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RESEARCH ARTICLE

Biochar Addition in Flooded Paddy Soil for Ameliorating the Effects of Elevated Carbon dioxide (eCO_2)

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ABSTRACT

Improving soil organic carbon by biochar application is of concern in flooded rice soils. However, the effect of biochar on soil physico-chemical and biological properties under increasing levels of CO_2 is limited and needs investigation. The present study examined the response of soil carbon pool, and soil enzyme activities of rice upon application of biochar under elevated carbon dioxide (eCO_2) - 550±30 µmol.mol^{-1} level. The study was carried out in open-top chambers (OTC) under a rice-rice (*Oryza sativa* L.) cropping system in 2020. A set of OTCs at ambient CO_2 (415 ± 10 µmol mol^{-1} (aCO_2)) served as a check with and without biochar application. The fourth OTC maintained eCO_2 without the application of biochar. Rhizosphere soil samples from three critical crop stages *viz.*, active tillering, flowering, and maturity were analyzed for various physico-chemical and biological properties. Most soil biological parameters, specifically soil enzymes, β – glucosidase, urease, and phosphatases, exhibited significant changes during the flowering stage in biochar applied e(CO_2) treatment. The per cent increase of 46.8 %, 30.2 %, 18.75 %, and 51.2 % over ambient CO_2 in soil organic carbon (SOC), soil microbial biomass carbon (SMBC), oxidizable carbon (OC), and water-soluble carbon, respectively, was observed. This study emphasized that biochar can improve the soil C pool and enzyme activities under anticipated climate change.

Keywords: *Elevated carbon dioxide; Paddy; C pool; Soil enzymes; Biochar*

INTRODUCTION

The global phenomenon of climate change is menacing the globe over recent decades, whichis predictable to persist. The chief cause of climate change is an increase in the atmospheric concentration of CO_2, which has been increased steadily in the past decade and anticipated to get as high as 550 µmol.mol^{-1} by 2050 (IPCC, 2018). Further, the global temperature is projected to increase by 3.2 ℃ by 2100 (Guterres and Liu, 2020) and would have unfavorable impacts, including heat waves, agricultural drought, floods, and diminished crop yields. The dependence of agriculture on climate cycles and the productivity of crops would be afflicted. The increase in atmospheric CO_2 alters the C cycling in the terrestrial ecosystem with the concurrent increase in the magnitude of C fluxes in the ecosystem.

Paddy (*Oryza sativa* L.), a prime crop across the globe for food security (Tolba *et al*., 2020), is sensitive to climatic conditions. Climate change grimly impinges on world rice harvesting countries including China, India, and Indonesia to about 52%, contributing more than 70% of global rice output. These climatic changes affect the below ground and above ground processes, including nutrient availability, uptake, photosynthetic rate, crop yield etc., As CO_2 is a significant substrate for photosynthesis, its increased concentration at the

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cellular level increases photosynthesis by enhancing carboxylation and lowering oxygenation. Numerous strategies are being adapted to cope with climate changes, such as improving breeding strategies (Fita *et al*., 2015), effective fertilizer management (Linquist *et al*., 2012), use of conservative agriculture technologies, use of functional microbes (Geetha Thanuja and Karthikeyan, 2020; Davamani *et al*., 2020). Rice straw compost or farmyard manure was reported to minimize methane emission from a paddy field and improve soil fertility and crop productivity (Khosa *et al*., 2011). Amongst them, biochar application is suggested as supply-side technology that bestows in climate change mitigation (IPCC, 2014). Biochar has been proposed as an appropriate agent for improving carbon sequestration and greenhouse gas emission reductions (Pokharel *et al*., 2018; Castaldi *et al*., 2011). Biochar application plays a pivotal role in soil biochemistry of C and promotes the stabilization of SOC. It also influences the activities of soil enzymes by harboring habitat for soil microorganisms. A significant correlation between soil enzyme activities and SMBC, SOC have been reported (Zheng *et al*., 2019).

