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Effect of elevated CO_2 on chlorpyriphos degradation and soil microbial activities in tropical rice soil

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Abstract Impact of elevated CO_2 on chlorpyriphos degradation, microbial biomass carbon, and enzymatic activities in rice soil was investigated. Rice (variety Naveen, Indica type) was grown under four conditions, namely, chambered control, elevated CO_2 (550 ppm), elevated CO_2 (700 ppm) in open-top chambers and open field. Chlorpyriphos was sprayed at 500 g a.i. ha^{-1} at maximum tillering stage. Chlorpyriphos degraded rapidly from rice soils, and 88.4 % of initially applied chlorpyriphos was lost from the rice soil maintained under elevated CO_2 (700 ppm) by day 5 of spray, whereas the loss was 80.7 % from open field rice soil. Half-life values of chlorpyriphos under different conditions ranged from 2.4 to 1.7 days with minimum half-life recorded with two elevated CO_2 treatments. Increased CO_2 concentration led to increase in temperature (1.2 to 1.8 °C) that played a critical role in chlorpyriphos persistence. Microbial biomass carbon and soil enzymatic activities specifically, dehydrogenase, fluorescien diacetate hydrolase, urease, acid phosphatase, and alkaline phosphatase responded positively to elevated CO_2 concentrations. Generally, the enzyme activities were highly correlated with each other. Irrespective of the level of CO_2, short-term negative influence of chlorpyriphos was observed on soil enzymes till day 7 of spray. Knowledge obtained from this study highlights that the elevated CO_2 may negatively influence persistence of pesticide but will have positive effects on soil enzyme activities.

Keywords Climate change · Enzyme activity · Persistence · MBC · DHA

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Introduction

The rising levels of anthropogenic greenhouse gases (particularly carbon dioxide) led to unwanted consequences in agroecosystems. The combined land and ocean surface temperature increased 0.65 to 1.06 °C for the period of 1880 to 2012 as per the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC 2014). Increases in temperature and atmospheric CO_2 alter the relationship between plants and herbivores through complex mechanisms (Harrington et al. 1999). Managing these herbivores will be a great challenge under changing climatic situations. Persistence of pesticides may change, and it can cause a setback in pest management. Researchers predicted climate change variables mainly temperature, precipitation, and CO_2 will influence on the rate of degradation of pesticides in soil and in plant. For example, soil and aquatic concentrations of pesticides might reduce due to

a combination of increased volatilization and degradation (Bailey 2004; Benitez et al. 2006). The half-life of pesticides in soils might decrease by 60 % with 10 °C increase in temperature (Bloomfield et al. 2006). Increased CO_2 concentration will have influence on soil rhizospheric region by changing crop-soil-microbial interaction. Increased CO_2 might not directly influence pesticide degradation as reported by Manna et al. (2013) for azoxystrobin, but might play a major role in microbial degradation of pesticides.

Chlorpyriphos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothionate] is a broad spectrum organophosphorus insecticide, registered for application to more than 40 different food commodities worldwide. Hua et al. (2009) reported that chlorpyriphos residues in soil had a temporary or short-term inhibitory effect on soil microbes. Singh et al. (2002) reported that the effects from chlorpyriphos on the soil microbial characteristics were either very small or insignificant. However, little information is available to confirm these assumptions on chlorpyriphos. On the other hand, there may be few soil microbial parameters which may accelerate the degradation of chlorpyriphos. Sikora et al. (1990) reported a correlation between soil phosphatase activity and degradation of organophosphorus insecticides.

Soil enzyme activities are "sensors" of soil microbial functioning, play important role in the soil fertility, and are the indicators of soil quality. Earlier researchers reported that elevated CO_2 had positive effect on soil enzyme activities like FDA and dehydrogenase activity (DHA) (Bhattacharyya et al. 2013; Li et al. 2010). Few other researchers reported minimum or no effect of CO_2 on soil enzymatic activities (Bazot et al. 2006; Lesaulnier et al. 2008). However, studies on the effect of elevated CO_2 in rice crop had suggested that microbial biomass carbon (MBC) was significantly higher in rice soils grown under elevated CO_2 environment than the plant maintained at ambient CO_2 (Inubushi et al. 2011; Bhattacharyya et al. 2013). Manna et al. (2013) reported elevated CO_2 did not affect dehydrogenase, fluorescein diacetate, and acid phosphatase activity in rice soil, whereas Bhattacharyya et al. (2014) reported that acid and alkaline phosphatase activity was significantly higher under elevated CO_2 compared to the open field rice soil. Increased available C input into the soil under elevated CO_2 stimulates soil microbes in tropical rice soil, which leads to increased soil enzymatic activities (Bhattacharyya et al. 2014).

The major objective of our research was to know the influence of elevated CO_2 on soil enzymatic activities on chlorpyriphos degradation. Simultaneously, we studied the effect of chlorpyriphos on MBC and soil enzymes.

Materials and methods

Reagents and solvents

Chlorpyriphos standard was purchased from Sigma-Aldrich, India. Analytical grade chemicals and solvents were used for routine work, and for gas liquid chromatography (GLC) analysis, analysis grade solvents from Merck, India, were used.

Experimental site

The study site was situated at the experimental farm of the National Rice Research Institute, Cuttack (20° 27′ 10″ N, 85° 56′ 9″ E; 24 m above mean sea level), in the eastern part of India. The soil was an Aeric Endoaquept with sandy clay loam texture (25.9 % clay, 21.6 % silt, 52.5 % sand), bulk density 1.40 Mg m^{-3}, cation exchange capacity 15.3 cmol(+)kg^{-1}, total C 0.79 %, and total N 0.077 %.

