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Effects of Rice-Husk Biochar on Nitrogen Retention, Nitrification, and Plant Nitrogen Uptake in Decontaminated Sandy Soils in Fukushima, Japan

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Abstract

Decontaminated sandy soils in Fukushima are characterized by low fertility and high nitrogen (N) loss, requiring effective nutrient management strategies. This study evaluated the effects of rice husk biochar on N dynamics, focusing on ammonium (NH_4^+) retention, nitrification, and plant N availability, using column, incubation, and pot experiments with decontaminated Fukushima soil. A significant interaction between biochar application and time indicated that biochar-applied soils showed different patterns in NH_4^+ and nitrate leaching over the experimental period. Incubation results showed that biochar reduced net nitrification rates (−18.1%) and tended to reduce the abundance of ammonia-oxidizing bacteria DNA. These effects may be attributed to the porous structure and adsorption properties of biochar. In the pot experiment, co-application of biochar with organic amendments (manure and kudzu) reduced plant N uptake by 9.6% and 9.0%, respectively, compared with their sole application. This indicates a trade-off between N retention and plant availability, particularly during the initial stage after biochar application. These findings highlight the importance of carefully balancing N retention and availability when applying biochar and organic amendments in low-fertility soils.

Keywords: biochar; nitrogen dynamics; ammonium retention; nitrification; nitrogen leaching; decontaminated soil; Fukushima; sandy soil; organic amendments

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1. Introduction

As a result of the 11 March 2011 earthquake and tsunami, large areas of farmland in Fukushima, the eastern part of Japan, were contaminated with radioactive materials, leading to evacuation orders and the suspension of agricultural activities [1]. To restore agricultural production, large-scale decontamination measures were implemented, including the removal of approximately 5 cm of topsoil [2]. Although effective in reducing radiocesium levels, this process also removed fertile soil rich in organic matter and nutrients. The replacement soils, often collected from nearby mountainous areas, are typically sandy, low in organic matter, and have poor nutrient retention capacity [3,4]. Consequently, restoring soil fertility and improving nutrient management in these decontaminated soils are critical challenges for agricultural recovery.

In such degraded sandy soils, nitrogen (N) dynamics are particularly important because N is the most important nutrient in agricultural production, and low organic matter and poor structure can result in substantial N losses mainly through leaching [5,6]. In particular, rapid nitrification—the microbial oxidation of ammonium (NH_4^+–N) to nitrate (NO_3^-–N)—can increase NO_3^-–N leaching and reduce N use efficiency [6]. Therefore, strategies to retain NH_4^+–N and regulate nitrification are essential for improving N availability and minimizing environmental losses in these systems.

Biochar, a carbon-rich material produced by pyrolysis of biomass, has been widely studied as a soil amendment due to its high porosity and chemical stability [7–9]. These properties can improve soil pH, water-holding capacity, and CEC [8,9]. In addition, biochar has been reported to influence N dynamics by retaining NH_4^+–N through adsorption and ion exchange processes and by altering microbial activity, including nitrification [10]. However, the magnitude and mechanisms of these effects vary depending on the types of biochar and soil properties, and remain insufficiently understood, particularly in decontaminated sandy soils in Fukushima.

Organic amendments such as manure are commonly used not only to supply nutrients and improve soil physicochemical properties but also to increase nutrient retention and crop productivity, particularly in degraded or coarse-textured soils [11–13]. Manure provides essential nutrients, including N, phosphorus, and potassium, and can enhance microbial activity and soil aggregation [14–16]. Kudzu (*Pueraria montana* var. *lobata)*, a fast-growing leguminous species widely distributed and treated as a vigorous weed in Japan, has also attracted attention as a potential organic resource due to its high biomass production, N fixation capacity, and relatively low C/N ratio [17]. Its rapid decomposition and potential to enhance nutrient cycling may be beneficial in low-fertility soils. Previous studies have shown that co-application of biochar with organic amendments such as manures can affect soil N dynamics and nutrient availability in soils [18,19], but the effects of co-application of biochar and kudzu or leguminous plant materials have rarely been studied, and their interactions in relation to soil N dynamics remain unclear.

Therefore, this study aimed to elucidate the effects of rice husk biochar on N transformation in decontaminated soil from Fukushima, Japan. We hypothesized that rice husk biochar would enhance NH_4^+–N retention and reduce N leaching through its porous structure, while also influencing soil nitrification. To test these hypotheses, we first characterized the porous structures of rice husk biochar using scanning electron microscopy. Then, a column experiment was conducted to evaluate NH_4^+ retention and N leaching. An incubation study was conducted to test the effects of biochar on net nitrification rates. In addition, the interactions between biochar and organic amendments (manure and kudzu) were further evaluated in relation to plant biomass and N uptake of komatsuna (*Brassica rapa* var. *perviridis*).

