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Long term application of herbicides on soil microbial demography in rice - rice cropping sequence of North-East India

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ABSTRACT

Field study was carried out to determine the long term effect of herbicide application on soil microbial community in rice- rice cropping sequence in acid soil of North-East India. Treatment comprised of absolute control with one hand weeding, butachlor + 2,4-D with 100% NPK through chemical fertilizer, butachlor + 2,4-D with 75% NPK through chemical fertilizer and 25% through organic source, butachlor + 2,4-D rotated with pretilachlor with 100% NPK through chemical fertilizer and butachlor + 2,4-D rotated with pretilachlor with 75% NPK through chemical fertilizer 25% through organic source. Result revealed that, after 14 years of continuous use of herbicide and organic input along with recommended dose of fertilizer application demonstrated significant increase in the activity of acid phosphatase. The effect of herbicide application was more prominent with sole chemical fertilizer than with organic manure for 25% N fertilizer replacement. Dehydrogenase activity in soil was increased following herbicide application up to 14 days after that it again decreased more prominent with addition of organic manure than with chemical fertilizer. No characteristic trend of urease activity was observed after application of herbicide. Significant inhibition of respiration was observed after application of herbicide up to 14 days followed by gradual recovery afterwards. Microbial biomass carbon in soil was significantly enhanced by application of organic manure for 25% N fertilizer substitution. Under rice-rice cropping system, application of herbicide showed temporary decline in microbial population and enzyme activities up to 14 days. Application of organic manure for 25% N fertilizer substitution significantly enhanced the microbial population and enzyme activities as compared to sole application of chemical fertilizers. Further application of butachlor rotated with pretilachlor resulted in higher microbial population as well as enzyme activities.

Key words: Demography, Herbicide, Microbial community, Rice-Rice sequence, Soil enzyme, Soil respiration

Weed management is an ever-present challenge to crop production. Weeds have the potential to reduce resources that would otherwise provide nourishment to growing crops or interfere with planting or harvesting operations. Due to negative impacts of weed in crop production, it should be aimed at reducing its populations usually through mechanical disturbance or chemical applications. Since weed management through mechanical and cultural methods is not feasible due to morphological similarity between the crop and weeds and scarcity of labour in the peak period of transplanting, weed management through herbicide practices is more promising for farmers. Butachlor and pretilachlor, member of the chloroacetanilide group of herbicides, are used for the selective control of annual weeds in rice fields. Both are most commonly used herbicides to control a wide range of annual grass and broad-leaf weeds. However application of such chemicals may exert an effect upon the soil microbes (Wardle and Parkinson1990) and their biological activities. An unintended consequence of the application of herbicides is that it may lead to significant changes in the soil microbial population and their activities thereby influencing the microbial ecological balance in the soil (Saeki and Toyota 2004) and affecting the productivity of soils. For optimizing and sustaining natural resources, the soil microbial population play an important role in carbon stock, nutrient cycling, organic matter decomposition which in turn affect soil fertility and plant growth.

Application of herbicides may affect the non-target organism including soil microorganisms is the major concern with the use of xenobiotic compounds in rice ecosystem (Latha and Gopal 2010). A large portion of these chemicals accumulates in the top layer soil where most of the microbiological activities occur. As a result, there is a loss in microbial biodiversity and that can affect the functional stability of the soil microbial community and soil health. Many soil enzymes can be used as indicators of soil quality

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for sustainable management because they are sensitive to ecological stress and land management (Benedetti and Dilly 2006). Evidence of the stimulation effect of herbicides on soil biochemical properties has been earlier reported (Garcia-Ruiz *et al*. 2008), even if herbicides are not designed to directly interact with soil enzymes. Long term use of herbicide with or without organic matter may alter the soil organic carbon, which ultimately affect the soil health. Microbes like bacteria, fungi, algae, protozoa and some nematodes have significant role in sustaining soil and crop productivity. Various soil microbial processes, enzymatic activities are affected by the time and application of herbicide. In cognizance with the above, a study was carried out to determine the long term effect of herbicide application on soil microbial community in rice-rice cropping sequence.

