Universidad del País Vasco · Euskal Herriko Unibertsitatea

ZTF-FCT Zientzia eta Teknologia Fakultatea · Facultad de Ciencia y Tecnología

FACULTY OF SCIENCE AND TECHNOLOGY. LEIOA

FINAL DEGREE PROJECT IN BIOTECHNOLOGY

THE INFLUENCE OF PRO-ENVIRONMENTAL AGRICULTURAL PRACTICES ON CARBON CONTENT AND THE ACTIVITY OF RELATED ENZYMES (β-GLUCOSIDASE, CELLULASE AND INVERTASE) IN SOIL AFTER FOUR YEARS OF WINTER WHEAT MONOCULTURE

Alumn: ***Carracedo Lorenzo, Zulema***

Date: June 2016

Director
Dr. Anna Piotrowska-Długosz

Codirector
Dr. Sonia Bañuelos Rodríguez

Academic Year
2015/16

INDEX

1. INTRODUCTION

Monoculture is a widely used agricultural practice that consists in producing or growing genetically similar, or essentially identical plants, over large areas, year after year. It has been shown that monocultures produce high yields as the plants grow without the pressure of other species and with uniform plant structure (http://www.chgeharvard.org/topic/biodiversity-and-agriculture). However, monocultures are selected for specific conditions and when these conditions change there is a high risk of losing the entire crop. For that reason, several agricultural practices have been proposed in order to prevent the worsening of soil properties under long-term monocultures (http://www.chgeharvard.org/topic/biodiversity-and-agriculture).

The quality of the soil depends not only on its natural composition, but also on the changes caused by the human use and agricultural practices (Pierce and Larson, 1993). Thus monocultures, among many other agricultural practices, can be responsible of the worsening of the physical, chemical and biological properties of the soil (Fauci and Dick, 1994; Melero et al., 2006). The actual concern for the sustainability of soil quality has promoted the development of pro-environmental agricultural practices whose intention is to reduce the negative impact of soil management, including monocultures (Piotrowska and Wilczewski, 2014). These environmental practices that prevent the loss of organic matter include the addition of organic matter, such as straw, the use microbial fertilizers and the use different tillage systems (simple plowing; grubber after harvest tillage; pre-sowing plowing; direct sowing of winter wheat after no-till). However, proper organic management appears to be the most effective practice.

Tillage system causes a great perturbation in soil environment (Madejon *et al.,* 2007; Karaca *et al.,* 2011). The use of one tillage system or another varies in soil quality, as it alters soil physicochemical, hydrological, microbiological and biochemical properties, hence influences soil microbial community diversity and the production of soil enzymes. Karaca *et al.* (2011) proved that tillage also affects nutrient levels in soil and its availability, distribution of organic matter in the soil profile, soil water and oxygen content and soil fertility. Tillage exposes more soil organic matter to microbial attack causing a rapid loss of soil organic matter and in the end a decline of crop productivity, an increase of soil erosion, a reduction in soil biological activity and, in the long term, to a decrease in the sustainability of soil (Valarini *et al.,* 2002). For that reason, it has become more common the used of no-plough tillage and direct sowing, that consists in leaving a large amount of post-harvest residues on the soil surface. This type of tillage has some advantages compared with conventional tillage. Some studies have proven that it has organizational and economic advantages (Jaskulski *et al.,* 2012) and that it could beneficially affect the physical (Lepiarczyk *et al.,* 2007, Tian *et al.* 2013, Topa *et al.,* 20014), chemical (Lenart and Sławiński 2010, Swedrzyńska *et al.,* 2013) and biological properties of the soil (Swedrzyńska *et al.,* 2013). In addition, no-till or reduced tillage have the potential to conserve SOC (soil organic carbon) by reducing mineralization (Abdalla *et al.,* 2013) and enhancing soil aggregation (Six *et al.,* 2000). Nevertheless, it has some disadvantages too: it often leads to an increase in soil density and is the cause of an impediment in its emergence, reduction in the development of the root system and plant yields (Małecka *et al.,* 2012, Haliniarz *et al.,* 2013). Due to that disadvantages the yields may not differ significantly between conventional tillage and no-till tillage (Wesołowski *et al.,* 2011, Haliniarz *et al.,* 2013).

The straw management is important in agriculture, as its beneficial used maintaining soil quality has been recognized (Chander *et al.,* 1997). Several studies have shown that straw has a high content of organic materials and soil nutrients so it could be a natural organic fertilizer which may replace chemical fertilizers (Dick *et al.,* 1988; Duiker and Lal, 1999; Saroa and Lal, 2003; Tan *et al.,* 2007; Bakht *et al.,* 2009) that are harmful to the environment. Straw management practices can influence the soil C sequestration rates since soil C contents depends on the input and decomposition rates of organic matter in the soil. The straw addition to the field can increase soil aggregation and accumulation of SOC. It has also been shown that straw has significant effects in improving the activity levels of soil enzymes (Garg and Bahl, 2008). However, the effects of the straw retention are partly influenced by climate and soil conditions (Powlson *et al.,* 2011; Curtin and Fraser, 2003), so it is not clear the extent to which the addition or not of straw affects soil quality.

The loss of the soil organic matter may be solved with the addition of bio-fertilizers as there is evidence to suggest that they accelerate the transformation of organic matter by increasing soil biology activity and help to liberate available nutrient for plants. Bio-fertilizers are substances that contain different microorganisms that increase the availability and intake of mineral nutrients for plants. They probably help to liberate some nutrient such as available phosphorous through nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant hormones, production of antibiotics and biodegradation of organic matter in the soil (Sinha *et al.,* 2014). However, the microbial composition of the bio-fertilizers remains unknown, making it difficult to evaluate their effectiveness (Schenck zu Schweinsberg-Mickan and Muller, 2009). This situation has created a discussion between the defenders and opponents of their efficiency. The followers of bio-fertilizers state that those compounds balance nutrient supply, enhance soil structure and encourage the growth of beneficial microorganisms, among other benefits. Meanwhile, the opponents say that nutrient release rate is too slow to meet the requirements of the crop, that the nutrient composition of the compost is highly variable and that their cost is higher to the cost of traditionally used chemical fertilizers (Mishra and Dash, 2014).

The so-called "effective microorganisms-EM)" is one of the most used bio-fertilizers (Schenck zu Schweinsberg-Mickan and Muller, 2009). It has been proved to improve the quality of the soil, increasing not only the soil organic matter, but also the plant growth and yield (*Emiko,* 2003). Researchers have made efforts to find methods to analyze the effect of the addition of biomass and bio-fertilizers in the soil.

