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Use of soil enzyme activity in assessing the effect of No-Till in the South of Russia

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Abstract. The activity of 11 enzymes (catalase, dehydrogenases, peroxydases, polyphenoloxidases, ascorbateoxidase, ferrireductase, β-fructofuranosidase, amylase, urease, phosphatase and protease) was assessed under conditions of steppe zone in the south of Russia when using different tillages. Winter wheat and sunflower are main crops in these soils. Moreover, chickpea, coriander, lint, barley, corn and some other crop are cultivated here in the crop rotation duration 6 years. Enzyme activity was compared in soils of 15 fields with long-term no-till (NT) versus to 15 fields with conventional tillage (CT). The researches were held along the whole Haplic Chernozem Loamic at a depth of 0–65 cm. Special attention was paid to top soil (0–10 cm), which is directly subject to the mechanical effect. The carbon cycle enzyme (β-fructofuranosidase) activity was the most sensitive indication for NT use. In top soil the enzyme activity was greater by 16–35% at NT versus to CT. Activity of this enzyme reduces by 28–293% when soil depth increasing in both the tillages. Enzymes of different classes had different behaviours in soils depending on season, crops and tillage thanks to biochemical nature. Hydrolases and oxidoreductases were assessed by the indices characterizing soil condition and health. For this purpose geometric mean by hydrolase activity (GME_{hd}) and geometric mean by oxidoreductase (GME_{ox}), as well as integral index of biological soil condition (IIBC) were used. Index GME_{ox} in soil under sunflower reduced by 16% in summer versus to spring. Thereby, hydrolase index GME_{hd} reduced by 60%. At NT activity of oxidoreductase was lower by 10 and 13%, and activity of hydrolase was increased by 12 and 14% versus to CT. Soil IIBS values at NT increased by 18–35% at average within three years (2016–2018). The use of NT technology contributes to an increase in the activity of hydrolases and soil quality due to the conservation of moisture in the soil.

Key words: bioindicator, biological activity, soil enzymes, No-Till, soil health indexes.

INTRODUCTION

Biological indicators are often used as sensitive indicators of soil fertility under different tillages and the degree of its degradation under anthropogenic impacts (Trasar-Cepeda et al., 2008; Burns et al., 2013; Minnikova et al. (2019a, 2019b, 2019c)). The soil enzyme activity is an important diagnostic criterion during assessment of soil quality at different types of man-caused impact on soils (Trushkov et al., 2019; Kazeev et al., 2020). The study of the enzymatic activity of soils is important: characterization of the metabolic potential of soils, quality and fertility, assessment of resistance to various natural and anthropogenic influences (Shukla & Varma, 2011; Akimenko et al., 2014; Loeppmann et al., 2016). Enzymes serve as indicators of the cycle of carbon (β-fructofuranosidase, amylase), nitrogen (urease, protease), phosphorus (phosphatase). These cycles play a key role in assessing soil quality and maintaining soil health. The activity of soil enzymes is important in assessing the quality of soils and diagnosing their ecological state (Hugh, 2012; Burns et al., 2013; Kazeev et al., 2015; Kolesnikov et al., 2019).

During conventional tillage (CT) fruitful soils obtain new properties, the morphogenetic statuses and soil-formation factors are changed (Azarenko et al., 2020). One of the processes resulting in reformation of tillable chernozem is tillage practices (Minnikova et al., 2017, Minnikova et al., 2018; Mokrikov et al., 2019). The nutrient cycle without the appropriate fertilizer element returning in the form of fertilizers is violated due to change in soil microbiota that affects soil organic matter pool. Therefore, mouldboard plowing causes decrease of humus level and reduction of soil enzyme activity (Dadenko et al., 2014; Garbuz et al., 2016; Sharkov et al., 2016; Zhelezova et al., 2017). In case of no-till (NT) as resource-saving analogy of the conventional tillage, soil dysfunction is significantly reduced that protects soil from degradation towards resistivity in combination with the crop rotation. The number of macroaggregates is an important microbiological process for control mechanism and organic substance maintenance (Beare et al., 1994; Dick et al., 1984; Gupta & Germida, 1988). When using various tillages with deep moldboard plowing, nitrogen and carbon cycles are violated in soil that causes change in microbial biomass and soil enzyme activity (Franchini et al., 2007; Paz-Ferreiro & Fu, 2016; Hlisnikovský et al., 2020). Due to maintenance or destruction of organic matters content the enzyme activity can be a powerful indicator of soil agrogenic transformation and remediation processes (Lagomarsino et al., 2009; Mancinelli et al., 2013; Marinari et al., 2015; Vilny, 2015; Shirokikh et al., 2017; Bai et al., 2018). The soil biochemical activity is a good index for assessment of tillage effect on the structure and microbiological activity of the soils. Under influence of second crop residues organic-matter degradation processes are intensified and carbon cycle enzyme (dehydrogenases, fluorescein diacetate hydrolysis, β-glycosidase, alkaline phosphatase) activity is increased. In case of no-till the soil structure is improved that causes increase in content of organic matter and microbial biomass (Garbuz et al., 2016; Sharkov et al., 2016; Mokrikov et al., 2020).

