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Influence of Soil Amendment on Iron Reduction in Paddy Field and Crop Improvement

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Abstract: In the present investigation, laboratory incubation experiment and field trials were done to assess the effect of organic soil amendment including acetate, rice straw and charcoal at different levels on temporal changes of microbially reducible iron, evaluate plant root oxidase activity and crop performance in both simulated and field conditions. The contents of reduced iron in alluvial soil supplemented with acetate were in the range of 3.5 to 23 μmol g^{-1} soil as compared to the laterite soil. The addition of rice straw stimulated the process of iron reduction, as evident from the increased contents of reduced Fe(II) till 14 days of incubation. However, application of charcoal along with glucose showed inhibitory effect on Fe(II) production. Extensive field trial studies suggested that control plants and addition of charcoal at varying levels led to concomitant decreases in the iron reduction potential and α-naphthylamine oxidase activities. The crop performance was evaluated by grain yield of rice plants. Results clearly showed high yield content and harvest index when amended with 10 Mg ha^{-1} charcoal, highlighting that organic amendment significantly improve plant nutrient uptake and its availability by reducing sorption and leaching activity.

Key words: Soil amendment, rice straw, charcoal - Fe(III) reduction, crop yield.

Introduction

Agricultural productivity decline in tropical climates are often attributed to poor soil fertility and agronomic practices. Though chemical fertilizer application has been extensively limited, the improvement for crop production are adversely affected due to abiotic stresses including, top soil erosion; soil acidity; salinity; decrease of soil organic matter; depletion of macro and micronutrients and deteriorating soil physical properties [23, 16].

Rice (*Oryza sativa* L.) is considered as a major staple food grain for nearly two-third of the global population [24]. As compared to other crops, the high nutritional and calorie content makes rice a major commodity across the globe, including tropical countries like India. However, either due to poor management, nutrient deficiency or toxicity, yield production reduces drastically in about 100 million hectares of land subjected to rice cultivation [1]. Although, several soil applications including biochar and compost have been proposed as soil amendments to increase soil organic carbon (SOC) levels and soil fertility, application of compost including rice straw may influence increase of pH, SOC and available micronutrients in paddy soil.

The addition of different substrates rich in carbon content usually enhance microbial activities and contribute in improving the fertility of soils.

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As charcoal forms a significant proportion of organic carbon content of many soils, its unique ability to adsorb metal ions and organic compounds has been extensively used in water treatment processes for the recovery of metals and the removal of organic wastes and micro-pollutants [4]. The strongly adsorptive nature of activated or artificial charcoal is due to its large surface area as well as its high porosity and reactive chemical structure. The immobilization of metals through their interaction with natural charcoal may be a significant sink for trace elements in soils. Studies have suggested that, the combined application of compost and biochar in maize crop had a synergistic positive effect on nutrient content, soil bulk density and water holding capacity of the soil under field conditions [3,11]. Generally, there is no adequate information, on the interaction effects of charcoal and associated amendments rich in carbon content on iron dynamics and rice cultivation in agricultural soils. Therefore, the objectives of the present study was to assess the effect of different amendments including acetate; rice straw and charcoal at different levels on kinetics of iron reduction; evaluate associated factors including plant root oxidase activity and crop performance in both laboratory and field conditions.

Materials and methods

Experimental site and soil amendment

The experiment was conducted in field plots at Central Rice Research Institute, Cuttack during wet season (June-November, 2012). The soil at the farm site was a Typic Haplaquept (deltaic alluvium). After the field was flooded, ploughed, puddle thoroughly and leveled, rice plants (30-day-old seedlings of *cv Naveen*) were transplanted in early June, 2012 in a randomized block design in field plots (8.8 m x 2.2 m) separated by levees. The fertilizers were applied at the rates of 60:40:40 ($N:P_2O_5:K_2O$) kg ha^{-1} and the plots were continuously submerged to a depth of 5 ± 2 cm during crop growth. The bio-wastes from the dairy of institute farm were composted to obtain composted manure and applied as 5 Mg ha^{-1}. In addition, charcoal amendment was done at rates of 1 Mg ha^{-1}, 2 Mg ha^{-1}, 10 Mg ha^{-1}. In a follow-up experiment, effect of different carbon rich substrate was examined using alluvial soil under laboratory incubation.