Soil enzymes are sensors for the functioning of soil microbes and physicochemical characteristics (Sparling 1997). Being the catalyst for chemical reactions, it is also associated with the decomposition rate and turnover of the C pool. eCO_2 tends to cause a high rate of decomposition and release of nutrients facilitating microbial activities. This enhanced activity has a profound effect on soil enzymes, whose interpretation needs caution and an alternative strategy to sustain the environment. Thus the interaction of biochar with flooded paddy soil characteristics for the future climate would be an option of adaption. With this background, the study was intended to investigate the various C pools, namely, SOC, water-soluble organic carbon (WSOC) and soil microbial biomass carbon (SMBC), and soil enzymes (acid phosphatase, alkaline phosphatase, soil dehydrogenase, β-glucosidase, fluoresein diacetate hydrolysis, and urease) upon the addition of biochar under simulated CO_2 condition.

MATRIALS AND METHODS

Experimental location and climate

The study site is situated at the wetland forms of Tamil Nadu Agricultural University, (11° 005' 13" N, 76° 93' 045" E), Tamilnadu, India. The location has characteristics of sub-tropical climate with a mean precipitation of 710 mm. The maximum and minimum temperature of the study site is 36.7°C and 17.9°C.

Open top chambers

UV protected open-top chambers (OTC) (diameter 5m and height 4.3m) (M/s. Genesis technologies, India) made up of multi-layered polycarbonate sheets were used for CO_2 enriched experiments. SCADA (Supervisory Control And Data Acquisition) software provided with a data logger has been installed to obtain information on the temperature, relative humidity, and CO_2 level in each OTC. The system exhibited and record the actual and required CO_2 level in each OTC. A feedback control loop passing through programmable logic controllers monitored the said parameters. The CO_2 was purged into the OTC, through perforated polyvinyl tubes based on sensor data.

Crop management and treatments

The field experiment was conducted under a rice-rice cropping system during *rabi* season 2020. The land was prepared thoroughly with a tractor-drawn rotovator (single tillage) and flooded 2-3 days before transplanting. Rice seedlings (var CO51) of 20 d old have been transplanted at a spacing of 25 x 25 cm with one seedling per hill. The crop was raised following recommended agronomic practices and fertilizers as per Crop Production Guide 2020 (Directorate of Agriculture). Every experimental plot received 150: 50:50 Kg NPK.ha^{-1}. N and K have been applied in the form of urea and muriate of phosphate in three equal splits viz, basal tillering, panicle initiation, and heading stages. The treatments were distributed in a complete randomized block design with three replications. The application of biochar was beased on the NAS recommended application rates for ricecultivation (NAS, 2010).

T_1- Chamber with ambient CO_2 (aCO_2)

T_2- Chamber with ambient CO_2 + biochar (1 tonne.ha^{-1}) (aCO_2 + B)

T_3- Chamber with elevated CO_2 (eCO_2)

T_4- Chamber with elevated CO_2 + biochar (1 tonne.ha^{-1}) (eCO_2 + B)

The concentration of CO_2 in ambient and elevated was 415 ± 10 µmol mol^{-1} and 550 µmol mol^{-1} respectively, which was maintained throughout the cropping season.

Biochar characteristics

The biochar used in the study was prepared from paddy straw feedstock at a pyrolysis temperature of approximately 350-400 °C. The quantity of C, H , N and S in the biochar were 52.2%, 3.9%, 1.8% and 0.3%, respectively.

Soil sampling

The properties of the soil prior to the treatment were analyzed and are given in the table. 1. For the collection of rhizosphere soil, the plants were uprooted carefully and the soil adhering to the roots (rhizosphere soil) was collected at three critical stages viz, active tillering, flowering and maturity, with three replications for each treatment. The fresh samples collected were divided into two parts. One part of the sample was stored at 4 ℃ for biochemical analysis, while another part was air-dried, sieved through 2 mm mesh, and stored in closed plastic containers for physico-chemical analysis.