2. Materials and Methods

2.1. Soil and Amendment Materials

Soil used in all experiments was collected from a farmer's field in Nagadoro, Iitate Village, Fukushima Prefecture, Japan (37°39′54″ N, 140°43′36″ E) in April 2024. The site had been decontaminated prior to 2022 by removing the surface soil and covering the scraped layer with clean sandy soil. The field has remained fallow with no agricultural activity since March 2011. The region is characterized by a humid temperate climate. Soil was air-dried and sieved using a 2 mm sieve, then used for experiments.

The soil was classified as loamy sand, with sand, silt, and clay contents of 85.1%, 10.4%, and 4.5%, respectively, as determined by the gravimetric method. Additional physicochemical properties of the soil are presented in Table 1. Soil pH was measured in 1:2.5 (*w*/*w*)

soil-to-water suspensions using a glass electrode pH meter (LAQUAact D-72, Horiba Ltd., Kyoto, Japan). Electrical conductivity (EC) was determined in 1:5 (*w*/*w*) suspensions using an EC meter (MM-41DP, TOA Electronics Ltd., Aichi, Japan). Cation exchange capacity (CEC) was determined using the ammonium acetate method (Schollenberger method). Total carbon (TC) and total N (TN) concentrations were measured using a combustion analyzer (MT-700, Yanaco Analytical Instruments Co., Tokyo, Japan). The concentrations of NH_4^+–N and NO_3^-–N were determined colorimetrically after extraction with 1 M KCl solution (1:5, dry soil:solution).

Table 1. Physicochemical properties of soil, rice husk biochar, and manure used in this study.

	pH	EC dS m^{-1}	CEC cmolc kg^{-1}	TC g kg^{-1}	TN	NH_4^+-N mgN kg^{-1}	NO_3^--N	Sand %	Silt	Clay
Fukushima soil	6.3	0.05	21.4	18.7	1.5	38.3	7.7	85.1	10.4	4.5
Rice husk biochar	9.5	2.9	80.0	369	4.4	2.5	0.5	ND	ND	ND
Cow dung manure	8.4	11	60.6	283	18	55	303	ND	ND	ND

Abbreviations: EC: electric conductivity, CEC: cation exchange capacity, TC: total carbon, TN: total nitrogen, ND: not determined.

Rice husk biochar used in all experiments was produced in Miyagi Prefecture and purchased from a local supplier. This biochar was produced using an industrial-scale pyrolysis system operated at approximately 400–500 °C (average approximately 450 °C) and had a pH of 9.5. Proximate analysis showed that the biochar consisted of 6.1% moisture, 41.4% ash, 24.3% volatile matter, and 28.2% fixed carbon [20]. Cow dung manure, for the plant growth experiment, produced in the Iitate region, was also used. The properties of these materials are summarized in Table 1. Kudzu biomass (leaves and stems) used for the plant growth experiment was collected from the experimental field of Chiba University, air-dried, ground, and applied to the pots. The TC and TN contents of the kudzu biomass were 458 g C kg^{-1} and 32 g N kg^{-1}, respectively.

2.2. Biochar Characterization

The surface morphology of rice husk biochar was observed by scanning electron microscopy (SEM, SU3500, Hitachi, Tokyo, Japan). Elemental composition of the rice husk biochar was analyzed using X-ray fluorescence (XRF) spectroscopy (XRF-1800, Shimadzu, Kyoto, Japan). The analysis provided semi-quantitative information on inorganic elemental composition, particularly for major elements such as Si, K, and Ca.

2.3. Column Experiment

A 10-day column experiment was conducted to evaluate mineral N leaching from soil. Air-dried soil (400 g) was adjusted to 40% of its water-holding capacity (WHC) by adding ultrapure water and pre-incubated for 7 days at 25 °C in the dark. This pre-incubation process was intended to enhance microbial activity in the soils before treatment application, although it may have resulted in microbial community conditions that differed from those under field conditions at the time of biochar application. After pre-incubation, rice husk biochar was added at a rate of 5% (*w*/*w*) and thoroughly mixed with the soil. This application rate is unrealistically high, but we aimed to clearly evaluate the effects of biochar on decontaminated soils. The soil with and without rice husk biochar (control vs. biochar) was packed into rigid polyvinyl chloride (PVC) columns (15 cm in height, 55 mm inner diameter) to achieve uniform bulk density. A stainless-steel mesh (250 µm aperture) was attached to the bottom of each column, and a filter paper was placed above the mesh to prevent soil loss. The columns were replicated three times for control and biochar treatments, respectively.

At the start of the experiment (day 0), ultrapure water was added to each column until 50 mL of leachate was collected from the bottom. Over the next 10 days, 50 mL of solution was applied every 24 h after collecting leachates from the previous day. Ultrapure water was applied on days 1–3 and 5–10, while a solution containing mineral N was applied on day 4. The solution applied on day 4 contained 19 mg each of NH_4^+–N and NO_3^-–N. Mineral N was applied as a pulse input to clearly evaluate the effects of biochar on mineral N retention and leaching under controlled conditions. The experiment was conducted at 25 °C under controlled laboratory conditions. The amounts of NH_4^+–N and NO_3^-–N leaching were determined on a daily basis using colorimetric methods.