Study of selected physico-chemical properties of soils

The physico-chemical characteristics of the soil samples used in the present investigation, as highlighted in Table 1 were determined using the following methods: Soil pH was determined in 1:1.25 soil to water suspension using glass electrode pH meter [7]. The maximum water holding capacity (WHC) of soils was determined by the Keen-Raczkowski method [14]. Electrical conductivity (EC) was determined in supernatant of 1:1.25 soil to water suspension using conductivity bridge [7]. The organic carbon content of the soil samples was estimated by the Walkley-Black's wet-oxidation method [7].

Effect of acetate on microbial iron reduction in soils

The soil samples in 20 g portions were placed in incubation bottles, mixed with sterile aqueous solution of acetic acid (CH_3COOH) at 50 mM, 100 mM, 200 mM and 250 mM CH_3COO at 1:1.25 soil to solution ratio, and then, incubated under anaerobic condition at 30 ± 2°C. At an interval of 2 day, the contents in triplicate samples were mixed thoroughly, and the Fe(II) content was estimated colorimetrically using ferrozine [19].

Effect of addition of rice straw on iron reduction in soils

The soil samples were placed in 20 g portions in incubation bottles, mixed with air dried, powdered rice straw at 0.05 %, 0.25 %, 0.5 % and 1 % and incubated under flooded condition. The Fe(II) iron content was analyzed at an interval of 2 days using ferrozine [19].

Effect of addition of charcoal on microbial iron reduction in soils

A laboratory incubation study was conducted to assess the effect of charcoal amendment on microbial iron reduction in alluvial and laterite soil. Samples in 20 g portions were placed in incubation bottles, amended with charcoal at varying rates (1 Mg ha^{-1}, 2 Mg ha^{-1}, and 10 Mg ha^{-1}) at 1:1.25

Table 1. Physico-chemical properties of soil sample used in the present study

Soil		pH	EC ($ds\ m^{-1}$)	Organic carbon (%)	Maximum water holding capacity (%)	Total N (%)	CEC [c mol (+) kg^{-1}]	Soil fractions (%) Clay	Silt	Sand
Alluvial-CRRI	(A-CRRI)	6.62	0.79	1.12	43.70	0.07	18.00	32.00	27.00	41.00
Laterite-Bhubaneswar	(L-BBSR)	5.89	0.82	0.71	32.45	0.06	11.63	9.00	11.20	79.80

soil to water ratio and incubated under anaerobic condition at 30 ± 2°C. A control experiment was also set up without charcoal amendment. At an interval of 2 days, the contents in triplicate samples were mixed thoroughly and reduced Fe(II) iron content was estimated colorimeterically in a spectrophotometer (Specord 200, Analytika Jena).

Study of iron reduction kinetics in roots of rice cultivar as influenced by charcoal amendment under field conditions

At a periodic interval of 10 days, plant sample was uprooted mechanically, washed thoroughly using deionized water, blotted dry and cut uniformly of 1 cm size. Root samples were then treated with 25 ml portions of basal nutrient solution (BNS) comprising of NH_4NO_3 (5 mM), K_2SO_4 (2 mM), $CaCl_2$ (4 mM), $MgSO_4.7H_2O$ (1.5 mM), KH_2PO_4 (1.3 mM), Fe(III)-EDTA (100 µM), H_3BO_4 (10 µM), $ZnSO_4.7H_2O$ (1 µM), $CuSO_4.5H_2O$ (1 µM), $MnSO_4.H_2O$ (5 µM), $Na_2MoO_4.\ 2H_2O$ (0.5 µM) and $CoSO_4.7H_2O$ (0.2 µM). The sample was incubated for 2 hours under continuous shaking, followed by centrifugation at 3000 rpm (REMI RM12C microcentrifuge) for 5 min. An aliquot of 200 µl was reacted with 0.1 % ferrozine reagent (wt/v), and absorbance was recorded at 562 nm (Spectronic 20D+, Spectronic Inc., USA). Root samples were kept in an oven to obtain dry weight, and used in calculation for determining the mean concentration value.

