spring, II term – heading growth stage of spring barley, III term – after harvesting of spring barley (in each year in the same way as for samples for chemical analysis).

Microbiological analysis was performed on the basis of Koch's plate method and involved determining (using

selective substrates in four replications) the numbers of colony forming units (CFU g^{-1} DM of soil) of heterotrophic bacteria, oligotrophic and copiotrophic bacteria, actinobacteria, and fungi. Estimation of CFU number of the above-mentioned microorganisms is a measure of the intensity of their current metabolic activity.

The number of heterotrophic bacteria was determined on Merck standard agar (3 g yeast extract; 5.0 g peptone from casein (free from fermentable carbohydrates); 5 g sodium chloride; 12 g agar, 11 H_2O), after 5-6 days of incubation at 28°C [15]. Oligotrophic bacteria were counted on diluted nutritive broth (0.1g peptone, 0.1g beef extract, 0.05 g sodium chloride, 20g agar, 11 H_2O) at 28°C after 21 incubation days [16]. Copiotrophic bacteria were determined on nutritive broth (10g peptone, 10g beef extract, 5 g sodium chloride, 20 g agar, 11 H_2O) at 28°C after seven days of incubation [16]. Fungi were determined on Martin substrate (1 g KH_2PO_4 , 0.5 g $MgSO_4$, 5 g peptone, 10 g glucose, 3.3 ml Bengal, 0.1 g chlortetracycline, 25 g agar, 1 l H_2O) for five days at 24°C [17], and numbers of actinobacteria were assessed on a selective Pochon substrate (0.05 g asparagine, 0.1 g nystatin, 2 g starch, 5g K_2HPO_4 , 2.5g $MgSO_4 \cdot 7H_2O$, 2.5g NaCl, 0.05g $MnSO_4 \cdot 5H_2O$, 0.05 g $Fe_2(SO_4)_2 \cdot 5H_2O$, 25g agar, 11 H_2O) following plate incubation for seven days at 26°C [18].

The performed examination of the soil enzymatic activity in conditions of different tillage systems was based on the determination of the activities of dehydrogenases, acid and alkaline phosphatase, urease, protease, and catalase (in four replications).

The activity of dehydrogenases was identified by the spectrophotometric method, using as substrate 1% TTC (2,3,5- triphenyltetrazolinum chloride), after 24-hour incubation in 30°C at pH 7.4. Triphenylformazan (TPF) was produced, extracted with 96% ethanol and measured spectrophotometrically at 485 nm. Enzyme activity was expressed in μmol TPF $\cdot kg^{-1}$ DM of soil $\cdot 24h^{-1}$ [19].

The activity of acid phosphatase was determined using p-nitrophenylphosphate sodium as substrate, after one hour incubation at 37°C with 400 nm wavelength. Enzyme activity was expressed in μmol NP $\cdot g^{-1}$ DM of soil $\cdot h^{-1}$ [20].

Urease activity was determined using urea as substrate, after one hour incubation at 37°C with 410 nm wavelength. Enzyme activity was expressed in μg N- NH_4^+ $\cdot g^{-1}$ DM of soil $\cdot 18 h^{-1}$ [21].

Protease activity was determined by the Ladd and Butler method [22], with measurement of the concentration of tyrosine released by soil after 1 h incubation at 50°C with a TRIS-HCl (pH 8.1) casein solution. The tyrosine concentration was measured at 578 nm. Protease activity was expressed in μg tyrosine $\cdot g^{-1}$ DM of soil $\cdot 2h^{-1}$. Catalase activity (CAT) in the soil was determined by means of titration [23]. The soil with 0.3% H_2O_2 solution was incubated for 20 minutes and then 1.5 M H_2SO_4 was added. The resulting solution was titrated with 0.02 M $KMnO_4$. The catalase activity was expressed as μmol H_2O_2 $\cdot g^{-1}$ DM of soil $\cdot min^{-1}$.

The results were tested by using standard variance analysis (ANOVA) for the randomized complete block.

Mean separations were made for significant effects with LSD and Tukey tests at probability $P \leq 0.05$.