This work gives brief results for the comparative assessment of long-term use of NT under conditions of the steppe zone of Russia in the Rostov Region within the last 5 years. This resource-saving and soil protective technology is widely used in the world, but it is poorly known in Russia. It allows gathering steadily heavy yields of agricultural crops even under conditions of unstable watering within the risks farming in agriculture zone. Particularly under such conditions NT shows its advantages versus to conventional moldboard plowing (CT) and other tillage with soil turbating (Riley et al., 1994; Soane et al., 2012; Gristina et al., 2018). The CT with plowing result in dehumification and reduction of the biological activity for chernozem in the south of Russia (Dadenko et al., 2014; Azarenko et al., 2020). On the contrary, NT has a positive effect on the reserve of fertilizer elements and soil physical properties (Minnikova et al., 2018; Trofimova et al., 2018; Kravtsova et al., 2019; Mokrikov et al., 2019).

The research gap of our study to the assessment of soil enzyme activity in assessing the effect of NT in the South of Russia. NT technology is reserved moisture versus CT and increases the activity of soil enzymes of different classes. Analyze the change in the activity of hydrolases and oxidoreductases under different processing technologies. Assess soil quality using condition indices and IIBC.

MATERIALS AND METHODS

Site Description. The research objects were fields with NT and CT. The experimental fields have been processing within 10 years by NT and are located in the south of the steppe zone in Russia. The researched area exceeds 5.5×10^3 ha. Within the researched area ordinary chernozems are commonly spread, which genesis, properties and fertility are well-known from the literature source (Valkov et al., 2008, 2012). In total pair wise comparison of 30 fields with different tillages (15 fields with NT and 15 fields with CT) were researched. The areas with NT were compared with CT areas at a distance of 50–100 m from each other. The major part of the crop production areas are occupied with grain crops (winter wheat and barley) - 49%, grain maize - 10%, sunflower and permanent legume grasses (melilot, lucerne, sainfoin, tare) - 10%, grain legume (chickpea, lentil, pea) - 8%, winter crucifers (false flax, rape, mustard) - 6.5%, coriander - 6%, lint - 4%, false saffron - 2.5%, buckwheat - 2.0%, permanent grasses - 2%.

The agricultural crops were seeded during NT by the following machines: tractor Buhler Versatile 2375 + Great Plains NTA 3510 (10.7 m) and Case Magnum 315 + Great Plains NTA 3510 (10.7 m), all crops were seeded with row width of 19.1 cm. As per the articles when using NT fuel consumption was 26 L ha^{-1}, during conventional tillage with moldboard plowing - 74.1 L ha^{-1} (Mokrikov et al., 2017, Minnikova et al., 2018; Mokrikov et al., 2019).

Experiment Methodology. As a result of complex environmental researches 45 parameters were determined characterizing ecological state of soils in 30 fields with NT and CT. Soils were researched 3–5 times per a season in 2017–2020. Samples were taken from different depths 0–10, 10–20, 20–30, and 50–60 cm. The mixed samples for the surface soil layer were obtained from 5 individual samples taken at a distance of 3–5 m from each other along the diagonal. The soil was dried out in the shade, free from roots and organic residues, sieved through 1 mm screen and analysed within several days after sampling in 3–9-fold analytical replication. The work represents the results of the enzyme activity analysis for 2016–2020.

In 2016 the large-scale comparative diagnostics of soil ecological and biological condition by activity of 11 enzymes of oxidoreductase and hydrolase classes were performed (Table 1). Within the next 4 years quantity of researched enzymes were restricted with the most informative indices well proven within the first year of the our research (2016). Comparison of the activity for separate enzymes is not always informative. Thus, for assessment of soil quality and condition by the indices of soil enzyme activity of each class the geometric means were calculated for enzymes of each class in absolute units (Hinojosa et al., 2004). The geometric means (GME_{ox}, GME_{hd}) were used as a single index number for integration of all soil enzyme activity indices being similar by nature, but having different ranges of variation.