Table 1. Properties of experimental soil

Soil properties	Value
Sand (%)	30.5
Silt (%)	32.7
Clay (%)	31.4
Bulk density (g cm^{-3})	1.17
pH	8.5
EC(dSm^{-1})	0.63
Available N (kg ha^{-1})	240.62
Available P (kg ha^{-1})	28.2
Available K (kg ha^{-1})	282.33

Analysis of soil nutrients

Alkaline permanganate method is followed for the estimation of nitrogen content and expressed as kg ha^{-1} (Subbaiah and Asija, 1956). The available phosphorus content is estimated by using Olsen's extractant method (Olsen, 1954). Soil sample is extracted with neutral normal ammonium acetate, pH 7.0, and the available potassium in the filtrate is analyzed using a flame photometer (Stanford and English, 1949) and the values expressed in kg ha^{-1}.

Analysis of soil enzymes

The colorimetric method is used to assess the activity of soil dehydrogenase using the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) to 2,3,5-tetraphenylformazon (TPF) as given by (Casida *et al.*, 1964). β- glucosidase activity of the soil is examined by the method of Eivazi and Tabatabai, (1988) as an indicator of C-substrate utilization. Fluorescein diacetate (FDA) hydrolysis is carried out in field moist soil as described (Schnürer and Rosswall, 1982). Urease activity is determined by the method given by Tabatabai and Bremner (1972). Soil acid and alkaline phosphatase are analyzed using p-nitrophenyl phosphate disodium as the substrate (Tabatabai and Bremner, 1969).

Analysis of soil C pools

The organic carbon present in the soil sample is analyzed by the wet chromic acid digestion method (Walkley and Black, 1934). Water-soluble and oxidizable carbon was estimated by the methods given by Haynes and Swift (1990) and Blair *et al.* (1995), respectively. Biomass carbon is determined by the modified fumigation-incubation technique. Biomass C is calculated by using the below formula:

$$MBC\ (\mu g/g) = (F_c\text{-}UF_c) / K_c$$

Where, F_c – CO_2 flush from fumigated soil; UF_c - CO_2 produced by the control soil; K_c - 0.45 (Proportion of microbial C evolved as CO_2).

Statistical analysis

The datasets were analyzed for analysis of variance and separated by Duncan's Multiple Range test (DMRT) at 0.05 level of probability using statistical software SPSS 16.0 (Statistical software for social science) and to analyze the simple correlation between soil enzymes and carbon pool, Pearson correlation (r) was applied.

RESULTS AND DISCUSSION

Carbon is the prime nutrient in determining paddy growth and productivity. In contrast with croplands, paddy soils hold an imperative C pool comprising more than 20% SOC per hectare. However, the paddy SOC stocks are resolved by natural factors and agronomical management (Liu *et al.*, 2021). Application of biochar in our significantly influenced paddy soil carbon pool and the effect was higher at elevated CO_2. The order of SOC in increasing trend was observed as aCO_2 < aCO_2 +B < eCO_2< eCO_2 + B (Fig 1A), however aCO_2 +B and eCO_2 were on par.

Under elevated CO_2, biochar application has significantly increased SOC content by 46.8%. The same increasing pattern was observed on SMBC (Fig 1B). The microbial biomass C ranged from 150-217 mg kg^{-1}. The oxidizable C and water-soluble C recorded maximum in biochar applied plot under eCO_2 followed by aCO_2. (Fig 1 C and D). aCO_2 recorded the lowest in both OC and WSC. The significant difference at the flowering stage was displayed by SMBC and WSC, while the significance was par in SOC and OC. However, the flowering stage showed higher values than active tillering and

maturity. Studies by Bhattacharya *et al*. (2012) have shown to increase the carbon storage in paddy soil upon application of rice straw along with urea. The changes in soil properties including SOC, SMBC upon biochar addition in the paddy investigated here, were in accordance with studies in non-paddy soil (Jin, 2010; Jones *et al*., 2011). Generally, the response of soil biological properties as affected by biochar application and the initial soil properties. The increase in MBC in this study is in analogy to that in other rice paddies across sites in south China (Chen *et al*., 2015). However, submerged soil witnesses slower decomposition of organic matter than aerobic soil, which remains in flooded soil for years. Labile carbon pools namely SMBC, WSC, OC, readily mineralizable C and SOC were reported to increase upon exposure to eCO_2 (Padhy *et al*., 2020), analogous to our study.

