

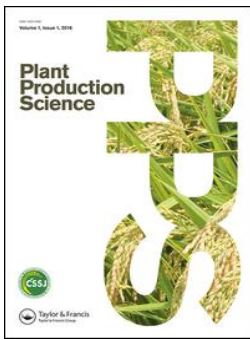


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著者	Koyama Shinichi, Hayashi Hisayoshi
journal or publication title	Plant production science
volume	20
number	2
page range	176-182
year	2017
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URL	http://hdl.handle.net/2241/00146347

doi: 10.1080/1343943X.2017.1290506





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To cite this article: Shinichi Koyama & Hisayoshi Hayashi (2017) Rice yield and soil carbon dynamics over three years of applying rice husk charcoal to an Andosol paddy field, Plant Production Science, 20:2, 176-182, DOI: [10.1080/1343943X.2017.1290506](https://doi.org/10.1080/1343943X.2017.1290506)

To link to this article: <http://dx.doi.org/10.1080/1343943X.2017.1290506>



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Accepted author version posted online: 03 Feb 2017.
Published online: 17 Feb 2017.



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Rice yield and soil carbon dynamics over three years of applying rice husk charcoal to an Andosol paddy field

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ABSTRACT

Rice husk charcoal (RC) produced from the pyrolysis of rice husk (RH) can be one of the cost-effective biochars for use in rice-based farming systems. This study investigated changes in rice yield and soil carbon sequestration over three years of RC application to an Andosol paddy field. The treatments were RC application at 0.02, 0.2, and 2 kg m⁻² (RC0.02, RC0.2, and RC2, respectively), RH application at 0.2 kg m⁻² (RH0.2), and a control with no RC or RH application (CONT). The results showed that RC2 increased culm length by 4% and straw weight by 14% on average over the three years. These increases in plant growth coincided with a higher level of silicon uptake by the rice plants, although they did not significantly affect grain yield. The soil carbon content was progressively increased by RC2 over the three years, whereas it was not significantly affected by RC0.02 or RC0.2. A considerable amount (>72%) of the applied carbon with RC2 remained in the soil by taking account of its downward movement below the 10 cm layer of the paddy field after three consecutive years of RC application. We conclude that rice husk charcoal application to Andosol paddy fields is an effective option for increasing carbon sequestration. Furthermore, the increase in silicon uptake by rice plants suggests that rice husk charcoal can also be functioning as a silicon fertilizer.

ARTICLE HISTORY

Received 30 July 2016
Revised 15 January 2017
Accepted 23 January 2017

KEYWORDS

Andosol; biochar; C/N ratio; mineralization; rice husk; silicon; soil carbon sequestration

CLASSIFICATION

Agronomy & Crop Ecology

Introduction

Biochar is a carbonaceous product of the thermo-chemical conversion of biomass under oxygen-limited conditions (Crombie et al., 2013). It has a highly porous structure, and biochar application is thus considered to improve soil physical properties such as bulk density, water holding capacity, and hydraulic conductivity (Hardie et al., 2014). However, the effect of biochar application on crop productivity varies depending on soil type, fertility, and moisture content, as well on the type of biochar applied to the soil (Lehmann et al., 2009). Some studies have reported that biochar application can increase crop yield by improving soil physicochemical (Glaser et al., 2002; Lehmann & Rondon, 2006) and biological (Lehmann et al., 2011) properties, whereas others have observed a reduction in grain yield due to nitrogen (N) limitation caused by the high carbon to nitrogen ratio (C/N ratio) of biochar (Asai et al., 2009).