Results and Discussion

Weather Conditions

Table 1 presents monthly mean temperatures and the sum of precipitation over the study period. In the 2011growing season, spring was warm but dry. Rainfall from March to June oscillated below long-term means, and April and May were particularly dry. In these months, the rainfall constituted 36.6 and 45.1% of the long-term mean, respectively, which as a consequence was the cause of inhibiting and reducing the emergence of spring barley, and of the slower rate of plant development. In 2012, spring vegetation continued under favorable thermal conditions – especially rainfall. In May rainfall was 34% higher, and in June and July more than 2.5-times higher than in the long-term period. This year may be considered as favorable both for spring barley emergence and its development. The third year of research was characterized by unfavorable thermal conditions in March, as the mean temperature was below 0°C, at -2.5°C. Such conditions contributed to a significant delay in the sowing time of spring barley, which shortened its growing season. Further spring barley development continued under favorable thermal and moisture conditions. Rainfall in May, June, and July oscillated above the mean from the long-term period, and June was a particularly humid month. The last year of research had the most favorable weather conditions for growth, development, and yield of spring barley. Temperature and rainfall were above or on

Table 1. Mean daily temperatures of air and sum of precipitation in spring vegetation period of spring barley in 2011-14 and 1961-2010.

Year	Vegetation period					Mean or sum
	March	April	May	June	July	
Mean temperatures (°C)						
2011	3.1	11.7	14.1	18.6	17.9	13.1
2012	5.7	8.8	14.8	16.0	19.2	12.9
2013	-2.5	8.0	14.4	17.3	20.1	11.5
2014	6.6	10.5	13.1	16.1	21.5	13.6
1961-2011	2.9	7.9	13.2	16.6	18.2	11.8
Sum of precipitation (mm)						
2011	25.0	13.9	34.0	52.6	175.4	300.9
2012	20.0	22.9	77.2	163.0	197.6	480.7
2013	12.0	15.4	69.8	125.3	67.3	289.8
2014	39.1	37.2	57.1	64.1	81.2	278.7
1961-2011	40.4	38.0	57.4	61.8	77.5	275.1

a similar level when compared with the means from the long-term period.

Chemical Properties of Soil

The impact of the applied soil tillage systems on selected soil chemical properties is presented in Table 2 and Fig. 1. One of the effects of the application of limited soil cultivation, in particular the complete abandonment of tillage (NT), was a significant increase – in comparison with CT – of organic C and total N in soil. This referred, especially, to the topsoil layer of 0 to 10 cm (Table 2), in which organic C and N contents were 12.2% and 6.5% higher in RT and 25.0% and 10.8% higher in NT in comparison with CT, respectively (Fig. 1). Since the influence of the tillage system was greater with respect to organic C than total N, the C:N ratio also increased together with the reduction in soil cultivation. This positive effect of both ploughless systems (NT and RT) observed in the 0-10 layer of soil translated into the entire topsoil thickness, i.e., 0-20 cm, despite the fact that in the 10-20 cm layer, differences between tillage systems were smaller and statistically significant only with respect to the content of organic C. The recorded interrelationships were in agreement with our earlier research results concerning the long-term application of soil tillage simplifications [24-27] as well as those obtained by other researchers [4, 28-29] in shorter experiments.

In addition, simplifications in soil cultivations also contributed to a decline in soil pH (Table 2). When RT was applied, pH was 0.36 units lower in the near-surface soil layer (0-10 cm), while in NT conditions by 0.90 units in comparison with CT. The trend was similar in the deeper

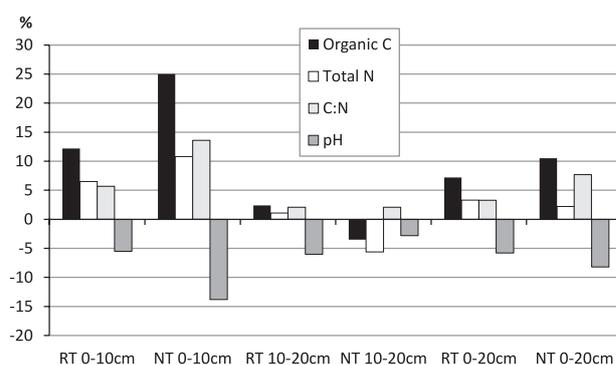


Fig. 1. Impact of simplifications in soil tillage (reduced tillage – RT and no-tillage – NT) on carbon and nitrogen contents as well as soil pH in different soil layers (0-10 cm, 10-20 cm, and 0-20 cm) in comparison with conventional tillage (CT).

soil layer (10-20 cm), but the differences were smaller and statistically non-significant. It is worth noting that, irrespective of the employed tillage system, soil pH values in the deeper layer were higher than in the near-surface layer. In the entire analyzed topsoil thickness treated jointly (0-20 cm), and statistically significant differences in soil pH were recorded only between CT and NT.

