EFFECTS OF GREEN MANURING ON SOIL ENZYME ACTIVITY

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ABSTRACT

In this research, the effects of faba bean *(Vicia faba* L.*)* used for green manure and forage production as a winter catch crop on some soil biological properties were investigated. The field experiments were conducted in Çarşamba Plain located in the north region of Turkey. The "green manuring-maize-wheat" crop rotation with a randomized complete block with four replications was repeated in two separate years. The treatments used to investigate the management effect on soil enzyme activity were: (GM1-GM4) burying the whole above ground faba bean plant as green manure, (GM5-GM8) burying the under ground-stubbles of plants and (C1-C2) control. Different nitrogen doses of 0, 60, 120, 180 kg N ha^{-1} for maize, and 0, 50, 100 and150 kg N ha^{-1} for wheat were applied following the green manuring. The results revealed that both types of green manure applications provided an increase in urease and dehydrogenase contents of soils compared to the control. The urease (UA) and dehydrogenase (DH) activities were higher with above ground parts of faba been application when compared to application of the underground stubbles. The activity of the UA and DG were significantly ($P<0.05$) influenced at the end of the maize harvest in the GM amended soils. At the end of the maize harvest, significantly ($P<0.05$) highest UA activity (205.1 μg N 1^{-1} g dry soil) was observed in the GM1 treatment compared to the control soil. While, the lowest UA activity was recorded in the GM5 (178.4 μg N 1^{-1} g dry soil).

KEYWORDS:
Green manure, maize, wheat, urease, dehydrogenase

1. INTRODUCTION

Frequent soil tillage operations and cropping for centuries have resulted in degradation of soils and considerably lowered the biological productivity. The current agricultural systems have also more detrimental effects because of numerous heavy plowing and intensive mineral fertilizing. The sustainable cropping system should improve the physical, chemical and biological soil properties. The system increases organic matter and fertility, and reduces the potential of soil erosion [1].

The use of green manure (GM) is a good management practice which stimulates soil microbial growth and activity with subsequent mineralization of plant nutrients, and therefore increases soil fertility and quality. Incorporation of GM into soil is immediately followed by an increase in soil microorganisms that decompose the fresh material. Upon degradation of plant materials, plant nutrients held within the GM are released and made available to the succeeding crop. Microbial activity in the soil also leads to the formation of mycelium and viscous materials which benefit the soil health by increasing the soil structure (i.e. by aggregation). The further aeration of soil enhances the ability of GM crops root systems penetrating to the compacted soils. The amount of humus also increases with higher rates of decomposition, which is beneficial for the growth of the crop succeeding the GM crop.

Enzymes play key roles in the nutrients cycling in nature, their activity is sensitive to agricultural practices and they are considered as an index of soil fertility [2]. Among all enzymes in the soil environment, dehydrogenases (DG) are the most important and extensively used indicators of overall soil microbial activity [3], because they occur intracellular in all living microbial cells [4]. DG play a significant role in the biological oxidation of soil organic matter [5]. Soil urease (UA) is an extracellular enzyme involved in the hydrolysis of urea-type substrates, and its activity is important for transformation of urea fertilizer. UA activity is widely used in monitoring the soil quality for the evaluation of management practices [6].

The activity of soil microorganisms is strongly linked to the activity of enzymes. Soil management practices including crop rotations, fertilization, tillage and crop residue placement influences the activity of soil enzymes [7]. Analyses of the soil enzyme activities provide information on biochemical processes occurring in the soil that are important for soil quality and healthy.

The objective of this study was to evaluate the effects of GM *(Vicia faba* L.*)* on DG and UA soil enzyme activities in maize-wheat rotation system.

