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Alterations of the Chemical Compositions, Surface Functionalities, and Nitrogen Structures of Cage Layer Chicken Manure by Carbonization to Improve Nitrogen Bioavailability in Soil

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Abstract: Carbonization of cage layer chicken manure (CLCM) can improve its bio-recalcitrance which might improve nitrogen (N) bioavailability in soil. However, temperature(s) to exhibit appropriate variations in the chemical makeup of the manure during carbonization in order to achieve this objective is unknown. In this study, we investigated the alterations in chemical compositions, surface functionalities, and N speciation initiated by different carbonization temperatures (350, 500, and 650 °C) and the effects of these alterations on N bioavailability in soil. The objective was to identify suitable temperature condition(s) for the conversion of CLCM into a carbonized product of appropriate bio-recalcitrance that is capable of improving N bioavailability in soil more than the un-carbonized CLCM. The results showed an increased bio-recalcitrance of the manure with increasing carbonization temperatures due to drastic changes in the chemical makeup and accumulation of heterocyclic aromatic N structures. Subsequently, these alterations in the chemical makeup and state of the organic N species in the manure affected N bioavailability in soil. Notably, N bioavailability of CLCM and benefits on plants were improved when soil was supplied with CLCM that was altered at 350 °C. With these observations, we concluded that alterations in chemical and surface structural compositions and N speciation at 350 °C are optimum for instituting the required bio-recalcitrance to CLCM in order to improve N bioavailability in soil for plants.

Keywords: cage layer chicken manure; carbonization; N availability; bio-recalcitrance; chemical properties; mineral nitrogen; N speciation; surface functional groups

1. Introduction

The importance of livestock manure as a source of nutrient for crop productivity has long been acknowledged. Chicken manure (CM), and especially cage layer chicken manure (CLCM), is a concentrated plant food that contained the highest nitrogen (N) content when compared to other livestock manure commonly used as nutrient source [1]. CM has been reported to have several positive effects on crop growth, yield, and soil fertility improvement [2,3]. The high N value of CLCM is due to the absence of litter and the relatively high crude protein content in the diet formulation [4]. However, this high quality coupled with a very low C/N ratio creates a condition of rapid decomposition of the manure, which can cause negative environmental and agronomic effects [5]. For instance, the excess

release of N during the rapid decomposition of the manure in soil will lead to surface and ground water contamination through N losses by runoff and leaching [6,7]. Furthermore, it can also lead to the release of greenhouse gases into the atmosphere through volatilization of N related gases [8]. This rapid decomposition and losses of N from the soil systems following application of the manure negatively affect N bioavailability [5]. Abbasi et al. [9] reported that a greater portion of the organic N of CM was mineralized within the first one to two weeks after application. Similarly, Chescheir et al. [10] also reported depletion in mineral N availability shortly (one to two weeks) after application of CM to soils. The rapid decomposition and reduction of N in soil after application of CLCM will cause early nutrient deficiency which will then necessitate additional nutrient application times if good growth and yield of crops are to be maintained. This will increase cost of production. However, in resource constraint farming systems where farmers cannot afford to apply fertilizer severally, the overall effects of such early nutrient deficiency is a poor growth of plants and excessive yield reduction.

A common recommended practice to reduce the negative environmental impacts caused by substantial N loss from CM and to improve N bioavailability after application of the manure in soil is to apply the manure during planting [9]. However, application of the manure at planting is very risky since it has a high caustic nature, odor, and the huge release of gaseous forms of N can negatively affect the growing plants [11]. Hence, the application of the manure for several weeks before planting in order to reduce the negative effects is the ideal and common practice [12]. Therefore, it is generally critical to efficiently utilize the fresh manure in crop production without either causing huge negative effects to the environment and/or to the growing plants [5]. Thus, immediate processing of the manure is required in order to eliminate the harmful pathogens and caustic effects associated with it while also preserving the nutrient property during storage and after application as fertilizer to soil [5].

Consequently, for efficient utilization of CLCM as N source for crop production while reducing its negative environmental effects, converting the manure into a more stable form is imperative in order to improve N bioavailability in soil. Basically, biological (composting/fermentation) and thermal treatments are two popular methods to convert the manure into a more stable and storable product [13]. Among these two methods, thermal treatment of CM by carbonization offers superior ability in the elimination of pathogens, reduction of waste stream volume, and elimination of odor [14]. Furthermore, the processing of CM by carbonization requires minutes to hours instead of days to weeks or even months required for biological treatments like composting/fermentation [14].

Generally, the thermal treatment of N-rich organic materials can result in alterations of the chemical and structural compositions, together with transformation of labile aliphatic compounds to heterocyclic aromatic structures [15,16]. These transformations in the material facilitate greater resistance to biological degradation in soil and consequently result in sustainable N bioavailability due to increased stability with specific chemical structures [15,16]. Therefore, alterations of the chemical makeup of CLCM by carbonization could be a promising practice to increase the bio-recalcitrance of the manure, which could improve N bioavailability in soil. However, during the carbonization process, nutrients susceptible to volatilization such as N are partially lost during the process [17]. Furthermore, the thermal treatment results into the formation of highly stable aromatic N structures and deformation of certain N functionalities on the surface of the manure [18]. As such, excessive N losses and the conversion of the remaining N to heterocyclic aromatic compounds can compromise the N supply ability of the manure [18]. However, other studies revealed that the N mobilized during thermal treatments of N-rich materials can actually be bio-degradable to improve N retention and bioavailability in soil [15,16]. Accordingly, we infer that there could be optimum alterations during carbonization of CLCM, which when instituted in the manure can increase its bio-recalcitrance and improve N retention and bioavailability in soil. Also, if the optimum alterations could result in increased N bioavailability in soil, then it may be possible that the N loss that occurs during the carbonization process could be compensated for by this increased N bioavailability.

But, the carbonization conditions required to institute these optimum alterations that could stabilize and sustain N bioavailability from CLCM in soil is unknown. Furthermore, even though

the carbonization process of CM is an age old practice with a huge body of literature on the effects of carbonization parameters on the physicochemical properties of CM, and the effects of carbonized CM on the improvement of soil fertility and crop productivity [19–21], there have been no studies to the best of our knowledge which have specifically studied the variations in chemical compositions, structural formations, and N speciation instituted during carbonization at different temperature conditions particularly to identify appropriate conditions whose alterations will result in a suitable bio-recalcitrance that will help to improve N bioavailability in soil for crops.

N is the most important plant nutrient but is most times deficient in soils due to its high mobility and thus affects soil productivity. The bioavailability of N from CM and its carbonized products is an important factor for elucidating the agronomic impact of converting the manure to carbonized products. In fact, the fertilizer value of any “fertilizer material” is ultimately governed by the ability of the material to make nutrients bioavailable in soil for plants. Therefore, systematic investigation is required in order to ensure optimum alterations are instituted to the manure during carbonization in order to make the manure more stable but at the same time capable of compensating for the N loss through increased N retention and bioavailability in soil.

Therefore, the objective of this study was to investigate the alterations in chemical compositions, surface functionalities, and N speciation initiated by different carbonization temperatures and their influence in affecting N bioavailability from CLCM in soil. This was done to identify an appropriate temperature condition capable to transform the easily decomposable manure into medium-term bio-recalcitrance material for N bioavailability improvement after soil application.

Such studies will be necessary to provide useful information that will assist farmers and organic fertilizer producers during the carbonization of CLCM for the production of an environmentally safe organic N fertilizer capable of N supply in the medium to long-term.