Study of α-naphthylamine-oxidase activity in roots of rice cultivar as influenced by charcoal amendment in soil under field conditions

The oxidizing of power of rice roots in a field study was determined by using α-naphthylamine (α NA) as described by Ota [13]. Portions of 1 g thoroughly washed root samples was added to 100 ml incubation bottles containing 25 ml of α-naphthylamine solution (20 ppm) and shaken in an orbital shaker at 125 rpm for 2 hours. An aliquot of 2 ml was treated with equal volume of sulphanilic acid (1 %) and $NaNO_2$ (1 M). The sample was left for the development of color and absorbance was recorded at 545 nm using a spectrophotometer. The α-naphthylamine oxidase activity was expressed as µg α-naphthylamine oxidized g^{-1} roots (d.w).

Estimation of plant biomass

The estimation of plant biomass (fresh and dry weight) from field plots was done by drying the plant portions in a hot air oven at 70°C for 2-3 days. The mean of all values was reported as t ha^{-1} based on spacing of hills.

Estimation of yield parameters

In field experiments, grain and straw yields were estimated during post mature and harvesting stage. All samples were sundried and recorded as t ha^{-1}. The harvest index was calculated by using the formula:

Harvest index (%) = [(Grain wt.) / (Grain wt + Straw wt)] x 100

Statistical analyses

All analyses were carried out on basis of three replicates. The data were analyzed statistically using analysis of variance (ANOVA) procedure. Duncan's new multiple range test (DMRT) was employed to assess the differences between the treatment means. The treatment effects were declared as significant at 5 % probability levels.

Result and discussion

Changes in concentration of reducible soil iron as influenced by acetate amendment

In anoxic rice soils, acetate is a major fermentation product, and the disappearance of added acetate and the product of $^{14}CO_2$ from [2-^{14}C] acetate are associated with the accumulation of Fe(II) [8]. The effect of acetate on microbial iron reduction was evaluated using alluvial and laterite soil. Acetate was added at 100 mM and 250 mM and the changes in the contents of reduced iron were comparatively significant. In alluvial soil, additions of acetate at 100 mM increased the contents of reduced iron and were more pronounced till 20 day of incubation, while at higher concentration it reduced significantly and was comparable with both 100 mM and control samples (Fig 1A). The contents was in the range of 3.5 to 23 µmol g^{-1} soil as compared to laterite soil, the addition apparently depressed the availability of reduced [Fe(II)] (Fig 1B). The obtained results clearly correlate that available Fe (II) accumulation in soil do not exceed as more as 100 µmol g^{-1} soil as studied earlier by Nealson and Myers [12].

Microbially reducible iron content in soil as affected by incorporation of rice straw

Rice among the world's major food crops has the widest adaptability to soil and hydrological conditions. Because of its semiaquatic nature, rice grows in totally or temporarily waterlogged soils. After harvest, rice straw is used as a source of organic matter for soil amendment. In the present investigation, rice straw at 1 % (w/w) level was used, and iron reduction was monitored regularly. In alluvial soil, addition of rice straw did not in-