Experimental design

A pot experiment in complete randomized design was set up with four treatments, namely, (i) unchambered control, i.e., open field (outside; 394 ± 10 mol mol^{-1} CO_2); (ii) chambered control (OTC; 394 ± 10 mol mol^{-1} CO_2); (iii) elevated CO_2 (CO_2 at 550 ppm, 550 ± 30 mol mol^{-1} CO_2); and (iv) elevated CO_2 (CO_2 at 700 ppm, 700 ± 30 mol mol^{-1} CO_2). The experiment was conducted in circular shaped, UV-shielded open-top chambers (OTCs; diameter 4 m and height 3 m) (M/S Neogenesis Engineering, Thane, Maharashtra, India). The elevated CO_2 concentrations were maintained daytime throughout the crop growing period in rice-rice ecosystem. Atmospheric temperature was measured automatically through a sensor.

Rice (var. Naveen, Indica type) seeds were sown in soils of respective treatments in December, 2012. Twenty-five-day-old seedlings were transplanted in plastic pots (20 cm × 20 cm). Soils for the experiment were obtained from the respective OTC chambers. Two seedlings were planted in each pot

containing 4 kg soil, and a total of 204 pots were maintained as three chambered and one unchambered treatments. Chlorpyriphos was applied at maximum tillering stage to each pot at recommended dose (500 g a.i. ha^{-1}) that corresponded to 0.9 mg per pot. Chlorpyriphos was mixed with minimum quantity of acetone followed by mixing in water to apply on crops using a manual hand sprayer. Among each set, 24 pots were treated with chlorpyriphos while 27 other pots were maintained as untreated control. Pots were maintained with 4–5 cm standing water, and water loss was supplemented daily. Recommended agronomic package of practices were followed to raise the crops. Pots were removed at regular intervals on 0 (before spray), 1, 3, 5, 7, 15, 21, 31, and 40 days after pesticide spray for extraction of chlorpyriphos residues from soil and to determine its effect on different soil enzyme activities.

Chlorpyriphos extraction and analysis

Three pots from each group of pesticide-treated and untreated were removed for analysis on each sampling day. Soil sample was collected from the rhizospheric region and were mixed thoroughly. Chlorpyriphos was extracted from fifty grams soil (oven dry basis) using acetonitrile (50 + 30 + 20 mL) by dipping and shaking method. Acetonitrile fractions were pooled and clean up was done by adding a pinch of activated charcoal followed by drying over anhydrous Na_2SO_4 and concentrated for further analysis.

Chlorpyriphos was analyzed in Agilent 6820 gas chromatograph (GLC) equipped with capillary column, HP-I (15 m × 0.53 mm × 0.5 μm) and electron capture detector (ECD). The operating parameters of the instrument were as follows: oven temperatures 150 °C (2 min) → 5 °C min^{-1} → 200 °C (3 min) → 10 °C min^{-1} → 250 °C (2 min), injection port at 200 °C and detector at 300 °C. Flow rate of nitrogen (carrier gas) was 30 mL min^{-1}, but through column, it was 1 mL min^{-1} and injection was done in splitless mode. Under these operating conditions, the retention time of chlorpyriphos was found to be 14.45 min. Limit of detection of the method was ≤0.0025 μg g^{-1} soil (S/N, 3). The recovery of the chlorpyriphos from soil was more than 87 %.

Microbial biomass carbon and soil enzyme activities

Soil MBC was measured by modified chloroform fumigation extraction method (Vance et al. 1987). A set of fresh soil samples was fumigated with chloroform for overnight under dark condition. Without the addition of chloroform, another set of soil samples was kept under

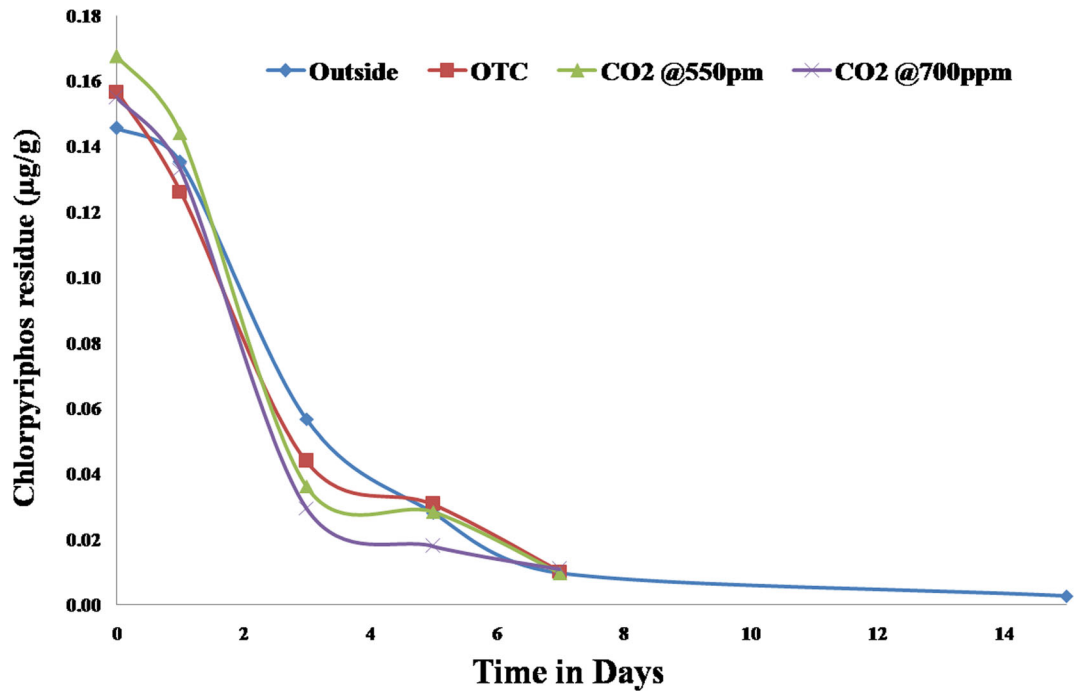

Fig. 1 Persistence of chlorpyriphos from rice planted soil under different elevated CO_2 conditions