2.4. Incubation Experiment

A 56-day incubation experiment was conducted to evaluate N transformation in soil with and without biochar. Air-dried soil (100 g) was placed in plastic bags and adjusted to 40% of its WHC. The soil was pre-incubated for 7 days at 25 °C in the dark prior to biochar addition. Three replicates were prepared for both control and biochar treatments. After pre-incubation (day 0), rice husk biochar was added at a rate of 5% (*w*/*w*), which is the same rate as the column experiment, to designated treatments and thoroughly mixed with the soil. Soil moisture was then adjusted to 60% of WHC. Ammonium sulfate was applied to all the treatments at a rate of 95 mg N kg d.s.$^{-1}$. The bags were sealed with silicone stoppers to minimize moisture loss and incubated at 25 °C in the dark. Soil moisture was maintained by weighing the samples weekly and replenishing evaporated water with ultrapure water. The bags were periodically opened (every 2–3 days) to allow gas exchange and prevent oxygen limitation during incubation.

Soil samples were extracted using a 1 M KCl solution at 0, 7, 28, and 56 days after biochar application to determine the concentrations of NH_4^+–N and NO_3^-–N concentrations and to calculate net nitrification rates (NNR). NNR was calculated as the increase in NO_3^-–N concentration over time using the following equation [21]:

$$NNR = ([NO_3^-–N]_{t2} - [NO_3^-–N]_{t1})/(t2 - t1)$$

where $[NO_3^-–N]_{t1}$ and $[NO_3^-–N]_{t2}$ represent NO_3^-–N concentrations (mg N kg d.s.$^{-1}$) at the beginning (t1) and end (t2) of each incubation period (days), respectively. NNR values were calculated from adjacent sampling intervals [22]. Therefore, partial dependence among intervals cannot be completely excluded.

2.5. qPCR Analysis

During the incubation study described in Section 2.4, soil DNA was extracted from every soil sample using Extrap® Soil DNA Kit Plus Ver. 2 (BioDynamics Laboratory Inc., Tokyo, Japan) immediately after sampling. The abundances of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) DNA were quantified by quantitative PCR (qPCR) targeting the *amoA* gene.

For AOA, the amoA19F and amoA643R primers were used [23]. For AOB, the amoA1F and amoA2R primers were initially tested [24] using soil-derived DNA as template; however, amplification of the target gene was unsuccessful, possibly due to sequence variation in the primer-binding regions. Therefore, new primers (amoA-1F: 5′-TACGTGCGCCTGATCG-3′; amoA-2R: 5′-TTGCCAAAGTACCACCATAC-3′) were designed within conserved regions of the *amoA* gene, suggested by Sanger sequence analysis of the PCR amplicons of the AOB's *amoA* gene. ATCC25196 (accession number U91603) reported by Morimoto et al. (2011) [25] was used as a reference sequence for the *amoA* gene of AOB.

Quantitative PCR was performed using a StepOnePlus Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) with THUNDERBIRD® Next SYBR™ qPCR Mix (TOYOBO, Osaka, Japan). Standard curves were generated using serial dilutions of purified PCR products, ranging from 10^3 to 10^8 copies for AOA and 10^4 to 10^7 copies for AOB. Amplification efficiencies were 91% for AOA and 109% for AOB, which fall within the commonly accepted technical range for qPCR assay (90–110%), with coefficients of determination (R^2) of 0.979 and 0.988, respectively. Melt curve analysis after the qPCR showed a single major peak for each primer pair, including the newly designed primers for AOB, indicating the specificity of the qPCR assay.

2.6. Plant Growth Experiment

A pot experiment was conducted to evaluate the effects of organic amendments and their co-application with rice husk biochar on komatsuna growth and N uptake. Five treatments were established: control (no amendment), manure, biochar + manure, kudzu, and biochar + kudzu, replicated three times.

Each Wagner pot (1/5000a; surface area: 0.02 m^2; depth: 15 cm; volume: 3 L) was filled with 4.5 kg of air-dried soil. The same rice husk biochar used in the column and incubation experiments was applied at a rate equivalent to 2 t ha^{-1} in the pot experiment, which is within a field-realistic range reported in previous agricultural studies [26,27]. This application rate was substantially lower and more practically realistic than those used in the column and incubation experiments. Kudzu biomass (leaves and stems) was collected from the experimental field of Chiba University, air-dried, ground, and applied to the soil. The application rate of kudzu biomass was 3.75 t ha^{-1}, corresponding to a N input of 120 kg N ha^{-1}. The amounts of manure and kudzu were adjusted to supply equivalent total N inputs across treatments. Nine komatsuna seeds were sown per pot on 19 August 2024. During the 35 days of the plant growing experiment, the mean air temperature was 28.0 °C, with mean daily maximum and minimum temperatures of 32.0 °C and 24.5 °C, respectively. Pots were irrigated daily with deionized water and maintained under ambient greenhouse conditions with natural light (estimated total sunshine duration: approximately 180 h) without any artificial light. At harvest on 23 September 2024, plant growth was evaluated based on aboveground biomass and N uptake. Root biomass was not evaluated because the decontaminated sandy soil became highly compacted during cultivation, making complete and reliable root recovery difficult.

