

Article

Higher Biochar Rate Can Be Efficient in Reducing Nitrogen Mineralization and Nitrification in the Excessive Compost-Fertilized Soils

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Abstract: The effects of a high biochar rate on soil carbon mineralization, when co-applied with excessive compost, have been reported in previous studies, but there is a dearth of studies focusing on soil nitrogen. In order to ascertain the positive or negative effects of a higher biochar rate on excessive compost, compost (5 wt. %) and three slow pyrolysis (>700 °C) biochars (formosan ash (*Fraxinus formosana* Hayata), ash biochar; makino bamboo (*Phyllostachys makino* Hayata), bamboo biochar; and lead tree (*Leucaena leucocephala* (Lam.) de. Wit), lead tree biochar) were applied (0, 2 and 5 wt. %) to three soils (one Oxisols and two Inceptisols). Destructive sampling occurred at 1, 3, 7, 28, 56, 84, 140, 196, 294, and 400 days to monitor for changes in soil chemistry. The overall results showed that, compared to the other rates, the 5% biochar application rate significantly reduced the concentrations of inorganic N ($\text{NO}_3^- - \text{N} + \text{NH}_4^+ - \text{N}$) in the following, decreasing order: lead tree biochar > bamboo biochar > ash biochar. The soil response in terms of ammonium and nitrate followed a similar declining trend in the three soils throughout the incubation periods, with this effect increasing in tandem with the biochar application rate. Over time, the soil $\text{NO}_3^- - \text{N}$ increased, probably due to the excessive compost N mineralization; however, the levels of soil $\text{NO}_3^- - \text{N}$ in the sample undergoing the 5% biochar application rate remained the lowest, to a significant degree. The soils' original properties determined the degree of ammonium and nitrate reduction after biochar addition. To reduce soil $\text{NO}_3^- - \text{N}$ pollution and increase the efficiency of compost fertilizer use, a high rate of biochar application (especially with that pyrolyzed at high temperatures (>700 °C)) to excessively compost-fertilized soils is highly recommended.



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1. Introduction

Biochar plays an important role in determining the N availability in soil, despite the low content of available N, as it may directly or indirectly influence N immobilization and mineralization, as well as nitrification [1]. The potential applicability of biochar in decreasing soil N losses was generally lower in tropical regions than in temperate regions [2], probably due to these areas' high precipitation and warm temperature, which result in notable soil erosion, nutrient leaching, and the rapid decomposition of soil organic matter. The high precipitation levels (annually > 2500 mm) and warm temperature (annually around 23~25 °C) are the two major detriments of Taiwan's agricultural soils. Farmers in Taiwan usually apply a greater amount (2~5 wt. %) of compost during intensive cultivation periods for short-term leafy crops, because of the relative low N mineralization rate and the low levels of nutrients in this compost compared with chemical and complete fertilizer. Therefore, the excessive levels of applied compost could have negative environmental impacts, such as eutrophication (N and P) and acidification (N), which are similar to the consequences of the inefficient use of N and P [3]. In order to establish more efficient N

fertilizer use, co-application with biochar is a potentially beneficial strategy [4–6]. Besides this, at specific rates of biochar application, mixing biochar with manure could potentially benefit producers who utilize manure and yet still observe increasing soil NO_3^- – N pollution [7].

Several studies have reported the effects of a higher biochar application rate on N mineralization and immobilization. In the study of Ippolito et al. [8], a hardwood-based fast-pyrolysis biochar was applied (0, 1, 2 and 10 wt. %) to calcareous soil without manure. The authors suggested that the 10 wt. % biochar application rate would not be suitable for crop growth, since excessive applications dramatically lower soil NO_3^- – N concentrations and prevent NO_3^- – N from accumulating over time. Additionally, as compared to the 0, 1, and 2 wt. % biochar treatments, the 10 wt. % biochar application rate, when supplemented with a 2% manure application rate, likely allowed for some net mineralization and nitrification of the manure N, but limited excessive soil NO_3^- – N accumulation [7]. A pot incubation experiment was conducted for 84 days, with ten different soils and four rates of wood chip-based biochar (0.5, 2, 4 and 8%). Hailegnaw et al. [9] stated that the biochar's effect on soil ammonium was inconsistent and insignificant in most of the incubated soils at all biochar rates. The biochar rate-dependent increase in N immobilization suggested the potential ability of biochar to promote N retention [10], and the authors suggested that the applications of wheat straw and biochar alone and in combination ($22.5 \text{ t} \cdot \text{ha}^{-1}$ and $45 \text{ t} \cdot \text{ha}^{-1}$) reduced soil mineral N content in comparison to the control treatment. This was further reinforced by the negative net N mineralization and immobilization. In addition, in a previous study [11], lead tree biochar (700 °C) was shown to reduce the available total inorganic N (NO_3^- – N + NH_4^+ – N) by 6%, 9% and 19%, on average, under the 0.5%, 1.0% and 2.0% treatments, respectively.

Clough et al. [12] summarized their variable observations of the effects of the addition of biochar to soils on soil inorganic nitrogen, which included the slower mineralization of the biochar materials than the uncharred biomass, reduced or increased net N mineralization, the absence of an effect on mineralization, a small effect on dissolved organic N, a lack of an effect on soil N immobilization, and the promotion of immobilization. The important factors in terms of both soil and biochar include pyrolysis temperature (high or low) and feedstock source (ranging from herbaceous to woody materials, and/or from animal (poultry litter, cow dung) to plant origin) [13]. However, the co-application of biochar with excessive compost manure has not been extensively studied, especially in Taiwan, despite the fact that excessive levels of compost are already commonly applied to agriculture soils here, and there is also an interest in applying soil amendments (such as biochar) to attain efficient compost fertilizer use and reduced levels of soil NO_3^- – N pollution. Additionally, after 400 days of incubation, the negative effect (reduced CO_2 release) that accompanied the increasing biochar application rates (2 wt. % and 5 wt. %) was eliminated when the biochar was co-applied with excessive compost. Furthermore, in general, the potentials of the three biochars (ash, bamboo and lead tree) for C sequestration were similar in the three studied soils [14]. Besides this, it is possible that the factors of biochar feedstock source and varied rates of biochar application can contribute to the positive or negative effect of the added labile organic C source (i.e., excessive compost) on the soil N dynamics. Therefore, it is imperative to delineate the response of the N dynamics to different biochar feedstocks, biochar rates, and soils, especially in soils with excessive compost. Our study aimed to fill gaps in the existing biochar research. Thus, similarly to a previous study [14], here, a 400-day laboratory incubation study was conducted with three well-characterized biochars mixed with three soil types under constant laboratory incubation conditions. The objectives were to (1) evaluate the effect of the biochar feedstock and rate on the nitrate and ammonium content in three soils with diverse properties, and define the most restricting soil factor, and (2) compare the N mineralization (ammonification and nitrification) and immobilization, in order to study the interaction between soil and biochar following the co-application of excessive compost, which may lead to increased or decreased effects and natural changes in N mineralization. We hypothesized that an excessive level biochar

application (e.g., 5 wt. %) would have a negative effect on N mineralization (or a positive effect on N immobilization) even in the presence of excessive compost, and lead to efficient compost fertilizer use and reduced soil NO_3^- – N pollution.

