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Influence of straw degrading microbial compound on wheat straw decomposition and soil biological properties

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The main challenge to successful use of wheat straw as soil amendment is its slow decomposition rate as compared to other crop residues. In order to solve this problem and increase farmers' acceptability, inoculation of straw amended soil with microbial compounds to hasten decomposition is being promoted in China. In this experiment, the effect of three types of industrially produced composite microbial system (Renyuan shengwu (RW), Taigubio (TB) and China green health (GH)) were investigated under two straw placement options (incorporation I and layered L) on wheat straw decomposition and soil biological properties. The result indicates significant differences for all the parameters measured at $p \leq 0.05$. RW had highest total CO_2 emission during the incubation period (61 days). There was a correlation between total CO_2 emission and cellulase activity ($R^2 = 0.81$) and dehydrogenase activity ($R^2 = -0.66$). Placement option had effect on soil total organic carbon (TOC), microbial biomass carbon (MBC), fungi, actinomycetes and bacteria population, and cellulase activity. Both inoculation and straw returning to the soil increased TOC, micro flora and cellulase activity. The results show that direct inoculation of straw amended soil could improve straw decomposition rate and soil biological properties if suitable microbial strains are used.

Key words: Cellulase, dehydrogenase, composite microbial compound, wheat straw, straw placement.

INTRODUCTION

Straw incorporation into soil is being promoted as an alternative way of managing crop residues. The recycling of crop residues has the potential to convert the surplus farm wastes to a useful product so as to meet up with the nutrient requirement of crops. It also maintains the soil physical, chemical and biological conditions and improves the overall ecological balance of the crop production system (Krishna et al., 2004). In India alone, wheat straw constitutes 27% of the total crop residue per year. In China, wheat is sown in approximately 20 x 10^6 ha annually, generating about 90 x 10^9 tons of wheat straw that could be returned to the field (Cai and Alimujiang, 2009; Li et al., 2012). Wheat has been categorized as one of the exhaustive feeders of soil nutrients, and under double cropping system, it heavily depletes soil of its nutrient content. If this residue is not returned, it may mean mining of soil's major nutrients elements which in turn could lead to net negative balance and multi-nutrient deficiencies in crops (Tripathi et al., 2006).

Incorporation of crop residues with high C:N ratio into soil could enhance microbial immobilization of N and thus

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reduce nitrate leaching (Shindo and Nishio, 2005; Huang et al., 2008), but could also substantially increase the production of N_2O and CO_2. The other disadvantage of cereal straw incorporation into soil in addition to nitrogen immobilization is the presence of high lignin and cellulose which are not easily decomposed (Wang and Bakken, 1997). After harvest, wheat residue incorporated into soil interferes with the seed drill operation. To solve the problem, farmers resort to *in situ* burning of crop residues in order to achieve smooth farm operation (Krishna et al., 2004; Abdulla, 2007) and immediate sowing of next crop (Li et al., 2012). This act leads to loss of huge biomass and environmental pollution. Among the proposed solutions to problems associated with the usage of straw as soil amendment is the inoculation with a cellulose degrading microbial compound to hasten decomposition.

It has been established that microorganisms are crucial to the functioning of ecosystem and contribute to the biogeochemical cycles (Goyal and Sindhu, 2011) such as nutrient cycling and organic matter decomposition. Some beneficiary microorganisms have been used in transformation and remediation of polluted soil (Dejonghe et al., 2001; Juhanson et al., 2009) leading to improved soil fertility and crop productivity (Weyens et al., 2009; Sindhu et al., 2011), and enhancement of straw composting (Abdulla, 2007; Goyal and Sindhu, 2011). However, the direct inoculation of straw incorporated into soil to increase straw decomposition rate is uncommon (Li et al., 2012). Further, the use of cellulose degrading microbial compound has not been practiced in this research area. According to van Veen et al. (1997), obstinacy of the soil ecosystem which acts as a buffer against soil inoculants is the main limiting factor to soil inoculation. In China, the use of microbial inoculants to enhance straw decomposition is recently being introduced, however, most inoculants have been produced and used blindly without examination of their activities through scientific experiment (Li et al., 2011), and possible consequences on soil properties. To optimize the potential of these products, proper investigation is required since their survival and catabolic activity is the key to successful inoculation.