Table 1. List of determined enzymes and methods of determination

No.	List of enzymes (EC)	Method of determination
	Class of Oxidoreductases	
1.	H_2O_2: H_2O_2-oxidoreductase, EC 1.11.1.6.	by the volume of decomposed oxygen during the decomposition of hydrogen peroxide
2.	TPP : NAD (F) - oxidoreductase, EC 1.1.1	for the reduction of tetrazolium salts to formazan
3.	NAD (F) • H_2: Fe_2O_3 – oxidoreductases, EC 1.6.99	by the amount of reduced ferric iron
4.	O-diphenol: oxygen – oxidoreductase, EC 1.10.3.1	on the oxidation of hydroquinone to quinones
5.	quinone: H_2O_2 – oxidoreductase, EC 1.11.1.7	on the oxidation of hydroquinone to quinones
6.	L - ascorbate: oxygen oxidoreductase, EC 1.10.3.3	by the difference between the amount of residual ascorbic acid and the amount of formed dehydroascorbic acid (DHAA)
	Class of Hydrolases	
7.	invertase, sucrose, EC 3.2.1.26	by the amount of glucose during the hydrolysis of sucrose, colorimetrically using Felling's reagent
8.	β-amylase: 1,4 - α - glucan - maltohydrolase, EC 3.2.1.2	by the amount of glucose during start hydrolysis
9.	urea – amidohydrolase, EC 3.5.1.5.	by the amount of ammonia with Nessler's reagent, with hydrolysis of carbamide
10.	phosphohydrolases of monoesters of orthophosphoric acid, EC 3.1.3.1-2	by the amount of inorganic phosphorus during hydrolysis of sodium phenolphthalein phosphate
11.	peptide – hydrolases, EC 3.4.4	by the number of amino acids during proteolysis of casein

The more index value is the better soil capability to function and maintain the plant performance.

The geometric mean of the oxidoreductase activity by formula 1:

$$GME_{ox} = \sqrt[6]{CAT \times DHG \times ASC \times PER \times PHEN \times FER} \quad (1)$$

CAT – activity of catalase, 1 mL O_2 × 1 g soil^{-1} × 1 min^{-1}; DHG – activity of dehydrogenases, mg TPP × 10 g soil^{-1} × 24 hours^{-1}; ASC – activity of ascorbatoxidases, mg DHAA × 1 g soil^{-1} × 24 hours^{-1}; PER – activity of peroxidases, mg 1,4 benzoquinone × 1 g soil^{-1} × 30 min^{-1}; $PHEN$ – activity of polyphenoloxidases, mg 1,4 benzoquinone × 1 g soil^{-1} × 30 min^{-1}; FER – activity of ferrioreductase, mg Fe_2O_3 × 100 g soil^{-1} × 48 hours^{-1}.

The geometric mean of the hydrolase activity by formula 2:

$$GME_{hd} = \sqrt[5]{INV \times UR \times PHOS \times AM \times PRL} \quad (2)$$

INV – activity of β-fructofuranosidase (invertase), mg of glucose × 1 g soil^{-1} × 24 hours^{-1}; UR – activity of urease, mg NH_3 × 1 g soil^{-1} × 24 hours^{-1}; $PHOS$ – activity of phosphatase, mg P_2O_5 × 100 g soil^{-1} 1 hour; AM – activity of amylase, mg glucose × 1 g soil^{-1} × 24 hours^{-1}; PR – activity of protease, mg glycine × 1 g soil^{-1} × 24 hours^{-1}.

Moreover, for assessment of enzyme activity the previously proven integral index of biological soil condition ($IIBC$) was used (Kazeev et al., 2016). This index allows combining a great amount of soil enzyme activity indices.

Specific values of each NT soil enzyme were calculated versus to values of CT soils by formula 3:

$$B_1 = \frac{B_{NT}}{B_{CT}} \times 100\% \qquad (3)$$

Then values of the integral index of soil biological activity were calculated by formula 4:

$$IIBC = \frac{B}{B_{max}} \times 100\% \qquad (4)$$

where B – average score point for activity of all enzymes; B_{max} – maximum score point for activity of all enzymes.

For assessment of high correlation ratio between enzyme activity indices and soil moisture content correlation factors were calculated.

Data were statistically processed using Python 3.6.5 program, Matpolib package.

