agronomy

Article

Nitrogen Fertilizer Reduction Combined with Biochar Application Maintain the Yield and Nitrogen Supply of Rice but Improve the Nitrogen Use Efficiency

Chuanchuan Ning [1,2,3], Rui Liu [1,2,3], Xizhi Kuang [1,2,3], Hailang Chen [1,2,3], Jihui Tian [1,2,3] and Kunzheng Cai [1,2,3,*]

1 Guangdong Provincial Key Laboratory of Eco-Circular Agriculture, South China Agricultural University, Guangzhou 510642, China
2 College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China
3 Key Laboratory of Tropical Agro-Environment, Ministry of Agriculture, South China Agricultural University, Guangzhou 510642, China
* Correspondence: kzcai@scau.edu.cn

check for updates

Citation: Ning, C.; Liu, R.; Kuang, X.; Chen, H.; Tian, J.; Cai, K. Nitrogen Fertilizer Reduction Combined with Biochar Application Maintain the Yield and Nitrogen Supply of Rice but Improve the Nitrogen Use Efficiency. *Agronomy* **2022**, *12*, 3039. https://doi.org/10.3390/agronomy12123039

Academic Editors: Othmane Merah, Purushothaman Chirakkuzhyil Abhilash, Magdi T. Abdelhamid, Hailin Zhang and Bachar Zebib

Received: 27 October 2022
Accepted: 29 November 2022
Published: 1 December 2022

Abstract: Excessive application of nitrogen (N) fertilizer will cause many adverse consequences in paddy fields, especially for the reduction in N use efficiency. Biochar can be used to replace part of N fertilizer for rice production. A field experiment of 2-year/four-season was conducted to investigate the effects of N fertilizer reduction combined with rice straw biochar application on rice yield, soil fertility, and N use efficiency. The experiment contained six treatments: No N application (CK), customary N application (N100), 20% N reduction (N80), 20% N reduction + biochar (N80+BC), 40% N reduction (N60), and 40% N reduction + biochar (N60+BC). Compared with N100, N reduction alone had no significant impact on the number of tillers and aboveground biomass of rice, exceptfor N60 which slightly reduced grain yield, while biochar incorporation tended to obtain higher tillers, aboveground biomass, and grain yield of rice compared with N reduction alone. The average contribution of biochar to grain yield on the basis of N80 and N60 were 5.8% and 7.7%, respectively. Notably, biochar incorporation further improved the agronomic N efficiency (54.5–309.4% over N100) and apparent N recovery (25.7–150.5% over N100) on the basis of N reduction. Furthermore, biochar application could not only maintain N nutrition level of rice, but also improve soil fertility mainly by increasing soil pH and organic matter. Therefore, integrated application of mineral N fertilizer and biochar is a feasible nutrient management measure to increase rice yield and soil fertility, and improve N use efficiency in paddy ecosystem.

Keywords: straw biochar; rice yield; N dynamics; N uptake; paddy

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops that feeds more than half of the world's population [1]. Nitrogen (N), as one of the most basic macroelements, participates in many important physiological processes, such as the synthesis of proteins, nucleic acids, phospholipids, and chlorophyll, and plays a dominant role in rice production [2,3]. Therefore, mineral N fertilizers including urea, ammonium sulfate, ammonium chloride, etc. are often excessively applied in paddy fields [4]. China is one of the largest consumers of N fertilizer in the world, and its N fertilizer input accounts for about 32% of the world's total consumption [5]. Long-term excessive use of mineral N fertilizer not only causes adverse effects on rice, such as lodging, aggravation of pests and diseases, and decrease in yield and quality [6,7], but also contributes to soil compaction, acidification, and reduction in organic matter content [8,9]. Furthermore, excessive use of N fertilizer leads to a series of environmental problems, such as water eutrophication, groundwater pollution, and greenhouse gas emission [10–12], which has become a major concern.

With the improvement in people's awareness of agricultural product safety and environmental protection, reducing N fertilizer application on the premise of ensuring crop yield has become a common requirement for sustainable agriculture [13]. Many studies have shown that appropriate N fertilizer reduction will not affect crop yield but help improve the N use efficiency, which can save production costs and ease the environmental load [14,15]. However, improper N fertilizer reduction will inevitably harm crop yield. For instance, Su et al. found that 50% N reduction reduced maize yield by 7.7% through a 4-year field experiment in barren soil areas [16]. Tian et al. showed that rape yields were decreased by 11.7–31.7% under 20–40% N reduction through a 2-year field experiment [17]. Therefore, whether N reduction is feasible depends on the amount of N reduction and the N level of the soil itself. Notably, even in the paddy with a high N level, if the soil is always in a negative N surplus, N reduction may not affect rice yield in the short term, but it will inevitably cause a decrease in rice yield with the continuous rice planting.

At present, the integrated application of inorganic and organic resources has been an attractive and effective way of nutrient management in the field [18]. In addition to organic fertilizers (straw, manure, green manure, etc.), which are often applied in combination with chemical fertilizers [19,20], biochar, a by-product of agricultural waste, is being increasingly used in agricultural production [21,22]. Biochar is an aromatic and carbon-rich solid substance produced by the pyrolysis of biological residues at high temperature under anaerobic conditions, and it has the characteristics of high stability, high porosity, large specific surface area, and strong adsorption capacity [23,24]. Previous studies have indicated that the application of biochar in the agro-ecosystem is beneficial in many aspects. In most soils, especially acidic soils, biochar addition can increase soil pH and macroaggregate ratio, reduce soil bulk density, and increase soil organic matter and available nutrient content, thereby promoting crop growth and increasing yield [21,25,26]. Due to the strong adsorption capacity and stability, the application of biochar helps in sequestering carbon in the soil and thus reducing greenhouse gas emissions [27,28], and at the same time, it also plays a role in alleviating the accumulation of heavy metals and organic pollutants in crops [29–31].

Numerous studies have shown that the application of biochar has a positive effect on soil N level, crop N uptake, and N use efficiency of fertilizer, while this effect is closely related to the raw material, amount, and time of biochar application, as well as soil properties [32–34]. On the one hand, owing to its porosity, high cation exchange capacity, and rich functional groups, biochar can adsorb N and other nutrients in the soil, reduce N leaching loss and ammonia volatilization [32,35], and thus improve crop N uptake and N use efficiency [33,36]. On the other hand, application of biochar can affect soil pH, temperature, humidity, as well as the composition and activity of microorganisms, thereby affecting soil N mineralization, which increases soil N content and availability [37,38]. Furthermore, biochar can promote the expression of functional genes and the increase in enzymes activity involved in N cycling in the soil, and accelerate the turnover of soil N pools [34,39].

South China is an important double-cropping rice production area in China due to its advantages in water and heat resources. In this region, the phenomenon of excessive use of N fertilizer is relatively serious, which causes soil nutrient imbalance and low N use efficiency (about 30–35%) [40]. In recent years, with the promotion of mechanization, the majority of rice straw are directly returned to the field. Straw returning is undoubtedly an important nutrient management measure, but it may bring some unfavorable factors, such as aggravating rice diseases and pests, affecting rice growth, and polluting water environment [41,42]. Therefore, straw biochar to replace part of N fertilizer is an ideal method for rice production. Although various field experiments have been conducted to research the dynamic of N due to biochar application around the world [43,44], it is still scarce for understanding the N use efficiency of fertilizer and the contribution of biochar to rice yield under different N fertilizer reductions combined with biochar application in the paddy field.

In the present study, we hypothesized that N fertilizer reduction combined with biochar can maintain rice yield and soil nutrient fertility but improve the N use efficiency of fertilizer, and the effects of biochar on N use efficiency and yield contribution depend on the amount of N fertilizer reduction. Therefore, a field experiment over 2-year/four-season of N fertilizer reduction combined with or without straw biochar were conducted to investigate and evaluate the effects of different fertilization regimes on rice yield, soil fertility, and N use efficiency of fertilizers, which will provide scientific evidence for N fertilizer reduction and biochar utilization in paddy field.

2. Materials and Methods

2.1. Study Site

The field trial was located on the rice farm at South China Agricultural University (113°21′ E, 23°9′ N), Tianhe District, Guangzhou, Guangdong Province, China. This area has a subtropical monsoon climate, and the annual average temperature is 20 to 22 °C and total rainfall is approximately 1720 mm. The rainfall and average temperature per month during the experimental years are shown in Figure 1. The season for rice cultivation is from April to November, and usually divided into early rice season (April to July) and late rice season (August to November). The soil type in the trial field is Stagnic Anthrosols, which develops from the diluvial and alluvial deposit of granite, and the initial properties of the surface soil (0–0.2 m) were as follows: pH 5.88, CEC 5.25 cmol·kg^{-1}, 36.36 g·kg^{-1} organic matter, 2.65 g·kg^{-1} total N, 0.58 g·kg^{-1} total P, 11.07 g·kg^{-1} total K, 2.70 mg·kg^{-1} ammonium N, 4.96 mg·kg^{-1} nitrate N, 21.60 mg·kg^{-1} available P, and 73.42 mg·kg^{-1} available K.