Soil organic carbon (SOC) is an important soil property considered as one of soil quality indicators and has direct and indirect relationship with soil physical, chemical and biological properties. Other biological soil quality indicators include microbial biomass carbon (MBC) and soil respiration (Suman et al., 2006; Marriot and Wander, 2006), soil microbial population and soil enzyme (Eldor, 2007). For decades, studies have concentrated on soil microflora, ignoring the influence of extracellular enzymes produced by microorganisms on the decomposition of organic matter. Soil enzymes catalysis several reactions of soil microorganisms, play a role in stabilization of soil structure, decomposition of organic wastes, organic matter formation, and provide early indication of changes in soil and agricultural management (Tabatabai, 1994; Dick et al., 1994; Kandeler et al., 2006; Gelsomino et al., 2006; Joachim et al., 2008).

The present study was conducted with the aim of examining the effects of three types of cellulose degrading microbial compounds on decomposition of wheat straw returned into soil and subsequent effects on soil TOC, MBC, microbial population, cellulase and dehydrogenase activity.

MATERIALS AND METHODS

Sampling

The soil used in this incubation experiment was sampled (0 to 20 cm) in May, 2012 from the experimental farm of Northwest A&F University, Yangling, Shaanxi province, China. The soil can be classified as Eum-Orthic Anthrosol (Udic Haplustalf in the U.S. soil taxonomy) (soil survey staff, 2010). The local climate is semi-humid but prone to drought, an average annual temperature of 12.9°C and an average annual rainfall of 620 mm. The soil pH is 7.94 (1:2.5 soil: water ratio), 14.4 g/kg TOC, 4.4 g/kg total N, 12.36 mg/kg Olsen P, 166 mg/kg K, the DTPA extractable Fe, Cu, Zn and Mn are 3.22, 0.56, 0.40 and 8.96 mg/kg, respectively, 65.1 g/kg $CaCO_3$ and 69.8 mg/kg total Zn. The soil was sieved (2 mm) and stored for 2 weeks in polyethylene bags at room temperature until the beginning of the experiments. The wheat straw was obtained from the same field following wheat harvest, air-dried and chopped into pieces of <1 cm.

Incubation procedure

Three commercially produced microbial compounds consisting of combination of different fungi and bacteria strains were used. The products were:

1. Renyuan shengwu (RW): this consists of bacteria, filamentous fungus and Saccharomycetes.
2. Taigubio (TB): comprises of *Bacillus substilis*, *Aspergillus oryzae* and *Trichoderma harzianum*.
3. China green health (GH): comprises of Bacillus, *Trichoderma viride* and Saccharomycetes.

The experiment consisted of nine treatments: (1) soil only (NT), (2) soil amended with wheat straw only (WSI), (3) wheat straw and soil only arranged in layers (WSL), (4) wheat straw incorporated soil + renyuan shengwu (RWI), (5) wheat straw and soil arranged in layers + renyuan shengwu (RWL), (6) wheat straw incorporated soil + Taigubio (TBI), (7) wheat straw and soil arranged in layers + Taigubio (TBL), (8) wheat straw incorporated soil + China green health (GHI), and (9) wheat straw and soil arranged in layers + China green health (GHL). The soil only and straw and soil only (SWI and SWL) treatments were set as controls. The two straw placement methods used in this experiment were incorporation and layering. In straw-soil layered treatments, 279 g soil and 11.4 g wheat straw were arranged in layers. There were three layers of soil, each layer was 1 cm deep and was alternated with two layers of straw to the depth of 5 cm each. For straw incorporation treatments, the same amount of soil and straw used in layered treatments were thoroughly mixed together in the incubation pots. To each pot, corresponding microbial product was applied according to the recommendation of the company. TB and GH were applied at the rate of 30 kg/ha assuming 5300 kg straw was returned to the soil while RW recommended 530 kg/ha at the same straw rate. The inoculants were applied directly on the straw and