MATERIALS AND METHODS

The study area was located at 26°44ˈN and 94°12ˈE and at an altitude of 91.0 m above mean sea level. The climatic condition of the site is sub-tropical having humid summer and cold winter with total annual rainfall 2124 mm. The soil of the experimental site was sandy clay loam in texture. The experiment was laid down in randomized block design (RBD) replicated thrice with five treatment combinations *viz*. absolute control with one hand weeding, butachlor + 2,4-D with 100% NPK through chemical fertilizer, butachlor + 2,4-D with 75% NPK through chemical fertilizer, 25% through organic source, butachlor + 2,4-D rotated with pretilachlor with 100% NPK through chemical fertilizer and butachlor + 2,4-D rotated with pretilachlor with 75% NPK through chemical fertilizer, 25% through organic source. The plot of each treatment was 100 sq m in size.

Field trials were carried out for 14 years (2001-2015) with two rice crops in rice-rice cropping sequence during winter and summer season. Two sources of nitrogen (N) *viz*. inorganic to supply 100% N and organic (FYM) to supply 25% N were used as per the treatment. Weed management treatments include hand weeding, pre-emergence, herbicides in combination and pre emergence herbicides in rotation. Crop varieties used in the sequence were '*Ranjit (TTB 101-17)*' and '*Luit*'. Recommended dose of fertilizer *i.e.*, 40:20:20 and 60:20:40 N: P_2O_5: K_2O for autumn and winter rice, respectively were applied as per-recommended practice. The organic manure was applied to each crop in the respective treatment to substitute 25% of N fertilizer. During 2001 to 2005, FYM was used and there after vermicompost 2 t/ha (total N 1.4-1.5%, P 1.0-1.1% and K 1.5-1.8%) is being used as organic manure for partial substitution of N-fertilizer. However, the amount of P and K supplied through organic manure was not adjusted and recommended doses were applied through mineral fertilizers. Observations were made on soil biological properties at the end of the 26th and 27th rice crop (winter – autumn rice). (two crops in a year)

The classical serial dilution technique was used for enumeration of *Azotobacter* and PSB by spread plate technique on Burks media and Pikovskaya's solid media, respectively. Rhizosphere soil sample of 1g was suspended in 9 ml water blank, followed by serially diluted up to 10^4 and 10^5. Aliquots of 100 µl of 10^4 and 10^5 dilutions were spread over the solidified media in triplicates and plates were incubated at 30±2°C for phosphate solubulizing bacteria (PSB) while nitrogen fixing bacteria (NFB) plates were incubated at 35±2 °C for 3 days. The microbial population were estimated as colony forming unit (cfu) per gram soil on dry weight basis and transformed to log cfu/g.

Microbial biomass carbon (MBC) was determined by chloroform fumigation extraction technique following the method of Vance *et al*. (1987). Field moist soil (25 g) was fumigated with ethanol free chloroform at 25 °C for 24 hours. After fumigation, chloroform vapours were removed by repeated evacuation. The soil samples were then extracted with 100 ml 0.5M K_2SO_4 (1:4 soil: K_2SO_4). Controls were prepared by extracting soils without fumigation. The soil suspension was filtered through Whatman No 42 filter paper. Dehydrogenase (DH) activity was determined by the reduction of triphenyltetrazolium chloride (TTC) to triphenyl-formazan (TPF). Briefly, field moist soil (1g) was treated with one ml of 3% TTC, and then incubated at 28°C for 24 hours. To account for any abiotic TTC reductions, sterile controls consisted of autoclaved soil (121°C, 20 min. on three consecutive days) were used. Spectrophotometer blanks for both autoclaved and non-autoclaved treatments consisted of soil and TTC replaced with millipore water. The optical density at 485 nm was compared to that of triphenylformazan standards. DH activity was expressed on dry weight as ìg TPF/g dry soil /24hr. Phosphomonoesterase (PMEase) activities involve the use of an artificial substrate, *p*-nitrophenyl phosphate (*p*-NPP). The product of phospho-monoesterase activity, *p*-nitrophenol, a chromophore under alkaline conditions were detected colorimetrically following the method of Tabatabi and

Bremner (1969) and expressed as ìg *p*-nitrophenol/g dry soil/hr. Urease activity in soil was estimated by incubating the soil sample with (hydroxymethyl) aminomethane (THAM) buffer, Urea solution and toluene at 37°C for two hours with 2.5M KCl solution containing a Urease inhibitor (Ag_2SO_4) and steam distilled with MgO.