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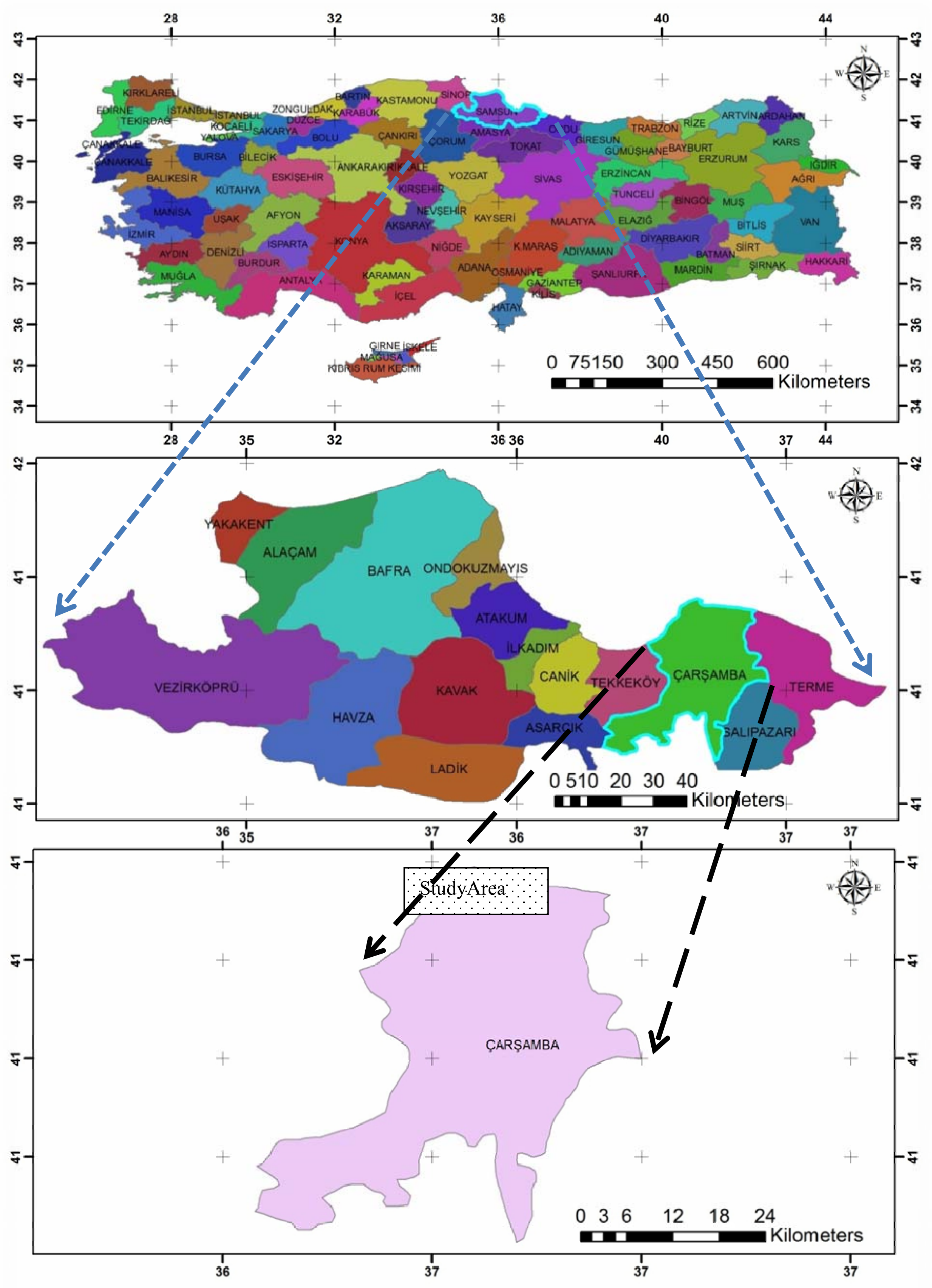

FIGURE 1 - Study Area

2. MATERIAL AND METHODS

The research was carried out in Çarşamba Plain located in the Central Black Sea region of Turkey. It is 35 km to the eastern of the city of Samsun (Figure 1).

The climate is semiarid, and the long-term annual mean temperature, relative humidity, and total annual precipitation in the area are 14.2 °C, 73.9% and 680.0 mm, respectively. During the rotation periods in 2000-02 and in 2001-03, an average temperature of 15.0 and 14.9 °C, total precipitation of 615.8 and 667.2 mm, and average humidity of 72.3% and 73.0% were recorded, respectively. The data related to the some soil physical and chemical properties are given in Table 1. In general, the experimental soils were silty clay loam in texture, slightly alkaline in reaction (pH), and moderately calcareous. The soils showed no signs of salinity problem, and had a low/moderate organic matter content. Available P content was at low, and available K content was sufficient for crop production (Table 1).