It has been found that there is a relationship between the soil biological activity and the soil quality and fertility, so that long-term soil management requires being controlled from the point of view of its biological activity (Feng *et al.,* 2003). Soil ecosystem is inhabited by a large number of microorganisms who carried out organic matter transformation and are the major source of soil enzymes (Said and Kpomblekou-A, 2009). Indeed, soil microbial biomass, through the decomposition of organic matter, releases nutrients into plant available forms, and degrades toxics. Schloter (2003) reported that soil microbial biomass and activity is an important aspect of soil quality. That is why microbial biomass is considered a sensitive parameter used to analyze changes in organic matter composition of the soil (Brookes, 1995). In addition, traditionally used physical and chemical parameters of soil quality represent slowly changes in soil characteristics, such as soil structure or organic matter pool and nutrient balance (Shukla and Varma, 2011); whereas microbial biomass let us to measure changes in soil features in a short frame of time, which is more useful from the point of view of humans. Two microbial indices have been suggested to monitor soil quality changes: the microbial biomass C, N and P and soil microbial biomass.

Soil enzymes are involved in the cycling of the most important nutrients (C, N, P and S) and are constantly being synthesized, stored, inactivated and/or decomposed in the soil (Tabatabai, 1994; Dick, 1997). Dick (1997) found that among the reactions catalyzed by soil enzymes are the decomposition of organic inputs, transformation of native soil organic matter, released of inorganic nutrients, N_2 fixation, nitrification, denitrification and detoxification of xenobiotics. As their activity can be affected by the agricultural practices, they have also been suggested as an index of soil microbial activity and fertility (Benitez *et al.,* 2000). There are several reasons why they are considered sensitive indicators of soil quality: they measure main microbial reactions involving nutrient cycles in soil; they respond rapidly to changes in both, natural and anthropogenic factors; and they are easily measured (Shukla and Varma, 2011) and produce reproducible results (Klaus Schaller, 2009). Moreover, they predict changes in soil environment (Shukla and Varma, 2011), allowing us to take action before the damage is done.

For all that reasons, studying the interactions among soil enzymes and organic matter transformation will help us first to understand better the activity of the soil ecosystem; and second, to design new agricultural practices to gain more productive crops while at the same time being respectful with the environment.

1.1. SOIL ENZYMES INVOLVE IN C CYCLE

1.1.1. β-glucosidase

β-glucosidase is one of the most abundant enzymes in the soil (Eivazi and Tabatabai, 1988; Tabatabai, 1994). It catalyses the hydrolysis and biodegradation of various glucosides present in plant debris decomposing in the ecosystem, having a key role in soils (Ajwa and Tabatabai, 1994; Martinez and Tabatabai, 1997). The final product of the reaction that catalyse this enzyme is glucose, an important C energy source of life for microorganisms of soil (Esen, 1993).

β-glucosidase is used as a soil quality indicator. It has been demonstrated that it can provide evidence of changes in organic carbon long time before it can be measured using other routine methods (Dick, 1994; Dick *et al.,* 1996; Wick *et al.,* 1998). It is very sensitive to changes in pH and soil management practices and it is inhibited by heavy metal contamination. The sensitivity to pH changes can be used as a biochemical indicator for measuring ecological changes that are a result of soil acidification in situations that affect the activity of β-glucosidase (Utobo and Tewari, 2014).

For all of that, it is crucial to understand β-glucosidase and its activity and factors that affect it in the ecosystem in order to improve the soil management.

1.1.2. Invertase

The most abundant sugar in plants is sucrose, a disaccharide of glucose and fructose. This sugar has an unusual characteristic; it does not contain free anomeric carbon atoms. So it does not act as a reducing sugar, making its hydrolysis easier than in other disaccharides.

Invertase or saccharase is the enzyme which catalyses the hydrolysis of sucrose into its two disaccharydes, D-glucose and D-fructose, and has been extensively studied because of its widespread distribution in plants and soil (Ross, 1983; Frankenberger and Johanson, 1983). This enzyme is abundant in microorganism, animals and plants and it has an optimum activity in soil at pH 5.0-5.6 and temperature 50ºC.

Some studies have found a significant correlation between invertase activity and the amount of organic carbon in the soil, while other did not find significant correlation (Alef and Nannipieri, 1995).

Understanding the invertase and its relationship with soil carbon would be useful in order to use it as a parameter of soil quality.

1.1.3. Cellulase

Cellulose is a linear polymer of D-glucose with β(1-4) glucosidic linkages and is the most abundant structural polymer of plant cell walls; what is more, it the most abundant organic compound in the biosphere. For this reason, hydrolization of the cellulose by soil microorganisms is an important process in the degradation of plant debris into glucose, cellobiose and high molecular weight oligosaccharides (Alef and Nannipieri, 1995).

Cellulases is the system of enzymes which catalyses the hydrolysis of cellulose. This system is form of three enzymes: endo-β-1,4-glucanases which randomly cleave glucosidic linkages along non-crystaline parts of cellulose; exo-β-1,4-glucanases which binds to crystalline cellulose and cleave celluloligosaccharides from the non-reducing ends of cellulose molecules; and β-glucosidases which release glucose from celluloligosaccharides and aryl-β-glucosides (Alef and Nannipieri, 1995). Richmond (1991) found out that the majority of soil cellulases come from the plant debris added on it, and that only a little proportion come from microorganisms of soil.

In agricultural soils the degradation of cellulose is a slow process in which cellulases can be affected by several factors that includes temperature, soil pH, water and O_2 contents, the chemical structure of organic matter/plant debris and its location in the soil profile horizon (Deng and Tabatabai, 1994; Alef and Nannipieri, 1995), quality of organic matter and soil mineral elements (Deng and Tabatabai, 1994; Arinze and Yubedee, 2000) and the trace elements from fungicides (Deng and Tabatabai, 1994; Petkar and Rai, 1992; Arinze and Yubedee 2000; Atlas *et al.*, 1978; Vicent and Sisler, 1968).

Due to the importance of cellulases in the recycling of cellulose, the most abundant polymer of the biosphere, it would be useful to understand this enzyme in order to apply as a predictive tool in soil fertility programmes (Das and Varma, 2011).

2. OBJECTIVE

The aim of this project was to determine the effect of different tillage systems, straw and biofertilizer application on the carbon content and the activity of the C-related enzymes such as β-glucosidase, invertase and cellulase.

3. METODOLOGY

3.1. THE EXPERIMENT AND SOIL SAMPLING

The study is based on a three-factor field experiment in a dependent lay-out of equivalent sub-blocks (split-plot, split-block) with three repetitions. The influence of the following factors were investigated: I factor- different tillage practices (grubber after harvest tillage + grubber in autumn; simple plowing, grubber after harvest tillage; pre-sowing plowing; direct sowing of winter wheat after no-till); II factor - straw management (straw that was removed; crumbled straw that was left), III factor – bio-fertilizer (Effective Microorganisms – EM, control without EM). Soil samples are collected from the Ap horizon (0-27 cm) 4 time a year 2015 (April, June, August, November) to determine seasonal variation of studied properties. A bio-fertilizer was applied in a dose of 40 dm^3 ha^{-1} every year (2011-2015) always after winter wheat harvest.