Rice husk charcoal processed from pyrolysis of rice husk is considered to be one of the most cost-effective biochars for use in rice-based farming systems (Ogawa & Okimori, 2010). Rice husk is generated at rice processing facilities

as a by-product after collecting, drying, and dehulling paddy rice. The utilization of rice husk can be a feasible and sustainable option compared to the other woody biomass that sparsely exists in the natural environment. Approximately two million tons of rice husk, equivalent to 22% of brown rice weight (Ogawa et al., 1988), is annually produced in Japan, although about one-fifth of it is still unused (Agriculture, Forestry & Fisheries Research Council, 2014). With the development of modern bioenergy technologies, the coupling of bioenergy production from rice husk and the use of rice husk charcoal in rice production has a potential to offer environmental and agronomic advantages in a sustainable manner (Shackley et al., 2012). Haefele et al. (2011) reported that rice husk charcoal application at 41.3 Mg ha⁻¹ increased rice yield by 16–35% depending on conditions such as water holding capacity, available N level, and cation exchange capacity (CEC) in three soil types, including Gleysols, Nitisols, and Acrisols. We conducted a pot experiment (based on Wagner's pots with a surface area of 0.02 m²) using Andosol paddy soil and found that rice husk charcoal application at 40 g pot⁻¹ increased silicon (Si) uptake of rice plants by 250% and

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brown rice yield by 30% (Koyama et al., 2016). The study as well as a field experiment (Koyama et al., 2015) revealed that rice husk charcoal-derived carbon (C) remained in the soil even after rice cultivation without a significant increase in methane (CH₄) emissions. Andosol paddy soil is characterized by a relatively high C content (Takata et al., 2013) and low levels of CH₄ emitted under anaerobic conditions (Yagi & Minami, 1990), among the Japanese soil types. The potential residual time of biochar in soil is reported to depend on the feedstock and the pyrolysis temperature used for producing the biochar as well as on soil conditions, but it ranges from hundreds to thousands of years (Lehmann et al., 2009; Wang et al., 2015). For example, Knoblauch et al. (2011) estimated the carbon mineralization rate of rice husk charcoal in two soil types according to the U.S. soil taxonomy (Typic Fluvaquents and Aquic Udifluvents). They found that 31–54% of the C added as rice husk was mineralized, whereas only 8.5% of the C added in the form of rice husk charcoal was mineralized to CO₂ under anaerobic conditions after almost three years of laboratory incubation. Rice husk charcoal application may thus be an effective option for enhancing rice production and simultaneously increasing soil C sequestration. The objective of the present study is to evaluate the effects of applying rice husk charcoal to an Andosol paddy field on the growth and yield of rice plants, and on soil physicochemical properties with a particular emphasis on C sequestration, over a period of three years.

Materials and methods

Experimental design

A three-year field experiment was conducted from 2012 to 2014 under a conventional rice farming system in an irrigated paddy field at the Agricultural and Forestry Research Center (AFRC), University of Tsukuba, Japan (36°12'N, 140°09'E, 25 m above sea level). The soil at this site was classified as a Haplic Andosol by the Food and Agriculture Organization of the United Nations (FAO) and the United Nations Educational, Scientific and Cultural Organization (UNESCO) system (FAO-UNESCO, 1990). The treatments comprised three rates of rice husk charcoal (RC) application, 0.02, 0.2, and 2 kg m⁻² (RC0.02, RC0.2, and RC2, respectively), one rate of rice husk (RH) application, 0.2 kg m⁻² (RH0.2), and a control without the application of RC or RH (CONT). RC0.02 is equivalent to the average yield of RC from paddy fields in Japan, since the weight of RH is about 22% of that of brown rice (Ogawa et al., 1988) and yielding percentage of RC is 25% as compared to the RH weight (Senoo et al., 2009). We included higher RC application rates, up to RC2, on the assumption that increasing the amount of RC applied would increase

soil C sequestration. RH0.2 was included to compare the effects between carbonized and un-carbonized rice husk applications. The RC, which was produced by pyrolysis at 350–400 °C for 15 min, was purchased from Pros Co. Ltd., Nagano, Japan. Approximately 45% of the feedstock, on a volume basis, remains as RC after the carbonization process. The RH was collected from a country elevator near the experimental site.