The initial values were for *Azotobacter* count 18.0 x10^4cfu gm, PSB population 16.0x10^4cfu gm, dehydrogenase activity 110.6 µg TPFg soil 7 days, phosphomonusterase activity 270.86 µg p-nitrophenol g soil h, urease activity 210.72 µg NH_4 g soil 2h, soil respiration 1.65 µg CO_2 –C g dw hr, microbial biomass carbon 365.5 µg g soil .

The technique of analysis of variance was used in RBD for statistical analysis of data obtained from various treatment studies. For statistical significance the difference between the treatment means was tested with DMRT test.

RESULTS AND DISCUSSION

Azotobacter and PSB population

The *Azotobacter* and PSB populations in soil with rotational application of pre-emergence herbicide *i.e.* butachlor in autumn rice and pretilachlor in winter rice showed relatively higher values compared to one that was continuously subject to butachlor application (**Table 1**). This might be due to the fact that butachlor is known to have more inhibitory effect to microbial population and soil enzyme activities than pretilachlor (Latha and Gopal 2010). Significant variation in *Azotobacter* population were recorded in different treatments. The population count of *Azotobacter* were significantly higher in farmers practice with one hand weeding in each crop and with the combination of herbicide and organic manure for partial N fertilizer substitution. Moreover, a higher *Azotobacter* and PSB populations in soil was observed during autumn season over the winter season. On the contrary, highest inhibition recorded with the sole application of chemical fertilizer. Inhibitory effects of butachlor and pretilachlor on population of nitrogen fixing bacteria in rice rhizosphere had been reported (Barman *et al.* 2009) and confirm to the observation of the present study. *Azotobacter* population decreased sharply up to 14 days after application of herbicide after which it showed an increasing trend. Highest inhibition recorded with the sole application of chemical fertilizer. The significant reduction in the population of *Azotobacter* in herbicide treated plots may be ascribed to the negative effect of herbicides on *Azotobacter* (Barman *et al.* 2009). Similar trend was observed with the PSB population. Most of the herbicides exhibited detrimental influence on soil microflora upto 15 days after application which was recovered later on. Balasubramanian and Sankaran (2001) also reported initial suppression of soil microflora on herbicide application in different soils. The toxic effect of herbicides normally appears immediately after the application when their

Table 1. Population dynamics of *Azotobacter* and PSB in soil during autumn and winter rice as affected by herbicide application in rice-rice sequence (cfu x 10^4/g)

Treatment	Autumn					Winter				
	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA
Azotobacter										
Farmers practice (one hand weeding)	25.00^{a}	28.00^{a}	25.33^{a}	27.00^{a}	25.33^{a}	17.66^{a}	22.67^{a}	19.00^{a}	18.00^{a}	20.00^{a}
Butachlor + 2,4-D (100% NPK TCF)	13.67^{c}	9.67^{d}	10.67^{c}	12.67^{d}	14.67^{c}	12.33^{c}	8.33^{c}	8.67^{d}	11.67^{d}	14.00^{d}
Butachlor + 2,4-D (75% NPK TCF 25% TOS)	18.33^{b}	14.00^{b}	15.00^{b}	17.67^{c}	20.67^{b}	14.67^{bc}	9.67^{c}	9.67^{b}	12.67^{c}	17.33^{b}
Butachlor + 2,4-D rotated with pretilachlor (100% NPK TCF)	14.67^{c}	11.67^{c}	10.33^{c}	13.00^{d}	17.67^{c}	13.00^{bc}	9.00^{c}	9.33^{c}	11.67^{cd}	15.00^{d}
Butachlor + 2,4-D rotated with pretilachlor (75% NPK TCF, 25% TOS)	19.00^{b}	14.33^{b}	15.67^{b}	19.00^{b}	23.00^{ab}	15.00^{b}	10.00^{b}	11.67^{b}	16.00^{b}	17.67^{c}
PSB										
Farmers' practice (one hand weeding)	20.67^{a}	21.33^{a}	19.67^{a}	19.00^{a}	22.00^{a}	15.00^{a}	17.00^{a}	14.00^{a}	16.00^{a}	17.33^{a}
Butachlor + 2,4-D (100% NPK TCF)	10.33^{d}	6.33^{c}	7.00^{d}	11.00^{e}	13.33^{e}	7.33^{d}	4.33^{d}	3.67^{d}	7.33^{e}	10.00^{e}
Butachlor + 2,4-D (75% NPK TCF 25% TOS)	13.00^{c}	9.67^{b}	8.67^{c}	12.67^{c}	17.67^{c}	10.00^{c}	7.67^{b}	8.33^{b}	11.33^{c}	14.00^{c}
Butachlor + 2,4-D rotated with pretilachlor (100% NPK TCF)	11.00^{d}	7.00^{bc}	7.33^{d}	11.67^{d}	15.67^{d}	8.33^{d}	6.00^{c}	5.67^{c}	9.67^{d}	12.33^{d}
Butachlor + 2,4-D rotated with pretilachlor (75% NPK TCF , 25% TOS)	14.67^{b}	11.67^{b}	11.33^{b}	15.00^{b}	20.00^{b}	12.00^{b}	8.00^{b}	9.33^{b}	13.00^{b}	15.67^{b}