2.7. Statistical Analysis

Statistical analyses were conducted using R (version 4.5.3). For the column experiment, linear mixed-effects models were fitted using the lmer function in the lme4 package to evaluate the effects of treatment, time, and their interaction. Experimental units were included as random effects to account for repeated measurements over time. *p*-values for fixed effects were obtained using the lmerTest package based on Satterthwaite's approximation. For the incubation experiment, data were analyzed using a two-way repeated-measures ANOVA with treatment as a between-subject factor and time as a within-subject factor. Gene copy numbers obtained by qPCR were log 10-transformed prior to statistical analysis to improve normality and homogeneity of variance. In addition, differences between treatments at specific time points were evaluated using Welch's *t*-test. For the plant growth pot experiment, data were analyzed using two-way ANOVA, with biochar application and fertilizer type (manure vs. kudzu) as fixed factors (control was excluded from the statistical analysis to detect the effects of biochar). Statistical significance was determined at $p < 0.05$. The relatively small number of replicates ($n = 3$) in each experiment may have limited the statistical power to detect subtle treatment differences.

3. Results

3.1. Rice Husk Biochar Traits

SEM observations revealed that the rice husk biochar exhibited a highly porous and heterogeneous structure with complex pore networks (Figure 1a–c). At higher magnification (5000×), pore structures of approximately 10 μm in diameter were observed, along with much smaller pores (Figure 1c). The rice husk biochar contained a high carbon content (369 g C kg^{-1}, Table 1). XRF analysis showed that silicon was the dominant detected element (84.2%), followed by potassium (11.0%) and calcium (1.68%), indicating the silica-rich and ash-derived nature of the rice husk biochar, although the elemental values should be interpreted as semi-quantitative estimates.

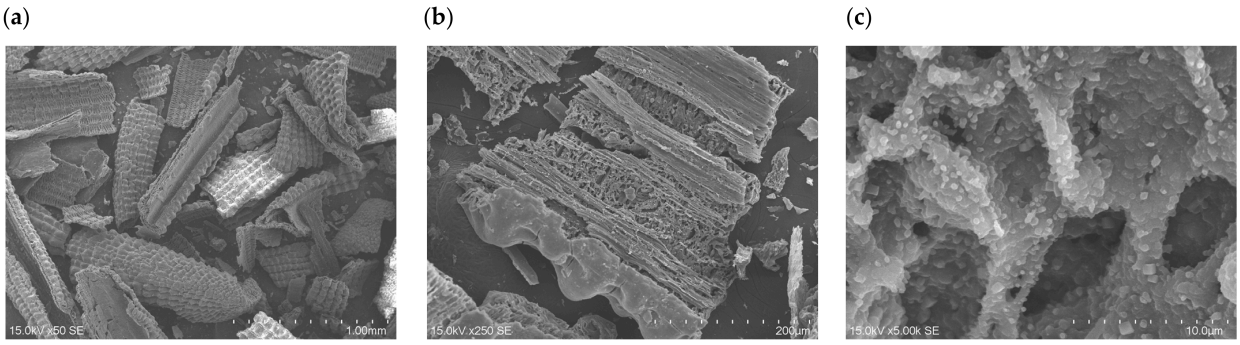


Figure 1. SEM images of rice husk biochar at different magnifications: 50× (**a**), 250× (**b**), and 5000× (**c**), showing the surface morphology and pore structure.

3.2. Nitrogen Leaching and Retention

Although the biochar effect in column experiment was not significant (p = 0.056 for NH_4^+–N, p = 0.684 for NO_3^-–N), a significant biochar × time interaction on NH_4^+–N ($p < 0.001$) and NO_3^-–N (p = 0.034) leaching was observed, indicating that mineral N leaching changed differently over time between treatments (Figure 2a,b). In particular, after day 4, NH_4^+–N leaching tended to be lower in the biochar-treated soil over 10 days (−75.7%; Figure 2a). A similar temporal pattern was observed for NO_3^-–N leaching, which increased less in the biochar treatment compared with the control (−24.2% in biochar treatment; Figure 2b).