Greater soil acidification in RT and NT was probably associated with the intensification of biochemical processes in the soil environment caused by enhanced mineralization of accumulated organic matter. Soil acidification is also affected by such transformation products of organic matter as, for example, CO₂ or low molecular organic acids whose quantity depends on the metabolic activity of microorganisms and enzymes secreted by them [4, 30].

Table 2. Some chemical properties of soil under conventional tillage, reduced tillage, and no-tillage systems at different depths of soil (mean 2011-14).

Parameter	Tillage systems	Soil layer		
		0-10 cm	10-20 cm	0-20 cm
Organic C (g·kg ⁻¹)	Conventional tillage	8.20	8.50	8.35
	Reduced tillage	9.20	8.70	8.95
	No-tillage	10.25	8.20	9.23
	LSD_{0.05}	0.69	0.54	0.59
Total N (g·kg ⁻¹)	Conventional tillage	0.93	0.90	0.92
	Reduced tillage	0.99	0.91	0.95
	No-tillage	1.03	0.85	0.94
	LSD_{0.05}	0.07	n.s.	n.s.
C:N	Conventional tillage	8.8	9.4	9.1
	Reduced tillage	9.3	9.6	9.4
	No-tillage	10.0	9.6	9.8
	LSD_{0.05}	0.61	n.s.	0.58
pH in 1 M KCl	Conventional tillage	6.53	6.82	6.68
	Reduced tillage	6.17	6.41	6.29
	No-tillage	5.63	6.63	6.13
	LSD_{0.05}	0.43	n.s.	0.51

n.s. – not significant

The performed analyses of all results show that the influence of the soil tillage system on soil chemical properties was most evident in the uppermost soil layer and was caused primarily by differences in the distribution of organic matter in the soil profile as well as variations in aeration and, consequently, soil moisture content. Organic C and total N accumulated in the soil derived from living and dead plant tissues (aboveground and underground) as well as other soil organisms. The balance of these chemical elements was also considerably influenced by processes taking place in the soil with the assistance of microorganisms affected, among others, by soil aeration. With respect to N, mineral fertilization with this element is also important as well as its translocation in the soil profile [29]. Ploughing overturns and partially mixes the topsoil and loosens it and, simultaneously, aerates and dries it. The aboveground plant parts get mixed with the deeper layer of the soil profile. The result is rapid disruption of the ecological homeostasis of the topsoil layer and – on

the basis of those transformed ecological conditions — new ecological systems are developed. Non-inversion tillage systems (RT and NT) restricted to loosening and mixing of the topsoil layer mix this layer and plant residues intensively and significantly increase its aeration and, at the same time, do not directly affect deeper soil layers. However, the NT system basically does not interfere with the soil layer system and the aboveground plant residues remain almost totally intact on the soil surface [24, 31]. On the other hand, in NT conditions, numbers of earthworms as well as other oligochaetes in soil can be several times higher than in other cultivation systems [32-33]. These invertebrates relocate considerable quantities of organic matter deposited in the surface to deeper soil layers.

Size of Soil Microorganism Populations

The applied soil tillage systems as well as dates of analyses exert a significant influence on the microorganism

Table 3. Effect of term and tillage systems on the number of soil microorganisms (CFU·g⁻¹ DM of soil) in barley cultivation (mean 2011-14).

