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Refinement of soil biological properties with biochar addition in sweetcorn

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Abstract
Modern agricultural practices resulted in soil pollution and deleterious effect on soil enzymes and population which plays crucial role in maintaining soil properties. To mitigate this, ecologically safe and user friendly methods should be adopted. By keeping this in view a field experiment was conducted with seven treatments *viz.*, control (no fertilizers) (T_1), RDF (T_2), RDF+Azophos (T_3), 75% RDF+biochar @ 5 t ha^{-1} (T_4),75% RDF+biochar @ 5 t ha^{-1}+Azophos (T_5), 75% RDF+FYM @ 5 t ha^{-1} (T_6), 75% RDF+FYM @ 5 t ha^{-1}+ Azophos (T_7) in Randomised Block Design (RBD). Addition of organics showed a significant influence on the soil enzyme activity and population. Urease, alkaline phosphatase and dehydrogenase activities were higher in biochar treatments than that of remaining treatments, whereas acid phosphatase activity was significantly superior in FYM applied treatments. Vigorous proliferation of bacterial and actinomycetes populations were observed in FYM supplied treatments whereas fungal colonies were more in biochar applied soils.

Keywords: biochar, FYM, RDF, azophos

Introduction
Environmental pollution is of serious concern, as modern agriculture in the name of yield maximization either directly or indirectly affecting the environment. Burning of farm wastes has become a common phenomenon, despite the awareness of their value as fodder or an important carbon source to the soil, mainly due to lack of disposal mechanism and high labour requirement. Carbon sequestration is very important to minimize the load of oxides of carbon in atmosphere, the best alternative is making biochar with farm wastes and using the same as source of organic carbon in soil. Biochar is a fine grained, carbon rich, porous product remaining after plant biomass has been subjected to thermo-chemical conversion process (pyrolysis) at low temperature (~350 ^{0}C – 600 ^{0}C) in an environment with little or no oxygen. Biochar can be prepared from wide range of biomasses and the properties of biomass influences biochar properties. Current study maize stalk was used for biochar preparation.

Materials and Methods
A field study in Agricultural college farm, Bapatla, Andhra Pradesh to study the interaction effect of organics such as biochar and FYM with inorganic fertilizers and Azophos. The experimental soil was clay loam in texture, slightly alkaline reaction, low in organic carbon, low in available nitrogen (179 kg ha^{-1}), medium in available phosphorus (25.71 kg ha^{-1}). Biochar, FYM and Azophos were applied to the field according to the treatments one week before sowing. Soil population and enzyme activities were estimated by following procedures.

Bacteria: The enumeration of total bacteria in fresh soil samples was carried out by following serial dilution plate count technique (Dhingra and Sinclair, 2000) using nutrient agar medium (agar agar-20 g; beef extract-3 g; peptone-5 g; water - 1 litre).

Fungi: The enumeration of total fungi in the fresh soil samples of all treatments was carried out by following the standard serial dilution plate technique using Martins Rose Bengal Agar for fungi (Martin, 1950) [17].

Actinomycetes: Enumeration of total actinomycetes was done by using Khusters nutrient agar medium.

Urease activity: Urease activity was measured by estimating the ammoniacal nitrogen in soil

Suspension by steam distillation method. 0.2 mL of toluene, 9 mL THAM buffer and 1 mL of 0.2M urea solution were added to 5g soil, swirled for few seconds and incubated at 37ºC for two hours. Then the solution was made upto 50mL with KCl-Ag_2SO_4 solution and estimated the ammoniacal nitrogen, expressed as µg NH_4^+-N g^{-1} soil h^{-1} (Kapoor and Paroda, 2007) [4].

Phosphatase activity: Phosphatase activity was measured by estimating concentration of paranitrophenol (PNP) produced due to the hydrolysis of the substrate p- nitrophenol phosphate by phosphatase enzyme (Tabatabai and Bremner, 1972) [12]. One gram of fresh soil sample was treated with 0.2 mL of toluene, 4.0 mL of modified universal buffer (pH 6.5 for acid phosphatase and 11.0 for alkaline phosphatase activity) and 1.0 mL para nitrophenyl phosphate solution and incubated at 37 ^{0}C for an hour. Then to that 1mL of 0.5 M $CaCl_2$ and 4.0 mL of 0.5M NaOH were added, filtered and the yellow colour intensity of the filtrate was measured at 440 nm with blue filter. The enzyme activity was expressed as µg of p nitrophenol produced g^{-1} soil h^{-1}.