RESULTS AND DISCUSSION

Climate and moisture reserve in the soil with different tillages: Within the researched period the climatic conditions differed from average long-term ones by total amount of precipitations (Fig. 1).

Precipitations laid down within the vegetation season for 2016–2018 exceeded the climatic rate by 66, 35 and 21%. Based on the precipitation amount 2016 and 2017 were humid due to precipitations in spring.

In 2018 since April till June there was less amount of precipitation by 79–83%, than within previous years (Mokrikov et al., 2019). The temperature conditions were closer to the climatic norm; the average annual air temperature was from 12.1 °C in 2016 to 11.6 °C in 2018. The soil temperature was determined by the air temperature and according to the researches it was changed by loggers in soil within a year. For understanding features of thermal conditions within the researched area accumulating temperature sensors - loggers Thermochron DS1921 were positioned. The sensors were located at three depths 10, 20 and 30 cm. The reading frequency is every 6 hours. This allowed assessing the temperature features in top mostly biogenic layer with maximum root expansion of agricultural plants.

As a result it was detected that the surface layer (0–10 cm) has significantly higher temperature fluctuations than at depths of 20 and 30 cm from 21.3 to 31.5 °C. While depth increasing daily temperature fluctuations were less intensive. Temperatures of 0 °C and only by 3–4 °C lower in the soil at a depth of 10 cm prevail since November till the beginning of March. At a depth of 20 cm negative temperatures are settled for 3 winter months. At a depth of 30 cm low temperatures occur within the restricted time since the end of January till the end of February.

Temperature below 10 °C, which is taken as a temperature of active biological processes, at a depth of 10 cm was since the middle of October till the beginning of April. Moreover, achievement of this level was noted in the middle of November during temporary warming. At a depth of 20 cm the border below 10 °C was overcome since the first half of October till the beginning of April. At a depth of 30 cm temperature of

10 °C being optimum for the biological processes was settled only by the beginning of May due to long-term and chilly spring.

