INFLUENCES OF RICE HUSK BIOCHAR (RHB) ON RICE GROWTH PERFORMANCE AND FERTILIZER NITROGEN RECOVERY UP TO MAXIMUM TILLERING STAGE

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ABSTRACT

A pot study was carried out to investigate the effects of rice husk biochar addition on rice growth performance and fertilizer nitrogen recovery. The biochar effect was studied by using ¹⁵N labelled fertilizer urea (10 atom% ¹⁵N), as isotopic tracer, until maximum tillering stage (75 days after sowing). Rice husk biochar (RHB) was applied at rates of 0, 5, 10 and 20 Mg ha⁻¹ and laid in randomized complete block design with four replications. The result showed that biochar application significantly improved soil chemical properties (pH, total C, total N, and available P) compared to control treatment. Biochar addition increased number of tiller and root dry matter weight up to 4% and 35%, respectively, compared to un-amended pot. Likewise, application of biochar significantly increased N, P and K uptake by 3%, 19% and 33%, respectively, as compared to the nutrient uptake from the control treatment. Biochar treatment had no significant impact on fertilizer nitrogen recovery in aboveground biomass, in the range of 41% and 42%, in comparison to the control. However, nitrogen fertilizer recovery in soil significantly increased by 47% over the control at application rate of 20 Mg ha⁻¹ RHB. Increased fertilizer N recovery in soil possibly reduced N losses to the environment from volatilization and denitrification processes. Total ¹⁵N fertilizer recovery also found increase at highest application of RHB biochar with an increment of 16%. In general, addition of biochar appeared to enhance crop growth performance but its effect on fertilizer N recovery in plant requires further study up to maturity of rice plant.

Keywords: Rice husk biochar; ¹⁵N isotopic tracer, nitrogen recovery, organic soil, rice

INTRODUCTION

The Malaysia population growth is estimated at 31.7 million persons in 2016 (Department of Statistics Malaysia, 2016). To meet the challenges, more grains need to be produced with minimal environmental deterioration and fertilizer cost. The key factor which should be of concern is the efficient use of nitrogen (N) fertilizer such as urea in rice cultivation in Malaysia. According to Liu et al., (2010a), the global average N efficiency rate of 59% indicated that nearly two-fifths of N inputs were lost in ecosystems by various ways. High N fertilizer rates applied in rice cultivation are due to high losses of N (mainly gaseous losses) which was earlier reported to be more than 55% (Zhu and Chen 2002; Xu et al., 2012). Nitrogen losses are through NH₃ volatilization and denitrification, leaching and

runoff with floodwater (Xing et al., 2002; Lin et al., 2007; Tian et al., 2007).

As reported by Fageria and Baligar (2001), low fertilizer nitrogen use efficiency in lowland rice might due to N loss from leaching, volatilization, surface runoff and denitrification in the soil-flood water system. Roelcke et al. (2004) and Zou et al. (2009) reported that higher N fertilizer application at rates of over 200 kg N ha⁻¹ were used for rice cultivation in eastern China so as to ensure high productivity. The optimum N rate for rice production is different across countries, such as 120 kg N ha⁻¹ in clay loam in Malaysia (Puteh et al., 2014), 175 kg N ha⁻¹ in clay loam soil of Pakistan (Manzoor et al., 2006), 150 to 250 kg N ha⁻¹ in soils of China (Peng et al., 2006), 122 kg N ha⁻¹ for Gangetic soils of India (Kumar et al., 2010). Thus, there has been increasing attention towards fertilizer N recovery, which is defined as the ratio of the N output or uptake by crops to fertilizer N input (Liu et al., 2010b; Qiao et al., 2012).

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Most recently attention has been given to the use of biochar in cultivation of crops including rice to reduce nutrient losses and improve yield. However, in Malaysia, documented reports on influence of biochar on N fertilizer recovery using ^{15}N labelled fertilizer as isotopic tracer in rice is greatly lacking. According to Petter et al. (2016), application of biochar has improved the efficiency of N use in upland crops four times higher compared to un-amended plot. However, some studies showed that no significant effect was detected on the application of biochar toward fertilizer N recovery. Due to limited ^{15}N labelled fertilizer, a pot experiment under controlled environment was carried out using the isotopic dilution technique ^{15}N -labelled

fertilizer with aim of assessing the effectiveness of rice husk biochar (RHB) in improving fertilizer N (urea) uptake by rice (*Oryza sativa*) plant and retention in soil up to maximum tillering stage, 75 days after sowing (DAS).