2. Materials and Methods

2.1. Sample Source, Preparation, and Carbonization

Fresh CLCM that was used in the experiments was obtained from the Layer section in the poultry farm of the Field Science Centre, Gifu University. The manure was immediately dried in an electric oven at 65 °C for 24 h and then stored in air tight plastic prior to carbonization. Dried CLCM was placed in a ceramic crucible and carbonized in a muffle furnace at 350, 500, and 650 °C with a heating rate of 5–10 °C per minute. The temperatures of 350, 500, and 650 °C were selected to represent slow, fast, and very fast carbonization, respectively [22]. All the carbonizations for the various temperature conditions were done in duplicate each for a period of 1 $\frac{1}{2}$ hours. At the end of the carbonization, the samples were cooled in the furnace and later transferred to a vacuum desiccator for further cooling. The duplicate samples for each treatment were mixed together and milled using mortar and pestle and sieved with a 2 mm mesh. The samples were placed in air tight plastic containers and stored for characterization, incubation, and pot experiments. For brevity, hereinafter, the un-carbonized CLCM will be referred to as CM; CLCM carbonized at 350, 500, and 650 °C, will be referred to as CCM350, CCM500, and CCM650, respectively. Samples for characterization were freeze dried for 24 h.

2.2. Chemical Compositions of CM and CCM Samples

The pH was measured in a 2 M KCL solution (1:10 w/v) using a compact pH meter AS-211 (AS ONE Corporation, Osaka, Japan). Elemental C, H, and total N abundances were determined by a JMA 102 auto sampler CHN elemental analyzer (J-Science Lab Co., Ltd., Kyoto, Japan), and Oxygen content was determined by difference. Atomic H/C (aromaticity) and O/C (stability) ratios were calculated from the elemental results. NH_4^+ -N was determined by steam distillation [23] and NO_3^- -N using a compact nitrate meter AS-211 (AS ONE Corporation, Osaka, Japan).

2.3. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

A JASCO 4100 spectrometer (JASCO Corporation, Tokyo, Japan) was used to determine the organic functional groups of CM and each CCM sample. The FTIR spectra were recorded at a wavelength ranging from 4000 to 400 cm^{-1} and a resolution of 2 cm^{-1} . A cylindrical piston was used to prepare a potassium bromide pellet that contained 1% (w/w) of manure under high pressure and vacuum. The spectrum of the pure potassium bromide (KBr) pellet was recorded before every measurement for spectra correction.

2.4. X-ray Photoelectron Spectroscopy (XPS) Analysis

Nitrogen functionalities in CM and CCM samples were characterized with XPS using the Quantera SXM-GX scanning X-ray microprobe spectrometer (Ulvac-PHI.inc., Kanagawa, Japan) after mounting the samples on double-sided carbon tapes. A monochromatic $\text{AlK}\alpha$ (1486.6 eV) X-ray source operating at 25 W was used with a spot size of 100 μm in diameter at an electron take-off angle of 45°. Spectra were acquired in constant analyzer energy mode, with pass energies of 120 and 55 eV for survey and narrow regions, respectively. All XPS spectra were obtained under identical conditions. The XPS spectra were fitted based on Gauss–Lorentzian line shapes with Shirley type background subtraction (MultiPak 8.2 software- Ulvac-PHI.inc., Kanagawa, Japan). The fitted areas of peaks reflect the relative contents of different N-containing compounds.

2.5. Laboratory Incubation and Greenhouse Pot Studies for N Bioavailability Test

N bioavailability from CM, CCM350, CCM500, CCM650, and control (soil alone without manure) were examined in a laboratory aerobic incubation study conducted at 30 °C for 35 days. Triplicate 100 g (oven dried basis) of air dried 2 mm mesh-sieved soil for each treatment were weighed in a 400 mL plastic container. The initial basic chemical properties of the soil used for the study are presented in Appendix A Table A1. Each treatment was applied to the soil so that a N content equivalent to 120 kg N/ha was supplied. After that, the soil and amendment for each treatment were mixed thoroughly. Water was added to each container to bring the soils to 60% water holding capacity. The initial weight of all the samples was recorded, then covered with pin hole parafilm, placed in an incubator in a completely randomized design. At weekly intervals, all the samples were removed from the incubator, aerated, and their moisture contents adjusted gravimetrically. At days 0, 7, 14, 21, 28, and 35 of incubation, triplicate samples of each treatment were removed from the incubator and assessed for mineral N content (NH_4^+ -N and NO_3^- -N). NH_4^+ -N was measured from a 2 M KCl extract by steam distillation [23], while NO_3^- -N was determined from a 0.5 M K_2SO_4 extract by nitration of salicylic acid method [24] as reported in Logah et al. [25] using a U-1800 spectrophotometer (Hitachi High Technology Corporation, Omuta, Japan). When concentrations of nitrate in the samples were suspected to be higher than the standard curve range, they were diluted before analysis. At each sampling date, the mineral N content of the control soil (Appendix B Figure A1) was subtracted from each treatment at that particular date.

The bioavailability of N for plants was investigated in a greenhouse pot experiment using *Brassica rapa* komatsuna (Japanese mustard spinach) as a test crop. Three treatments (CM, CCM350, and control) based on the outcome of the incubation study were used for this experiment. CM and CCM350 were mixed thoroughly with 500 g 2 mm mesh-sieved soil at a rate of 120 kg N/ha (the recommended N rate in Gifu prefecture for the test crop *Brassica rapa* komatsuna). The pots were then arranged in a completely randomized design with three replications. Four seeds of *Brassica rapa* komatsuna were planted in each pot, ten days after planting, thinning to 1 seedling was done. All the pots were watered every day and weeds were removed as soon as sported. At 35 days after planting, growth parameters including plant height, number of leaves, shoot dry matter yield, and the total nitrogen content in the plants tissue were evaluated.

2.6. Statistical Data Analysis

The data obtained from the characterization of the chemical compositions, mineral N bioavailability, and plant growth experiments were checked for their fitness to normal distribution and homogeneity of variance distribution with the Kolmogorov–Smirnov and Levene’s tests, respectively. The tests revealed that all the collected data satisfied the ANOVA assumptions except for the H/C atomic ratio, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and total mineral N (TMN) data of the characterization experiment and the number of leaves of the plant growth experiment. All the data suitable for parametric analysis were analyzed by one-way ANOVA using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA). Significant means were separated using Tukey’s honest significant difference post-hoc comparison. The parameters that violated the ANOVA assumption were analyzed by the Kruskal–Wallis test using SigmaXL, version 8 (SigmaXL, Inc., Kitchener, ON, Canada).

3. Results

3.1. Alterations in Chemical Properties, Aromaticity, and Stability of CM during Carbonization at Different Temperatures

Elemental contents (N, C, H, and O) of CM were altered with increasing carbonization temperature (Table 1). N, H, and O contents significantly decreased with increasing carbonization temperature. The CM was the greatest in terms of the composition of these elements (N, H, and O) while CCM650 was the least. Numerical increase in the accumulation of carbon was observed with increasing carbonization temperature but the carbon results were not statistically significant. Elemental atomic ratios (H/C and O/C ratios) significantly decreased as the temperature of carbonization increased, while a significant increase was observed for the C/N ratio. The C/N ratio of the CM rose from 3.99 to 5.36, 9.07 and 10.85 in CCM350, CCM500, and CCM650, respectively. The mineral N ($\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$) contents drastically reduced as the temperature of carbonization increased such that at 500 °C the presence of mineral N was very minimal and was not detected for CCM650 (Table 1). Significantly greater pH values were observed for the CCM samples over the CM. The pH values graduated from 6.8 for CM to 8.6 for CCM350, 10.0 for CCM500, and 10.7 for CCM650 (Table 1).