dark condition. MBC was extracted using 0.5 M K_2SO_4 from both fumigated and nonfumigated soil and followed by UV-visible spectrophotometric determination at 280 nm (Paul et al. 2009). DHA was determined by reduction of triphenyl tetrazolium chloride (TTC) (Casida et al. 1964). Soil sample was treated with $CaCO_3$ and TTC and incubated for 24 h at 37 °C. The triphenyl formazan (TPF) was extracted from the reaction mixture with methanol and assayed at 485 nm. FDA hydrolase activity was measured by the potassium phosphate buffer method followed by extraction with chloroform/methanol (2:1 *v*/*v*) as described by Adam and Duncan (2001). Soil samples were treated with potassium phosphate buffer (pH 7.6) and FDA. FDA hydrolase activity was assayed at 490 nm. Urease activity was determined by the nonbuffer method of Zantua and Bremner (1977). The amount of residual urea was extracted using 2 M KCl-PMA solution. Coloring agent comprising of acidified diacetylmonoxime and thiosemicarbazide was added to the extract. Urease activity was assayed at 527 nm. Alkaline and acid phosphatase activity was assayed by treating 1 g of soil sample with 0.2 mL of toluene, 4 mL of modified universal buffer (pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase) and 1 mL of *p*-nitrophenyl phosphate solution (Eivazi and Tabatabai 1977; Juma and Tabatabai 1977). After 1 h of incubation at 37 °C, 0.5 M $CaCl_2$ and 0.5 M NaOH solution was added. The suspension was filtered, and the color intensities of filtrates were measured at 420 nm.

Statistical analysis

The degradation rate of chlorpyriphos in soil was fitted to a first-order kinetic model. The rate constant (k) was determined using the algorithm $C_t/C_0 = e^{-kt}$, where C_0 is the amount of chlorpyriphos in the soil at time zero and C_t is the amount of chlorpyriphos in the soil at time t. Linear regression (ln (C_t/C_0) of the chemical data and time) was used to calculate the time in which the chlorpyriphos concentration in the soil was reduced by 50 % (Hoskin 1961).

Data were analyzed following analysis of variance (SAS Software packages, SAS EG 4.3), and means of treatments were compared based on Tukey's minimum significant difference test (HSD) at 0.05 probability level. Correlation coefficients among traits were determined using SAS software packages, SAS EG 4.3. Two-way ANOVA was performed using SAS 9.3 to know the

Table 1 MBC in pesticide-treated and pesticide-nontreated soils (μg g^{-1} soil)

Treatment	0 days	1 day		7 days		15 days		21 days		30 days		40 days	
		No-P	P	No-P	P	No-P	P	No-P	P	No-P	P	No-P	P
Outside	120.33b	122.11b	89.74b	159.20c	110.21bc	175.92b	166.37b	156.24b	178.42b	118.54c	180.13b	130.43c	142.93c
OTC	110.56b	120.21b	108.84ab	163.87c	94.93c	189.35b	164.25b	164.98b	201.18b	119.99c	182.49b	152.18bc	148.63c
CO_2 at 550 ppm	145.89ab	160.80a	121.81ab	217.09b	141.62ab	231.41b	252.70a	260.29a	279.00a	176.95b	276.86a	177.36ab	204.27b
CO_2 at 700 ppm	161.71a	167.52a	146.10a	288.43a	158.66a	296.52a	279.43a	261.08a	283.57a	222.01a	297.26a	194.90a	253.03a
CV(%)	10.75	5.47	12.99	2.88	12.59	9.4	8.06	4.14	11.42	7.13	9.61	8.22	6.25
SE(d)	11.82	6.37	12.36	4.86	12.99	17.13	14.18	7.12	21.95	9.27	18.37	10.98	9.55
Tukey HSD at 5 %	40.91	22.06	42.81	16.84	44.98	59.32	49.11	24.66	76.00	32.12	63.60	38.03	33.07

Means with at least one letter common are not statistically significant using Tukey's honest significant difference

No-P without chlorpyriphos-treated soils, *P* with chlorpyriphos-treated soils

effects of pesticide, time, and their interaction on soil microbial activities.

Results and discussion

Dissipation of chlorpyriphos

Dissipation of chlorpyriphos in rice soil is represented under four different treatments as Fig. 1. We could not observe difference among treatments in dissipation pattern of chlorpyriphos from the rice-planted soils. However, the rate of dissipation was slower in open field treatment. Chlorpyriphos recovered from rice soil after 2 h of spray was in the tune of 0.146–0.168 μg g^{-1}. By day 5, 88.4 % of initially applied chlorpyriphos was dissipated from the soil maintained under elevated CO_2 at 700 ppm, whereas the amount of chlorpyriphos dissipated from the treatments under elevated CO_2 at 550 ppm, ambient CO_2 in OTC and open field soil were 83, 80.4, and 80.7 %, respectively. After 15 days of spray, chlorpyriphos was not detected from rice grown soil under both elevated CO_2 concentrations and ambient CO_2 in OTC.

