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COMPARISON OF SOME SOIL ENZYMATIC ACTIVITIES IN LUVISOL OF CONSERVATION AND CONVENTIONAL TILLAGE IN A MODEL EXPERIMENT

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Abstract

The effect of soil tillage operation on soil biological properties has not been extensively studied in Hungary. We investigated some soil biological enzymatic activities (dehydrogenase, β-glucosidase, and phosphatase) of a Luvisol, treated by different tillage, management intensities, i.e., conservation tillage (CT), fully conventional tillage with mouldboard ploughing every year (PT), and moderately conventional tillage with shallow and deep ripping intermittently in every two years (BR). A pot experiment was carried out in climate-controlled growth chamber for six weeks as a model experiment, of using the composite soils (0-20 depth) with the three types of tillage intensity. Our finding suggested, that adding of the crop residues might increase the soil organic matter content, that is reflected by the high concentration of labile carbon in the CT soil. The greater intensified soil aeration at the conventional tillage operation, contributed to the much higher dehydrogenase activity in the PT and the BR soil. Otherwise, the higher aeration of soil resulted a decreased β-glucosidase activity in the conventional tillage (BR) soil. The high phosphorus availability of soil correlated by the lowest phosphatase enzymatic activity and the improved available P ratio in CT soil, indicating the inhibition of phosphatase activity. The soil biological enzymatic activities was shown to be affected by the presence of different substrates at the three management practices.
Keywords: *Labile carbon, dehydrogenase, β-glucosidase, phosphatase, mineralization*

ENZIM AKTIVITÁSOK ÖSSZEHASONLÍTÓ VIZSGÁLATA TALAJKÍMÉLŐ ÉS HAGYOMÁNYOS MŰVELÉSŰ MODELLKÍSÉRLETBEN LUVISOLS TALAJOKON

Összefoglalás

Vizsgáltuk a Luvisol talajbiológiai, enzim-aktivitását különböző talajművelési intenzitások mellett, azaz kímélő (CT), teljesen hagyományos (PT) és mérsékelt-hagyományos talajművelési gyakorlat (BR) mellett modell-kísérleti háttérrel. A klímaszabályozott növényszobában hat héten át tenyészedényes kísérletet végeztünk a háromféle talajművelésből származó talaj felhasználásával. Eredményeink azt mutatták, hogy a növényi maradványok hozzáadása növeli a talaj szervesanyag-tartalmát, amit a CT talajban lévő labilis szén magas koncentrációja is tükrözött. A hagyományos talajművelés mellett az erősebb talajművelés valószínűleg hozzájárult a PT és a BR talajok magasabb dehidrogenáz enzim aktivitásához. A nagyobb-fokú művelés csökkentette a β-glükozidáz enzim aktivitását is a hagyományos művelésű talajban (BR). A magas foszfor hozzáférhetőség a foszfatáz enzim aktivitás csökkenését váltotta ki a rendelkezésre álló foszfor mennyiségével összhangban a CT talajban. Megállapítottuk, hogy a

talajok biológiai aktivitását a rendelkezésre álló szubsztrátok jelenléte befolyásolja a különböző művelésű talajokban, szoros összefüggésben a talajok nedvességtartalmával.
Kulcsszavak: *labilis szén, dehidrogenáz, β-glükozidáz, foszfatáz, mineralizáció*

Introduction

Creating a favorable environment for root growth and seedbed preparation are the main purposes of soil tillage in agriculture. Mouldboard ploughing and disc-harrowing are the common techniques in tillage operation called conventional tillage. The investigation by KHAN et al. (2017); AHUJA et al. (1998) indicated the alteration of soil physical properties under conventional tillage, such as the lower bulk density, higher aeration and total porosity, and the changing of pore size distribution. On the other hand, many studies have reported that long-term conservation tillage practice results in soil structure deterioration and reduces soil aggregate- and organic matter content (KLADIVKO, 2001; ROPER et al., 2010; ZHENG et al., 2018). Soil organics and the related and enhanced soil-biological activities are crucial in considering soil health parameters among the agri- and horti-cultural conditions (VERMANN et al., 2021).