Table 1. Elemental concentration, atomic elemental ratios, and basic chemical properties of chicken manure and chicken manure thermally altered at different temperatures.

Samples	CM	CCM350	CCM500	CCM650
Elemental Concentration				
(%) <i>n</i> = 3				
N	7.74a (±0.05)	6.46b (±0.21)	3.45c (±0.19)	3.07c (±0.13)
C	30.92a (±0.65)	34.59a (±0.33)	31.23a (±0.99)	33.35a (±1.41)
H	3.89a (±0.15)	2.48b (±0.02)	1.25c (±0.06)	0.85c (±0.06)
O	32.08a (±0.75)	19.99b (±0.56)	16.83bc (±1.22)	11.60c (±1.60)
Atomic elemental ratios				
H/C	1.51a (±0.03)	0.86b (±0.01)	0.49c (±0.01)	0.30d (±0.01)
O/C	0.78a (±0.03)	0.43b (±0.02)	0.41c (±0.04)	0.26d (±0.05)
Basic chemical properties				
C/N ratio (%)	3.99d (±0.11)	5.36c (±0.13)	9.07b (±0.26)	10.85a (±0.11)
$\text{NH}_4^+\text{-N}$ (mg/100 g)	156.62a (±0.58)	1.68b (±0.00)	0.70bc (±0.00)	0.00c (±0.00)
$\text{NO}_3^-\text{-N}$ (mg/100 g)	12.80a (±0.75)	3.01b (±0.75)	0.00c (±0.00)	0.00c (±0.00)
Total MN (mg/100 g)	169.43a (±0.21)	4.69b (±0.75)	0.70c (±0.00)	0.00c (±0.00)
Organic N (%)	7.51a (±0.05)	6.45b (±0.21)	3.45c (±0.19)	3.07d (±0.13)
pH	6.8d (±0.03)	8.6c (±0.06)	10.0b (±0.00)	10.7a (±0.00)

“*n*” is the number of replicate determined for mean calculation and = 3; means in the same rows with different letters were significantly different at $\alpha = 0.05$; N = nitrogen, C = carbon, H = hydrogen, O = oxygen, MN = mineral nitrogen; values in parentheses are the standard error of the means; CM = chicken manure; CCM350, CCM500, and CCM650 means chicken manure carbonized at 350, 500, and 650 °C, respectively.

Clarifying the condensation degree of the aromatic structures formed, the stability of the manure at different temperatures of carbonization and the nature of reaction that took place during the carbonization process, the atomic H/C and O/C ratios were plotted in a Van Krevelen [26] diagram (Figure 1). The atomic H/C and O/C ratios of the plot showed that the major reaction that took place during carbonization of CM at 350 °C was dehydration. However, there was a slight shift away from the dehydration region in the plot as the temperature of carbonization increased, showing that at elevated temperatures a combination of dehydration, loss of carbon dioxide, or carbon monoxide, together with loss of functional groups containing H and O (for instance, hydroxyl, carboxyl), could have been responsible for any principal changes that occurred in the material.

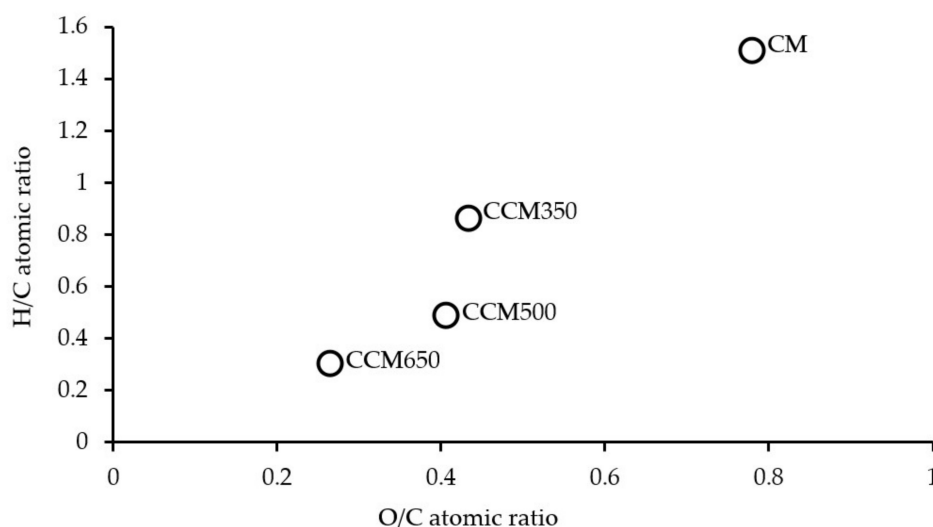


Figure 1. Van Krevelen diagram of chicken manure and chicken manure thermally altered at different temperatures. CM: chicken manure; CCM350, CCM500, and CCM650 represent manure thermally altered at 350, 500, and 650 °C, respectively.

3.2. Transformation of Nitrogen Functional Groups during Carbonization of CM at Different Temperatures

FTIR spectra of the CM and different CCM samples are presented in Figure 2, while the proposed assignments of peaks are shown in Table 2. The spectrum of CCM350 showed a slight variation to the spectrum of CM. All signals present in the CM were still present after carbonization of the manure at 350 °C except for slight variations in the intensities of some of their peaks. For instance, a more intense band was observed for CCM350 at 1600 cm^{-1} than for CM. In addition, a broader stretch band was observed at 1060–1000 cm^{-1} for CM than for CCM350. On the other hand, CCM500 and CCM650 showed vast variation in organic functional groups to that of the CM and the CCM350 samples. First, the broad absorption band detected at around 3600–2500 cm^{-1} which was attributed to a mixture of free amino acid groups (N-H vibration) and hydroxyl groups (OH) was only present in CM and CCM350. The intensity of the broad band noticed for the CCM500 at the range of around 3600–3000 (maximum peak at 3437 cm^{-1}) was ascribed to OH groups, although there could be some amount of free amino acid groups within this peak. The band, however, almost completely disappeared in the spectrum of CCM650. The aliphatic C-H methylene assignment around 2925 faded with increase in temperature. The bands found around 1665–1650 cm^{-1} can be attributed to amide band I (C=O). The proposed amide band I decreased with increasing temperature such that it was absent in spectra of CCM500 and CCM650. The weak band observed at around 1600 cm^{-1} could be assigned to aromatic C=C vibrations of lignin. The intensity of this peak increased with increasing temperatures up to CCM500 but was absent in CCM650. Peaks observed at 1433, 1431, 1426, and 1417 cm^{-1} are assigned to CH_2 units in biopolymers. The intensity of this band increased with increasing carbonization temperature. FTIR spectra of CM and CCM350 showed a medium stretch band at 1060–1000 cm^{-1} but this band was

transformed to a very sharp peak in spectra of CCM500 and CCM650. For CM and CCM350, this band could be attributed to the stretching mode of the C-N group, an indication of amine groups, while the sharp vibration observed for CCM500 and CCM650 could be due to the vibration of C-O in polysaccharides. C-H bond in aromatic and hetero-aromatic compounds can be assigned to the wagging vibration of band between 800 and 600 cm^{-1} . The intensity of these peaks increased with increasing temperature, with the CCM500 showing the highest intensity.