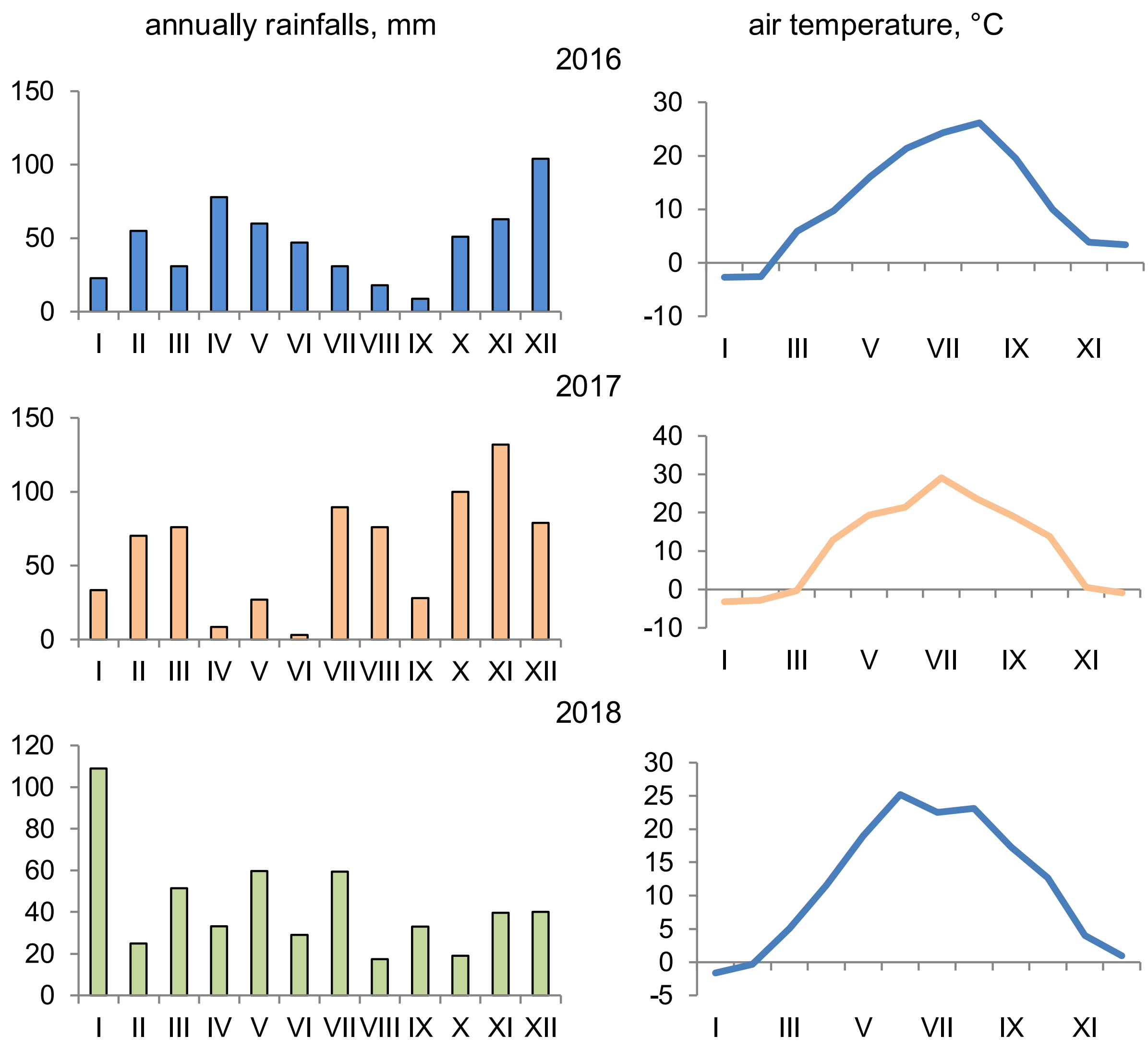

Figure 1. Changes in annually rainfalls and air temperature (2016–2018).

As Fig. 2 shows, at NT since September till November moisture conservation by 19–92% was detected versus to CT.

In general within 5 years of observations the soil moisture content with NT was higher by 22% versus to CT. This is conditioned by fewer amounts of process operations and availability of after harvesting residues at the soil level. They keep the snow cover in winter and contribute to moisture accumulation.

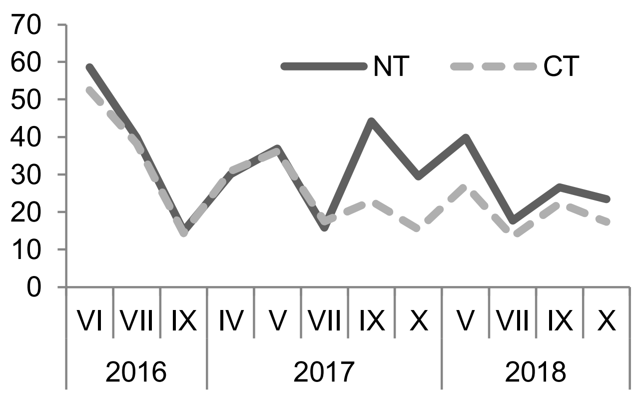

Figure 2. Dynamics of soil moisture NT for the period from June 2016 to November 2018 in the top soil, % versus CT.

Change in the enzyme activity of soils NT versus to CT. Change in the activity for enzymes of oxidoreductase and hydrolase classes at NT versus to CT is given in Fig. 3. The activity of oxidoreductase (peroxydases, polyphenoloxidases and ferrireductase) changes versus to CT both towards stimulation by 8–44% and inhibiting (dehydrogenases, catalase and ascorbateoxidase) by 8–15% versus to CT.

