Effects of Fertilization and Tillage on Soil Biological Parameters

Khosro Mohammadi

Abstract- This study was conducted to determine the best tillage and fertilization system for soil microbial community in sunflower production. Experiments were arranged in a split plot based on randomized complete block design with three replications. Main plots consisted of tillage systems including the no-tillage (T1), minimum tillage (T2) and conventional tillage (T3). Six strategies of fertilization 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 subplots. Results showed that the addition of compost and farmyard manure increased the soil microbial biomass. No tillage system increased MBC compared to other tillage systems. The activities of all enzymes were generally higher in the N4 treatment. The activity of phosphatase and urease tended to be higher in the no-tillage treatment compared to the T1 and T2 treatments.

Keywords- Compost, Enzyme activity, Farmyard manure, No tillage.

I. INTRODUCTION

Regarding to the physical, chemical and biological attributes of the soil, there are divers ecological niches to grow different microorganisms. Therefore soil protection and balance development between all characteristics of the soil is the key factor to ensure optimum microbe growth and sustainable utilization of the agricultural ecosystems. Conventional sunflower production utilizing tillage, commercial fertilizer applied through pesticides, and irrigation can improve the grain yield. However, this intensive production system also can degrade soil biological quality [21]. Alternative systems have been developed that use renewable organic resources and minimize tillage to build soil organic matter and enhance soil quality. Fertilization is one of the soil and crop management practices, which exert a great influence on soil biological quality [3]. Farmyard manure and compost are organic sources of nutrients that also have been shown to increase soil organic matter and enhance soil quality. It is well known that organic amendments, such as plant residues, manures and composts have a number of benefits in soil physical and biological properties. Many reports have also revealed different aspects of biology of soils amended with organic matters, including the number of general microorganisms [4, 19], biomass of bacteria and fungi [1, 11], enzyme activities [8] and biochemical properties [12, 18].

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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. Alternation to no-tillage or increased cropping intensity increases microbial biomass C (MBC) in response to increase nutrient reserves and improved soil structure and water retention [2].

Enzyme activities have been indicated as soil properties suitable for use in the evaluation of the degree of alteration of soils in both natural and agroecosystems. The objective of this study was to determine the effects of conservation management practices, such as no-tillage, reduced tillage and organic fertilizers on microbiological soil indicators in a sunflower field under Mediterranean conditions in Kurdistan province of Iran.

II. MATERIALS AND METHODS

2.1. Site description and experimental design

This research was conducted at the Islamic Azad University of Sanandaj (11°45' lat. N; 30°47' long. E, 1400 m above sea level) in Kurdistan province of Iran in 2011. The dominant soil type is Inceptisol. The annual temperature averages 18 °C and the annual rainfall averages 512 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 (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) plots, 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 fertilizers were
determined according to soil test analysis. Soil texture was clay loam (28% sand, 42% clay and 30% silt) with 0.8% organic matter and a pH of 7.6. The farmyard manure and compost were also analyzed for chemical and nutrients properties. Farmyard manure, compost and chemical fertilizers were added to plots before sowing sunflower. In conventional tillage (CT) and minimum tillage (MT) plots chemical and organic fertilizers was applied and then incorporated with tillage, while for NT plots, fertilizers were surface applied on the plots. Urea fertilizer was applied equally two times before sowing and at flowering.

Sunflower seeds planted on April 12, 2011. Main plot size was of 15×20 m and spaces between main plots were three meters. The field was irrigated three times with a 4 day interval for the better germination of seeds. The plots were irrigated to maintain moisture level of 0.33 bars (i.e. field capacity) (moisture level was measured using Tensiometers installed in the field).

2.2. Soil sampling

Soil for microbiological analysis was sampled in all plots. Soil samples were collected in crop rhizosphere at flowering stage of sunflower. 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.

