American-Eurasian J. Agric. & Environ. Sci., 12 (3): 377-383, 2012
ISSN 1818-6769

Canola Traits and Some Soil Biological Indices in Response to Fertilizer and Tillage Management

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Abstract: This study describes the effects of fertilization and tillage methods on soil microbial community and canola traits. A field experiment was carried out in 2009-10 growing seasons. Experiments were arranged in a split plot based on randomized complete block design with three replications. Main plots consisted of no tillage (T1), minimum tillage (T2) and conventional tillage (T3). Six strategies for obtaining the basal fertilizer requirement including (N1): farmyard manure; (N2): compost; (N3): chemical fertilizers; (N4): farmyard manure + compost; (N5): farmyard manure + compost + chemical fertilizers and (N6): control were arranged in sub plots. Results showed that the activities of all enzymes were generally higher in the N4 treatment than in the unfertilized and chemical fertilizer treatments. The phosphatase, catalase and urease activities in the N3 treatment were significantly lower than in the FYM and compost treatments. The activity of all enzyme activity tended to be higher in the NT treatment. The highest leaf N, P and K containing grain and grain yield was obtained from N5 treatment. Applying CT system caused to a reduction in grain yield as compared with chisel plowing.

Key words: Enzyme activity · Compost · Farmyard manure · Tillage

INTRODUCTION

To understand soil fertility and nutrient management in tillage systems, it is need to recognize the unique conditions in these systems that influence nutrient behavior and management. One of the most important functions of conservation tillage systems is the maintenance of crop residues on the soil surface to protect the soil from erosion. To achieve proper residue maintenance, there must be limited soil mixing [1]. If crop residues are not incorporated or mixed with the soil, then fertilizer, manure and limestone also will not be mixed with the soil. Lack of incorporation can have a major impact on the behavior and management of nutrients. Producers traditionally have depended on tillage to mix immobile nutrients such as phosphorus with the soil, thus moving them into the primary rooting zone of crops. With conservation tillage systems this movement does not occur. For nitrogen sources that contain urea, lack of incorporation can result in substantial nitrogen losses from volatilization [2]. Fertilization is one of the soil and crop management practices, which exert a great influence on soil quality [3]. Farmyard manure (FYM) and compost are organic sources of nutrients that also have been shown to increase soil organic matter and enhance soil quality. The farmyard manure is a significant source of nutrients on many farms in Iran. For farmyard manure to be effectively utilized as a source of nutrients, the content and behavior of the nutrients in farmyard manure must be known. Farmyard manure nutrient content is best determined by analyzing a representative sample of the farmyard manure. Nutrient behavior in farmyard manure varies with the nutrient. Thus, management considerations for farmyard manure use in conservation tillage also vary.

Microbial communities perform necessary ecosystem services, including nutrient cycling, pathogen suppression, stabilization of soil aggregates and degradation of xenobiotics. Soil microbial biomass, activity and community structure have been shown to respond to agricultural management practices. Soil microbial properties have a strong correlation with soil

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health. Some research has already suggested the favorable effects of conservation tillage practices and organic fertilizers on soil enzyme activities [2]. Inorganic fertilizers had relatively less effect on soil microbial biomass and activities than organic fertilizers [4-7]. Fertilization also results in microbial community shifts in soils [8, 9].

The objective of this study was to determine the short-term effects of conservation management practices, such as no-tillage, reduced tillage and organic fertilizers on microbiological soil quality indicators and canola traits under Mediterranean conditions in Kurdistan province of Iran.