Dehydrogenase Activity: Dehydrogenase activity in the soil sample was determined by following the procedure as described by Klein *et al*. (1971) [5]. One gram of air-dried soil was taken in an air tight screw capped test tube (15 ml capacity). To that 0.2 ml of 3 per cent 2, 3, 5- triphenyl tetrazolium chloride (TTC) solution was added in each of the tubes to saturate the soil. In each tube 0.5 ml of 1% glucose solution was added. The bottom of the tube was gently tapped to drive out all trapped oxygen and thus a water seal is formed above the soil. Ensure that no air bubbles are formed. Tubes were Incubated at 28±0.5^{0}C for 24 hours. After incubation 10 ml methanol was added and shaken vigorously. The contents were allowed to stand for 6h. Clear pink coloured supernatant solution was withdrawn and readings were taken with spectrophotometer at a wavelength of 485nm (blue filter). The amount of triphenyl formazan (TPF) formed was extrapolated from the standard curve drawn in the range of 10µg to 90µg TPF ml^{-1}. The results were expressed as µg TPF h^{-1} g^{-1}soil.

Results and Discussions

1. Soil microbial population

a. Soil bacteria

The results of bacterial population presented in table 1 indicated a significant influence of imposed treatments on bacterial population. The higher values at different crop growth stages were recorded in treatments T_6 and T_7 *viz.*, 75% RDF + FYM @ 5 t ha^{-1} and 75% RDF + FYM @ 5 t ha^{-1} + Azophos. In biochar treatments numerical increase in the bacterial population than that of control was observed at all stages of the crop growth. At knee high stage lowest (25 $\times 10^6$ CFU g^{-1}) bacterial population was noticed in control treatment, where as the highest (39.79 $\times 10^6$ CFU g^{-1} soil) in the treatment supplied with 75% RDF + FYM @ 5 t ha^{-1} + Azophos which was at par with the treatments supplied with 75% RDF + FYM @ 5 t ha^{-1} and RDF + Azophos and significantly superior to rest of the treatments. At tasseling the discernible range of bacterial population was in between 27.43 $\times 10^6$ CFU g^{-1} (control) and 40.57 $\times 10^6$ CFU g^{-1} (75% RDF + FYM @ 5 t ha^{-1} + Azophos). Bacterial population in the treatments supplied with FYM was significantly superior to rest of the treatments. At harvest highest bacterial population (37.81$\times 10^6$ $CFUg^{-1}$) was observed in 75% RDF + FYM @ 5 t ha^{-1} + Azophos which was at par with the treatment supplied with 75% RDF + FYM @ 5 t ha^{-1} and significantly superior to remaining treatments. The lowest (26.31 $\times 10^6$ CFU g^{-1}) bacterial population was recorded in control. Increase in the bacterial population on FYM addition was due to the increased supply of nutrients and more organic matter content. These results were in confirmation with Lalfakzuala *et al*., 2008. Increase in the bacterial population on biochar addition might be due to the volatile matter content in the biochar which was utilised by bacteria (Ouyang *et al*., 2014) [9].

Table 1: Influence of biochar on microbial population in soil (CFU g^{-1} soil)

Treatments	Bacteria ($\times 10^6$)			Fungi ($\times 10^3$)			Actinomycetes ($\times 10^4$)		
	Knee high	Tasseling	Harvest	Knee high	Tasseling	Harvest	Knee high	Tasseling	Harvest
T_1 - Control	25.00	27.43	26.31	3.30	4.20	3.90	3.70	4.00	3.80
T_2 - RDF	32.30	33.13	32.13	4.30	5.33	4.93	4.33	4.90	4.73
T_3 - RDF + Azophos	32.39	33.33	32.52	4.50	5.40	5.00	4.40	4.97	4.90
T_4 - 75% RDF + biochar @ 5 t ha^{-1}	30.32	31.33	31.08	6.61	7.83	7.11	3.63	3.73	3.51
T_5 - 75% RDF + biochar @ 5 t ha^{-1} + Azophos	31.13	32.52	31.66	6.74	7.87	7.32	3.74	3.87	3.64
T_6 - 75% RDF + FYM @ 5 t ha^{-1}	39.71	40.41	36.52	5.46	6.50	5.91	5.01	5.93	5.67
T_7 - 75% RDF + FYM @ 5 t ha^{-1} + Azophos	39.79	40.57	37.81	5.60	6.71	6.11	5.24	6.17	5.74
SEm±	2.36	1.65	1.15	0.30	0.37	0.29	0.21	0.26	0.19
CD @ 0.05	7.26	5.09	3.55	0.92	1.15	0.88	0.63	0.80	0.58
CV (%)	12.38	8.39	6.12	9.88	10.29	8.64	8.29	9.37	7.11