mixed together. In treatments where straw was mixed with soil, the microbial inoculants were first mixed with the straw before incorporation into the soil. The soil water content was adjusted to 80% of the field capacity, the C:N ratio of the straw was also adjusted to 28 using urea (applied as water solution) while non amended soil received equivalent amount of deionised water. To each pot, small baker containing 20 ml 2M NaOH was fitted to trap evolved CO_2, covered with nylon sheet and incubated at 25°C for 61 days. CO_2 sampling was carried out every 24 h from day 2 to 8, every other day from day 10 to 31, thereafter it was sampled on 35th, 39th, 43rd, 50th and 61st days during the incubation. Pots were opened for about 5 min on each sampling day to maintain proper aeration, also NaOH in the bakers were changed. Amount of CO_2 absorbed was determined by titration of NaOH against 1 M HCl after precipitation with excess $BaCl_2$. At the completion of the incubation, the soil was divided into two; one part was kept in polyethylene bags at temperature of 4°C, while the other part was air-dried. The wet soil was used in determination of MBC, microbial population and soil enzyme activity. Each part of the soil was sieved to remove undecomposed straw before the commencement of the soil analyses.

Analytical procedures

Soil microbial carbon was determined by fumigation extraction procedure (Brookes et al., 1985; Vance et al., 1987). Total N was determined using Kjeldahl digestion follow by automated titration against H_2SO_4 after which total N was calculated (Jackson, 1967). Total organic carbon was analyzed by dichromate wet oxidation method (Walkley and Black, 1934). Soil microflora was determined by spread plate method. The culture media used are peptone beef extract for bacteria, potato dextrose agar (PDA) for fungi and Gause 1 cultural medium for actinomycetes. Dehydrogenase activity by Chloride Triphenyl Tetrazolium (TTC) assay and cellulase activity by 3,5-dinitro-salicylic acid assay (Guan SongYin, 1986).

Statistical analysis

All the results are the means of four replicates. The data were analyzed using one way ANOVA and means separated using Duncan Multiple Range test (DRMT) at $p \leq 0.05$. SPSS statistical package 17.0 and Microsoft excel were used to analyze data.

RESULTS

CO_2 emission

The total CO_2 production among the treatments was significant at $p \leq 0.05$. Straw addition significantly increased CO_2 production as compared to the control NT, and the increase ranged from 72.5% in WSL to 78% in RWI. All inoculated straw treatments (except TB) increased CO_2 evolution over pots treated with straw only (WSI and WSL). Among the treatments, RWI and RWL were significantly higher than others in terms of total CO_2 production, followed by GHL which was not significantly different from GHI. The differences in total CO_2 produced in pots inoculated with GH and uninoculated straw treatments (WSI and WSL) were not significant, indicating that application of the product had no significant effects on amount of CO_2 released from the decomposing wheat straw in this experiment. Also, placement method had no effect on the total CO_2 produced per straw amended soil whether inoculated or not. The amount of wheat straw carbon mineralized as CO_2 were not statistically different when comparing the two placements options for all the straw amended soils. In this research, microbial product RW effectively aided straw decomposition as compared to TB and GH. The order of performances among the treatments were RW > GH > WS > TB > NT (Figure 1).