The treatments were arranged in a randomized block design with three replicates. Each 1.6 m × 2 m plot was separated from its neighbors by vinyl chloride resin levees with a height of 15 cm above ground and a depth of 15 cm inserted in the soil, in order to avoid soil movement and root penetration into the other treatments. In each plot, the same treatment was continued for three years. Tilling practice was carried out using hand tools, such as shovel and three-prong digging hook. Puddling and leveling were done with a small cultivator (MKC31; Maruyama MFG. Co., Inc. Tokyo Japan) and rake for preventing contamination between the treatments. Basal compound fertilizer was applied to all the plots at a locally recommended rate of 80, 80, and 53 kg ha⁻¹ of N, P₂O₅, and K₂O, respectively, 7 days before transplanting (DBT) in the 1st year, 8 DBT in the 2nd year, and 13 DBT in the 3rd year. RC or RH, according to the treatment being applied, was manually incorporated to a soil depth of approximately 10 cm. The RC or RH application was carried out at the same time of basal fertilizer application in the 1st year (7 DBT), and at the timing of spring plowing in the 2nd year (67 DBT) and the 3rd year (62 DBT).

Crop management and yield measurements

Four rice seedlings (*Oryza sativa* L. cv. Nipponbare) per hill were transplanted into the field at the 3–4 leaf stage with a spacing of 25 cm × 20 cm on 26 May 2012, 26 May 2013, and 24 May 2014. A local water regime for irrigated paddy fields was practiced; it included midseason drainage for a period of about 10 days at the end of July every year, followed by intermittent flooding until three weeks before the point of harvest in each season. The aboveground parts of the rice plants were harvested at the maturity stage, which was attained 127 days after transplanting (DAT) on 30 September 2012, 140 DAT on 13 October 2013, and 140 DAT on 11 October 2014. The harvested plants were air-dried for 2 weeks. The lengths of the culm and the panicle in the longest culm, straw weight, panicle weight, and panicle number were measured from 12 to 15 hills per plot. Five hills that had the average panicle weight and number for their plot were then selected for further determination of yield and yield components. Fully ripened grains were separated from threshed grains using a saline solution with a specific gravity of 1.06. Yield was defined as the weight of

fully ripened grains with a water content of 14%. The other plants harvested were separated into panicle and straw fractions. The dry weight of each fraction was determined after drying in an oven at 80 °C for 48 h. The daily mean air temperature and precipitation during the three years were obtained from a meteorological station located at the AFRC. During the periods from rice transplantation to harvest in 2012, 2013, and 2014, the mean air temperature was 24.4, 24.0, and 24.6 °C, and the total precipitation was 499, 520, and 617 mm, respectively. The mean solar radiation collected from the observatory of Japan Meteorological Agency located within 9 km from the experimental site was 18.0, 16.3, and 16.1 MJ m⁻², respectively, during the same periods.

Physicochemical analysis of soil, RC and RH

Physicochemical properties of paddy soil were measured after each rice growing season; on 30 September 2012, 20 November 2013, and 11 October 2014. Three soil phases in each plot were calculated from two samples collected at the top 10 cm layer by applying a soil three-phase meter (Daiki Rika Kogyo Co., Ltd. Saitama, Japan) to a 100 ml undisturbed soil core (FV-478; Fujiwara Scientific Co., Ltd. Tokyo Japan). Soil bulk density was determined from the mass of the solid phase after oven-drying at 105 °C for 48 h. The bulk density of RC and RH was measured by gently tapping a container five times and topping up the contents to the brim (Cooperband, 2002). Another soil sample, composed of five cores taken from the top 10 cm layer of each plot with a hand shovel, was air-dried and sieved (<2 mm) for chemical analysis. Soil pH (soil:H₂O = 1:2.5) and electrical conductivity (EC, soil:H₂O = 1:5) were determined using a pH meter (HM-30R; DKK-TOA Corp., Tokyo, Japan) and an EC meter (CM-30R; DKK-TOA Corp., Tokyo, Japan), respectively. To measure the pH and EC of the RC and RH, a 1:10 ratio of the material to deionized water (weight:volume) was adopted due to the low bulk densities of RC and RH. The total N and C content of the soil, RC, and RH were