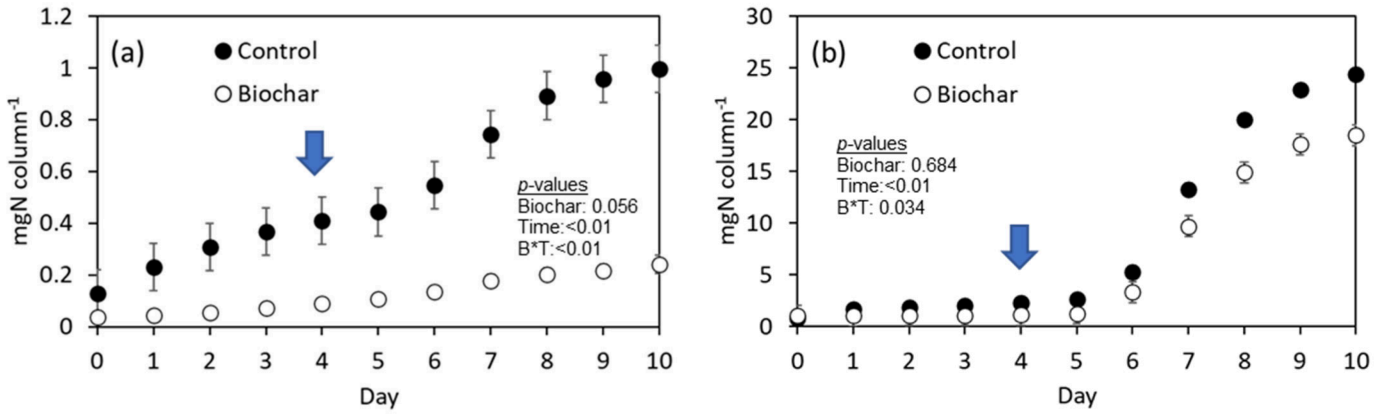


Figure 2. Cumulative leaching of NH_4^+–N (**a**) and NO_3^-–N (**b**) during the 10-day column experiment as affected by rice husk biochar application. Initially, the soil columns contained 15.32 mg NH_4^+–N and 3.1 mg NO_3^-–N. On day 4 (blue arrow), 19 mg of NH_4^+–N and 19 mg of NO_3^-–N were added to the solution. p-values for the effects of biochar, time (day), and their interaction (biochar × time) were obtained from the linear mixed-effects model. Values are expressed as mean ± SE (n = 3).

The total NH_4^+–N leaching relative to the total available NH_4^+–N pool (initial NH_4^+–N + applied NH_4^+–N) was 2.9% and 0.7% in the control and biochar treatments, respectively. The total NO_3^-–N leaching relative to available NO_3^-–N pool (initial NO_3^-–N + applied NO_3^-–N) were 110.6% and 83.9% in the control and biochar treatments, respectively. Overall, retained mineral N (sum of NH_4^+–N and NO_3^-–N) over total mineral N (initial mineral N + applied mineral N) was estimated to be 54.9% and 66.7% in the control and biochar treatments, respectively.

3.3. Net Nitrification Rates and Microbial Abundance

During the 56-day incubation experiment, NH_4^+–N concentrations decreased while NO_3^-–N concentrations increased over time (Figure 3a,b). Even though statistically not significant, biochar application resulted in lower concentrations of both NH_4^+–N and NO_3^-–N compared with the control. At day 0, mineral N concentrations were measured immediately after mixing soil with biochar and adding NH_4^+–N solution, and differences between treatments were already observed. During the incubation period, NO_3^-–N concentrations increased, whereas biochar-treated soils consistently showed lower NO_3^-–N concentrations than the control throughout the experiment (Figure 3b).

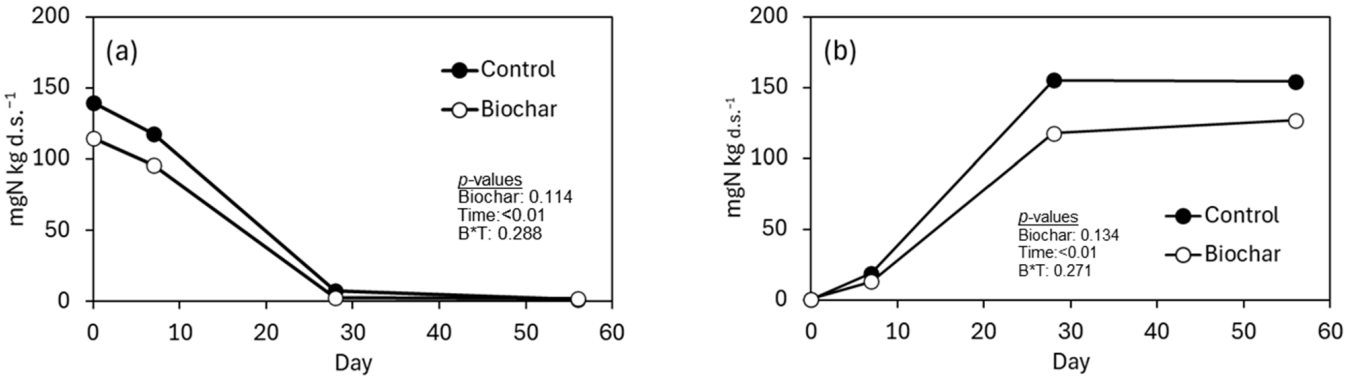


Figure 3. Concentrations of NH_4^+–N (**a**) and NO_3^-–N **(b)** in soils during the 56-day incubation experiment as affected by biochar application. At the start of the experiment, soils were amended with 95 mg N kg^{-1} as NH_4^+–N. *p*-values for the effects of biochar, time (incubation day), and their interaction (biochar × time) were obtained from the repeated measures ANOVA. Values are expressed as mean ± SE (n = 3).