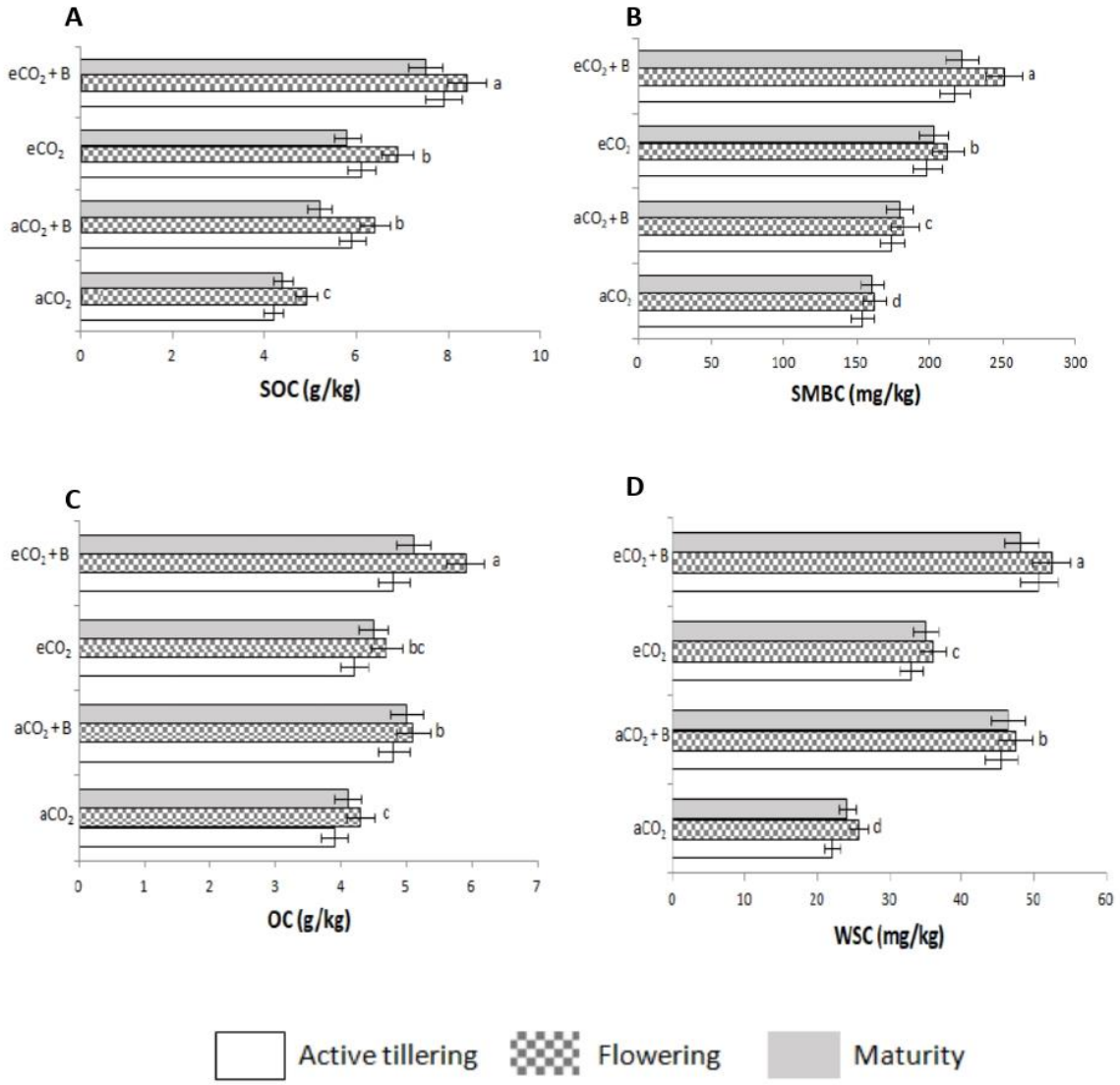

Figure 1. Impact of CO_2 and biochar on changes of soil carbon pools at different growth stages. Data represent mean (n=3) and error bars indicate the standard error. For each panel, different letters denote the significant difference within the treatments at P<0.05 to DMRT. SOC-soil organic carbon; SMBC- Soil Microbial biomass Carbon; OC-Oxidizable carbon; WSC-Water-Soluble Carbon.

Soil enzyme activity

Yuan *et al*., (2006) described the effect of eCO_2 in enhanced soil enzyme activities due to CO_2-induced carbon entering the soil. The assayed enzymes were correlated with the organic carbon of the soil since these parameters tend to increase considerably upon increasing returns of organic residues. In analogy, Graham and Haynes, (2005) reported the significant correlation between organic carbon content and soil enzymes. Paddy soil under elevated CO_2 coupled with biochar application has significantly influenced soil enzyme activities (Fig 2A-F). In the present study, maximum dehydrogenase (33.3 µg TPF g^{-1} soil day^{-1}), β – glucosidase (61.3 µg pNP g^{-1}), FDA (56.3 µg fluorescein $g^{-1}h^{-1}$), acid phosphatase (42.5 µg pNP g^{-1}), alkaline phosphatase (46.3 µg pNP g^{-1}) and urease (226.8 µg $g^{-1}h^{-1}$) activity was evidenced in plots applied with biochar under eCO_2. SOC serves as a substrate for several soil enzymes and protects from the complex formation with clay and humus (Tatabai, 1994). A significant increase in dehydrogenase was observed in biochar applied soil under elevated conditions. Alef and Nannipieri, (1995) reported an increase in soil dehydrogenase activity due to increased bacterial population and activity since they are correlated with active cells and linked to labile organic matter.