analyzed using the dry-combustion method with an NC analyzer (NC-220F; Sumika Chemical Analysis Service, Ltd., Osaka, Japan). Soil C sequestration (kg m⁻²) was calculated using the following equation:

$$\text{Soil C sequestration} = (C_{\text{tre}} \times 0.1 \times BD_{\text{tre}}) - (C_{\text{cont}} \times 0.1 \times BD_{\text{cont}})$$

where C_{tre} denotes the soil C content in the treatment plot after rice cultivation (g kg⁻¹ dry soil), 0.1 is the soil depth in meters corresponding to the top 10 cm layer, and BD_{tre} is the bulk density of the treatment (Mg m⁻³). C_{cont} and BD_{cont} is the soil C content (g kg⁻¹ dry soil) and bulk density (Mg m⁻³) of the CONT plots in the same year of the treatment, respectively. In the 3rd year of the experiment, soil samples from a depth of 10–20 cm were also collected in order to estimate the translocation of soil C during the experiment.

Dried rice straw was ground to a fine powder (<2 mm) using a cutting mill (SM-100; Retsch GmbH, Haan, Germany). The total N concentration of the rice straw was determined using the same method as that used for soil analysis. The Si concentration of the straw, RC, and RH was determined using gravimetric analysis, as described by Saito et al. (2005). The rice straw from the 2nd year's harvest, RC, and RH were digested in a mixture of sulfuric acid (H₂SO₄) and hydrogen peroxide (H₂O₂) in order to measure potassium (K), calcium (Ca), and magnesium (Mg) concentrations using an atomic absorption photometer (Z-2300; Hitachi High-Technologies Corp., Tokyo, Japan). The uptake of nutrients by rice plants was calculated by multiplying the nutrient concentrations by the weight per unit area of the rice straw. Table 1 lists the physicochemical characteristics of the field soil before the experiment, and of the RC and RH used for the experiment.

Statistical analysis

Statistical analyses were conducted using the JMP 8 software package (SAS Institute Japan Inc., Tokyo, Japan). A

Table 1. Physicochemical properties of the paddy soil before commencing the experiment, and of the rice husk charcoal and rice husk applied.

Physicochemical property	Paddy soil		Rice husk charcoal		Rice husk	
		(SD)		(SD)		(SD)
Bulk density (g cm ⁻³)	0.61	(0.06)	0.12	(0.01)	0.11	(0.00)
pH	5.80	(0.00)	9.19	(0.76)	6.59	(0.52)
EC (mS cm ⁻¹)	0.11	(0.00)	0.52	(0.15)	0.66	(0.15)
Total C (g kg ⁻¹)	73.2	(0.29)	396	(34.4)	369	(10.6)
Total N (g kg ⁻¹)	5.42	(0.03)	3.40	(0.56)	3.26	(0.15)
C/N ratio	13.5	(0.05)	118	(9.66)	116	(1.46)
Si (g kg ⁻¹)	na		215	(24.8)	106	(13.1)
K (g kg ⁻¹)	na		8.35	(1.19)	4.36	(0.16)
Ca (g kg ⁻¹)	na		2.18	(0.24)	0.97	(0.02)
Mg (g kg ⁻¹)	na		0.65	(0.04)	0.33	(0.10)

Notes: EC, electrical conductivity; N, nitrogen; C, carbon; Si, silicon; K, potassium; Ca, calcium; Mg, magnesium and na, not analyzed. pH and EC were measured in a material to water solution at a ratio of 1:10. Figures in parentheses indicate standard deviation ($n = 3-6$).

Table 2. Growth, yield, and yield components of rice plants as affected by three years of application of rice husk charcoal (RC) or rice husk (RH).