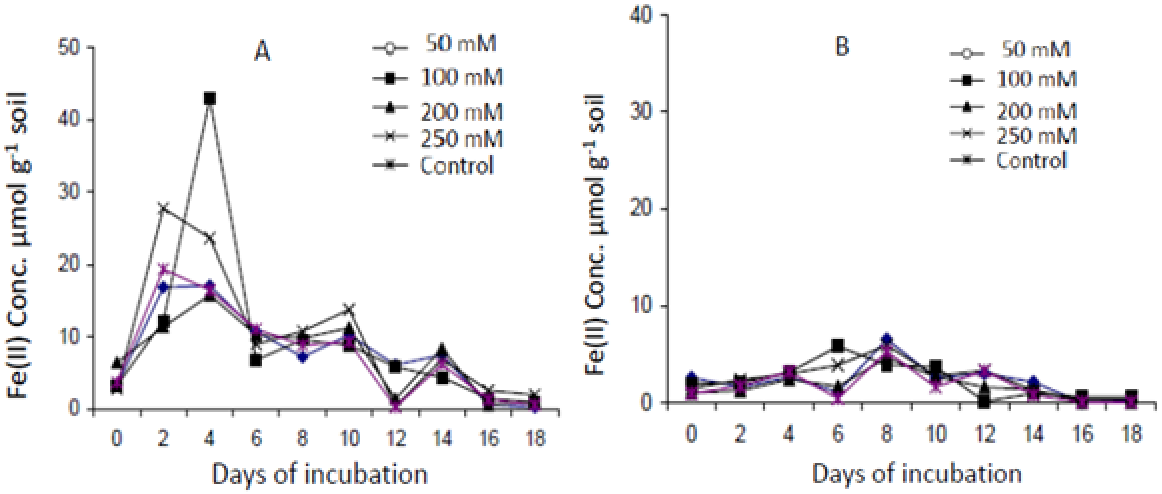

Fig. 1. Concentration of reduced iron expressed as µmol g^{-1} soil (A-alluvial, B-laterite) at different days of incubation amended with acetate (Control, 50 mM, 100 mM, 200 mM and 250 mM)

crease the dissolved iron contents, although it stimulated the process of iron reduction, as evident from the increased contents of reduced [Fe(II)] till 32 days of incubation (Fig 2A), whereas in the laterite soil the peak concentration was observed on 28 day of incubation (Fig 2B). The contents of reduced iron in all the samples ranged between 1.5 to 18.5 µmol g^{-1}soil. According to Tadano and Yoshida [20], acetic acid is the dominant organic acid produced in the early stage of decomposition processes, whereas Kyuma [9] highlighted the oxidation of acetic acid occur simultaneously with the reduction of Fe(III). In addition, earlier reports have also clearly demonstrated the role of heterotrophic microorganisms and reduction processes involving the use of carbon sources as potential electron donor.

Influence of charcoal amendment in soil iron reduction

The addition of charcoal to alluvial soil led to non-significant decreases in reduced iron [Fe(II)] concentrations till 12 days of incubation (Table 2). Extended incubation of soil samples amended with higher amounts of charcoal, especially at 2 Mg ha^{-1}and 10 Mg ha^{-1}, inhibited the production

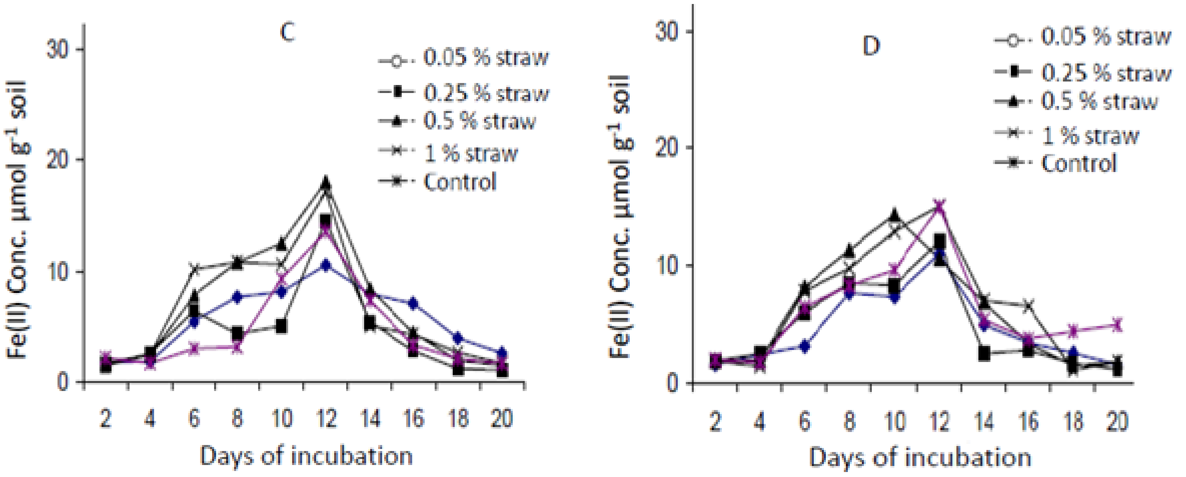

Fig. 2. Concentration of reduced iron expressed in µmol g^{-1} soil (C-alluvial, D-laterite) at different days of incubation amended with rice straw (0.05 %, 0.25 %, 0.5 % and 1 %)