3.2. DETERMINATION OF MICROBIAL BIOMASS C

A fumigation-extraction method was used to estimate microbial biomass C (MBC) with extractable C converted to microbial C using a standard factor (Kc =0.38) (Vance *et al*., 1987). Soil sample was placed in desiccator with wet tissue paper and the beaker with 25 ml of chloroform with a few boiling chips. The desiccator was evacuated until the chloroform has boiled vigorously for 2 minutes. Than the desiccators were incubated in the dark at 25^{o}C for 24 h. After incubation chloroform was removed by repeated (six-fold) evacuation. Both fumigated and unfumigated soil samples were then extracted with 0.5 M K_2SO_4 for 30 minutes and analysed for soluble C (Vance *et al*., 1987). All extracts are stored at –15^{o}C prior to analysis. The ratio MBC /C_{ORG} (%) was also calculated (Anderson and Domsch 1989).

3.3. DETERMINATION OF ORGANIC CARBON AND TOTAL NITROGEN

The content of organic carbon (C_{org}) and nitrogen (N_{tot}) was determined with the analyser Vario Max CNS (Elementar, Germany). Determination of C_{org} and N_{tot} is necessary, since both these parameters as well as C_{org}/N_{tot} is essential indicator of organic matter transformation intensity (mineralization, humification). Mineralization and humification are processes, which directly influence the content of soil organic matter in soils.

3.4. DETERMINATION OF THE DISSOLVED FORMS OF CARBON (DOC) AND NITROGEN (DNt)

The extraction of dissolved organic carbon (DOC; DNt) was performed with 0.004 M $CaCl_2$ for one hour, at the ratio of the soil to extraction solvent of 1:10 (w/v). The content of DOC was assayed with the analyser Multi N/C 3100 Analityk Jena (Germany). The DOC (DNt) content was expressed in mg $C(N)\cdot kg^{-1}$ of d.w. of the soil sample and as a percentage share in the TOC (Nt) pool.

3.5. DETERMINATION OF OTHER CHEMICAL PROPERTIES

Soil pH (in water and in 1 M KCl) was measured using the potentiometric method in 1:2.5 soil:solution suspensions. Soil moisture was analyzed using drying-weighing method.

3.6. ENZYMATIC ASSAYS

Cellulase (CEL) and invertase (INV) activities were assayed as reported by Schinner and von Mersi (1990). Field-moist soil was placed in an Erlenmeyer flask (50 mL) and treated with acetate buffer (2 M, pH 5.5) and CMC solution (carboxymethylcellulose, 0.7% w/v) for CEL activity and sucrose (1.2%) for INV activity, mixed well and incubated for 24 (CEL activity) and 3 (INV activity) hours at 50^{o}C. After the incubation the resulting soil suspension was filtrated and 1 mL of filtrate was diluted with water and mixed thoroughly. Later 1 mL of diluted filtrate was placed into glass tubes and 1 mL of reagent A (anhydrous sodium carbonate and potassium cyanide) and 1 mL of regent B (potassium ferric hexacyanide) were added and mixed well. Then, the tubes were boiled in a water bath (100^oC, 15 minutes). Reducing sugar released during the incubation period caused the reduction of potassium hexacyanoferrate (III) in an alkaline solution After cooling 5 mL of reagent C (ferric ammonium sulphate, sodium dodecyl sulphate, concentrated H_2SO_4) was added, mixed well and allowed to stand at 20^oC for 60 minutes for colour development. Reduced potassium hexacyanoferrate (II) reacted with ferric ammonium sulphate in an acid solution to form a complex of ferric hexacyanoferrate (II), which was determined spectophotometrically at 690 nm. The control was prepared by adding substrate solutions after the incubation but immediately before filtration.

β-glucosidase activity (GLU) was measured as described by Eivazi and Tabatabai (1988). Briefly, 1 g of soil was incubated with 4 mL of buffer (MUB, pH 6.0) and substrate (p-Nitrophenol-B-glucoside solution – PGN, 25mm) in reaction flasks for 1 h under continuous stirring. Concentrations of *p*-nitrophenol were determined by direct sample reading at 400 nm after alkalinisation a Tris/NaOH buffer (pH 10.0) and $CaCl_2$. To prepare the controls, the PGN was added at the end of the incubation before adding the $CaCl_2$ and Tris/NaOH buffer.

3.7. STATISTICAL ANALYSIS

A three-way analysis of variance (ANOVA) was performed to determine the effect of examined factors on the properties studied. In the case of significant *F*-tests, differences between the group means were assessed using the Tukey test ($p \leq 0.05$). Simple regressions were done to show the relationship among the properties studied. Pearson correlation analysis was done to show the relationship among the properties studied. All of the statistical analysis were conducted using Statistica 8.1 for Windows software.

4. RESULTS AND DISCUSSION

4.1. SOIL ENZYMATIC ACTIVITIES

Enzymatic activity was studied for three factors: I factor – different tillage practices (1. grubber after harvest tillage + grubber in autumn; 2. grubber after harvest tillage + simple plowing; 3. pre-sowing plowing; 4. farmyard manure (FYM) + first plowing + pre-sowing plowing; 5. direct sowing of winter wheat after no-till), II factor – straw management (crumbled straw that was left – A; straw that was removed – B), III factor – bio-fertilizer (with effective microorganisms – EM, control without EM – WEM) (**Figures 1-3**).

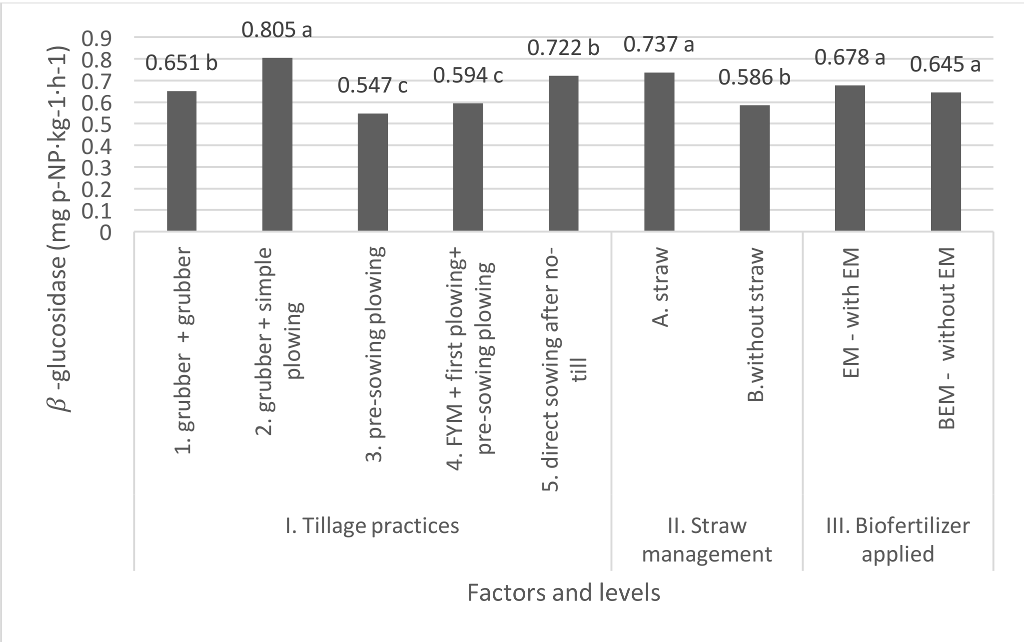

Figure 1. Effect of different tillage practices, straw management and bio-fertilizer application on the average values of the β-glucosidase activity.

