Meat and bone meal biochar can effectively reduce chemical fertilizer requirements for crop production and impart competitive advantages to soil

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Abstract

Safe and effective circulation of nutrient-rich meat and bone meal (MBM) could become a carbon-based alternative to limited chemical fertilizers (CFs). Therefore, MBM biochars (MBMCs) were produced at 500, 800, and 1000 °C to evaluate their effects on plant growth, nutrient uptake, and soil characteristics. The results revealed that MBMC produced at 500 °C (MBMC500) contained the maximum amount of C, N, and phytoavailable P. All additional MBMC doses with recommended CF increased sorghum shoot yield (6.7–16%) and significantly improved P uptake. Additional experiments were conducted with decreasing doses of CF (100–0%) with or without MBMC500 (7 t/ha) to quantify its actual fertilizing value. MBMC500 showed the capability to reduce CF requirement by 20% without compromising the optimum yield (by 100% CF) while increasing pH, CEC, total-N, available-P, Mg, and microbial population of post-harvest soil. Although a $\delta^{15}N$ analysis confirmed MBMC500 as a source of plant N, a reduction in N uptake by MBMC500 + 80% CF treatment compared to 100% CF might have limited further sorghum growth. Thus, future studies should concentrate on producing MBMC with better N utilization capability and achieving maximum CF reduction without negative environmental impacts.

Keywords: Biochar-based fertilizer, Waste management, Soil quality, Nutrient recycling, Environmentally friendly fertilizer, Resource recovery

1. Introduction

High-yielding modern agriculture depends mainly on the widespread use of chemical fertilizers for crop production (Tilman et al., 2002). However, continuous application of these fertilizers to soil is not a sustainable practice due to their limited future availability and energy-intensive production processes. These fertilizers also have severe environmental impacts including water quality degradation, smog formation, eutrophication, and greenhouse gas emission (Vitousek et al., 2009). Moreover, even with the continuous application of these fast-release fertilizers, nutrient use efficiency (NUE) for plants is very low, and the soil remains depleted of nutrients due to rapid loss to the environment (Baligar et al., 2001). For a sustainable food production system, nutrients lost from the soil should be returned to close the nutrient loop within the agroecosystem, while also maintaining soil health. Therefore, the safe and efficient circulation of nutrient-rich agricultural waste could be a reasonable carbon-based alternative to synthetic fertilizers and might help the soil to sustain its productivity with reduced environmental impacts (Anae et al., 2021; Rosemarin et al., 2020).

Among agricultural wastes, meat and bone meal (MBM) has one of the highest amounts of essential plant nutrients, especially N, P, and Ca (Jeng et al., 2006; Zwetsloot et al., 2015). MBM is a by-product of animal rendering industries, mainly contains livestock carcasses, leftover fats, and meat residues, and is produced in huge quantities. In Europe alone, 18 million tonnes were produced in a single year (Makara et al., 2015). It was historically used in cattle feed preparation (Cascarosa et al., 2012). However, since the 1980s, the use of MBM has been restricted around the world due to its potential risk to public health and the environment (Vamvuka et al., 2018). For example, the use of MBM was severely constrained in Japan as a result of the bovine spongiform encephalopathy (BSE) epidemic outbreak (Yamamoto et al., 2016). However, considering the reduction of BSE risk and the potential of MBM to be used as a nutrient-rich resource, countries later approved its use as a fertilizer after appropriate pretreatments. Appropriate treatments should necessarily eliminate pathogens, antibiotics, and BSE agents, and make the resulting product environmentally friendly.

Thermochemical conversion of waste biomass into biochar has been repeatedly credited to be a safe technology to remove pathogens, denaturing hazardous chemicals, and stabilizing biomass (Anae et al., 2021). Previous studies have also confirmed the inactivity of BSE agents (prions, the major concern for MBM pretreatment) by treatment at ≥ 180 °C (Taylor and Woodgate, 2003; Yoshioka et al., 2013). Therefore, carbonizing MBM to biochar could become a practical method for safely utilizing this nutrient-rich bioresource in agroecosystems. Additionally, biochar application has many other agronomic benefits to soil including carbon sequestration, and increased water holding capacity, pH, cation exchange capacity, and nutrient retention (Lehmann and Joseph, 2015; Ok et al., 2018). Thus, thermochemically treated MBM (MBMC) might impart additional soil health benefits while being used as an environmentally friendly organic fertilizer.

Although several studies have confirmed biochar’s potential to supply essential plant nutrients (Hossain et al., 2020; Piash et al., 2021), the degree of nutrient conservation during synthesis and effective utilization by plants is controlled by many factors including the biochar production conditions, dose and soil type (Igalavithana et al., 2016; Ok et al., 2018). For example, the N content of chicken manure biochar and the release rate in soil decrease with increasing production temperature (Piash et al., 2021), whereas the P content in manure biochar increases (Sun et al., 2018). Applying the optimum biochar dose is also important since it can have both positive and negative effects on plant growth (Chan et al., 2007; Asai et al., 2009). However, studies have claimed that biochar alone cannot support optimum plant growth since it does not contain sufficient amounts of major nutrients (Ma and Matsunaka, 2013). Rather, the co-application of biochar with chemical fertilizers can significantly improve plant growth, increase NUE, and reduce environmental loss and pollution (Shi et al., 2020). Since biochars like MBMC possess a significant amount of different essential nutrients (N, P, K, Ca, etc.), it is expected that with other agronomic benefits it will help to reduce the chemical fertilizer (CF) requirement for crop production. However, to our knowledge, no previous study has empirically quantified nutrient-rich biochar's potential to partially substitute for CF while maintaining the optimal crop yield. This quantification could be the essential first step toward the effective circulation of nutrient-rich resources.

Therefore, this study hypothesized that nutrient-rich MBMC produced at an ideal temperature and applied at an optimum dose can significantly reduce the hazardous CF requirement without compromising the crop yield. To test this hypothesis, two separate experiments were conducted. The objective of the first experiment was to identify the best MBMC production conditions and application dose for optimum plant growth. The second experiment aimed to determine the percentage of CF that can be substituted by MBMC application while maintaining the current production goal.