Table 2. Effect of charcoal amendment on iron reduction (expressed as µmol Fe(II) produced g^{-1} soil) in an alluvial soil (A-CRRI) under laboratory conditions

Treatment	Days after transplanting									
	2	4	6	8	10	12	14	16	18	20
Control	0.79^{a}	1.35^{a}	1.72^{a}	2.34^{ab}	2.63^{bc}	3.56^{bc}	2.60^{b}	2.58^{c}	1.54^{b}	0.87^{c}
^{x}+ 1 Mg ha^{-1}	0.51^{a}	1.05^{a}	1.47^{a}	1.65^{ab}	1.76^{bc}	1.93^{c}	2.70^{bc}	2.08^{bc}	1.54^{b}	0.59^{b}
^{y}+ 2 Mg ha^{-1}	0.37^{a}	1.07^{a}	1.39^{a}	1.90^{b}	1.72^{ab}	1.63^{bc}	1.00^{a}	0.96^{c}	1.00^{ab}	0.46^{d}
^{z}+ 10 Mg ha^{-1}	0.34^{a}	1.03^{a}	1.60^{b}	1.81^{ab}	1.95^{bc}	1.37^{bc}	0.81^{c}	0.91^{c}	0.71^{ab}	0.43^{b}

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT (Duncan's Multiple Range Test) *

^{x} Charcoal was added at 2 mg g^{-1} soil to represent 1 Mg ha-^{1}

^{y} Charcoal was added at 20 mg g^{-1} soil to represent 2 Mg ha^{-1}

^{z} Charcoal was added at 200 mg g^{-1} soil to represent 10 Mg ha^{-1}

of reduced iron [Fe(II)]. Considerable decrease in reduced iron concentration was observed only after 12 days of incubation. A similar trend of charcoal inhibitory effects was also observed in the laterite soil (Table 3). The amount of Fe(II) production was not completely inhibited by the amendment process, at least during the initial period after mixing with charcoal. Significant decreases in reduced iron [Fe(II)] concentration were observed only after a period of 14 days. In a follow-up experiment, the effects of glucose in combination with charcoal and humic acid on Fe(II) production were studied. Application of charcoal along with glucose showed inhibitory effect on Fe(II) production (Table 4). In the field trial experiment, the effects of charcoal additions to the alluvial soil planted with rice (cv. *Naveen*) were studied. When analyzed for the iron reduction potential of these plants, there were significant differences due to the charcoal application (Table 5). Charcoal application led to significant decreases in the iron reduction potential of rice plants and these potentials were dependent on the amounts of charcoal added. It was quite evident that higher the addition of charcoal in the field plots, lesser was the reduction potential of roots. Extensive studies have shown that the occurrence of excess Fe (II) in soil solution often result in iron toxicity problems in paddy fields [17, 18, 2]. Thus, large concentrations of ferrous Fe in soil solution

Table 3. Effect of charcoal amendment on iron reduction (expressed as µmol Fe(II) produced g^{-1} soil) in a laterite soil (L-BBSR) under laboratory conditions

Treatment	Days after transplanting									
	2	4	6	8	10	12	14	16	18	20
Control	7.21^{a}	8.07^{a}	1.72^{a}	2.34^{ab}	2.63^{bc}	3.56^{bc}	2.60^{b}	2.58^{c}	1.54^{b}	0.87^{c}
^{a}+ 1 Mg ha^{-1}	3.33^{a}	4.05^{a}	1.47^{a}	1.65^{ab}	1.76^{bc}	1.93^{c}	2.70^{bc}	2.08^{bc}	1.54^{b}	0.59^{b}
b+ 2 Mg ha^{-1}	1.37^{a}	2.07^{a}	1.39^{a}	1.90^{b}	1.72^{ab}	1.63^{bc}	1.00^{a}	0.96^{c}	1.00^{ab}	0.46^{d}
^{c}+ 10 Mg ha^{-1}	2.04^{a}	3.03^{a}	1.60^{b}	1.81^{ab}	1.95^{bc}	1.37^{bc}	0.81^{c}	0.91^{c}	0.71^{ab}	0.43^{b}