Dissipation data from all of the four treatments fitted well in first order kinetics. The coefficient of determination value ranged from 0.94 to 0.99. Half-life values determined from first-order kinetics found to be 2.4 to 1.7 days. In open field soil, chlorpyriphos had better half-life of 2.4 days as compared with soils under chambered OTC, elevated CO_2 at 550 ppm and elevated CO_2 at 700 ppm which registered 1.8, 1.7, and 1.7 days, respectively. Dissipation pattern of chlorpyriphos from the rice-planted soils under different conditions were similar except the higher rate of degradation for elevated CO_2-treated soils. The experiment was conducted with the hypothesis that elevated CO_2 will increase the atmospheric temperature which will lead to increased volatilization loss of pesticides. In addition to this, better microbial growth under elevated CO_2 will have influence in microbial degradation of pesticides. We observed an increase of 1.2–1.8 °C temperature in elevated CO_2 chambers. The role of increased temperature in pesticide degradation has already been ascertained earlier. Zhang et al. (2012) reported the half-life of 1.35 days for the dissipation of chlorpyriphos from soil under paddy field conditions. Bloomfield et al. (2006) used a simulation model and predicted that for every 10 °C increase in temperature, the half-life of pesticides

Table 2 Probability values of two-way ANOVA for the effects of treatment, time and their interaction on the measured parameters

Enzymatic parameters	Source	DF	Outside	OTC	CO_2 at 550 ppm	CO_2 at 700 ppm
MBC	Pesticide	1	0.8453	0.7417	0.6381	0.7128
	Time	6	<0.0001	<0.0001	<0.0001	<0.0001
	Pesticide × time	6	0.0003	0.0319	0.0217	0.0011
DHA	Pesticide	1	0.1842	0.8098	0.0850	0.0583
	Time	6	0.0004	<0.0001	<0.0001	<0.0001
	Pesticide × time	6	0.0032	0.0057	0.0266	0.0316
FDA	Pesticide	1	0.9333	0.3452	0.8194	0.3936
	Time	6	<0.0001	<0.0001	<0.0001	<0.0001
	Pesticide × time	6	0.5831	0.1444	0.0634	0.1276
Acid phosphatase	Pesticide	1	0.5312	0.2502	0.4651	0.0329
	Time	6	0.0003	<0.0001	<0.0001	<0.0001
	Pesticide × time	6	0.2436	0.7717	0.0442	0.0870
Alkaline phosphatase	Pesticide	1	0.1052	0.0022	0.0373	0.0449
	Time	6	<0.0001	<0.0001	<0.0001	<0.0001
	Pesticide × time	6	0.3903	0.0644	0.0042	0.0001
Urease	Pesticide	1	0.0267	0.0616	0.0740	0.0231
	Time	6	<0.0001	<0.0001	<0.0001	<0.0001
	Pesticide × time	6	0.1265	0.9904	0.0012	0.0021

MBC microbial biomass carbon, *DHA* dehydrogenase activities, *FDA* fluorescien diacetate hydrolase activities

in soil might decrease by 60 %. In another study, Bailey (2004) examined that increased degradation of isoproturon in warmer soils. Additionally, increased water temperature was also found to increase the degradation rate of several phenyl-urea pesticides (Benitez et al. 2006). Direct role of elevated CO_2 on pesticide degradation cannot be proved here. Increased temperature in elevated CO_2 chambers compared to unchambered control helped in chlorpyriphos degradation. Manna et al. (2013) reported that elevated CO_2 did not have any significant effect on the persistence of azoxystrobin in rice-planted soil, but they reported that elevated CO_2-treated soil had less half-life of azoxystrobin compared to unchambered control.

Microbial biomass carbon and soil enzyme activities

MBC in chlorpyriphos-treated and chlorpyriphos-nontreated soils under lowland rice ecosystem were investigated over a time. It has been found that irrespective of pesticide spray, MBC content was higher in both the elevated CO_2 treatments compared to control chambered and nonchambered rice soils throughout the investigation. MBC content was recorded 161–296 μg g^{-1} in chlorpyriphos-nontreated soil, whereas it was 146–297 μg g^{-1} in chlorpyriphos-treated soil under elevated CO_2 at 700 ppm condition (Table 1). Under elevated CO_2 at 550 ppm, MBC content was recorded 145–260 μg g^{-1} in chlorpyriphos-nontreated soil, whereas it was 121–260 μg g^{-1} in chlorpyriphos-treated soil. MBC content in soils under ambient OTC and unchambered soil recorded maximum of 201 and 180 μg g^{-1} of soil, respectively. MBC content in chlorpyriphos-treated soil did not differ from the untreated soils at the end of the experiment. But, it has been observed that, up to sixth days of spray, MBC content was less in treated soil than the nontreated soil. Beyond that, negligible effect of chlorpyriphos was ascertained. However, time, i.e., crop growth stages have influence on MBC content in soil ($p < 0.0001$). Interaction between time and pesticides had influence on MBC content in soil in all the four treatment conditions (Table 2).