2. Materials and methods

2.1. Soil collection and preparation

The soil used in this study was collected from an arable field (0–15 cm, cereals are usually grown) of Hokkaido University (43°04'23"N, 141°20'14"E), Japan. According to the Hokkaido Research Organization (2002), the soil is classified as Japanese brown lowland soil (Haplic Fluvisol). Once in the laboratory, the soil was air-dried, ground to pass through a 2-mm sieve, and stored in plastic bags for further analyses. The physicochemical properties of the collected soil were determined before starting the experiments (Table S1). Due to the difference in collection time, soil characteristics varied slightly. In brief, the soil had a loamy texture and the pH was 5.9–6.2. The soil contained on average 0.0255 mg g^{-1} NO_3-N, 0.005 mg g^{-1} NH_4-N, 0.293 mg g^{-1} available-P, and 0.577 mg g^{-1} exchangeable-K.

2.2. Feedstock collection and production of biochar

MBM was collected from a livestock business cooperative in Hokkaido, Japan. The feedstock contained mostly cattle MBM (~80%) and the rest (~20%) was swine MBM. Before biochar production, the feedstock was dried at 105 °C for 24 h. It was heated at 10 °C/min and pyrolyzed for 1 h in a muffle furnace (FO810, Yamato Scientific, Japan) at three different temperatures (500, 800, and 1000 °C) and designated MBMC500, MBMC800, and MBMC1000, respectively. The muffle furnace has the ability to restrict air entry during heating, which ensures an oxygen-limited condition in the furnace for proper carbonization. After pyrolysis, the biochars were ground to pass through a 2-mm sieve and stored in airtight bags.

2.3. Pot experiments

2.3.1. Pot experiment to evaluate the effect of MBMC type and dose

The first pot experiment was conducted to evaluate the effect of MBMC production temperature and dose on plant growth, nutrient uptake, and the physicochemical properties of post-harvest soil. The experiment included 6 different treatments, having 5 replicates each (Table S2). The control treatment contained only recommended (Hokkaido Agriculture Department, 2015) chemical fertilizers (ammonium sulfate, calcium superphosphate, and potassium sulfate). The next three treatments included CF and additional MBMC (3.5 t/ha) produced at 1000, 800, and 500 °C, to elucidate the appropriate carbonization temperature. The last two treatments included different amounts of MBMC500 (1.75 and 7.0 t ha^{-1}) with CF to determine the appropriate dose.

Sorghum (*Sorghum bicolor* (L.) Moench), a fast-growing major feed-grain in Japan was grown in Neubauer pots in a greenhouse regulated at 26 °C. Each pot received the same amount of CF, and a predetermined amount of MBMC to soil. Distilled water was used to irrigate the pots once a day and 60% of the maximum water-holding capacity of the soil was maintained. The pots did not have any holes, so no soil or water could leach out. Five sorghum seeds were initially sowed, and after 7 days, the weakest one was removed so that each pot had 4 sorghum seedlings. At 37 days following sowing, the sorghum shoots were harvested, washed with distilled water, and dried completely at 65 °C. Dry yield, and total N, P, K, Ca, and Mg contents of the shoots were determined later. Samples were also collected from the pots to investigate the chemical properties of the post-harvest soil.

2.3.2. Second pot experiment to evaluate the potential of MBMC to reduce CF use

A second pot experiment similar to the previous one with decreasing CF content was conducted to determine the potential of MBMC500 to reduce the required CF. This experiment was conducted with 12 treatments, having 5 replications each (Table S3). The first six treatments received 100%, 80%, 60%, 40%, 20%, and 0% of recommended CF without any MBMC, whereas the last six treatments received a similar amount of CF together with 7.0 t/ha (the most effective dose in the first pot experiment) MBMC500. The agronomic practices were the same as in the first pot experiment. At 37 days after sowing, sorghum shoots and roots were harvested and immediately washed with distilled water. The shoots and roots were analyzed for dry shoot yield, dry root yield, and total N, P, K, Ca, and Mg contents. A post-harvest soil analysis was also conducted.

2.4. Analysis of materials

2.4.1. Characterization of soil, biochar, and plants

Biochar yield (%) was determined from the mass ratio of dry biochar to biomass, multiplied by 100. The pH of the soil and MBMC samples was determined using a glass electrode (JIS Z 8802). The exchangeable K, Ca, and Mg content in fresh and post-harvest soil was measured by atomic absorption spectrophotometry after extraction with 1 M ammonium acetate. The available P, NO_3-N and NH_4-N were determined by Truog's method, the hydrazine reduction procedure, and the indophenol method, respectively (Gilbert et al., 2009; Hayashi et al., 1997; Selmer-Olsen, 1971). The total CHN content in MBMCs and plants was determined using a CHN analyzer (Exeter Analytical CE440, Exeter Analytical Inc.). The elemental composition (P, K, Ca, Mg) of MBMC and plant shoots and roots was determined by inductively coupled plasma mass spectrometry (ICP-

MS; ELAN DRC-e, PerkinElmer) after HNO_3 digestion. The available phosphorus in biochars was extracted by 0.1 M KCl followed by analysis with ICP-MS (Xu et al., 2013). All the chemicals (For example: $C_2H_7NO_2$, HNO_3, KCl) used in this study for extraction, digestion or spectrophotometric analysis of the samples were of analytical grade (Wako pure chemical).

2.4.2. Nitrogen stable isotope ratio mass spectrometry

To determine the source of nitrogen taken up by sorghum, a nitrogen stable isotope analysis was performed. The nitrogen stable isotope ratio for each MBMC500, CF, raw soil, and harvested plant samples was measured using an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific). The nitrogen was separated from each sample through complete oxidation at 1000 °C and reduction at 680 °C (Flash EA 1112, Thermo Fisher Scientific), and the sample was then introduced into the mass spectrometer with helium gas. The results are expressed as $\delta^{15}N$ (parts per thousand), and calculated as follows:

$$\delta^{15}\mathrm{N} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000$$

where R_{sample} stands for the relative abundance of ^{15}N ($^{15}N/^{14}N$) in a sample, and R_{standard} is the relative abundance of ^{15}N in atmospheric N_2.

2.4.3. Microbial diversity analysis

The raw and post-harvest soils were analyzed for changes in microbial diversity by 16S rRNA gene sequencing. Microbial genome DNA extraction, two-step PCR reaction and purification for 16s metagenomic sequencing, and operational taxonomic unit (OTU) clustering were performed following the methods described in Piash et al. (2022).