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT (Duncan's Multiple Range Test) *

Table 4. Effect of selected soil amendment on iron reduction (expressed as µmol Fe(II) produced g^{-1} soil) produced in an alluvial soil incubated under laboratory conditions

Treatment	Days after transplanting									
	2	4	6	8	10	12	14	16	18	20
Control	0.38^{a}	0.69^{a}	1.10^{a}	2.62^{ab}	2.93^{bc}	2.26^{bc}	1.37^{b}	1.23^{c}	1.17^{b}	1.49^{c}
^{a}+ Glucose	10.67^{a}	10.74^{a}	11.78^{a}	15.41^{ab}	16.25^{bc}	19.25^{c}	17.54^{bc}	15.62^{bc}	14.62^{b}	12.93^{b}
^{b}+ Humic acid	0.54^{a}	1.07^{a}	2.39^{a}	4.90^{b}	6.72^{ab}	4.67^{bc}	4.00^{a}	2.96^{c}	1.54^{ab}	0.95^{d}
^{a}+ Charcoal	0.51^{a}	1.05^{a}	1.47^{a}	1.65^{ab}	1.76^{bc}	1.93^{c}	2.70^{bc}	2.08^{bc}	1.54^{b}	0.59^{b}
+ Glucose + Humic acid	5.76	6.03	11.60	14.31	17.05	14.37	12.81	10.91	5.17	3.59
^{c}+ Glucose + Charcoal	8.56^{a}	9.03^{a}	11.10^{b}	12.31^{ab}	11.05^{bc}	10.37^{bc}	7.81^{c}	5.91^{c}	3.37^{ab}	1.87^{b}

In a column, mean followed by a common letter are not singnificantly different at the 5 % level by DMRT (Duncan's Multiple Range Test).

[a] Glucose was added at 4 mg C g^{-1} soil

[b] Humic acid was added at 10 mM

[c] Charcoal was added at 20 mg g^{-1} to represent 1 Mg ha^{-1}

may occur either when iron is mobilized in situ by microbial reduction of ferric iron [15].

Influence of charcoal amendment on α-naphthylamine-oxidase activity in roots of rice cultivar

Likewise, the activities of α-naphthylamine oxidase in roots were significantly reduced due to the addition of charcoal (Table 6). Higher the activities of α-naphthylamine oxidase in roots, more it can contribute to the oxidation of Fe(II) produced by the iron reducing microorganisms. Although the deposition of primarily of Fe(III)-oxides on the root surface can occur by both abiotic and biotic Fe(II) oxidation, the later can be attributed in part to microaerophilic Fe(II)-oxidizing bacteria (FeOB), at least from the evidence obtained from other wetland plants [22,21] and those reported from the present study. Since rice plants can support both the iron-reducing and iron-oxidizing microorganisms, the reduction potential and the α-naphthylamine oxidase activities can be directly related. This was apparently observed with control plants and the addition of charcoal led to concomitant decreases in the iron reduction potential and α-naphthylamine oxidase activities.

Yield components in rice cultivar as affected by addition of charcoal under field conditions

The grain yield of rice plants in field plots treated with 10 Mg ha^{-1} was highest, followed by that of 2 Mg ha^{-1} and 1 Mg ha^{-1} charcoal treatments (Table 7). Although straw yield was not significantly different among the treatments, the harvest index was highest in the treatment with 10 Mg of charcoal ha^{-1}. The results of present study suggest that the application of charcoal can retard the iron reduction process in soils, without adversely affecting grain yield. Therefore, organic soil amendments improved plant nutrient uptake and its availability by reducing sorption and leaching [10, 6].