Conservation tillage leaves ~30% of the litter on the surface of the soil is the system that harmonizes soil protection with the demands of the crop, soil, and climate (BIRKÁS et al., 2014; BOGUNOVIC et al., 2019). Conservation tillage increases the soil organic matter that plays an important role in the quantity, diversity, and activity of soil microorganisms in the upper soil depth (CHOUDHARY et al., 2018; PEIGNÉ et al., 2018; SZABÓ et al. 2022). In addition, the minimum soil disruption in conservation tillage enhances the stability of the rhizosphere bacterial community (WANG et al., 2017). Conservation tillage has been implemented in Hungary for over three decades (BIRKAS et al., 2017). Some researchers have compared the effect of conservation and conventional tillage on soil properties, including soil water content, penetration resistance, crumb ratio, and crusted area (BOGUNOVIC et al., 2019); soil physical properties, earthworm abundance, and crop yield (DEKEMATI et al., 2019). Several previous long-term studies that emphasize soil erosion, nutrient loss, and nitrogen use efficiency in different tillage intensity has also been reported by MADARÁSZ et al. (2011, 2021), JAKAB et al. (2017), JUG et al. (2019). The investigation to figure out the effect of different tillage intensities on soil biological enzymatic activities has not been widely carried out in Hungarian Luvisol, whereas this soil type dominates the arable land for agriculture in Hungary.

We assessed the labile carbon and the activity of soil enzymes of the Luvisol soil to find out how the tillage intensity influences some of the soil biological activity, assessed by the investigating some enzyme functioning.

Materials and methods

Soil treatments

This study used the Luvisol soil (IUSS WORKING GROUP WRB, 2014) collected from Szentgyörgyvár and Baranya, Hungary, in the 2020 spring season. The soil material was sampled at 0-20 cm depth by the composite method. Each composite sample comprised of minimum four random sampling points representing the area of the three types of soil tillage intensity (Table 1).

Table 1. Types of soil tillage intensity and origine of the soils, used in the model experiment.

Type	Description
Conservation tillage (CT)	Non-inversion reduced tillage (8–12 cm depth), leaving ~30 % of the soil surface covered with crop residues, and a cultivator (8–10 cm depth) to weed control in every year.
Fully conventional tillage (PT)	Mouldboard ploughing (up to 25–30 cm depth) in every year.
Moderately conventional tillage (BR)	Shallow and deep ripping (up to 15 cm and 45 cm depth) intermittently in every two years.

Five hundred grams of CT, PT, and BR soils were packed into plastic pots. Three seeds of corn (*Zea mays L.*) were sowed and frequently irrigated. The seven days after sowing and the young plants have emerged, thinning out was conducted to select a plant with the best growing. The nutrient treatments of using microbial biofertilizer industrial products was used, by applying Bact-Inoc in 15 l ha^{-1}, Myc-Inoc in 10 kg ha^{-1} dosage, and No-Inoc established in the soil of each tillage intensity (products and industrial producers are available at the authors).

A completely randomized design (CRD) that consisted of five pots for each nutrient treatment was employed in this experiment. So, there were fifteen pots totally for each soil tillage intensity. The treated pots are then arranged in the tray and kept in the plant growth chamber for about six weeks. The air temperature was set for an average of 25 °C. An air conditioner unit was employed to assist the air circulation in the chamber. For sunlight replacement, two units of LED UV grow light lamps were installed above each tray and lighted on for 24 hours. The soil moisture content of all pots was maintained to 100% of the field capacity condition.

Soil analysis

A composite soil sample was taken from each nutrient treatment pot, and soil water content was measured. Further, the soil is kept in the fridge at 4 °C until soil enzymes analysis to maintain the soil fresh. The other part of the composite samples was dried at room temperature (20 °C) for labile carbon and available P analysis. Labile C was measured by the $KMnO_4$ oxidation method (WEIL et al., 2003). A 1 g air-dried soil was reacted in $KMnO_4$ and shaken. Samples were then measured spectrophotometrically at 565 nm wavelength. The potential available P was determined by 0.03 M NH_4F and 0.1 M HCl extraction methods (BRAY & KURTZ, 1945).

Dehydrogenase activity (DHA) was assessed by the optimizing method of VERES et al. (2013). 1 ml soil solution (the soil - water ratio is 1:10) was reacted with triphenyl tetrazolium chloride (TTC) and then incubated for 24 hours at 30 °C. Methanol was used to terminate the enzymatic reaction. The mixture then was centrifuged in the mixture to obtain the soil supernatant. The DHA was measured spectrophotometrically at 546 nm wavelength.