Faba bean (*Vicia faba* L.) was used as the green manuring material and some characteristics of GM crop used are given in Table 2.

In this research, the "green manuring-maize-wheat" crop rotation system was examined and field trials of the same crop rotation were repeated in two separate years.

In this study the following treatments were used: (GM1-GM4) burying the whole above ground faba bean plants as GM, (GM5-GM8) burying the under ground-stubble of the plants and (C1-C2) control. Different N doses of 0, 60, 120, 180 kg N ha^{-1} for maize, and 0, 50, 100 and 150 kg N ha^{-1} for wheat were applied following the green manuring (Table 3).

Conventional system (A treatment without GM, N, P and K fertilization): In the first period field experiment, 160 kg N ha^{-1} and 120 kg P_2O_5 ha^{-1} were applied for maize and for wheat 200 kg N ha^{-1} and 90 kg P_2O_5 ha^{-1}. Since the soils were rich in potassium, K was not applied. In the second period field experiment, 160 kg N ha^{-1}, 120 kg P_2O_5 ha^{-1} and 200 kg K_2O ha^{-1} were applied for maize and for wheat 200 kg N ha^{-1}, 190 kg P_2O_5 $ha^{-1}$and 100 kg K_2O ha^{-1}. Mineral N application rates were determined based on the agronomic N requirement of maize and wheat [8].

The experiment was conducted using a complete randomized block design with four replications in 40 plots, each measuring 25.2 m^2 (4.2 m x 6.0 m), with a separation strip of 1 m between them. In both rotation periods, the faba bean seeds were sown by hand during the first week of November. Row spacing was 30 cm, and faba bean seeds inoculated with bacteria breed of *Rhizobium leguminosarum* before sowing.

The applications of green manuring were made when faba bean was at 75% of blooming. The green manures were surface broadcasted (April 18, 2001, and April 26, 2002) and incorporated to a 20-cm depth by chisel plowing and disking at the day after application. Maize sowing was made with a combined seeder and 30 kg of seeds were sowed per ha on May 22, 2001 and May 14, 2002. Seeds were sown in longitudinal rows with intra- and inter-row spacing of 25 and 70 cm, respectively. Wheat sowing was

TABLE 1 - Some physical and chemical properties of the experimental soil.

Soil parameters	Values		Soil parameters	Values	
	1st period trial material	2nd period trial material		1st period trial material	2nd period trial material
Clay, %	34.16	35.20	Electrical Conductivity EC) dS m^{-1}	0.645	0.412
Silt, %	54.84	55.16	Calcareous ($CaCO_3$) %	5.9	8.3
Sand, %	10.99	9.64	Organic Matter, %	2.77	1.77
Textural Class	SiCL	SiCL	Available P kg P_2O_5 ha^{-1}	43	41
pH	7.83	7.88	Available K kg K_2O ha^{-1}	540	310

TABLE 2 - Characteristics of GM crop used in the study.

Properties	Units	Values	
		1st period trial material (2000-02)	2nd period trial material (2001-03)
Root-stubble dry matter weight	kg ha^{-1}	995	814
Total dry biomass laid to rest (underground parts-stubble-above ground parts)	kg ha^{-1}	3499	2581
Total N (underground parts-stubble)	kg ha^{-1}	16.5	17.3
Total N (underground parts-stubble-above ground parts)	kg ha^{-1}	100.4	101.4
Organic C (underground parts-stubble)	%	33.52	32.30
Organic C (above ground parts)	%	46.23	46.61
Total N (underground parts-stubble)	%	1.66	2.12
Total N (above ground parts)	%	3.35	4.76
C/N (underground parts-stubble)		20.2	15.2
C/N (above ground parts)		13.8	9.8

TABLE 3 - The treatments applied in the field experiments.