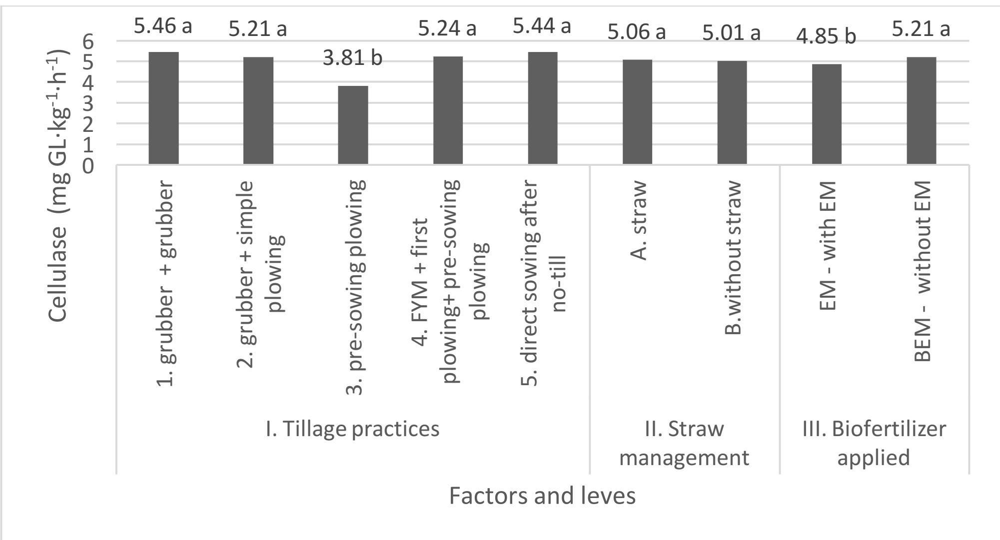

Figure 2. Effect of different tillage practices, straw management and bio-fertilizer application on the average values of cellulase activity.

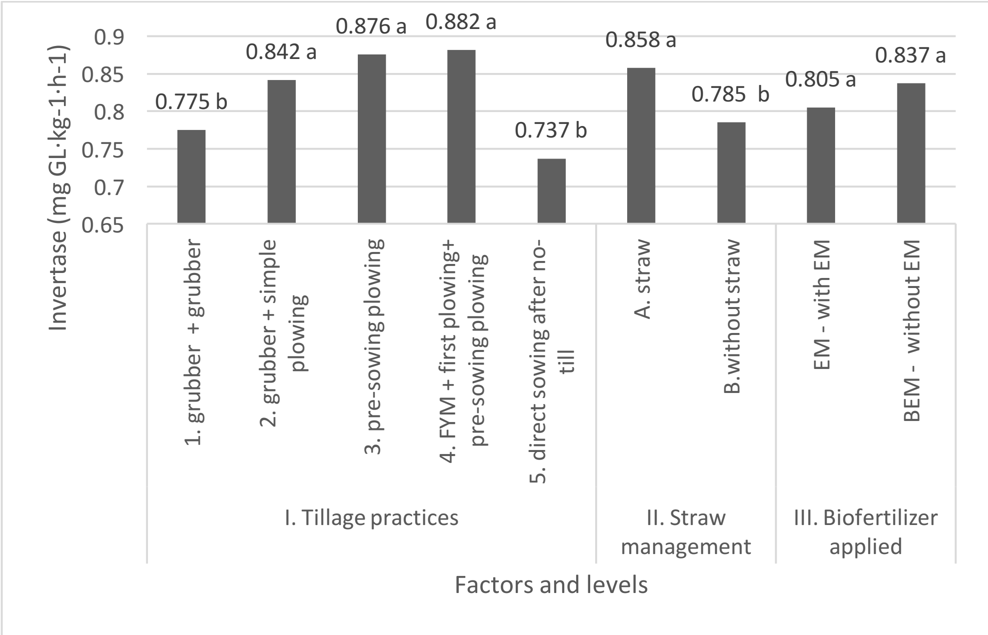

Figure 3. Effect of different tillage practices, straw management and bio-fertilizer application on the average values invertase activity.

Tillage practices was shown to affect soil quality (Pierce and Larson, 1993). Activity of soil enzymes are used as the predictive tools in soil fertility (Benitez *et al.,* 2000). In this study, different tillage practices has demonstrated to affect the activity of soil enzymes, as well as soil carbon content.

As shown in **Figure 1**, among the five different tillage systems used, grubber after harvest tillage + simple plowing gave significantly higher activity for GLU activity (0.805 mg p-NP$\cdot$kg$^{-1}\cdot$h^{-1}). However, the second highest activity was under direct sowing of winter wheat after no-till, suggesting that maybe minimum tillage systems will also be adequate. The activity under farmyard manure (FYM) + first plowing + pre-sowing plowing was one of the lowest ones (0.547 mg p-NO$\cdot$kg$^{-1}\cdot$h^{-1}), while another study (Gopinath *et al.,* 2007) found that GLU activity was significantly higher when farmyard manure was added compared with the mineral fertilizer and unamended check treatments.

In **Figure 2** is shown the CEL activity of soil. In respect of tillage practices, there was not a significant variation in the enzymatic activity among grubber after harvest tillage + grubber in autumn, grubber after harvest tillage + simple plowing, farmyard manure (FYM) + first plowing + pre-sowing plowing and direct sowing of winter wheat after no-till, which could indicate that there is always enough cellulose in the soil, so the CEL works at high rate anyway. However, pre-sowing plowing gave considerably less activity (3.81 mg GL·kg^{-1}·h^{-1}) as compare to the other tillage systems.

As shown in **Figure 3**, INV activity was significantly higher when pre-sowing plowing (0.876 mg GL·kg^{-1}·h^{-1}), farmyard manure (FYM) + first plowing + pre-sowing plowing (0.882 mg GL·kg^{-1}·h^{-1}) and grubber after harvest tillage + simple plowing was carried out than when grubber after harvest tillage + grubber in autumn (0.775 mg GL·kg^{-1}·h^{-1}) and direct sowing of winter wheat after no-till (0.737 mg GL·kg^{-1}·h^{-1}) was done. On the contrary, other studies (Jin *et al.,* 2009; Mikanowa *et al.,* 2009) showed that INV activity was significantly higher under subsoiling with mulch and no-till with mulch than under conventional tillage systems.