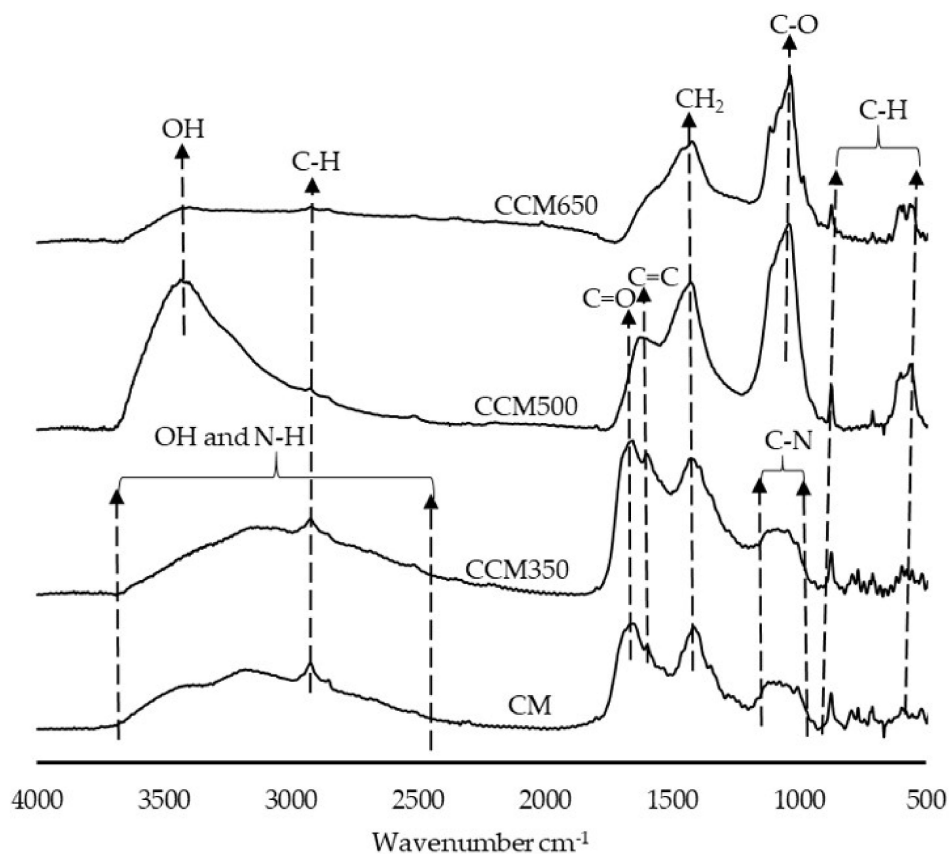


Figure 2. FTIR spectra of chicken manure and chicken manure thermally altered at different temperatures. CM: chicken manure; CCM350, CCM500, and CCM650 represent manure thermally altered at 350, 500, and 650 °C, respectively.

Table 2. Peak positions in the FTIR spectra of the present study and their proposed assignments.

Peak Positions cm^{-1}	Proposed Assignments	References
3600–2500	Amino acid hydrohalides group (N-H vibration)	[27]
3600–3000	OH bond in water, carboxyl, and hydroxyl group	[28]
1665–1650	Amide band I (C=O vibration)	[29]
1600	Aromatic C=C vibration (lignin carbohydrate)	[30]
1435–1415	CH ₂ units in biopolymer	[31]
1060–1000	Amines (C-N stretching) or polysaccharides (C-O vibration)	[32]
800 and 600	C-H bonds (aromatic C-H out-of-plane deformation)	[33]

In summary, the organic functional groups of CM were greatly altered during carbonization at various temperatures. The variations in the organic functional groups of CCM350 from CM were mild, but huge alterations were observed in the organic functional groups of CM following carbonization at 500 °C and 650 °C. Notable changes involve the degradation of the amino acid group of CM, the increase in C=C and C-O bonding, drastic increase in the OH group, and the possible formation stable aromatic compounds.

3.3. Speciation of N Forms during Carbonization of CM at Different Temperatures

The peak positions, their proposed assignments and symbols as to how these peaks will be referred to in this section and discussion section of this paper for the sake of brevity are presented in Table 3. Figure 3a–d represents the N 1s XPS spectra of CM, CCM350, CCM500, and CCM650, respectively. The relative N 1s peak areas are presented in Figure 4. In the spectrum of CM (Figure 3a), two peaks were found, which are assigned to N-I and N-X groups. From Figure 4, it can be seen that in CM the N-I peak is the dominant peak (94.76%) while the N-X peak accounts for only 5.24%. When CM was carbonized at 350 °C, the N functionality results (Figure 3b) showed that the N-I peak area declines giving a percent relative peak area of 70.18% (Figure 4). Further, the N-X peak disappeared completely but two new functionalities emerged, which corresponded to N-II and N-Q groups. N-II and N-Q in CCM350 had a percent relative peak area of 9.33% and 20.48%, respectively (Figure 4). The decrease in N-I and the introduction of new and/or increase in existing heterocyclic N forms continues as the temperature of carbonization increased. In CCM500, the N-I forms decreased to 47.71% while the N-Q forms previously introduced at 350 °C increased to 26.99% (Figure 4). A new form of N (N-III) appeared and it accounted for 25.29% of the relative peak area. The N-II peak was absent at this temperature (Figure 3c). After heating the manure at 650 °C (Figure 3d), N-I totally disappeared, making the N-III peak to be the main N form (55%) in the resulting carbonized manure (Figure 4). This is followed by the introduction of a new N functionality (N-IV) with relative peak area of 39.99% and the reappearance of the N-X peak (5.01%).

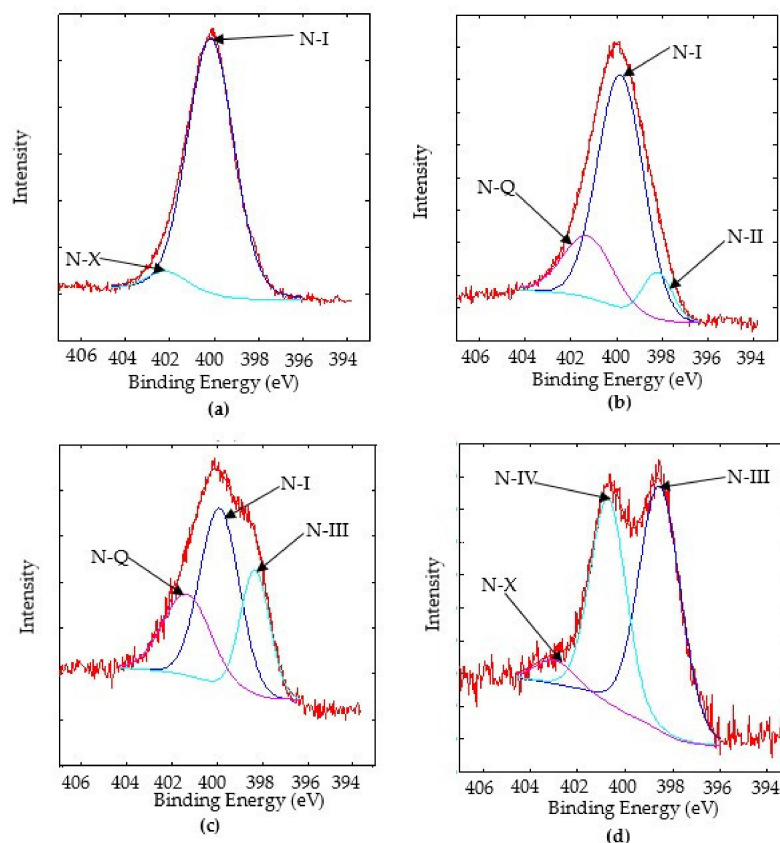


Figure 3. N 1s spectra for (a) un-carbonized chicken manure, (b) chicken manure carbonized at 350 °C, (c) chicken manure carbonized at 500 °C, and (d) chicken manure carbonized at 650 °C. Peak assignment: N-I (Protein-N, Amino-N, Amide-N, nitrile), N-II (Amine-N), N-III (Pyridine-N), N-IV (neutral imines), N-Q (Quaternary-N), and N-X (Oxidized-N).