Representative sequences were compared with the EzBioCloud 16S database (https://www.ezbiocloud.net/) for phylogenetic estimation.

2.5. Statistical analysis

Statistical analysis of data was carried out using R (Version 3.6.2). To assess the differences in sorghum yield, nutrient uptake, and post-harvest soil properties in the first experiment, a Tukey-HSD test was used. Welch's *t*-test was performed to compare the effects of MBMC (applied with or without the same CF dose) on mean sorghum yield, nutrient uptake, $\delta^{15}N$ value, and post-harvest soil properties (second experiment). To verify the effect of MBMC in reducing CF input, Dunnett's test was used to compare sole CF treatment (100%) and all other CF (0–100%) treatments with MBMC.

3. Results

3.1. Characteristics of meat and bone meal biochars (MBMCs)

The characteristics of MBMCs produced at three different temperatures are shown in Table 1. More than 40% of the MBM was converted to biochar and the biochar yield (48.40–42.67%) decreased with increasing pyrolysis temperature. In all the MBMCs, the Ca and P content was relatively high among the macronutrients essential for plants. As the carbonization temperature of MBMC increased, the P and Ca content also increased, whereas the C, H, and N content decreased significantly. The amount of total P contained in MBMC was around 4–5%, whereas the KCl-P (phytoavailable) content was <0.15%, indicating the limited solubility of most P forms. It should also be noted that, although the total P content increased with production temperature, the KCl-P content decreased significantly. All MBMCs were alkaline (pH 9.05 for MBMC500, 9.74 for MBMC800, and 10.58 for MBMC1000), and the pH increased with carbonization temperature.

Table 1. Characteristics of MBMCs produced at different temperatures. The data shows the mean value for three replicates (n=3).

Biochar	Yield (%)	Elemental composition							pH
		C	H	N	P (KCl-P)	K	Ca	Mg	
	(% db)								(-)
MBMC500	48.40	22.39	1.03	3.96	4.14 (0.13)	0.39	14.24	0.27	9.05
MBMC800	42.99	20.59	0.28	3.01	4.26 (0.08)	0.37	14.12	0.27	9.74
MBMC1000	42.67	18.55	0.08	1.44	5.07 (0.04)	0.39	15.26	0.30	10.58

KCl-P: potassium chloride extractable phosphorus; db: dry basis

3.2. Effects of MBMC type and dose on plant growth, nutrient uptake, and post-harvest soil properties

3.2.1. Effect on plant growth and nutrient uptake

In the first pot experiment, the effect of combined MBMC and CF application was investigated. It was found that the addition of MBMC together with CF increased the sorghum shoot yield (dry mass) by 6.7–16.0% compared to the CF-only treatment (Fig. 1a). A dose of 7 t/ha MBMC500 proved to be the best treatment for increasing shoot yield, although the yield differences were statistically insignificant. No significant yield difference was observed among the MBMCs produced at different carbonization temperatures.

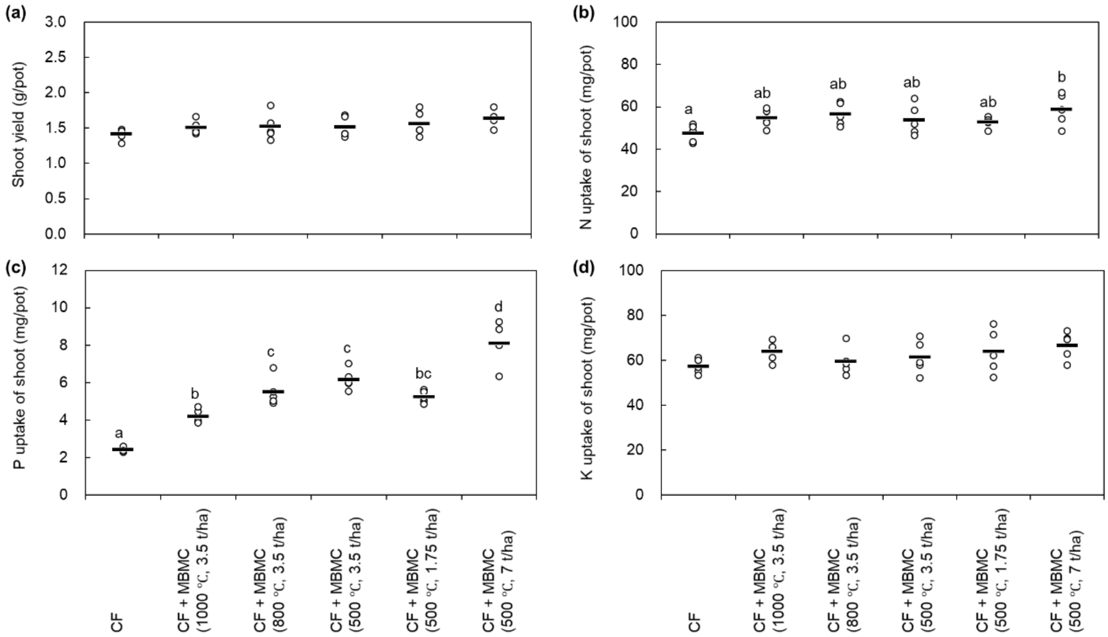

Fig. 1. Effect of MBMC type and dose on sorghum shoot yield (a), N (b), P (c), and K (d) uptake in first pot experiment. White circles represent the data for one replicate, and black bars are the mean for each treatment (n = 5). The letters indicate significant differences among the treatments at $p < 0.05$ according to the Tukey-HSD test.

The nutrient content of the sorghum shoots grown using MBMC was higher than that of the sorghum produced by 100% CF (Fig. 1b–d). In particular, the phosphorus uptake for all pots with MBMC was significantly higher than that for the CF-only pot (Fig. 1c). The highest dose (7 t/ha) of MBMC500 resulted in the maximum P uptake (234% higher than for CF-only treatment). Although the total P was highest in MBMC1000, the lowest amount of P was taken up using this treatment. Both the N and K uptake were highest for plants grown with the maximum dose (7 t/ha) of MBMC500 (Fig. 1b,d); however, only the N uptake was significantly higher (24%) than that for the CF-only treatment.