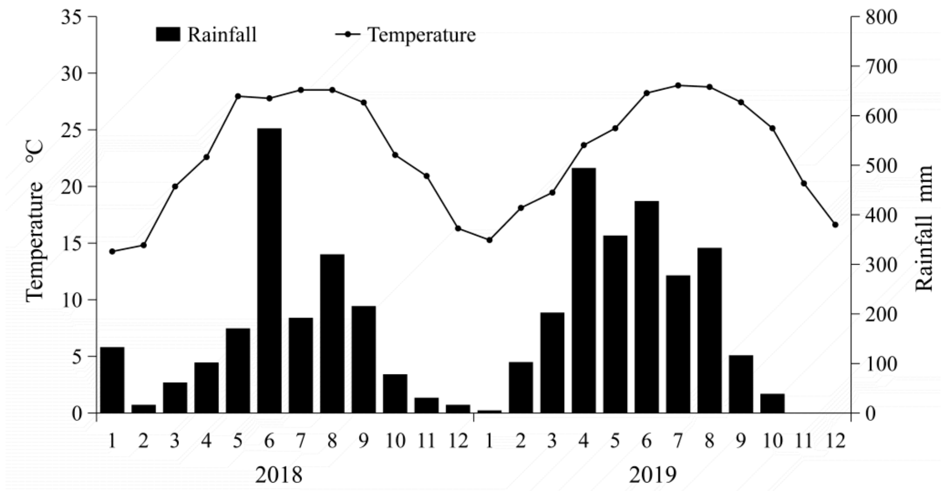

Figure 1. Rainfall and average temperature per month during the experimental years (2018, 2019). Numbers 1–12 in the X axis represent the 12 months in a year.

2.2. Experimental Material

The rice variety tested was Hua-hang 31, a high-quality variety provided by the College of Agriculture, South China Agricultural University. The biochar used in the experiment was produced by Jin-He-Fu Agricultural Company in Liaoning Province, China. It was made from the slow pyrolysis of rice straw at 600 °C, with the following properties: pH 9.04, 50.55% C, 1.79% H, 1.89% N, 0.54% P, 0.89% K, 0.17% S, C/N 26.79, and C/H 28.30.

2.3. Experimental Design

Field trial was conducted in 2018–2019 including four rice planting seasons. There were six treatments with three replicates: (1) Local customary N application rate, 180 kg·ha^{-1}, N100, (2) 20% N reduction, 144 kg·ha^{-1}, N80, (3) 20% N reduction with biochar, N80+BC,

(4) 40% N reduction, 108 kg·ha^{-1}, N60, (5) 40% N reduction with biochar, N60+BC, and (6) no N and biochar application, CK. Treatments were arranged in a randomized complete block design. When rice seedlings grew the fourth leaf, they were transplanted to the experiment field with a row spacing of 0.2 m. The field plot area of each treatment was 15 m^2 (3 × 5 m), and each plot was separated by a 30-cm-high soil dam.

For treatments with biochar, only in the early season of each year, 15 t·ha^{-1} of biochar was mixed into the cultivated soil 7 d before rice transplanting. For all treatments, urea, calcium superphosphate, and potassium chloride were applied as N, P, and K fertilizers, respectively. Each plot received 75 kg·ha^{-1} of P_2O_5 and 120 kg·ha^{-1} of KCl in each planting season. P fertilizer was applied as base fertilizer before rice transplanting, and N fertilizer and K fertilizer were applied half on the 7th and 14th day after transplanting, respectively. In addition, all soil dams were covered with plastic film to inhibit the growth of weeds, insecticides and fungicides were used in the early stage of pests and diseases, irrigation and drainage were carried out according to the actual conditions.

2.4. Rice Plant Sampling and Analysis

At the ripening stages of rice in each season, two hills of rice plants in each plot were sampled. Rice plants were cut with a sickle from the ground and the number of effective tillers were recorded, and then rapidly divided into stems, leaves, and spikes. Rice stems, leaves, and spikes were baked first at 105 °C for 0.5 h, and then baked to a constant weight at 80 °C. The dried stems, leaves, and spikes were weighed and summed to obtain the aboveground dry biomass of rice. Then, stems, leaves, and spikes were ground separately to measure N concentration using the Kjeldahl method. The aboveground N uptake in sampled rice was equal to the sum of the product of the N concentration and the dry biomass of each part. The aboveground dry biomass and N uptake of rice per hectare were obtained according to the sampled rice and the number of rice per hectare.

2.5. Soil Sampling and Analysis

After rice plants were sampled, soil samples (0–20 cm depth) were collected using a soil auger. Three soil samples were collected from each plot and mixed into one composite sample. The soil samples were air-dried and then sieved through 1 and 0.15 mm meshes to measure pH, cation exchange capacity (CEC), organic matter, available P, available K, total N, ammonium N, and nitrate N.

Soil pH was measured in the suspension of 5 g of soil and 25 mL of 1 mol·L^{-1} KCl solution. Soil CEC was determined using the $BaCl_2$-$MgSO_4$ method [45]. Soil organic matter was determined using the Walkley-Black method [46]. Soil available P was determined with colorimetry by extracting 5.0 g of soil with 100 mL of 0.5 mol·L^{-1} $NaHCO_3$ [47]. Soil available K was determined by a flame atomic absorption spectrometry by extracting from 5.0 g of soil with 50 mL of 1.0 mol·L^{-1} NH_4OAc [48]. Soil total N was determined using the Kjeldahl method. Soil ammonium N and nitrate N were determined colorimetrically using a spectrophotometer [49]. Ammonium N was reacted with salicylate and sodium hypochlorite solution to produce a blue compound and measured at 660 nm. Nitrate N was reduced to nitrite by hydrazine in an alkaline solution with a copper catalyst, reacted with sulfanilamide to form a pink compound, and measured at 550 nm.

2.6. Yield and N Fertilizer Use Efficiency

The remaining rice in the plot was harvested and threshed manually, and grain yield per plot was dried and weighed. The contribution of biochar to grain yield on the basis of different N fertilizer levels wascalculated as the difference in the average yield between treatments with and without biochar [50], as follows:

$$\text{The contribution of biochar to grain yield (\%)} = (Y_b - Y_0)/Y_0 \times 100 \quad (1)$$

where Y_b and Y_0 are the average grain yield (kg·ha^{-1}) under treatments with and without biochar, respectively.

N use efficiency of fertilizers was assessed according to the following series of indexes. Agronomic N efficiency (AE_N) was calculated as follows [51]:

$$AE_N\ (kg \cdot kg\ N^{-1}) = (Y_N - Y_{CK})/N \quad (2)$$

where Y_N and Y_{CK} are the grain yield ($kg \cdot ha^{-1}$) under treatment with N fertilizer and CK, respectively. N is the N fertilizer amount ($kg \cdot ha^{-1}$) in the N fertilizer plot.

Apparent N recovery (AR_N) was calculated as follows [52]:

$$AR_N\ (\%) = (U_N - U_{CK})/N \times 100 \quad (3)$$

where U_N and U_{CK} are the N uptake ($kg \cdot ha^{-1}$) of aboveground rice under treatment with N fertilizer and CK, respectively. N is the N fertilizer amount ($kg \cdot ha^{-1}$) in the N fertilizer plot.

N partial factor productivity (PFP_N) was calculated as follows [51]:

$$PFP_N\ (kg \cdot kg\ N^{-1}) = Y_N/N \quad (4)$$

where Y_N is the grain yield ($kg \cdot ha^{-1}$) under treatment with N fertilizer. N is the N fertilizer amount ($kg \cdot ha^{-1}$) in the N fertilizer plot.

2.7. Statistical Analysis

The results are presented as mean ± standard error. The data were statistically analyzed using SPSS 18.0 by one-way ANOVA and Duncan's method for multiple comparisons at a 5% significance level. Moreover, Pearson correlation and partial correlation coefficients were analyzed using SPSS 18.0. All figures were constructed using Origin 8.0.

3. Results

3.1. Effects of Fertilization Regimes on the Aboveground Biomass, Grain Yield, and N Uptake of Rice

The number of tillers, aboveground dry biomass, and grain yield of rice were higher in late season than in early season, and higher in 2019 than in 2018 (Table 1). N fertilizer reduction alone (N80, N60) or combined application with biochar (N80+BC, N60+BC) had no significant impact on the number of tillers and the aboveground dry biomass of rice in general compared with N100, and in 2018, application of biochar (N80+BC, N60+BC) increased the number of tillers of rice compared with N fertilizer reduction alone. Similarly, in the late season of 2018, application of biochar tended to obtain higher aboveground dry biomass of rice compared with N fertilizer reduction alone. During the trial, compared with N100, 40% N reduction (N60) slightly reduced grain yield, and combined application with biochar increased the grain yield compared with N fertilizer reduction alone. Notably, the grain yields under N80+BC and N60+BC were even higher than under N100, and the increase rate reached 0.3–18.1%. In addition, no significant difference in grain yield was observed between N80+BC and N60+BC.

As shown in Figure 2, a large difference was observed in the contribution of biochar to grain yield in different planting seasons, and it was higher in early season than in late season, and higher in 2018 than in 2019. During the trial, the contribution of biochar to grain yield on the basis of 40% N reduction (15.8%, 3.7%, 10.0%, and 3.4% in the four seasons, respectively) was always higher than on the basis of 20% N reduction (13.3%, 3.2%, 6.9%, and 2.2% in the four seasons, respectively). The average contributions of biochar to grain yield in the four seasons on the basis of 20% and 40% N reduction were 5.8% and 7.7%, respectively.

Furthermore, N uptake in aboveground rice showed significant differences among different planting seasons, with a similar trend to aboveground dry biomass of rice (Figure 3). Undoubtedly, N uptake in aboveground rice under CK was significantly lower than under other treatments. In late season of 2018 and early season of 2019, N fertilizer reduction alone (N80, N60) resulted in a slight reduction in N uptake in aboveground rice compared with N100, and combined application with biochar increased N uptake in aboveground rice compared with the corresponding N fertilizer reduction, especially N80+BC, it was observed to have the highest N uptake in aboveground rice.

Table 1. Effects of N fertilizer reduction combined with biochar application on the number of tillers, aboveground dry biomass, and grain yield of rice.