Table 5. Iron reduction potential of roots of rice (cv. Naveen) as influenced by addition of charcoal under field conditions

Treatment	Days after transplanting							
	10	20	30	40	50	60	70	80
Control	10.99^{a}	28.72^{a}	41.44^{a}	55.74^{a}	101.50^{a}	110.49^{a}	107.38^{a}	109.50^{a}
+ 1 Mg ha^{-1}	5.26^{b}	13.40^{b}	15.94^{b}	14.25^{b}	46.13^{b}	23.58^{b}	32.13^{b}	24.87^{b}
+ 2 Mg ha^{-1}	4.72^{b}	12.12^{b}	13.51^{b}	19.82^{b}	39.37^{b}	28.31^{b}	35.69^{b}	18.42^{b}
+ 10 Mg ha^{-1}	4.81^{b}	8.08^{b}	12.79^{b}	13.52^{b}	35.80^{b}	30.89^{b}	26.49^{b}	19.02^{b}

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT (Duncan's Multiple Range Test) *

Table 6. α-Naphthylamine oxidase activity of roots of rice (cv. Naveen) as influenced by addition of charcoal under field conditions

Treatment	Days after transplanting							
	10	20	30	40	50	60	70	80
Control	295.3^{a}	360.2^{a}	492.9^{a}	358.8^{a}	423.7^{a}	485.3^{a}	308.7^{a}	419.6^{a}
^{x}+ 1 Mg ha^{-1}	171.2^{b}	197.5^{b}	232.0^{b}	138.3^{b}	134.0^{b}	248.2^{b}	191.0^{b}	279.4^{b}
^{y}+ 2 Mg ha^{-1}	154.2^{b}	130.7^{b}	273.6^{b}	183.9^{b}	157.4^{b}	222.1^{b}	136.6^{b}	250.2^{b}
^{z}+ 10 Mg ha^{-1}	103.9^{b}	196.1^{b}	222.5^{b}	139.0^{b}	151.6^{b}	207.4^{b}	167.2^{b}	249.2^{b}

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT (Duncan's Multiple Range Test) *

Table 7. Plant biomass of rice (cv. Naveen) as influenced by addition of charcoal under field conditions

	Days after transplanting		
Treatment	**Grain**	**Straw**	**Harvest index**
Control	3.83	6.70	36.34
+ 1 Mg ha^{-1}	4.28	7.18	37.36
+ 2 Mg ha^{-1}	4.50	7.30	38.14
+ 10 Mg ha^{-1}	4.74	7.26	39.47

Conclusion

The results of this study highlight the impact of application of different organic amendments on soil iron reduction processes, including improving the quality of paddy soil, and in promoting rice growth and harvest. Soil applications including biochar and compost though increase soil organic carbon (SOC) levels and soil fertility, application of compost including rice straw relatively influence physico-chemical properties like pH, cation-exchange capacity and available micronutrients in paddy soil. The contents of different fractions of iron including reduced form in analyzed samples ranged between 1.5 to 18.5 µmol g^{-1} soil and rice cultivar (cv. *Naveen*) show high yields with the application of charcoal at the rate of 10 Mg ha^{-1}. The positive effects of organic amendments on soil properties and crop yield can be correlated relatively greater in at the site with lower soil fertility, resulting into toxicity. As crop production in the country is either irrigated or patially rain-fed, localized biogeochemical cycling of nutrients as influenced by soil microorganisms affects available nutrient pool for plant growth and development. The amendment of different organic carbon substrates like acetate, decomposed rice straw compost-charcoal mixes to soil may have stabilizing effects especially on easily degradable components of the compost. Therefore, organic amendments in a farming system can improve soil biophysico-chemical properties, maintain satisfactory crop yield, reduce the cost-production and enhance long-term sustain-ability of the production system.

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References

1. **Asch, F., Becker, M., Kpongor, D.S. (2005).** A quick and efficient screen for tolerance to iron toxicity in lowland rice. Journal of Plant Nutrition and Soil Science. 168: 764-773.
2. **Engel, K., Asch, F. and Becker, M. (2012).** In Vivo Staining of Reduced Iron by 2,2' Bipyridine in Rice Exposed to Iron Toxicity. Journal of Plant Nutrition and Soil Science. 175: 548-552.
3. **Fischer, D., Glaser, B. (2012).** Synergisms between compost and biochar for sustainable soil amelioration. In: Kumar, S.E. (Ed.), Management of Organic Waste. In Tech, Shanghai, China. 167-198.
4. **Gustafsson, J.P., Pechova, P., Berggren, D. (2003).** Modeling metal binding to soils: the role of natural organic matter. Environ. Sci. Technology. 37: 2467-2774.
5. **He, J.Z., Liu, X.Z., Zheng, Y. (2010).** Dynamics of sulfate reduction and sulfate-reducing prokaryotes in anaerobic paddy soil amended with rice straw. Biology and Fertility of Soils. 46: 283-291.
6. **Inal, A., Gunes, A., Sahin, O., Taskin, M., Kaya, E. (2015).** Impacts of biochar and processed

poultry manure applied to a calcareous soil, on the growth of bean and maize. Soil Use Management. 31: 106-113.