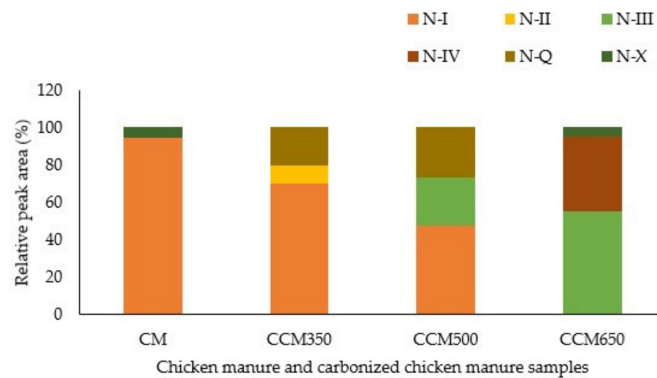


Figure 4. Relative XPs peak area of N species in chicken manure and chicken manure thermally altered at different temperatures. CM: chicken manure; CCM350, CCM500, and CCM650 represent manure thermally altered at 350, 500, and 650 °C, respectively. Peak assignment: N-I (Protein-N, Amino-N, Amide-N, nitrile), N-II (Amine-N), N-III (Pyridine-N), N-IV (neutral imines), N-Q (Quaternary-N), and N-X (Oxidized-N).

Table 3. Binding energies of Group N 1s.

Forms	Symbol	BE (eV)	References
Protein-N, Amino-N Amide-N, Nitrile	N-I	399.8 ± 0.3	[34,35]
Amine-N	N-II	398.2 ± 0.1	[36]
Pyridinic-N	N-III	398.3–398.8	[37,38]
Neutral imine	N-IV	400.7 ± 0.1	[36]
Quaternary-N	N-Q	401.0–401.4	[39,40]
Oxidized-N	N-X	402–404	[40]

3.4. N Bioavailability from CM and Thermally Altered CCM Samples in Incubation Study

Availability of N from CM, CCM350, CCM500, and CCM650 during a 35-day incubation study significantly differs depending on the carbonization temperature (Figure 5). CCM500 and CCM650 had significantly the lowest N availability compared to CCM350 and CM throughout the study period. From day zero up to day 21, the mineral N release for CCM350 followed an increasing stepwise trend, while from day 21 the mineral N release from CCM350 started decreasing up to the end of the study period (Figure 5). In the case of the CM, there was an initial increase in N release from the CM soils within the first seven (7) days of incubation, but mineral N release from CM became fairly constant within day 7 to 14 and a decreasing trend was observed from day 14 up to the end of the incubation period so that the N release from CCM350 at day 21 and 28 was significantly higher ($p < 0.05$) than the release from CM-amended soils (Figure 5).

The cumulative mineral N release throughout the study period is presented in Figure 6. CCM350 accumulated significantly ($p < 0.05$) more mineral N (27.93 mg/100 g soil) than all the other treatments followed by the CM (15.31 mg/100 g soil), which was significantly ($p < 0.05$) higher than CCM500 and CCM650. CCM500 (1.90 mg/100 g soil) and CCM650 (0.31 mg/100 g soil) accumulated the lowest mineral N contents and were not significantly different from each other (Figure 6).

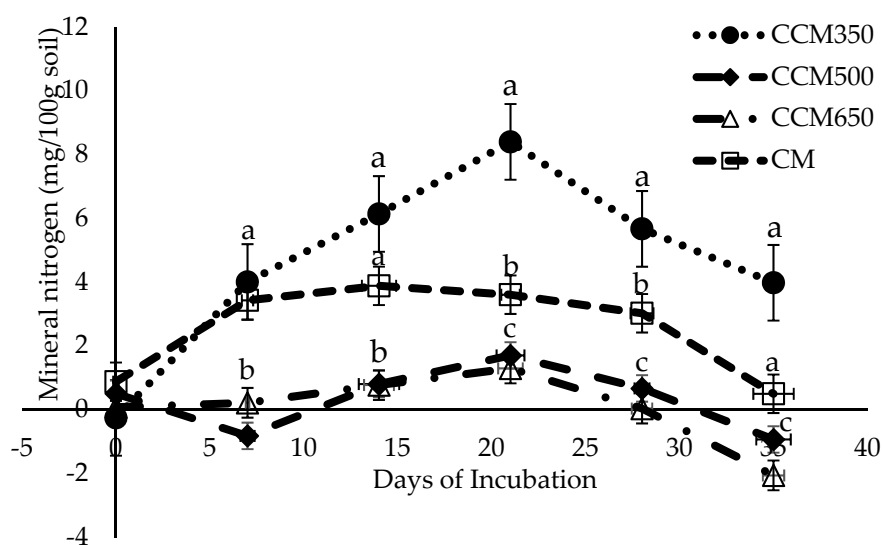


Figure 5. Mineral nitrogen availability during the decomposition of chicken manure and chicken manure thermally altered at different temperatures. Means with different letters on a particular sampling days were significantly different at $\alpha = 0.05$. CM: chicken manure; CCM350, CCM500, and CCM650 represent manure thermally altered at 350, 500, and 650 °C, respectively. Error bars are standard error of the means ($n = 3$).

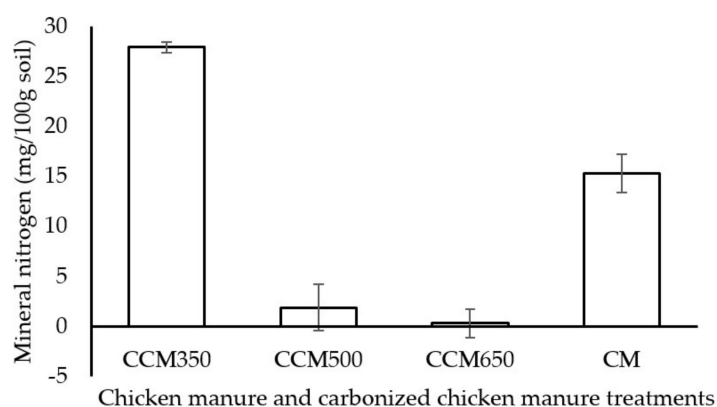


Figure 6. Cumulative mineral nitrogen accumulated in soil during 35 days of decomposition of chicken manure and chicken manure thermally altered at different temperatures. CM: chicken manure; CCM350, CCM500, and CCM650 represent manure carbonized at 350, 500, and 650 °C, respectively. Error bars are standard error of the means ($n = 3$).

3.5. N Bioavailability from CM and Thermally Altered CCM350 in Pot Study for Plants

Figure 7a–d shows the effects of CM, CCM350, and control soil on plant height, number of leaves, shoot dry matter, and total tissue N content, respectively, of the test crop (*Brassica rapa komatsuna*). *Brassica rapa komatsuna* growth parameters were significantly ($p < 0.05$) improved by CCM350 over the CM and the control soil. Plant height of the CCM350 increased by 13.5% and 82.4% more than CM and the control, respectively. The greatest number of leaves was observed in CCM350, which was significantly higher than CM and the control whose number of leaves were less than that of CCM350 by 16% and 36%, respectively. At harvest, the plants grown in soils treated with CCM350 produced higher dry matter yield as compared to plants grown in soils treated with CM and the control. The result showed that the application of CCM350 improved the dry matter yield of *Brassica rapa komatsuna* by 33.2% and 184.9% more than the CM and the control, respectively. CCM350 had a positive and significant influence in improving the quality (total N content in plant tissue) of *Brassica rapa komatsuna*

more than the control ($p < 0.05$) but not more than CM ($p > 0.05$) (Figure 7d). The N content that was observed in the plant tissues grown in a pot fertilized with CM was observed to be numerically higher, but not statistically significant ($p > 0.05$) from the control (Figure 7d).