Values with same letters are not statistically significant, DAA- Days after application; TCF - Through chemical fertilizer; TOS - Through organic source

concentration in soil is highest. Later on, microorganisms take part in degradation process and herbicide concentration in soil and their toxic effects decrease (Radivojevic *et al*. 2004).

Soil enzymes

Organic acids produced during decomposition of organic manure tend to reduce soil reaction, which enhanced the enzyme activity (Reddy and Reddy 2009). Recovery of enzyme activities after the initial inhibition associated with the increased availability of nutrients due to the degradation of herbicides was reported by Ismail *et al.* (1998). Significant increased in dehydrogenase activity was recorded in flooded rice soils treated with herbicide with or without organic manure (**Table 2**). Significant increase in dehydrogenase activity with application of butachlor was recorded highest being at 14 days after application and that decreased gradually. The result is in accordance with the result of Vandana *et al*. (2012). Addition of organic manure for partial substitution of N fertilizer significantly increased dehydrogenase activity in soils than application of inorganics alone because of substitution of organic sources increased the availability of substrate for dehydrogenase activity (Chaudhury *et al*. 2005). Dehydrogenase activity is known to have strong correlation with organic carbon content and thus addition of organic manure resulted in more soil dehydrogenase activity in the study (Madejon *et al.* 2007). The anaerobic microorganisms could effectively degrade butachlor in paddy soil. Since dehydrogenase is mostly produced by anaerobic microorganisms under anaerobic condition, such as sulphate-reducing bacteria, this might have led to increase in dehydrogenase activity. Tejada and Gonzalez (2009) reported that the increased in dehydrogenase activity in submerged soil attributed to the increase in the anaerobic microbial population and shift from aerobic to anaerobic microbes ones after a soil is flooded. The applied organic sources were able to provide sufficient nutrition for proliferation for microbes and their activities in terms of soil dehydrogenase activity. A balanced amount of NPK and organic manures will increase the enzyme activity (Joa 2010).

Phosphatases are often measured because of their importance in phosphorus cycles (Aon and Colaneri 2001). Apart from being good indicators of soil fertility, phosphatase enzyme plays a key role in the soil system (Dick *et al*. 2000). PMEase is an enzyme of agronomic value because it hydrolyses compounds of organic P and transforms them into inorganic P. The activity of acid phosphatase in soil is significantly affected by herbicide application compared to farmers practice with one hand weeding. Application of herbicide resulted in inhibition of acid phosphatase activity and increased after 14 days of application of herbicide. The PMEase activity was highest in control plots that received only one hand weeding at 40 days after transplanting (DAT) and decreased in soil with application of herbicide with or without organic matter (**Table 3**). The result was in accordance with Wang *et al*. (2008). The increased acid phosphatase activity in organic manure treatments might be due to the added quantity of organic matter, which in turn increased organic carbon and nitrogen (Kadlag *et al*. 2008).

The activity of urease as influenced by the herbicide treatments (**Table 4**) recorded higher value with nutrient application as compared to control. It was observed that the significantly higher urease activity levels in herbicide treatments than the control for all the periods. Vandana *et al.* (2012) also reported that application of butachlor did not change the urease activity despite an initial increase during first ninety days after application. Among the herbicide treated soil, increase in urease activity with organic manure addition may be due to the positive effect of organic

Table 2. Activity of dehydrogenase enzyme in soil during autumn and winter rice as affected by herbicide application in rice-rice sequence (µg TPF g/dry soil/7 days)