Planting Seasons	Treatments	Number of Tillers	Aboveground Dry Biomass (kg·ha^{-1})	Grain Yield (kg·ha^{-1})
Early season in 2018	N100	8.3 ± 0.3ab	10,609.7 ± 459.1a	4488.9 ± 228.1bc
	N80	7.5 ± 0.1ab	10,281.3 ± 266.6a	4422.2 ± 121.9bc
	N80+BC	9.2 ± 0.3a	11,244.1 ± 519.3a	5011.1 ± 100.8ab
	N60	8.2 ± 0.3ab	10,692.9 ± 225.6a	4577.8 ± 127.8bc
	N60+BC	9.3 ± 0.3a	11,277.5 ± 226.1a	5300.0 ± 33.3a
	CK	7.0 ± 0.2b	8796.2 ± 105.6a	3933.3 ± 19.2b
Late season in 2018	N100	11.2 ± 0.2a	15,146.3 ± 208.5ab	5766.7 ± 112.8a
	N80	10.0 ± 0.2ab	13,909.1 ± 214.4b	5766.7 ± 106.0a
	N80+BC	11.3 ± 0.3a	16,750.9 ± 259.6a	5950.0 ± 140.9a
	N60	10.5 ± 0.2ab	15,544.5 ± 254.8ab	5666.7 ± 58.8a
	N60+BC	10.8 ± 0.2ab	14,756.3 ± 187.5b	5877.8 ± 32.1a
	CK	9.2 ± 0.2b	11,217.7 ± 269.9c	4988.9 ± 115.7b
Early season in 2019	N100	10.7 ± 0.4a	15,874.3 ± 446.7a	5277.8 ± 90.5ab
	N80	10.8 ± 0.4a	15,521.6 ± 287.1a	5155.6 ± 61.2ab
	N80+BC	10.0 ± 0.1a	16,131.3 ± 286.0a	5511.1 ± 39.0a
	N60	10.3 ± 0.1a	14,751.5 ± 148.6a	5011.1 ± 89.1b
	N60+BC	9.8 ± 0.3a	15,643.9 ± 202.6a	5511.1 ± 121.9a
	CK	8.7 ± 0.2a	12,422.8 ± 216.4b	4488.9 ± 55.9c
Late season in 2019	N100	12.7 ± 0.5a	19,601.4 ± 239.2a	6388.9 ± 167.2a
	N80	13.0 ± 0.1a	20,614.1 ± 260.2a	6466.7 ± 33.3a
	N80+BC	13.5 ± 0.4a	20,465.5 ± 294.5a	6611.1 ± 122.4a
	N60	13.3 ± 0.4a	19,366.5 ± 205.3a	6200.0 ± 77.8a
	N60+BC	12.3 ± 0.3a	20,362.8 ± 310.7a	6411.1 ± 157.7a
	CK	11.3 ± 0.3a	15,549.3 ± 447.9b	5522.2 ± 105.0b

N100, 180 kg N·ha^{-1}; N80, 144 kg N·ha^{-1}; N80+BC, 144 kg N·ha^{-1} + biochar; N60, 108 kg N·ha^{-1}; N60+BC, 108 kg N·ha^{-1} + biochar; CK, no N and biochar application. Different small letters indicate significant differences among different treatments at the same planting season ($p < 0.05$).

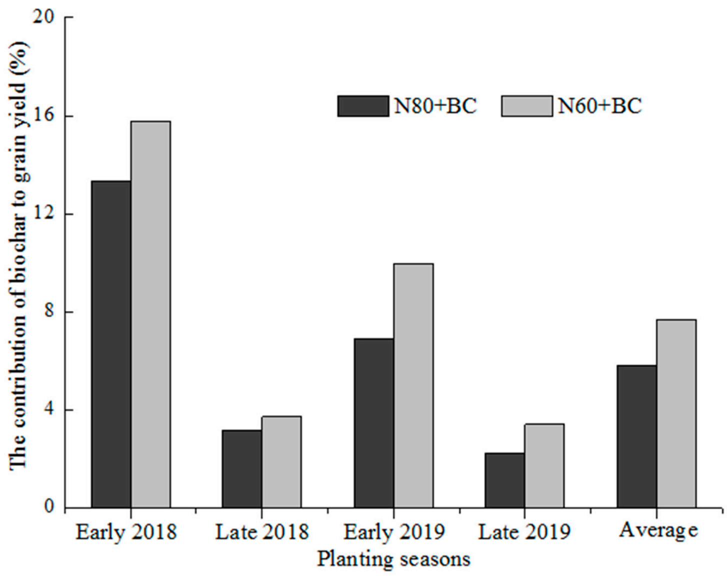

Figure 2. The contribution of biochar to rice grain yield on the basis of different N fertilizer levels. "The contribution" was calculated as the average grain yield of three repeated plots under treatments with or without biochar. N80+BC, 144 kg N·ha^{-1} + biochar; N60+BC, 108 kg N·ha^{-1} + biochar. "Average" refers to the average of the four seasons.

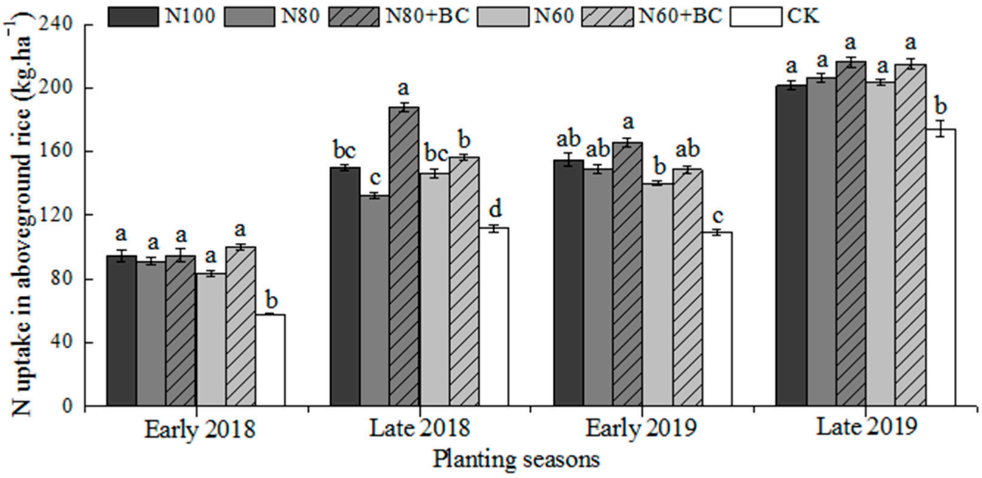

Figure 3. Effects of N fertilizer reduction combined with biochar application on N uptake in aboveground rice. N100, 180 kg N·ha^{-1}; N80, 144 kg N·ha^{-1}; N80+BC, 144 kg N·ha^{-1} + biochar; N60, 108 kg N·ha^{-1}; N60+BC, 108 kg N·ha^{-1} + biochar; CK, no N and biochar application. Different small letters indicate significant differences among different treatments at the same planting season ($p < 0.05$).

3.2. Effects of Fertilization Regimes on Soil Nutrient Fertility

Throughout the trial period, compared with N100, N fertilizer reduction alone (N80, N60) had no significant impacts on soil pH, CEC, organic matter, available P, and available K (Table 2). No matter compared to N100 or corresponding N fertilizer reduction, combined application with biochar (N80+BC, N60+BC) also had no significant impacts on soil CEC, available P, and available K in general. In the late season of 2018 and 2019, soil pH under the treatments with biochar (N80+BC, N60+BC) was significantly higher than under other treatments (Table 2). Compared with N100, N80+BC and N60+BC increased soil pH by 0.49 and 0.10 units, respectively in late season of 2018, and increased soil pH by 0.30 and 0.37 units, respectively in late season of 2019. In 2019, soil organic matter under treatments with biochar (N80+BC, N60+BC) was significantly higher than under other treatments (Table 2). For example, in early season, N80+BC and N60+BC increased soil organic matter by 13.3% and 27.7% compared with N100, respectively. In late season, they increased soil organic matter by 28.0% and 28.1% compared with N100, respectively.

Soil total N, ammonium N, and nitrate N fluctuated greatly in different planting seasons (Figure 4). However, during the trial, N fertilizer reduction or combined application with biochar had no significant impacts on soil N overall. In late season of 2018, N80 significantly reduced soil total N by 19.8% compared with N100. In early and late seasons of 2018, N80+BC significantly increased soil total N by 35.4% and 31.7% compared with N80, respectively (Figure 4a). There was no significant difference in soil ammonium N among different treatments (Figure 4b). In early season of 2018, N fertilizer reduction (N80, N60) or combined application with biochar (N80+BC, N60+BC) was observed to have higher soil nitrate N compared with N100, especially N80 and N60. In early season of 2019, N fertilizer reduction (N80, N60) had no significant impacts on soil nitrate N compared with N100, and combined application with biochar (especially N80+BC) resulted in a significant increase in soil nitrate N compared with other treatments (Figure 4c).

Table 2. Effects of N fertilizer reduction combined with biochar application on soil pH, CEC, organic matter, available P, and available K.