2. Materials and Methods

2.1. Soils, Biochars, and Compost Characteristics

Three study soil samples, including Pingchen (Pc) soil (red earth, slightly acidic Oxisols (SAO)), Erhlin (Eh) soil (fluvo-aquic soil, mildly alkaline Inceptisols (MAI)), and Annei (An) soil (fluvo-aquic soil, slightly acid Inceptisols (SAI)), were collected from Taoyuan county, Changhua county and Tainan county, located in northern, central and southern Taiwan, respectively. Composite bulk soil samples were collected from the cultivated layers (0–15 cm). Eight to ten soil cores (30 cm × 30 cm) were taken randomly and mixed homogeneously at each site. The soil was air-dried at room temperature and then ground to pass through a 2 mm sieve before its use in incubation. The SAO soil was clay-textured with a pH level of 6.1, and the MAI soil and SAI soil were clay loam-textured with pH levels of 7.5 and 6.5, respectively. The main raw materials (>50 wt. %) of the studied compost were poultry manure (mostly chicken) and livestock manure (mostly swine), as well as a commercial product (organic fertilizer; Tianluo Composting Plant, Changhua, Taiwan) certified by the government and often used by farmers. The characteristics of the three studied soils and the compost were analyzed and described in previous studies [15].

The feedstocks of the three studied biochars were the stems and branches of formosan ash (*Fraxinus formosana*) (ash biochar, A), makino bamboo (*Phyllostachys makino*) (bamboo biochar, B), and lead tree (*Leucaena leucocephala* (Lam.) de. Wit) (lead tree biochar, L), and the charring was performed in an earth kiln that was produced by the Forest Utilization Division, Taiwan Forestry Research Institute, Taipei, Taiwan. The highest temperature achieved in the kiln at the end of carbonization was over 750 °C. The biochars were homogenized and ground into a mesh of <2 mm for analysis. The characteristics of the studied biochars, including results of pH, electrical conductivity (EC), cation exchangeable capacity (CEC), elemental analysis, Fourier transform infrared spectra (FTIR), X-ray diffraction (XRD) patterns and scanning electron microscopy (SEM) observations, have been analyzed and described in previous studies [14].

2.2. Extracting Water-Soluble Biochar N and Analyses

Water-extractable organic carbon (WEOC), as well as the pH and EC of the extracted substance, have been studied and discussed in the previous study [14]. In the current study, the water-extractable NO_3^- – N and NH_4^+ – N concentrations were determined in these extracts using the same extractive method by Luo et al. [16]. In brief, the biochar sample was gently shaken with de-ionized water (water/biochar ratio 10:1) for 30 min, and the water extracts were filtered through 0.45 mm pore-size nylon membrane filters (Whatman®, Maidstone, England) and collected. This extraction process was repeated 5 times for each biochar sample with three replicates. The inorganic N concentrations (NO_3^- – N and NH_4^+ – N) were determined colorimetrically using an automated flow injection analysis with an O-I-Analytical Aurora Model 1030W (O.I. Corporation/Xylem, Inc., College Station, Texas, USA). For the NO_3^- – N and NH_4^+ – N analyses, the colorimetric reagents were prepared as described by Henriksen and Selmer-Olsen [17], and by Krom [18], respectively.

2.3. Nitrogen Incubation Experiment

For each soil, the incubation experiment comprised seven treatments with five replicates ($n = 105$): (1) natural soil + 5% compost (control); (2) soil + 5% compost + 2% ash biochar (A2); (3) soil + 5% compost + 5% ash biochar (A5); (4) soil + 5% compost + 2% bamboo biochar (B2); (5) soil + 5% compost + 5% bamboo biochar (B5); (6) soil + 5% compost + 2% lead tree biochar (L2); and (7) soil + 5% compost + 5% lead tree biochar (L5). Then, 5 wt. % compost was applied to test excessive application, as in previous studies [11,14,15,19]. The use of 5% manure compost is not economically viable for most farmers, but that was

not the focus of the present work. Soil, biochar and compost were added to each jar, and a spoon was used to thoroughly mix the samples. The control soil samples were identically mixed. Ten sets of incubation vessels were prepared for ten sampling repetitions. Then, 25 g of mixed soil sample was placed into each 30 mL plastic container, and these were subsequently placed into 500 mL plastic jars. The jars were sealed and incubated at 25 °C. The soil moisture content was adjusted to 60% of field capacity before incubation, and this was maintained throughout the experiment via repeated weighing. The soils were destructively sampled at 1, 3, 7, 28, 56, 84, 140, 196, 294 and 400 days after incubation, consistently with previous C mineralization processes [14].

The destructive sampling of soil samples was conducted at 1, 3, 7, 28, 56, 84, 140, 196, 294 and 400 days for the measurement of soil pH, 1N KCl extractive inorganic N ($\text{NO}_3^- - \text{N}$ and $\text{NH}_4^+ - \text{N}$), total carbon (TC), total nitrogen (TN), total phosphorus (TP) and Mehlich-3 extractive nutrients (P, K, Ca, Mg, Fe, Mn, Cu, Pb, and Zn). The analysis methods were as described in the previous study [11,15,19].