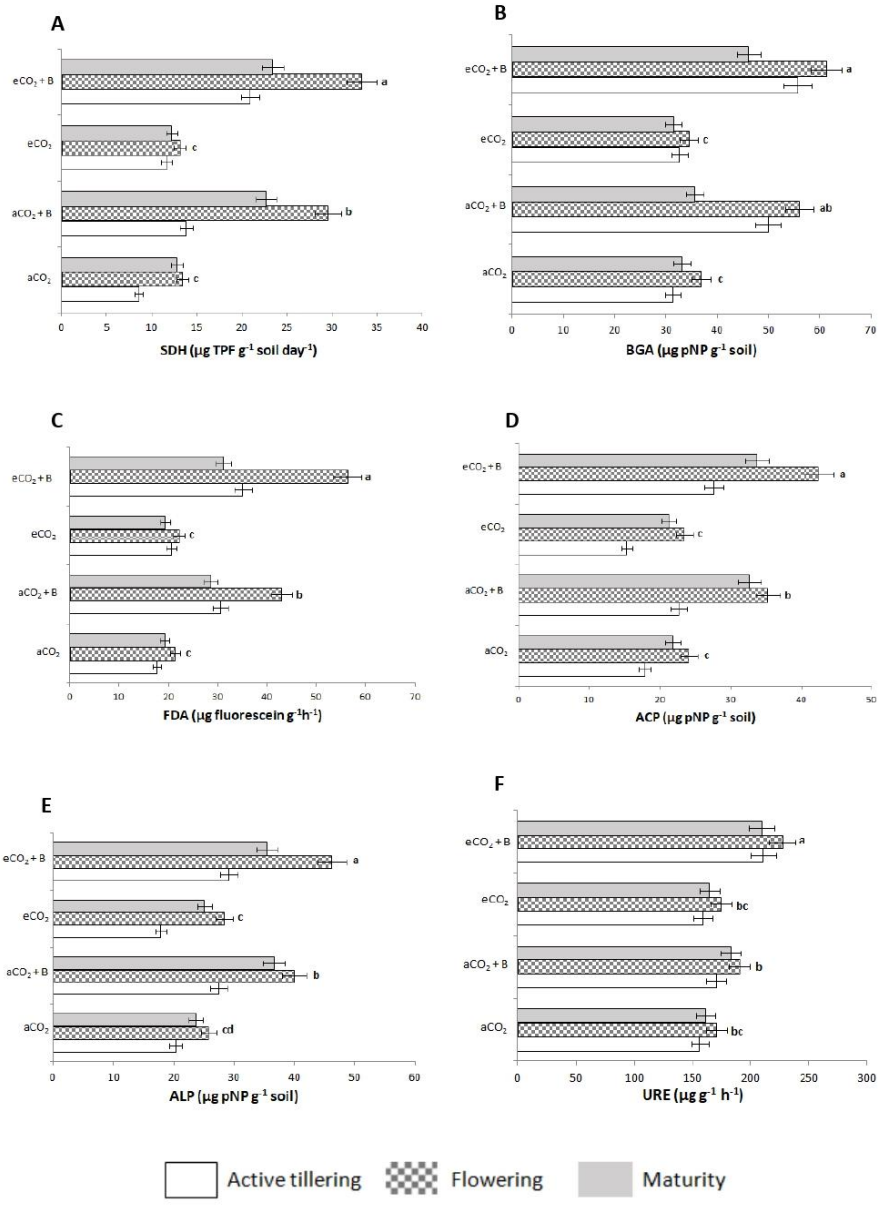

Figure 2. Impact of CO_2 and biochar on changes in soil enzyme activities at different growth stages. Data represent mean (n=3) and error bars indicate the standard error. For each panel, different letters denote the significant difference within the treatments at P<0.05 to DMRT. (SDH- Soil dehydrogenase; BGA-β glucosidase; FDA-Fluorescein diacetate hydrolysis; ACP-Acid Phosphatase; ALP- Alkaline phosphatase, URE-Urease).

The soil dehydrogenase activity was significantly differentfrom biochar applied aCO_2 and eCO_2, while the difference was not noticed in β – glucosidase. Chen *et al*. (2015) have also reported an increase in acid and alkaline phosphatase with the addition of biochar which corresponds to the current results. Higher root biomass and exudates under eCO_2 enhance the availability of soil P, wherein P is linked to SOM by ester bonds which is made available by the action of phosphatase. In the current study, carbon input by means of biochar and eCO_2 may have increased phosphatase activity. Of all the observed rice crop stages, the flowering stage was significantly different from the maturity and active tillering stage due to biochar application. However, no significant difference was observable between active tillering and maturity, specifically on eCO_2 + B.