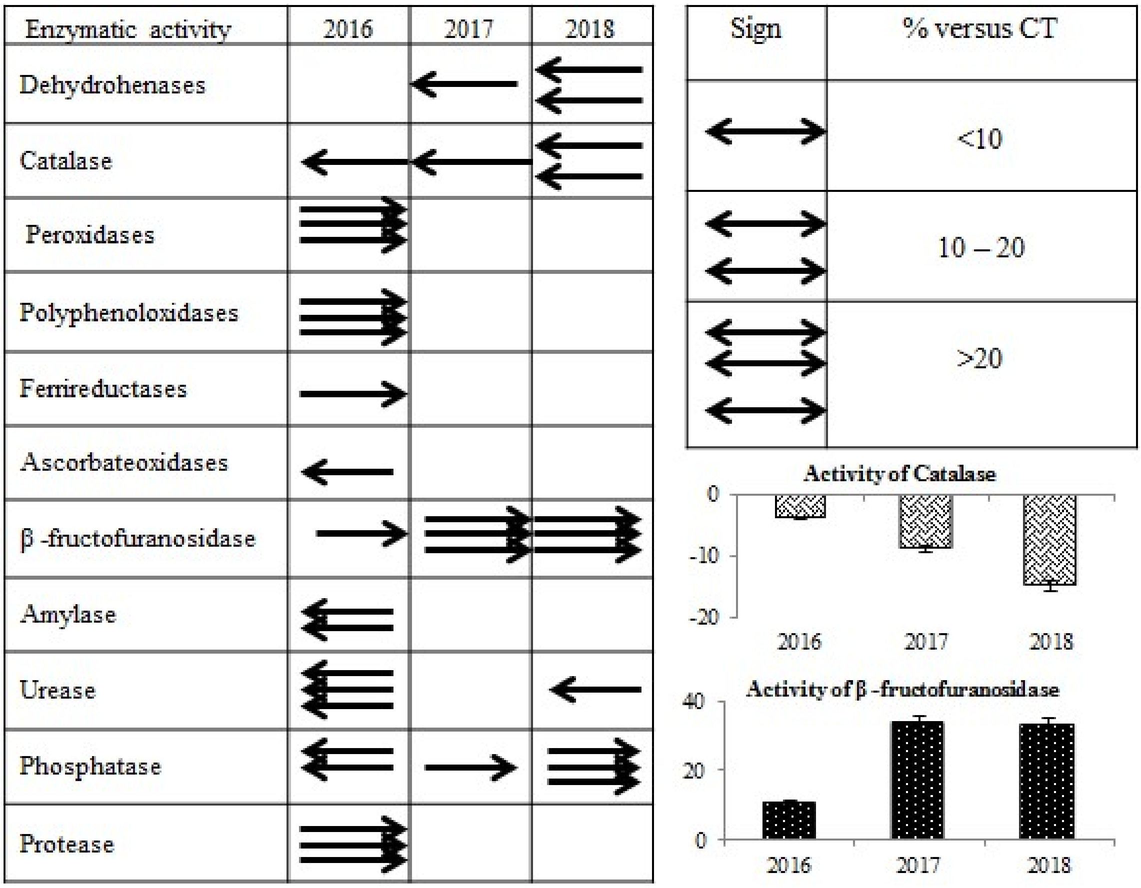

Figure 3. Estimation of the enzymatic activity of soils under NT, % versus to CT.

The activity of hydrolase class enzymes was changed by other principle. Thus, the activity of carbon cycle enzymes (β-fructofuranosidase and amylase) was compared in 2016 (1 year of research). It is determined that NT reduces the amylase activity by over 30%, but stimulates the activity of β-fructofuranosidase by 10%. Thereby, in 2017 and 2018 during the research more stimulation of β-fructofuranosidase activity was obtained by 33–34% versus to CT. Such an effect is conditioned by increase in concentration of organic matters in top soil due to agricultural crop organic residues digestion.

Multistage changes β-fructofuranosidase activity of soil under different tillages. The specialized change in the activity of the major part of oxidoreductase (catalase and dehydrogenases) at NT didn't differ from CT versus to hydrolase. Among hydrolases β-fructofuranosidase is the most sensitive to tillage tehnologies. The activity of β-fructofuranosidase as a carbon cycle enzyme depends on the carbon content in the soil and the accessibility of its forms (Fig. 4). The enzyme activity reduces at lower levels during all tillage types by 81–293% at NT, by 28–152% at CT. In 0–10 cm layer the enzyme activity was higher by 16–27% at NT (in April, May, September and

October). In July within the period of maximum moisture lack in the soil and in the absence of precipitations the actual difference of the β-fructofuranosidase activity from CT was not detected. Within spring months in the beginning of the intensive vegetation different results are obtained.