Term (A)	Tillage system (B)	Soil layer									
		0-10 cm					10-20 cm				
		Bacteria (n·10 ⁵)	Oligo-trophs (n·10 ⁵)	Copio-trophs (n·10 ⁵)	Actino-myces (n·10 ⁵)	Fungi (n·10 ⁴)	Bacteria (n·10 ⁵)	Oligo-trophs (n·10 ⁵)	Copio-trophs (n·10 ⁵)	Actino-myces (n·10 ⁵)	Fungi (n·10 ⁴)
I	CT	33.88	37.59	20.83	24.07	15.21	35.83	47.07	29.74	29.30	12.97
	RT	45.10	53.68	27.87	36.25	20.16	29.91	46.51	27.40	29.38	12.58
	NT	40.76	40.79	29.57	38.56	14.51	25.52	39.61	23.42	40.17	7.90
II	CT	28.72	29.58	19.64	36.25	9.27	39.86	23.87	18.74	36.04	6.82
	RT	41.26	27.05	16.24	47.77	16.68	26.55	35.45	18.64	33.51	6.62
	NT	37.92	22.10	18.53	45.50	15.45	14.22	23.84	26.42	32.51	8.29
III	CT	23.26	23.82	14.70	25.53	11.34	21.02	26.04	20.15	19.26	8.06
	RT	27.58	31.87	14.51	24.71	12.95	19.16	27.80	18.90	12.51	8.99
	NT	26.26	28.06	20.04	21.03	19.25	17.44	22.64	17.72	15.59	7.04
LSD_{0.05}											
A/B		3.461	3.055	2.915	4.545	2.400	3.522	3.698	3.285	4.920	1.430
B/A		3.694	3.010	2.784	4.062	2.145	3.438	4.239	3.434	4.359	1.400
Average in term											
I		39.91	44.02	26.09	33.01	16.63	30.42	44.40	26.86	32.95	11.15
II		35.97	26.24	18.13	43.17	13.80	26.87	27.72	21.27	34.02	7.24
III		25.70	27.92	16.42	22.76	14.51	19.21	25.49	18.92	15.79	8.03
LSD_{0.05} (A)		3.236	2.504	1.965	2.245	1.189	2.562	4.046	2.916	2.301	1.050
Average in tillage system											
CT		28.62	30.33	18.39	27.62	11.94	32.24	32.33	22.88	28.20	9.28
RT		37.98	37.53	19.54	36.30	19.60	25.21	36.59	21.64	25.13	9.40
NT		34.98	30.31	22.17	35.03	16.40	19.06	28.69	22.52	29.42	7.74
LSD_{0.05} (B)		2.498	2.205	2.104	3.280	1.732	2.542	2.669	n.s.	3.550	1.032

n.s. – not significant

counts differentiation in both analyzed soil layers (Table 3), and there were interactions between these factors. Population size of the majority of microorganism groups were most numerous on the first date of analysis. Also, differences between the applied tillage systems were observed to be most conspicuous on the same date.

Irrespective of the date, nearly all analyzed groups of microorganisms (bacteria, oligotrophs, actinomycetes, and fungi) were most numerous in the soil subjected to reduced tillage (RT), and only copiotrophs were more abundant in conditions of direct sowing (NT). Numbers of soil microorganisms in RT and NT conditions did not always differ from one another in a manner that was statistically significant, although nearly always they were significantly higher in comparison with the soil subjected to CT. Exceptionally, and only with respect to single dates, did a situation occur in which microorganisms were most numerous in CT (oligotrophs and copiotrophs on the second date and actinomycetes on the third date).

In deeper soil layers (10-20 cm), the impact of the soil tillage system on numbers of microorganisms was smaller and less unequivocal. The tillage system was found to most strongly modify the total number of bacteria, which were most numerous when the CT system was employed, whereas their smallest numbers were recorded in conditions of NT. Numbers of oligotrophs were very similar as in the 0-10 cm layer; their highest numbers occurred when RT was applied. On the other hand, numbers of copiotrophs were very unstable on individual dates and tillage systems and, therefore, mean differences between experimental treatments with respect to this group of microorganisms turned out to be small and statistically non-significant. Also, in the case of numbers of actinomycetes and fungi found in the deeper soil layers, the effect of the tillage system varied considerably depending on the date of analysis.

The observed stimulating impact of the RT system on numbers of microorganisms in the topsoil can probably be attributed to the fact that shallow mechanical cultivation mixes soil with organic matter accumulated on the soil surface derived from the fore crop, simultaneously aerating this layer and creating better conditions for the development of microorganisms. This relationship has already been observed many times, both in our experiments as well as by other researchers, and was explained mainly by differences in organic matter distribution in the soil depending on the applied soil tillage [8, 25-26, 34]. Moreover, apart from environmental nutrient availability, higher soil moisture content in the no-tillage system in comparison with ploughing – observed both in dry and wet years – and greater soil aeration in RT conditions in comparison with NT is, undoubtedly, one of the primary factors that positively impacts numbers of microorganisms [3, 35].