The calculated net nitrification rate (NNR) peaked between day 7 and day 28 (Figure 4). The average NNR of all measured time points was significantly lower in the biochar treatment than in the control (p = 0.002). The average NNR over the 56-day incubation period was 2.75 and 2.25 mg N kg^{-1} day^{-1} in the control and biochar treatments, respectively, corresponding to an 18.1% reduction due to biochar application.

Consistent with the NNR results, the abundance of ammonia-oxidizing bacteria (AOB) DNA, expressed as gene copy numbers per gram of dry soil, tended to be lower in the biochar treatment than in the control (Figure 5a, p = 0.062 on 28 day, p = 0.109 on 56 day). In contrast, the abundance of ammonia-oxidizing archaea (AOA) DNA was much lower overall and was not affected by biochar application (Figure 5b).

3.4. Plant Nitrogen Uptake as Affected by Biochar Application

Compared with the control, organic amendments such as manure and kudzu increased komatsuna biomass and N uptake (Figure 6a,b). However, co-application of biochar with these organic amendments significantly reduced plant biomass and N uptake compared with their sole application (Figure 6a,b, p = 0.025 for biomass, p = 0.046 for plant N up-

take). Biochar application reduced N uptake by 9.6% and 9.0% in the manure and kudzu treatments, respectively.

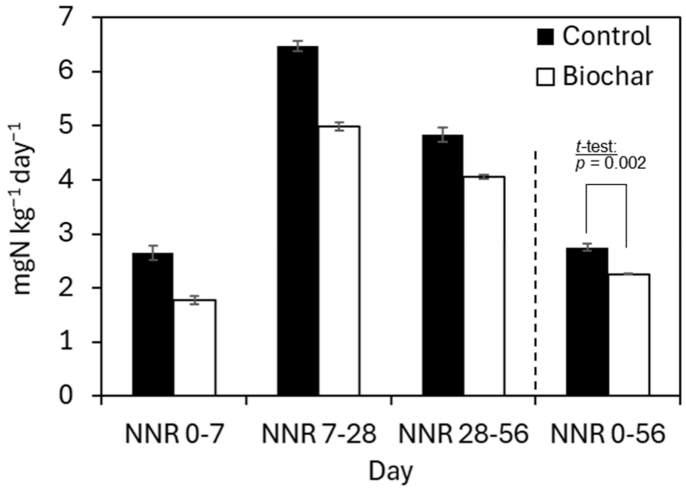


Figure 4. Net nitrification rates (NNRs) in soils during the 56-day incubation experiment as affected by biochar application. The NNR was calculated based on the increase in NO_3^-–N concentrations over specified time intervals. The effects of biochar were tested using a *t*-test using an average of 0–56 days. Values are expressed as mean ± SE (n = 3).

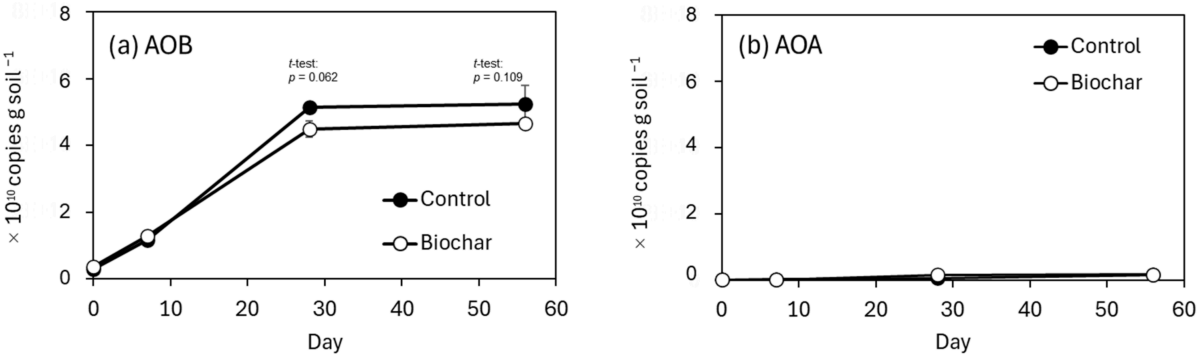


Figure 5. Abundance of ammonia-oxidizing bacteria (AOB) (**a**) and ammonia-oxidizing archaea (AOA) (**b**) DNA, expressed as gene copy numbers per gram of dry soil, during the 56-day incubation experiment as affected by biochar application. Differences between treatments at specific time points were evaluated using Welch's *t*-test, and the corresponding *p*-values are shown in the figure. Values are expressed as mean ± SE (n = 3).