Total soil organic carbon (TOC) and microbial biomass carbon (MBC)

Significant differences were found among the treatments with respect to both TOC and MBC. Straw amendment significantly increased total soil organic matter above the NT. The percentage increase ranged from 26.06 to 45.39% in WSI and TBL pots, respectively. Highest TOC (17 g/kg) was recovered from TBL but was not statistically different from GHL, RWI and RWL (Table 1). Straw placement influenced amount of straw carbon accumulated in the soil. All the treatments had higher TOC in straw-soil layered soils than their corresponding treatments where straw were mixed with the soil. This shows the potential of straw placement method to sequester carbon especially from decomposing wheat straw returned directly into soil. Straw inoculation generally increased soil TOC as compared to the controls (NT and WS); however, the increase was more pronounced in TB and RW than GH except under soil-straw layered placement option. Comparing the three inoculants, more organic carbon was accumulated in RW amended soil under the two placement options, and the difference between RWI and RWL was not so large as compared to TB and GH (Table 1).

The increase in MBC ranged from 15.66 to 138.34 mg/kg in straw amended soils as compared to NT. Among the treatments, RWL had highest value but was not statistically different from WSI. Inoculation was not able to increase MBC over treatment with straw only except in RWL. The effect of straw placement option was apparently seen in that WS, RW and TB had higher MBC in soil-straw layered treatments than their corresponding straw incorporated soils, whereas the reverse was the case for GH. Among the treatments, the order of performances with respect to MBC increment was WS > RW > TB > GH > NT (Table 1).

Soil microflora

At the end of the incubation period, the population of bacteria, actinomycetes and fungi were determined, and they were all significant at $p \leq 0.05$. Straw amendment increased population of soil bacteria over the control NT. Inoculation significantly increased bacteria population as compared to treatments with straw only (Table 2). In soils

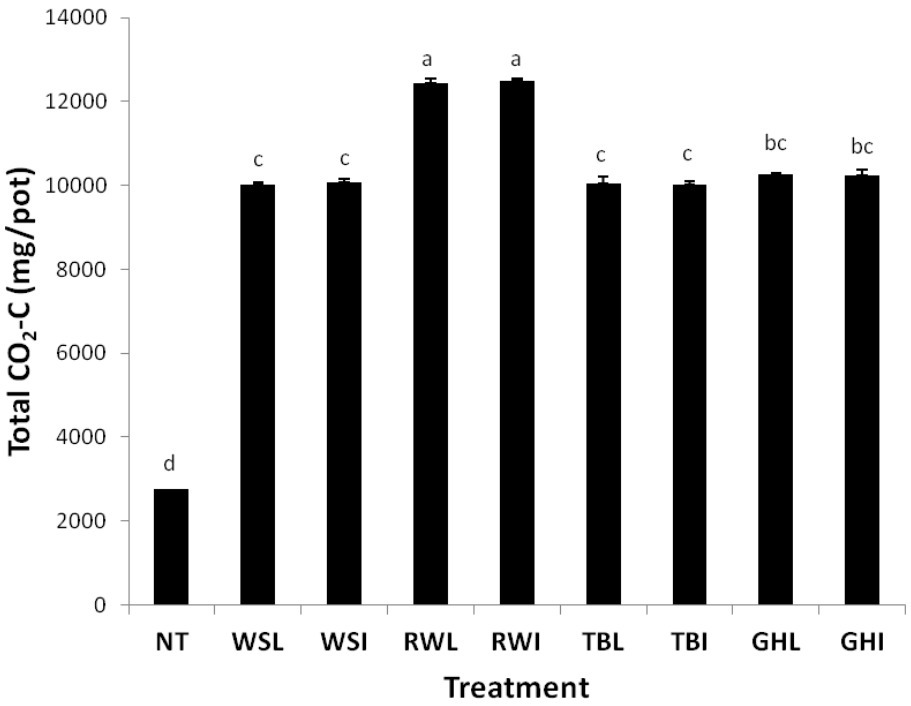

Figure 1. Total CO_2 emission from the soil supplied with wheat straw and cellulose degrading microbial products. The treatment NT means soil only; WSL is straw-soil layered pot; WSI is straw incorporated into soil; RWL, TBL and GHL are soil treated with the three microbial compounds where straw and soil are arranged in layers while RWI, TBI and GHI are their corresponding treatments with straw incorporated into soil. CO_2 sampling was carried out every 24 h from day 2 to 8, every other day from day 10 to 31, thereafter it was sampled on 35th, 39th, 43rd, 50th and 61st days during the incubation.