The **β-glucosidase activity** was assessed by SINSABAUGH et al. (1999). 1 ml of soil-water solution (1: 20) in a test tube was reacted to PNP-β, while the other two tubes were reacted with the Na-acetate buffer. The tubes were then incubated for 2 hours at 30 °C. A Tris-hydroxymethyl (aminomethane) (pH 12) and $CaCl_2$ solution were added to stop the enzymatic reaction. The supernatant was obtained by centrifuging the tubes. A spectrophotometer was employed to measure the activity of β-glucosidase at 410 nm.

A similar procedure was used in determining **soil phosphatase activity**. The method was based on the amount of P-nitrophenol (PNP) converted from p-nitrophenyl-phosphate

($PNP\text{-}PO_4$). The $CaCl_2$ and NaOH solutions are added to this measurement to stop the enzymatic reaction. A spectrophotometer was operated at 410 nm to measure the soil phosphatase activity SINSABAUGH et al. (1999).

Data analysis

We compared the Luvisol soil under different tillage intensities (CT, PT, and BR), where the nutrient treatments *viz*. Bact-Inoc, Myc-Inoc, and No-Inoc as the replication. The ANOVA and Pearson correlations test were employed using IBM SPSS statistics for windows, version 27.0 (IBM Corp., 2019). All analyses were carried out with a significant p-value of 0.05. In addition, the assumption test, normality and heterogeneity of variance were checked for the data. The heterogeneity of variance for the activity of dehydrogenase, β-glucosidase, and phosphatase was "violated" with a p-value < 0.05; therefore, we performed the post-hoc test by the Games-Howell method.

Results

Tillage intensity significantly affected the measured soil biological enzymatic activities in our experiment. The ANOVA result of studied soil biological activities is shown in Table 1.

Table 1. The ANOVA table for means of assessed soil biological and enzymatic activities in the modell experiment with luvisol and various management intensities

Soil biological activities	df	Mean Square	F	Sig.
Labile Carbon	2	46100.646	21.533	0.000
Dehydrogenase	2	13.097	11.548	0.001
β-glucosidase	2	2.205	8.241	0.004
Phosphatase	2	54.348	7.556	0.005

Labile Carbon

Labile carbon concentration was significantly higher in the CT soil (460.54 ± 36.77 mg kg^{-1}) followed by BR (394.73 ± 42.02 mg kg^{-1}) and PT soil (317.55 ± 57.48 mg kg^{-1}) (Fig. 1). This circumstance indicated that high organic matter input by the crop residue increase the soil carbon content and it confirmed the previous results by BONGIORNO et al. (2019); MALOBANE et al. (2020); KOTROCZÓ et al. (2022).Our finding also suggested that the more intensive tillage practice, the lower soil organic carbon concentration. It could be explained that conventional tillage practice damages the soil structure. In addition, the temperature in the growth chamber was very proper to the activity of soil microorganisms. This condition boosted the soil organic matter decomposition, resulting in organic carbon losses through the air and the water (LAJTHA et al., 2018; BILANDŽIJA et al., 2017; CHOWANIAK et al., 2020; JUHOS et al., 2021).

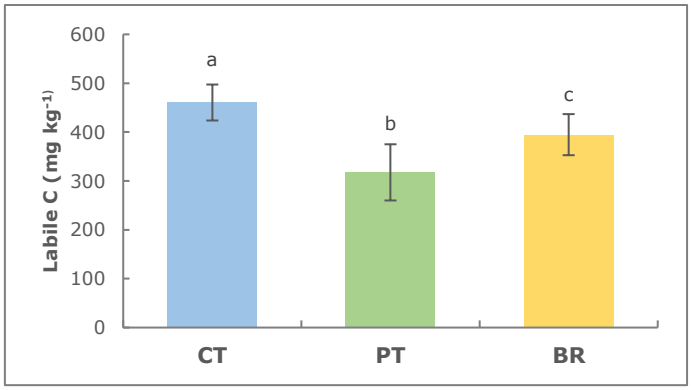

Figure 1. Labile carbon concentration of soil under different tillage intensities. The different lowercase letter (a and b) means a significant difference in soil tillage intensity ($p < 0.05$).

Dehydrogenase activity

The highest activity of dehydrogenase occurred in the BR soil (3.09 ± 1.82 TPF µg g^{-1} dry soil) compared to CT and PT soil (0.34 ± 0.07 and 0.77 ± 0.24 TPF µg g^{-1} dry soil consecutive) (Fig. 2). DHA associates with soil moisture and reflects the microbial redox system and the oxidative activities of the soil (WEAVER et al., 2012; WOLINSKA & STEPNIEWSK, 2012). Tillage operation in the BR and PT soil has improved the soil porosity implying a lower soil water holding capacity than the CT soil. Henceforth, potentially, the BR and PT soil to get dry faster than CT soil triggering the DHA increment.