MATERIALS AND METHODS

Experimental site and soil characteristics

A pot study was carried out at Field 10, Universiti Putra Malaysia (UPM), Selangor in a rain shelter. The soil used in this study was collected from Kampung Tengah, Pasir Panjang (3°35'27.6" N 101°04'10.5" E), Selangor. Characteristics of the soil are as given in Table 1.

Table 1: Selected chemical properties of the soil before treatment and RHB properties (n=3)

Chemical properties	Initial soil (non-amended soil)	RHB
pH (soil = 1: 2.5; biochar = 1: 50)	5.52	7.40
Total Carbon, (g kg ⁻¹)	131.13	431.20
Total Nitrogen, (g kg ⁻¹)	6.62	6.80
Available P, (mg kg ⁻¹)	125.20	-
Extractable K, (mg kg ⁻¹)	28.00	-
Extractable Ca, (mg kg ⁻¹)	3221.40	-
Extractable Mg, (mg kg ⁻¹)	156.00	-
CEC, (cmol (+)kg ⁻¹)	34.25	-
Total ash, (%)	-	41.00
Volatile, (%)	-	43.30
Total P, (g kg ⁻¹)	-	1.55
Total K, (g kg ⁻¹)	-	2.92
Total Ca, (g kg ⁻¹)	-	0.85
Total Mg, (g kg ⁻¹)	-	0.87

- not determine

Experimental layout and treatment

The RHB used was collected from a small RHB producer, SENDI Enterprise at Sekinchan, Selangor. The experiment was laid out in a randomized complete block design with four biochar application rates (0, 5, 10 and 20 Mg ha⁻¹ RHB). Each treatment was replicated four times. The RHB rates was selected according to the study conducted by Masulili et al. (2010) which also used a similar source of RHB in rice cultivation on acid sulphate soil, Kalimantan, Indonesia. The

results showed that application of biochar seems to response with application of 5 Mg ha⁻¹ RHB and produce the maximum grain yield up to 10 Mg ha⁻¹ RHB. Treatments were set up in slope-sided plastic pot 37.5 cm in height and 35 cm in diameter at the top and 26.5 cm at the base. Biochar was incorporated into soil two weeks before the rice seeds were sown. Twenty-one seeds of variety MR 263 was used and sown through direct broadcasting. After one week, thinning of seedlings was carried out to sixteen seedlings per pot based on calculation of field

application where seeds were broadcasted at 200 g for 25 m². The water depth was maintained at 5 cm above soil surface which started at 15 DAS and then drained at 7 days before harvesting at 75 DAS on the rice maximum tillering stage. Submerged soil pH and plant growth were recorded every 15 days starting from day 15 DAS to determine the effect of RHB amendment on soil pH and height improvement. Fertilization of rice crop was according to recommended rate and procedure by Malaysian Agricultural Research and Development Institute (MARDI). The ¹⁵N-labelled urea (10% atom excess) was used as the only chemical N fertilizer application and functioned as isotopic tracer. Fifteen days after sowing, N at rate of 70 kg N ha⁻¹ (2.65 g ¹⁵N labelled urea pot⁻¹) was added as a basal dose. Likewise, triple super phosphate was applied at rate of 47.2 kg P ha⁻¹ (1.0 g TSP pot⁻¹), while muriate of potash was applied at rate of 39 kg K ha⁻¹ (0.62 g MOP pot⁻¹) at 3 different stages (15, 30 and 55 DAS).