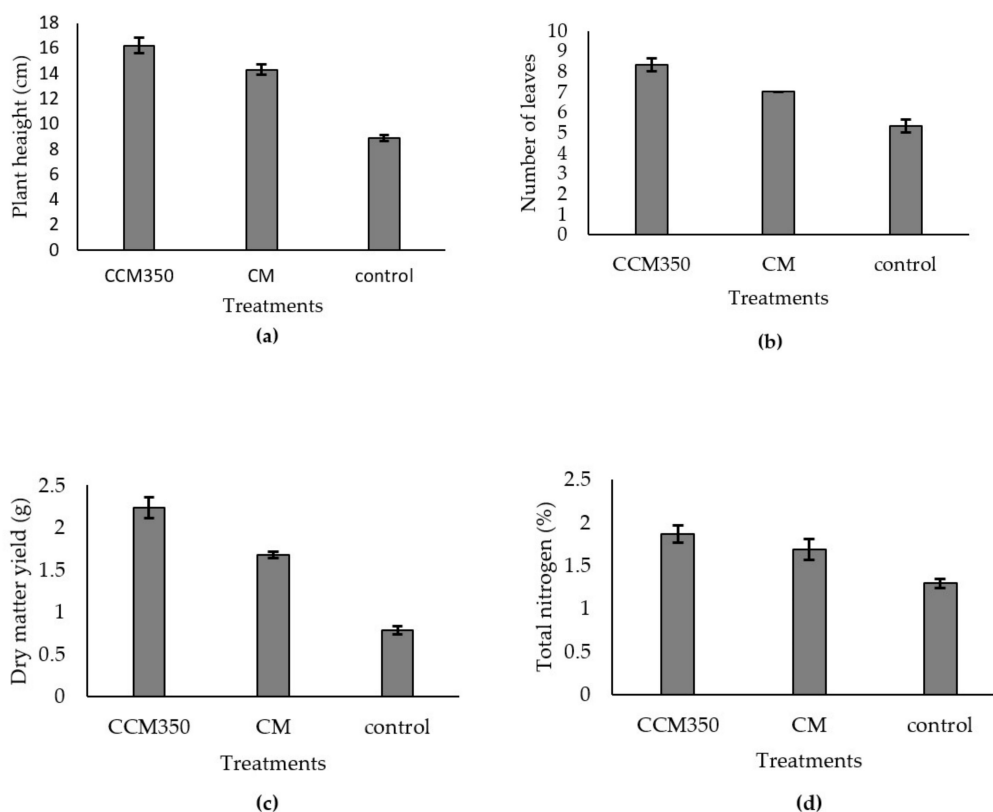


Figure 7. (a) Plant height, (b) number of leaves, (c) dry matter yield, and (d) plant total nitrogen of *Brassica rapa komatsuna* fertilized with CM, CCM350, and control soil. CM: chicken manure; CCM350 represent manure thermally altered at 350 °C. Error bars in figures are standard error of the means ($n = 3$).

4. Discussion

4.1. Effects of Carbonization Temperatures on Alterations of Chemical Properties, Aromaticity, and Stability of CM

Our results of the increased alterations in the chemical properties of CM with increased carbonization temperatures conform to well-known progressions [41–45] in which elemental compositions (N, H, and O) and mineral N components are removed with increasing temperature of heating (Table 1). The reduction in the elemental N, together with inorganic N contents, H and O with increasing carbonization temperature can be attributed to dehydration and continuous release of volatile compounds containing N, H, and O [20,41]. The decrease in N as temperature increased could also be tentatively due to deamination and degradation of amino acids and amino sugars. Decreased in H and O contents with increasing carbonization temperatures indicated ongoing dehydration, dehydrogenation, dehydroxylation, and condensation during the carbonization process, as also observed and indicated by Paneque et al. [46]. This indication is consistent with the Van Krevelen diagram of the atomic H/C and O/C ratios (Figure 1) which showed a trend corresponding to the abovementioned ongoing reactions with increasing carbonization temperatures. The C content tended to increase numerically when the manure was carbonized at 350 °C and later decreased as the temperature of carbonization increased. Overall, however, the C alterations were not statistically significant, which is in disagreement with a

report by Al-Wabel et al. [47] but is in line with Zornoza et al. [48] who recorded insignificant changes in the C content with increasing temperature during the carbonization of several organic wastes including CM. The insignificant changes in C concentrations with different temperature conditions obtained in this study, could be due to the high content of volatile materials in CM, which could have been rapidly lost at the initial stage of the carbonization process [48]. The decrease in the total N content with increasing temperature even with insignificant changes in the total C content during carbonization resulted into an increased C/N ratio as carbonization temperature increased. Carbonization of organic materials at increasing temperature conditions is mostly associated with an increased C/N ratio [17,19,49,50]. However, irrespective of the increased C/N ratio observed with increase in temperature, the values of the C/N ratios of the CM and all the CCM samples were below the critical value (20) for N immobilization during decomposition of organic materials [51]. Hence, both CM and CCM samples were expected to mineralize N depending on sufficient N and C bioavailability. There was an increment in ash content with increasing carbonization temperature (result not shown), which might have led to a systematic increase of the pH of CM as the carbonization temperature increased. The pH increment with increasing temperature is consistent with previous studies [19,42,48].

The atomic H/C and O/C ratios were used for preliminary clarification of the condensation degree of the aromatic structures formed and stability of the manure carbonized at different temperatures (Table 1). Increasing the temperature from zero to 650 °C, the H/C and O/C atomic ratios decreased. The decrease of the H/C ratio with rising temperature signals the formation of aromatic compounds with various degrees of resistance to biological decomposition and is in line with the H/C and O/C atomic ratio results reported by Bavariani et al. [49] during carbonization of CM. With continuous decrease in atomic H/C and O/C ratios as the temperature increased, it showed that the stability and the presence of heterocyclic aromatic N compounds will be in the order of CCM650 > CCM500 > CCM350. The high atomic H/C ratio of CM (>1) opposed the possibility for significant portion of aromatic N compounds within the manure but indicates the presence of carbohydrate, protein, and other easily decomposable organic N forms. The possible formation of heterocyclic aromatic compounds during carbonization could be attributed to the dehydration and degradation of carbohydrate and proteinaceous structures during thermal treatments of organic materials [52].