2.4. Statistical Analysis

Statistical analyses (the calculation of means and standard deviations, and differences of means) were performed using the Statistical Analysis System (SAS) 9.4 package (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). Arithmetic means of the pH, inorganic N ($\text{NO}_3^- - \text{N}$, $\text{NH}_4^+ - \text{N}$), TC, TN, TP, and available nutrients were calculated from the results at each consecutive measurement date. The net N mineralization, ammonification, and nitrification during the selected period were calculated by subtracting the inorganic N ($\text{NO}_3^- - \text{N} + \text{NH}_4^+ - \text{N}$), $\text{NH}_4^+ - \text{N}$, and $\text{NO}_3^- - \text{N}$ concentration of the initial soil from that of the incubated soil. Net N mineralization, ammonification, and nitrification rates were determined as the changes in the sizes of the inorganic N ($\text{NO}_3^- - \text{N} + \text{NH}_4^+ - \text{N}$), $\text{NH}_4^+ - \text{N}$, and $\text{NO}_3^- - \text{N}$ pools, respectively, over time. Three-way analysis of variance (ANOVA) was used to examine differences in the selected soil parameters of N mineralization among soils, biochars and rates. A repeated measure multivariate analysis of variance (MANOVA) was used to test the effects of biochar feedstocks, soils, addition rates, and their interactions on inorganic N concentrations and available nutrients in each incubation period. The feedstocks, addition rates and soils served as between-subject factors, and incubation time served as the within-subject factor. The repeated measure MANOVA was carried out using the general linear model (GLM) procedure. The results were analyzed by analysis of variance (one-way ANOVA) to test the effects of each treatment. Significantly different means were compared via least significant difference (LSD), based on a t-test with a 5% probability level. The values presented in the graphs and the text are means \pm 1 standard deviation (SD). The Pearson correlation coefficient (r) was calculated, and principal component analysis (PCA) was performed using SAS 9.4 software.

3. Results

3.1. Water-Soluble Extracts of Biochar

The results of the five repetitions of water extraction are shown in Figure 1. Five washes extracted most of the water-extractable nitrogen ($\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$), and the results of the cumulative $\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$ were 5.65 and 2.30, 5.04 and 5.69, and 8.59 and 1.00 mg kg^{-1} biochar⁻¹, for ash, bamboo and lead tree biochar, respectively. The study of Luo et al. [16] indicated that the cumulative $\text{NH}_4^+ - \text{N}$ concentrations of *Miscanthus giganteus* straw biochar pyrolyzed at 350 and 700 °C after five washes were 1.75 and 0.18 $\text{mg NH}_4^+ - \text{N kg}^{-1}$ biochar, respectively, but extractable $\text{NO}_3^- - \text{N}$ was not detected in the water extracts. The concentrations of cumulative total inorganic N (TIN) ($\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N}$) in ash, bamboo and lead tree biochar were 7.95, 10.7 and 9.59 mg kg^{-1} biochar, respectively. As the biochar was added to the soil at rates equivalent to 2% and 5% of the total soil organic N, this gave 0.16 and 0.40, 0.21 and 0.54, and 0.19 and 0.48 $\text{mg water-extractable N kg}^{-1}$ soil for ash, bamboo and lead tree biochar, respectively. According to the previous study [14], the cumulative water-extractable organic carbon (WEOC) levels were 138, 189,

and 35 mg C kg⁻¹ biochar, for ash, bamboo and lead tree biochar, respectively. In the current study, bamboo biochar application gave rise to more water-extractable C and N (NH₄⁺-N + NO₃⁻-N).

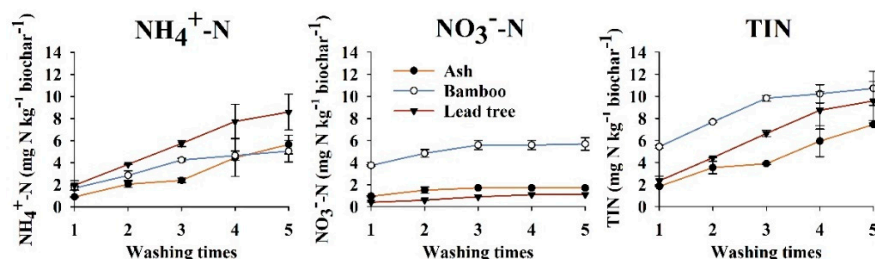


Figure 1. The concentrations of cumulative ammonium (NH₄⁺-N), nitrate (NO₃⁻-N), and total inorganic nitrogen (NH₄⁺-N + NO₃⁻-N) extracted with water from ash, bamboo, and lead tree biochar over 5 repetitions. Error bars represent the standard deviation of the mean ($n = 3$).

3.2. Changes in Nitrogen Mineralization

The soil NH₄⁺-N concentrations in all treatments of SAO soil were generally increased from Day 1 to Day 3 (with the highest concentration of 74.6 mg kg⁻¹ in L2), and sharply decreased from Day 3 to Day 28; thereafter, the concentrations were less variable, and approximately maintained at about 5 mg kg⁻¹ up to the end of incubation (Figure S1a). However, in the MAI and SAI soils, the highest concentration was on Day 1, and this sharply decreased from Day 1 to Day 7. Thereafter, the concentrations were approximately maintained at about 3 mg kg⁻¹ up to the end of incubation. The highest concentration at Day 1 was found in the control (18.3 mg kg⁻¹) and in B5 (54.6 mg kg⁻¹) for the MAI and SAI soils, respectively, which was lower than in the SAO soil. At the end of the incubation period, this effect was mostly nullified because the content of ammonium in the control treatment decreased to the same level as that observed when the biochar was added. In addition, the soil NH₄⁺-N concentrations were significantly affected by the soil, the rate, the incubation duration, and all the possible interactions between these factors ($p < 0.001$ – 0.0001) (Table 1).

Table 1. Significance (p value) of repeated measures MANOVA results for selected soil parameters under different woody biochars (Biochar), soil types (Soil) and biochar application rates (Rate) after 400 days of N incubation ¹.

Source of Variation	Df ¹	NH ₄ ⁺ -N	NO ₃ ⁻ -N	TIN	pH	TC	TN	C/N
Between-subject effect								
Soil	2	*** ²	***	***	***	*	***	***
Biochar	2	ns	***	***	***	***	***	***
Rate	2	***	***	***	***	***	***	***
Soil × Biochar	4	***	*	ns	***	*	***	*
Biochar × Rate	4	ns	***	***	***	ns	***	ns
Soil × Rate	4	***	***	*	***	ns	**	***
Within-subject effect								
Time	9	***	***	***	***	***	***	***
Time × Soil	18	***	***	***	***	*	***	ns
Time × Biochar	18	***	***	***	***	***	***	***
Time × Rate	18	***	***	***	***	***	***	***
Time × Soil × Biochar	36	***	*	ns	**	**	***	***
Time × Soil × Rate	36	***	***	***	***	ns	**	ns
Time × Biochar × Rate	36	**	***	***	***	***	**	**

¹: df = degree of freedom; TIN = total inorganic nitrogen (TIN = NH₄⁺-N + NO₃⁻-N); TC = total carbon; TN = total nitrogen; ns = not significant. ²: * significant at $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$.