Planting Seasons	Treatments	pH	CEC ($cmol \cdot kg^{-1}$)	Organic Matter ($g \cdot kg^{-1}$)	Available P ($mg \cdot kg^{-1}$)	Available K ($mg \cdot kg^{-1}$)
Early season in 2018	N100	6.03 ± 0.03a	5.18 ± 0.15a	30.37 ± 0.98a	23.67 ± 0.96a	52.92 ± 0.49a
	N80	6.10 ± 0.02a	5.36 ± 0.12a	27.91 ± 0.71ab	20.75 ± 1.76a	69.92 ± 4.88a
	N80+BC	6.11 ± 0.03a	5.43 ± 0.12a	26.77 ± 0.31ab	19.83 ± 1.11a	72.83 ± 5.69a
	N60	6.02 ± 0.03a	5.21 ± 0.19a	27.17 ± 0.79ab	20.67 ± 0.97a	70.11 ± 0.48a
	N60+BC	5.93 ± 0.05a	5.61 ± 0.03a	24.88 ± 0.81b	21.75 ± 1.04a	58.44 ± 4.26a
	CK	5.92 ± 0.01a	5.11 ± 0.15a	28.29 ± 0.77ab	22.92 ± 0.39a	70.50 ± 5.56a
Late season in 2018	N100	5.13 ± 0.03ab	4.99 ± 0.06a	36.99 ± 0.21ab	20.08 ± 0.81ab	37.35 ± 2.17a
	N80	5.11 ± 0.13b	4.93 ± 0.11a	36.42 ± 1.11ab	17.54 ± 1.24b	43.17 ± 3.28a
	N80+BC	5.62 ± 0.13a	5.35 ± 0.09a	32.07 ± 0.74bc	19.21 ± 0.99ab	46.03 ± 3.58a
	N60	5.19 ± 0.02ab	5.19 ± 0.18a	35.29 ± 1.30abc	19.09 ± 0.87ab	41.08 ± 0.58a
	N60+BC	5.23 ± 0.05ab	5.42 ± 0.12a	39.80 ± 2.14a	21.14 ± 1.12ab	47.56 ± 1.72a
	CK	5.15 ± 0.07ab	4.85 ± 0.11a	29.38 ± 0.65c	24.17 ± 0.56a	40.86 ± 0.72a
Early season in 2019	N100	5.30 ± 0.06a	6.13 ± 0.13a	31.68 ± 0.70c	15.67 ± 0.97bc	40.99 ± 1.00a
	N80	5.43 ± 0.08a	5.74 ± 0.18a	29.58 ± 1.08c	14.38 ± 0.69c	44.99 ± 3.47a
	N80+BC	5.63 ± 0.12a	6.15 ± 0.20a	36.51 ± 1.33ab	21.92 ± 0.59a	47.71 ± 0.46a
	N60	5.30 ± 0.00a	6.14 ± 0.02a	31.91 ± 0.90bc	17.83 ± 1.02bc	51.74 ± 1.04a
	N60+BC	5.37 ± 0.02a	5.87 ± 0.09a	40.47 ± 0.47a	18.54 ± 0.35ab	48.03 ± 1.65a
	CK	5.23 ± 0.11a	5.90 ± 0.02a	28.80 ± 0.15c	15.92 ± 0.33bc	47.07 ± 4.04a
Late season in 2019	N100	4.90 ± 0.03b	6.13 ± 0.20ab	30.47 ± 0.31b	23.00 ± 0.53a	46.98 ± 1.18a
	N80	4.83 ± 0.04b	4.25 ± 0.26b	31.30 ± 0.33b	21.38 ± 0.58a	53.97 ± 4.57a
	N80+BC	5.20 ± 0.03a	7.22 ± 0.25a	38.99 ± 0.08a	24.46 ± 0.82a	56.50 ± 1.85a
	N60	4.93 ± 0.04b	5.38 ± 0.48ab	30.09 ± 0.59b	21.08 ± 0.89a	51.31 ± 3.09a
	N60+BC	5.27 ± 0.05a	5.40 ± 0.48ab	39.03 ± 0.36a	24.38 ± 1.10a	52.12 ± 0.51a
	CK	4.80 ± 0.06b	5.37 ± 0.24ab	30.62 ± 0.79b	23.42 ± 0.19a	52.70 ± 3.77a

N100, 180 kg $N \cdot ha^{-1}$; N80, 144 kg $N \cdot ha^{-1}$; N80+BC, 144 kg $N \cdot ha^{-1}$ + biochar; N60, 108 kg $N \cdot ha^{-1}$; N60+BC, 108 kg $N \cdot ha^{-1}$ + biochar; CK, no N and biochar application. Different small letters indicate significant differences among different treatments at the same planting season ($p < 0.05$).

3.3. Effects of Fertilization Regimes on N Use Efficiency of Fertilizers

Figure 5 clearly showed that N fertilizer reduction or combined application with biochar significantly increased the N use efficiency of fertilizers. Compared with N100, N fertilizer reduction (N80, N60) slightly increased the AE_N, although the difference did not reach a significant level; combined application with biochar (N80+BC, N60+BC) further increased the AE_N compared with N fertilizer reduction alone; compared with N100, N80+BC and N60+BC greatly increased the AE_N, the increase rate under N80+BC reached 54.5–142.5%, whereas under N60+BC it reached 71.1–309.4% (Figure 5a). Similarly, N fertilizer reduction slightly increased the AR_N compared with N100, and combined application with biochar (N80+BC, N60+BC) further increased the AR_N; compared with N100, N80+BC and N60+BC increased the AR_N by 25.7–150.5% and 44.0–148.7%, respectively (Figure 5b). PFP_N increased significantly with N fertilizer reduction, N80 and N60 increased it by 22.1–26.5% and 58.2–70.0% compared with N100, respectively. However, combined application with biochar (N80+BC, N60+BC) had similar PFP_N compared with the corresponding N fertilizer reduction; compared with N100, N80+BC and N60+BC increased the PFP_N by 29.0–39.5% and 67.3–96.8%, respectively (Figure 5c).

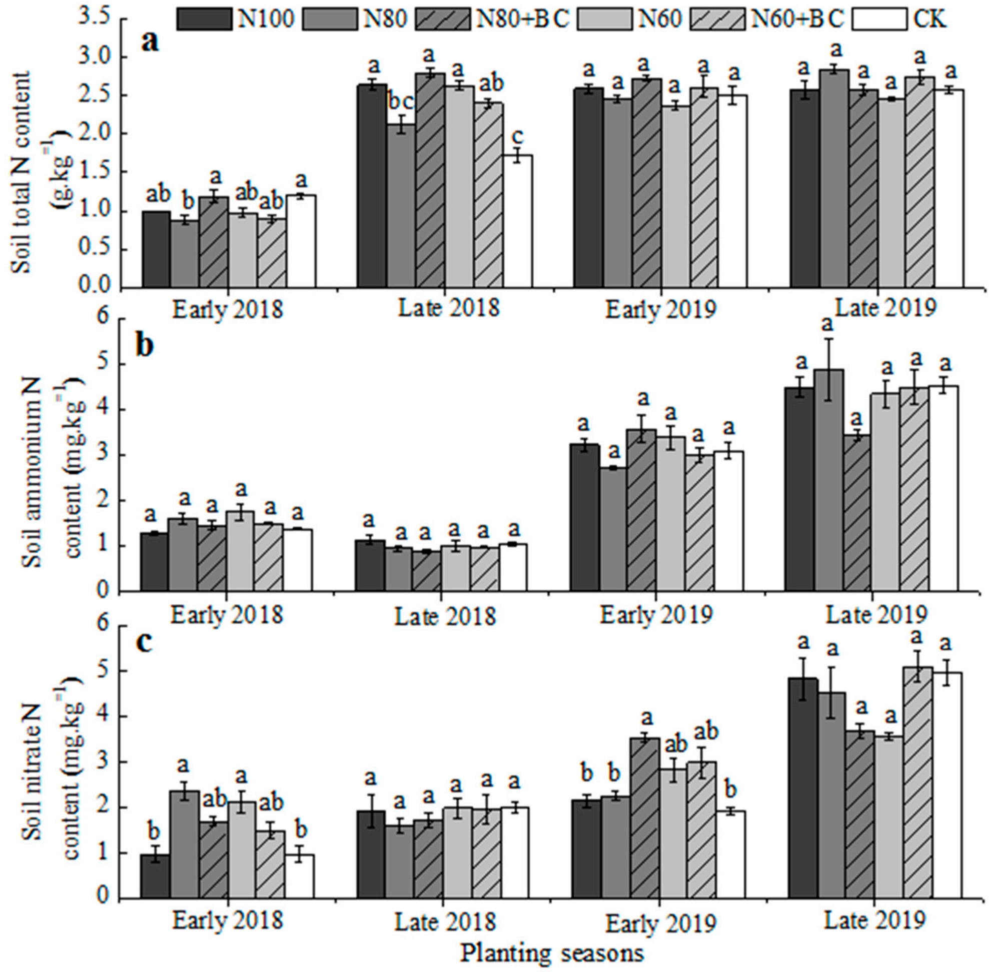

Figure 4. Effects of N fertilizer reduction combined with biochar application on total N (**a**), ammonium N (**b**), and nitrate N (**c**) in the soil. N100, 180 kg N·ha^{-1}; N80, 144 kg N·ha^{-1}; N80+BC, 144 kg N·ha^{-1} + biochar; N60, 108 kg N·ha^{-1}; N60+BC, 108 kg N·ha^{-1} + biochar; CK, no N and biochar application. Different small letters indicate significant differences among different treatments at the same planting season ($p < 0.05$).

According to the Pearson correlation analysis, AE_N was positively correlated with SOM, N uptake, and grain yield, and negatively correlated with NH_4^+-N; AR_N was correlated with all test parameters ($p < 0.01$), which was positively correlated with pH, CEC, SOM, N uptake, and grain yield, and negatively correlated with TN, NH_4^+-N, and NO_3^--N; there was no correlation between PFP_N and all test parameters (Table 3). According to partial correlation analysis, AE_N was positively correlated with NH_4^+-N and grain yield, and negatively correlated with pH and NO_3^--N; AR_N was positively correlated with CEC, NH_4^+-N, N uptake, and grain yield, and negatively correlated with TN and NO_3^--N; PFP_N only had a positive correlation with grain yield (Table 3).