Several studies have demonstrated that the addition of straw is beneficial for maintaining soil quality as it increase organic matter, enzyme activities and can replaced chemical fertilizers (Chander *et al.,* 1997; Dick *et al.,* 1988; Garg and Bahl, 2008). The results obtain in this study indicate that straw addition significantly increase GLU activity (**Figure 1**). This result is supported by previous studies (Gopinath *et al.,* 2007; Liu *et al.,* 2010) that reported that GLU activity was higher with organic amendments. It appears logical as GLU is synthesized by soil microorganisms in the presence of suitable substrate, so if there is a proper source of C, like straw, microorganisms will produce more GLU. The same happened to INV activity (**Figure 3**), which was significantly higher with straw addition compared to the situation when the straw was removed . This result was supported by Zhang *et al.* (2016), who had demonstrated that straw incorporation improve activity levels and that incorporation of high rate of straw had more enzymatic activity that low rate incorporation of straw. In the case of CEL (**Figure 2**), the addition of straw did not have a significant effect on its activity, which was only 0.05 mg $GL \cdot kg^{-1} \cdot h^{-1}$ higher when the straw was left than when it was not left.

Regarding the III factor, GLU and INV activities did not shown significant variation between the application of bio-fertilizer and the absence of it (**Figure 1, and 3**). GLU was a little bit higher under bio-fertilizer application. INV activity was a little bit lower under bio-fertilizer application, whereas a previous study (Wang et al. 2016) pointed out that the application of seaweed fertilizer, another type of bio-fertilizer, to replant soil resulted in highest INV activity under replant condition. CEL activity shown significantly higher activity when bio-fertilizer was used than when it was not applied (**Figure 2**).

4.2. SOIL MICROBIAL BIOMASS C, DISSOLVED ORGANIC C AND DOC/C_{ORG} %

Soil microbial biomass C (MBC), dissolved organic C (DOC) and DOC/C_{org} % were studied for the same factors than enzymatic activities (**Figures 4-6**).

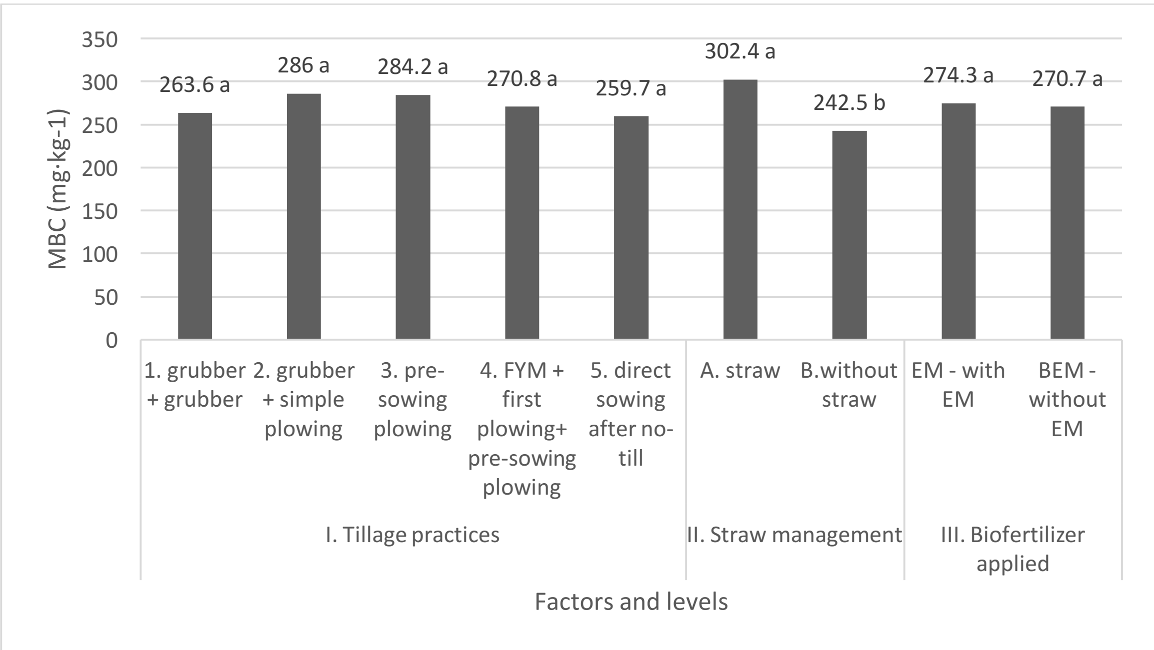

Fig. 4. Effect of different tillage practices, straw management and bio-fertilizer on the average values of the microbial biomass carbon (MBC).

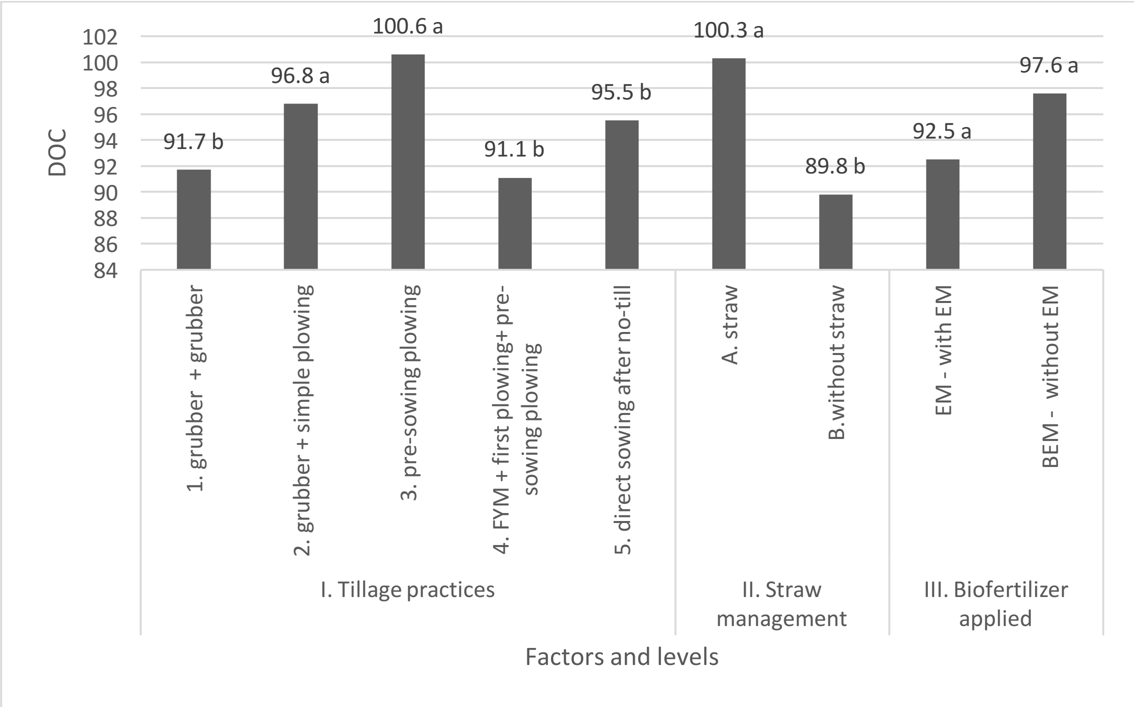

Fig. 5. Effect of different tillage practices, straw management and bio-fertilizer application on the average values of the dissolved organic carbon (DOC).