Treatment	Autumn					Winter				
	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA
Farmers practice (one hand weeding)	118.3^{e}	123.1^{e}	128.4^{e}	125.8^{d}	121.8^{d}	108.5^{e}	115.1^{e}	103.8^{e}	106.7^{d}	110.6^{c}
Butachlor + 2,4-D (100% NPK TCF)	133.7^{d}	138.8^{d}	144.5^{d}	134.7^{c}	127.8^{c}	126.7^{d}	131.0^{d}	136.0^{d}	124.4^{c}	119.5^{b}
Butachlor + 2,4-D (75% NPK TCF 25% TOS)	142.7^{b}	149.7^{b}	152.5^{b}	139.7^{b}	135.8^{b}	136.5^{b}	143.6^{b}	147.2^{b}	134.8^{b}	128.7^{a}
Butachlor + 2,4-D rotated with pretilachlor (100% NPK TCF)	137.5^{c}	144.5^{c}	147.4^{c}	135.7^{c}	128.6^{c}	129.7^{c}	134.1^{c}	138.7^{c}	125.7^{c}	120.7^{b}
Butachlor + 2,4-D rotated with pretilachlor (75% NPK TCF , 25% TOS)	145.7^{a}	154.5^{a}	156.7^{a}	143.7^{a}	139.7^{a}	140.7^{a}	147.6^{a}	151.1^{a}	138.5^{a}	130.2^{a}

Values with same letters are not statistically significant, DAA- Days after application; TCF - Through chemical fertilizer; TOS - Through organic source

manure in soil. Urease being a urea degrading enzyme, and does not mediate the degrading pathway of the herbicides and probably therefore remained unaffected by the herbicides. It appears that the herbicides were inert to urease producing microbes and so as to the urease activity. Mineral fertilization resulted in the lowest urease activity presumably by the small amounts of organic residues left in the soil (Balezentiene and Kilimas 2009). Though in general, application of chemical fertilizers stimulated the growth and multiplication of microorganisms, increased dosage was found to inhibit the survival of microbe due to osmotic stress created by fertilizers (Bharathi *et al.* 2011). A significant increase in soil enzyme activity of urease (78 ìg of NH_4^+/ g/ day), in unfertilized control plot was followed by 75% of RDF of NPK at 30 DAS. There is further reduction in enzyme activity when150% of RDF of NPK was applied due to the negative impact of higher dose of chemical fertilizers alone on survival of microorganisms.

Butachlor application on respiration showed a temporary inhibition within the earlier period (14 Days) after treatment and followed by a recovery during the later period in paddy soil (**Table 5**). The results are in conformity with the findings of Barman *et al.* (2009). The decline in the microbial population following herbicide application might have inhibited the respiration. It was observed that application of organic manure reduced the inhibition than application of sole chemical fertilizers. The higher enzyme activity in herbicide applied plots with organic manure addition may be ascribed to the significantly higher microbial population and activity compared to the plots with only chemical fertilizers (Bohme *et al.* 2005). The positive effect of organic manure addition may be ascribed to the significantly higher microbial population and activity compared to the plots with only chemical fertilizers. (Gu *et al.* 2009) or supply of substrates such as carbohydrates and amino acids (Bohme *et al.* 2005), which provide important sources of nutrients for microorganisms in the rhizosphere.

The microbial biomass of microorganisms is one of the important properties of ecological studies, which can be related to parameters describing microbial activity and soil health (Bolter *et al.*, 2006). The rising MBC accretion, established *Azospirillum* and PSB populations were extensively correlated with the PMEase activity signifying that compost and

Table 3. Activity of phosphatase enzyme in soil during autumn and winter rice as affected by herbicide application in rice-rice sequence (µg p-nitrophenol/g/dry soil/h)

Treatment	Autumn					Winter				
	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA
Farmers practice (one hand weeding)	288.2^{a}	285.6^{a}	290.4^{a}	287.6^{a}	294.7^{a}	245.8^{a}	242.3^{a}	249.9^{a}	250.7^{a}	246.8^{a}
Butachlor + 2,4-D (100% NPK TCF)	255.7^{e}	248.2^{e}	242.6^{e}	259.7^{e}	267.0^{e}	202.5^{e}	190.5^{d}	188.2^{d}	206.0^{e}	219.7^{d}
Butachlor + 2,4-D (75% NPK TCF 25% TOS)	265.0^{c}	255.3^{c}	252.3^{c}	268.7^{c}	275.7^{c}	210.4^{c}	204.5^{c}	202.0^{c}	222.3^{c}	234.4^{b}
Butachlor + 2,4-D rotated with pretilachlor (100% NPK TCF)	258.5^{d}	250.5^{d}	245.6^{d}	262.5^{d}	269.4^{d}	204.6^{d}	187.7^{e}	189.1^{d}	210.7^{d}	225.5^{c}
Butachlor + 2,4-D rotated with pretilachlor (75% NPK TCF , 25% TOS)	269.3^{b}	258.4^{b}	256.7^{b}	272.5^{b}	280.6^{b}	212.2^{b}	210.4^{b}	205.6^{b}	225.0^{b}	237.6^{b}