MATERIALS AND METHODS

This study was conducted at the Islamic Azad University of Sanandaj in Kurdistan province of Iran during 2009-10 growing seasons. The dominant soil type is Inceptisol. The annual temperature averages 19°C and the annual rainfall averages 526 mm. Experiments were arranged in the split plot based on randomized complete block design with three replications. Main plots consisted of no tillage (T1), minimum tillage (MT) (disk harrowing with average depth of 15 cm + one shallow disk harrowing) (T2) and conventional tillage (moldboard plowing with average depth of 30 cm + two shallow disks followed by secondary tillage with a soil grubber and harrow for seedbed preparation) (T3). In no tillage (NT), crop residues cut by the combine were chopped and spread evenly with a combine-attached chopper. NT plots were seeded with a NT seed drill. Sub-plots were six strategies of supplying the basal fertilizer requirements of canola, including (N1): 30 t FYM ha^{-1}; (N2): 15 t compost ha^{-1}; (N3): 100 kg triple super phosphate ha^{-1} + 150 kg Urea ha^{-1}; (N4): 15 t FYM ha^{-1} + 7.5 t compost ha^{-1}, (N5): 10 t FYM ha^{-1} + 5 t compost ha^{-1} + 50 kg triple super phosphate ha^{-1} +75 kg Urea ha^{-1} and (N6) Control (without fertilizer). Expectation values of basal fertilizers were determined according to soil test analysis. Soil texture was clay loam (29% sand, 41% clay and 30% silt) with 0.81% organic matter and a pH of 7.5. The FYM and compost were also analyzed for chemical and nutrients properties (Table 1). FYM, compost and chemical fertilizers were added to plots before sowing canola. For conventional tillage (CT) and MT chemical fertilizer or organic fertilizers was applied and then incorporated with tillage, while for NT treatments, fertilizers were surface applied on the plots. Urea fertilizer was applied equally two times before sowing canola and flowering.

Canola seeds planted on September 18, 2009. Main plot size was of 15×20 m and spaces between main plots were three meters. The field was irrigated twice with a 7-9 day interval for the better germination of seeds. The field was also irrigated at stemming and flowering along with fertilization and at podding and grain filling. Weeds removed by hand in all plots.

Soil for microbiological analysis was sampled in canola plots. Soil samples were collected in crop rhizosphere at flowering stage of canola growth. Plants were excavated from four random 0.5-m lengths of a row from each plot. Loose soil was shaken off the roots and the soil that adhered strongly to the roots was carefully brushed from the roots and kept as rhizosphere soil. The four rhizosphere samples from each plot were combined, passed through a 2-mm sieve and stored at 4°C until required for analysis.

Activity of soil catalase was determined according to Ladd [10], using H_2O_2 as substrate, in units of H_2O_2 decompositon g^{-1} dry soil over 30 min (mg H_2O_2 (30 min^{-1}) g^{-1} d.m. soil). To measure alkaline (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2) enzymes p-nitrophenyl phosphate disodium (0.115 M) were used as the substrate [11]. Soil samples (1 g) were treated with 2ml of 0.5 M sodium acetate buffer with a pH of 5.5 (using acetic acid) [12] and 0.5 ml of substrate and were incubated at 37°C for 90 min. Cooling at 2°C for 15 min inhibited the reaction. The treated samples were then mixed with 2 ml of 0.5M NaOH and 0.5 ml of 0.5 M $CaCl_2$ (to inhibit the enzyme reaction) and centrifuged at 4000 rpm for 5min. Using spectrometry at 398 nm the produced p-nitrophenol was measured [13]. Urease (EC 3.5.1.5) activity was measured using 0.5 M urea as a substrate in 0.1 M phosphate buffer at pH 7.1 [14]. The NH_4^+- N produced by urease activity was determined using a flow injection analyzer (FIAStar, Tecator, S). To account for the NH_4^+-N fixation by soils, NH_4^+-N solutions with concentrations in the range of those released by urease activity was incubated with these spoils. All enzyme activities values were calculated based on of oven-dry (105°C) weight of soil.

Table 1: Chemical characteristics of farmyard manure and compost applied to the soil

Characteristic	pH	N	P	K	Ca	Mg	S	Cu
		(%)			(ppm)			
FYM	7.45	0.47	0.49	0.31	2745	1100	34	25
Compost	7.21	0.78	1.15	0.51	1950	1890	384	295

The phosphorus and nitrogen content of matured seeds was determined by vanado molybdate phosphoric acid yellow colour method and Microkjeldahl method, respectively [15]. Also, the potassium content was determined by Flame Photometer model-EEL. At harvest time harvest, grain yield was evaluated from an area of 2×2.5 m^2 in each sub plot.