2.3. Microbial biomass carbon (MBC)

The MBC was determined on a 15-g oven-dry equivalent field-moist soil sample (sieved to <5mm) by the chloroform fumigation extraction method. In brief, organic C from the fumigated (24 h) and non-fumigated (control) soil were quantified by a TOC/TN analyzer (Model: TOC-Vcpn and TNM-1, Shimadzu Corp., Kyoto, Japan). The non fumigated control values were subtracted from the fumigated values. Biomass C was determined using the following formula: MBC = (C in fumigated soil - C in unfumigated soil)/k, where k = 0.45 [27]. Each sample had duplicated analyses, and results are expressed on a moisture-free basis.

2.4. Soil enzyme activities

Protease (EC 3.4.21-24) activity was determined according to Kandeler [7]. To measure alkaline (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2) enzymes [14] p-nitrophenyl phosphate disodium (0.115 M) were used as the substrate. 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 [17]. Dehydrogenase activity was determined by the reduction of triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) as described by Serra-Wittling et al. [23] with modifications. All enzyme activities values were calculated based on of oven-dry (105 °C) weight of soil.

2.5. Statistical analysis

Using SAS (SAS Institute 2003) data were subjected to analysis of variance, including combined analysis. 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 traits (P < 0.05). In addition correlation coefficients among soil enzymes and MBC were also determined.

III. RESULTS AND DISCUSSION

3.1. Microbial biomass-C in soil

The results indicated statistically significant (p < 0.05) differences in the level of MBC in the soil between various methods of tillage and fertilization. There were no significant differences between interaction effect of tillage and fertilization on MBC. The pattern of variation of MBC in the soil during the two years of study was similar. The addition of compost or FYM, significantly (p < 0.05) increased the soil MBC in comparison to the chemical fertilizer and the control. Higher levels of MBC in compost treated soil could be due to greater amounts of biogenic materials like mineralizable nitrogen, water soluble carbon and carbohydrates. Integrated use of chemical fertilizers and organic matter (N5) brings in more MBC in soil compared to their single application (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MBC (µg)</th>
<th>Protease (µg)</th>
<th>Acid phosphatase (µg)</th>
<th>Alkaline phosphatase (µg)</th>
<th>Urease (µg)</th>
<th>Dehydrogenase (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal fertilizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FYM (N1)</td>
<td>278.4 c</td>
<td>86.5 c</td>
<td>167.4 b</td>
<td>2987.3 b</td>
<td>49.6 a</td>
<td>60.1 b</td>
</tr>
<tr>
<td>Compost (N2)</td>
<td>312.6 c</td>
<td>94.6 bc</td>
<td>169.2 b</td>
<td>3001.4 b</td>
<td>44.4 b</td>
<td>62.9 ab</td>
</tr>
<tr>
<td>Chemical fertilizer (N3)</td>
<td>196.3 d</td>
<td>87.1 c</td>
<td>158.1 c</td>
<td>2676.8 c</td>
<td>28.8 c</td>
<td>21.2 d</td>
</tr>
<tr>
<td>FYM + Compost (N4)</td>
<td>409.5 b</td>
<td>110.3 a</td>
<td>226.6 a</td>
<td>3314.4 a</td>
<td>49.8 a</td>
<td>63.8 a</td>
</tr>
<tr>
<td>FYM + Compost + Chemical (N5)</td>
<td>691.2 a</td>
<td>96.2 b</td>
<td>169.2 b</td>
<td>2879.1 bc</td>
<td>29.4 c</td>
<td>53.7 c</td>
</tr>
<tr>
<td>Control (N6)</td>
<td>89.3 c</td>
<td>73.1 d</td>
<td>41.8 d</td>
<td>2658.7 c</td>
<td>27.9 c</td>
<td>20.8 d</td>
</tr>
</tbody>
</table>

Mean values in each column with the same letter(s) are not significantly different using LSD tests at 5% of probability.
Similar observations were recorded by Leita et al. [9]. Fertilizers may meet up the demand of mineral nutrition required by the microbes but not that of carbon, which is a major component of microbial cells. Integrated application of organic and inorganic materials provides a balanced supply of mineral nutrients as well as carbon. NT system increased MBC compared to other tillage systems (Fig 1). Conventional tillage decreases soil organic matter and soil structure, and it is due to decrease soil microbial communities. Madejon et al. [13] observed that conservation tillage increased MBC and microbial activities. Along with microbial biomass changes, one might also expect shifts in microbial community structure to occur due to the temporal increase in microbial niche, water retention or reduced physical disturbance with no-tillage.