3.2.2. Effect on post-harvest soil properties

The physicochemical properties of post-harvest soil from the first experiment are shown in Table S4. The pH of CF-treated soil was the lowest (~5.0), whereas the pH was higher (on average by 0.5 units) for all MBMC-treated soils. The post-harvest pH for the MBMC500-treated soil (7 t/ha) exhibited the maximum value (5.7). Similarly, the total N, available P, Ca, Mg content, and CEC of soils were higher for MBMC-treated pots than for CF-only pots, and the maximum values were achieved for the highest MBMC500 dose. The amount of NH_4-N tended to decrease with the application of MBMC (by 22.6% to 67.7%). In particular, the NH_4-N concentration in the CF + MBMC500 (7 t/ha) treated pot was significantly lower (67.7%) than that for the CF-only pot.

3.3. Effect of reduced CF dose and MBMC500 input

3.3.1. Effect of reduced CF dose and MBMC500 input on plant growth

In the second pot experiment, shoot yields increased by 2.7 to 25.1% by co-application of CF and MBMC500 relative to the CF-only case (Fig. 2a). In contrast, additional MBMC500 application with decreased CF (up to 40% CF) reduced the root yield (Fig. 2b). However, MBMC incorporation without CF or with 20% CF increased the root yield. In general, additional MBMC500 application (7 t/ha) with CF did not significantly increase the shoot yield or decrease the root yield. However, MBMC application alone (0% CF) increased both the shoot and root yields significantly compared to the non-fertilized control (Fig. 2).

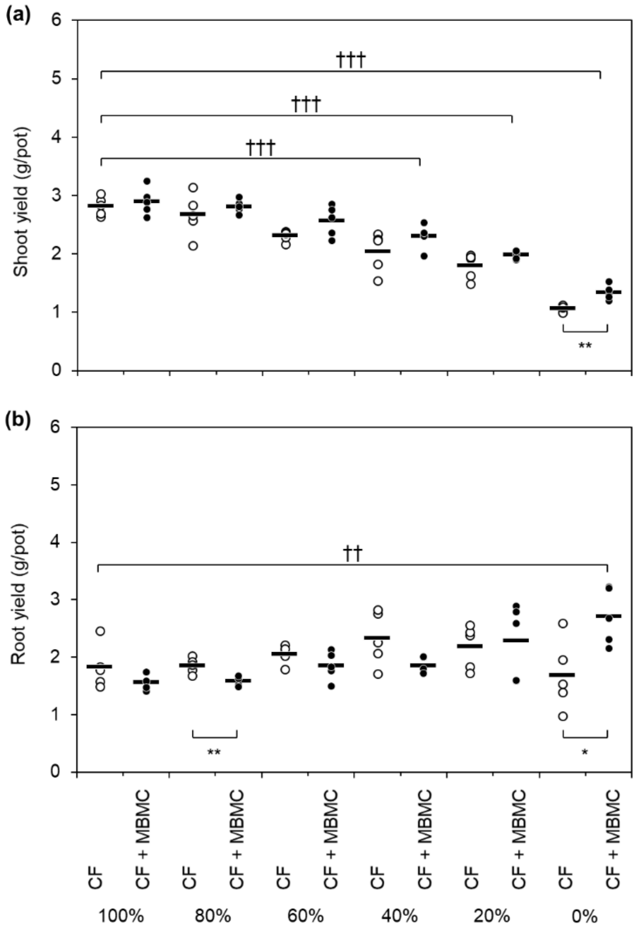

Fig. 2. (a) Shoot and (b) root dry yields of sorghum in second experiment. Black horizontal bars represent the mean (n = 5) yield for each treatment. The white or black circles represent the distribution of data. Daggers (†) or asterisks (*) between the two treatments indicate significant differences according to the Dunnett or Welch *t*-test, respectively. Single, double, or triple symbols between two treatments mean a significant difference at the 0.05, 0.01, or 0.001 level, respectively.

3.3.2. Effect of reduced CF dose and MBMC500 input on nutrient uptake by sorghum

In the second pot experiment with reduced CF input, the N uptake of sorghum shoots did not increase significantly for MBMC500-treated pots compared to the CF-only case, except for 0% CF (Fig. 3a). The N uptake of sorghum roots decreased by 7.4–12.8% for

MBMC treatments with high CF doses (80–100%), whereas it increased by 33.8–332.9% for low CF doses (0–60%) compared to the cases without MBMC (Fig. 3b). Overall, N uptake was not significantly affected by MBMC500 application. It also had no significant impact on K uptake in shoots and roots (Fig. 3e, 3f). Interestingly, P uptake by both shoots and roots increased significantly by MBMC500 application, increasing by 57.1–104.5% and 158.8–759.5%, respectively, compared to the CF-only cases (Fig. 3c, 3d). Interestingly, for MBMC500 application with increasing CF dose, the relative P uptake of shoots increased (Fig. 3c) whereas that of roots decreased (Fig. 3d).