The soil C pool and enzyme activity of flooded paddy soil were significantly correlated (Table.2). A correlation study revealed a highly significant ($p<0.01$) relationship between acid phosphatase with alkaline phosphatase (r=0.98**), soil organic carbon with water soluble carbon (r=0.88**), and soil dehydrogenase (r=0.87**). The least correlation was observed in fluorescein diacetate hydrolysis (r= 0.19) with acid phosphatase.

CONCLUSION

The elevated CO_2 has a positive effect on soil carbon pool, and enzyme activities. However, it could be enhanced with the application of biochar under anticipated climate change in the future. Further studies should focus on the application rate and the type of biochar feedstock used in preparation. The biochar application rate and the type may indirectly influence the soil microbial activity and related biological properties. The study evidently illustrated the potential of biochar to be employed to sustain rice productivity. On the other hand, further investigation is suggested to study the interactive long-term effect of biochar and eCO_2 on the projected climate change and agricultural needs.

Ethics statement

No human or animal subjects were involved in this research.

Originality and plagiarism

Authors ensure the submission of original works.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required.

Author contributions

Idea conceptualization-SK, Experiments- KGT , Guidance –SK,DB,SM,KC. Writing original draft – KGT, Writing- reviewing &editing - SK

REFERENCES

Alef, K., & Nannipieri, P. (1995). *Methods in applied soil microbiology and biochemistry* (No. 631.46 M592ma). Academic Press,.