Soil sampling and analysis

After harvest, the soil samples were collected at three different points using a small hand auger to determine the soil characteristics (soil pH, total carbon (C), total N, available phosphorus (P), extractable bases (potassium (K), calcium (Ca) and magnesium (Mg)) and cation exchange capacity (CEC) after harvest. The soil samples collected were air dried, ground and sieved (≤2 mm) before analysis. The soil pH (H₂O) was determined using a pH meter (Eijkelamp Waterproof Portable Meter 18.52.01) with 1:2.5 ratio soil solutions (with ionized water). Soil total C and total N were determined using dry combustion (Dumas method) using LECO TruMac CNS analyser (LECO TRuMac CNS Analyser). The soil available P was extracted based on Bray and Kurtz (1945) method. The extractable P concentration was determined

using the Auto-analyser (AA; LACHAT Instrument QuickChem FIA+8000 SERIES). Soil extractable bases (K, Ca and Mg) and soil CEC was determined using ammonium acetate pH 7 method. The soil exchangeable bases were determined using atomic absorption spectrophotometer (AAS, Perkin Elmer AAnalyst 400), while soil CEC was analysed using the Auto-analyser (AA).

Plant tissue sampling and analysis

The rice plants were harvested at 75 DAS. Biomass freshweight (DMWs), number of tillers (NT), root dry matter weight (DMWr) and plant height were recorded. After harvest, roots were washed free from the soil with a medium force spray of water to keep the root systems intact. The subsamples of plants and roots were then weighed and oven-dried for 72 h at 65 °C until it reached a constant weight. Dried plant samples were ground and sieved through 0.25 mm sieve and digested with wet digestion method (Wolf, 1982). Plant tissue was analysed for total P, K, Ca and Mg using AAS. Meanwhile, the total N of rice plant tissues was determined using LECO TruMac CNS analyser. Plant nutrients uptake was then calculated by multiplying the nutrient concentration obtained with the dry matter weight of rice crop.

For ¹⁵N analysis, an organic and inorganic nitrogen conversion using Kjeldahl-Rittenberg method into nitrogen gas form is requisites for the determination of ¹⁵N/¹⁴N ratios in the plant and soil samples. The atom percentage of ¹⁵N of rice straw and soil were analysed using the optical emission spectrometer (Model NOI7 Fischer). The quantity of N derived from the labelled fertilizer (*Ndff*), N yield (NU), ¹⁵N labelled fertilizer N yield (FNY) and N recovery using ¹⁵N labelled urea fertilizer in plants and soils are calculated according to the following equation:

Percentage of ¹⁵N labelled fertilizer recovery in aboveground biomass

Fraction of N derived from ¹⁵N labelled fertilizer (%*Ndff_{plant}*) is calculated as below:

$$\%N_{dff_{plant}} = \frac{\text{atom } \%^{15}\text{N excess in above ground biomass}}{\text{atom } \%^{15}\text{N excess fertilizer}} \times 100 \quad (1)$$

$$\text{Total N yield (g pot}^{-1}\text{)} = \% \text{ N} \times \text{Dry matter yield, (g pot}^{-1}\text{)} \quad (2)$$

$$\text{N fertilizer yield, (g pot}^{-1}\text{)} = \% \text{Ndff} \times \text{Total N yield, (g pot}^{-1}\text{)} \quad (3)$$

Percentage of ^{15}N labelled fertilizer recovery in soil

Fraction of ^{15}N labelled fertilizer ($\% \text{Ndff}_{\text{soil}}$) is calculated as below:

$$\% \text{ } ^{15}\text{N} \text{ labelled fertilizer recovery} = \frac{\text{Amount of N fertilizer yield}}{\text{Amount of N added to each pot}} \times 100 \quad (4)$$

in above ground biomass

Nitrogen recovery is a quantitative measure of the actual uptake of fertilizer N by the plant in relation to the amount of N added to the soil as fertilizer. A common form of expression of fertilizer N use efficiency (NUE) is plant recovery of the added fertilizer.

Statistical analysis

Data collected were analysed with SAS 9.1 software (SAS Institute Inc., USA). One-way analysis of variance (ANOVA) was performed to determine the significance effects of RHB treatments in crop growth parameters, soil properties and nitrogen recovery. The significant differences between means were determined by Least Significant Difference (LSD) test at $p \leq 0.05$. Single linear regression analysis was performed to examine the effect of RHB on rice yields, nutrient uptake and soil properties. Pearson correlation coefficients were calculated to determine the relationship between straw dry matter weight and soil properties, rice nutrient uptake using at $P = 0.05$ using SAS 9.2 software (SAS Institute Inc., USA).