Values with same letters are not statistically significant, DAA - Days after application; TCF - Through chemical fertilizer; TOS - Through organic source

Table 4. Activity of urease enzyme in soil during autumn and winter rice as affected by herbicide application in rice-rice sequence (µg NH_4 g/soil/2h)

Treatment	Autumn					Winter				
	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA
Farmers practice (one hand weeding)	270.73^{e}	276.77^{e}	288.48^{d}	295.76^{c}	281.55^{d}	234.55^{d}	243.76^{d}	225.39^{e}	242.76^{c}	251.72^{e}
Butachlor + 2,4-D (100% NPK TCF)	285.54^{c}	292.62^{c}	268.50^{e}	278.58^{d}	267.59^{e}	226.70^{e}	255.87^{c}	267.76^{c}	236.71^{d}	247.80^{d}
Butachlor + 2,4-D (75% NPK TCF 25% TOS)	315.40^{a}	329.39^{a}	340.49^{a}	311.43^{b}	303.46^{b}	290.63^{a}	281.89^{b}	296.75^{a}	276.69^{b}	287.75^{a}
Butachlor + 2,4-D rotated with pretilachlor (100% NPK TCF)	279.04^{d}	287.64^{d}	294.79^{c}	265.76^{e}	292.43^{c}	242.79^{c}	230.57^{e}	254.61^{d}	229.61^{e}	265.64^{c}
Butachlor + 2,4-D rotated with pretilachlor (75% NPK TCF , 25% TOS)	309.65^{b}	301.62^{b}	317.03^{b}	331.65^{a}	336.82^{a}	280.83^{b}	289.50^{a}	280.62^{b}	295.83^{a}	281.50^{b}

Values with same letters are not statistically significant, DAA - Days after application; TCF - Through chemical fertilizer; TOS - Through organic source

Table 5. Soil respiration during autumn and winter rice as affected by herbicide application in rice –rice sequence (µg CO_2 –C g/dw/hr)

Treatment	Autumn					Winter				
	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA
Farmers practice (one hand weeding)	1.83[a]	1.75[a]	1.82[a]	1.74[a]	1.76[a]	1.56[a]	1.43[a]	1.65[a]	1.49[a]	1.47[a]
Butachlor + 2,4-D (100% NPK TCF)	1.17[e]	1.11[e]	1.08[e]	1.22[e]	1.33[d]	0.98[e]	0.89[e]	0.87[e]	1.06[e]	1.16[e]
Butachlor + 2,4-D (75% NPK TCF 25% TOS)	1.42[c]	1.33[c]	1.25[b]	1.56[c]	1.67[b]	1.25[c]	1.16[c]	1.13[c]	1.32[c]	1.34[c]
Butachlor + 2,4-D rotated with pretilachlor (100% NPK TCF)	1.27[d]	1.18[d]	1.15[d]	1.32[d]	1.41[c]	1.07[d]	0.97[d]	0.96[d]	1.23[d]	1.25[d]
Butachlor + 2,4-D rotated with pretilachlor (75% NPK TCF, 25% TOS)	1.58[b]	1.50[b]	1.22[c]	1.65[b]	1.76[a]	1.34[b]	1.27[b]	1.24[b]	1.38[b]	1.43[b]

Values with same letters are not statistically significant, DAA - Days after application; TCF - Through chemical fertilizer; TOS - Through organic source

Table 6. Microbial biomass carbon in soil during autumn and winter rice as affected by herbicide application in rice–rice sequence (µg/g)