As evident from the diagram shown in Fig. 2, in conditions of RT, the total number of bacteria in the 0-10 cm soil layer was by 32.7% higher, of oligotrophs by 23.7%, of copiotrophs by 6.25%, of actinomycetes by 31.4% and of fungi by up to 64.1% in comparison

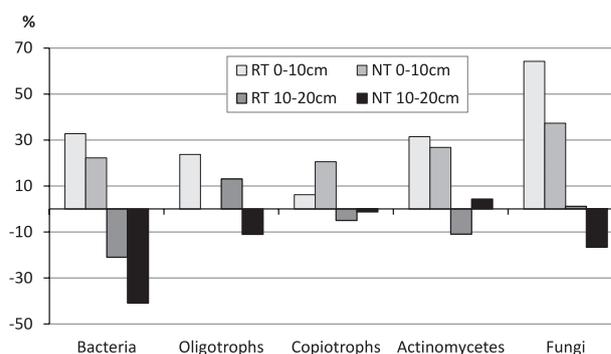


Fig. 2. Impact of simplifications in soil tillage (reduced tillage – RT and no-tillage – NT) on the number of soil microorganisms in different soil layers (0-10 cm, 10-20 cm) in comparison with conventional tillage (CT).

with the CT system. In NT conditions, these numbers were higher in comparison with the CT system, although these differences were somewhat smaller than in the RT system and, in the case of oligotrophs, were not found. Low oligotroph susceptibility to a supply of fresh organic matter, at their simultaneous considerable capability for survival in competitive conditions (e.g., declining amounts of nutrients), is characteristic for this group of soil microorganisms [29, 36-37].

The observed very poor response of copiotrophs to tillage systems was fairly surprising. It is true that in the NT conditions, they were more abundant (by about 20%) in the shallower soil layer than in the remaining systems, but even here differences were not very high, especially compared to other groups of microorganisms. Usually, this group of microorganisms, characteristically for zymogens, positively responds to organic matter accumulated in conditions of application of no-tillage systems in the soil surface layer [24-26, 37-38]. On the other hand, however, it should be noted that the NT system does not supply organic matter rapidly. Rather, it is accumulated systematically in the soil surface layer, which does not have to stimulate any particular increase of copiotrophs number, which takes place in conditions of a rapid supply of organic matter [36].

Interesting information can also be inferred from proportions of oligotrophs to copiotrophs found in individual experimental treatments (Table 4). The value of O:C (oligotrophs:copiotrophs) ratio, sometimes even referred to as the index of soil biological equilibrium [39], changed in the course of barley vegetation from 1.50 to 3.47, reaching its highest mean value on date III, i.e., after plant harvest. The prevalence of oligotrophs over copiotrophs was observed in all tillage systems and in both analyzed soil layers, although the coefficient always reached the highest values in RT conditions and the mean advantage over the remaining systems amounted to 30%. According to Weymann-Kaczmarek [39], such domination is essential to maintain constant levels of soil organic matter and bears witness to sustained soil biological equilibrium.

Table 4. The ratio between the number of oligotrophs to copiotrophs (O:C) and BIF (biological index of fertility; mean 2011-14).

Term	Tillage system	Soil layer					
		0-10 cm		10-20 cm		0-20 cm	
		O:C	BIF	O:C	BIF	O:C	BIF
I	CT	1.97	0.22	1.59	0.47	1.78	0.35
	RT	2.05	0.90	1.82	0.21	1.94	0.56
	NT	1.50	1.20	1.91	0.36	1.71	0.78
II	CT	1.61	0.55	1.61	0.97	1.61	0.76
	RT	2.16	1.50	2.17	0.45	2.17	0.98
	NT	1.92	1.42	1.29	0.51	1.61	0.97
III	CT	1.67	0.39	1.41	0.61	1.54	0.50
	RT	3.47	0.71	1.61	0.28	2.54	0.50
	NT	1.80	0.88	1.63	0.27	1.72	0.58
LSD_{0.05} (A/B) (B/A)		0.50 0.56	n.s.	0.42 0.42	0.15 0.16	0.38 0.40	n.s.
I		1.84	0.77	1.77	0.34	1.81	0.56
II		1.90	1.16	1.69	0.64	1.79	0.90
III		2.31	0.66	1.55	0.39	1.93	0.52
LSD_{0.05} (A)		0.53	n.s.	0.37	n.s.	n.s.	0.32
CT		1.75	0.39	1.54	0.68	1.64	0.54
RT		2.56	1.04	1.87	0.31	2.21	0.68
NT		1.74	1.17	1.61	0.38	1.68	0.77
LSD_{0.05} (B)		0.36	0.26	0.30	0.09	0.36	n.s.

n.s. – not significant

Actinomycetes and fungi demonstrated the strongest response to decreased soil tillage (RT and NT systems) by increasing their numbers in the shallow soil layer. At the same time, in the deeper soil layer, their quantities differed only slightly in relation to those found in CT. This, no doubt, was the result of their role. Both these groups of microorganisms are very active mineralisers of organic matter deposited in soil and play a key role in the turnover of energy and matter in the environment [40].