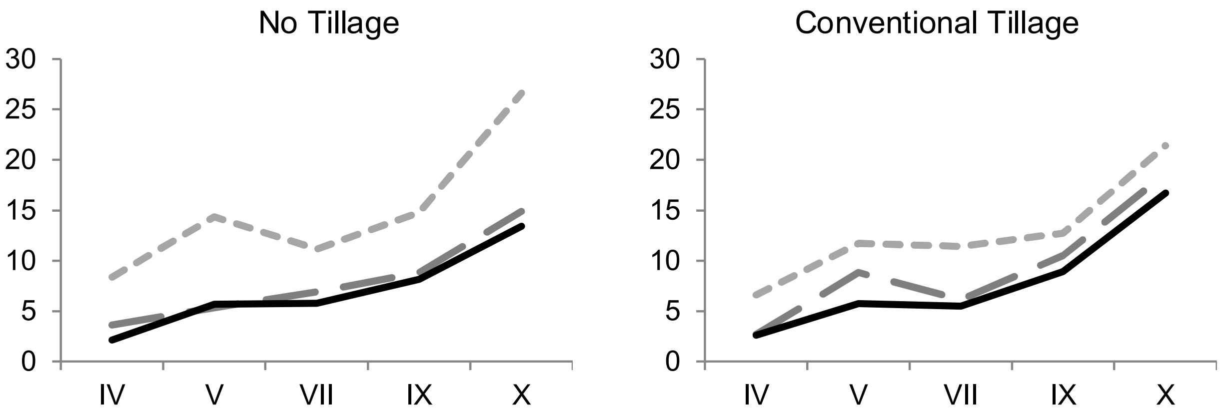

Figure 4. Change in β-fructofuranosidase activity under different tillage, mg of glucose × 1 g soil^{-1} × 24 hours^{-1}.

In April at a depth of 25–35 cm increase in the enzyme activity by 35% was increased versus to CT. On the contrary in May inhibiting of the enzyme activity by 40% was observed. In September and October at this depth difference from CT varied from 16 to 20%. This is conditioned by the fact that at NT the surface root layer is kept. Thus, the β-fructofuranosidase activity was stimulated by moisture content increase. In underlying layers the activity was increased to 40%. At a depth of 55–65 cm differences from CT were detected only in April and October by 19–20% higher than within similar months at CT.

Assessment of soil condition using soil health quality indexes. For assessment of the soil condition by all enzyme activity indices the indices based on geometric mean by each enzyme class (oxidoreductases and hydrolases) were used. As a result of soil enzyme activity determination the soil quality index was calculated. According to data of the each class enzyme activity the geometric mean was calculated for all hydrolases (GME_{hd}) and all oxidoreductases (GME_{ox}). Table 2 shows change in the geometric mean for 11 enzymes of hydrolase class (β-fructofuranosidase, amylase, urease and phosphatase and protease) and oxidoreductase class (dehydrogenases and catalase, peroxydases, polyphenoloxidases, ascorbateoxidase) during the research to 2016.

It was detected that in July versus to May reduction in GME_{ox} values by 16% and for hydrolases reduction in GME_{hd} values by 60% were observed. Moreover, the index values for oxidoreductase is less by 2–4 times versus to hydrolase. Reduction in the hydrolase activity is directly associated with change in the soil moisture content due to less amount of precipitation in

Table 2. Geometric mean for hydrolases (GME_{hd}) and oxidoreductases (GME_{ox}) of soils with binary crops to 2016

month	May	July	September
GME_{ox}	4.8	4.0	3.9
GME_{hd}	16.7	6.2	9.0

Note: GME_{ox} – geometric mean activity of oxidoreductases (n = 6); GME_{hd} – geometric mean of hydrolases activity (n = 6).

July and August. In September, the hydrolase activity became higher, than in July by 46%, but less, than at the beginning of vegetation. This is associated with autumn precipitations. For oxidoreductase activity this dependence is less expressed.

When comparing the tillages for 2016–2018 the following results were obtained at average per each season. Table 3 shows the results of the geometric mean calculation for hydrolases (β-fructofuranosidase, urease and phosphatase) and oxidoreductases (dehydrogenases and catalase) 2016–2018.

Table 3. Geometric mean for hydrolases (GME_{hd}) and oxidoreductases (GME_{ox}) of soils with different tillage during the vegetation seasons of 2016–2018

(numerator – NT, denominator – CT)			
year	2016	2017	2018
GME_{ox}	$\frac{14.8}{15.1}$	$\frac{4.7}{5.1}$	$\frac{14.1}{16.2}$
GME_{hd}	$\frac{10.1}{10.6}$	$\frac{2.2}{1.9}$	$\frac{5.1}{4.5}$

Note: GME_{ox} – geometric mean activity of oxidoreductases (n = 6); GME_{hd} – geometric mean of hydrolases activity (n = 6).

In comparison with sunflower seeding NT of different agricultural crops had stimulating impact or didn't have impact. Only within 2017–2018 the oxidoreductase activity at NT was decreased by 10 and 13% versus to CT. On the contrary for hydrolases GME_{hd} was higher by 12 and 14% in 2017 and 2018 accordingly. Such common trends of the activity as oxidoreductase and hydrolase were conditioned increased moistening at the beginning of vegetation and subsequent non uniform amount of precipitations within the season of 2016. In 2017 and 2018 according to the data of Table 3 amount of precipitations (in mm) was less, than in 2016 that determined optimum conditions and favourably effected the activity of the soil enzymes, and, thus, the soil ecological condition.

