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Full Length Article

Short-term Effect of Biogas Residue on some Soil Enzymes Activities under Maize and Clover Growth in a Semiarid Ecosystem

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Abstract

The increases in anaerobically digested organic wastes in the world created a greater attention to evaluate the impact of the residue on soil properties. The purpose of this experiment was to evaluate anaerobically digested cattle manure on some soil chemical and biological properties under maize and clover production. Biogas residue was applied as 20, 40 and 60 t ha^{-1} (BGR20, BGR40 and BGR60) to the plots with a control (including only chemical fertilizers). Soil samples were taken from 0-15 and 15-30 cm depths at the end of growing season of maize and clover (October). Soil organic carbon (SOC) slightly increased with biogas residue while the increase was greater in microbial biomass carbon (MBC). However, microbial biomass nitrogen (MBN) was constant through the treatments and only significant difference occurred between the plants as greater in clover plots. Dehydrogenase enzyme activity was similar in maize plots and slightly increased in clover BGR40 compared to control. β-glucosidase enzyme activity significantly increased with the amendment of biogas residue compared to control, but there is no significant change between the rates of biogas residue. Alkaline phosphatase enzyme responded greatly to the doses of biogas residue than the others. The ratio of MBC/SOC enhanced with biogas residue however, the contrasting effect for dehydrogenase enzyme activity per microbial biomass was observed. These findings indicate that the response of soil enzymes on biogas waste can vary depending on the processing of organic waste. The activities of soil enzymes are sensitive to the characteristic of organic materials. © 2017 Friends Science Publishers

Keywords: Biogas residue; Dehydrogenase enzyme; β-glucosidase enzyme; Alkaline phosphatase enzyme

Introduction

Anaerobically digestion of organic wastes recently increased in the world due to use as an energy source and it has significant potential to reduce global warming and climate change (Clemens *et al.,* 2006). Soil microbial biomass and soil enzymes are known as an important factor of soil quality and nutrient cycling processes (Rezaei *et al.,* 2015). Soil management practices can influence soil properties and various soil enzymes, which can be used as sensitive indicators of soil quality. Agricultural areas shows large degree of spatial variability in soil physicochemical properties (Vasseur *et al.,* 2013). This may alter the activity and composition of soil organisms (Schipanski and Drinkwater, 2012).

Measurement of soil microbial biomass shows no information about microbial activity. Soil enzymes are important for sustaining nutrient availability and their activities depend on soil microbial activity. Therefore, the activities of soil enzymes are indicators of soil degradation since this includes information about microbial status and soil physicochemical conditions of both the present and the past. Enzymatic activities of soil can be stabilized by clay-humus complexes, which play a major significant role in the depolymerisation of various polymeric macromolecules (Burns *et al.,* 2013).

Properties of organic residue may also impact microbial community structure and activity (Reed and Martiny, 2013). The availability of most limiting nutrients can be enhanced by the activity of soil enzymes (Allison *et al.,* 2011). Some studies indicated that enzyme activity and microbial biomass can vary high both seasonal and interannual (Sardans *et al.,* 2008). In semiarid regions, enhancing microbial community is a challenge in dryland cropping systems due to limited biomass production as a result of low rain and high ambient temperatures (Liebig *et al.,* 2006). The drought effect on soil decreases enzyme activities manly in summer and changes nutritional quality of enzyme substrate (Sardans and Penuelas, 2010). The reduction in soil enzyme activity can result in a decline on N released from organic matter and thus a decrease in the plant N uptake (Sardans and Penuelas, 2010).

The activity of soil enzyme will provide information about soil productivity. β-glucosaminidase enzyme can degrade chitin and has been correlated with N mineralization in soils (Tabatabai *et al.,* 2010).

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β-glucosaminidase is sensitive to cropping systems and involves in the degradation of plant components such as cellobiose, melobiose, and chitin (Sotomayor-Ramirez *et al.*, 2009). Phosphates enzyme transfers the phosphate groups from nucleic acid and contributes to the P-cycle (Acosta-Martinez *et al.*, 2011). Soil phosphates activity involves the mineralization and transformation of P in soil and provides some information about plant productivity. Dehydrogenase presents information about the electron transport system to remove the oxidative substrate, and has been correlated with the oxygen uptake and organic substrate removal rates. The purpose of this study was to determine the different rates of anaerobically digested cattle manure (biogas residue) on some soil chemical (pH, EC, and organic carbon) and biological properties (β-glucosaminidase, dehydrogenase, and phosphates) under maize and clover production in the semiarid ecosystem of northern Turkey.

