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Response of soil enzymes to elevated CO_2 and temperature in weeds associated with rice-wheat cropping system

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ABSTRACT

Biological properties of the soil have often been proposed as early and sensitive indicators of soil ecological stress or other environmental changes. In the present investigation, the soil samples were collected from weeds associated with rice- wheat cropping system from open- top chambers to assess the effect of elevated CO_2 and temperature on soil enzymes. In *Rabi* season, higher activity of FDA hydrolysis rate was noticed in wild oat with 26.5 (µg fluorescein/g/h) at elevated CO_2 + ambient temperature levels. Dehydrogenase activity was higher at enrichment of CO_2 for *P. minor* with 35.9 (µg TPF/g soil/24 h). Urease significantly was higher in enrichment of CO_2+ elevated temperature with wild oat 34.6 (µg NH_4/g soil/24 h) followed by wheat (31.5µg NH_4/g soil/24 h) with elevated CO_2 + ambient temperature. In *Kharif* season, enrichment of CO_2 concentration in rhizosphere of *Echinochloa crusgalli* recorded with higher FDA hydrolysis rate (19.8 µg fluorescein/g/h), dehydrogenase activity (39.8 µg TPF/g soil/24 h) and urease activity (45.6 µg NH_4/g soil/24 h) respectively. We found the carbon dioxide enrichment significantly increased the soil enzymes like dehydrogenase, fluorescein diacetate (FDA) hydrolysis and urease activity in weeds rhizosphere than the crops.

Key words: Elevated CO_2, Elevated temperature, Open top chambers, Rice, Soil enzymes, Wheat

Indian agriculture has done remarkably well by increasing food production from 50 Mt in 1951 to 252 Mt in 2011-2012 assuring food security to the nation. However, perceived impacts of climate change could adversely affect the food output. Over the last two decades, global warming was easily dismissed as the natural fluctuation in an ever changing global climate. Although there exists natural fluctuations in climate, human induced changes are predominantly during the current global warming trend (Stager 2012). Carbon dioxide is one of the major contributors of greenhouse gases which, being a primary substrate for photosynthesis, may have a significant impact on plant metabolism that directly affect the overall plant growth and soil health. Soil enzyme activities are the indicators of soil microbial functioning, soil physicochemical conditions and nutrient cycling in soils. The use of soil enzymatic activities as possible indicators of changes in below ground processes is induced by CO_2 enrichment. Many factors can influence the activity of soil enzymes. Temperature changes can affect microbial diversity and enzymatic activity directly. A source of microbial substrates for enzymatic decomposition could be rhizodeposition and secretion of soluble root exudates. Microbes could have various positive and negative feedback responses to temperature. Whether, changes in microbial processes lead to a net positive and negative feedback for greenhouse gas emission in unclear. The reason is that microbes live in very diverse communities that that interact with organisms and the environments in complex ways. It is important and there is an urgent need for more empirical knowledge that will fundamentally improve our ability to predict feedbacks of the terrestrial carbon cycle in agricultural ecosystems to anticipated climate change.

Climate change factors such as elevated atmospheric carbon dioxide (CO_2) can exert significant impacts on soil microorganisms and the ecosystem level processes they mediate. Therefore, the present investigation was carried out to study the effects of elevated CO_2 and temperature concentrations on soil enzyme activities in the weeds associated with rice-wheat ecosystem in open- top chambers (OTCs).

MATERIALS AND METHODS

Field experiment was laid out to study the changes of enzyme activity in veritsols under open top chambers (OTCs) to study the effect of elevated CO_2 and temperature on soil enzymes, at crop physiological maturity stage during *Kharif* and *Rabi* season (2013-14). The treatments included i) ambient CO_2 + ambient temperature ii) ambient CO_2 + elevated temperature iii) elevated CO_2 + ambient

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temperature iv) elevated CO_2 + elevated temperature in open top chambers (OTCs) placed in ICAR-Directorate of Weed Research, Jabalpur. The elevated temperature was maintained by putting infrared lamps inside the OTC chambers and was controlled by the automatically calibrated temperature sensors. Air temperature and relative humidity was monitored continuously at every 5 min interval in each OTC chamber by temperature– humidity-calibrated sensors. During sampling, the crops were gently pulled out, and loose soil was shaken off the roots and the soil that adhered strongly to the roots was carefully brushed and sampled as rhizosphere soil. All samples were performed in triplicate, and were used to quantify the soil enzymes.

Dehydrogenase activity was assayed by the method of Casida *et al*. (1964). Moist soil samples (4 g) were placed in 16 x 150 mm^2 test tubes to which was added 1 ml of 3% aqueous solution of 2,3,5-triphenyl tetrazolium chloride, 40 mg $CaCO_3$ and 2.5 ml distilled water. The contents of each tube were then mixed with a glass rod and incubated for 24 h at 37°C. Triphenyl formazan (TPF) was extracted by transferring the soil with the aid of methanol from each tube to a funnel plugged with absorbent cotton and the colour intensity determined in a spectrophotometer at a wave length of 485 nm. The dehydrogenase activity was expressed as µg TPF formed/g soil/24h.