Factor	Culm length (cm)	Panicle length (cm)	Aboveground weight (gDW m ⁻²)		Grain-straw ratio (%)	Panicle number (m ⁻²)	Spikelet number (panicle ⁻¹)	Percentage of ripened grains (%)	1000-grain weight (g)	Grain yield (g m ⁻²)
			Panicle	Straw						
<i>Treatment</i>										
CONT	82.1 ab	19.5	605.9	722.0 b	81.3 a	351.2	76.4	81.9	27.6	602.0
RC0.02	82.2 ab	20.0	584.1	696.4 b	82.3 a	339.7	76.9	82.2	27.7	589.9
RC0.2	81.5 b	19.9	574.5	709.5 b	78.1 ab	335.9	77.4	81.6	27.5	579.1
RC2	85.3 a	19.8	639.4	822.3 a	73.6 b	360.2	80.8	79.1	27.4	625.7
RH0.2	83.3 ab	19.6	582.9	717.7 b	78.9 ab	343.8	76.6	80.8	27.7	582.7
<i>Application year</i>										
1st year	80.3 b	20.8 a	612.0 a	685.9 b	81.3 a	347.6 b	78.4 a	76.4 c	28.1 a	582.9 b
2nd year	87.7 a	19.3 b	639.5 a	879.0 a	72.9 b	401.6 a	74.8 b	82.0 b	27.2 b	667.7 a
3rd year	80.6 b	19.2 b	540.7 b	635.9 b	82.3 a	289.3 c	79.6 a	85.0 a	27.5 b	537.0 b
ANOVA										
Treatment	*	NS	NS	**	**	NS	NS	NS	NS	NS
Application year	**	**	**	**	**	**	**	**	**	**
Interaction	NS	**	NS	NS	NS	NS	NS	NS	NS	NS

Notes: Panicle and straw weight indicated on a dry weight basis. 1000-grain weight and grain yield were adjusted to have a moisture content of 14%.

**, * and NS denote significantly different at $p < 0.01$, $p < 0.05$ and not significant, respectively, according to analysis of variance (ANOVA).

Means followed by the same letter within each column are not significantly different at the 5% level in Tukey's HSD test.

two-way analysis of variance (ANOVA) was applied to analyze the effects of RC treatment, application year and their interaction on rice growth and yield, nutrient concentration and uptake by rice plant, and soil physicochemical properties. RC treatment and application year were considered fixed effects in the analysis. Multiple comparisons among the different treatments were performed using Tukey's honest significant difference (HSD) test at the 5% level.

Results

Growth, yield, and yield components of rice plants

Of the growth and yield parameters analyzed, only culm length, straw weight, and grain-straw ratio showed significant differences among the treatments according to ANOVA (Table 2). The culm length and straw weight in RC2 increased by 4 and 14%, respectively, as compared to those of the CONT on the average of the three years. The increases in straw weight led to decrease in grain-straw ratio in RC2 irrespective of application year. The other parameters, including panicle length, panicle weight, panicle number, spikelet number, percentage of ripened grains, 1000-grain weight, and grain yield were not significantly affected by the treatments. Significant differences were observed among the application years in all the growth and yield parameters, although there was no consistent relationship between those parameters and the number of RC or RH application year. The interaction between treatment and application year was significant only for panicle length, and this was caused by the variation in the response of this parameter to RC treatment depending on the year. There were no significant differences between RC0.2 and RH0.2 for any of the growth and yield parameters.