7. **Jackson, M.L. (1973).** Soil Chemical Analysis, Prentice-Hall of India Private Limited, New Delhi.
8. **Kamura, T., Takai, Y., Ishikawa, K. (1963).** Microbial reduction mechanism of ferric iron in paddy soils. Soil Sci & Plant Nutrition Japan. 9: 171-175.
9. **Kyuma, K. (2004).** Paddy Soil Science. Kyoto University Press and Trans Pacific Press. Melbourne. Australia. 279.
10. **Lehmann, J., da Silva, J.P., Steiner, C., Nehls, T., Zech, W., Glaser, B., (2003).** Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil. 249: 343-357.
11. **Liu, J., Schulz, H., Brandl, S., Miehtke, H., Huwe, B., Glaser, B. (2012).** Short-term effect of biochar and compost on soil fertility and water status of a Dystric Cambisol in NE Germany under field conditions. Journal of Plant Nutrition and Soil Science. 175: 698-707.
12. **Nealson, K.H., Myers, C. (1992).** Microbial reduction of manganese and iron: new approaches to carbon cycling. Appl. Environ. Microbiol. 58: 439-443.
13. **Ota, Y. (1970).** Diagnostic methods for the measurement of root activity in rice plant. Jpn. Agric. Res. Quart. 5(3): 1-6.
14. **Piper, C.S. (1966).** Soil and Plant Analysis. Hans Publisher, Bombay.
15. **Ponnamperuma, F.N. (1972).** The Chemistry of Submerged Soils. Advances in Agronomy. 24: 29-96.
16. **Regassa, H., Agegnehu, G. (2011).** Potentials and limitations of acid soils in the highlands of Ethiopia: a review. In: Mulatu, B., Grando, S. (Eds.), Barley Research and Development in Ethiopia. ICARDA. Addis Ababa, Ethiopia. 103-112.
17. **Sahrawat, K.L. (2004).** Iron Toxicity in Wetland Rice and the Role of Other Nutrients. Journal of Plant Nutrition. 27: 1471-1504.
18. **Sahrawat, K.L. (2010).** Reducing Iron Toxicity in Lowland Rice with Tolerant Genotypes and Plant Nutrition. Plant Stress. 4: 70-75.
19. **Stookey, L.L. (1970).** Ferrozine-a new spectrophotometric reagent for iron. Anal. Chem. 42: 779-781.
20. **Tadano, T. and Yoshida, T. (1978).** Chemical changes in submerged soils and their effect on rice growth. In. Soils and Rice. IRRI. Los Banos, The Philippines. 399-420.
21. **Weiss, J.V., Emerson, D., Megonigal, J.P. (2004).** Geochemical control of microbial Fe(III) reduction potential in wetlands: comparison of the rhizosphere to nonrhizosphere soil. FEMS Microbiol Ecol. 48: 89-100.
22. **Weiss, J.V., Emerson, D., Backer, S.M., Megonigal, J.P. (2003).** Enumeration of Fe(II)-oxidizing and Fe(III)-reducing bacteria in the root zone of wetland plants: Implications for a rhizosphere iron cycle. Biogeochemistry. 64: 77-96.
23. **Zelleke, G., Agegnehu, G., Abera, D., Rashid, S. (2010).** Fertilizer and Soil Fertility Potential in Ethiopia: Constraints and Opportunities for Enhancing the System. International Food Policy Research Institute (IFPRI), Washington, DC, USA.
24. **Zhou, Z., Robards, K., Helliwell, S., Blanchard, C. (2002).** Ageing of stored rice: changes in chemical and physical attributes. Journal of Cereal Science. 35: 65-78.