Materials and Methods

Experimental Site and Design

The study was conducted in 2014 at the experimental station of Gaziosmanpaşa University, Tokat district (40°18′ N and 36°34′ E), Turkey. The average annual precipitation and temperature of the study area are around 430 mm and 11.9°C respectively, and most of the precipitation occurs in winter and spring seasons. The experiment was conducted on Kanal series which is classified as clay loam (Typic Ustorthent). Four treatments including a control (three replicates each) and two plants maize (*Zea mays indentata)* and clover (*Medicago sativa*) – were established in split plot design with total 24 plots (5 × 3 m).

Experimental Treatments

Anaerobically digested cattle manure (biogas residue) were applied to the rates of 20, 40, and 60 t ha^{-1} (BGR20, BGR40, and BGR60, respectively) and the control plots had only chemical fertilizers based on the requirements of plants. The chemical properties of biogas residue were presented in Table 1. Biogas residue was applied to the surface of plots and mixed to 10 cm of soil. Maize and clover were planted on March 15. Soil samples were taken from 0-15 and 15-30 cm depths using a hand probe (2.5 cm diameter) at the end of growing season (October). Soil samples sieved using 2 mm-sieve and stored at 4°C until analysis. Some soil physical, chemical, and biological properties were determined in the samples.

Soil Chemical and Biological Analysis

Soil pH and electric conductivity were measured in the air-dried soil using a glass electrode (soil:water ratio, 1:2.5).

Table 1: Chemical properties of biogas residue

pH	7.3 ± 0.2
Organic C (g kg^{-1})	276.7 ± 6.8
Organic matter (g kg^{-1})	245.3 ± 5.3
Total N (g kg^{-1})	17.5 ± 0.8
Available P (g kg^{-1})	2.32 ± 0.2
C:N	14.7 ± 0.7

The mean value ± standard error (n = 3)

Soil organic carbon (SOC) content was determined using the modified Walkley-Black wet oxidation method (Nelson and Sommer, 1982). Microbial biomass C (MBC) was estimated by the fumigation-incubation method (Horwath and Paul, 1994). A alkaline solution (NaOH) was used for trapping CO_2 during incubation period. The solution was titrated with 0.5 M HCl after precipitation of the carbonates using $BaCl_2$ solution. Microbial biomass N (MBN) was determined by extracting 2 M KCl solution at the end of incubation (Keeney and Nelson, 1982).

Enzyme activities in soil were determined by the procedure described by Tabatabai (1994). Dehydrogenase enzyme activity was measured using the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenylformazon (TPF). Alkaline (pH 11) phosphatase activity was described by determination of the phenol release during 1 h at 37°C incubation period. β-glucosidase enzyme activity was assayed by determination of *p*-nitrophenol released by β-glucosidase after 1 h of incubation of soil samples at 37°C with pnitrophenyl-β-D-glucopyranoside (PNG) in modified universal buffer (pH 6.0) as the substrate.

Statistical Analysis

The analysis of variance (ANOVA) was performed for all measurements. All the values were the mean of three replications and standard errors were also determined for the comparison of soil properties. Significant statistical differences for the doses and depths were established by Duncan's test at $\alpha = 0.05$.

Results

The effects of biogas residue on some soil properties were presented in Table 2. The effect of biogas residue on soil reaction was not significant ($p > 0.05$). Soil pH ranged from 8.1 to 8.5 at the end of plant growth. However, soil pH significantly increased in clover plots compared to maize plots ($p<0.05$). Electric conductivity (EC) under different rates of biogas residue was similar up to 30 cm depth. The only significant difference occurred between maize and clover plots as being greater in clover ($p<0.05$). The greater EC in clover plots could be the result of lower leaching and translocation of ions from deeper depth to the surface by evaporation. The application of biogas residue increased inorganic N content compared to control, but the highest inorganic N content was measured as 510 mg kg^{-1} N at 15 to 30 cm depth of BGR20.

Table 2: The effect of biogas residue on soil pH, electric conductivity (EC), and inorganic N contents under maize and clover growth