RESULTS

Effect of RHB amendment on soil chemical characteristics

Table 2 represent the average of soil chemical properties as influenced by different RHB application rates after harvest. Biochar amendment significantly increased soil pH at all RHB application rates after harvest. Soil pH

showed a tendency to increase with increasing biochar application rates and increased by 0.6 and 1.3 units, respectively, as compared to the treatment with no biochar. The highest soil pH value was observed in the pot treated with maximum biochar application rates, while the lowest value was recorded in the control pot. However, soil total C increased significantly only at application of 5 Mg ha^{-1} RHB relative to control, while no significant changes was observed at rates of 10 Mg ha^{-1} and 20 Mg ha^{-1} . In particular, compared to the corresponding control, soil total N significantly increased by 18% and 38% with application of 10 and 20 Mg ha^{-1} RHB, respectively, in comparison to the corresponding control. Thus, the soil total N showed an increase in biochar treated pot with increasing rates of biochar application. Soil available P was significantly affected by all biochar application rates with an increment of 14% to 21% in comparison to control. The highest soil available P was observed in the pot with treatment of 5 Mg ha^{-1} RHB, while the lowest value was recorded in the control pot. As for soil extractable bases, biochar addition significantly increased soil extractable K only at high RHB application rate of 20 Mg ha^{-1} as compared to control pot, while did not change significantly at rate of 5 Mg ha^{-1} and 10 Mg ha^{-1} . However, no significant differences were observed in extractable bases (Ca and Mg) and soil CEC in biochar treated pot compared to untreated pot (Table 2).

Table 2. Selected soil chemical properties as influenced by different RHB application rates at harvest (75 DAS)

Parameters	Biochar rate, Mg ha ⁻¹			
	0	5	10	20
pH	5.41b* (±0.06)	5.88a (±0.19)	5.91a (±0.19)	6.09a (±0.25)
Total C, (g kg ⁻¹)	144.4b (±11.92)	157.3a (±19.77)	139.7b (±12.24)	129.7c (±11.51)
Total N, (g kg ⁻¹)	5.0c (±0.50)	5.3c (±0.69)	5.9b (±1.19)	6.9a (±0.80)
Available P, (mg kg ⁻¹)	132.34b (±6.87)	159.43a (±7.63)	156.32a (±7.17)	150.72a (±8.67)
Extractable K, (mg kg ⁻¹)	33.54b (±4.90)	37.46b (±1.57)	40.70b (±5.05)	90.92a (±0.30)
Extractable Ca, (mg kg ⁻¹)	1846a (±79.12)	1906a (±151.70)	1814a (±107.41)	1699a (±50.25)
Extractable Mg, (mg kg ⁻¹)	150a (±22.04)	146a (±17.95)	149a (±19.53)	132a (±10.27)
CEC, (cmol (+) kg ⁻¹)	29.55a (±3.31)	31.05a (±2.87)	31.23a (±3.08)	32.45a (±2.84)

* Means with different letters indicates significantly difference between treatment at $p \leq 0.05$ level by lsd test and $\pm$ value indicate mean standard deviation

Influence of RHB on crop growth performance

In this study, RHB application was observed to increase the DMWs, plant height and root dry matter weight (Table 3). Rice straw dry matter weight was significantly affected by biochar application rates of 10 and 20 Mg ha⁻¹, but not in the 5 Mg ha⁻¹ RHB, compared to corresponding control ($p \leq 0.05$). The highest DMWs were observed in the pot treated with 20 Mg ha⁻¹ RHB with an increment of 12% as compared to control pot which is recorded the lowest value of DMWs. As for number of tillers, it was increased significantly at all RHB application rates with an increment up to 12%

as compared to the control treatment. A higher number of tiller was recorded at application rate of 5 Mg ha⁻¹ RHB, whereas control recorded the lowest. However, a significant increase in plant height was observed only at harvest (75 DAS) compared to control treatment (Figure 1). As expected, DMWr with RHB treatment was statistically higher than those from the control ($p \leq 0.05$), indicating that RHB amendment are essential for better root development with an increment up to 46%. The highest DMWr was observed at application rate of 20 Mg ha⁻¹ RHB, while control was recorded the lowest DMWr value.