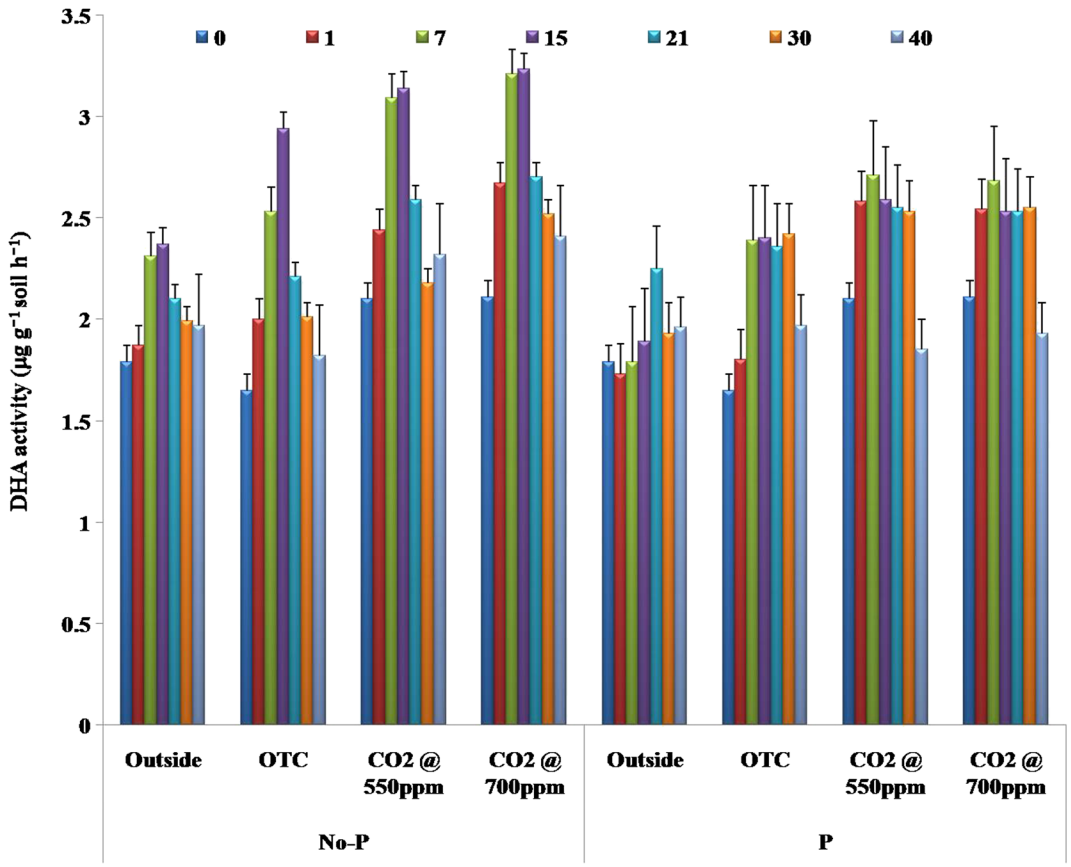

Fig. 2 DHA in pesticide-treated and pesticide-nontreated soils under elevated CO_2 conditions (the *error bars* indicate standard deviations)

Among the different microbial parameters, soil microbial biomass (MBC) is considered to be responsible for regulating nutrient cycling (Singh et al. 1989) and is closely linked to the primary productivity of an ecosystem (Marcel et al. 2008) and soil health (Sparling 1997). Impacts on MBC are of great importance to understand the below ground processes in soils exposed to high CO_2 (Drigo et al. 2008). This increase could be attributed to more soil exudates in rice grown under elevated CO_2 environment (Hill et al. 2007). Inubushi et al. (2011) reported that microbial biomass carbon in rice grown in elevated CO_2 environment was significantly higher than rice soil maintained at ambient CO_2. In our study, we observed more number of tiller (data not shown) in CO_2 elevated treatments compared to both the controls. This may lead to increased secretion of root exudates, thereby MBC content in soil.

DHA in rice soils maintained under different CO_2 environments is presented in Fig. 2. DHA varied according to the different CO_2 environments in chlorpyriphos-nontreated soils. In case of chlorpyriphos-treated soils, DHA did not vary among the treatments during the experiment time. Highest DHA activities recorded in both CO_2-enriched soils compared to both control soils irrespective of pesticide treatment. Crop duration had an effect on DHA activities ($p \leq 0.0004$). It was found that DHA activities were maximum after 6–14 days of spray, which coincide with panicle initiation, irrespective of different CO_2 environments. In our present study, increased in the DHA activity under elevated CO_2 conditions suggest impact of climate change drivers on the soil microbial activity. The activity of dehydrogenase is exclusively intracellular and can function only within viable cells. It is considered as an indicator of the oxidative metabolism in soils (Wlodarczyk et al. 2002). Earlier, Inubushi et al. (2010) and Manna et al. (2013) studied dehydrogenase activity in rice soils under elevated CO_2 and reported that there was no significant difference in the dehydrogenase activity of these soils. However, Das et al. (2011) reported increased dehydrogenase activity in rice soils incubated at elevated CO_2 in a laboratory incubation study. Increased DHA activities may be due to higher deposition of carbon material around the root zone.