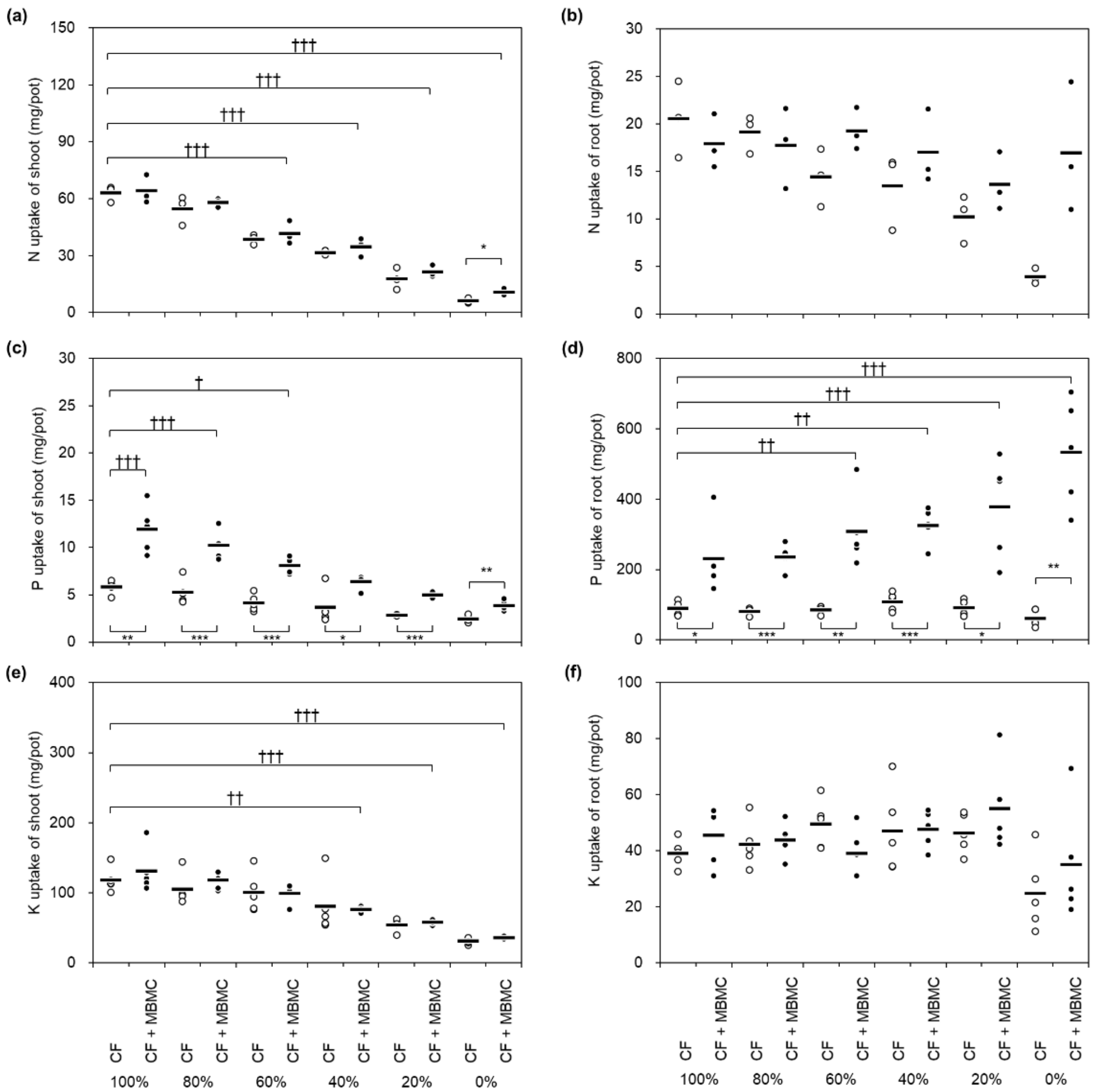

Fig. 3. (a) N, (c) P, (e) K uptake of sorghum shoots, and (b) N, (d) P, (f) K uptake of sorghum roots in second experiment. Horizontal black bars represent the mean ((a) and (d): n = 3; (b), (c), (e), and (f): n = 5) nutrient uptake for each pot. The white or black circles represent the distribution of data. Daggers (†) or asterisks (*) between two treatments indicate significant differences according to Dunnett or Welch t-test, respectively. Single, double, or triple symbols between two treatments mean a significant difference at the 0.05, 0.01, or 0.001 level, respectively.

3.3.3. Source of N in sorghum plants

The $\delta^{15}N$ content in plants increased as the CF content was reduced both for MBMC-treated and CF-only pots (Fig. 4). For the same amount of CF, the $\delta^{15}N$ value for MBMC-treated pots was significantly higher than those for CF-only pots (except 80% CF). For 60–100% CF, the $\delta^{15}N$ values were close to that for the CF itself (-9.14‰). In contrast, the $\delta^{15}N$ values for low CF doses (0–40%) were close to that for the soil (7.26‰) or MBMC500 (7.38‰). This implies that MBMC500 directly contributes to plant N uptake, and the ratio of N uptake from MBMC increases with decreasing CF content.

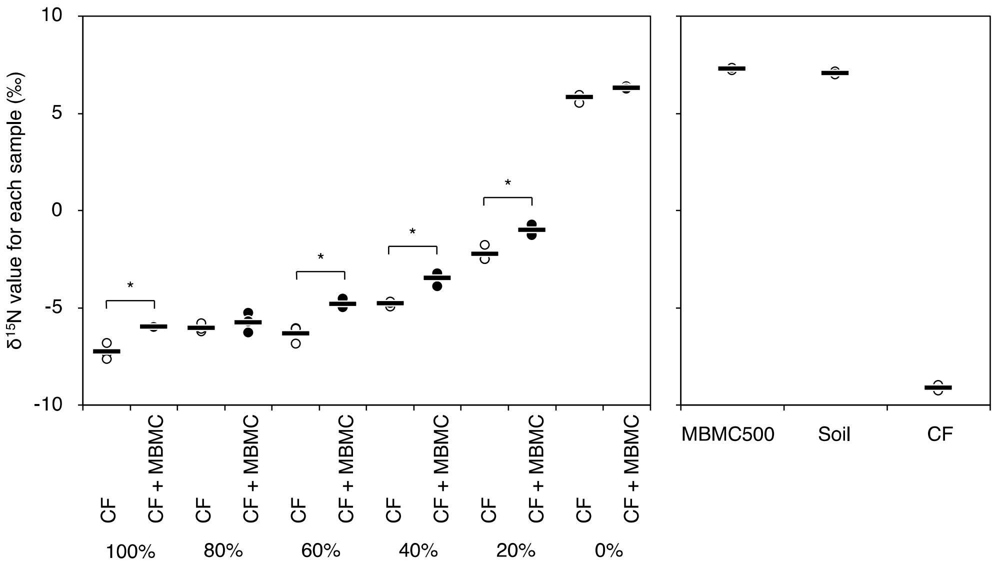

Fig. 4. $\delta^{15}N$ for harvested sorghum shoots, MBMC500, soil, and CF used in second experiment. Horizontal bars represent the mean ($n = 3$) $\delta^{15}N$ value in the analyzed samples. The white or black circles represent the distribution of data. Asterisks (*) between two treatments indicate significant differences according to Welch's t-test at $p < 0.05$.

3.3.4. Effect of reduced CF dose and MBMC500 on microbial diversity of soil

MBMC500 co-application with different CF doses increased the total number of identified OTUs (microbial count) in the post-harvest soils compared to the CF-only cases, except for 100% CF (Fig. 5). Interestingly, in both situations (with and without MBMC), treatments with 60% CF resulted in the highest microbial populations. Further analyses were attempted to identify nitrifying and phosphate solubilizing bacteria (PSBs) to compare the effect of MBMC500 on their diversity; however, no significant difference was observed among the treatments through 16S rRNA gene sequencing.