Treatment	Autumn					Winter				
	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA	0 DAA	7 DAA	14 DAA	30 DAA	60 DAA
Farmers practice (one hand weeding)	387.34[c]	382.39[c]	419.42[c]	399.61[c]	410.41[c]	374.51[c]	367.35[c]	358.79[c]	355.19[c]	375.15[c]
Butachlor + 2,4-D (100% NPK TCF)	332.66[e]	319.49[e]	313.61[e]	330.26[e]	339.76[e]	299.57[e]	280.67[e]	270.57[e]	289.44[e]	304.21[e]
Butachlor + 2,4-D (75% NPK TCF 25% TOS)	452.59[b]	442.33[b]	439.39[b]	459.41[b]	461.32[b]	430.61[b]	425.29[b]	405.34[b]	412.86[b]	429.34[b]
Butachlor + 2,4-D rotated with pretilachlor (100% NPK TCF)	365.47[d]	359.21[d]	350.47[d]	368.53[d]	379.38[d]	337.62[d]	328.63[d]	318.45[d]	340.38[d]	322.49[d]
Butachlor + 2,4-D rotated with pretilachlor (75% NPK TCF, 25% TOS)	498.43[a]	479.58[a]	478.43[a]	486.49[a]	506.79[a]	468.48[a]	463.47[a]	444.25[a]	449.53[a]	458.19[a]

Values with same letters are not statistically significant, DAA - Days after application; TCF - Through chemical fertilizer; TOS - Through organic source

enriched compost plays an important role in protecting and maintaining soil enzymes in their active forms (Saha *et al.* 2008). The effect of butachlor on MBC was found to be highest on 14th day (**Table 6**). Increase in MBC in some herbicide treated soil may be due to the fact that some of the herbicides acting as the source of nutrients (Cook and Hutter 1981) in which case they significantly affect microbial growth and multiplication. However, the effect of herbicides is usually short-term and minor, when compared with natural, spatial and temporal variation in soil microbial biomass. The decrease in MBC may be due to the adsorption of small amount of pesticides on organic matter that mask the effects of these agrochemicals on soil microbial biomass, and subsequently led to hydrolysis of microbial cells (Jayamadhuri and Rangaswamy 2005). Herbicides affect various soil microbial processes (Johen and Drew 1977), inhibit decomposition (Grossbard and Wingfield 1978), which depends upon the type and rate of application that can alter the microbial biomass quantitatively and qualitatively in both short-term and long-term (Anderson and Armstrong 1981).

Microbial population in the rhizosphere soil of transplanted *Kharif* rice decreased after herbicide application but, there was no long term adverse effect on the microbial population of the soil. Butachlor 1.5 kg/ha and pretilachlor 0.75 kg/ha are harmful to the given soil in terms of total microbial activity, however addition of easily degradable source of organic carbon with recommended dose of fertilizer to the soil prior to butachlor and pretilachlor application could nullify this adverse impact on soil microorganisms as well as on physico-chemical properties of soil.

REFERENCES

Aon MA and Colaneri AC. 2001. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied Soil Ecology* **18**: 255-270.

Balasubramanian K and Sankaran S. 2001. Effect of pendimethalin on soil micro-organisms. *Indian Agriculturist* **45**: 93-98.

Balezentiene L and Kilimas E. 2009. Effect of organic and mineral fertilizers and land management on soil enzyme activities. *Agronomy Research* **7**(1): 191–197.

Barman KK, Shrivastava E and Varshney JG. 2009. Effect of butachlor on total microbial activity, *Azotobacter* and phosphate solubilizing fungal population. *Indian Journal of Weed Science* **41**: 27–31.

Benedetti A and Dilly O. 2006. Introduction. pp. 3–14. In: *Microbiological Methods for Assessing Soil Quality,* (Eds. Bloem J, Hopkins, D H and Benedetti A), CABI Publishing, Wallingford, UK.

Bharathi JM, Balachandar D, Narayanan R and Kumar K. 2011. Impact of fertigation on soil microbial community and enzyme activities cropped with maize under precision farming system. *The Madras Agricultural Journal* **98**: 84–88.

Bolter, M, Bloem J, Meiners K and Moller R. 2006. Enumeration and biovolume determination of microbial cells. pp. 93–107. *In Microbiological Methods for Assessing Soil Quality* (Eds. Bloem J, Hopkins, DH and Benedetti A), CABI Publishing, Wallingford, UK.

Chaudhury J, Mandal UK, Sharma KL, Ghosh H and Mandal B. 2005. Assessing soil quality under long-term rice-based cropping system. *Communications in Soil Science and Plant Analysis* **36**: 1141-1161.