No significant difference in FDA activities was observed among the different CO_2 treatments in pesticide-treated soils over time (Fig. 3). After 14 days of spray, untreated soil recorded maximum FDA activity of

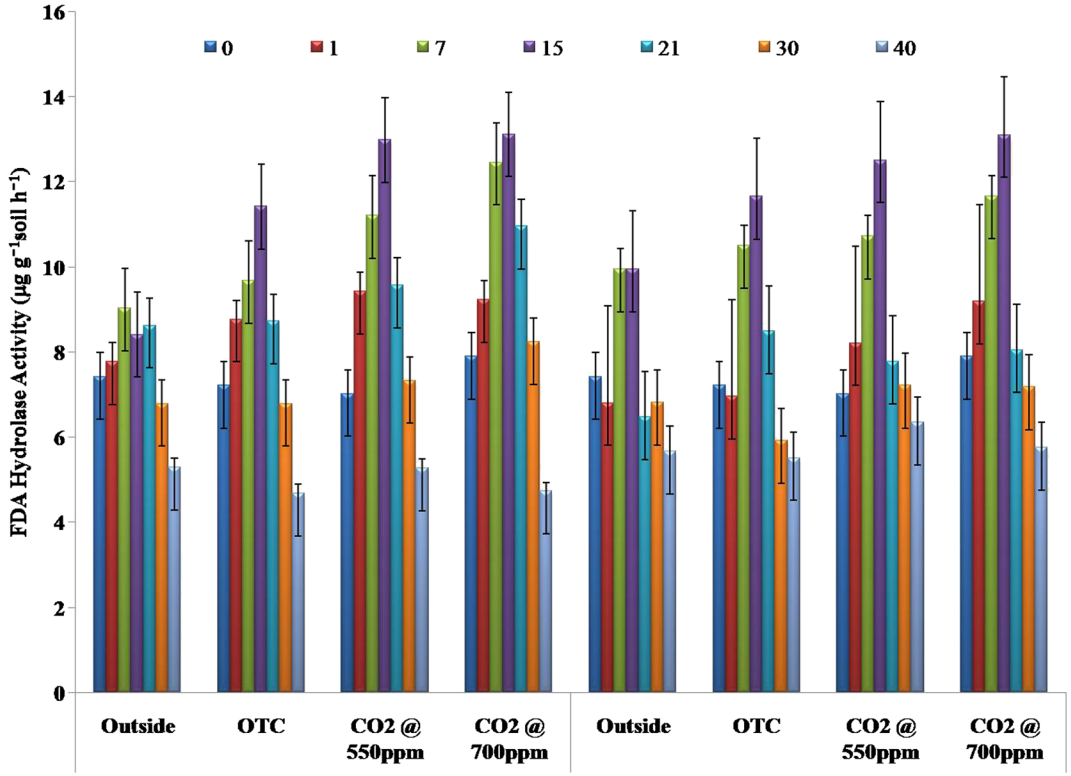

Fig. 3 FDA in pesticide-treated and pesticide-nontreated soils under elevated CO_2 conditions (the *error bars* indicate standard deviations)

Table 3 Urease in pesticide-nontreated and pesticide-treated soil (μg g^{-1} soil h^{-1})

Treatment name	0 days	1 day		7 days		15 days		21 days		30 days		40 days	
		No-P	P	No-P	P	No-P	P	No-P	P	No-P	P	No-P	P
Outside	336.73c	349.25b	339.59b	353.67c	346.79b	382.80b	368.09b	362.58b	360.16b	367.89c	364.53c	355.25ab	338.67
OTC	349.75b	355.69ab	345.39b	357.65bc	351.30b	390.32ab	390.92a	385.61a	382.10a	380.63b	375.29bc	341.66b	348.13
CO_2 at 550 ppm	348.79b	356.33ab	357.98ab	372.56ab	367.95a	390.73ab	385.32ab	384.62a	383.80a	383.62ab	385.04ab	367.82a	341.90
CO_2 at 700 ppm	360.70a	363.16a	365.12a	385.17a	370.53a	407.08a	390.16a	384.81a	387.01a	392.11a	393.12a	363.30ac	359.07
CV(%)	1.11	0.93	1.93	1.60	1.56	1.77	1.88	1.18	0.49	0.99	1.39	1.47	2.35
SE(d)	3.15	2.709	5.56	4.80	4.56	5.67	5.89	3.66	1.52	3.09	4.30	4.29	6.64
Tukey HSD at 5 %	10.92	9.37	19.25	16.61	15.80	19.63	20.40	12.68	5.28	10.71	14.90	14.86	NS

Means with at least one letter common are not statistically significant using Tukey's honest significant difference

No-P without chlorpyriphos-treated soils, *P* with chlorpyriphos-treated soils

13.12 μg g^{-1} soil h^{-1} in elevated CO_2 at 700 ppm. Chlorpyriphos did not affect the FDA activity in pesticide-treated soils under different CO_2 treatments. But, among the different CO_2 treatments, there were significant differences till 21 days of spray in pesticide-nontreated soil. We observed no significant differences in the interaction between pesticide and duration of application. Das et al. (2011) reported that FDA hydrolysis activity increased significantly under elevated CO_2.