Nutrient concentration and uptake by rice plants

To dissect the cause of increase in rice straw biomass, nutrient analyses were carried out (Table 3). The ANOVA indicated that there were significant differences in N, Si, and Mg concentrations among the treatments. The N and Mg concentrations decreased gradually as the RC application rate increased. The rice plants in RC2 had significantly lower N and Mg concentrations than those in CONT. However, N and Mg uptakes by rice plants did not significantly differ among the treatments because their lower concentrations were compensated by the larger rice straw biomass in RC2. On the other hand, Si concentration increased as the RC application rate increased. The rice plants in RC0.2 and RC2 significantly increased Si concentration by 123 and 170%, respectively, as compared to those in CONT on the average of the three years. As a result, Si uptake by rice plants increased with higher rates of RC application. Si uptake in RC2 was nearly twice (195%) as high as that in CONT. Significant differences among the application years were observed in both concentrations and uptakes of N and Si due to the differences in plant growth during the three years. The interaction between treatment and application year was not significant in both concentrations and uptakes of N and Si.

Soil physicochemical properties and C sequestration

The ANOVA showed significant differences in bulk density, porosity, total C content, and C/N ratio among the treatments (Table 4). Bulk density decreased significantly by the RC2 treatment, although porosity did not show statistical differences at 5% level in Tukey's HSD test. Among the application years, the bulk density was the lowest and the porosity was the highest in the 3rd year of experiment,

Table 3. Nutrient concentration and uptake in mature rice straw as affected by rice husk charcoal (RC) or rice husk (RH) application.

Factor	Concentration (%)					Nutrient uptake (g m ⁻²)				
	N	Si	K	Ca	Mg	N	Si	K	Ca	Mg
<i>Treatment</i>										
CONT	0.60 a	4.92 c	1.28	0.32	0.110 ab	4.34	34.9 b	11.3	2.81	0.97
RC0.02	0.57 ab	5.56 bc	1.28	0.30	0.111 a	3.91	38.5 b	10.4	2.48	0.91
RC0.2	0.54 ab	6.07 b	1.30	0.32	0.109 ab	3.81	42.6 b	11.1	2.71	0.93
RC2	0.50 b	8.35 a	1.30	0.24	0.095 c	4.13	68.2 a	12.6	2.37	0.91
RH0.2	0.56 ab	5.82 bc	1.30	0.24	0.108 b	3.96	41.5 b	11.3	2.13	0.94
<i>Application year</i>										
1st year	0.63 a	6.20 ab	na	na	na	4.33 a	43.1 b	na	na	na
2nd year	0.51 b	5.66 b	1.29	0.28	0.107	4.47 a	50.1 a	11.3	2.50	0.93
3rd year	0.52 b	6.58 a	na	na	na	3.29 b	42.1 b	na	na	na
<i>ANOVA</i>										
Treatment	*	**	NS	NS	**	NS	**	NS	NS	NS
Application year	**	**	–	–	–	**	**	–	–	–
Interaction	NS	NS	–	–	–	NS	NS	–	–	–

Notes: na, not analyzed as the K, Ca, and Mg concentrations were analyzed only in the 2nd year of experiment.

**, * and NS denote significantly different at $p < 0.01$, $p < 0.05$ and not significant, respectively, according to analysis of variance (ANOVA).

Means followed by the same letter within each column are not significantly different at the 5% level in Tukey's HSD test.

Table 4. Effects of three years of application of rice husk charcoal (RC) or rice husk (RH) on paddy soil physicochemical properties.

Factor	Bulk density (Mg m ⁻³)	Porosity (%)	pH	EC (mS m ⁻¹)	TN (g kg ⁻¹)	TC (g kg ⁻¹)		C/N ratio
						0–10 cm	10–20 cm	
<i>Treatment</i>								
CONT	0.70 a	72.6 a	5.91	88.4	4.08	54.0 b	49.8 b	13.2 c
RC0.02	0.68 ab	73.5 a	5.96	85.8	3.99	53.2 b	49.2 b	13.3 c
RC0.2	0.70 a	72.5 a	5.99	85.5	4.07	56.6 b	50.6 ab	13.9 b
RC2	0.65 b	73.9 a	5.92	86.0	4.16	79.6 a	59.1 a	19.1 a
RH0.2	0.69 a	73.0 a	5.99	82.5	4.05	54.9 b	47.6 b	13.5 bc
<i>Application year</i>								
1st year	0.71 a	71.6 b	5.91 b	82.5 b	4.23 a	59.6 ab	na	14.1 c
2nd year	0.71 a	72.1 b	5.92 ab	71.7 b	3.95 b	57.6 b	na	14.6 b
3rd year	0.63 b	75.7 a	6.03 a	102.9 a	4.03 b	61.7 a	51.3	15.2 a
<i>ANOVA</i>								
Treatment	**	*	NS	NS	NS	**	**	**
Application year	**	**	*	**	**	**	–	**
Interaction	NS	NS	NS	NS	NS	**	–	**