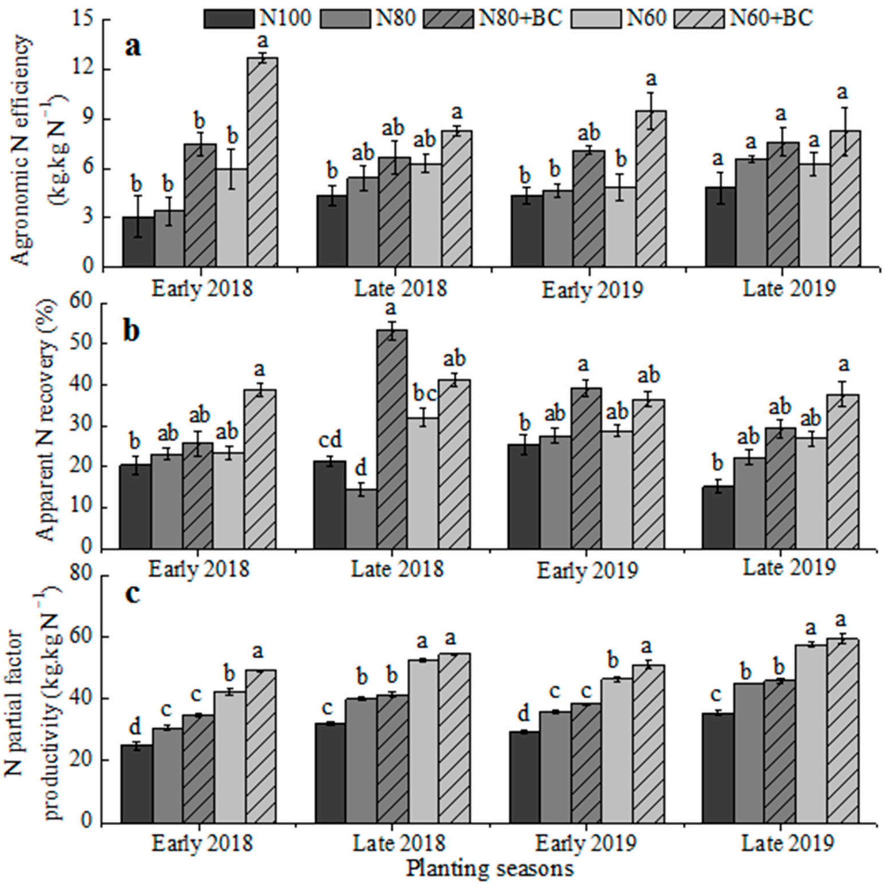

Figure 5. Effects of N fertilizer reduction combined with biochar application on N use efficiency of fertilizers. (**a**) Agronomic N efficiency, AE_N; (**b**) Apparent N recovery, AR_N; (**c**) N partial factor productivity, PFP_N. N100, 180 kg N·ha^{-1}; N80, 144 kg N·ha^{-1}; N80+BC, 144 kg N·ha^{-1} + biochar; N60, 108 kg N·ha^{-1}; N60+BC, 108 kg N·ha^{-1} + biochar. Different small letters indicate significant differences among different treatments at the same planting season ($p < 0.05$).

Table 3. Pearson correlation coefficients and partial correlation coefficients between the parameters of N use efficiency and other relevant parameters during the experiment.

Parameters of N Use Efficiency	Pearson Correlation Coefficient							
	pH	CEC	SOM	TN	NH_4^+-N	NO_3^--N	N Uptake	Grain Yield
AE_N	0.249	0.190	0.289 *	−0.251	−0.261 *	−0.195	0.320 *	0.375 **
AR_N	0.870 **	0.570 **	0.891 **	−0.835 **	−0.871**	−0.591 **	0.883 **	0.911**
PFP_N	0.144	−0.024	0.146	−0.140	−0.101	0.136	0.164	0.204
	Partial correlation coefficient							
	pH	CEC	SOM	TN	NH_4^+-N	NO_3^--N	N uptake	Grain yield
AE_N	−0.491**	0.143	−0.182	−0.216	0.325 *	−0.367 **	0.092	0.683 **
AR_N	−0.203	0.316 *	0.205	−0.443 **	0.455 **	−0.383 **	0.496 **	0.298*
PFP_N	−0.061	−0.121	−0.061	−0.041	0.124	0.085	−0.012	0.349 **

AE_N, agronomic N efficiency; AR_N, apparent N recovery; PFP_N, N partial factor productivity. SOM, TN, NH_4^+-N, NO_3^--N refer to the content of organic matter, total N, ammonium N, and nitrate N in the soil, respectively. N uptake and grain yield refer to the aboveground N uptake and grain yield of rice, respectively. ** Correlation is significant at $p < 0.01$; * Correlation is significant at $p < 0.05$.

4. Discussion

4.1. Biochar Incorporation Improve the Grain Yield of Rice

Although N plays an important role in rice growth and yield formation, many previous studies have shown that appropriate and short-term N reduction will not affect rice [15,53]. Meanwhile, as a friendly soil conditioner, biochar is widely reported to have positive effects on rice growth and yield, especially in acidic and sandy soils [25,27]. In fact, in our study, compared with N100, N fertilizer reduction alone (N80, N60) or combined application with biochar (N80+BC, N60+BC) had no significant impact on the number of tillers, aboveground dry biomass, and aboveground N uptake of rice (Table 1 and Figure 3). Given that ammonium N and nitrate N contents were relatively low in our field, we speculate that the surface application of heavy N fertilizer may lead to low N use efficiency, and in this case, appropriate N reduction did not affect rice growth and N uptake. The reason why combined application with biochar did not promote rice growth and N uptake compared with N100 is probably due to the fact that effectiveness of biochar often relies on multiple applications in the field as confirmed by the research of Major et al. [54]. Another reason is that combined application with biochar was based on N reduction, and the available N supplied was different from N100. However, it could still be seen that combined application with biochar tended to obtain higher number of tillers, aboveground dry biomass, and N uptake of rice compared with corresponding N reduction (Table 1 and Figure 3), which indicated that 15 t·ha^{-1} of biochar incorporation in our trial was able to promote rice growth and N uptaketo a certain extent. As described above, long-term application of biochar may be more beneficial for rice production [54]. However, in short-term trials, many studies have shown that rice growth and N uptake under biochar addition can exhibit an advantage over the one under no biochar addition [36,55], which is mainly due to the comprehensive performance of biochar in the soil, such as improving soil structure and properties, increasing soil available nutrients [21,25].

Inconsistent with rice biomass, during the trial, 40% N reduction (N60) resulted in a slight decrease in grain yield, and biochar incorporation increased the grain yield compared with N fertilizer reduction alone (Table 1), which seems to indicate that grain yield is more dependent on N fertilizer or biochar compared with biomass. The reason may be that grain yield is mainly formed in the later stage of rice growth, and excessive N reduction easily causes N shortage in this period, and is not conducive to yield formation. In contrast, biochar can adsorb N and delay the fertilizer efficiency of N in soil [32], thereby increasing N supply in this period, but the detailed mechanism still needs further study. Moreover, the studies by Huang et al. and Dong et al. confirmed that biochar with N reduction could significantly increase rice yield [43,55]. Apart from optimizing soil properties and increasing soil fertility, biochar incorporation may also induce N transfer from leaves to spikes, which is beneficial to protein transformation and increase the grain yield of rice [56,57].

Notably, the contribution of biochar to grain yield in early seasons was clearly higher than in late seasons, which was the opposite of the grain yield (Figure 2). Regardless of the fact that biochar was only applied in the early seasons, it was clear that biochar could play a better role in unfavorable environments, and stabilize rice yield. During the trial, the contribution of biochar to grain yield on the basis of 40% N reduction was always higher than on the basis of 20% N reduction (Figure 2), which also indicated that biochar could play a better role in stabilizing grain yield in the case of N shortage. Apart from increasing N utilization of rice, biochar may enhance the resistance of rice under adverse conditions, for example, Yuan et al. found that biochar can interact with the proteins related to abscisic acid in rice, thus improving the ability to resist chilling stress [58]. Furthermore, the role of silicon cannot be ignored, since rice straw biochar contains a higher content of silicon, which can help rice resist biotic and abiotic stresses [59,60]. In addition, the number of tillers, aboveground dry biomass, aboveground N uptake, and grain yield of rice fluctuated greatly in different planting seasons (Table 1and Figure 3), which was mainly due to climate difference (Figure 1). In this region, there was more rainfall in the early season than in the later season, especially in the ripening stage of rice, which was not conducive to the yield formation. In particular, in 2018, the local area was frequently hit by typhoons, resulting in rice growth and yield which is not as good as in 2019.

4.2. Biochar Incorporation Maintain N Balance and Improve Soil Fertility

Nutrient balance, improved fertility, and environmentally harmless are important goals of sustainable farmland. In addition to reducing environmental load, proper N fertilizer reduction and biochar application can increase soil fertility and nutrient balance, but this often requires a long-term process [15,21,30]. A meta-analysis showed that N addition had a significant effect on soil pH, and current N addition reduced global pH by an average of 0.26 [61]. Tian et al. found that the application of 150 kg N ha^{-1} could produce a significant increase in soil organic C content compared with 225 kg N hm^{-2}, after 7 years of the same management practices [62]. In the present study, throughout the trial period, compared with N100, N fertilizer reduction alone (N80, N60) had no significant impacts on soil pH, CEC, organic matter, available P, and available K (Table 2). It is seen in the agro-ecosystem, short-term reduction in N fertilizer was not sufficient to change soil physicochemical properties except for soil N levels.