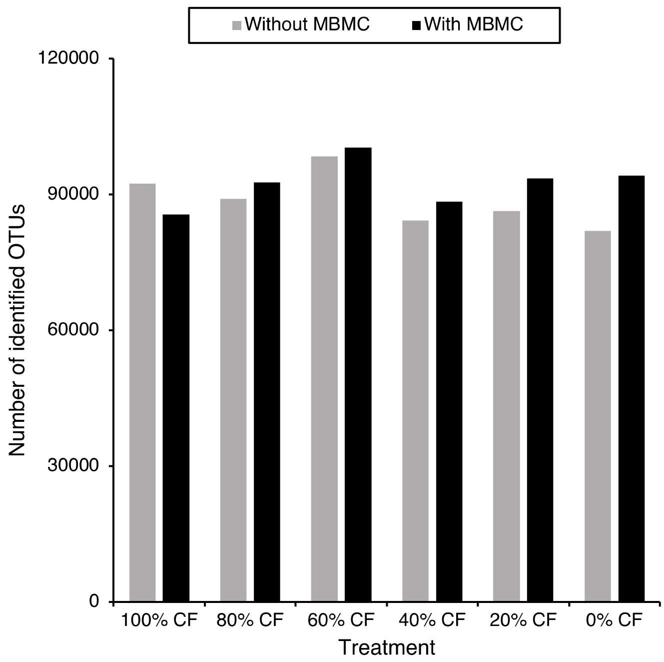

Fig. 5. Number of identified OTUs (n=1) in post-harvest soil affected by CF dose with and without MBMC500 treatment.

3.4. Capability of MBMC500 to effectively reduce chemical fertilizer requirement and provide agronomic benefits to soil

Table 2 compiles the changes in biomass yield, nutrient uptake, and nutrient content for post-harvest soils as a result of MBMC500 application for different CF doses (0–100%) compared to 100% CF. The shoot yield produced by 80% CF with MBMC500 (7 t/ha) was almost the same as that for 100% CF, implying the potential of MBMC to decrease the CF requirement by up to 20%. A 40% reduction in CF with co-applied MBMC500 resulted in an insignificant decrease in shoot yield, although it considerably decreased shoot N uptake. Sorghum shoot yields decreased by 9.2–52.7% for MBMC treatments with lower CF doses (from 60% to 0%), and significantly decreased after reducing the CF dose by more than 60% (40% CF). Compared with 100% CF, root yields were not reduced significantly up to 80% reduction of CF (Fig. 2b). The MBMC500 treatment with 80% CF showed superior nutrient uptake and post-harvest soil properties without compromising shoot yield. For example, the soil pH (0.7 unit), NO_3-N (247.6%), available P (699.2%), and exchangeable Mg (9.3%) increased significantly together with 75% more P uptake by sorghum shoots and 12% more K uptake by sorghum roots compared to the 100% CF treatment. For a 20% reduction of CF, there is an 8.1% decline in shoot N and a 13.7% decline in root N, which is insignificant compared to the 100% CF case. NH_4-N in post-harvest soil also declined for all MBMC500 treatments relative to the fertilized control (100% CF).

Table 2. Change (%) in biomass yield, soil properties, and nutrient uptake by sorghum shoots and roots caused by MBMC treatments (n=5) compared to 100% CF treatment.

Treatments	Parameters															
	Biomass yield		Post-harvest Soil Properties								Nutrient uptake of shoots			Nutrient uptake of roots		
	Shoots	Roots	pH	CEC	NH4-N	NO3-N	Av-P	Ex-K	Ex-Ca	Ex-Mg	N	P	K	N	P	K
	g/pot		(-)	$cmol_c$ kg^{-1}	mg/g						mg/pot					
100% CF + MBMC500	2.7%	-14.8%	0.53 ***	5.1% *	-32.6% **	249.2% **	706.2% ***	11.9% *	13.6% **	11.0% **	1.7%	104.9% ***	10.7%	-12.8%	158.8%	16.6%
80% CF + MBMC500	-0.4%	-13.0%	0.60 ***	1.9%	-53.6% ***	247.6% **	699.2% ***	1.7%	-2.1%	9.3% **	-8.1%	75.3% ***	-0.4%	-13.7%	163.9%	12.2%
60% CF + MBMC500	-9.2%	1.1%	0.67 ***	4.2% *	-67.1% ***	217.5% **	692.6% ***	6.7%	4.4%	8.3% *	-33.9% ***	38.5% *	-16.5%	-6.1%	245.5% **	-0.0%
40% CF + MBMC500	-18.4% ***	1.3%	0.83 ***	2.9%	-77.6% ***	36.5%	623.9% ***	2.2%	0.0%	9.1% **	-45.0% ***	10.3%	-35.5% **	-17.2%	264.8% **	22.1%
20% CF + MBMC500	-29.7% ***	25.3%	1.17 ***	2.2%	-67.8% ***	-6.3%	658.4% ***	18.6% ***	0.6%	12.2% ***	-66.0% ***	-14.6%	-51.1% ***	-33.5%	324.1% ***	40.6%
0% CF + MBMC500	-52.7% ***	48.4% **	1.47 ***	3.3%	-51.2% ***	-30.2%	540.4% ***	22.9% ***	-9.0% *	11.5% ***	-82.9% ***	-33.6%	-69.6% ***	-17.3%	496.2% ***	-10.3%

CEC: cation exchange capacity; Av: available; Ex: exchangeable; *: Significant at $p < 0.05$ level; **: Significant at $p < 0.01$ level; ***: Significant at $p < 0.001$ level, according to Dunnett test.