4.2. Effects of Carbonization Temperatures on Alteration of Surface Functionalities in CM

The surface functionalities of CM were greatly altered during carbonization of the manure at various temperatures (Figure 2). The majority of the organic functional groups on the surface of CM degraded with increasing carbonization temperature except for those indicating stability and aromaticity. The variations in the organic functional group of CM and CCM350 were mild, but huge alterations were observed in the organic functional groups between CM, CCM500, and CCM650. The amino-N group degraded with increasing carbonization temperature, and by 650 °C the peak for the amino-N group was absent. This could be the main reason for the significant reduction in the N content of CCM with increasing temperature, as speculated earlier. The disappearance of almost all the peaks proposed to be the hydroxyl group for CCM650, indicating the degradation of the organic OH groups at this high temperature. Hossain et al. [33] reported that the organic OH group is not stable and therefore can be lost at very high temperatures. The reduction in the aliphatic C-H methylene band with increasing temperatures is in line with [53], which is attributed to hydrolysis of aliphatic chain and dissolution. The increase in C=C and C-O bonds as the temperature increased could be as a result of the condensation of carbon matrix within the manure during the thermal treatment as reported by Koutcheiko et al. [38]. From Figure 2, it was noticed that amine peaks for CM and CCM350 were transformed in CCM500 and CCM650 to peaks that represented C-O chains in polysaccharides. The C-H bonds, which are an indication of aromaticity, increased with increasing carbonization temperature. The result of the FTIR analysis of the different samples is in agreement with the results obtained for their respective elemental analysis and their elemental atomic ratios, suggesting that as the temperature of carbonization increased, the cracking of atoms and rebuilding of aromatic rings takes preference.

Our result is in line with Daramy et al. [18] who reported substantial alterations and losses in surface functional groups of CM upon carbonization at increasing temperatures.

4.3. Effects of Carbonization Temperatures on Alteration of N Structures on the Surface of CM

Carbonization of CM at different temperatures resulted in changes at the molecular level as a result of volatilization of functional groups, functional groups cleavage, condensation of macromolecules, and interaction with the environment. Comparison of XPS spectra of the CM with CCM350, CCM500, and CCM650 proved that the dominant N-I aliphatic proteinaceous-N functionalities in CM transformed steadily to heterocyclic aromatic N forms which is especially noted in CCM650 (Figure 3a–d).

When the manure was carbonized at 350 °C, two new forms of N appeared (N-II and N-Q) while the N-I functionality decrease and the N-X groups disappeared. The formation of the two new compounds is possible due to the thermal cracking and decomposition of the N-I functionalities and the transformation of the aliphatic N-I groups into N-II and that of N-Q through polymerization and cyclization [37]. The N-X group is nitrogen associated with oxides, but the oxygen content in the manure was decreased with carbonization (Table 1). It is therefore obvious to suspect the disappearance of the N-X functionality on bond disruption between the nitrogen and the oxides which might have led to the formation of new N compound. Kelemen et al. [54] also reported a decrease in oxygen associated N compounds following the loss of organic oxygen species when coal was pyrolyzed under mild condition (673 K).

With an increase in temperature from 350 °C to 500 °C, the forms and yields of the heterocyclic-N compounds (N-III and N-Q) increased while the N-I compounds continued to decrease and the N-II group that previously appeared in the manure after carbonization at 350 °C disappeared. This indicates further thermal cracking of the N-I groups and polymerization of the N-II compounds which led to the formation of more heterocyclic-N structures. In addition, the increase of the N-Q compounds when the carbonization temperature was raised to 500 °C could be that nitrogen was incorporated into the interior of the developing graphene-like char structure substituting for carbon atoms due to condensation of the carbon matrix [40,55]. Our result is similar to that of Zhang et al. [56] who observed that the intermediate amine-N compounds previously formed during mild thermal treatment of sewage sludge further transformed to contribute to the pool of heterocyclic-N through the secondary cracking reactions as the heating temperature increased. This result is also consistent with the results of the FTIR analysis in this study (Figure 2) which showed that the amine-N peak for CCM350 was transformed in CCM500 and CCM650 to structures assigned to C-O chains in polysaccharides.

As heating proceeded to 650 °C, all the remaining N-I nitrogen forms were eventually converted to N-III and N-IV forms. The N-Q nitrogen form developed during carbonization at 350 and 500 °C also disappeared in CCM650. Surprisingly, the N-X nitrogen forms reappeared in CCM650. The increase in N-III and N-IV can be attributed to the aforementioned conversion of the aliphatic N-I groups to heterocyclic-N forms by polymerization and cyclization [37], and also by the disruption of hydrogen bonding in certain N-Q structures as the high thermal treatment might have caused depolymerization leading to cleavage of some thermally stable bonds [57]. N-IV could have also developed through the dehydrogenation of N-II functionality, especially when there was continuous loss of hydrogen with increasing carbonization temperature (Table 1). The reappearance of the N-X in CCM650 can be attributed to the oxidation of N-III groups as a result of exposure to the environment during sample preparation and/or during storage. Chars produced under severe conditions are highly reactive towards oxidation as compared to mild temperature carbonized ones, even at room temperature [58]. This could also explain the reason for the absence of the N-X compounds in CCM350 and CCM500 despite being prepared and stored under the same condition after carbonization.

In general, carbonization led to speciation of the organic N in CM towards heterocyclic aromatic N forms with increasing temperature. The thermal decomposition of proteinaceous compounds, amides, and nitriles (N-I) was the dominant reaction occurring during the transformation of the organic N of CM subjected to heat, particularly at the evaluated temperatures in our experiment. Disruption of bonds between nitrogen and oxygen atoms also took place. During this evolution of the organic N in CM at different carbonization temperatures, key intermediates were amine-N (N-II) and quaternary-N (N-Q) forms, which are subsequently converted to pyridinic N forms (N-III) and neutral imines (IV) by bond cleavage and dehydrogenation.

4.4. Effect of Thermal Induced Chemical Alterations of CM on N Bioavailability in Soil for Plants

In the incubation study conducted to reveal the effect of compositional and structural alterations with N speciation caused by thermal treatments at different temperatures on the sustainable improvement of N bioavailability in soil, it was observed that the application of CM and CCM350 resulted in similar N availability in the first two weeks of incubation. Thereafter, the mineral N started decreasing in CM-amended soils as the organic N in the manure might have been quickly decomposed due to its high labile nature. Rapid decomposition of the organic N of CM in soil is a characteristic property of the manure, which has also been noted by Amanullah et al. [5] and Abbasi et al. [9]. In contrast, CCM350 maintained N bioavailability in soil at an increasing level of up to day 21 of incubation, making the quantity of mineral N in CCM350-amended soils to be significantly higher than CM at day 21 and 28 of the incubation study (Figure 5). The relatively fast but gradual availability of N for a longer period from CCM350 as opposed to CM suggested that the organic N pool of CCM350 was gradually decomposed. This is due to the increased bio-recalcitrant caused by the reformation in the chemical compositions, structural functionalities, and the conversion of part of the labile proteinaceous N forms to amines and highly stable heterocyclic aromatic N structures in CCM350 during carbonization. To date, studies about the transformation of N in CM during carbonization and its specific role in N bioavailability improvement in soils are rare. However, the thermal modification of the chemical formation of other N-rich organic materials by introducing N species with aromatic nature, occlusion of organic N by aromatic structures, and decrease in labile N forms have been reported to increase resistance to decomposition making them available in soil for longer nutrient supply [15,16].