Notes: EC, electrical conductivity; TN, total nitrogen; TC, total carbon; C/N ratio, carbon to nitrogen ratio; na, not analyzed. Total carbon contents of paddy soil at a depth of 10–20 cm were measured only in the 3rd year of experiment. C/N ratio was calculated using total C and N contents at the top 10 cm layer of paddy soil.

**, * and NS denote significantly different at $p < 0.01$, $p < 0.05$ and not significant, respectively, according to analysis of variance (ANOVA).

Means followed by the same letter within each column are not significantly different at the 5% level in Tukey's HSD test.

suggesting cumulative effects of RC application on the physical properties of paddy soil. Total C content of paddy soil increased significantly by the RC2 treatment. The relationship between the number of application year and soil C sequestration showed a strong positive correlation in the case of RC2, whereas soil C sequestration was not clearly detected when the rates of RC or RH application were 0.2 kg m⁻² or less (Figure 1). The regression coefficient for RC2 indicated that 0.365 kg m⁻² of C was sequestered annually in the top 10 cm of paddy field after the 1st year of RC application. Similarly, C/N ratio increased in proportion to the rate of RC application (Table 4). The interaction between treatment and application year was significant in total C content and C/N ratio because the difference of soil C content in RC0.02, RC0.2, and RH0.2 from CONT was marginal and varied depending on the

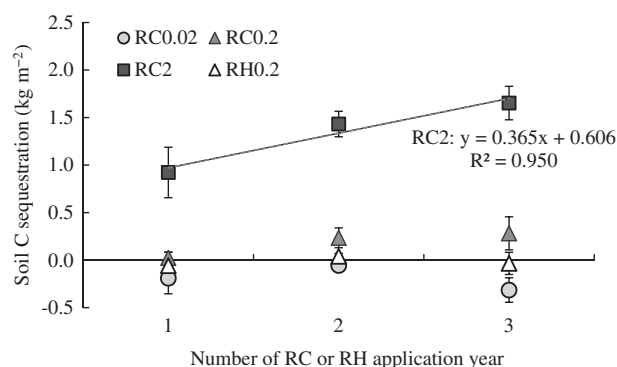


Figure 1. Increase in soil carbon content compared to that in the control plots over three years of rice husk charcoal (RC) or rice husk (RH) applications. Vertical bars indicate standard errors of three replicates. The regression equation shown in the figure was calculated from the data from RC2 plots.

application year. At the end of the experiment, the lower soil layer (10–20 cm) of the RC2 plots accumulated a higher C content than those in the other treatments probably due to the cultivation management regime used and the root growth of rice plants. The ANOVA results in soil pH, EC, and N content showed significant differences only among the application years. However, cumulative effects of RC application on these chemical parameters were unclear because the values in the CONT plots also varied depending on the application year. With respect to soil physico-chemical properties, there were no significant differences between RC0.2 and RH0.2.