The final rate of NH₄⁺-N mineralization showed an insignificant difference between the control and the biochar treatments in the three soils. (Table 2). The sums of NH₄⁺-N

concentrations at 1 week and 57 weeks indicated that the SAO soil contained a significantly higher concentration than the SAI soil and the MAI soil, and the differences between the control and biochar-amended treatments significantly narrowed with increasing biochar addition (Table 3). At weeks 1 and 57, the A5 and B5 treatments in the SAO soil, the B5 and L5 treatments in the MAI soil, and the A5, L2 and L5 treatments in the SAI soil showed significant reductions in ammonification (negative value). However, the reduction in ammonification was fast; it clearly occurred in the first week, and gradually continued to the end of incubation. The sum of the $\text{NH}_4^+ - \text{N}$ concentrations in the first week was 81 (A5 treatments) ~86% (control and B5 treatment) of the total sum at 57 weeks for the SAO soil, and was 38 (B5) ~50% (control) and 71 (B5) ~75% (control) for the MAI and SAI soils, respectively.

Table 2. Final rate of $\text{NH}_4^+ - \text{N}$ mineralization, $\text{NO}_3^- - \text{N}$ mineralization and net N mineralization at the end of the incubation time.

Soil	Treats ¹	Rate of $\text{NH}_4^+ - \text{N}$ Mineralization ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$)	Rate of $\text{NO}_3^- - \text{N}$ Mineralization ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$)	Rate of net N ² Mineralization ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$)
SAO	Control	−0.089 a ³	0.835 a	0.739 a
	A2	−0.106 a	0.706 b	0.600 b
	A5	−0.106 a	0.618 b	0.492 bc
	B2	−0.106 a	0.710 b	0.604 b
	B5	−0.102 a	0.676 b	0.554 b
	L2	−0.108 a	0.658 b	0.550 b
	L5	−0.108 a	0.500 c	0.394 c
MAI	Control	−0.008 a	0.741 a	0.713 a
	A2	−0.006 a	0.578 b	0.492 b
	A5	−0.004 a	0.518 b	0.494 b
	B2	−0.008 a	0.600 b	0.572 ab
	B5	−0.004 a	0.508 b	0.504 b
	L2	−0.006 a	0.524 b	0.496 b
	L5	−0.004 a	0.332 c	0.328 c
SAI	Control	−0.073 a	0.684 a	0.553 a
	A2	−0.088ab	0.538 ab	0.454 ab
	A5	−0.078 ab	0.512 b	0.432 ab
	B2	−0.098 ab	0.540 ab	0.444 ab
	B5	−0.122 b	0.482 b	0.358 ab
	L2	−0.076 ab	0.514 b	0.438 ab
	L5	−0.090 ab	0.394 b	0.308 ab

¹ A = ash biochar, B = bamboo biochar, L = lead tree biochar, 2 = 2% addition, 5 = 5% addition; ² Net N = ($\text{NH}_4^+ - \text{N}$ + $\text{NO}_3^- - \text{N}$); ³ Means (n = 5) compared within a column of the same soil followed by a different lowercase letter are significantly different at $p < 0.05$ using a one-way ANOVA.

Table 3. Significance test of $\text{NH}_4^+ - \text{N}$, $\text{NO}_3^- - \text{N}$, and total inorganic N (TIN) ($\text{NO}_3^- - \text{N} + \text{NH}_4^+ - \text{N}$) at 1 week and 57 weeks.

Soil	Treats ¹	$\text{NH}_4^+ - \text{N}$				$\text{NO}_3^- - \text{N}$				TIN			
		1 week ²		57 weeks		1 week		57 weeks		1 week		57 weeks	
		Mean \pm SD (mg·kg ⁻¹) ²	Change (%) ³	Mean \pm SD (mg·kg ⁻¹) ²	Change (%) ³	Mean \pm SD (mg·kg ⁻¹) ²	Change (%) ³	Mean \pm SD (mg·kg ⁻¹) ²	Change (%) ³	Mean \pm SD (mg·kg ⁻¹) ²	Change (%) ³	Mean \pm SD (mg·kg ⁻¹) ²	Change (%) ³
SAO	Control	180 \pm 5.9 a ⁴		210 \pm 6.0 a		62.6 \pm 8.8 e		1387 \pm 22 a		242 \pm 10 a		1597 \pm 23 a	
	A2	168 \pm 5.6 b	−6	202 \pm 6.4 a	−4	76.8 \pm 7.4 cde	23	1313 \pm 21 b	−5	245 \pm 9.1 a	1	1515 \pm 24 b	−5
	A5	143 \pm 2.5 e	−21	176 \pm 4.5 c	−16	78.8 \pm 2.0 bcd	26	1138 \pm 13 d	−18	222 \pm 2.5 b	−9	1314 \pm 16 d	−18
	B2	159 \pm 6.2 c	−11	191 \pm 5.1 b	−9	63.6 \pm 3.4 e	1	1246 \pm 27 c	−10	223 \pm 5.2 b	−8	1437 \pm 27 c	−10
	B5	149 \pm 8.6 d	−17	173 \pm 9.4 c	−18	62.6 \pm 8.8 e	−0.1	1045 \pm 50 e	−25	212 \pm 17 b	−13	1218 \pm 53 e	−24
	L2	174 \pm 5.6 ab	−3	204 \pm 5.7 a	−3	66.8 \pm 7.0 de	7	1148 \pm 17 d	−17	241 \pm 8.5 a	−1	1352 \pm 18 d	−15
MAI	L5	162 \pm 5.9 c	−10	193 \pm 5.8 b	−8	62.4 \pm 6.4 e	−1	862 \pm 23 h	−38	224 \pm 8.0 b	−8	1055 \pm 26 f	−34
	Control	22.3 \pm 5.1 j		44.8 \pm 7.0 g		75.9 \pm 16 cde		1003 \pm 42 f		98.1 \pm 16 gh		1048 \pm 42 f	
	A2	18.8 \pm 2.6 jk	−16	43.2 \pm 4.0 g	−4	88.2 \pm 7.8 abc	16	857 \pm 20 h	−15	107 \pm 7.8 fg	9	901 \pm 20 g	−14
	A5	19.4 \pm 3.2 jk	−13	46.4 \pm 4.2 g	3	99.4 \pm 6.8 a	31	793 \pm 14 jk	−21	119 \pm 8.3 f	21	840 \pm 17 h	−20
	B2	19.8 \pm 3.9 jk	−12	45.8 \pm 5.9 g	2	67.8 \pm 11 de	−11	853 \pm 27 h	−15	87.6 \pm 11 h	−11	899 \pm 31 g	−14
	B5	15.4 \pm 2.8 k	−32	40.2 \pm 1.9 gh	−10	78.6 \pm 5.0 bcd	4	726 \pm 36 lm	−28	94.0 \pm 4.2 gh	−4	767 \pm 35 j	−27
SAI	L2	19.4 \pm 1.2 jk	−13	39.2 \pm 2.4 gh	−13	76.6 \pm 8.3 cde	1	733 \pm 13 lm	−27	95.8 \pm 8.9 gh	−2	772 \pm 14 ij	−26
	L5	14.8 \pm 4.5 k	−34	33.8 \pm 5.4 h	−25	74.4 \pm 14 cde	−2	523 \pm 60 n	−48	89.4 \pm 12 h	−9	557 \pm 62 l	−47
	Control	68.8 \pm 4.0 f		92.2 \pm 6.8 d		87.6 \pm 12 abc		922 \pm 36 g		156 \pm 11 c		1015 \pm 35 f	
	A2	63.8 \pm 1.8 fg	−7	86.0 \pm 2.0 de	−7	83.2 \pm 6.5 bc	−5	836 \pm 43 hj	−9	147 \pm 5.7 cde	−6	922 \pm 43 g	−9
	A5	54.6 \pm 11 hi	−21	72.8 \pm 14 f	−21	93.2 \pm 17 ab	6	765 \pm 36 kl	−17	148 \pm 10 cde	−6	838 \pm 35 h	−17
	B2	64.2 \pm 3.9 fg	−7	88.4 \pm 6.1 de	−4	83.6 \pm 9.2 bc	−5	808 \pm 44 ij	−12	148 \pm 13 cde	−6	896 \pm 46 g	−12
	B5	59.2 \pm 2.7 gh	−14	83.8 \pm 4.9 e	−9	93.2 \pm 7.8 ab	7	705 \pm 26 m	−24	153 \pm 7.9 cd	−2	788 \pm 27 ij	−22
	L2	52.8 \pm 3.1 i	−23	73.8 \pm 4.2 f	−20	83.6 \pm 12 bc	−5	737 \pm 20 lm	−20	136 \pm 11 e	−13	811 \pm 19 hi	−20
	L5	49.0 \pm 2.9 i	−29	66.0 \pm 4.5 f	−28	89.6 \pm 12 abc	2	537 \pm 21 n	−42	139 \pm 13 de	−11	604 \pm 23 k	−41