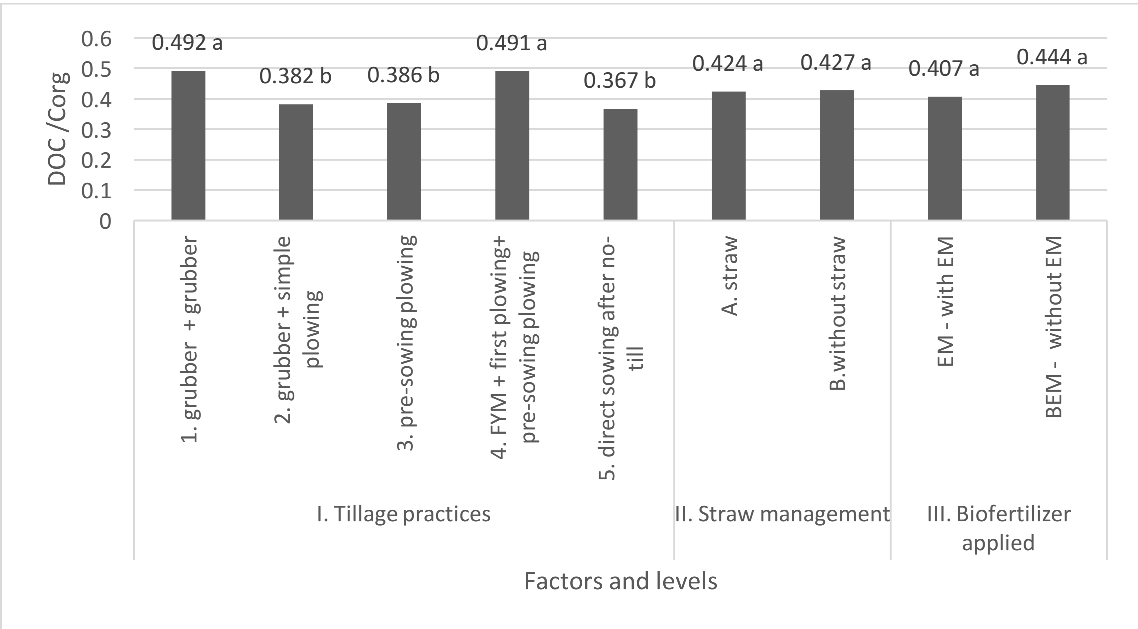

Fig. 6. Effect of different tillage practices, straw management and bio-fertilizer application on the average values of the DOC / Corg %.

As shown in **Figure 4**, tillage practices did not significantly affect MBC content. The highest amount of MBC occurred when grubber after harvest tillage + simple plowing was done (286 $mg \cdot kg^{-1}$), but it was not too different to the quantity reached with pre-sowing plowing (284.2 $mg \cdot kg^{-1}$). The lowest quantity took placed with direct sowing of winter wheat after no-till (259.7 $mg \cdot kg^{-1}$). In regard to DOC quantity, **Figure 5** shown that there is a significant higher amount with pre-sowing plowing (100.6 units) and grubber after harvest tillage + simple plowing (96.8 units) compare to the other tillage systems. The lowest one with grubber after harvest tillage + grubber in autumn (91.7 units) and farmyard manure (FYM) + first plowing + pre-sowing plowing (91.1 units). As shown in **Figure 6**, the ratio of DOC/Corg is significantly higher when grubber after harvest tillage + grubber in autumn and farmyard manure (FYM) + first plowing + pre-sowing plowing are performance compare to the other tillage practices. The percentage is the lowest when direct sowing of winter wheat after no-till is done.

Soil MBC concentration (**Figure 4**) was significantly higher when straw was added (302.4 $mg \cdot kg^{-1}$), than when that it was removed (242.5 $mg \cdot kg^{-1}$). However, the study of Debosz *et al.* (1999) demonstrated that MBC concentration display marked temporal variability, suggesting that MBC concentration variations could be driven by climatic factors and by crop growth, and not only by the presence or absence of straw. DOC quantity (**Figure 5**) was significantly lower when the straw was removed (89.8 units) that when it was not (100.3 units). The ratio of DOC/Corg was not significantly affected by the presence or absence of straw.

The addition of bio-fertilizers, fertilizers that contain living microorganisms, can help to maintain or increase the content of organic matter in soil. Analysis of variance suggested that there is not significant difference between the used or not of bio-fertilizer in MBC content, DOC and the ratio of DOC/C_{org} (**Figures 4, 5 and 6**). In this study the DOC concentration was a little bit lower when the bio-fertilizer was added (**Figure 5**). This meets the results of Dębska *et al.* (2016), whose study shown that after 3 years of UGmax bio-fertilizer application the DOC concentration in soil decreased.

4.3. CORRELATION BETWEEN THE STUDIED PROPERTIES

Table 1. Pearson correlation coefficients between C related soil enzyme activities and chemical/microbial soil properties.

	CEL	INV	GLU	MBC	CORG G/KG	DOC	C/N	MBC/ C_{ORG}	MBC/ MBN	$C_{DISOLVED}$/ C_{ORG}%	PH KCL	PH H_2O	MOISTURE %
MOISTURE %													
PH H_2O													-0.01
PH KCL												-0.01	0.24
$C_{DISOLVED}$/ C_{ORG}%											-0.13	0.17	0.02
MBC/MBN										0.12	-0.14	0.08	0.1
MBC/C_{ORG}									0.2	**0.63**	-0.09	0.14	0.19
C/N								**-0.33**	-0.01	**-0.27**	-0.02	-0.16	**0.26**
DOC							-0.13	0.13	0.11	0.35	0.05	0.07	**0.34**
C_{ORG} G/KG						-0.02	**0.57**	**-0.74**	-0.05	**-0.6**	0.12	-0.16	**0.32**
MBC					0.15	0.16	0.19	0.5	0.24	0.08	0	0.02	**0.62**
GLU				**0.46**	0.19	0.23	0.14	0.15	0.09	-0.09	-0.18	-0.2	**0.39**
INV			**0.26**	0.5	0.02	0.17	0.1	0.3	0.05	0.2	-0.1	0.07	**0.43**
CEL		0.13	**0.46**	0.14	-0.07	-0.06	-0.12	0.16	0.02	0.08	**-0.4**	-0.23	**-0.26**

CEL - celullase activity (mg $GL \cdot kg^{-1} \cdot h^{-1}$), GLU - β-glucosidase activity (mg p-$NP \cdot kg^{-1} \cdot h^{-1}$), INV – invertase activity (g $GL \cdot kg^{-1} \cdot h^{-1}$), MBC – microbial biomass carbon ($mg \cdot kg^{-1}$), MBN – microbial biomass nitrogen ($mg \cdot kg^{-1}$).

Positive correlation coefficients exists between soil moisture %, C/N and C_{org} (**Table 1**). C_{org} has negative correlation with MBC/ C_{org} and C_{dis}/C_{org}, and MBC/ C_{org} and C_{dis}/C_{org} have positive correlation between them. GLU has positive correlation with INV and CEL, but INV and CEL do not have correlation between them. GLU and INV had positive correlation with moisture %, whereas CEL has negative correlation. GLU had positive correlation with MBC, agreeing with the study of Turner *et al.* (2002) in which they found a strong correlation ($P < 0.001$) between those two parameters. Moisture % has a strong correlation with MBC. In this study there is no correlation between DOC and MBC, whereas in the study of Shuang *et al.* (2015) there was correlation ($P < 0.05$).