Bhattacharyya, P., K.S. Roy, S. Neogi, T.K. Adhya, K.S. Rao, and M.C. Manna. 2012. "Effects of rice straw and nitrogen fertilization on greenhouse gas emissions and carbon storage in tropical flooded soil planted with rice. *Soil. Tillage Res.,* **124**:119-130.

Blair, G., R. Lefroy, and L. Lisle. 1995. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. *Aust. J. Agric. Res.*, **46 (7):**1459-1466.

Casida LEJ, Klein DA, Santoro T (1964) Soil dehydrogenase activity. *Soil Sci.,* **98(6)**: 371-376.

Table 2. Correlation matrix between soil enzymes and carbon pool

	ACP	ALP	BGA	SDH	FDA	URE	SOC	MBC	OC	WSC
ACP	1									
ALP	0.98**	1								
BGA	0.42*	0.43*	1							
SDH	0.37**	0.41**	0.82**	1						
FDA	0.19	0.26	0.73**	0.85**	1					
URE	0.67**	0.67**	0.79**	0.72**	0.57**	1				
SOC	0.32*	0.38**	0.81**	0.87**	0.84**	0.60**	1			
MBC	0.57**	0.60**	0.67**	0.74**	0.76**	0.73**	0.67**	1		
OC	0.50**	0.53**	0.61**	0.75**	0.68**	0.72**	0.72**	0.75**	1	
WSC	0.33*	0.38*	0.76**	0.88**	0.80**	0.62**	0.88**	0.73**	0.75**	1

Castaldi, S., M. Riondino, S. Baronti, F.R. Esposito, R. Marzaioli, F.A. Rutigliano, F.P. Vaccari, and F. Miglietta. 2011. Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. *Chemosphere.,* **85 (9)**:1464-1471.

Chen, J., X. Liu, L. Li, J. Zheng, J. Qu, J. Zheng, X. Zhang, and G. Pan. 2015. Consistent increase in abundance and diversity but variable change in community composition of bacteria in topsoil of rice paddy under short term biochar treatment across three sites from South China. *Appl. Soil Ecol.,* **91**:68-79.

Davamani, V., E. Parameswari, and S. Arulmani. 2020. Mitigation of methane gas emissions in flooded paddy soil through the utilization of methanotrophs. *Sci. Total Environ.,* **726**:138570.

Eivazi F, Tabatabai MA (1988). Glucosidases and galactosidases in soils. *Soil Biol. Biochem.,* **20(5):** 601-606.

Fita, A., A. Rodríguez-Burruezo, M. Boscaiu, J. Prohens, and O. Vicente. 2015. Breeding and Domesticating Crops Adapted to Drought and Salinity: A New Paradigm for Increasing Food Production. *Front. Plant Sci.*, **6**.

Geetha Thanuja, K., and S. Karthikeyan. 2020. Exploring bio-mitigation strategies to reduce carbon footprint in wetland paddy system. *Bioresour. Technol. Rep.,* **12**:100557.

Graham, M. H., & Haynes, R. J. (2005). Organic matter accumulation and fertilizer-induced acidification interact to affect soil microbial and enzyme activity on a long-term sugarcane management experiment. *Biol. Fertil. Soils.*, **41(4)**: 249-256.

Guterres, A., and Z. Liu. 2020. The Sustainable Development Goals Report 2020. *United Nations Intergovermental Organization: New York, NY, USA*.

Haynes, R.J., and R.S. Swift. 1990. Stability of soil aggregates in relation to organic constituents and soil water content. *J Soil Sci.*, **41** (1):73-83.

IPCC. 2014. *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Edited by Rajendra K. Pachauri et al. Geneva, Switzerland: IPCC.

IPCC. 2018. *Global Warming of 1.5 °C: An IPCC Special Report on the impacts of global warming of 1.5° C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*. Edited by Valérie Masson-Delmotte et al.