In addition, the net cumulative mineral N accumulated during the 35-day incubation study was significantly higher in CCM350 soils than for CM-amended soils (Figure 6). This further indicated that CCM350 has superior potential as organic N fertilizer than CM. It is important to note that the chemical makeup of CCM350 was only weakly altered by the thermal treatment at this temperature, and the carbonized manure at 350 °C contained substantial quantities of protein and amines N which can be easily decompose and supply immediate mineral N, but the presence of the highly stable heterocyclic N structures together with the increased C/N ratio and excessive dehydration could have occluded N, limiting the rapid microbial accessibility to this material. This thereby instituted a medium-term bio-recalcitrant to CCM350, thus making N bioavailability to be gradual but continuous for a longer period than CM. This might have led to the accumulation of more mineral N in soil fertilized with CCM350. Paneque et al. [46] reported that even though pyrolysis of N-rich organic materials may result in N losses, this N loss may be outbalanced by the sequestration of the remaining N in heterocyclic forms, which can increase the pool of organic N with medium-term biochemical recalcitrance. In the case of the CM, however, the majority of the organic N was in the labile form and was less stable and not aromatic. Therefore, the N in CM can be rapidly decomposed and diminished from the soil system, and hence might have decreased the accumulation of N as compared to CCM350 (Figure 5). Mineralized N in the soil may take several paths after decomposition of the material; they may remain in the soil and contribute to the net mineralized N pool, or be immobilized by microbes and formed part of their biomass or maybe denitrified and lost from the soil as either nitrous oxide (N₂O) or dinitrogen (N₂) [59]. These sets of conditions are most likely to be highest for manure with high labile N content and a very low C/N ratio [59]. The results obtained for the CCM350 clearly showed that

despite about 16.5% loss of the original total N in CM during carbonization at 350 °C, the CCM350 compensated for the N loss through prolonged N retention and increased N bioavailability in soil after application of each amendment at the same rate. In line with our results, Yu et al. [60] also reported improved N bioavailability in soil for carbonized CM produced at 350 °C than for the un-carbonized CM. Similarly, Sikder and Joardar [61] found that carbonization of CM in the range of 300 °C produced a material that had superior N bioavailability in soil than the un-carbonized CM. Song and Guo [41] stated that CM carbonized at temperatures of ≤ 350 °C can supply more N to soil in comparison to the CM feedstock, and this N could be gradually available due to their low water extractability.

A significantly low availability of N was obtained for CCM500 and CCM650 throughout the study period (Figure 5). Their low N mineralization potential is attributed to the enormous losses of N together with the presence of highly stable organic N compounds tightly held in heterocyclic aromatic structures as shown in Table 1, Figure 3c,d and Figure 4. Furthermore, CCM650 for instance, had an atomic O/C ratio (0.26) (Table 1) that is close to “0.2”, which is the region where materials that have undergone vast structural changes such that they have no relic structures from the original biomass are classified [62]. The surface functional groups of CCM650 also showed substantial variations from the original CM, with the more striking one being the non-existence of the band that is proposed to be the free amino acid group (Figure 2). The inability of CCM500 and CCM650 to decompose and release significant N in soil during the incubation study corroborate with findings of Daramy et al. [18] who stated that above temperatures of 375 °C, the N supply ability of CM will be highly compromised. Surprisingly, irrespective of the 47.71% N-I content in CCM500, it did not significantly decompose and release mineral N more than CCM650 which contain no amount of N-I groups during the incubation. However, a result in line with this has been previously reported by Steiner et al. [63], reporting that the N in CM carbonized at 500 °C is not bioavailable. The most possible explanation for the inability of CCM500 to decompose and release a significant amount of N in this study could be that the carbon content in CCM500 was less bioavailable due to their participation in the formation of the heterocyclic aromatic compounds. Organic carbon bioavailability plays an important role in regulating the mineralization and immobilization of N during the decomposition of manure [64,65].

As a result of their low potential as N source observed in the laboratory incubation study, CCM500 and CCM650 were excluded for further evaluation in the greenhouse pot experiment, having concluded that these materials are unsuitable for the purpose of this study.

When CM, CCM350, and control (soil without addition of fertilizer source) were evaluated for their effects on plant productivity, the results showed that CCM350 stimulated the growth (plant height, number of leaves, and shoot dry matter yield) of *Brassica rapa* komatsuna more than CM and the control (Figure 7a–c). The order of stimulation of plant growth was CCM350 > CM > control. The quality (total N content of plant tissue) was not significant for CCM350 and CM but was significantly higher in CCM350-amended plants than the control. The plants quality in CM pots was not significantly different from the control soil. Tagoe et al. [66] conducted experiments to evaluate the beneficial effects of carbonized and un-carbonized CM on crop productivity, and they reported that the growth and yield of several leguminous crops were enhanced for the CCM than the CM and the control. Similarly, Yu et al. [60] reported superior plant growth in CCM fertilized soils in comparison to soils that were fertilized with CM.

The growth of plants, accumulation of biomass, and uptake of N from soils depend on the bioavailability and the ability of the plant to uptake N in the soil systems. Results of the superiority of plants in CCM350 over CM in terms of growth and biomass accumulation obtained in the pot experiments was an indication of more bioavailable N in CCM350-amended soils than CM-amended soils and the control. This validates the results of the laboratory incubation study. This outcome is agriculturally, economically, and environmentally beneficial considering the fact that CCM350 is medium-term bio-recalcitrant and its decomposition can increase N bioavailability in soil for a longer time for effective growth of plants.

5. Conclusions

Carbonization of CLCM at increasing temperatures resulted in increased alterations in the chemical makeup of the manure. Part or the entire labile organic N forms are also converted into more stable heterocyclic N forms (pyridine N, neutral imine, and quaternary N) as the temperature increased. Subsequently, these alterations in the chemical makeup and state of the organic N species in the manure affected the organic N bioavailability in soil. Notably, N bioavailability of CLCM and its effect on plants were improved when the manure was carbonized at 350 °C. Although about 16.5% of the total N of the manure was lost during carbonization at 350 °C, the medium-term bio-recalcitrance of the organic N in CCM350 caused by the alterations in the chemical compositions and the transformation of part of the labile N to heterocyclic aromatic N structures improved the stability and ability of CCM350 to retain and make more N bioavailable in soil as compared to the un-carbonized CLCM after application of both of the amendments at the same N rate as shown in the incubation and plant growth studies. Furthermore, we noted that excessive losses of the total N together with huge transformation of labile organic N to highly stable heterocyclic aromatic N structures during carbonization affected N bioavailability in soil negatively as shown for the manure that were carbonized at 500 and 650 °C, respectively. Therefore, appropriate cautions should be taken to keep N losses and transformation during the carbonization process at a minimum but at the same time select suitable temperature conditions that will institute appropriate compositional alterations for attaining medium bio-recalcitrance which will facilitate increased N retentions and bioavailability after application of carbonized CLCM to soil. For this purpose, our results showed that the speciation of N and chemical alterations that occurred in CLCM at 350 °C is ideal to institute the required bio-recalcitrance for sustainable N bioavailability in soil for plants.

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

Appendix A

Table A1. Basic chemical properties of the soil used for the experiment.

Parameters	Amount of Nutrient
Total N (%)	0.1
Total C (%)	1.1
C/N ratio	9.4
NH ₄ ⁺ -N (mg/100 g)	0.82
NO ₃ -N (mg/100 g)	1.84
Organic N (%)	0.11
pH	6.7

Appendix B

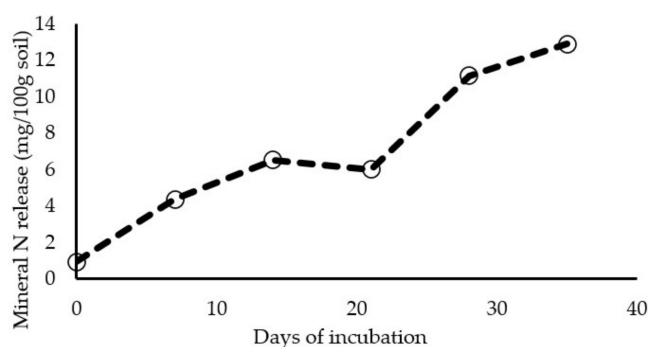


Figure A1. N availability from the control soil during the incubation study.

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