In contrast, although combined application with biochar (N80+BC, N60+BC) had no significant impacts on soil CEC, available P, and available K overall, no matter compared to N100 or corresponding N fertilizer reduction, combined application with biochar significantly increased soil pH and organic matter content, especially in the late season of 2019 (Table 2). Numerous studies have demonstrated consistent evidence that biochar incorporation can increase soil pH, CEC, organic matter, and available nutrients content [25,63,64]. Ultimately, of course, the increased magnitude depends on the raw material, application amount and time of biochar, and soil properties. In our study, biochar incorporation had no significant impacts on soil CEC, available P, and available K overall, excluding the above factors, the reason may also be related to the behavior of rice. In fact, biochar tends to have a large CEC, and after it is applied to the soil, the surface functional groups will be oxidized to obtain higher CEC, thereby resulting in an increase in soil cation adsorption capacity [65,66]. Moreover, P and K in biochar are highly soluble [67,68], and biochar can also adsorb P and K in soil and reduce P and K leaching, thereby facilitating the increase in soil available P and K [69]. Therefore, the response of soil CEC, available P, and available K to biochar application requires long-term monitoring. In our study, the pH of biochar was significantly higher than soil, and repeated application of biochar would undoubtedly increase soil pH; similar results were obtained by Zhang et al. [70]. Meanwhile, biochar contained rich organic carbon, and its repeated application could also increase soil organic matter. Moreover, biochar could promote the formation of organic matter by adsorbing organic small molecules in the soil and enhancing their polymerization on the surface [71]. In particular, soil pH under all treatments showed a slight decrease with the planting season, and considering the same situation under CK, it was more likely caused by climatic factors, such as rainfall (Figure 1), which also requires long-term monitoring. Nevertheless, the increases in pH and organic matter under biochar application were critical for the improvement in soil properties and fertility in the long term.

In our study, the dynamics of soil N, especially available N, should be the focus of our content. However, during the trial, N fertilizer reduction or combined application with biochar had no significant impacts on soil total N, ammonium N, and nitrate N overall (Figure 4). Moreover, Oladele et al. found that N fertilizer reduction alone had no significant impacts on soil total N, ammonium N, and nitrate N, but 12 t·ha^{-1} of biochar with N reduction resulted in a significant increase in soil N content [72]. In our study, it is easy to understand that short-term N fertilizer reduction followed by surface application was difficult to change soil N level. With regard to why combined application with biochar did not increase soil N levels, some scholars also obtained similar results, for example, Luo et al. indicated that biochar amendment did not significantly influence soil ammonium N, but nitrate N was related to the application amount of biochar and soil type [73]. Nguyen et al. conducted a meta-analysis and found that 95% of cases of biochar amendment within 1 year reduced soil ammonium N and nitrate N regardless of experimental conditions [74]. It can be seen that the effects of biochar on soil N levels are a comprehensive interaction between biochar and environmental factors. In addition to the fact that N carried by biochar was mainly in the form of organic N and low availability [75], the type and application rate of biochar, experimental stage, soil properties, and even climate factors may all affect the experimental results. Nevertheless, previous studies have shown that biochar application has a positive effect on the increase in soil N content and availability by adsorbing N from soil and reducing N leaching, as well as increasing N mineralization [32,38,65]. Therefore, the response of soil N to N fertilizer reduction and biochar application still needs further monitoring.

4.3. Biochar Incorporation Improve the N Use Efficiency of Fertilizers

At present, agronomic N efficiency (AE_N), apparent N recovery (AR_N), and N partial factor productivity (PFP_N) are the main parameters for evaluating N fertilizer use efficiency in farmland. Several studies indicated that proper N fertilizer reduction or combined with biochar application could significantly improve the N use efficiency of fertilizer [15,76,77]. According to the formulas, the results of three parameters of N use efficiency depended on the rice yield (or N uptake by rice) and the amount of N fertilizer application under different treatments. In our study, as described above, N fertilizer reduction alone (N80, N60) did not significantly reduce the yield and N uptake of rice (Table 1 and Figure 3), but the amount of N provided by fertilizer was reduced, thus it caused a slight increase in AE_N and AR_N of N fertilizer (Figure 5a,b), which was in agreement with the results obtained by Qiao et al. [53]. Meanwhile, combined application with biochar tended to obtain higher rice yield and N uptake compared with corresponding N reduction (Table 1 and Figure 3), and then further improved AE_N and AR_N of N fertilizer (Figure 5a,b). Furthermore, Huang et al. found that the 3-year application of biochar had little effect on rice N uptake, but increased the AE_N by 7–11% in the third year [36]. In contrast to AE_N, the yield from PFP_N did not subtract the yield under CK, thus PFP_N showed a significant increase with N fertilizer reduction, but combined application with biochar only had similar PFP_N compared with the corresponding N fertilizer reduction (Figure 5c). Notably, in our study, the N introduced by biochar was not calculated, since this part of N has a relatively low leaching rate [78] and is mainly in the form of organic N with low availability [38,75]. In summary, in our study, N fertilizer reduction could improve the N use efficiency of fertilizer, and biochar incorporation further improved it to a certain extent, but the detailed mechanism for improving N use efficiency needs ^{15}N isotope tracer to study the interaction of biochar-fertilizer N-rice N uptake.

In addition, according to the Pearson and partial correlation analysis, it can be seen that N use efficiency of fertilizer was closely related to grain yield and N uptake of rice, and it is also positively correlated with soil CEC and organic matter to a certain extent, which indicates that an increase in soil CEC and organic matter could improve N use efficiency of fertilizer (Table 3). In particular, the relationship between N use efficiency and soil NH_4^+-N was inconsistent under the two analyses, which is likely caused by the autocorrelation effect between soil NH_4^+-N and other parameters (Table 3). After eliminating the autocorrelation effect, according to the partial correlation analysis, we observe that N use efficiency of fertilizer was positively correlated with soil NH_4^+-N and negatively correlated with soil NO_3^--N overall, which may be attributed to the fact that biochar was more inclined to adsorb NH_4^+-N than NO_3^--N [79], resulting in more NH_4^+-N remaining in the soil, but the detailed mechanism needs further study. In summary, our study emphasizes the importance of N fertilizer reduction combined with biochar application in enhancing the N use efficiency of fertilizer, thereby reducing the application of mineral N fertilizer in paddy fields, and in turn, reducing the environmental load.

5. Conclusions

Through a 2-year/four-seasonfield experiment, whether biochar can replace part of mineral N fertilizer was well evaluated. Although some parameters fluctuated widely in different planting seasons, the present results still showed that 20% N reduction had no significant impact on rice yield, while 40% N reduction resulted in a slight decrease in rice yield. In contrast, N reduction combined with biochar application could not only improve the N use efficiency of fertilizer and increase rice yield (even 0.3–18.1% over N100), but also could improve soil fertility by increasing soil pH and organic matter, indicating that it is a feasible nutrient management measure, which will have important practical significance for sustainable rice production. Furthermore, rice yield, N use efficiency of fertilizer, and soil N dynamic in response to integrated application of mineral N fertilizer and biochar requires more long-term monitoring, and the mechanism for improving N use efficiency deserves to be further clarified.

Author Contributions: Conceptualization, R.L. and K.C.; methodology, R.L. and C.N.; software, C.N., R.L. and J.T.; validation, C.N. and R.L.; formal analysis, C.N.; investigation, R.L., X.K. and H.C.; resources, R.L. and C.N.; data curation, C.N. and K.C.; writing—original draft preparation, C.N.; writing—review and editing, J.T. and K.C.; visualization, C.N.; supervision, K.C.; project administration, K.C.; funding acquisition, K.C. All authors have read and agreed to the published version of the manuscript, and C.N. and R.L. contributed equally to this manuscript.

Funding: This research was funded by the National Science and Technology Support Program of China, grant number 2012BAD14B00 and the Science and Technology Project of Guangdong Province, China, grant number 2012A020100003.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Acknowledgments: We thank Fucheng You (a local worker) for his reliable work at the farm for this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tsukaguchi, T.; Nitta, S.; Matsuno, Y. Cultivar differences in the grain protein accumulation ability in rice (*Oryza sativa* L.). *Field Crop. Res.* **2016**, *192*, 110–117. [CrossRef]
2. Ladha, J.K.; Reddy, P.M. Nitrogen fixation in rice systems: State of knowledge and future prospects. *Plant Soil* **2003**, *252*, 151–167. [CrossRef]
3. Dhillon, A.K.; Sharma, N.; Dosanjh, N.K.; Goyal, M.; Mahajan, G. Variation in the nutritional quality of rice straw and grain in response to different nitrogen levels. *J. Plant Nutr.* **2018**, *41*, 1946–1956. [CrossRef]
4. Gil-Ortiz, R.; Naranjo, M.Á.; Ruiz-Navarro, A.; Atares, S.; García, C.; Zotarelli, L.; San Bautista, A.; Vicente, O. Enhanced agronomic efficiency using a new controlled-released, polymeric-coated nitrogen fertilizer in rice. *Plants* **2020**, *9*, 1183. [CrossRef] [PubMed]
5. Ata-Ul-Karim, S.T.; Zhu, Y.; Cao, Q.; Rehmani, M.I.A.; Cao, W.; Tang, L. In-season assessment of grain protein and amylose content in rice using critical nitrogen dilution curve. *Eur. J. Agron.* **2017**, *90*, 139–151. [CrossRef]
6. Zhang, W.; Wu, L.; Wu, X.; Ding, Y.; Li, G.; Li, J.; Weng, F.; Liu, Z.; Tang, S.; Ding, C.; et al. Lodging resistance of japonica rice (*Oryza Sativa* L.): Morphological and anatomical traits due to top-dressing nitrogen application rates. *Rice* **2016**, *9*, 31. [CrossRef]
7. Hu, X.F.; Cheng, C.; Luo, F.; Chang, Y.Y.; Teng, Q.; Men, D.Y.; Liu, L.; Yang, M.Y. Effects of different fertilization practices on the incidence of rice pests and diseases: A three-year case study in Shanghai, in subtropical southeastern China. *Field Crop. Res.* **2016**, *196*, 33–50. [CrossRef]
8. Blanchet, G.; Gavazov, K.; Bragazza, L.; Sinaj, S. Responses of soil properties and crop yields to different inorganic and organic amendments in a Swiss conventional farming system. *Agric. Ecosyst. Environ.* **2016**, *230*, 116–126. [CrossRef]
9. Ying, J.Y.; Li, X.X.; Wang, N.N.; Lan, Z.C.; He, J.Z.; Bai, Y.F. Contrasting effects of nitrogen forms and soil pH on ammonia oxidizing microorganisms and their responses to long-term nitrogen fertilization in a typical steppe ecosystem. *Soil Biol. Biochem.* **2017**, *107*, 10–18. [CrossRef]
10. Liu, X.; Zhang, Y.; Han, W.; Tang, A.; Shen, J.; Cui, Z.; Vitousek, P.; Erisman, J.W.; Goulding, K.; Christie, P. Enhanced nitrogen deposition over China. *Nature* **2013**, *494*, 459–462. [CrossRef]
11. Tayefeh, M.; Sadeghi, S.M.; Noorhosseini, S.A.; Bacenetti, J.; Damalas, C.A. Environmental impact of rice production based on nitrogen fertilizer use. *Environ. Sci. Pollut. Res.* **2018**, *25*, 15885–15895. [CrossRef]