4. Discussion

4.1. Effect of carbonization temperature on nutrient content and speciation of MBMC

Among the major macronutrients, the phosphorus and calcium content of MBMCs was substantially higher than any other plant- or manure-based biochars manufactured at similar production temperatures (Ippolito et al., 2020). For example, the average P content of biochars made from wood, crops, grass, and manure (or biosolids) is 0.4, 0.8, 2.01, and 2.57%, respectively, whereas the average Ca concentration is 2.63, 2.07, 4.59, and 5.27%. Comparatively, the much higher P and Ca content (at least 4.14 and 14.12%) of MBMC biochars indicates their potential to be good nutrient sources. A previous study suggested that raw MBM (dairy cattle) contains a considerable amount of N (10.52%) and P (4.07%) (Takahashi et al., 2003). Carbonization can cause loss and transformation of some of these nutrients, affecting the nutrient recovery rate. The decreasing KCl-P content in MBMCs with carbonization temperature can be explained by the transformation of organic P into crystalline (hydroxyapatite) calcium phosphates (Zwetsloot et al., 2015). The higher KCl-P content in low-temperature MBMCs is an indication of more P availability for plants (Xu et al., 2013). Additionally, the conservation of N in MBMCs also decreased significantly with increasing production temperature, which is in line with the previous study on animal manure biochars (Piash et al., 2021). This is due to substantial N release in gaseous form (N_2, NH_3, NO_x) with increasing pyrolysis temperature (Ippolito et al., 2015). The carbonization temperature did not have a significant effect on the other two macronutrients (K and Mg) analyzed. Therefore, where conservation of major macronutrients (N, P, K, Ca, Mg) and their phytoavailable forms (especially P) are concerned, a lower carbonization temperature (≤ 500°C) should be considered as long as it can effectively eliminate pollution and human health risks. A lower production temperature (500°C) has also been seen to yield more biochar with higher carbon content.

4.2. MBMC production temperature and dose affect plant growth, nutrient uptake, and post-harvest soil

The increased sorghum yield (6.7 to 16%) using MBMC can be attributed to two beneficial aspects of this biochar. First, it adds a considerable amount of nutrients (N, P, and Ca mainly) to the soil which improves nutrient uptake by sorghum plants. Particularly, P uptake for all MBMC-treated pots was significantly higher than the fertilized control (CF) due to the above-mentioned reason. Among the different MBMC types and doses, MBMC500 applied at 7 t/ha resulted in maximum P uptake due to its highest P availability (KCl-P). Additionally, crystalline hydroxyapatites in MBMCs (produced at higher temperatures) are acid-soluble (Zwetsloot et al., 2015), and therefore MBMC800 and MBMC1000 could also contribute to higher P uptake since the post-harvest soil pH was slightly acidic. MBMCs also contributed to better N uptake; however, it was only significant when the highest N-containing MBMC500 was applied at 7 t/ha, which indicates the limited potential of MBMCs with high N content (1.5–4%) to enhance plant N uptake.

Second, MBMC conditions the soil environment to become more suitable for plant growth. For example, the post-harvest soils treated with MBMC had a more favorable soil reaction (pH) compared to the CF-only treatment. This increased soil pH was also associated with the increased availability of P, Ca, Mg, NO_3-N, and improved CEC. This implies that the combination of MBMC and CF not only improves nutrient uptake but also the quality of the soil, which will provide sustainable benefits for plant growth. Chen et al. (2020) also reported that these two factors were responsible for enhanced plant growth using pig-carcass biochar.

MBMC500 applied at 7 t/ha showed the best plant growth and post-harvest soil properties because it had the highest N and phytoavailable P content, and better pH and CEC. This also implies that the plant growth rate increases linearly with MBMC dose, which is in line with a previous study involving sheep bone biochar (Azeem et al., 2021). However, some researchers have suggested that a much larger dose of biochar may result in decreased crop yield and N uptake, due to N immobilization caused by the high C/N ratio in the applied biochar (Asai et al., 2009; Lehmann et al., 2003). For this reason, excessive application of MBMC may adversely affect crop growth, so the MBMC dose must be set carefully.

Therefore, the second experimental study to quantify the potential of MBMC to reduce CF requirements was conducted with the best-performing MBMC500 at the highest dose (7 t/ha), which resulted in increased plant growth without any negative side effects.

4.3. MBMC500 to reduce chemical fertilizer requirement

4.3.1. Sustained sorghum shoot and root growth with lower CF dose

It is apparent from the results that MBMC500 application with 80% of the recommended CF dose could produce a sorghum shoot yield similar (-0.4%) to that for 100% CF. This implies that nutrient-rich MBMC500 applied at 7 t/ha can reduce CF input by 20% without compromising the optimum yield. Previously, increased maize growth was reported using a similar bone biochar (500 and 800 °C) with a much higher dose (2.5–10% w/w) without any CF (Azeem et al., 2021). In contrast, this study successfully quantified the plant growth benefits of MBMC500 with a practical dose. This study confirmed MBMC500's capability to reduce the CF requirement when applied for the first time. MBMC might have residual effects and continue to reduce the CF requirement in the next growing season. However, this short-term study could not evaluate any residual benefits. Although this study found a slight decrease in root yield after MBMC application, other studies reported an increase in root growth (Azeem et al., 2021). The decrease in this study can be explained by additional nutrients supplied by biochar application that decreased the need for root elongation for N, P, and K uptake (Forde and Lorenzo, 2001). However, the decrease would not adversely affect above-ground biomass production, but rather increase the shoot:root ratio. Overall, the increase in sorghum shoot yield (2.7–25.1% compared to CF without MBMC500) by additional MBMC500 can be attributed not only to direct nutrient input (Biederman and Harpole, 2013; Lehmann et al., 2003; Uzoma et al., 2011), but also an increase in biological activity (Fig. 5), and subsequent nutrient release for plant uptake (Thies and Rillig, 2009).

4.3.2. MBMC500 improved nutrient uptake of plants

Although the co-application of MBMC500 with CF did not significantly increase the sorghum yield compared to the CF-only treatment, it could produce relatively nutritious crops. For example, P uptake by sorghum shoots and roots was substantially higher when MBMC500 was applied with all doses of CF, probably due to Ca-P dissolution in an acidic soil environment and subsequent uptake (Piash et al., 2022). Substantial P remained available (540–700% higher than CF-only treatment) even in post-harvest soil, which could be utilized in the next growing season, indicating a long-term benefit of MBMC application.