3.2. Soil enzyme activities

The activities of all enzymes varied significantly in different fertilization methods. Only, urease activity was significantly affected by the two-way interactions of fertilizers × tillage. The pattern of variation of enzyme activity in the soil during the two years of study was similar; however, urease activity was higher in the first year. The activities of all enzymes were generally higher in the N4 treatment than in the unfertilized and chemical fertilizer treatments (Table 1). There were no differences in phosphatase activity between the compost treatment and the FYM treatments. The dehydrogenase, phosphatase and urease activities in the N3 treatment were significantly lower than in the FYM and compost treatments. As shown in table 1, 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. Taraﬁdar and Marschner [25] 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. [16] reported that addition of the organic matter maintained high levels of phosphatase activity in soil during a long term study. Giusquiani et al. [5] 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. The authors concluded that because the nitrogen fertilizers used in the experiments contained NH₄⁺ and that the reaction products of urease being NH₃, 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 [16]. This is also indicated by the strong correlation of protease, acid phosphatase and urease with microbial soil C concentrations. Only alkaline phosphatase activity showed statistically non-significant, correlations with MBC (Table 2). Compost application increased dehydrogenase activity (Table 1). Stronger dehydrogenase activity in compost applied plots may be due to higher organic matter content [26]. Marinari et al. [15] reported that a higher level of dehydrogenase activity was observed in soil treated with compost and farmyard manure compared to soil treated with mineral fertilizer. 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 dehydrogenase activity [16]. These results were similar to our finding that dehydrogenase in rhizosphere soil of N2 treatments was average three times higher than that of mineral fertilizer (N3) treatments. 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.

<table>
<thead>
<tr>
<th>MBC</th>
<th>Protease</th>
<th>Acid phosphatase</th>
<th>Alkaline phosphatase</th>
<th>Urease</th>
<th>Dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBC</td>
<td>1</td>
<td>0.671 **</td>
<td>0.674 **</td>
<td>0.671 **</td>
<td>1</td>
</tr>
<tr>
<td>Protease</td>
<td>0.873 **</td>
<td>0.712 **</td>
<td>0.783 **</td>
<td>0.703 **</td>
<td>0.783 **</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>0.665 **</td>
<td>0.632 **</td>
<td>0.733 **</td>
<td>0.674 **</td>
<td>1</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0.389 ns</td>
<td>0.523 **</td>
<td>0.249 ns</td>
<td>0.523 **</td>
<td>0.674 **</td>
</tr>
<tr>
<td>Urease</td>
<td>0.332 ns</td>
<td>0.733 **</td>
<td>1</td>
<td>0.512 **</td>
<td>1</td>
</tr>
<tr>
<td>Dehydrogenase</td>
<td>0.322 ns</td>
<td>0.523 **</td>
<td>0.249 ns</td>
<td>0.523 **</td>
<td>1</td>
</tr>
</tbody>
</table>

ns and **: not significant, significant at 1% of probability, respectively.
Report of Nayak et al. [20] 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 [24, 26]. Results indicated statistically significant (p < 0.05) differences in the enzyme activity in the soil between various methods of tillage. The activity of acid, alkaline phosphatase and protease tended to be higher in the NT treatment compared to the MT and CT treatments. However, activity of urease and dehydrogenase were similar in NT and MT treatments (Fig 1). Finding of Jin et al. [6] 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.

![Fig. 1. Effect of tillage practices on MBC (A), protease (B), acid phosphatase (C), alkaline phosphatase (D), urease (E), and dehydrogenase (F) activity in soil.](image)

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Urease activity under T1N4 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 [10].

IV CONCLUSION

The present study provides information on soil microbial biomass dynamics and bio catalytic activities as influenced by organic and inorganic fertilization in canola production conditions. The results demonstrate that microbial biomass and soil enzyme activity is sensitive in discriminating between organic fertilizers and inorganic fertilizer application on a short-term basis. Soil microbial biomass and 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.

REFERENCES