No	Treatments	
GM1	GM application (UPSAGP)	+ for maize 0 kg ha^{-1} N, for wheat 0 kg ha^{-1} N
GM2	GM application (UPSAGP)	+ for maize 60 kg ha^{-1} N, for wheat 50 kg ha^{-1} N
GM3	GM application (UPSAGP)	+ for maize 120 kg ha^{-1} N, for wheat 100 kg ha^{-1} N
GM4	GM application (UPSAGP)	+ for maize 180 kg ha^{-1} N, for wheat 150 kg ha^{-1} N
GM5	GM application (UPS-FBH)	+ for maize 0 kg ha^{-1} N, for wheat 0 kg ha^{-1} N
GM6	GM application (UPS-FBH)	+ for maize 60 kg ha^{-1} N, for wheat 50 kg ha^{-1} N
GM7	GM application (UPS-FBH)	+ for maize 120 kg ha^{-1} N, for wheat 100 kg ha^{-1} N
GM8	GM application (UPS-FBH)	+ for maize 180 kg ha^{-1} N, for wheat 150 kg ha^{-1} N
C1	Conventional system (Fertilizer-NPK)	
C2	Control (a control without application of neither green manure nor fertilization)	

C: Control, UPSAGP: Underground parts-stubble-above ground parts, UPS-FBH:Underground parts-stubble-Faba bean harvested.

TABLE 4 - UA and DG activities in soils collected at the end of crops harvest in the rotation system of green manuring-maize-wheat (First and second experimental period).

Treatments	UA (μg N 1^{-1} g dry soil)				DG (μg TPF 1^{-1} g dry soil)			
	At the maize harvest		At the wheat harvest		At the maize harvest		At t the wheat harvest	
	Experimental period		Experimental period		Experimental period		Experimental period	
	1.	2.	1.	2.	1.	2.	1.	2.
GM1	205.1 a*	335.1	226.3 bc**	30.8 bc**	455.0 a*	268.1 ab*	---	241.2 a*
GM2	200.1 ab	323.5	261.0 ab	39.3 a	419.7 ab	258.9 abc	---	164.3 b
GM3	197.1 ab	323.4	266.5 a	37.9 a	389.2 ab	251.0 abc	---	154.4 bc
GM4	187.7 bcd	318.3	233.0 abc	37.9 a	346.3 b	303.6 a	---	145.6 bcd
GM5	178.4 d	310.6	217.0 c	29.4 bc	397.2 ab	238.9 abcd	---	134.6 cd
GM6	192.3 abcd	310.5	200.5 c	33.7abc	394.3 ab	191.5 cd	---	141.2 bcd
GM7	186.4 bcd	317.3	224.9 c	31.5 bc	404.5 ab	227.2 bcd	---	140.4 bcd
GM8	193.1 abc	305.0	234.0 abc	34.9 ab	391.0 ab	237.3 abcd	---	154.1 bc
C1	190.3 bcd	286.1	163.5 d	30.6 bc	326.8 bc	169.7 d	---	120.5 d
C2	178.8 cd	298.3	113.8 e	28.9 c	232.7 c	163.3 d	---	123.6 d

*: P≤0.05, Means followed by the same letter in a column are not statistically different,
**: P≤ 0.01, Means followed by the same letter in a column are not statistically different,
UA: Urease activity, DG: Dehydrogenase activity

made with an aerobic combined grain seeder and 180 kg of seeds were sowed per ha with 14 cm row spacing on November 27, 2001 and November 19, 2002, respectively.

Soil samples (0-20 cm) for biological parameters were collected from each plot (4 replicates per plot) with a gauge auger at the end of the maize and wheat harvesting, soil samples were sieved (2 mm) and stored at 4 ^{o}C following the drying at room temperature. The harvesting dates were for maize October 02, 2001 and September 26, 2002, for wheat July 10, 2002 and July 07, 2003, respectively of crop rotation periods. .

UA activity was assayed with the method of Kandeler and Gerber [9].

DG activity was measured using the modified method of Thalmann [10].

Statistical analysis was conducted using the MSTAT-C statistical software. The F test was applied to examine the statistical significance of differences among treatments. Statistical analysis of the two years data was done at 1% or 5% level of probability using Duncan's Multiple Range Test (DMRT) to test the difference between the individual means [11].