The determination of fluorescein diacetate (FDA) hydrolysis (Schnurer and Rosswall 1982) was carried out in 2 g of field moist soil, where 15 ml of 60 mM potassium phosphate buffer (pH 7.6) was added. 100 µl of substrate solution was added to samples. After 20 min of incubation, the reaction was stopped by adding 15 ml of chloroform: methanol (2: 1). After filtering the solutions (Whatman No. 42), absorbance was measured at 490 nm on a UV Visible Spectrophotometer and calculated from the standard graph of fluorescein. The FDA hydrolysis rate was expressed as µg flurescein/g/h.

Urease activity was assayed by the method of Bremner and Mulvany (1978). Ten gram of dry and sieved soil was taken in a 100 ml volumetric flask. To this 1.5 ml of toluene was added, mixed well and incubated for 15 min. Then 10 ml of 10% urea solution and 20 ml of citrate buffer were added, mixed thoroughly, stoppered and incubated for 3 h at 37^0 C. Then the volume was made up to 100 ml with distilled water, mixed by shaking immediately. The contents were filtered through Whatman No.1 filter paper and 1ml of filtrate was pipetted out into 50 ml volumetric flask. To this 9 ml of distilled water, 4 ml of phenate and 3 ml of NaOCl were added, mixed well and allowed to stand for 20 min. The volume was made up to 50 ml and mixed well. The bluish green colour developed was read at 630 nm. The concentration of urease in the sample was obtained from the standard graph using diammonium sulphate. Urease activity was expressed µg NH_4/g soil/24h.

All the data were subjected to statistical analysis with softwares, SPSS (Kirkpatrick and Feenay 2005) and Microsoft Excel for Windows 2007 add-ins with XLSTAT Version 2010.5.05 (XLSTAT, 2010). Statistically significant differences between the treatments were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5 % significance level.

RESULTS AND DISCUSSION

Alterations in microbial mineralization and nutrient cycling may control the long-term response of ecosystem to elevated CO_2, because microorganisms are regulators of decomposition, and understanding of microbial activity is so crucial. Elevated CO_2 concentration can affect extracellular enzyme activities in several ways.

In *Rabi* 2013, of the three enzymes examined, dehydrogenase, FDA and urease activities were significantly higher in soils under elevated CO_2 than in ambient. In this study, the increased air temperature in vertisols condition coupled with higher amount of C substrates through rhizodeposits geared up the rate of enzyme activity under elevated CO_2 + temperature condition. In the present study, higher activity of FDA hydrolysis rate noticed in wild oat with 26.5 (µg fluorescein/g/h) at elevated CO_2 - ambient temperature levels. Fluorescein diacetate (FDA) hydrolysis rate are the widely accepted as an accurate method for measuring total microbial activity in aerobic soils. Because they mediated simultaneously by protease, esterase and lipase and thereby reflect the activities of these enzymes in soil (Adam and Duncan 2001). Since 90% of energy flow in a system passes through microbial decomposers and heterotrophic microorganisms are predominant in soil, FDA hydrolysis is thought to reflect overall soil microbiological activity (Schnurer and Rosswall 1982). The activity of dehydrogenase is considered as an indicator of the oxidative metabolism in soils and the microbiological activity, because it is extremely intracellular and able to function within the viable cells.

Dehydrogenase activity was higher at enrichment of CO_2 for *P. minor* with 35.9 (µg TPF/g soil/24 h) followed by wild oat (34.8µg TPF/g soil/24 h). Maximum dehydrogenase activities were found at

the panicle initiation stage and it declined sharply at maturity (Sarathambal *et al.* 2015). Urease the key enzyme hydrolyses urea to ammonia and CO_2 had significantly higher in enrichment of CO_2-elevated temperature with wild oat 34.6 (µg NH_4/g soil/24 h) followed by wheat (31.5 µg NH_4/g soil/24 h) with elevated CO_2 and ambient temperature. The enzyme activities in soil were strongly influenced by exposure to elevated CO_2 as well as by the interactive effect of the elevated CO_2 and temperature throughout the plant growth stages. Moorhead and Linkins (1997) suggested that elevated CO_2 altered the soil enzyme characteristics in a tussock tundra ecosystem.

The microorganisms in soils play a key role in the responses of ecosystem to global climate changes, as they regulate the dynamics of organic matter deposition and plant nutrient availability. However, the increasing atmospheric CO_2 concentrations will indirectly influence microbial populations in soils, through increased root biomass, total rhizodeposition, chemical composition of plant tissues and root exudates probably change when CO_2 is enriched, because CO_2 concentration in soil is much greater than the atmospheric CO_2 (Yue *et al.* 2007).