5. CONCLUSION

The research shown that enzyme activities and MBC, DOC and DOC/C_{org} % are in different degree sensitive to various tillage practices as well as to the use or not of straw and bio-fertilizers. The direction of changes was however different for each property studied.

There were ambiguous changes of studied properties as regards the five studied tillage methods. Soil after grubber after harvest tillage + single plowing shown significantly higher activity for the three enzymes tested (β-glucosidase, cellulase and invertase). MBC and DOC was also significantly higher when this type of tillage was used. Moreover, the addition of FYM and first plowing + pre-sowing plowing seems to be a good tillage practice, since it increased all studied properties (except GLU activity). Therefore, they might be the most recommended tillage systems to achieve the highest soil biological activity and finally the highest yield in monoculture crops.

The use of straw increased the activity of studied enzymes (except CEL) and the content of soil carbon, suggesting that the straw can be a good natural fertilizer, which influenced beneficially soil properties.

Bio-fertilizer did not have any effect on soil studied properties, except CEL activity which was decreased, so its use might not be reasonable in this case.

Regarding to Pearson correlation results, moisture percentage of soil appears to be specially correlated to the enzymes tested as well as to the carbon content in soil, suggesting that moisture is important factor influencing soil biological activity.

6. BIBLIOGRAPHY

Abdalla, M., Osborne, B., Lanigan, G., Forristal, D., Williams, M., Smith, P., Jones, M. 2013. Conservation tillage systems: a review of its consequences for greenhouse gas emissions. Soil Use Manag. 29: 199–209.

Alef, K., Nannipieri, P. 1995. Methods in Applied Soil Microbiology and Biochemistry. Academic Press.

Arinze, A.E., Yubedee, A.G. 2000. Effect of Fungicides on *Fusarium* Grain Rot and Enzyme Production in Maize (Zea *mays* L.). Glob. J. Appl. Sci. 6(4): 629-634.

Atlas, RM; Pramer, D; Bartha, R. 1978. Assessment of pesticide effects on non-target soil microorganisms. Soil Biol. Biochem. 10: 231-239.

Bakht, J., Shafi, M., Jan, M.T., Shah, Z. 2009. Influence of crop residue management: cropping system and N fertilizer on soil N and C dynamics and sustainable wheat (Triticum aestivum L.) production. Soil Tillage Res. 104: 233–240.

Benitez, E., Melgar, R., Sainz, H., Gómez, M., Nogales, R., 2000. Enzyme activities in the rhizosphere of pepper (*Capsicum annuum L.)* grown with olive cake mulches. Soil Biol. Biochem. 32: 1829–1835.

Brookes, P.C. 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. Biol. Fertil. Soils. 19: 269–279.

Chander, K., Goyal, S., Mundra, M.C., Kapoor, K.K. 1997. Organic matter, microbial biomass and enzyme activity of soils under different crop rotations in the tropics. Biol. Fertil. Soils. 24: 306–310.

Curtin, D., Fraser, P. 2003. Soil organic matter as influenced by straw management practices and inclusion of grass and clover seed crops in cereal rotations. Aust. J. Soil Res. 41: 95–106.

Das, S. K., Varma, A. 2011. Role of Enzymes in Maintaining Soil Health. - In: Shukla, G., Varma, A. (eds.) Soil Enzymology, Soil Biology 22, Springer-Verlag Berlin Heidelberg USA.

Debosz, K., Rasmussen, P.H., Pedersen, A.R. 1999. Temporal variations in microbial biomass C and cellulolytic enzyme activity in arable soils: effects of organic matter input. Appl Soil Ecol 13: 209-218.

Dębska, B., Długosz, J., Piotrowska-Długosz, A., Banach-Szott, M. 2016. The impact of a bio-fertilizer on the soil organic matter status and carbon sequestration—results from a field-scale study. J Soils Sediments. DOI 10.1007/s11368-016-1430-5.

Deng, S.P., Tabatabai, M.A. 1994. Cellulase Activity of Soils. Soil Biol Biochem. 26:1347–1354.

Dick, R.P. 1997. Soil Enzyme Activities as Integrative Indicators of Soil Health. - In: Pankhurst, C. E., Doube, B. M., Gupta, V.V.S.R. (eds.) Biological Indicators of Soil Health, CABI Publishing. USA.

Dick, RP. 1994. Soil enzyme activities as indicators of soil quality. In: Doran JV, Coleman DC, Bezdicek DF, Stewart BA (Eds.). Defining Soil Quality for a Sustainable Environment, Soil Science Society of America, American Society of Agriculture, Madison, pp. 107-124.

Dick, RP; Breakwell DP; Turco RF. 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Methods for Assessing Soil Quality, vol. 9. Soil Sci. Soc. Am. Madison, WI pp. 9-17.

Dick, R., Rasmussen, P., Kerle, E. 1988. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biol. Fertil. Soils. 6: 159–164.

Duiker, S., Lal, R. 1999. Crop residue and tillage effects on carbon sequestration in a Luvisol in central Ohio. Soil Tillage Res. 52: 73–81.

Eivazi, F; Tabatabai, MA. 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20: 601-606.

Esen, A. 1993. β-glucosidases: overview. In: Esen A. (Ed.) β- glucosidases and molecular biology. American Chemical Society, Washington, DC, pp. 9-17.

Fauci, M.F., Dick, R.P. 1994. Soil microbial dynamics: short- and long-term effects of inorganic and organic nitrogen. Soil Sci. Soc. Am. J. 58: 801–806.

Feng, Z., Motta, A.C., Reeves, D.W., Burmester, C.H., van Santen, E., Osborne, J.A. 2003. Soil microbial communities under conventional-till and no-till continuous cotton systems. Soil Biol. Biochem. 35: 1693–1703.

Frankenberger, W.T., Jr., Johanson, J.B., 1983. Factors afecting invertase activity in soils. Plant Soil. 74: 313-323.

Garg, S., Bahl, G. 2008. Phosphorus availability to maize as influenced by organic manures and fertilizer P associated phosphatase activity in soils. Bioresource Technol. 99: 5773–5777.

Gopinath, K.A., Saha, S., Mina, B.L., Pande, H., Kundu, S., Gupta, H.S. 2007. Influence of organic amendments on growth, yield and quality of wheat and on soil properties during transition to organic production. Nutr. Cycl. Agroecosyst. 82: 51–60.

Haliniarz M., Bujak K., Gawęda D., Kwiatkowski C. 2013. Response of spring wheat to reduced tillage systems and to different levels of mineral fertilization. Acta Sci. Pol. Agricultura. 12(3): 13-24

Hamido, A.K., Kpomblekou-A. 2009. Cover crop and tillage effects on soil enzyme activities following tomato. Soil Till. Res. 105: 269-274.