Treatment		pH		EC		Inorganic N	
	depth	maize	clover	maize	clover	maize	clover
	cm			mS cm^{-1}		mg kg^{-1} N	
Control	0-15	8.2±0.1a	8.5±0.1a	0.289±0.08a	0.314±0.06a	110±17a	100±16a
	15-30	8.2±0.1a	8.4±0.1a	0.289±0.06a	0.318±0.03a	260±12b	310±24c
BGR20	0-15	8.2±0.2a	8.5±0.1a	0.306±0.11a	0.312±0.07a	380±22c	460±27d
	15-30	8.1±0.0a	8.5±0.2a	0.298±0.07a	0.321±0.05a	460±25d	510±38d
BGR40	0-15	8.1±0.1a	8.5±0.1a	0.288±0.09a	0.328±0.02a	270±13b	360±31c
	15-30	8.1±0.2a	8.5±0.2a	0.315±0.07a	0.302±0.05a	340±24c	310±29c
BGR60	0-15	8.2±0.1a	8.5±0.1a	0.288±0.01a	0.323±0.03a	260±19b	460±37d
	15-30	8.1±0.1a	8.5±0.0a	0.295±0.00a	0.302±0.01a	140±21a	240±26b
Treatment		ns		ns		*	
Plant		***		*		ns	
Depth		ns		ns		ns	
Treatment × plant		ns		ns		ns	
Plant × depth		ns		ns		ns	
Treatment × plant × depth		ns		ns		ns	

BGR20 – application of biogas residue 20 t ha^{-1}; BGR40 – application of biogas residue 40 t ha^{-1}; BGR60 – application of biogas residue 60 t ha^{-1}. Mean ± standard error. *, **, *** – significant at the 0.05, 0.01 and 0.001 probability levels, respectively. ns – not significant. Different letter in each column indicates significant difference ($p < 0.05$)

Table 3: The effect of biogas residue on soil organic carbon (SOC), microbial biomass carbon (MBC), and microbial biomass N (MBN) under maize and clover growth

Treatment		SOC		MBC		MBN	
	depth	maize	clover	maize	clover	maize	clover
	cm	%		mg kg^{-1} C		mg kg^{-1} N	
Control	0-15	0.98±0.02a	0.66±0.07ab	94±16a	87±11a	18±5a	36±4a
	15-30	0.81±0.01bc	0.58±0.05a	65±21a	79±9a	11±3a	34±3a
BGR20	0-15	1.03±0.04a	0.93±0.04d	160±19c	214±21c	12±4a	45±6a
	15-30	0.84±0.03b	0.71±0.07b	147±25bc	124±16b	10±3a	37±5a
BGR40	0-15	0.88±0.06b	0.96±0.02d	198±31c	309±31e	19±5a	47±4a
	15-30	0.79±0.03c	0.59±0.04a	123±14b	284±24de	15±3a	45±4a
BGR60	0-15	0.87±0.05b	0.81±0.02c	346±35e	332±33e	22±6a	46±3a
	15-30	1.08±0.08a	1.01±0.04d	267±29d	256±17cd	18±3a	37±3a
Treatment		*		*		ns	
Plant		ns		ns		**	
Depth		ns		*		ns	
Treatment × plant		ns		ns		ns	
Plant × depth		ns		ns		ns	
Treatment × plant × depth		ns		ns		ns	

BGR20 – application of biogas residue 20 t ha^{-1}; BGR40 – application of biogas residue 40 t ha^{-1}; BGR60 – application of biogas residue 60 t ha^{-1}. Mean ± standard error. *, **, *** – significant at the 0.05, 0.01 and 0.001 probability levels, respectively. ns – not significant. Different letter in each column indicates significant difference ($p < 0.05$)

This result also indicated a greater N uptake by maize than clover, which resulted generally higher inorganic N content at all doses. Inorganic N content generally increased from surface to the deeper depth except the treatment of BGR60. The increasing rate of biogas residue enhanced N leaching from surface to 15-30 cm depth. However, the lower N content at 15 to 30 cm depth of BGR60 can be attributed to greater dissolved organic C which may stimulate denitrification below the surface soil.

Soil organic carbon (SOC) content significantly increased with the application of biogas residue ($p<0.05$) (Table 3). The highest SOC was obtained at 15-30 cm depth of maize plot (%1.08). These findings indicated a smaller change in SOC content in a short period of time. In addition, SOC content was similar in maize and clover plots. Soil microbial biomass C (MBC) was significantly affected by biogas residue (Table 3). The enhancing rate of biogas residue significantly increased MBC ($p<0.05$). Generally, the increasing rate of biogas residue stimulated MBC with the highest value of 346 mg C kg^{-1} in BGR60. MBC was similar in the both plant type ($p>0.05$), but significantly decreased below the surface soil ($p<0.05$). Microbial biomass N (MBN) was generally greater in clover planted plots compared to maize ($p<0.05$) and the highest MBN was 47 mg N kg^{-1} measured at 0-15 cm depth of BGR40 (Table 3). On the other hand, the response of MBN on the different rates of biogas residue was not significant in this study ($p>0.05$). The high MBN in clover plots might be the effect of higher inorganic N content stimulates N immobilization by soil microorganisms. The ratio of MBC:SOC constantly

increased from control to the highest dose of biogas residue except for 15 to 30 cm depth (Fig. 1). The lowest MBC/SOC was 0.96 in control and the highest one was 4.81 at in BGR40.