Table 1. Soil enzyme activity of wheat and associated weed species under OTCs as influenced by elevated CO_2 and temperature

Level of CO_2/temperature treatment	Crop / weed	Soil enzyme activity		
		Fluorescein diacetate hydrolysis rate (µg fluorescein/g/h)	Dehydrogenase (µg TPF/g soil/24 h)	Urease (µg NH_4/g soil/24 h)
Ambient CO_2 + ambient temperature	Wheat	18.9^{bcd}	25.6^{cd}	25.9^{bcd}
	P. minor	16.5^{de}	24.3^{d}	26.5^{bcd}
	Wild oat	14.6^{ef}	27.8^{cd}	24.6^{cd}
Elevated CO_2 + ambient temperature	Wheat	21.5^{b}	26.9^{cd}	31.5^{ab}
	P. minor	20.6^{bc}	35.9^{a}	20.9^{d}
	Wild oat	26.5^{a}	34.8^{ab}	29.5^{abc}
Ambient CO_2 + elevated temperature	Wheat	15.2^{def}	22.3^{d}	25.2^{cd}
	P. minor	12.3^{f}	23.6^{d}	22.3^{d}
	Wild oat	11.5^{f}	24.5^{dg}	21.5^{d}
Elevated CO_2+ elevated temperature	Wheat	15.6^{def}	26.9^{cd}	30.6^{abc}
	P. minor	16.8^{cde}	28.7^{bcd}	21.8^{d}
	Wild oat	14.6^{ef}	31.5^{abc}	34.6^{a}

Values represent mean of three replications and values followed by the same letter in each column are not significantly different from each other as detected by DMRT (p £ 0.05)

Table 2. Soil enzyme activity of rice and associated weeds under OTCs as influenced by elevated CO_2 and temperature

Levels of CO_2/temperature treatment	Type of cultivar	Soil enzyme activity		
		Fluorescein diacetate hydrolysis rate (µg fluorescein/g/h)	Dehydrogenase (µg TPF/g soil/24 h)	Urease (µg NH_4/g soil/24 h)
Ambient CO_2 + ambient temperature	MRWR27	9.6^{ef}	26.5^{d}	39.1^{ab}
	W1R4	8.9^{efg}	27.6^{bcd}	33.9^{d}
	C9	7.6^{fg}	18.9^{g}	36.5^{c}
	EC	8.7^{efg}	32.4^{b}	36.2^{c}
Elevated CO_2 + ambient temperature	MRWR27	12.3^{d}	28.6^{bc}	39.8^{c}
	W1R4	15.6^{c}	29.8^{bc}	36.9^{c}
	C9	11.2^{de}	19.5^{g}	42.9^{ab}
	EC	18.5^{ab}	39.8^{a}	45.6^{a}
Ambient CO_2 + elevated temperature	MRWR27	6.5^{g}	25.8^{d}	23.6^{f}
	W1R4	9.6^{ef}	20.6^{g}	29.8^{e}
	C9	8.7^{efg}	22.3^{e}	32.8^{d}
	EC	6.9^{g}	18.9^{g}	33.6^{d}
Elevated CO_2 + elevated temperature	MRWR27	12.8^{d}	22.3^{e}	34.6^{d}
	W1R4	18.2^{ab}	18.9^{h}	29.5^{e}
	C9	16.4^{bc}	16.9^{i}	28.9^{e}
	EC	19.7^{a}	34.8^{b}	35.5^{d}

Values represent mean of three replications and values followed by the same letter in each column are not significantly different from each other as detected by DMRT (p £ 0.05); MRWR27-weedy rice; W1R4-wild rice; C9-cultivated rice; EC- *Echinochloa crusgalli*

Repeated soil sampling during *Kharif* 2014, revealed an enhancement of enzyme activities under elevated CO_2 levels (Table 2). Whereas among the rice associated weeds tested, *Echinochloa crusgalli* rhizosphere responds positively to elevated CO_2 and temperature. In the present study, higher FDA hydrolysis rate noticed in *E. crusgalli* with 19.8 and 18.5 (µg fluorescein/g/h) in elevated CO_2 - elevated temperature and in elevated CO_2 - ambient temperature levels respectively. Dehydrogenase activity was higher at enrichment of CO_2 for *E. crusgalli* with 39.8 (µg TPF/g soil/24 h) followed by elevated CO_2-elevated temperature 34.8 (µg TPF/g soil/24 h). Urease the key enzyme hydrolyses urea to ammonia and CO_2 had significantly higher in enrichment of CO_2-ambient temperature with *E. crusgalli* 45.6 (µg NH_4/g soil/24 h) followed by cultivated rice (C9) 42.9 (µg NH_4/g soil/24 h). Earlier studies conducted by Sardans *et al*. (2008) in soil warming experiment also observed higher enzyme activities under soil warming depending upon the seasonal variations. Similar to our findings, activities of the dehydrogenase, FDA hydrolysis, and urease increased under elevated temperature with CO_2 enrichment resulted in higher microbial activities and increased rate of C mineralization (King *et al*. 2004, Naidu and Sarathambal 2012). A further source of microbial substrates for enzymatic decomposition could be rhizodeposition derived from turnover of fine roots, root hairs and secretion of soluble root exudates; and turnover of rhizosphere-associated microbial biomass. Rhizodeposition was roughly doubled in elevated compared with the ambient CO_2 treatment over the last 4 years of the experiment (Pendall *et al*. 2004). Since rhizodeposition and newly formed roots enlarged the pool of easily available substrates mainly in the weed species like *E. crusgalli*, wild oat, *P. minor*, increased enzyme production in this soil than the crops.

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