12. Cui, N.; Cai, M.; Zhang, X.; Abdelhafez, A.A.; Zhou, L.; Sun, H.; Chen, G.; Zou, G.; Zhou, S. Runoff loss of nitrogen and phosphorus from a rice paddy field in the east of China: Effects of long-term chemical N fertilizer and organic manure applications. *Glob. Ecol. Conserv.* **2020**, *22*, e01011. [CrossRef]
13. Liu, J.; Ma, K.; Ciais, P.; Polasky, S. Reducing human nitrogen use for food production. *Sci. Rep.* **2016**, *6*, 30104. [CrossRef]
14. Robertson, G.P.; Paul, E.A.; Harwood, R.R. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. *Science* **2000**, *289*, 1922–1925. [CrossRef]
15. Yang, G.; Ji, H.; Liu, H.; Feng, Y.; Guo, Z. Nitrogen fertilizer reduction in combination with Azolla cover for reducing ammonia volatilization and improving nitrogen use efficiency of rice. *PeerJ* **2021**, *9*, e11077. [CrossRef]
16. Su, W.; Ahmad, S.; Ahmad, I.; Han, Q. Nitrogen fertilization affects maize grain yield through regulating nitrogen uptake, radiation and water use efficiency, photosynthesis and root distribution. *PeerJ* **2020**, *8*, e10291. [CrossRef]
17. Tian, X.; Li, Z.; Wang, L.; Wang, Y.; Li, B.; Duan, M.; Liu, B. Effects of biochar combined with nitrogen fertilizer reduction on rapeseed yield and soil aggregate stability in upland of purple soils. *Int. J. Environ. Res. Public. Health.* **2020**, *17*, 279. [CrossRef]
18. Ning, C.C.; Gao, P.D.; Wang, B.Q.; Lin, W.P.; Jiang, N.H.; Cai, K.Z. Impacts of chemical fertilizer reduction and organic amendments supplementation on soil nutrient, enzyme activity and heavy metal content. *J. Integr. Agric.* **2017**, *16*, 1819–1831. [CrossRef]
19. Baghdadi, A.; Halim, R.A.; Ghasemzadeh, A.; Ramlan, M.F.; Sakimin, S.Z. Impact of organic and inorganic fertilizers on the yield and quality of silage corn intercropped with soybean. *PeerJ* **2018**, *6*, e5280. [CrossRef]
20. Geng, Y.; Cao, G.; Wang, L.; Wang, S. Effects of equal chemical fertilizer substitutions with organic manure on yield, dry matter, and nitrogen uptake of spring maize and soil nitrogen distribution. *PLoS ONE* **2019**, *14*, e0219512. [CrossRef]
21. Omondi, M.O.; Xia, X.; Nahayo, A.; Liu, X.; Korai, P.K.; Pan, G. Quantification of biochar effects on soil hydrological properties using meta-analysis of literature data. *Geoderma* **2016**, *274*, 28–34. [CrossRef]
22. Win, K.T.; Okazaki, K.; Ookawa, T.; Yokoyama, T.; Ohwaki, Y. Influence of rice-husk biochar and Bacillus pumilus strain TUAT-1 on yield, biomass production, and nutrient uptake in two forage rice genotypes. *PLoS ONE* **2019**, *14*, e0220236. [CrossRef] [PubMed]
23. Mohan, D.; Sarswat, A.; Ok, Y.S.; Pittman, C.U., Jr. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent-a critical review. *Bioresour. Technol.* **2014**, *160*, 191–202. [CrossRef] [PubMed]
24. Duwiejuah, A.B.; Abubakari, A.H.; Quainoo, A.K.; Amadu, Y. Review of biochar properties and remediation of metal pollution of water and soil. *J. Health Pollut.* **2020**, *10*, 200902. [CrossRef] [PubMed]
25. Peng, X.; Ye, L.L.; Wang, C.H.; Zhou, H.; Sun, B. Temperature-and duration-dependent rice straw-derived biochar: Characteristics and its effects on soil properties of an Ultisol in southern China. *Soil Tillage Res.* **2011**, *112*, 159–166. [CrossRef]
26. Lu, S.G.; Sun, F.F.; Zong, Y.T. Effect of rice husk biochar and coal fly ash on some physical properties of expansive clayey soil (Vertisol). *Catena* **2014**, *114*, 37–44. [CrossRef]
27. Lai, W.Y.; Lai, C.M.; Ke, G.R.; Chung, R.S.; Chen, C.T.; Cheng, C.H.; Pai, C.W.; Chen, S.Y.; Chen, C.C. The effects of woodchip biochar application on crop yield, carbon sequestration and greenhouse gas emissions from soils planted with rice or leaf beet. *J. Taiwan Inst. Chem. Eng.* **2013**, *44*, 1039–1044. [CrossRef]
28. Plaza, C.; Giannetta, B.; Fernández, J.M.; López-de-Sá, E.G.; Polo, A.; Gascó, G.; Méndez, A.; Zaccone, C. Response of different soil organic matter pools to biochar and organic fertilizers. *Agric. Ecosyst. Environ.* **2016**, *225*, 150–159. [CrossRef]
29. Jones, D.L.; Edwards-Jones, G.; Murphy, D.V. Biochar mediated alterations in herbicide breakdown and leaching in soil. *Soil Biol. Biochem.* **2011**, *43*, 804–813. [CrossRef]
30. Uchimiya, M.; Wartelle, L.H.; Klasson, K.T.; Fortier, C.A.; Lima, I.M. Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. *J. Agric. Food Chem.* **2011**, *59*, 2501–2510. [CrossRef]
31. Wang, Q.; Chen, L.; He, L.Y.; Sheng, X.F. Increased biomass and reduced heavy metal accumulation of edible tissues of vegetable crops in the presence of plant growth-promoting Neorhizobium huautlense T1-17 and biochar. *Agric. Ecosyst. Environ.* **2016**, *228*, 9–18. [CrossRef]
32. Malińska, K.; Zabochnicka-Świątek, M.; Dach, J. Effects of biochar amendment on ammonia emission during composting of sewage sludge. *Ecol. Eng.* **2014**, *71*, 474–478. [CrossRef]
33. Fiorentino, N.; Sánchez-Monedero, M.A.; Lehmann, J.; Enders, A.; Fagnano, M.; Cayuela, M. Interactive priming of soil N transformations from combining biochar and urea inputs: A 15N isotope tracer study. *Soil Biol. Biochem.* **2019**, *131*, 166–175. [CrossRef]
34. Liang, J.; Tang, S.; Gong, J.; Zeng, G.; Tang, W.; Song, B.; Zhang, P.; Yang, Z.; Luo, Y. Responses of enzymatic activity and microbial communities to biochar/compost amendment in sulfamethoxazole polluted wetland soil. *J. Hazard Mater.* **2020**, *385*, 121533. [CrossRef]
35. Zheng, H.; Wang, Z.; Deng, X.; Herbert, S.; Xing, B. Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. *Geoderma* **2013**, *206*, 32–39. [CrossRef]
36. Huang, M.; Fan, L.; Chen, J.; Jiang, L.; Zou, Y. Continuous applications of biochar to rice: Effects on nitrogen uptake and utilization. *Sci. Rep.* **2018**, *8*, 11461. [CrossRef]
37. Zavalloni, C.; Alberti, G.; Biasiol, S.; DelleVedove, G.; Fornasier, F.; Liu, J.; Peressotti, A. Microbial mineralization of biochar and wheat straw mixture in soil: A short-term study. *Appl. Soil Ecol.* **2011**, *50*, 45–51. [CrossRef]
38. Clough, T.J.; Condron, L.M.; Kammann, C.; Müller, C. A review of biochar and soil nitrogen dynamics. *Agronomy* **2013**, *3*, 275–293. [CrossRef]