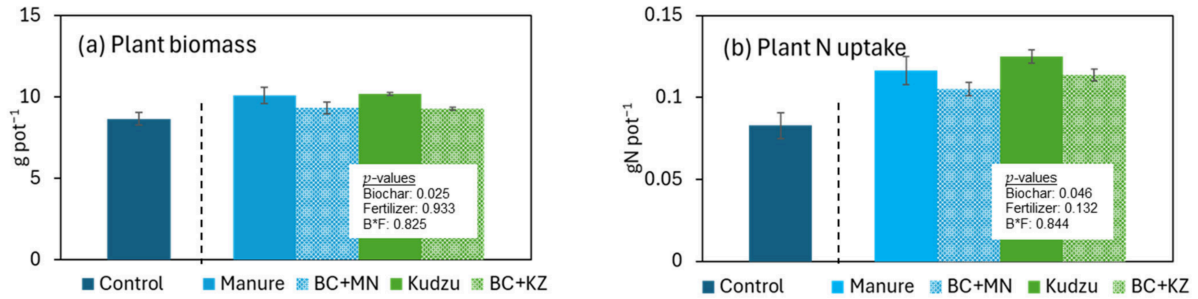


Figure 6. Plant biomass (**a**) and nitrogen uptake (**b**) of komatsuna cultivated under different amendment treatments. Data are presented as mean ± standard error (SE). BC: biochar; MN: manure; KZ: kudzu. Error bars represent SE. *p*-values for the effects of biochar, fertilizer, and their interaction (biochar × fertilizer) were obtained from the 2-way ANOVA. The control is shown in Figure 6 for descriptive comparison only and was not included in the ANOVA analysis. Values are expressed as mean ± SE (n = 3).

4. Discussion

The present study aimed to evaluate the effects of rice husk biochar on N dynamics in decontaminated sandy soil from Fukushima, Japan. The results showed that biochar application significantly altered N dynamics, particularly by reducing NH_4^+–N leaching (Figure 2a) and nitrification rates (Figure 4). These effects may be related to the structural and physicochemical properties of the biochar.

SEM observations revealed that the rice husk biochar exhibited a highly porous and heterogeneous structure with well-developed pore networks across multiple scales (Figure 1a–c). Previous studies have reported that rice residue-derived biochar exhibits well-developed pores, including interconnected pores and tubular structures originating from plant tissues, as observed in SEM images [28–30]. In particular, Figure 1c showed open macropore structures (approximately 10–20 μm in diameter), consistent with those reported by Somboon et al. (2025) [29]. Smaller pores (<2 μm) were also observed in the same image. These hierarchical structures may be attributed to the decomposition of cellulose and hemicellulose and the relative enrichment of lignin-derived aromatic carbon [29]. Such hierarchical pore structures are commonly observed in biochar and suggested to influence water and nutrient dynamics in soils [7]. However, direct evidence linking these structural features to N retention was not obtained in the present study.

The column experiment showed that biochar application changed the patterns of NH_4^+–N leaching (Figure 2a), indicating strong NH_4^+–N retention by the biochar. Also, the incubation experiment showed a lower NH_4^+–N concentration in the biochar treatment immediately after mixing soil and biochar (Figure 3a, day 0). This may reflect rapid adsorption of NH_4^+–N onto biochar surfaces and pore structures. This is consistent with many previous studies reporting that biochar can retain NH_4^+–N through adsorption and ion-exchange mechanisms [7,8,31]. The pyrolysis temperature of rice husk biochar, typically ranging from approximately 250 to 600 °C, has been widely reported to influence its physicochemical properties and functions through changes in porous structure, surface functional groups, and aromaticity [32–34]. For example, Kang et al. (2024) [35] reported that rice husk biochar produced at an intermediate pyrolysis temperature (400 °C) showed effective NH_4^+ adsorption and reduced NH_3 volatilization. The characteristics of their biochar were generally consistent with those of the rice husk biochar used in the present study in terms of pyrolysis temperature and relatively high NH_4^+ adsorption capacity, which may be associated with the relatively high CEC of the rice husk biochar used in this study (83.0 cmolc kg^{-1}, Table 1).