b. Soil fungi

The fungal population was significantly influenced by the imposed treatments. Highest population observed in the biochar treated plots followed by FYM and the lowest was in control. Maximum population at knee high, tasseling and harvest stages were noticed in the treatment T_5 (6.74 $\times 10^3$, 7.87 $\times 10^3$ and 7.32 $\times 10^3$ $CFUg^{-1}$ soil respectively) and minimum population was noticed in the treatment T_1 (3.3 $\times$ 10^3, 4.2 $\times$ 10^3 and 3.9 $\times$ 10^3 CFU g^{-1} soil respectively). Numerical increase in the fungal population on biochar Application might be due to the adaptability of fungi to altered environmental conditions. It can degrade complex substrates and can extend their extra radical hyphae into biochar and can sporulate inside. Small labile pool of biochar might have stimulated the fungal growth. Significant increase in the most commonly occurring types of mycorrhizal fungi was noticed with the application of biochar (Blackwell *et al*., 2010) [1]. Watzinger *et al*., 2014 supported the increase in the fungal population as fungi can adapt altered environmental conditions and can degrade the biochar directly.

c. Soil actinomycetes

The results of actinomycetes population presented in table 1 indicated a significant influence of imposed treatments. The higher values at different crop growth stages were recorded in treatments T_6 and T_7 *viz.*, 75 % RDF + FYM @ 5 t ha^{-1} and 75 % RDF + FYM @ 5 t ha^{-1} + Azophos. Decline in the population was observed in the treatments T_4 and T_5 *viz.*, 75 % RDF + biochar @ 5 t ha^{-1} and 75 % RDF + FYM @ 5 t ha^{-1} + Azophos as compared to other treatments at all the stages of the crop growth. The highest values at knee high, tasseling and harvest (5.24×10^4, 6.17×10^4 and 5.74×10^4 CFU g^{-1} soil, respectively) were recorded in treatment which received 75% RDF + FYM @ 5 t ha^{-1} + Azophos (T_7) and the lowest (3.63×10^4, 3.73×10^4 and 3.51×10^4 CFU g^{-1} soil,, respectively) were recorded in 75% RDF + biochar @ 5 t ha^{-1} (T_4). Reduced actinomycetes population on biochar addition was registered which might be because of majority of the biochar carbon was largely unavailable to microbes. Singh *et al*., 2010 reported that the relatively recalcitrant nature of the carbon in the biochar may fail to enhance the microbial activity. Watzinger *et al*., 2014 [13] supported that actinomycetes were negatively affected because of biochar properties that confer much stability. In all the treatments supplied with Azophos (T_3, T_5 and T_7), slightly higher microbial population was noticed as compared to their respective treatments without Azophos (T_2, T_4 and T_6). The results were in confirmation with Ramalakshmi *et al*., 2008 [10].

2. Enzyme activity

a. Urease

The different treatments received lone inorganics and inorganics in combination with organics were on par with one another and were significantly superior to control. At knee high stage the urease activity ranged from 74 µg NH_4^+ g^{-1} $2h^{-1}$ (control) to 130 µg NH_4^+ g^{-1} $2h^{-1}$ (75% RDF + FYM @ 5 t ha^{-1} + Azophos). It was ranged from 86 µg NH_4^+ g^{-1} $2h^{-1}$ in the control to 156 µg NH_4^+ g^{-1} $2h^{-1}$ in T_5 (75% RDF + biochar @ 5 t ha^{-1} + Azophos) at tasseling stage. At harvest the range was between 78 µg NH_4^+ g^{-1} $2h^{-1}$ and 141 µg NH_4^+ g^{-1} $2h^{-1}$ in control and 75% RDF + biochar @ 5t ha^{-1} treatments, respectively. Higher urease activity on biochar and FYM addition might be due to more organic matter content, hence enhanced hydrolysis of urea. Increase in the urease activity on biochar addition was supported by Du *et al*., 2014 and on FYM addition by Singaram and Kamala kumari, 1995 [11].