3. RESULTS

The UA and DG enzymes of the first and second crop rotation experimental periods are presented in Table 4.

3.1 The first crop rotation experimental period UA and DG enzyme activities

The activity of the UA and DG enzymes were significantly (P<0.05) influenced at the end of the maize harvest in the GM amended soils (Table 4).

At the end of the maize harvest, significantly (P<0.05) highest UA enzyme activity (205.1 μg N 1^{-1} g dry soil) was observed in the GM1 treatment followed by GM2 (200.1 μg N 1^{-1} g dry soil), GM3 (197.1 μg N 1^{-1} g dry soil), GM8 (193.1 μg N 1^{-1} g dry soil), and GM6 (192.3 μg N 1^{-1} g dry soil) treatments compared to the control soil. The lowest UA enzyme activity was recorded in the GM5 (178.4 μg N 1^{-1} g dry soil) (Table 4).

In the same period, the evolution of DG activity was very similar to the UA enzyme. The highest values (455.0 μg TPF 1^{-1} g dry soil) were obtained when all component of faba bean was mixed to the soil without additional NPK application (GM1). However, the difference in DG enzyme activity between GM1 and other subjects were not statistically significant. The lowest DG enzyme activity (232.7 µg TPF 1^{-1} g dry soil) was recorded in the C2 plots (Table 4).

The urease activity was also strongly stimulated by GM addition at the wheat harvest. The stimulation of this enzyme was the highest in GM3 amended soils (266.5 µg N 1^{-1} g dry soil), followed by GM2 (261.0 µg N 1^{-1} g dry soil), GM8 (234.0 µg N 1^{-1} g dry soil), and GM4 (233.0 µg N 1^{-1} g dry soil) treatments respect to the control. The lowest UA enzyme activity (113.8 µg N 1^{-1} g dry soil) was recorded in the C2 plots. The statistical analyses revealed significant (P<0.01) differences for all treatments during the experimental period (Table 4).

The DG activity could not be detected in soil samples following the wheat harvest (Table 4).

3.2 The UA and DG enzyme activities during the second crop rotation

The effect of green manuring and NPK doses on UA activity was not significant at the maize harvest. However, compared to the control plots, the highest UA enzyme activities were obtained with green manuring application (Table 4).

The highest DG enzyme activity was recorded in the GM4 (303.6 µg TPF 1^{-1} g dry soil) treatment, and there was no significant differences between the DG enzyme activity of the GM1, GM2, GM3, GM5 and GM8 treatments at the maize harvest. The treatment of C1 and C2 plots resulted in the lowest DG enzyme activities (169.7 and 163.3 µg TPF 1^{-1} g dry soil, respectively). The differences in DG enzyme activities for all treatments during the experimental period was significant (P<0.05) (Table 4).

The UA enzyme activity at the wheat harvest was significantly (P<0.01) influenced by GM applications. The highest UA enzymes activity (39.3 µg N 1^{-1} g dry soil) was found in the GM2 treatments and was statistically similar to the GM3, GM4, GM8 and GM6 treatments. The minimum UA enzymes activity during both rotation periods occurred in the C2 plot (Table 4).

At the end of the wheat harvest, the highest DG enzyme activity (241.2 µg TPF 1^{-1} g dry soil), was obtained in GM1 treatment. The stimulation of this enzyme was the lowest in control treatments. Again, the statistical analyses showed significant (P<0.05) differences for all treatments at the wheat harvest (Table 4).