Cook AM and Hutter R. 1981. s-Triazines as nitrogen sources for bacteria. *Journal of Agricultural and Food Chemistry* **29**: 1135-1143.

Dick WA, Cheng L and Wang P. 2000. Soil acid and alkaline phosphatase activity as pH adjustment indicators. *Soil Biology and Biochemistry* **32**:1915–1919.

Garcia-Ruiz R, Ochoa V, Hinojosa MB and Carreira JA. 2008. Suitability of enzymeactivities for the monitoring of soil quality improvement in organic agricultural systems. *Soil Biology and Biochemistry* **40**: 2137–2145.

Grossbard E and Wingfield GI. 1978. Effect of paraquat, aminotriazole and glyphosate on cellulose decomposition. *Weed Research* **18**: 347-353.

Gu Y, Zhang X, Tu K and Costa F. 2009. Soil microbial biomass, crop yields, and bacterial community structure as affected by long term fertilizer treatments under wheat-rice cropping. *European Journal of Soil Biology* **45**: 239–246.

Ismail BS, Yapp KF and Omar O. 1998. Effects of metasulfuron-methyl on amylase, urease and protease activities in two soils, *Australian Journal of Soil Research* **36**: 449–456.

Jayamadhuri, R. and Rangaswamy, V. 2005. Influence of orghorous and carbamate insecticides on enzymatic activities of amylase, cellulase and invertase in two groundnut soil. *Nature, Environment and Pollution Technology* **4**: 385-393.

Joa JH, Moon DG, Won HY, Lim HC and Hyun HN. 2010. Effect of different fertilization practices on soil microbial activities and community structure in volcanic ash citrus orchard soils. pp. 38–40 In: *Proceedings of 19th World Congress of Soil Science, Soil Solutions for a Changing World*, Brisbane, Australia.

Kadlag AD, Jadav AB and Kale YB. 2008. Urease and acid phosphatase soil enzymes are influenced by vermicompost and scepter herbicide. *Journal of Maharastra Agricultural University* **33**: 148-151.

Latha PC and Gopal G. 2010. Influence of herbicides on cellulolytic, proteolytic and phosphate solubalizing bacteria. *International Journal of Plant Protection* **3**(1): 83–88.

Madejon E, Moreno F, Murillo JM and Pelegrn F. 2007. Soil biochemical response to long term conservation tillage under semi-arid Mediterranean conditions. *Soil and Tillage Research* **94**: 346–352.

Reddy UR and Reddy SM 2009. Phosphatase activity in soil as influenced INM in tomato onion cropping system. *Indian Journal of Agricultural Research* **43**: 289-283.

Saeki M and Toyota K. 2004. Effect of bensulfuron-methyl (a sulfonyurea herbicide) on the soil bacterial community of a paddy soil microcosm. *Biology and Fertility of Soils* **40**: 110–118.

Saha S, Mina BL, Gopinath KA, Kundu S and Gupta HS. 2008. Relative changes in phosphatase activities as influenced by source and application rate of organic composts in field crops. *Bioresource Technology* **99**: 1750–1757

Tabatabai M A and Bremner JM. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biologyand Biochemistry* **1**: 301–307.

Tejada M and Gonzalez JL. 2009. Application of two vermicompost on rice crop: effect of soil biological properties and rice quality and yield. *Agronomy Journal* **101**: 336–344.

Vance ED, Brookes PC and Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass. *Soil Biologyand Biochemistry* **19**: 703–707.

Vandana LJ, Rao PC and Padmaja G. 2012. Effect of herbicides and nutrient management on soil enzyme activity. *Journal of Rice Research* **5**: 1–2.

Walkley A and Black IA. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* **37**: 29–38.

Wang J, Lu Y, Ding H and Guoqing A. 2007. Effect of cadmium alone and in combination with butachlor on soil enzymes. *Environmental Geochemistry and Health* **29**: 395–403.

Wang QK, Wang SL and Liu YX. 2008. Responses to N and P fertilization in a young *Eucalyptus dunnii*plantation; microbial properties, enzyme activities, and dissolved organic matter. *Applied Soil Ecology* **40**: 484–490.

Wardle DA and Parkinson D. 1990. Effects of three herbicides on soil microbial biomass and activity. *Plant Soil* **122**: 21–28.