¹: A = ash biochar, B = bamboo biochar, L = lead tree biochar, 2 = 2% biochar addition, 5 = 5% biochar addition. ²: The first week value is the sum of destructive soil samples analysis at day 1, 3 and 7, and the 57-week value is the sum of ten repetitions of destructive soil samples analysis. ³: Percentage expressed as the difference in value (1 week and 57 weeks, respectively) between biochar amended treatments and the un-amended control treatment; 0% indicates no change in those properties due to biochar addition. ⁴: Means (n = 5) compared within a column followed by a different lowercase letter are significantly different at $p < 0.05$ using a one-way ANOVA.

On the other hand, the soil NO_3^- -N concentrations in all the treatments continuously increased throughout the course of the incubation, and peaked on Day 28 in the SAO and MAI soils and on Day 7 in the SAI soil. This indicates a small initial burst of nitrification, followed by a slight decrease on Day 56 in the SAO and MAI soils and on Day 28 and Day 56 for the SAI soil (Figure S1b). Thereafter, the soil NO_3^- -N concentrations sharply increased in the three soils, in the following order: SAO > MAI > SAI. The initial soil NO_3^- -N concentrations in the three biochar-amended soils were lower than the NH_4^+ -N concentrations. The NO_3^- -N concentration was affected by the soil, the addition of biochar, the addition rate, the incubation duration, and the interactions between these factors ($p < 0.01$ and 0.0001) (Table 1).

Compared to the control, the final rate of NO_3^- -N mineralization was significantly reduced following the biochar treatments for the three soils (Table 2). No significant differences between biochar rate could be found, except for the L5 treatment in the SAO and MAI soils, which had the significantly lowest value. In the first week, the total nitrate content significantly increased in the A2 and A5 treatments of the SAO and MAI soils (Table 3), suggesting an enhanced nitrification. However, after incubation, the sum of nitrate content at 57 weeks was significantly reduced with the increasing addition of biochar, especially for the lead tree biochar treatment. The nitrification levels in the L2 and L5 treatments were -17% and -38% , -27% and -48% , and -20% and -42% , for the SAO, MAI and SAI soils, respectively. However, in the first week, the total NO_3^- -N concentration was relatively lower than the ammonium content. The relative percentage of the sum in the first week compared to the final sum at 57 weeks was under 20%. For the SAO, MAI and SAI soils, the relative percentages were 5 (control)~7% (A5 and L5), 8 (control and B2)~14% (L5), and 9 (control)~17% (L5), respectively.

Furthermore, because the nitrate content was much higher than the ammonium content, the TIN (ammonium + nitrate) content was mostly attributed to the nitrate content. The soil TIN content displayed similar temporal changes to the nitrate content (Figure S1c). The TIN concentration was also affected by soil, the addition of biochar, the addition rate, the incubation duration, and all the possible interactions between these factors ($p < 0.01$ and 0.0001) (Table 1). The final rate of net N mineralization in the SAO and MAI soils was significantly reduced compared to the control, but the difference was insignificant in the SAI soil (Table 2). In the SAO and MAI soils, the significantly lowest value of net N mineralization rate in the L5 treatment suggests a significant effect on net N immobilization. In the first week, in the SAO soil, the differences between the control and the biochar-amended treatments significantly narrowed with the increasing levels of biochar addition under the ash biochar and lead tree biochar treatments (Table 3); however, there was a less significant difference in the MAI and SAI soils. Similar to the changes in the nitrate content, in the MAI soil, the sum TIN contents in the A2 and A5 treatments in the first week were significantly increased. After incubation, the total TIN showed a similar trend to the nitrate content, that is, a significant reduction with increasing biochar addition. Increasing the application rate of biochar increased the net immobilization (negative value), and the L5 treatment caused the greatest reductions (-34% , -47% , and -41% for SAO, MAI and SAI soils, respectively). The relative percentage of the sum in the first week to the total sum at 57 weeks was between 9 and 23%. For the SAO, MAI and SAI soils, these percentages were about 15 (control)~21% (L5), 9 (control)~16% (L5), and 15 (control)~23% (L5), respectively.