b. Acid phosphatase activity

Data pertaining to soil phosphatase activity presented in table 2 revealed a significant effect due to imposed treatments. Highest acid phosphatase activity was noticed in the soils treated with FYM and were at a par with soils supplied with 100% RDF. Significant diminution in the acid phosphatase activity was observed in biochar treated plots over control. At knee high stage highest (32.68 µg PNP g^{-1} h^{-1}) acid Phosphatase activitiy was observed in the treatments supplied with 75% RDF + FYM @ 5 t ha^{-1} + Azophos which was on par with the treatments received 75% RDF + FYM @ 5 t ha^{-1}, RDF with and without fertilizer and significantly superior to remaining treatments. Significant minimization (13.58, 14.14 µg PNP g^{-1} h^{-1}) in the enzyme activity was registered in the treatments supplied with biochar (T_4 and T_5) over control (18.17 µg PNP g^{-1} h^{-1}). At tasseling stage highest acid phosphatase activitiy (35.60 µg PNP g^{-1} h^{-1}) was noticed in treatments supplied with 75% RDF + FYM @ 5 t ha^{-1}+ Azophos followed by 75% RDF + FYM @ 5 t ha^{-1} (34.40 µg PNP g^{-1} h^{-1}) which were on par with T_2 and T_3 and significantly superior to remaining treatments. Treatments supplied with biochar (T_4 and T_5) registered significant reduction (18.49, 20.61 µg PNP g^{-1} h^{-1}) in the enzyme activity over control (21.03 µg PNP g^{-1} h^{-1}). At harvesting stage the highest acid phosphatase activity (34.35 µg PNP g^{-1} h^{-1}) was recorded in the soils supplied with 75% RDF + FYM @ 5 t ha^{-1} + Azophos, while the lowest (13.52 µg PNP g^{-1} h^{-1}) was found in treatment supplied with 75% RDF + biochar @ 5 t ha^{-1} (T_4).

Significant diminution of acid phosphatase activity was discernible in the soils supplied with biochar, probably due to more pH of biochar. It was supported by Masto *et al*., 2013 [8]. Increase in the acid phosphatase activity on FYM addition was because of more organic matter content.

c. Alkaline phosphatase activity

Significant improvement in the alkaline phosphatase activity was noticed in the soils treated with biochar (T_4 and T_5) and was significantly superior to all other treatments. The soil phosphatase activity (both acid and alkaline phosphatase activites) was maximum at tasseling stage and later decreased with advancement of crop growth in all treatments. The higher enzyme activity at tasseling could be due to the active growth of the crop with enhanced root activity and the release of extracellular enzymes in to soil solutions which resulted in higher rate of microbial populations and production of the enzyme. Alkaline phosphatase activity was more as compared to acid phosphatase activity. It might be due to stress response to unfavourable conditions during the warm period. At knee high stage higher alkaline phosphatase activity was observed in T_4 and T_5 (59.74 and 60.39 µg PNP g^{-1} $h^{-1,}$ respectively) which were on par with each other and significantly superior to remaining treatments. Minimum alkaline phosphatase activity was discernible in control (30.74 µg PNP g^{-1} h^{-1}). It was ranged from 33.31 µg PNP g^{-1} h^{-1} to 63.56 µg PNP g^{-1} h^{-1} in control and 75% RDF + biochar @ 5 t ha^{-1} + Azophos, respectively at tasseling stage. At harvesting stage the the highest alkaline phosphatase activity (58.01 µg PNP g^{-1} h^{-1}) was recorded in soils supplied with 75% RDF + biochar @ 5 t ha^{-1} + Azophos, while the lowest (32.62 µg PNP g^{-1} h^{-1}) was found in control.