Table 1. Total organic carbon and microbial biomass carbon of inoculated soil and controls under the two placement options after incubation.

Treatment	TOC (g/kg)	Increase in TOC over control (%)	MBC (mg /kg)	MBC due to straw application (mg /kg)
NT	9.37^{f}	-	79.12^{f}	-
WSL	13.44^{de}	30.28	180.97^{b}	101.85
WSI	12.67^{e}	26.06	200.03^{ab}	120.91
RWL	16.38^{ab}	42.80	217.46^{a}	138.34
RWI	16.06^{ab}	41.64	143.29^{c}	64.17
TBL	17.16^{a}	45.39	123.84^{cd}	44.72
TBI	13.33^{e}	29.72	119.93^{d}	40.81
GHL	16.85^{bcd}	44.38	112.35^{de}	33.23
GHI	14.96^{bc}	37.38	118.79^{d}	39.67

*Figures with the same letter in the same column are not significantly different at $p \leq 0.05$.

treated with WS, RW and TB, higher bacteria population was seen when straw was mixed with soil than when it was applied in layers; opposite was the case in GH soil. RWI had the highest bacteria count of 4.31 x 10^8 CFU g/dry soil follow by TBI and RWL with values of 3.89 x 10^8 CFU g/dry soil and 3.71 x 10^8 CFU g/dry soil, respectively. Among the microbial products, RW performed better than others and was significantly different from GH (Table 2). The order of performance of the three inoculants was GH < TB < RW.

For actinomycetes, the observation was similar to that of bacteria. Straw application significantly increased population of actinomycetes in all straw amended soil as compared to that of control NT. Inoculation increased population of actinomycetes over WS in RW and TB while WS was higher than microbial product GH. Straw placement method apparently had no effect on the amount of actinomycetes present in each treatment

Table 2. Soil microbial population as affected by the straw placement method and microbial inoculants at the completion of the incubation period.

Treatment	Bacteria (x10^8 CFU/g dry soil)	Actinomycetes (x10^6 CFU/g dry soil)	Fungi (x10^2 CFU/g dry soil)
NT	0.64^{g}	0.28^{f}	3.43^{d}
WSL	0.67^{g}	0.84^{e}	7.68^{c}
WSI	1.43^{fg}	2.13^{b}	1.33^{e}
RWL	3.71^{abc}	2.15^{b}	2.94^{de}
RWI	4.31^{a}	2.30^{ab}	1.20^{e}
TBL	3.29^{bcd}	2.67^{a}	16.99^{a}
TBI	3.89^{ab}	2.50^{ab}	1.17^{e}
GHL	2.88^{de}	1.20^{de}	9.25^{c}
GHI	2.1^{ef}	1.69^{c}	1.92^{de}

*Figures with the same letter in the same column are not significantly different.

because no definite pattern was observed for all the treatments. Among the treatments, TBL had the highest value but was not significantly higher than RWI and TBI. Comparing the inoculated straws, GH had the least values of 1.69 x 10^6 CFU g/dry soil and 1.20 x 10^6 CFU g/dry soil for GHI and GHL, respectively. For the three inoculants, TB treated soil had the highest value followed by RW (Table 2).

Straw returning to the soil did not completely increased fungi population over NT. Fungi population in the NT (3.43 x 10^2 CFU g/dry soil) was significantly higher than that of all straw incorporated treatments (Table 2). This shows that there was decrease in the initial population of fungi in these soils and that straw incorporation has potential to reduce fungi population in the soil under similar condition. In contrast, straw-soil layered treatments had higher values than NT, and were statistically higher than their corresponding straw incorporated treatments with the exception of RW, but the value was still higher under layered (2.94 x 10^2 CFU g/dry soil) than incorporated (1.20 x 10^2 CFU g/dry soil). With respect to layered treatments, the order of performance was TBL > GHL > WSL > RWL in terms of fungi population present in the soil at the end of the incubation period. The highest value was found in TBL while TBI and RWI had the least (Table 2).