Table 3. Effect of RHB amendment on shoot dry matter weight (DMWs), number of tiller and plant height

Biochar rate, Mg ha ⁻¹	Parameters		
	DMWs, (g pot ⁻¹)	No. of tiller	Root dry weight, (g)
0	44.62b (±0.89)	41.25b (±0.85)	21.82c (±2.54)
5	44.88b (±0.71)	46.25a (±0.96)	25.78b (±2.36)
10	49.97a (±1.30)	44.75a (±1.29)	29.15ab (±2.00)
20	50.02a (±1.64)	42.50a (±1.71)	31.87a (±1.87)

* Means with different letters indicates significantly difference between treatment at $p \leq 0.05$ level by lsd test and $\pm$ value indicate mean standard deviation

Figure 1: Plant height as affected by RHB application rates.

Nutrient uptake responses with biochar amendment

The pot study showed that N, P, K and Ca uptake statistically ($p < 0.05$) improved as a result of biochar application compared to control treatment (Table 4). Nitrogen and phosphorus uptake significantly increased ($p \leq 0.05$) by RHB application at rates of 10 and 20 Mg ha^{-1} , but not for 5 Mg ha^{-1} as compared to control treatment. The highest N and P uptake was recorded at 20 Mg ha^{-1} RHB application rates with an increment of 7% and 30%, respectively, compared to control which was recorded the lowest value. In particular,

potassium uptake was significantly different at all RHB application rates as compared to control treatment. The K uptake showed a tendency to increase with increasing RHB application rate with the highest value was recorded at application of 20 Mg ha^{-1} , whereas control was recorded the lowest. Additionally, Ca uptake was only significant ($p < 0.05$) at application rate of 10 Mg ha^{-1} , but not for 5 and 20 Mg ha^{-1} compared to corresponding control. However, RHB addition did not result in a significant change on Mg, Mn, Zn, Fe and Cu uptake as compared to control treatment (Table 4).

Table 4. Rice shoot nutrient uptake as influenced by different RHB application rates

Biochar rate, Mg ha^{-1}	Nutrient uptake, mg pot^{-1}								
	N	P	K	Ca	Mg	Mn	Zn	Fe	Cu
0	692.13c (± 2.47)	132.76c (± 4.71)	440.41c (± 56.36)	106.54b (± 7.52)	105.05a (± 2.59)	14.48a (± 2.20)	6.22a (± 1.35)	35.36a (± 7.25)	2.32a (± 2.18)
5	703.03bc (± 6.66)	138.39c (± 8.79)	647.03b (± 66.21)	106.12b (± 7.06)	96.8bc (± 6.73)	14.79a (± 4.46)	5.21a (± 0.14)	35.81a (± 5.49)	1.96a (± 0.62)
10	714.40b (± 15.24)	162.98b (± 5.06)	769.09a (± 47.03)	116.15a (± 3.11)	102.16ab (± 4.29)	14.11a (± 4.85)	5.50a (± 0.66)	32.85a (± 9.96)	1.60a (± 1.39)
20	740.45a (± 6.29)	172.63a (± 4.54)	846.95a (± 42.00)	94.98c (± 8.48)	94.13c (± 8.55)	15.92a (± 4.84)	5.07a (± 0.68)	37.83a (± 2.28)	1.87a (± 0.38)

* Means with different letters indicates significantly difference between treatment at $p \leq 0.05$ level by lsd test and $\pm$ value indicate mean standard deviation

Nitrogen components in aboveground biomass and soil as affected by RHB application

The experimental results for nitrogen components in aboveground biomass and soil such as nitrogen derived from fertilizer (*Ndff*), nitrogen derived from soil (*Ndfs*), total N yield (TNY), fertilizer nitrogen yield (FNY), ¹⁵N labelled fertilizer recovery and total ¹⁵N labelled fertilizer recovery (TLFR) are summarized in Table 5. As for aboveground biomass, application RHB showed significant increase in *Ndfs* only at application rate of 20 Mg ha⁻¹, while no significant changes at application rate of 5 Mg ha⁻¹ and 10 Mg ha⁻¹ as compared to control treatment. The results obtained showed that RHB application rate of 10 Mg ha⁻¹ and 20 Mg ha⁻¹ significantly improved TNY in aboveground biomass by 3% and 7%, respectively, compared to control treatment. However, there was no remarkable difference for *Ndff*, FNY and percent¹⁵N labelled fertilizer recovery in aboveground biomass (AGLFR) as compared to control pot. As for soil, RHB