3.3. Changes in Soil Properties

The soil pH, as well as the TC and TN contents and C/N, was significantly affected by the soil ($p < 0.01$ – 0.0001), the biochar ($p < 0.0001$), the addition rate ($p < 0.0001$), the incubation duration ($p < 0.0001$), and almost all the possible interactions between these factors ($p < 0.01$ – 0.0001) (Table 1). As shown in Figure S1d, the soil pH was the highest on Day 1 during the incubation period, then it gradually declined after Day 28 in the SAO soil, and more sharply after Day 56 in the MAI and SAI soils. In the first week, at Days 1, 3 and 7, the mean value of soil pH was the highest for all the treatments (Table 4). The pH significantly increased

with the increasing biochar application rate. The pH values of the A5 and B5 treatments in the MAI soil were the highest (pH 7.42), and the control, B2 and L2 treatments in the SAO soil were the lowest (pH 6.38 to 6.44). Throughout the 400-d incubation (57 weeks), the mean value (10 repetitions of destructive analysis) of pH was significantly lower in the control, A2 and B2 treatments in the SAO soil (pH 6.16 to 6.20), and significantly higher in the L5 treatment in the MAI and SAI soil (pH 7.03 and 6.98, respectively). The pH significantly increased with the increasing biochar rate only in the SAO soil, but mostly showed insignificant increases in the MAI and SAI soils. Compared with the control, the changes in pH between the biochar-amended treatment and the unamended control treatment indicated that there was a greater increase in the first week, but the pH gradually declined at the end of incubation test, indicating a reduction in the liming effect of biochar. However, the A5 treatment in the SAO soil, and the L5 treatment in the three soils, showed obvious increases (3% to 4%) at the end of the incubation test. In general, the mean soil pH between the three studied soils exhibited the following decreasing order: MAI > SAI > SAO. Within the three biochars, the liming effects exhibited the following decreasing order: lead tree biochar > ash biochar > bamboo biochar, for the SAO and SAI soils. The order was lead tree biochar > bamboo biochar > ash biochar for the MAI soil. In addition, within three soils, the following decreasing order obtained: SAI soil > SAO soil > MAI soil.

Table 4. Significant test of soil pH and C/N ratio at 1 week and 57 weeks.

Soil	Treats ¹	pH				C/N			
		1 Week ²		57 Weeks		1 Week		57 Weeks	
		Mean ± SD	Change (%) ³	Mean ± SD	Change (%) ³	Mean ± SD	Change (%) ³	Mean ± SD	Change (%) ³
SAO	Control	6.43 ±0.04 ^{1 4}		6.19 ±0.07 ij		9.6 ±1.09 k		8.2 ±0.41 j	
	A2	6.52 ±0.07 ij	1	6.20 ±0.01 ij	0.1	13 ±0.44 ij	33	12 ±0.38 fgh	44
	A5	6.62 ±0.06 h	3	6.35 ±0.02 g	3	20 ±1.24 de	113	18 ±0.74 bc	120
	B2	6.38 ±0.07 l	−1	6.16 ±0.02 j	−1	13 ±0.98 ij	34	13 ±0.66 f	56
	B5	6.50 ±0.02 jk	1	6.22 ±0.02 i	0.5	18 ±1.82 f	90	19 ±0.88 b	127
	L2	6.44 ±0.02 kl	0.3	6.28 ±0.02 h	1	16 ±2.34 g	64	13 ±0.82 fg	53
MAI	L5	6.58 ±0.05 hi	2	6.43 ±0.03 f	4	21 ±1.68 cd	124	18 ±0.69 bc	115
	Control	7.23 ±0.07 cde		6.85 ±0.04 cd		8.5 ±1.68 k		6.8 ±0.54 k	
	A2	7.30 ±0.04 bc	1	6.83 ±0.01 d	−0.2	15 ±0.80 g	82	11 ±0.21 gh	69
	A5	7.42 ±0.03 a	2	6.86 ±0.04 cd	0.2	23 ±1.54 b	175	18 ±0.93 bc	162
	B2	7.36 ±0.05 ab	1	6.92 ±0.03 b	1	12 ±0.70 j	41	11 ±0.60 hi	60
	B5	7.42 ±0.03 a	2	6.92 ±0.03 b	1	18 ±1.72 f	110	17 ±0.57 cd	151
SAI	L2	7.18 ±0.04 ef	−1	6.90 ±0.05 bc	1	12 ±0.52 j	40	10 ±0.19 i	50
	L5	7.28 ±0.08 cd	0.5	7.03 ±0.07 a	3	19 ±1.28 ef	125	16 ±1.05 d	140
	Control	7.05 ±0.04 g		6.73 ±0.06 e		8.6 ±0.82 k		7.0 ±0.39 k	
	A2	7.28 ±0.02 cd	3	6.84 ±0.08 d	2	15 ±1.55 gh	76	12 ±0.48 fg	74
	A5	7.28 ±0.06 cd	4	6.88 ±0.04 bcd	2	25 ±0.76 a	191	20 ±0.49 a	186
	B2	7.14 ±0.04 f	1	6.84 ±0.01 d	2	13 ±1.34 hij	56	14 ±3.13 e	99
	B5	7.22 ±0.05 de	2	6.86 ±0.03 cd	2	23 ±2.27 bc	166	21 ±1.20 a	197
	L2	7.14 ±0.04 f	1	6.88 ±0.04 bcd	2	14 ±1.78 ghi	64	12 ±0.83 fg	73
	L5	7.26 ±0.04 cd	3	6.98 ±0.01 a	4	20 ±1.58 de	132	19 ±0.40 b	163

¹: A = ash biochar, B = bamboo biochar, L = lead tree biochar, 2 = 2% biochar addition, 5 = 5% biochar addition. ²: The first week value is the sum of the results from the destructive soil sample analysis at Days 1, 3 and 7, and the 57 weeks value is the sum of the results of ten repetitions of the destructive soil samples analysis. ³: Percentage expressed as the difference in value (1 week and 57 weeks, respectively) between biochar-amended treatments and the unamended control treatment; 0% indicates no change in those properties due to biochar addition. ⁴: Means (n = 5) compared within a column followed by a different lowercase letter are significantly different at $p < 0.05$ using a one-way ANOVA.