Activity of the studied enzymes was significantly affected with the application of biogas residue ($p<0.05$) (Fig. 2). The plots exposed to biogas residue had generally greater enzyme activity than control. However, dehydrogenase activity only increased in clover plots with amendment of biogas residue while dehydrogenase enzyme activity was similar in maize plots. β-glucosidase activity significantly increased with the addition of biogas residue in both maize and clover plots compared to control ($p<0.05$) (Fig. 2). However, the differences between the doses of biogas residue was not significant on β-glucosidase activity in this study ($p>0.05$). The highest β-glucosidase activity was 51.1 mg PNP kg^{-1} h^{-1} in clover plot. Overall, the greater activities were measured at surface soil and β-glucosidase activity significantly decreased with depth ($p<0.05$). Alkaline phosphatase enzyme is influenced by biogas residue compared to control ($p<0.05$) (Fig. 2). The greater phosphates enzyme (503 mg PNP kg^{-1} soil h^{-1}) was measured at the surface of BGR40. However, there is no clear difference between the doses of biogas residue and the higher alkaline phosphatase activities were measured at BGR40 and BGR60. Phosphates enzyme activity decreased at 15-30 cm depth, which is similar to the previous soil enzymes.

Dehydrogenase activity per microbial biomass C was used as to estimate microbial metabolic activity. Dehydrogenase activity per microbial biomass C in the treatments (BGR20, BGR40, and BGR60) decreased compared to control ($p<0.05$) (Fig. 3). The lower dehydrogenase per microbial biomass C can be attributed to limited response of dehydrogenase enzyme activity on biogas residue as compared to MBC. Also, the lower increases of SOC in the first year of organic amendment and the changes in microbial community due to anaerobically digested residue are the most apparent reasons of the lower dehydrogenase activity per microbial biomass carbon.

Discussion

Biogas residue significantly increases MBC in soil, but decreases specific microbial growth rate, and results a change in microbial community to slower-growing microorganisms, because of limited availability of C related with the low labile C and more lignin in biogas residues (Chen *et al.*, 2012). Similarly, the four year field trial of biogas residue enhanced MBC and metabolically active microorganisms, compared to control (Odlare *et al.*, 2008). The increases in MBC/SOC ratio indicates a greater dynamic of soil organic C with the application of biogas residue, and is a clear indication of soil quality.

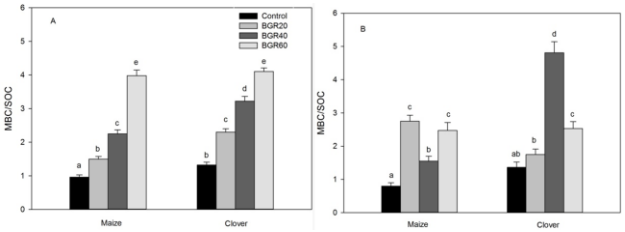

Fig. 1: Effects of biogas residue on microbial biomass C per soil organic carbon (MBC/SOC) at 0-15 (A) and 15-30 (B) cm depths under maize and clover production. Control – no biogas residue, BGR20 – biogas residue 20 t ha^{-1}, BGR40 – biogas residue 40 t ha^{-1}, BGR60 – biogas residue 60 t ha^{-1}. Vertical bars are standard error; not followed by the same letter indicate significant difference ($p < 0.05$)

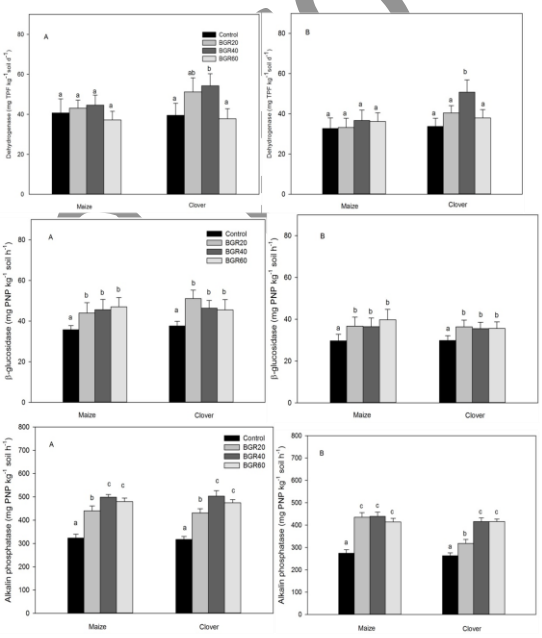

Fig. 2: Effects of biogas residue on dehydrogenase, β-glucosidase, and alkaline phosphatase activities at 0-15 (A) and 15-30 (B) cm depths under maize and clover production. Control – no biogas residue, BGR20 – biogas residue 20 t ha^{-1}, BGR40 – biogas residue 40 t ha^{-1}, BGR60 – biogas residue 60 t ha^{-1}. Vertical bars are standard error; not followed by the same letter indicate significant difference ($p < 0.05$)

The larger variation in MBC/SOC was observed at 15-30 cm depth compared to surface soil, which may relate to the movement of soluble organic C and nutrients from surface to the deeper depth.