application rate of 10 Mg ha⁻¹ and 20 Mg ha⁻¹ significantly increase TNY in soil with an increment of 18% and 37%, respectively, as compared to control. Thus, soil TNY seems to increase with increasing rates of biochar. There was also a significant RHB effect on soil FNY under application rate of 20 Mg ha⁻¹, while no statistically different at application rate of 5 Mg ha⁻¹ and 10 Mg ha⁻¹ relative to control. Interestingly, at harvest percent¹⁵N labelled fertilizer recovery in soil (SLFR) only showed positive response at higher RHB application at 20 Mg ha⁻¹ with an increment of 47% in comparison to corresponding control. However, there were no significant changes in *Ndff* and *Ndfs* in soil. As for total ¹⁵N labelled fertilizer recovery, RHB application was only significantly difference at application rate of 20 Mg ha⁻¹ as compared to control (Figure 2). However, total ¹⁵N fertilizer recovery seems to increase linearly with increasing RHB application rate.

Table 5. Influence of RHB amendment on *Ndff*, *Ndfs*, TNY, TN, FNY, ¹⁵N labelled fertilizer recovery, and total ¹⁵N labelled fertilizer recovery in aboveground biomass and soil for rice

Biochar rate (Mg ha ⁻¹)	Above ground biomass					Soil					Total ¹⁵ N labelled fertilizer recovery, (%)
	<i>Ndff</i> , (%)	<i>Ndfs</i> , (%)	TNY, (mg)	FNY, (mg)	¹⁵ N labelled fertilizer recovery (%)	<i>Ndff</i> , (%)	<i>Ndfs</i> , (%)	TNY, (mg)	FNY, (mg)	¹⁵ N labelled fertilizer recovery (%)	
0	39.49a	60.51b	692.13c	273a	40.97a	0.29a	99.71a	45.30c	130b	19.42b	60.39b
	(±0.92)	(±0.92)	(±2.47)	(±6.28)	(±0.94)	(±0.02)	(±0.02)	(±1.94)	(±14.68)	(±2.20)	(±1.54)
5	39.78a	60.22b	703.03bc	279a	41.93a	0.27a	99.72a	48.33c	132b	19.83b	61.76b
	(±0.38)	(±0.38)	(±6.66)	(±2.83)	(±0.42)	(±0.03)	(±0.04)	(±2.81)	(±9.07)	(±1.36)	(±1.53)
10	38.94a	61.07b	714.40b	278a	41.72a	0.25a	99.75a	53.30b	136b	20.34b	62.06b
	(±0.25)	(±0.25)	(±15.24)	(±6.60)	(±0.99)	(±0.01)	(±0.01)	(±3.05)	(±11.09)	(±1.66)	(±0.77)
20	37.50a	62.50a	740.45a	277a	41.64a	0.29a	99.70a	62.46a	191a	28.56a	70.2a
	(±0.30)	(±0.30)	(±6.29)	(±2.55)	(±0.38)	(±0.04)	(±0.05)	(±3.45)	(±14.09)	(±5.62)	(±5.44)

* Means with different letters indicates significant difference between treatment at 0.05 level by lsd test and ± value indicate mean standard deviation. (*Ndff*: N derived from ¹⁵N labelled fertilizer; TNY: Total N yield; FNY: fertilizer N yield). * *Ndfs*: N derived from soil.

*% *Ndfs*= 100 - % *Ndff*

Figure 2: Changes of ¹⁵N labelled fertilizer recovery in plant and soil under RHB amendment.

DISCUSSION

The characteristic of the soil plays an important role to ensure the plant's ability to absorb water and nutrients for better crop growth performance. Soil pH was significantly higher in all biochar amended soils than control treatment (Table 2). Thus, it might attribute to the alkalinity of the biochar use in this experiment which leads to greater increase in soil pH compared with control treatment. Soil pH was significantly correlated with soil total N and available P also explains why soil pH was the highest when biochar was added (Table 1). Similar changes in soil pH as the result of biochar application have been reported in previous studies, i.e. acid sulfate soil using RHB in West Kalimantan, Indonesia (Masulili et al., 2010) and incubation study on acid Ultisol using plant material biochar such as canola, rice, corn, wheat, mung bean, peanut, soybean, pea and faba bean straw (Yuan et al., 2011).