Using SAS [16] data were subjected to analysis of variance. Analysis of variance (ANOVA) was used to detect the treatment effects on measured variables and the least significant difference (LSD) were used to compare means of measured enzyme activities and microbial biomass carbon ($P < 0.05$).

RESULTS AND DISCUSSION

Soil Enzyme Activity: The activities of catalase, urease, acid and alkaline phosphatase varied significantly in different fertilization methods. Fertilization does not significantly effect on cellulases activity. Only, urease activity was significantly affected by the two-way interactions between fertilizers × tillage (Table 2). The activities of all enzymes were generally higher in the N4 treatment than in the unfertilized and chemical fertilizer treatments (Table 3). There were no differences in phosphatase and cellulases activity between the compost treatment and the FYM treatments. The phosphatase, catalase and urease activities in the N3 treatment were significantly lower than in the FYM and compost treatments. As shown in Table 3, alkaline and acid phosphatase generally increased with compost application. Increased phosphatase activity could be responsible for hydrolysis of organically bound phosphate into free ions, which were taken up by plants. Tarafdar and Marschner [8] reported that plants can utilize organic P fractions from the soil by phosphatase activity enriched in the soil-root interface. The observed increase in enzymatic activities due to organic fertilizers amendments are in accordance with previous studies. Martens *et al*. [17] reported that addition of the organic matter maintained high levels of phosphatase activity in soil during a long term study. Giusquiani *et al*. [18] reported that phosphatase activities increased when compost was added at rates of up to 90 t ha^{-1} and the phosphatases continued to show a linear increase with compost rates of up to 270 t ha^{-1} in a field experiment. Application of nitrogen fertilizers significantly decreased urease activity while addition of organic manure increased its activity. They also concluded that because the nitrogen fertilizers used in the experiments contained NH^{4+} and that the reaction products of urease being NH^{4+}, microbial induction of urease activity had been inhibited. The effect of organic amendments on enzyme activities is probably a combined effect of a higher degree of stabilization of enzymes to humic substances and an increase in microbial biomass with increased soil carbon concentration [17].

The enzyme activity in organic amendment soil increased by an average 2-4 fold compared with the unamended soil. Application of compost caused a significant increase in enzyme activity [17]. In addition, the higher organic matter levels in the compost treatments may provide a more favorable environment for the accumulation of enzymes in the soil matrix, since soil organic constituents are thought to be important in forming stable complexes with free enzymes. Soil factors, including redox potential (Eh) and pH can affect the rate of enzyme mediated reactions by influencing the redox status and ionization respectively, as well as solubility of enzymes, substrates and cofactors. In addition, some enzymes may predominate at specific pH levels. Application of compost and FYM caused a faster and higher reduction of soil and at the same time increased the soil pH. Report of Nayak *et al*. [19] showed that soil pH was lowest in the inorganic fertilizers amended plots and highest in compost amended plots. Soil dehydrogenase activity exhibited a strong negative relationship with Eh and a positive relationship with Fe^{2+} content, suggesting aeration status is the major factor determining the activity [20].

Results indicated statistically significant ($p<0.05$) differences in catalase, urease, acid and alkaline phosphatase in the soil among various methods of tillage (Table 2). The activity of all enzyme activity tended to be higher in the NT treatment compared to the MT and CT treatments. However, activity of catalase was similar in NT and MT treatments (Table 3). Finding of Jin *et al*. [1] has already suggested the positive effects of conservation tillage practices on soil enzyme activities. The generally higher enzyme activities in NT mainly resulted from the larger water availability in the plots rather than the better soil fertilities. Urease activity under T_1N_4 treatment in the two years of our study was the highest of all treatments. In this treatment co-application of compost and farmyard manure in no tillage system assemble good condition for urease activity. The higher bulk density could account for this difference. Enzyme activities were shown to be linearly related to soil bulk density [21].