Enzymatic Activity of Soil

Apart from the population size of different groups of soil microorganisms, one of the most useful indicators of current soil biological conditions, and even its fertility, is its enzymatic activity [41-43]. Interpretational possibilities of soil enzyme activities are also quite considerable and, so far, have not been completely recognized and defined.

Table 5 illustrates the impact of soil tillage systems on the activity of the examined enzymes in two layers of the soil profile. Also in this case, the applied analysis of variance revealed a significant influence of the experimental factors and the interaction taking place between them.

Soil enzymatic activity, similarly to numbers of soil microorganisms, depended to a considerable extent on the date of the performed analysis. However, while in

the case of microorganisms their greatest numbers were recorded on the first date of analyses (i.e., at the stem elongation growth stage of spring barley), the highest enzyme activities were observed on the second date (i.e., at the heading growth stage of spring barley). The only exceptions included catalase (in both layers of the soil profile) and acid phosphatase (in 10-20 cm layers), which were most active on the third date of analyses (after harvesting of spring barley). This can probably be attributed to the fact that numbers of soil microorganisms are closely associated with the presence in soil of organic matter derived from residues of fore crop plants. With the passage of time, the amount of this organic matter undergoes decomposition and decreases, which is why the highest size values of microorganisms were found on the first date, while the lowest were on the third date. Soil enzymatic activity correlated significantly with the effect of living plants, i.e., their condition or current status. The second date of analyses coincided with the period of the most intensive growth and development of barley plants (heading growth stage), which was connected, among others, with high quantities of root secretions as well as a greater demand of plants for mineral constituents. This explanation, in particular, fits very well to phosphatase activities, since these enzymes are to a considerable extent of plant origin. At the stem elongation growth stage and at the very beginning of heading, the demand for phosphorus

Table 5. Effects of term and tillage systems on enzymatic activity of soil in barley cultivation (mean 2011-14).

Term (A)	Tillage system (B)	Soil layer											
		0-10 cm						10-20 cm					
		Deh.	Ac.ph.	Al.ph.	Ure.	Prote.	Cat.	Deh.	Ac.ph.	Al.ph.	Ure.	Prot.	Cat.
I	CT	2.287	0.050	0.070	16.96	0.49	1.90	2.662	0.053	0.091	23.26	0.82	3,42
	RT	3.261	0.072	0.084	19.48	0.94	3.47	1.706	0.050	0.081	25.22	0.86	2,45
	NT	4.749	0.085	0.090	18.52	1.0	4.10	1.398	0.050	0.077	24.05	0.67	3,07
II	CT	3.036	0.068	0.071	31.96	1.60	5.87	3.316	0.063	0.074	37.70	1.61	6,67
	RT	4.934	0.090	0.104	33.87	1.74	7.27	1.552	0.056	0.085	44.92	1.71	4,16
	NT	5.540	0.122	0.116	33.98	1.97	3.30	1.616	0.059	0.085	37.24	1.52	6,16
III	CT	1.641	0.067	0.086	25.01	0.97	6.49	2.034	0.084	0.087	33.77	0.67	6,66
	RT	2.811	0.089	0.095	34.11	0.99	7.69	1.779	0.057	0.079	32.06	0.74	5,84
	NT	3.580	0.109	0.099	29.06	0.64	8.36	1.372	0.053	0.077	27.02	0.13	5,57
LSD_{0.05} (A/B)		0.541	0.005	0.005		0.223	0.586	0.319	0.009	0.004		0.320	0.553
LSD_{0.05} (B/A)		0.528	0.005	0.006	n.s.	0.268	0.514	0.231	0.009	0.004	n.s.	0.202	0.525
Average in term													
I		3.432	0.069	0.081	18.27	0.81	3.16	1.922	0.051	0.083	24.18	0.78	2.98
II		4.503	0.094	0.097	33.27	1.77	7.48	2.161	0.059	0.082	39.95	1.61	5.66
III		2.677	0.084	0.094	29.40	0.86	7.51	1.528	0.065	0.081	30.69	0.51	6.02
LSD_{0.05} (A)		0.394	0.004	0.005	2.054	0.268	0.256	0.296	0.007	0.034	0.320	n.s.	0.301
Average in tillage system													
CT		2.321	0.062	0.076	24.59	1.02	4.75	2.671	0.066	0.084	31.58	1.03	5.58
RT		3.667	0.084	0.097	29.15	1.22	6.14	1.479	0.054	0.082	34.06	1.10	4.15
NT		4.623	0.106	0.094	27.19	1.20	7.20	1.462	0.054	0.080	29.44	0.77	4.93
LSD_{0.05} (B)		0.390	0.004	0.004	3.112	0.161	0.423	0.231	0.007	0.028	0.202	n.s.	0.420