N uptake by sorghum shoots and roots increased most by co-application of MBMC500 and CF. This can be attributed to the mineralization of N species from MBMC and the retention of N (from CF) by biochar and subsequent slow release (Pandey et al., 2020). The significantly higher amount of NO_3-N in post-harvest soil following MBMC500 treatment with >60% CF also supports the claim of higher retention. Additionally, by a N isotope analysis (^{15}N), it was revealed that a significant portion of N taken up by sorghum was from MBMC500 when applied with CF. Therefore, MBMC500 applied at 7 t/ha can supply plant-available N. However, most previous studies conducted with or without ^{15}N labeling did not confirm biochar as a direct source of plant nitrogen (Chan et al., 2007; Fiorentino et al., 2019), perhaps due to its very low N content. In this study, N uptake from 80% CF + MBMC500 treatment was lower (~22%) than for 100% CF, despite a significant increase in soil NO_3-N content. Thus, reduced N uptake from MBMC treatment could become a limiting factor for crop growth. Furthermore, NH_4-N in post-harvest soil was seen to decrease significantly compared to CF-only treatment. One probable reason could be the increased volatilization of NH_3 as the soil pH increased upon MBMC application. However, the pH of the post-harvest soil remained acidic, which makes this argument disputable. Therefore, understanding the N dynamics after MBMC application by a time-dependent incubation study is essential to further understand its fertilizing value. Additionally, since a large amount of available P and K remained in post-harvest soil, better utilization of N in MBMC or supplementing with N fertilizer may result in a further CF reduction.

Since MBMC did not have a substantial amount of K, uptake was not much affected by the MBMC500 application.

4.3.3. Additional benefits of MBMC500 for post-harvest soil

Typically, the pH of post-harvest soil decreases due to the application of CF or the cultivation process (Goulding, 2016). In this study as well, soil pH declined after sorghum cultivation with CF. Soil acidification was also observed following MBMC treatment, but the degree of acidification was lower than that for CF treatments. In contrast, Ma and Matsunaka (2013) reported an increased soil pH after using dairy-carcass biochar. As described in section 4.1, calcium compounds can increase soil pH due to their ability to consume hydrogen ions during the dissolution process in soil. Suppressing soil acidification has additional benefits including better microbial activity and enhanced bioavailability of nutrients. Schneider and Haderlein (2016) reported that most P in soil is strongly bound to hydroxides and oxides of Fe, Al, and Mn under acidic conditions. Thus, a soil pH closer to neutral is beneficial for P availability. Since the pH for MBMC-treated soil was ~6.5, the amount of bioavailable P increased significantly compared to the CF-only treatment. In addition, the availability of N, K, Ca, and Mg generally increased, probably because of the increased soil pH (Steiner et al., 2007). Increased microbial diversity is believed to alter C mineralization and nutrient transformations (Singh et al., 2022) that can stimulate plant growth and defense mechanisms (Kolton et al., 2017). However, a much higher dose of biochar (>80 t/ha) can negatively affect soil microbial diversity (Singh et al., 2022), which necessitates identification of the appropriate biochar type and dose. The cation exchange capacity (CEC) of soil is crucial for plant growth since it helps to hold and supply essential anionic nutrients. In this study, MBMC application increased the CEC for post-harvest soil by 2–5% with or without the CF treatment, which is effective for retaining cationic nutrients like K, Ca, and Mg. All this evidence indicates MBMC's potential to increase and retain phyto-available nutrients in post-harvest soil, which will mitigate nutrient leaching and subsequent environmental pollution from agroecosystems.

Many previous studies have suggested that biochars with high C content could be promising and efficient materials for permanent C storage in soil (Shaaban et al., 2018). Azeem et al. (2021) reported an 89% increase in dissolved organic carbon and an 18% increase in total organic carbon for soil treated with 10% (w/w) sheep-bone biochar. In comparison, the MBMC500 used in the present study has double the amount of C (~22%) and therefore, is expected to increase the persistent organic carbon content of the soil. This increase in organic carbon as a climate-smart practice, especially in low-carbon soil, was reported to be one of the underlying factors behind plant growth promotion by animal-carcass biochar (Chen et al., 2020). Additionally, the increase in dissolved and persistent organic carbon in soil by a porous carbon-rich material is expected to impart many other additional benefits including higher water holding capacity, surface area, porosity, and microbial activity, together with reduced toxicity of pollutants (Chen et al., 2020; Haider et al., 2022; Lehmann and Joseph, 2015). Therefore, co-application of MBMC500 with CF can afford long-term benefits to soil resulting in a reduced burden on costly and hazardous synthetic fertilizers. Future studies should focus on determining the optimum production conditions for MBMC that maximize nutrient conservation and recycle the essential nutrients for plant growth.

5. Conclusion

This study quantified the potential of MBMC to become a safe and efficient organic fertilizer for plant growth, and found that it significantly reduced (by 20%) the CF demand for crop production. Its ability to supply a substantial amount of phytoavailable P for plant uptake and increase P availability in post-harvest soils makes it a promising substitute for synthetic phosphate fertilizers with reduced environmental impacts. However, despite MBMC500 acting as a N source, its combined use with 80% CF caused a reduction in N uptake, indicating its ambiguous N dynamics in soil. Clarifying such dynamics may help to utilize the full potential of MBMC for plant growth in the future and to further reduce CF requirements. Although this study opens a path for research involving the practical and effective circulation of nutrient-rich bioresources in agriculture, further studies are essential to reveal its economic feasibility and to mechanistically understand nutrient dynamics in the agro-environment. Validation of these positive results under field conditions with diverse nutrient-rich feedstocks and crops would help to establish a carbon-neutral, sustainable agricultural system that ensures an environmentally friendly circular bioeconomy for the future.

Acknowledgments

This research was funded by the Japan Livestock By-product Association. The authors are grateful to the Livestock Business Cooperative of Southern Hokkaido, Japan (Hokkaido Doh-nan Chikusan Jigyo Kyodoh-kumiai) for providing the MBM feedstock. We thank Associate Professor Dr. Toshihiro Watanabe of the Laboratory of Plant Nutrition at Hokkaido University for cooperation with the ICP-MS analysis. The authors would also like to thank the Instrumental Analysis Division of Hokkaido University for performing elemental analyses of samples.

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Highlights:

- Effective nutrient recycling capacities of different MBM biochars were compared.
- While the P content increased with production temperature, availability decreased.
- N-rich MBM biochar proved to be a direct source of plant N.
- Co-application of MBMC and chemical fertilizer imparts numerous agronomic benefits.
- Greater utilization of MBMC-N might completely replace synthetic fertilizers.