Positive effects of both the elevated CO_2 treatments were observed in soil urease activity (Table 3). Urease activity in untreated soil under CO_2 at 700 ppm treatment was maximum (407 μg g^{-1} soil h^{-1}) after 14 days of application. There was significant difference among the four treatments over the period of experimentation. Chlorpyriphos has played a role in soil urease activity in all the CO_2 treatments. Role of phosphatases in transformation of organic and inorganic phosphorous compounds in soil are already established in literature, and these activities are important factors in maintaining and controlling the rate of P cycling through soils. Acid and alkaline phosphatase activities of the soils are presented in Figs. 4 and 5. Both the elevated CO_2 treatments had effect on both acidic and alkaline phosphatise activity compare to both control. Highest acid phosphatase activity was observed in pesticide-nontreated soil under elevated CO_2 at 550 ppm in the tune of 44.75 μg g^{-1} soil h^{-1}, and minimum was observed in pesticide-treated soil under outside control in the tune of 20.51 μg g^{-1} soil h^{-1}. In case of alkaline phosphatase activity, highest activity was observed in pesticide-nontreated soil under elevated CO_2 at 700 ppm condition in the tune of 44.99 μg g^{-1} soil h^{-1} and minimum was observed in pesticide-treated soil under ambient OTC in the tune of 25.19 μg g^{-1} soil h^{-1}. Chlorpyriphos did not have any significant effect on acid phosphatase, but it has certain role in alkaline phosphatase activity in soils maintained under ambient OTC condition, elevated CO_2 at 550 and 700 ppm. There was significant interaction present between pesticide and time of application for treatments maintained under both elevated CO_2 treatments for alkaline phosphatase activity. Earlier research has indicated the possible inhibitory effect of the metabolites of chlorpyriphos, i.e., 3,5,6-trichloro-2-methoxy pyridine and 3,5,6-trichloropyridinol (TCP) on di-nitrogen-fixing bacteria and phosphate-solubilizing microorganism leading to short-term negative effect on urease and both phosphatase activities (Sardar and Kole 2005).

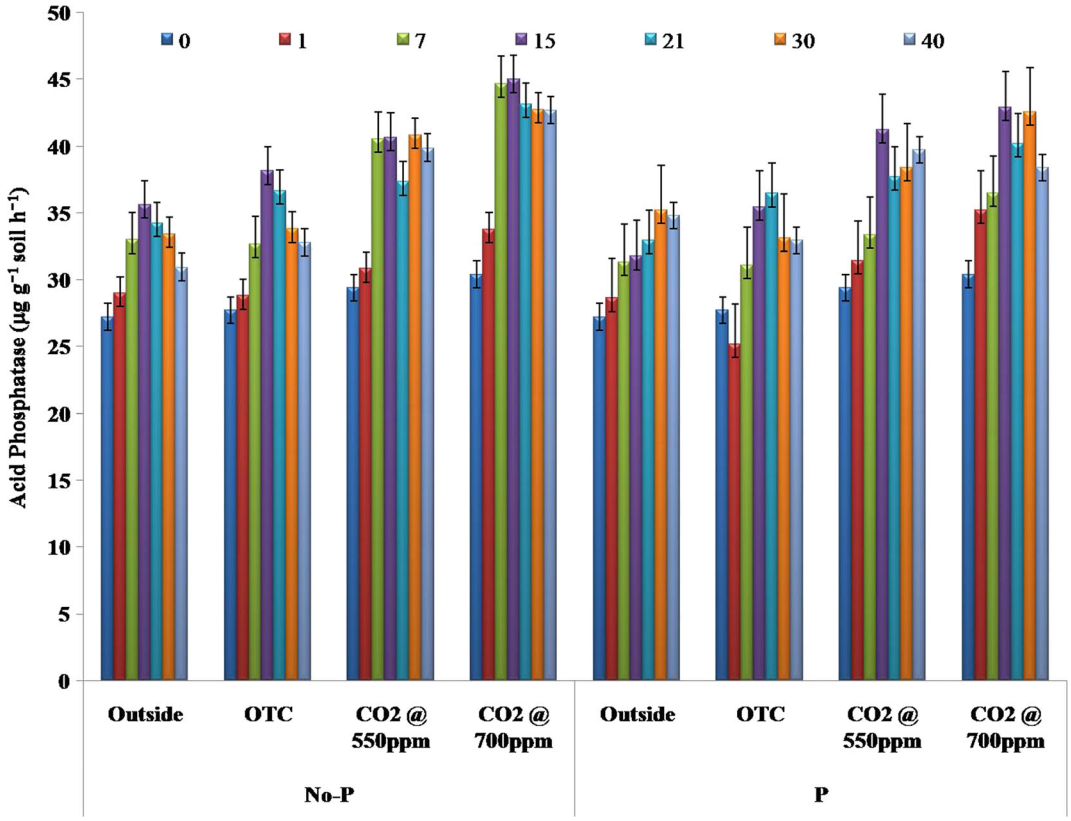

Fig. 4 Acid phosphatase activity in pesticide-treated and pesticide-nontreated soils under elevated CO_2 conditions (the *error bars* indicate standard deviations)

Degradation of chlorpyriphos by an alkaline phosphatase obtained from *Spirulina platensis* was reported by Thengodkar and Sivakami (2010). Increased phosphatase activities and urease activities under elevated CO_2 treatments supported by earlier reports (Kang et al. 2005; Das et al. 2011; Bhattacharyya et al. 2014). Phosphatase activities in wetlands under elevated CO_2 increased considerably as microbes in the soil might be stimulated to obtain more phosphate, resulting in higher phosphatase activity (Kang et al. 2005). In general, our experiment has observed better soil enzymatic properties under elevated CO_2 irrespective of pesticide treatments. It may be due to rhizospheric deposition of carbon materials led to better microbial activities.

Correlation among different enzymatic parameters was determined after 7 days of pesticide spray (table not included). It was found that except FDA, all other parameters were highly correlated and statistically significant. MBC was positively correlated with DHA (0.80), ALP (0.68), ACP (0.67), and urease (0.81). The correlation value between MBC and FDA was 0.25. Alkaline and acid phosphatases were highly correlated to each other with correlation value of 0.75. Both the phosphatase activities were similarly correlated with other soil microbial properties, namely, MBC, FDA, DHA, and urease.