The incorporation of RHB increased the soil total C (Table 2) ($p \leq 0.05$). The effect of

biochar on soil total C might have occurred due to its high carbon content (413.23 g kg^{-1} , Table 1). The differences between biochar treatments in this experiment are contradicting the findings of Jien and Wang, (2013) who reported biochar application did not exhibit a significant increase in SOC level even though biochar high total C content (78.3%). Additionally, application of RHB increased the soil total N, which response can only be observed at higher RHB application (10 and 20 Mg ha^{-1}). This effect may attribute to high N content of RHB (Table 1). This improvement of soil total N with biochar addition is in concordance with study by Zhang et al., (2010), who reported increase in soil total N with wheat straw biochar on clay soil in Jiangsu Province, China. Meanwhile, soil available P under RHB amendment significantly higher compared to the corresponding control ($p < 0.05$). The positive increase in soil available P may be attributed to high P concentration in RHB ash content (Steiner et al., 2009) and direct P supplied from the RHB (Wu et al., (2014).

Based on the results that has been obtained from this experiment, biochar application significantly increased root dry weight leading to the increased in straw dry matter weight and number of tiller (Table 3). The positive growth response might be attributed to the nutrient directly supplied by the biochar. This is in accordance with earlier studies that showed that an increases in root development in soils receiving rice-husk biochar treatment (Carter et al., 2013; Olmo et al., 2014). Positive yield as well as biomass responses resulting from biochar applications were reported repeatedly and attributed to various mechanisms, including the benefit from direct nutrient additions, improves nutrient availability, improves in root development, increases nutrient retention

through higher exchange capacity, improves soil physical characteristics, and positive effects on soil microorganisms (Glaser et al., 2002; Lehmann and Rondon, 2006; Jeffery et al., 2010). A similar effect was also reported by studies done by Lehmann et al. (2003); Lehmann et al. (2006); Steiner et al. (2007); and Van Zwieten et al. (2010) and often showed much higher increase rates. For all RHB treatments, the rice straw dry matter weight was positively correlated with total ^{15}N labelled fertilizer recovery, DMWr, soil pH, soil total C and total N, soil extractable K, phosphorous and potassium uptake (Table 6). However, DMWr was positively correlated with soil pH, soil total N, available P, soil extractable K, phosphorus and potassium uptake.

Table 6. Coefficients of correlation for aboveground biomass ^{15}N labelled fertilizer recovery, soil ^{15}N labelled fertilizer recovery, total ^{15}N labelled fertilizer recovery, soil properties (pH, TC, TN, avai. P), nutrient uptake (N, P, K) with four different RHB amendment rates

	AGLFR	SLFR	TLFR	DMWr	Soil pH	Soil total c	Soil total N	Avai. P	Soil Extrac. K	N uptake	P uptake	K uptake
DMWs	-0.009 ^{ns}	0.487 ^{ns}	0.516 [*]	0.683 ^{**}	0.622 [*]	-0.673 [*]	0.717 ^{**}	0.306 ^{ns}	0.716 ^{**}	0.343 ^{ns}	0.901 ^{***}	0.761 ^{**}
AGLFR		0.103 ^{ns}	0.190 ^{ns}	0.265 ^{ns}	0.305 ^{ns}	0.188 ^{ns}	0.167 ^{ns}	0.447 ^{ns}	0.034 ^{ns}	0.362 ^{ns}	0.109 ^{ns}	0.488 ^{ns}
SLFR			-0.651 ^{**}	0.471 ^{ns}	0.224 ^{ns}	-0.582 [*]	0.808 ^{***}	0.184 ^{ns}	0.779 ^{***}	0.501 [*]	0.585 [*]	0.458 ^{ns}
TLFR				0.523 [*]	0.304 ^{ns}	-0.553 [*]	-0.553 [*]	0.287 ^{ns}	0.783 ^{***}	0.566 [*]	0.639 [*]	0.562 [*]
DMWr					0.656 ^{***}	-0.290 ^{ns}	0.820 ^{***}	0.593 [*]	0.588 [*]	0.481 ^{ns}	0.717 ^{**}	0.811 ^{***}
Soil pH						-0.277 ^{ns}	0.555 [*]	0.601 [*]	0.602 [*]	0.520 [*]	0.616 [*]	0.849 ^{***}
Soil TC							-0.584 [*]	0.116 ^{ns}	-0.760 ^{***}	-0.348 ^{ns}	-0.709 ^{**}	-0.424 ^{ns}
Soil TN								0.336 ^{ns}	0.883 ^{***}	0.704 ^{**}	0.839 ^{***}	0.798 ^{***}
Soil avai. P									0.095 ^{ns}	0.350 ^{ns}	0.261 ^{ns}	0.603 [*]
Soil extrac. K										0.655 ^{**}	0.821 ^{***}	0.709 ^{**}
N uptake											0.507 [*]	0.694 ^{**}
P uptake												0.834 ^{***}