The extensive rate of biogas residue was not created a linear increase in dehydrogenase activity. The highest dehydrogenase enzyme activity was measured at BGR40 in maize plots. Dehydrogenase enzyme activity slightly decreased at 15-30 cm depth compared to surface soil.

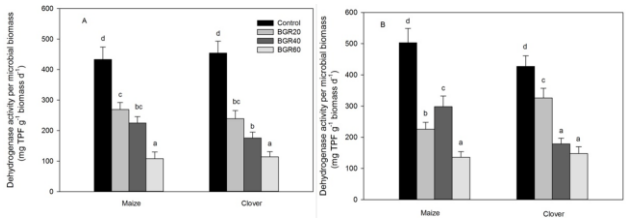

Fig. 3: Effects of biogas residue on dehydrogenase activity per microbial biomass at 0-15 (A) and 15-30 (B) cm depths under maize and clover production. Control – no biogas residue, BGR20 – biogas residue 20 t ha^{-1}, BGR40 – biogas residue 40 t ha^{-1}, BGR60 – biogas residue 60 t ha^{-1}. Vertical bars are standard error; not followed by the same letter indicate significant difference ($p < 0.05$)

Dehydrogenase is considered as a sensitive indicator of soil quality (Madejon *et al.,* 2007) and redox potential, and participates in microbial respiration (Paz-Ferreiro *et al.,* 2012). Dehydrogenase activity usually increases with the amendment of labile soil organic matter such as sewage sludge and biogas residue (Serra-Wittling *et al.,* 1996), however, contradict result similar to our findings have been indicated (Paz-Ferreiro *et al.,* 2012). High level of toxic elements such as Cu, As, Pb, Cd, Zn and Hg can significantly reduce many soil enzymes (Angelovicova *et al.,* 2015). The inhibitory compounds are organic in nature, possibly constitutive organic pollutants in anaerobic residue (Leven *et al.,* 2006). Several other researchers also indicated an increase in dehydrogenase activity under mineral N fertilizer (Chu *et al.,* 2007). Paz-Ferreiro *et al.* (2012) reported a significant decrease in β-glucosidase activity with the addition of sewage sludge compared to control. In this study, β-glucosidase slightly increased compared to control, but there is not an extensive increase in β-glucosidase activity with the addition of biogas residue. In a semiarid Mediterranean ecosystem through a year the greatest variation in soil β-glucosidase activity was observed and there was a great correlation with soil moisture content (Sardans and Penuelas, 2010). This indicates many environmental factors have significant impact on β-glucosidase enzyme activity.

The high enzyme activity in soil reflects the larger availability of nutrients. The application of biogas residue significantly increases the amount of phosphobacteria, silicate bacteria, ammonifiying bacteria, N-fixation bacteria and actinomycetes, but the amount of fungi was significantly inhibited (Hao *et al.,* 2011). Therefore, phosphates enzyme significantly increased with the amendment of biogas residue compared to the other enzymes ($p<0.05$). Similarly, mineral fertilization with pig manure significantly increases microbial biomass C and dehydrogenase activity per microbial biomass C (Luo *et al.,* 2015).

Conclusion

The application of biogas residue contributed limited change on soil organic carbon (SOC). However, the greater response was observed in microbial biomass carbon (MBC) with the increasing rate of biogas residue. Dehydrogenase enzyme activity was almost similar in the all treatments. The impact of biogas residue on β-glucosidase enzyme activity was greater than dehydrogenase enzyme activity; however, there was not a clear difference between the rates of biogas residue except for control. Clover plots had potentially higher enzyme activity than maize plots. The greatest response to biogas residue was observed in alkaline phosphate enzyme. Dehydrogenase enzyme activity per microbial biomass decreased with the increasing rate of biogas residue. The response of soil enzymes to biogas residue was limited in this study and soil enzymes showed a different tendency from each other. It can conclude that biogas residue display a lower mineralization rate than other organic fertilizers due larger amount of resistant organic materials to microbial decomposition.

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