Discussion

In a previous pot experiment, we found that RC application to Andosol paddy soil increased Si uptake by rice plants and hence increased aboveground biomass production in rice plants while promoting soil C sequestration (Koyama et al., 2016). The present field study showed significant increases in Si uptake (by 70%), culm length (by 4%), and rice straw weight (by 14%) over a period of three years when RC application rate was 2 kg m^{-2} (RC2), as compared to those of the CONT plants (Table 2 and 3). However, rice yield was not affected by the RC applications. It has been reported that Si deposits in rice plants have positive impacts on the growth by suppressing excessive transpiration, enhancing the light-intercepting structure of rice plants growing in a community, improving resistance to fungus and insect injuries, and increasing tolerance to lodging (Guntzer et al., 2012; Hossain et al., 1999; Ma et al., 1989). But the effects of additional Si application on Si concentration in rice plant differ depending on soil chemical properties such as available Si, Si adsorption capacity, contents of Si adsorbents, and the pH under flooded soil condition (Makabe-Sasaki et al., 2014). Imaizumi and Yoshida (1958) demonstrated that Si application had a greater impact on rice yield when the Si concentration of the rice plants was less than the critical level at 5.1% (equivalent to 11% SiO_2). Generally, rice plants grown in paddy fields were supplied with more Si from the larger volumes of rhizosphere soil and of irrigation water than those plants in the pot experiment. In addition, the historical record of the experimental field showed there were applications of calcium silicate as a soil amendment for several years before this experiment. These experimental conditions may have led to the relatively high Si concentration in the CONT plants (4.92%) and obscured any effects of RC application on rice yield. As Imaizumi and Yoshida (1958) argued that the Si concentrations in rice plants in approximately half of Japanese paddy fields were below the critical level (5.1%), it is most

likely that RC application increases rice yield in such paddy fields where Si is less available for the plants.

Soil C sequestration increased in proportion to the rate of RC application, and cumulatively with the number of RC application year (Table 4), indicating that the C derived from RC application remained in the paddy soil for more than three years. The significant increase in C/N ratios in the RC2 treatment may have caused immobilization of N in the soil and led to lower rice yields, as reported by Asai et al. (2009). However, growth parameters such as plant height, leaf SPAD value (data not shown), and panicle number did not differ significantly among the treatments. The rice plants were not subject to N limitation during the experiment because the C from RC was stable in paddy soils. The labile component of RC is considered to be minimal as we reported in the previous pot experiment (Koyama et al., 2016) that RC application to Andosol paddy soil proportionally increased soil C sequestration without stimulating CH_4 emission during rice cultivation. In the present field experiment, the soil C sequestration level for the RC2 plots was 1.65 kg m^{-2} in the top 10 cm of paddy soil after the 3rd consecutive year of RC application (Figure 1); while the estimated amount of C supplied from RC2 was 0.77 kg m^{-2} annually, as calculated based on the C content (396 g kg^{-1}) and the moisture content (3.0%) of RC at the time of application. Approximately 72% of the C derived from RC remained in the top 10 cm of the paddy soil. Furthermore, the analyses of the next lower layer (10–20 cm) of paddy field at the end of the experiment indicated a downward accumulation of some of the applied C from RC2 in the subsoil. This result matched that of Haefele et al. (2011), which found that 49–114% of the C supplied with rice husk charcoal remained in the topsoil depending on soil types and rice production systems while a considerable part of the applied C was also traced in the subsoil after three years. They demonstrated that soil conditions such as soil pH, moisture, temperatures, and clay mineral content affected soil organic matter mineralization. These soil conditions should be considered to determine the optimum RC application rates and frequencies for enhancing soil C sequestration across diverse soil types and rice production systems.

In conclusion, the results presented in this study provide important evidence that the C derived from RC remained in the Andosol paddy soil for more than three years. The increase in Si uptake by rice plants suggested RC application can also be functioning as a Si fertilizer. Longer-term studies are needed to confirm the cumulative effects of soil C derived from the lower rates of RC application and to evaluate various impacts of the increased Si contents on the growth of rice plants.

Acknowledgments

The authors wish to thank Dr. Kenji Tamura of Tsukuba University for his guidance on soil physicochemical analyses. The authors are also grateful to Ms. Keiko Sugawara and Mr. Kiyoshi Karube from AFRC for their assistance with the management of the experimental field.

Disclosure statement

No potential conflict of interest was reported by the authors.

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