At weeks 1 and 57, the values of the C/N ratio for the three soils all showed significant increases along with the increasing biochar rate (Table 4). Some of the C/N values at the 57th week are lower than in the first week, but not by much, suggesting that there is little C released and/or N lost after the first week. The contributions between the three biochars are not consistent in three soils; that is, L > B ≈ A in the SAO soil, A > B ≈ L in the MAI soil, and A ≈ B > L in the SAI soil. In addition, on average, the biochar treatments caused higher changes in the C/N values in the SAI soil, following by those in the MAI soil and the SAO soil. That is, the addition of biochar addition could result in greater C sequestration

and/or lesser N reduction in the SAI soil. On the contrary, in the SAO soil, some CO₂-C release and/or N mineralization occurred.

3.4. Principal Components (PCs) and Factor Correlation Coefficients

The current study used PCA to investigate the important components of the large data set at the beginning (1 week) and end (57 weeks) of the incubation, and the analysis variables included the mean value of soil pH, TC, TN, TP and C/N, and the sum of NH₄⁺-N, NO₃⁻-N, TIN, cumulative CO₂-C (abstracted from previous study [14]), and available nutrients content (Figure 2, Table 5). From the PCA, we determined that PC1 and PC2 explained 43.8% and 23.9%, and 46.7% and 23.4%, of the total variance in the soils, at the first week and the 57th week of incubation, respectively, accounting for 67.7% and 70.1% of the total variance. This indicates that all the possible information pertaining to soil properties, as related to N mineralization and nitrification, was contained in the first two PCs (Table 5).

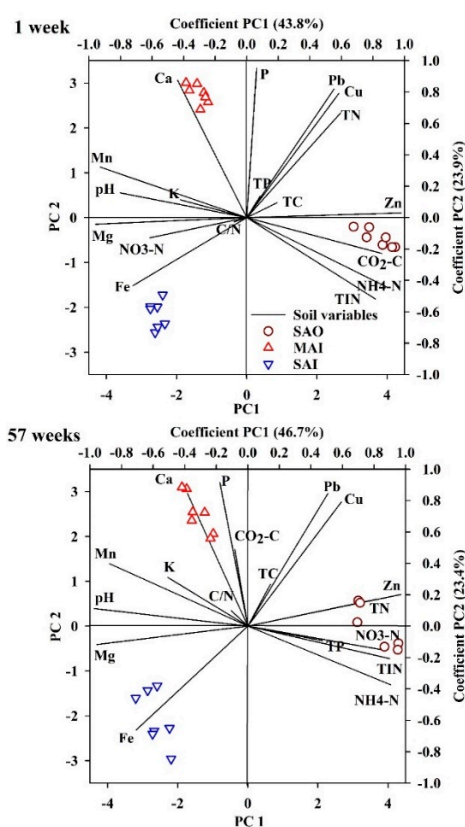


Figure 2. Principal component analysis at the first week and 57th week of the incubation period, based on soil chemical characteristics and cumulative CO₂-C, in the SAO, MAI and SAI soils. Each soil underwent seven treatments, and each treatment involved five replicates. The database of cumulative CO₂-C was abstracted from the previous study [14].

Table 5. Pearson correlation coefficients between measured parameters and principal components (PCs) (PC1 and PC2) at the 1st and 57th weeks.

Parameter ¹	1 week		57 weeks	
	PC1	PC2	PC1	PC2
Total variance	43.8%	23.9%	46.7%	23.4%
pH	−0.803 * ²	0.158	−0.973 *	0.112
NH ₄ ⁺ –N	0.887 *	−0.441 *	0.906 *	−0.374 *
NO ₃ [−] –N	−0.615 *	−0.129	0.855 *	−0.151
TIN	0.814 *	−0.518 *	0.899 *	−0.207
Cumulative CO ₂ –C	0.856 *	−0.228	−0.083	0.489 *
TC	0.193	0.095	0.144	0.267
TN	0.593 *	0.665 *	0.813 *	0.170
TP	0.085	0.136	0.472 *	−0.093
C/N	−0.080	−0.027	−0.106	0.099
P	0.063	0.953 *	−0.177	0.915 *
K	−0.420 *	0.113	−0.507 *	0.310
Ca	−0.439 *	0.875 *	−0.383 *	0.846 *
Mg	−0.959 *	−0.042	−0.956 *	−0.117
Fe	−0.722 *	−0.434 *	−0.707 *	−0.661 *
Mn	−0.927 *	0.321	−0.877 *	0.398 *
Cu	0.585 *	0.792 *	0.594 *	0.792 *
Pb	0.554 *	0.819 *	0.508 *	0.844 *
Zn	0.978 *	0.030	0.970 *	0.201

¹: TIN = total inorganic nitrogen; TC = total carbon; TN = total nitrogen; TP = total phosphorus. ²: The asterisks (*) after the data indicate the significant correlations analyzed by statistical analysis system (SAS) ($p < 0.0001$).

In the first week of incubation, PC1 showed significantly positive correlations with the content of NH₄⁺–N, the TIN, the cumulative CO₂–C, the TN, the Cu, the Pb and the Zn, and it had significantly negative correlations with the soil pH, NO₃[−]–N, K, Ca, Mg, Fe, and Mn. However, the TN, P, Ca, Cu and Pb contents were positively correlated with PC2, but the NH₄⁺–N, TIN and Fe were negatively correlated with PC2. At the 57th week of incubation, PC1 showed significantly positive correlations with the contents of NH₄⁺–N, NO₃[−]–N, TIN, TN, TP, Cu, Pb and Zn, and the pH, K, Ca, Mg, Fe and Mn had negative correlations with PC1. However, the cumulative CO₂–C, P, Ca, Mn, Cu and Pb contents were positively correlated with PC2, but the NH₄⁺–N and Fe were negatively correlated with PC2. As shown in Figure 2, the score plots of all the treatments were classified into three distinctive groups related to the coefficient of PC1, that is, the three soils. At the 1st and 57th weeks, the three soils could be distinctly separated, but the scores of the control and biochar treatments of three soils were close, and cannot be distinctively classified. These results suggest that in the current study, soil is an important factor influencing the effect of the biochar and its addition rate on C release, N mineralization and available nutrient status. In addition, in the first week, soil pH had significantly positive correlations with NO₃[−]–N, K, Ca, Mg, Fe and Mn, but negative correlations with NH₄⁺–N, TIN, cumulative CO₂–C, Cu, Zn and TN (Table S1). At 57 weeks, the soil pH showed significantly positive correlations with P, K, Ca, Mg, Fe and Mn, but negative correlations with NO₃[−]–N, NH₄⁺–N, TIN, Cu, Pb, Zn, TN, and TP. The soil pH may be the most important factor for in the current study in terms of regulating N mineralization (ammonification and nitrification) and immobilization, as well as influencing C mineralization and the soil available nutrients. Besides this, the soil C/N ratio in this study may be seen to have major effects on nitrification.