Table 2: Influence of biochar on enzyme activity in soil

Treatments	Urease (µg NH_4^+ g^{-1}2hrs^{-1})			Acid phosphatase (µg PNP $g^{-1}hr^{-1}$)			Alkaline phosphatase (µg PNP $g^{-1}hr^{-1}$)			Dehydrogenase (µg TPF $g^{-1}day^{-1}$)		
	Knee high	Tasseling	Harvest	Knee high	Tasseling	Harvest	Knee high	Tasseling	Harvest	Knee high	Tasseling	Harvest
T1	74	86	78	18.17	21.03	19.27	30.74	33.31	32.62	119	126	122
T2	109	127	122	31.63	33.13	30.24	39.64	41.70	40.60	144	151	147
T3	112	130	129	32.20	34.25	30.50	39.91	42.80	40.72	145	152	147
T4	126	155	141	13.58	18.49	13.52	59.74	62.59	57.43	173	184	175
T5	127	156	132	14.14	20.61	14.98	60.39	63.56	58.01	173	185	177
T6	124	150	138	32.52	34.40	34.06	49.36	50.42	48.56	169	178	174

T7	130	150	139	32.68	35.60	34.35	50.79	51.62	49.79	170	179	174
SEm±	8	11	9	1.17	2.18	1.11	2.86	2.25	2.47	7	8	6
CD (0.05%)	26	33	28	3.62	6.73	3.41	8.81	6.93	7.62	21	23	20
CV (%)	13	14	13	8.13	13.40	7.58	10.49	7.89	9.15	7	8	7

T_1 – Control T_4 - 75% RDF + biochar @ 5t ha^{-1} T_6 - 75% RDF + FYM @ 5 t ha^{-1}

T_2 – RDF T_5 - 75% RDF + biochar @ 5 t ha^{-1} + Azophos T_7 - 75% RDF + FYM @ 5 t ha^{-1} + Azophos

T_3 - RDF + Azophos

The increased phosphatase in the soils may also be due to more soil moisture content retained in organic treated plots for longer period creating favourable conditions for plant and microbial growth. Masto *et al*., 2013 [8] supported the increase in the alkaline phosphatase activity on biochar addition due to biochar properties.

d. Dehydrogenase activity

Dehydrogenase activity of soils was significantly influenced by the imposed treatments. Significant increase in the enzyme activity was noticed in T_5 and was on par with T_4 and T_7. Increment in the dehydrogenase activity from knee high to tasseling stage and decreased from tasseling to harvesting stage was recorded in all the treatments. At knee high stage highest (173 µg TPF $g^{-1}d^{-1}$) dehydrogenase activitiy was observed in the soils received 75% RDF + biochar @ 5 t ha^{-1} with and without Azophos inoculation which was at par with T_7 (75% RDF + FYM @ 5 t ha^{-1} + Azophos) and was significantly superior to remaining treatments. Minimum activity was observed in control (119 µg TPF g^{-1} d^{-1}). It was ranged from 126 µg TPF g^{-1} d^{-1} to 185 µg TPF g^{-1} d^{-1} in control and 75% RDF + biochar @ 5 t ha^{-1} + Azophos, respectively at tasseling stage. At harvesting stage the highest activity (177 µg TPF g^{-1} d^{-1}) was recorded in soils supplied with 75% RDF + biochar @ 5 t ha^{-1} + Azophos, while the lowest (122 µg TPF $g^{-1}d^{-1}$) was found in control. Higher activity of dehydrogenase in biochar amended soils might be due to the presence of volatile matter and available nutrients. Small labile fraction of organic carbon might decomposed and stimulated carbon mineralisation, thus enhanced the enzyme activity. Masto *et al*., 2013 [8] reported that the soils supplied with biochar showed the higher dehydrogenase activity coupled with lower respiration values. Ouyang *et al*., 2014 [9] supported the increase in the dehydrogenase activity over control due to higher volatile matter content in biochar. Increase in the dehydrogenase activity on FYM addition was due to increase in the available nutrients and microbial activity. Singaram and Kamala kumari (1995) [11] supported the increase in dehydrogenase activity on FYM addition which could be probably due to the increase in the microbial activity.

Conclusions

Continuous cultivation practices by modern agricultural methods leads to degradation of land, decrease in organic carbon and microbial growth in soil. To mitigate this among the possible options, better and feasible alternative is application of biochar. Field experiment was conducted to study the interaction effect of organics and inorganics on alteration of soil biological properties. Soil urease activity was comparable among the imposed treatments (T_2-T_7) and was significantly superior to control. Alkaline phosphatase and dehydrogenase activities in biochar treated plots were significantly superior to rest of the treatments. Highest acid phosphatase activity was noticed in FYM treatments and least in biochar imposed treatments. Bacterial and actinomycetes Populations in FYM treated plots were significantly superior to rest of the treatments. Fungal population in biochar treatments was recorded as significant to remaining.

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