Grain Nutrient (NPK): Basal fertilizers had a significant effect on grain nitrogen content, but tillage systems does not significant effects on nutrient uptake (NPK) by canola plants (Tables 2). The highest leaf N containing grain was obtained from N5 treatment. The main reason is that compost and farmyard manure can increase N availability to plant due to more nitrogen offered to plant. Hatch *et al*. [22] reported that incorporation of farmyard manure to the soil had beneficial effects of increasing biological nitrogen fixation, dry matter and N yields in red clover.

The results showed that different methods of soil fertility had a significant effect on grain phosphorus contents (Table 2). The highest grain P content was obtained from N5 treatment (Table 3). Increasing effect of combined application of compost and farm manure on soil enzymatic activity such as phosphatase and increasing P availability for plant has been reported by El-baruni and Olsen [23]. Chemical fertilizers (N_3) in comparison with compost and farmyard manure significantly increased grain P contents. Since phosphatases play an important role in nutrient P availability of organic manures, crop residue and phosphates activity, soil P availability appear to complement each other [24], therefore, providing P in rhizosphere can increase P uptake by plant. In addition, regarding to the importance of this element, the increase of P causes stimulating growth and increasing grain yield.

Basal fertilizers and biofertilizers had significant effect on grain potassium contents (Table 2). Combined application of basal fertilizers improved plant nutrition conditions. The highest grain K contents were obtained from N5 treatment. There is evidence that compost application increases potassium absorption in seeds [24]. The combined application of compost and seed inoculation with *Pseudomonas* increased the availability

Table 2: Effects of tillage and fertilization on soil enzyme activity and canola traits

Source of Variance	Catalase	Acid phosphatase	Alkaline phosphatase	Urease	Cellulases	Grain yield	Nitrogen	Phosphorus	Potassium
Block	**	n.s	*	n.s	n.s	*	**	*	**
Tillage	**	*	**	**	n.s	**	n.s	n.s	n.s
Fertilization	**	**	**	**	n.s	**	**	**	**
Tillage × Fertilization	n.s	n.s	n.s	**	n.s	n.s	n.s	n.s	n.s

n.s., * and ** are Non- significant, Significant at the 0.05 and 0.01 probability levels, respectively

Table 3: Effect of tillage and fertilization methods on soil enzyme activity

Treatment	Catalase (µg)	Acid phosphatase (µg)	Alkaline phosphatase (µg)	Urease (µg)	Cellulases (µg)
Fertilizers					
FYM (N1)	49.7 b	167.4 b	2987.3 b	49.6 a	13.1 a
Compost (N2)	69.9 a	169.2 b	3001.4 b	44.4 b	14.3 a
Chemical fertilizer (N3)	43.2 b	158.1 c	2678.4 c	27.8 c	12.7 a
FYM + Compost (N4)	73.6 a	226.6 a	3314.4 a	49.8 a	15.2 a
FYM + Compost + Chemical (N5)	54.7 b	169.2 b	3158.4 ab	39.4 b	13.4 a
Control (N6)	24.3 c	41.8 d	2658.7 c	27.9 c	12.1 a
Tillage					
No tillage (T1)	55.58 a	219.6	3645.8 a	45.6 a	13.52 a
Minimum tillage (T2)	53.34 a	169.5 b	3139.7 b	37.8 b	13.46 a
Conventional tillage (T3)	48.67 b	76.8 c	2113.6 c	36.1 b	13.42 a

Mean values in each column with the same letter(s) are not significantly different using LSD tests at 5% of probability

Table 4: Effect of fertilization and tillage methods on grain yield and NPK content