Jaskulski, D., Kotwica, K., Jaskulska, I., Piekarczyk, M., Osiński G., Pochylski, B., 2012. Elementy współczesnych systemów uprawy roli i roślin – skutki produkcyjne oraz środowiskowe. Fragm. Agron. 29(3): 61-70.

Jin, K., Sleutel, S., Buchan D., de Neve, S., Cai, D.X., Gabriels, D., Jin, J.Y. 2009. Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau. Soil Till. Res. 104: 115–120.

Karaca, A., Cema, C.C., Turgay, O.C., Kizilkaya, R. 2011. Soil Enzymes as Indicator of Soil Quality. - In: Shukla, Varma, G. A. (eds.) Soil Enzymology, Soil Biology 22, Springer-Verlag Berlin Heidelberg USA.

Lepiarczyk, A., Stępnik, K., Szylak, A. 2007. Wpływ systemów uprawy roli na niektóre właściwości fizyczne gleby pod wybranymi roślinami. Fragm. Agron. 24(1): 157-163.

Liu E., Yan C., Mei X., He W., Bing S.H., Ding L., Liu Q., Liu S., Fan T. 2010. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. Geoderma 158:173–180.

Madejon, E., Moreno, F., Murillo, J.M., Pelagrin, F. 2007. Soil Biochemical Response to Long-term Conservation Tillage under Semiarid Mediterranean Conditions. Soil Till. Res. 94:346–352.

Małecka, I., Blecharczyk, A., Sawinska, Z., Piechota, T., Waniorek, B. 2012. Plonowanie zbóż w zależności od sposobów uprawy roli. Fragm. Agron. 29(1): 114-123.

Mikanova O., Javurek M., Simon T., Friedlova M., Vach M. 2009. The effect of tillage systems on some microbial characteristics. Soil Till. Res. 105: 72–76.

Mishra, P., Dash, D. 2014. Rejuvenation of Biofertilizer for Sustainable Agriculture and Economic Development. J. Sust. Dev. 11: 41–6.1

Petker, AS; Rai, PK. 1992. Effect of fungicides on activity, secretion of some extra cellular enzymes and and growth of *Alternaria alternata*. Indian J. Appl. Pure. Biol. 7(1): 57-59.

Pierce, F.J., Larson, W.E. (1993): Developing Criteria to Evaluate Sustainable Land Management. - In: Kimble, J.M. (ed.) Proceeding of the VIII International Soil Management Workshop Utilization of Soil Survey Information for Sustainable Land use Sacramento, CA.

Piotrowska, A., Wilczewki, E. 2012. Effects of catch crops cultivated for green manure and mineral nitrogen fertilization on soil enzyme activities and chemical properties. Geoderma.189-190: 72-80.

Powlson, D.S., Glendining, M.J., Coleman, K., Whitmore, A.P. 2011. Implications for soil properties of removing cereal straw: results from long-term studies. Agron. J. 103: 279–287.

Ross, D.J. 1983. Invertase and amylase activities as influenced by clay minerals, soil-clay fractions and topsoils under grassland. Soil Biol. Biochem. 15: 287-293.

Saroa, G., Lal, R. 2003. Soil restorative effects of mulching on aggregation and carbon sequestration in a Miamian soil in central Ohio. Land Degrad. Dev. 14: 481–493.

Schaller, K. 2009. Soil Enzymes – Valuable Indicators of Soil Fertility and Environmental Impacts. Bulletin UASVM Horticulture. 66(2): 911-915.

Schenck zu Schweinsberg-Mickan, M., Müller, T. 2009. Impact of effective microorganisms and other biofertilizers on soil microbial characteristics, organic-matter decomposition, and plant growth. J. Plant Nutr. 172: 704-712.

Schloter, M., Dilly, O., Munch, J.K., 2003. Indicators for evaluating soil quality. Agric. Ecosyst. Environ. 98: 255–262.

Sinha, RK; Valani, D; Chauhan, K; Agarwal, S. 2014. Embarking on a second green revolution for sustainable agriculture by vermiculture biotechnology using earthworms: reviving the dreams of Sir Charles Darwin. Int. J. Agric. Health Saf. 1:50–64.

Six, J., Elliott, E.T., Paustian, K. 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32: 2099–2103.

Shuang, Z., Huicai, Z., Zhiqiang, J. 2015. Soil Microbiological and Biochemical Properties as Affected by Different Long-Term Banana-Based Rotations in the Tropics. Pedosphere. 25(6): 868–877.

Shukla, G., Varma, A., Soil Biology, 2011. Soil Enzimology, vol 22. Springer.

Swedrzyńska, D., Małecka, I., Blecharczyk, A., Swedrzyński, A., Starzyk J. 2013. Effects of various long-term tillage systems on some chemical and biological properties of soil. Pol. J. Envir. St. 22(6): 1835-1844.

Tabatabai, MA. 1994. Soil enzymes. In: Weaver RW, Angle JS, Bottomley PS (eds) Methods of soil analysis, part 2. Microbiological and biochemical properties. SSSA Book Series No. 5. Soil Sci. Soc. Am. Madison, Wis., pp. 775-833.

Tan, D., Jin, J., Huang, S., Li, S., He, P. 2007. Effect of long-term application of K fertilizer and wheat straw to soil on crop yield and soil K under different planting systems. Agr. Sci. China. 6: 200–207.

Utobo, EB., Tewari, L. 2014. Soil enzymes as bioindicators of soil ecosystem status. App. Ecol. Environ. Research. 13(1): 147-169.

Valarini, P.J., Cruz Díaz álvarez, M., Gascó, J.M., Guerrero, F., Tokeshi, H. 2002. Integrated evaluation of soil quality after the incorporation of organic matter and microorganisms. Braz. J. of Microbiol. 33: 35–40.

Vincent, PG., Sisler, HD. 1968. Mechanisms of antifungal action of 2, 4, 5, 6-tetrachloroisopathalonitrile. Physiol. Plant. 21: 1249-1264.

Wang, Y., Fu, F., Li, J., Wang, G., Wu, M., Zhan, J., Chen, X., Mao, Z. 2016. Effects of seaweed fertilizer on the growth of *Malus hupehensis* Rehd. seedlings, soil enzyme activities and fungal communities under replant condition. Eur. J. Soil Biol. 75: 1-7.

Wesołowski, M., Cierpiała, R., 2011. Plonowanie pszenicy ozimej w zależności od sposobu wykonania uprawy przedsiewnej. Fragm. Agron. 28(2): 106-118.

Wick, B., Kühne, RF., Vlek, PLG. 1998. Soil microbiological parameters as indicators of soil quality under improved fallow management systems in southwestern Nigeria. Plant Soil. 202: 97-107.

Zhang, P., Chen, X., Wei, T., Yang, Z., Jia, Z., Yan,. B., Han, Q., Ren, X. 2016. Effects of straw incorporation on the soil nutrient contents, enzyme activities, and crop yield in a semiarid region of China. Soil Till. Res. 160: 65–72.

http://www.chgeharvard.org/topic/biodiversity-and-agriculture, May 2016.