Cellulase and dehydrogenase activity

The control soil had the highest dehydrogenase value of 2.81 µg TPF/g/h and was significantly higher than all other treatments (Table 3). Straw amendment, the inoculants and the placement method had no effect on the dehydrogenase activity in this experiment. The correlation between dehydrogenase and total CO_2 emission was negative (R^2 = -0.66) while the relationship was positive for cellulase activity (R^2 = 0.81). Straw addition to soil significantly increased soil cellulase activity as compared to the NT. All the inoculants increased cellulase activity over WS treatments, the value ranged from 17.1 to 59.6% in treatments where straw was incorporated into soil, and from 18.5 to 28.4% in straw-soil layered pots. Cellulase activity was affected by the straw placement method. Except in RW, all straw-soil layered pots had higher cellulase activity than their corresponding pots containing mixture of straw and soil. Among the three inoculants, RWI had highest cellulase activity (22.53 µg glucose/g/h) followed by RWL (22.11 µg glucose/g/h), while the performances of TB and GH were similar. The amounts of cellulase activity recorded in inoculated straw amended soils were directly related to the total CO_2 released in those treatments in that, treatment with higher CO_2 release also had higher cellulase activity (Figure 1).

DISCUSSION

Effect of straw and inoculants on total CO_2 emission

As shown in Figure 1, straw addition increased soil respiration as compared to the control. The increase in soil respiration flux is associated with the activities of straw degrading microbes (Chen et al., 2007). The highest total CO_2 observed in RW (Figure 1) may be due to ability of the microorganisms present in this product to establish successfully following inoculation as compared to the TB and GH. Other possible reasons could be the potential of the inoculants to overcome the obstinacy of the native microorganisms in the experimental soil which usually act as a buffer against soil inoculants (van Veen,1997), or higher application rate (530 kg/5300 kg straw) recommended by the manufacturer as compared to TB and GH (30 kg/5300 kg straw). The non significant difference between TB and GH and uninoculated straw amended soil may be due to the inability of some or all microbes present in these products to establish in the presence of the native microorganisms, or rapid reduction in their size following inoculation thus hindered their activity (Li et al., 2012). It is also possible that the mineralized straw C was sequestered into soil as part of soil organic carbon or incorporated into microbial biomass. Further reason could be the presence of suitable

Table 3. Effect of the inoculants on dehydrogenase and cellulase activities on soil incubated with straw at the completion of the incubation period.

Treatment	Dehydrogenase activity (µg TPF/g soil /h)	Cellulase activity (µg glucose/g soil/h)
NT	2.81[a]	2.59[f]
WSL	2.17[bc]	15.84[d]
WSI	1.95[bc]	9.11[e]
RWL	2.26[b]	22.11[ab]
RWI	2.26[b]	22.53[a]
TBL	2.00[bc]	19.43[bc]
TBI	1.87[c]	11.62[e]
GHL	1.95[bc]	18.91[c]
GHI	2.09[bc]	10.99[e]

*Figures with the same letter in the same column are not significantly different at $p < 0.05$.

C:N ratio (28) which might have provided appropriate environment for the indigenous microbes at the expense of the introduced inoculants in TB and GH. Similar results were reported by Abro et al. (2011).