Quantity of chlorpyriphos present on day 1 of spray did not show any negative effect on soil enzymatic activities (table not included). We found nonsignificant but positive correlation between chlorpyriphos present on day 1 of spray and on soil enzymatic properties like DHA, FDA, ACP, and urease. However, ALP was negatively correlated (−0.26) with quantity of pesticide present on day 1 of spray. After 7 days of spray, we observed nonsignificant but negative correlation between quantity of chlorpyriphos present with soil enzyme activities like DHA, ALP, ACP, and urease. Microbial biomass carbon was negatively correlated with quantity of pesticide present on 7 days of spray. It means chlorpyriphos has some effect on soil enzyme activities for initial days of spray. However, there was no residual effect of pesticides on soil microbial activities

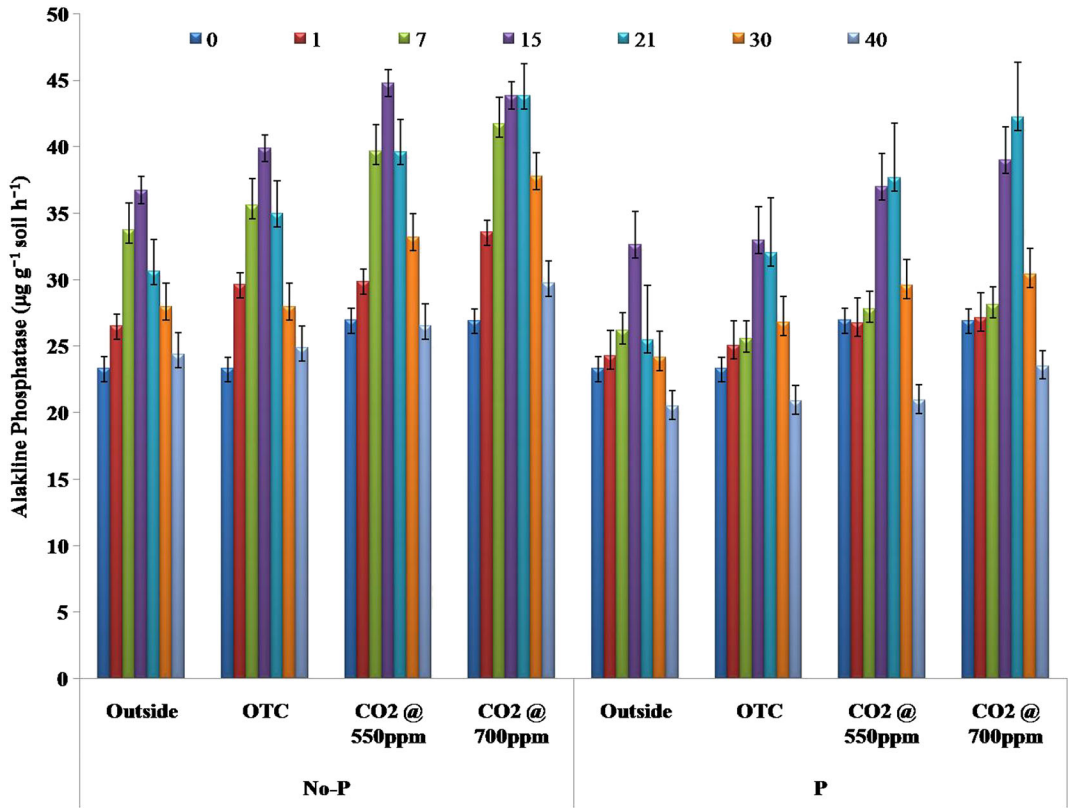

Fig. 5 Alkaline phosphatase activity in pesticide-treated and pesticide-nontreated soils under elevated CO_2 conditions (the *error bars* indicate standard deviations)

in later stage. Effect of chlorpyriphos on MBC was negligible in all the four treatments. Thus, when chlorpyriphos is used at recommended dose, it did not affect MBC in long run. Dutta et al. (2010) reported that application of chlorpyriphos at recommended field dose to agricultural soil is not likely to be detrimental to soil microbial activity. Repeated application of chlorpyriphos to the soil did not result in the development of a microbial population with the enhanced ability to degrade the pesticide (Singh et al. 2002). Earlier, Kumar (2011) reported that effect of chlorpyriphos on DHA depends on dose. Higher doses of chlorpyriphos will have prolonged negative effects on dehydogenase activity, whereas Menon et al. (2005) reported that chlorpyriphos and its metabolite had stimulatory effect on DHA. FDA was considered as a suitable tool for measuring the early detrimental effect of pesticides on soil microbial biomass (Nayak et al. 2007). Dutta et al. (2010) reported that chlorpyriphos at field rate did not influence FDA activity.

Conclusions

Increase in CO_2 concentration led to increase in atmospheric temperature which was responsible for reduced chlorpyriphos persistence. This may contribute toward environmental safety although it may lead to frequent use of pesticides to combat evolving pest scenario. The increase in root exudates under elevated CO_2 conditions improved the soil enzymatic activities and microbial biomass carbon. Chlorpyriphos had shown a transient negative effect on soil enzymes. This short-term study was for generating initial knowledge to assess the impact of elevated CO_2 on chlorpyriphos degradation, microbial biomass carbon, and enzymatic activities in rice soil. However, to have a realistic assessment of global warming on fate of pesticide, long-term studies are required.

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