Deh.- dehydrogenases ($\mu\text{mol T PF}\cdot\text{kg}^{-1}\text{ DM of soil}\cdot 24\text{h}^{-1}$); Ac.ph.- acid phosphatase ($\mu\text{mol PNP}\cdot\text{g}^{-1}\cdot\text{DM of soil h}^{-1}$); Al.ph.- alkaline phosphatase ($\mu\text{mol PNP}\cdot\text{g}^{-1}\cdot\text{DM of soil h}^{-1}$); Ure.- urease ($\mu\text{g N-NH}_4^+\cdot\text{g}^{-1}\cdot\text{DM of soil 18 h}^{-1}$); Prot.- protease ($\mu\text{g tyrosine g}^{-1}\cdot\text{DM of soil 2 h}^{-1}$); Cat.- catalase ($\mu\text{mol H}_2\text{O}_2\cdot\text{g}^{-1}\text{ DM of soil}\cdot\text{min}^{-1}$); n.s. – not significant.

is still high and available forms of phosphorus in soil are becoming increasingly exhausted. Then plant roots secrete phosphatase, which takes part in the mineralization of organic forms of phosphorus, i.e., making it available for plants [43, 47].

The effect of the applied tillage system on soil enzymatic activity depended on soil layer (Table 5, Fig. 3). In the near-surface soil layer (0-10 cm), all the examined enzymes exhibited a significantly higher activity in simplified systems (RT and NT) in comparison with CT. Dehydrogenases, acid phosphatase, and catalase were all most active in conditions of NT, whereas the remaining enzymes (alkaline phosphatase, urease, and protease) were most active when RT was employed.

On the other hand, in the case of soil at a depth of 10-20 cm, activities of the majority of enzymes (with the exception of urease), when simplified systems were applied (RT and NT), were distinctly lower than in the

near-surface soil layer, while in CT conditions they were found to be at a similar level and, in fact, were slightly higher. Therefore, in the deeper soil layer, dehydrogenases, acid, and alkaline phosphatase as well as catalases showed the highest activity in CT conditions and only urease and protease when RT was applied. The only enzyme whose activity failed to differ significantly in relation to the employed tillage system in this soil layer was protease.

Enzyme response to soil tillage simplification in comparison with CT was very noticeable, in particular in the case of dehydrogenases, but also of acid phosphatase and catalase. These enzymes behaved similarly to the majority of the examined groups of microorganisms, i.e., in conditions of the application of soil tillage simplifications, and their activity in the near-surface soil layer was several dozen percent higher than when the CT system was applied, while in the deeper soil layer it was distinctly lower.

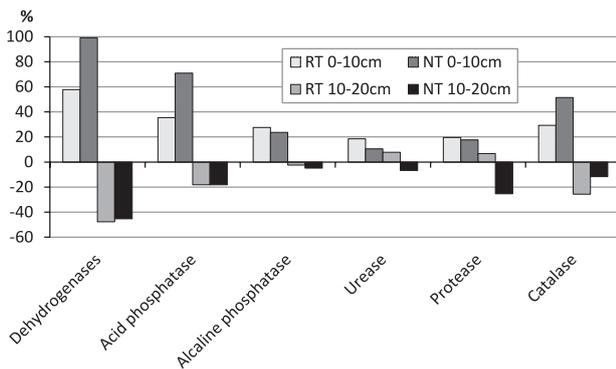


Fig. 3. Impact of simplifications in soil tillage (reduced tillage – RT and no-tillage – NT) on the enzymatic activity in different soil layers (0-10 cm, 10-20 cm) in comparison with conventional tillage (CT).