Within 2016–2018 in NT fields the integral index of the soil biological condition (IIBC) increased by 18–35% (Fig. 5). Only in one field IIBC remained unchanged: its level was close to CT within the whole observation period.

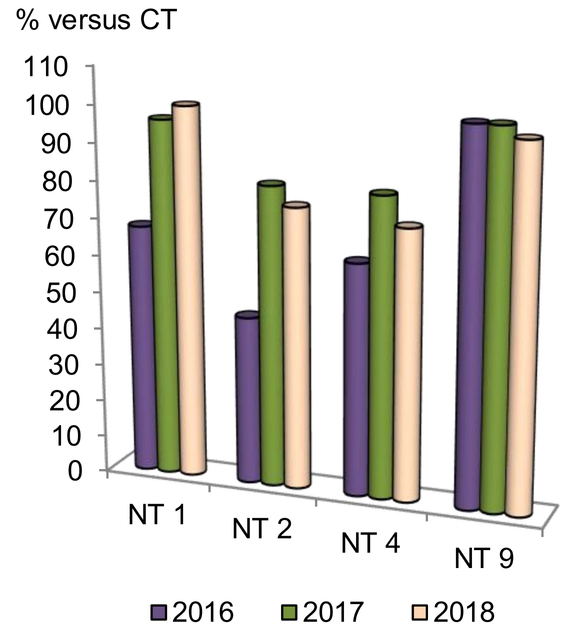

Figure 5. Change in the integral indicator of the biological state of soils by fields with NT for 2016–2019, % versus CT.

Within 2016–2018 during the investigation maximum increase of IIBC values was observed in NT 1 and NT 2 fields - 33 and 30% versus to CT. In NT 4 field the index value growth was 10%. Such an increase is used as indicator of soil condition improvement during NT use.

We conducted an experiment in the fields with the same crop - winter wheat, which showed a high sensitivity of enzymes of the hydrolase class, the values of which were generally higher on soils with NT versus to CT. The physical properties of soils such as moisture, temperature, density, penetration resistance have an indirect effect on the change in the enzymatic activity of soils. The activity of oxidoreductases, in comparison with hydrolases, changes to a lesser extent during agricultural use of arable land (Mokrikov et al., 2017; Azarenko et al., 2020). However, the possibility of using the activity of catalase and dehydrogenases in assessing the state of soils and the relationship with hydrothermal conditions and enzymatic activity in dynamics, taking into account crop rotation (Demina et al., 2016; Franke et al., 2018). The activity of enzymes from the class of oxidoreductases was less dependent on soil treatment than the activity of hydrolases (Roldan et al., 2005; Acosta-Martínez et al., 2008; Melero et al., 2009; Sinsabaugh, 2010; Papp et al., 2018). The activity of β-fructofuranosidase as an enzyme of the class of hydrolases is associated not only with root exudates, crop residues, but also with density, resistance to penetration, the amount of moisture in the soil, and pH (Vazquez et al., 2017). Similar results on the stimulation of hydrolase activity in different types of soil cultivation were obtained earlier by other authors. This is due to the fact that hydrolytic enzymes are involved in the metabolism of organic substances, an increased amount of which is formed on the soil surface during NT. As a result, the activity of these enzymes is associated with the organic carbon content in soils (Katsalirou et al., 2010; Gong et al., 2018). In the studied soils using the NT technology, an increase in the degree of organic matter humification and the content of permangate oxidized organic matter was found (Mangalassery et al., 2015; Hok et al., 2018). Along with an increase in soil moisture when using NT technology, an increase in the activity of hydrolases associated with hydrolytic processes of transformation of organic matter in soils is explained. The major indication of soil restoration at NT is soil organic matter accumulation and an increase of soil fertility caused by soil microbiota (Kudeyarov, 2015; Mokrikov et al., 2020).

CONCLUSIONS

It was found that the activity of enzymes in the chernozems of southern Russia depends on the season, soil moisture and depth of sampling, soil different tillage and the class of enzyme. The maximum change in enzymatic activity was noted in the top soil horizon. A decrease in soil moisture leads to inactivation of enzymes, regardless of the method of soil cultivation. The activity of hydrolase enzymes is recovered to a greater extent by soil moisture, and the activity of oxidoreductase enzymes depends more on soil temperature. Long-term use of NT technology leads to the increase of the activity of soil enzymes, especially the enzyme of the carbon cycle - β-fructofuranosidase.

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