Treatment	Grain yield (kg/ha)	Nitrogen (mg/g)	Phosphorus (mg/g)	Potassium (mg/g)
Fertilizers				
FYM (N1)	2405.6 d	41.38 d	8.31 c	28.33 e
Compost (N2)	2629.1 c	50.84 c	9.14 c	30.74 d
Chemical fertilizer (N3)	3161.9 b	69.11 b	14.43 b	40.93 b
FYM + Compost (N4)	3115.8 b	84.07 a	12.82 b	37.87 c
FYM + Compost + Chemical (N5)	4169.3 a	57.25 bc	19.05 a	42.85 a
Control (N6)	958.6 e	39.33 d	4.84 d	26.74 f
Tillage				
No tillage (T1)	2366.5 b	58.33 a	11.59 a	35.39 a
Minimum tillage (T2)	3613.9 a	54.56 a	10.82 a	34.78 a
Conventional tillage (T3)	2238.8 b	58.13 a	11.88 a	33.49 a

Mean values in each column with the same letter(s) are not significantly different using LSD tests at 5% of probability and uptake of minerals like P, Mn and K in seeds [24]. Canola grain yield was affected by different soil fertility systems. Microorganism's activity to excrete organic acids and phosphates could release elements from complexes presently in soil and increase nutrient availability to plants [25, 26].

Grain Yield: Base fertilizers comparison revealed that highest grain yield was obtained from N5 treatment (Table 3). For justification of this difference, it could be stated that along with meeting plant need to phosphorus, adding compost and farmyard manure to soil could provide micro elements for plant. Compost applied in the current study has been shown to contain elevated concentrations of micro elements including sulfur (S). Sulfur is one of the elements that canola shows positive response to it [27]. Moreover, it seems that organic fertilizers causes improving soil structure and optimizing root growth conditions by providing organic matter and nutrients.

Tillage systems significantly affected grain yield. Azim-Zade *et al*. [28] showed the same results on wheat under various tillage systems. The highest rate of grain yield (3613 kg/ha) was produced by applying MT that was statistically greater than which in CT and NT systems (Table 3). Applying CT system caused to a reduction of 38 percentages in grain yield as compared with minimum tillage system. Since CT system cause to soil moisture reduction, therefore this tillage system is not recommended under dry framing conditions. Increasing cone index and soil compaction are the main reasons of yield reduction. Soil compaction leads to restriction of root growth. Consequently water and nutrient uptake by roots will be confused and diminished. Schillinger [29] demonstrated that using NT system caused to lower production of wheat, barley and oat in comparison with CT system. Main reasons cited for lower yields under no-till system are reductions in plant density [30], increased weed infestation [31] and soil physical properties that limit crop growth [32]. The most probable cause of erratic stand establishment for no-till wheat treatment was poor soil-seed contact associated with the use of the drill for seeding into a layer of crop residue. On the other hand, Tarkalsona *et al*. [33] reported that application of NT system in a long term period led to indicative improvement in wheat productivity in comparison with CT system. No tillage system needs specific planting tools that cannot be find easily in Iran, therefore using chisel plow is more favored by farmers. Shams-Abadi and Rafiee [34] resulted that using chisel, leads to increase wheat production. Furthermore Quincke *et al*. [35] indicated sorghum production was higher under CT system comparing to No tillage system.

CONCLUSION

The present study provides information on soil microbial biomass dynamics and biocatalytic activities as influenced by organic and inorganic fertilization and tillage systems in canola production conditions. The results demonstrate that soil enzyme activity is sensitive in discriminating between organic fertilizers and inorganic fertilizer application on a short-term basis. Enzymatic properties were also closely related with the C inputs. Consistent distinctions in enzyme activities were observed between different tillage practices. These differences were most pronounced between no tillage at the one hand and conventional and reduced tillage at the other hand. According to the results of various characteristics of canola like as yield and minerals compound of grain, the T_2N_5 treatment could be suggested as superior treatment in this study. This treatment seems to be cost effective and environmentally sound; therefore, it could allocate our agro-ecosystems into sustainable agriculture. The more ecological approach to soil management has come from the sustainable development agenda in which the main concern with the maintenance of yield is closely associated with desires to conserve natural resources, including a greater value accorded to maintenance of biodiversity.

ACKNOWLEDGMENTS

The Sanandaj Agriculture Research Center for farm support and Islamic Azad University, Sanandaj Branch is acknowledged for financial support.

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