4. DISCUSSION AND CONCLUSIONS

The highest UA and DG levels were determined when all components of faba bean as green manure plant were mixed to the soil. The stimulation of enzymes was affected by different doses of N used in the crop rotation. However, the highest UA and DG enzyme activities were obtained when all components of faba bean was mixed to the soil with no addition NPK (GM1). This can be explained with C/N ratio of green manure material (Table 2). Therefore, the increase in soil microbial biomass-C and soil enzymatic activities varied depending on GM applied. The difference in C/N ratio of GM caused a variation of soil C/N ratio. Soil microorganisms should have a C/N ratio close to 8:1 for the optimum decomposition. The C/N ratio was reduced with the addition of N fertilizer. The decrease in C/N ratio can adversely affect the nutrition dynamics of living organisms. Microorganisms acquire enough C and N to maintain the required C and N ratio in their bodies. Dick et al. [12] reported similar results for enzymes involved in N cycle. The increasing rates of ammonia-based N fertilizer decreased the amidase and UA activities. Moreover, UA activity is sensitive to the inhibitory effects N fertilizer applications [13]. It is a known fact that the crude protein content of crops which is an important quality characteristic is positively affected with nitrogen application. In another study of this research; it was determined that the topics discussed had a very significant effect on the crude protein content of corn and wheat grains. In addition higher grain crude protein content was obtained by burying all parts of green manure applications compared to only stubble embedded applications [14].

Soil UA and DG activities were significantly higher in the green manuring system compared to the conventional system. The inhibition of enzyme synthesis by inorganic ions might result in the lowest enzyme activity in conventional soils. The increase in biological activity with GM applications significantly increased the UA activity. The GM added to the soil was used as a substrate source to UA enzyme which might also increase the UA. DG is an oxidoreductase that only exists in viable cells, and is considered a sensitive indicator of soil quality [15]. Higher levels of the activity of DG, a living microbial cellular enzyme, in GM amended-soils may have been the result of their organic carbon. Okur et al. [16] observed a significant relationship between organic C and DG activity. Studies comparing conventional and organic farming have reported an increase in UA, and DG activity in organically managed soils [16-18]. On the other hand, findings related to plant yields of green manure applications revealed that the highest corn grain yields were obtained from GM4 application in both rotation periods. However, the difference between GM3 and C1 applications were not statistically significant. Similar results were obtained for wheat yield when grown following the corn [14].

Although all applications were equal, significant differences were found between rotation periods. The difference between rotation periods probably resulted from the differences in temperature and precipitation and differences in total biomass. Other researchers have already reported that the activity of soil enzymes is also affected by temperature, soil type, soil moisture and aeration, soil pH, organic carbon and nitrogen contents, the presence of heavy metals, fertilization, and to large extent on agrotechnical measures [6].

The activity levels of DG and UA were higher when all components of GM mixed to the soil (GM1, GM2, GM3, and GM 4 treatments) compared with the treatments in which above ground parts of faba bean were harvested and removed and underground-stubble components (GM5, GM6, GM7, and GM8 treatments) were only mixed to the soils. The results are, of course, related to the amount of the organic materials added to the soil with green manuring.

The activity levels of the DG under maize conditions were higher compared to the wheat conditions throughout the maize-wheat crop rotation periods. When added to the soil, the simple structure organic C sources (monosaccharides, or glucose suchlike simple sugars) decompose in a short period of time. Microbial activity increases with the addition of the low C/N ratio organic residuals such as legumes [19-22]. The changes in temperature and moisture during harvest have affected the soil DG activity. Since month of October is more temperate and humid in October than the July, October is suitable to provide protoplasmic water for microbial activity. The results of current study agree with the previous published data that soil enzymes are more active at the end of spring and at the beginning of summer and autumn [23]. Yuan and Yue [4] found the highest DG level in autumn season and the lowest value of DG in winter time.

In the first rotation period, the UA enzyme activity was higher under wheat conditions compared with maize. The variation was probably caused from the origin of UA and DG enzymes. DG is an oxidoreductase and exists in viable cells [15]. DG activity decreases depending on the decreasing the microbial activity. Whereas, UA activity has extra cellular character and was rather constant under both green manuring and inorganic fertilization due to the adsorption and protection by organic complexes. In the second rotation period, the UA enzyme activity values were lower under wheat due to considerable differences in climate.

The results showed that application of GM improved the soil biological properties depending on type of application, amount and chemical composition of the GM. The application of faba bean as a winter cover crop can lead to an increase in soil enzymatic activity. The GM increased the soil UA and DG activities when compared to conventional fertilization. The increases in UA and DG activities were higher with the application of all components of faba bean as GM compared to the application of underground stubbles.

The authors have declared no conflict of interest

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