With respect to the dehydrogenase, these observations are in agreement with the reports in [43] as well as other researchers [34, 42, 44-46]. The higher activity of this group of enzymes observed in NT and RT (especially in its surface layer) in comparison with CT is attributed first and foremost to variations in the content of fresh organic matter as well as to different physicochemical properties developing in various layers of the soil profile under the influence of the applied tillage systems.

The accumulation of organic matter in RT and NT systems in the near-surface soil layer is also decisive for higher activities of phosphatases and plays an important role during processes, which make nutrients available for plants from forms that are inaccessible for them [25, 42]. Phosphatases belong to the group of enzymes most frequently examined in soils of different agroecosystems. Their activity is connected, among others, with soil colloids and humus substances whose levels do not undergo rapid changes and, therefore, are particularly useful for monitoring long-term changes associated with the intensity of agriculture [47-48].

In the presented studies, catalase also responded very strongly to simplifications in soil cultivation by increasing activity in the shallower soil layer and decreasing activity in the deeper layer in comparison with CT (Fig. 3). The reasons for such response, again, should be sought in the availability of fresh organic matter and its distribution in the soil profile, which is probably associated with the fact that catalase is an enzyme that can be found mainly in the cells of microorganisms using oxygen for respiratory processes [49]. There are many contradictory reports concerning factors influencing catalase activity in the soil profile. Koper and Piotrowska [41] reported high activity of the enzyme after wheat seedling emergence as well as in the rosette phase of rape, whereas other researchers failed to report any variation in seasonal catalase activity, maintaining that the activity of this enzyme was closely connected with the vegetation cover and depended on the root system of the cultivated plant [50].

The enzymes that exhibited the weakest response to tillage simplifications were protease and urease. The

activity of these enzymes is a sign of transformation intensity of nitrogen compounds in the environment and can serve as an indicator of its availability for plants in the tillage system [51]. Protease takes part in the mineralization process of organic nitrogen compounds, and their primary sources in soil are bacteria and fungi. In turn, urea hydrolysis to carbon dioxide and ammonia is catalyzed by ureases. This enzyme can be found in cells of higher plants and microorganisms, especially bacteria. A factor strongly limiting its activity is substrate availability – urea [43].

For years, in experiments evaluating soil quality, researchers have been looking for a universal indicator suitable for the fertility assessment of all soils irrespective of their specificity. When designing such an indicator, it is impossible to take into consideration all soil physical, chemical, and biological properties and selection is necessary. According to many scientists, soil enzymatic activity combined with its selected chemical properties reflect on its fertility as well as the intensity of processes taking place in it [41]. One such indicator is the biological index of fertility (BIF) – an algorithm determined on the basis of the activity of dehydrogenases and catalase ($BIF = [1.5DHA + 100kCAT]/2$, where k – the proportionality coefficient = 0.01) [52].

In the presented experiments, a particularly wide range of activity obtained for dehydrogenases and catalase indicates usefulness of this group of enzymes for the assessment of changes in soil environment under the influence of soil tillage systems. BIF calculated on the basis of the activity of these enzymes (Table 4) turned out to be significantly dependent on the soil tillage system, but only with respect to individual soil profile layers. In the near-surface soil layer (0-10 cm), BIF value increased together with limitations in soil cultivation, reaching its highest mean values of 1.04 and 1.17 in RT and NT conditions, respectively. On the other hand, in the 10-20 cm layer, BIF assumed the highest values in CT conditions. The observed positive impact of both ploughless systems in the 0-10 cm layer was so strong that it translated onto the entire analyzed topsoil thickness treated jointly (0-20 cm), although in this case, differences between the applied system were not statistically significant.

Conclusions

Soil quality is an integrated function of biological, chemical and physical properties of soil. Soil management practices significantly changed the basic soil properties. In this study a change of soil quality related to biological properties as a function of soil C management (reduced versus conventional tillage) occurred after 15 years, mainly at 0-10 cm depth. This shows how much time is needed for any management change to have an impact under these environmental conditions. The results of this experiment showed that reduced tillage and especially no-tillage increased total carbon, total nitrogen, the number

of microorganisms, and microbial activity compared with conventional tillage. It suggests that tillage has a significant impact on soil C cycling through its effects on soil microbial activity. A significant relationship between organic C and soil biological quality also suggests that a soil's biological quality can be used as an early indicator of soil quality. The contradictory results of other studies may be due to differences in soil properties, climatic characteristics, crop species, and their complex interactions, and more research is needed to assess these relationships.

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