* Means with different letters indicates significant difference between treatment at 0.05 level by lsd test. (DMWs: Shoot dry matter weight; AGLFR: Aboveground biomass ^{15}N labelled fertilizer recovery; SLFR: Soil ^{15}N labelled fertilizer recovery; TLFR: Total ^{15}N labelled fertilizer recovery; DMWr: Root dry matter weight; TC: Carbon; TN: Nitrogen; Avai. P: Available phosphorus; Extrac. K: Extractable potassium; P: phosphorus; K: Potassium).

Result obtained from this study indicated that RHB amendment could increase shoot N, P, K and Ca uptake. Thus, addition of RHB amendment into agricultural soil also reported to improve nutrient contents in soil as N, P and K which could enhance nutrient uptake and benefits the plant growth (Major et al., 2010). Apart from direct nutrient additions or nutrient retention with biochar application, it

was also reported to enhance crop productivity through improving soil quality, including soil porosity (Zhang et al., 2010) and alteration of soil microbial population and functions (Pietikainen et al., 2000). However, no statistical significance was detected for Mg Mn and Zinc in this study, which was contrary with the results of (Maru et al., 2015).

Nitrogen is one of the most important nutrients use for plant growth development. Plant mainly received nitrogen sources from fertilizer and soil, but also from N fixation by plant. The results obtained revealed the effect of biochar application are in accordance with previous studies which increase N availability to crops (Chan et al., 2007; Chan et al., 2008; Pan et al., 2009), N recovery and rice productivity in a long term monitored rice paddy in this same area (Pan et al., 2009) with biochar application. Furthermore, no significant difference in nitrogen recovery in aboveground biomass could be attributed to retention of N by biochar, volatilization of NH_3 , nitrification and denitrification (Liang et al., 2006; Steiner et al 2008; Cassman et al., 2012). However, the increased RHB application rates simultaneously increase soil N recovery (Table 5), which is an agreement with the previous studies (Clough and Condon, 2010; Cheng et al., 2012; Taghizadeh-Toosi et al., 2012) due to reduce N leaching and adsorb NH_3 with biochar application. In this pot study, there was no leaching, thus, labelled fertilizer N unaccounted losses of 29.8 to 39.6% may be attributed NH_3 volatilization and denitrification. Firestone et al, (1982) reported that 0 to 70% of applied fertilizer N was lost from agricultural soil due to crop management practices, soil chemical and physiological and climatic condition. In addition, total ^{15}N labelled fertilizer recovery was positively correlated with DMW_r , soil total C and N, soil extractable K and nutrient uptake (N, P, K).

CONCLUSION

Results indicate that RHB amendment enhanced fertilizer N recovery in soil by 47% which may be taken up by the plant at later stage of growth, but had no significant difference in fertilizer N recovery in aboveground biomass between with and without RHB. However, total ^{15}N fertilizer recovery was only significantly affected at 20 Mg ha^{-1} RHB application rate with an increment of 16%. Also, application of RHB improved soil chemical properties such soil pH, total C, total N, available P and extractable K of paddy soil. The RHB significantly increased shoot dry matter weight and root dry matter weight and N, P, and

K uptake by plant. Results of this study justified further investigation of the effect of RHB application on rice up to maturity stage.

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