β-glucosidase activity

β-glucosidase activity in the BR soil was smaller (0.34 ± 68 ± 0.48 TPF µg g^{-1} dry soil) than in CT and PT soil (1.62 ± 0.24 and 1.82 ± 0.71 TPF µg g^{-1} dry soil respectively) (Fig. 2). The more substrate availability by the crop residue application in the CT soil escalated the β-glucosidase activity (SINSABAUGH et al., 2008). ZHANG et al. (2011) stated that the soil water content also drove β-glucosidase activity. In the case of PT soil, even though the substrate was smaller than CT soil, the soil water content was probably higher than BR. Therefore, the activity of β-glucosidase was not inhibited by the lower soil water content as in the BR soil.

Phosphatase activity

The higher phosphatase activity (13.13 ± 0.86 µmol g^{-1} $hour^{-1}$) was recorded in the CT soil later on in PT (11.65 ± 3.97 µmol g^{-1} $hour^{-1}$) and BR soil (7.34 ± 2.24 µmol g^{-1} $hour^{-1}$) (Fig. 2). Microorganisms, plant, and other environmental factors impact the phosphatase activity. The phosphatase activity will decrease along with the decline of soil water content (SARDANS & PEÑUELAS, 2004). The good aeration in the conventional tillage soil (PT and BR) has led to the low soil water content hindering the phosphatase activity.

The amount of organic matter in CT soil corresponded to the higher mineralization (Fig. 2) that released a large concentration of ammonium and nitrate. These high nitrogen concentrations stimulate P mineralization (OLANDER & VITOUSEK, 2000;), proven by the higher concentration of available P in CT soil (85.25 mg kg^{-1}) than in PT and BR soil (38.61 and 44.80 mg kg^{-1} consecutively). The solubility of inorganic P also determines the activity of phosphatase. Therefore, the high concentration of inorganic P will inhibit the phosphatase activity (GIANFREDA et al., 2005). Our recent study showed a resemble situation, where the

phosphatase and available P ratio in CT soil was lower (5.4) than in PT and BR (8.2 and 8.6, respectively), indicating the inhibition of phosphatase activity by the higher phosphorus concentration in CT soil.

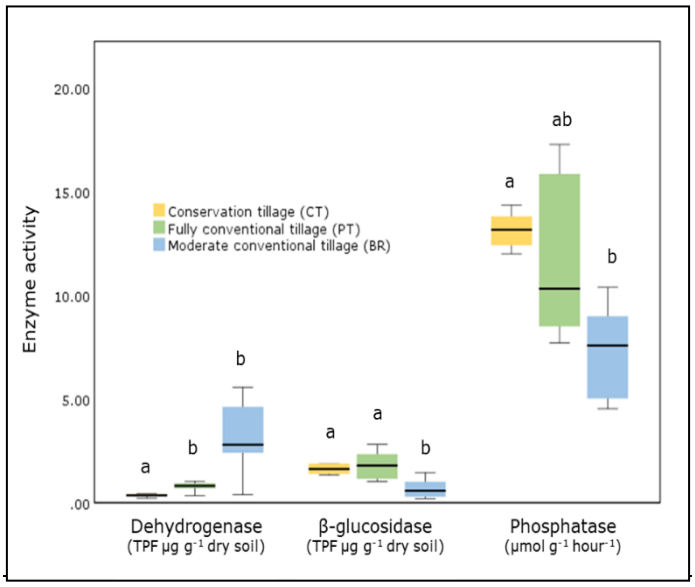

Figure 2. Soil enzyme activities (dehydrogenase, β-glucosidase and phosphatase) under different tillage intensities. The different lowercase letter (a and b) means a significant difference in soil tillage intensity ($p < 0.05$).

Conclusions

In this study, we investigated the soil biological activity, such as the labile carbon, and three different types of soil-enzymes across different tillage intensities of Luvisol soil, in a model experiment Our result highlighted low disruption of soil by tillage activity, and the abundance of crop residue in the conservation tillage soil elevated the labile carbon concentration. On the other hand, the disturbance of soil structure in the conventional tillage soil led to high soil porosity that associates with the water holding capacity. Finally, soil biological activity results from the soil's biochemical process depending on the soil substrate availability driven by the environmental factor.

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