Effect of straw and inoculants on soil carbon and biological property

Straw amendment and inoculation increased the soil TOC. An increase of 5 to 21.1 and 17.9 to 21.7% in straw-soil mixture and straw-soil layered treatments, respectively, as compared to the control was recorded in inoculated soils. The increase in soil carbon pool is likely associated with higher straw decomposition rate brought about by the activity of the inoculants. Li et al. (2012) also reported increase of 37 to about 52 g/kg when wheat straw powder was added to the soil. The higher TOC recorded in all straw-soil layered pots as compared to straw incorporated treatments suggests that C sequestration could increase when straw is covered with soil layer than when they are mixed together, and could help mitigate green house effect. Straw returning relatively increased soil MBC, but inoculation could not except in RWL (Table 1); however, this could not be entirely attributed to inoculation as it stands out as an isolated case. Cayuela et al. (2009) also reported a five-fold increase in MBC when soil was amended with crop residues.

Microbial community can be affected by changes in soil management options including introduction of organic matter or residue. In this work, inoculation increased bacteria population over that of control but the increase was more pronounced in straw incorporated treatments. This may be attributed to increase in the contact or interaction between soil and straw when both were mixed together as compared to layered arrangement. The result of this experiment shows an increase in the actinomycetes population as compared to NT. This noted increase might be due to the presence of abundant substrate (wheat straw) and suitable environment which enhanced multiplication of the organisms because they have high capacity to degrade cellulose and solubilize lignin (Ball et al., 1990; Tuomela et al., 2000). Influence of placement option was very vivid on fungi population in the straw amended soils after incubation (Table 2). The wide gap between the two placement methods is likely associated with the ability of fungi hyphae to grow extensively on the straw when it was arranged in layers as compared to condition where straw were scattered within the soil. The fungi population in the control soil was higher than that of RW and all straw incorporated treatments indicating a reduction in the initial population of fungi in these treatments following straw addition and/or inoculation. This may mean that the straw decomposition in these treatments was basically accomplished by bacteria and actinomycetes since most of these microfloras were found in straw-soil mixture than straw-soil layered treatments (Table 2).

Cellulase and dehydrogenase enzymes are parts of microbial metabolic substances which provide early information on changes in soil quality and can be assessed rapidly. Straw returning to the field and direct soil inoculation could not increase dehydrogenase activity over control in this work. It has been reported that the enzyme often is not correlated with other biological indicator such as oxygen consumption, CO_2 production or MBC (Dick, 1996), and its activity fluctuates as microbial activity does (Kandeler and Dick, 2007). Cellulose is the most abundant organic compound in the biosphere (Eriksson et al., 1990), but the availability of the carbon in these compounds depends on the ability of the cellulase to degrade residues (White, 1982). High cellulase activity than the control was recorded in this experiment when straw was returned to the soil. Straw returning produced an increase of 71.6 to 88.5% in cellulase activity over control. Inoculation also increased cellulase activity (Table 3), indicating an increase in the cellulose degradation due to the activities of the introduced micro-organisms. The initial cellulase activity of the microbial products

used in this study may also contribute to the observed increase in cellulase activity in the inoculated treatments. The result agrees with findings of Li et al. (2012) who reported that cellulase activity in the inoculated soil samples was higher than that of control samples. The high cellulase activity in RW treatments is likely associated with the increased presence of bacteria since it has low fungi population but higher cellulase activity as compared to others. The correlation between cellulase and microbial population was positive (R^2 = 0.68, 0.40 and 0.35 for bacteria, actinomycetes and fungi, respectively). Joshi et al. (1993) also reported that cellulase activity is potentially correlated with the fungal and bacterial population in the soil.

Conclusions

Direct inoculation of straw amended soil and placement methods used in this research seem to have little or no effect on total CO_2 released by the decomposing wheat straw within a short time except when large amount of inoculum was used as in RW. However, they had substantial influence on the soil organic carbon accumulation, soil microbial population and soil cellulase activity, indicating their ability to improve soil fertility due to enhancement of straw decomposition. Straw amendment increased soil MBC, while straw-soil placement method brought significance increase in TOC and fungi population. Also, there was relatively high positive correlation between total CO_2 produced and soil cellulase activity. Further research to investigate the potential of these composite microbial products is recommended.

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