Jin, H. 2010. Characterization of microbial life colonizing biochar and biochar-amended soils. PhD Doctoral Dissertation, Graduate School of Cornell University, Cornell University.

Jones, D.L., D.V. Murphy, M. Khalid, W. Ahmad, G. Edwards-Jones, and T.H. DeLuca. 2011. Short-term biochar-induced increase in soil CO_2 release is both biotically and abiotically mediated. *Soil Biol. Biochem.*, 43 **(8):**1723-1731.

Khosa, M.K., B.S. Sidhu, and D.K. Benbi. 2011. Methane emission from rice fields in relation to management of irrigation water. *J. Environ. Biol.*, **32 (2)**:169-172.

Linquist, B.A., M.A. Adviento-Borbe, C.M. Pittelkow, C. van Kessel, and K.J. van Groenigen. 2012. Fertilizer management practices and greenhouse gas emissions from rice systems: A quantitative review and analysis. *Field Crops Res.*, **135**:10-21. doi: https://doi.org/10.1016/j.fcr.2012.06.007.

Liu, Y., T. Ge, K.J. van Groenigen, Y. Yang, P. Wang, K. Cheng, Z. Zhu, J. Wang, Y. Li, G. Guggenberger, J. Sardans, J. Penuelas, J. Wu, and Y. Kuzyakov. 2021. Rice paddy soils are a quantitatively important carbon store according to a global synthesis. *Commun. Earth. Environ.*, **2 (1)**:154.

NAS, 2010. Recommended Application Amounts of Fertilizers for Crop Cultivation. National Institute of Agricultural Sciences, Rural Development Administration, p. pp16.

Olsen SR (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate: US Department of Agriculture.

Padhy, S. R., Bhattacharyya, P., Dash, P. K., Roy, K. S., Neogi, S., Baig, M. J., ... & Mohapatra, T. (2020). Enhanced labile carbon flow in soil-microbes-plant-atmospheric continuum in rice under elevated CO_2 and temperature leads to positive climate change feed-back. *Appl. Soil Ecol.*, *155*, 103657.

Pokharel, P., J.-H. Kwak, Y.S. Ok, and S.X. Chang. 2018. Pine sawdust biochar reduces GHG emission by decreasing microbial and enzyme activities in forest and grassland soils in a laboratory experiment. *Sci. Total Environ.*, **625**:1247-1256.

Schnürer J, Rosswall T (1982) Fluorescein Diacetate Hydrolysis as a Measure of Total Microbial Activity in Soil and Litter. *Appl. Environ. Microbiol.*, **43(6)**: 1256-1261.

Sparling, G. 1997. Soil microbial biomass, activity and nutrient cycling as indicators of soil health." In *Biological indicators of soil health*, 97-119. CAB International.

Stanford G, English L (1949) Use of the Flame Photometer in Rapid Soil Tests for K and Ca. *Agron J.,* **41(9)**, 446-447.

Subbaiah B, Asija G (1956) A rapid procedure for estimation of available nitrogen in soil. *Curr Sci.*, **25**, 259-260

Tabatabai, M.A. 1994. Soil Enzymes. In *Methods of Soil Analysis*, 775-833.

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil. Biol. Biochem**1***(4)**: 301-307.

Tabatabai, MA, Bremner JM (1972) Assay of urease activity in soils. *Soil. Biol. Biochem.*, **4(4),** 479-487.

Tolba, R.A., M.A. El-Shirbeny, S.M. Abou-Shleel, and M.A. El-Mohandes. 2020. Rice Acreage Delineation in the Nile Delta Based on Thermal Signature. *Earth Syst and Environ* **4 (1)**:287-296.

Walkley A, Black I (1934). Chromic acid titration for determination of soil organic matter. *Soil Sci.*, **63**, 251

Zheng, Y., X. Han, Y. Li, J. Yang, N. Li, and N. An. 2019. Effects of Biochar and Straw Application on the Physicochemical and Biological Properties of Paddy Soils in Northeast China. *Sci Rep.*, **9 (1):**16531.