39. Nelissen, V.; Saha, B.K.; Ruysschaert, G.; Boeckx, P. Effect of different biochar and fertilizer types on N_2O and NO emissions. *Soil Biol. Biochem.* **2014**, *70*, 244–255. [CrossRef]
40. Zhang, J.H.; Liu, J.L.; Zhang, J.B.; Zhao, F.T.; Wang, W.P. Effects of nitrogen application rates on translocation of dry matter and nitrogen utilization in rice and wheat. *Acta Agron. Sinica* **2010**, *36*, 1736–1742. [CrossRef]
41. Zhu, H.; Wang, Z.X.; Luo, X.M.; Song, J.X.; Huang, B. Effects of straw incorporation on Rhizoctoniasolani inoculum in paddy soil and rice sheath blight severity. *J. Agric. Sci.* **2014**, *152*, 741–748. [CrossRef]
42. Liu, Y.; Li, J.; Jiao, X.Y.; Li, H.D.; Hu, T.S.; Jiang, H.Z.; Mahmoud, A. Effects of biochar on water quality and rice productivity under straw returning condition in a rice-wheat rotation region. *Sci. Total Environ.* **2022**, *819*, 152063. [CrossRef] [PubMed]
43. Dong, D.; Feng, Q.; Mcgrouther, K.; Yang, M.; Wang, H.; Wu, W. Effects of biochar amendment on rice growth and nitrogen retention in a waterlogged paddy field. *J. Soil Sediment.* **2015**, *15*, 153–162. [CrossRef]
44. Amoakwah, E.; Arthur, E.; Frimpong, K.A.; Islam, K.R. Biochar amendment influences tropical soil carbon and nitrogen lability. *J. Soil Sci. Plant Nutr.* **2021**, *21*, 3567–3579. [CrossRef]
45. Hendershot, W.H.; Duquette, M. A simple barium chloride method for determining cation exchange capacity and exchangeable cations. *Soil Sci. Soc. Am. J.* **1986**, *50*, 605–608. [CrossRef]
46. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
47. Olsen, S.R.; Sommers, L.E. Phosphorus. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 2nd ed.; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; Soil Science Society of America: Madison, WI, USA, 1982; pp. 403–430.
48. Page, A.L.; Miller, R.H.; Keeney, D.R. *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*, 2nd ed.; American Society of Agronomy: Madison, WI, USA, 1982; pp. 595–624.
49. Chu, G.X.; Shen, Q.R.; Cao, J.L. Nitrogen fixation and N transfer from peanut to rice cultivated in aerobic soil in an intercropping system and its effect on soil N fertility. *Plant Soil* **2004**, *263*, 17–27. [CrossRef]
50. Tang, X.; Li, J.M.; Ma, Y.; Hao, X.Y.; Li, X.Y. Phosphorus efficiency in long-term (15 years) wheat–maize cropping systems with various soil and climate conditions. *Field Crop. Res.* **2008**, *108*, 231–237. [CrossRef]
51. Xu, C.L.; Huang, S.B.; Tian, B.J.; Ren, J.H.; Meng, Q.F.; Wang, P. Manipulating planting density and nitrogen fertilizer application to improve yield and reduce environmental impact in Chinese maize production. *Front Plant Sci.* **2017**, *8*, 1234. [CrossRef]
52. Vos, J. Nitrogen responses and nitrogen management in potato. *Potato Res.* **2009**, *52*, 305–317. [CrossRef]
53. Qiao, J.; Yang, L.; Yan, T.; Xue, F.; Zhao, D. Nitrogen fertilizer reduction in rice production for two consecutive years in the Taihu Lake area. *Agric. Ecosyst. Environ.* **2012**, *146*, 103–112. [CrossRef]
54. Major, J.; Rondon, M.; Molina, D.; Riha, S.J.; Lehmann, J. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil* **2010**, *333*, 117–128. [CrossRef]
55. Huang, M.; Yang, L.; Qin, H.; Jiang, L.; Zou, Y. Fertilizer nitrogen uptake by rice increased by biochar application. *Biol. Fert. Soils* **2014**, *50*, 997–1000. [CrossRef]
56. Noguera, D.; Barot, S.; Laossi, K.R.; Cardoso, J.; Lavelle, P.; de Carvalho, M.C. Biochar but not earthworms enhances rice growth through increased protein turnover. *Soil Biol. Biochem.* **2012**, *52*, 13–20. [CrossRef]
57. Ali, I.; Ullah, S.; He, L.; Zhao, Q.; Iqbal, A.; Wei, S.; Shah, T.; Ali, N.; Bo, Y.; Adnan, M. Combined application of biochar and nitrogen fertilizer improves rice yield, microbial activity and N-metabolism in a pot experiment. *PeerJ* **2020**, *8*, e10311. [CrossRef]
58. Yuan, J.; Meng, J.; Liang, X.; Yang, E.; Chen, W.F. Biochar's leacheates affect the abscisic acid pathway in rice seedlings under low temperature. *Front. Plant Sci.* **2021**, *12*, 646910. [CrossRef]
59. Guntzer, F.; Keller, C.; Meunier, J.D. Benefits of plant silicon for crops: A review. *Agron. Sustain. Dev.* **2012**, *32*, 201–213. [CrossRef]
60. De Tombeur, F.; Cooke, J.; Collard, L.; Cisse, D.; Saba, F.; Lefebvre, D.; Burgeon, V.; Nacro, H.B.; Cornelis, J.T. Biochar affects silicification patterns and physical traits of rice leaves cultivated in a desilicated soil (Ferric Lixisol). *Plant Soil* **2021**, *460*, 375–390. [CrossRef]
61. Tian, D.; Niu, S. A global analysis of soil acidification caused by nitrogen addition. *Environ. Res. Lett.* **2015**, *10*, 024019. [CrossRef]
62. Tian, J.; Lu, S.; Fan, M.; Li, X.; Kuzyakov, Y. Integrated management systems and N fertilization: Effect on soil organic matter in rice-rapeseed rotation. *Plant Soil* **2013**, *372*, 53–63. [CrossRef]
63. Glaser, B.; Wiedner, K.; Seelig, S.; Schmidt, H.P.; Gerber, H. Biochar organic fertilizers from natural resources as substitute for mineral fertilizers. *Agron. Sustain. Dev.* **2015**, *35*, 667–678. [CrossRef]
64. Bista, P.; Ghimire, R.; Machado, S.; Pritchett, L. Biochar effects on soil properties and wheat biomass vary with fertility management. *Agronomy* **2019**, *9*, 623. [CrossRef]
65. Liang, B.; Lehmann, J.; Solomon, D.; Kinyangi, J.; Grossman, J.; O'Neill, B.; Skjemstad, J.O.; Thies, J.; Luizão, F.J.; Petersen, J. Black carbon increases cation exchange capacity in soils. *Soil Sci. Soc. Am. J.* **2006**, *70*, 1719–1730. [CrossRef]
66. Jiang, T.Y.; Jiang, J.; Xu, R.K.; Li, Z. Adsorption of Pb (II) on variable charge soils amended with rice-straw derived biochar. *Chemosphere* **2012**, *89*, 249–256. [CrossRef] [PubMed]
67. Gaskin, J.W.; Speir, R.A.; Harris, K.; Das, K.; Lee, R.D.; Morris, L.A.; Fisher, D.S. Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status, and yield. *Agron. J.* **2010**, *102*, 623–633. [CrossRef]
68. Angst, T.; Sohi, S. Establishing release dynamics for plant nutrients from biochar. *GCB Bioenergy* **2013**, *5*, 221–226. [CrossRef]
69. Laird, D.; Fleming, P.; Wang, B.; Horton, R.; Karlen, D. Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma* **2010**, *158*, 436–442. [CrossRef]

70. Zhang, A.; Bian, R.; Pan, G.; Cui, L.; Hussain, Q.; Li, L.; Zheng, J.; Zheng, J.; Zhang, X.; Han, X. Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. *Field Crop. Res.* **2012**, *127*, 153–160. [CrossRef]
71. Liang, B.; Lehmann, J.; Sohi, S.P.; Thies, J.E.; O'Neill, B.; Trujillo, L.; Gaunt, J.; Solomon, D.; Grossman, J.; Neves, E.G. Black carbon affects the cycling of non-black carbon in soil. *Org. Geochem.* **2010**, *41*, 206–213. [CrossRef]
72. Oladele, S.O.; Adeyemo, A.J.; Awodun, M.A. Influence of rice husk biochar and inorganic fertilizer on soil nutrients availability and rain-fed rice yield in two contrasting soils. *Geoderma* **2019**, *336*, 1–11. [CrossRef]
73. Luo, C.Y.; Jiang, J.J.; Chen, W.; Han, F.P. Effect of biochar on soil properties on the Loess Plateau: Results from field experiments. *Geoderma* **2020**, *369*, 114323. [CrossRef]
74. Nguyen, T.T.N.; Xu, C.Y.; Tahmasbian, I.; Che, I.; Xu, Z.H.; Zhou, X.H.; Wallace, H.M.; Bai, S.H. Effects of biochar on soil available inorganic nitrogen: A review and meta-analysis. *Geoderma* **2017**, *288*, 79–96. [CrossRef]
75. Yan, L.; Xue, L.H.; Petropoulos, E.; Qian, C.; Hou, P.F.; Xu, D.F.; Yang, L.Z. Nutrient loss by runoff from rice-wheat rotation during the wheat season is dictated by rainfall duration. *Environ. Pollut.* **2021**, *285*, 117382. [CrossRef]
76. Van Zwieten, L.; Kimber, S.; Morris, S.; Chan, K.; Downie, A.; Rust, J.; Joseph, S.; Cowie, A. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant Soil* **2010**, *327*, 235–246. [CrossRef]
77. Petter, F.A.; de Lima, L.B.; Júnior, B.H.M.; de Morais, L.A.; Marimon, B.S. Impact of biochar on nitrous oxide emissions from upland rice. *J. Environ. Manag.* **2016**, *169*, 27–33. [CrossRef]
78. Knicker, H.; Skjemstad, J.O. Nature of organic carbon and nitrogen in physically protected organic matter of some australian soils as revealed by solid-state ^{13}C and ^{15}N nmr spectroscopy. *Aust. J. Agric. Res.* **2000**, *23*, 329–341. [CrossRef]
79. Liu, Z.Q.; He, T.Y.; Cao, T.; Yang, T.X.; Meng, J.; Chen, W.F. Effects of biochar application on nitrogen leaching, ammonia volatilization and nitrogen use efficiency in two distinct soils. *J. Soil. Sci. Plant. Nut.* **2017**, *17*, 515–528. [CrossRef]