Interestingly, NO_3^-–N leaching was also reduced by biochar application in this study by 24.2% (Figure 2b). On the other hand, NO_3^-–N leaching from the control exceeded 100% (Figure 2b), indicating active nitrification and substantial transformation of NH_4^+–N to NO_3^-–N during the experiment. These results suggest that the reduced NO_3^-–N leaching in the biochar treatment was partly associated with reduced nitrification. Supporting this interpretation, the incubation experiment showed that net nitrification rates (NNR) were significantly lower in the biochar-treated soils (Figure 4), with consistently lower NH_4^+–N and NO_3^-–N concentrations observed throughout the 56-day incubation period (Figure 3a,b). Previous studies on the effects of biochar on nitrification have reported conflicting results. Sun et al. (2017) [10] demonstrated that biochar application retarded the conversion of urea-N to NO_3^-–N in sandy soils, suggesting reduced nitrification rates. Other studies have suggested that reductions in NO_3^-–N leaching are not necessarily due to NO_3^-–N adsorption or nitrification reduction, but may instead be attributed to indirect mechanisms such as increased water-holding capacity and structural changes in soil [36,37]. Liu et al. (2024) [38] stated that biochar tends to increase nitrification activities through a meta-analysis of 95 studies. In the present study, it is difficult to attribute the reduction in

NO_3^-–N leaching to a single mechanism. Nevertheless, the observed decrease in NNR, together with the lower abundance of ammonia-oxidizing bacteria DNA (AOB; Figure 5a), suggests that reduced nitrification was likely one of the contributing factors, although qPCR-based abundance evaluation does not necessarily reflect the actual functional activity of ammonia oxidizers.

In contrast, the DNA of AOA abundance was much lower than that of AOB and was not affected by biochar application (Figure 5b). AOB abundance (4–5 $\times$ 10^{10} gene copies g^{-1} soil) greatly exceeded AOA abundance (1.5–3 $\times$ 10^8 gene copies g^{-1} soil), contrary to previous reports of AOA dominance in nutrient-poor soils [39], possibly because the relatively high NH_4^+–N input at the start of incubation in this study (95 mg N kg^{-1}) favored AOB, which may limit the direct applicability of these results to field conditions. Soil pH (6.3 in the present study) may be another factor influencing the abundance ratio between AOA and AOB, since AOA are often reported to dominate under more acidic soil conditions [36,40]. In addition, the AOB abundance observed in the present study was substantially higher than values commonly reported for agricultural soils [36,40]. Pratscher et al. (2011) [41] reported that AOA are generally adapted to low-nutrient environments and may be inhibited under high NH_4^+ fertilization conditions, whereas AOB can rapidly respond to elevated NH_4^+–N concentrations. The decontaminated soil used in this study was poor in organic matter and reported to have low microbial biomass [42]. Therefore, the relatively high NH_4^+–N input used in the present incubation experiment may have strongly stimulated AOB growth and activity, potentially contributing to the unusually high *amoA* gene copy numbers observed in this study.

Cow dung manure and kudzu application significantly enhanced komatsuna growth and N uptake, confirming their effectiveness as nutrient sources in decontaminated sandy soils (Figure 6a,b). The C/N ratios of the manure and kudzu used in this study were 15.7 and 14.3, respectively. These relatively low C/N ratios may have favored net N mineralization rather than strong microbial N immobilization, consistent with previous findings reported [43]. Co-application of biochar with these organic amendments significantly reduced komatsuna biomass and N uptake compared with their sole application (Figure 6a,b), indicating that biochar may reduce N availability to plants even though biochar enhances N retention in soil. The outcome of co-application of biochar and organic amendments is not consistent in many reports; increased N retention is commonly reported [44,45]; some studies report increased plant growth [18]; others report decreased plant growth [19]. In this study, the column experiment showed that rice husk biochar increased N retention by reducing mineral N leaching (Figure 2), whereas the plant growth pot experiment showed reduced plant N availability when biochar was co-applied with organic amendments (Figure 6b), indicating a trade-off between N retention and plant uptake. One possible explanation is that biochar retains NH_4^+–N within its pore structures and adsorption sites [37,46], thereby reducing its availability for plant uptake [47]. In addition, biochar may influence N mineralization by adsorbing dissolved organic compounds or mineral N released from organic amendments during decomposition [48]. The reduced N availability to plants observed in the present study occurred within the 35-day cultivation period, indicating that the negative effect of biochar on plant N uptake was expressed during the initial stage after biochar application under the present experimental conditions. One previous study showed that the combined application of biochar and organic manure did not initially improve soil N availability, but significantly increased N availability after the second and third repeated applications of organic matter [19]. Other studies have reported that the combined application of biochar improved N availability from as early as several weeks after biochar application [37,44]. Therefore, once nutrient adsorption sites on the

biochar become saturated, biochar may enhance N supply to plants through mineralized N derived from co-applied organic matter.

Overall, this study demonstrated that rice husk biochar significantly altered N dynamics such as mineral N leaching and net nitrification rates in decontaminated sandy soils, indicating its potential to improve nutrient management in low-fertility soils. However, biochar application also reduced plant N availability when co-applied with organic amendments, indicating a trade-off between N retention and plant uptake, particularly during the initial stage after biochar application. These findings suggest that while biochar is a promising amendment, careful management of N bioavailability and nutrient balance is essential when biochar is combined with other N sources for decontaminated soils in Fukushima.

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