4. Discussion

4.1. Impacts of Biochar on Nitrogen Mineralization, Immobilization and Nitrification

The potential mechanisms by which biochar affects the soil N cycle [20] include: (1) N adsorption or desorption by biochar to decrease or increase the soil inorganic N content; (2) changes in the soil mineralizable substrates' (i.e., labile organic compounds) content to impact the microbial processes of N mineralization or immobilization; (3) alterations in soil

properties (i.e., pH and aeration) to alter the balance between the processes of nitrification and denitrification. A biochar normally adsorbs more NH_4^+ via the acid functional groups and labile C presented on its surface, especially as regards low-temperature-pyrolyzed biochar in comparison with biochar produced at high temperatures [21]. Additionally, the removal rates of ammonium vary widely with feedstock and pyrolysis temperature, but no pyrolysis temperature trend has been observed [22]. Similar to soils, and illustrating its ability to electrostatically sorb or attract cations, the CEC of the biochar is generally cited as causing the adsorption of NH_4^+ onto the biochar and the observed reductions in NH_4^+ leaching [12]. However, increasing the pyrolysis temperature tends to cause a decrease in CEC, due to the increasing lignin and cellulose decomposition in the feedstock materials, and the removal and loss of organic functional groups (i.e., more volatile matter) at greater pyrolysis temperatures [8]. In the current study, the three high-temperature-pyrolysis biochars displayed more aromatic structures, fewer polar functional groups, and lower labile C (water extractable organic C) contents [14], as evidenced by the low CEC of the three biochars (8.46, 15.5, and 5.20 $\text{cmol (+) kg}^{-1} \text{ soil}^{-1}$, respectively). The acid functional group, as well as the labile C, could be expected to make less, or no, contribution to the immobilizing of N in the mineral soil in this study.

Besides this, several studies have indicated that significant NO_3^- adsorption occurs at higher pyrolysis temperatures [4,9,22,23], and there is variation in the degree of removal related to the species of feedstock used [22]. Since the surface area and micropore volumes followed different trends when compared to the process of NO_3^- adsorption that was observed, Kameyama et al. [4] considered that the adsorption of NO_3^- that occurs in high-temperature-pyrolysis biochar was due to the base functional groups, determined by the high pH (pH 8.7–9.8) of high-temperature-pyrolysis biochar, and was not a result of physical adsorption. Moreover, the removal of the O-carrying functional groups occurred at elevated temperatures of biochar production, and resulted in low polarity (i.e., low O/C ratio), contributing to the decline in surface functional groups with negative charge ($-\text{COO}-$, $-\text{COH}$ and $-\text{OH}$) at high production temperatures. This could reduce the sorption of ammonium, while increasing the sorption of nitrate [9]. The current study did not include data on the biochar sorption capacity of ammonium and nitrate, but the data in Table 3 might still suggest that high-temperature-pyrolysis biochar treatments cause greater reductions in nitrate concentration than in ammonium concentration at the end of incubation (57 weeks). Higher addition-rate treatments caused significant decreases in ammonium content at the 1st and 57th weeks, but nitrate content was only reduced at the 57th week. Comparing the three biochars, the effectiveness of a higher rate of application on the enhancing of N immobilization was measured in the following decreasing order: lead tree biochar > bamboo biochar > ash biochar. The feedstocks of the three studied biochars had a more significant influence on the reduction of nitrate content than ammonium content.

4.2. Effects of pH on Soil N Mineralization

In the 1st week, the mean value of the soil pH showed a significant increase with an increasing biochar rate in the three soils. However, at 57 weeks, a significant increase was observed only in the SAO soil, and an insignificant one in the MAI and SAI soils, indicating that the liming effect has been reduced (Table 4). Many reports have indicated that the inherent alkalinity of biochar could serve as a liming agent and leads to increased soil pH, especially for ameliorating the acidity of acidic soils [11,14,19,24–30]. At the end of incubation, the soil pH increased by 0.01–0.24, 0.01–0.18, and 0.11–0.26 pH units for the SAO, MAI and SAI soils, respectively, and by 0.01–0.16, 0.03–0.13 and 0.06–0.26 pH units for the ash, bamboo, and lead tree biochars, respectively. The meta-analysis results [20] indicated that, on average, the soil pH can be significantly increased by 8.78% in the biochar-amended soils; however, our study results indicated a significant increase by 4.0%. Increasing pyrolysis temperature causes the biochar to be more basic due to the removes of acidic functional groups and the increase of the ash content [8], and the formation of more alkaline minerals in the biochar [31]. Applying such biochar, with a high pH value and

containing calcite, could have a great neutralizing effect [32]. In this study, three biochars containing alkaline minerals that influence the pH and only limited acidic functional groups were pyrolyzed at similar high temperatures ($>700\text{ }^{\circ}\text{C}$) [14]. The pH values of the three biochars were 10.3, 10.6 and 9.9, for the A, B and L biochars, respectively. In addition, the total K, Ca and Mg contents were 1.59 g kg^{-1} , 4.96 g kg^{-1} and 0.48 g kg^{-1} for the A biochar, 2.18 g kg^{-1} , 0.51 g kg^{-1} and 0.30 g kg^{-1} for the B biochar, and 0.63 g kg^{-1} , 7.19 g kg^{-1} and 0.32 g kg^{-1} for the L biochar, respectively. The current study results indicated that the neutralizing effect of the three biochars was documented in the following decreasing order: $L > A > B$. The L biochar has a relatively lower pH, but a higher Ca content, indicating its greater neutralizing effect when applied to the soil, as evidenced by the significant increase in pH in the SAO and SAI soils (Table 4).

For many soil microbial oxidizers, Robertson and Groffman [33] indicated that the optimal pH range is 7–9. In addition, the N mineralization rate increased in the biosolids-amended soils as the pH value was raised within the acidic to slightly alkaline range ($<\text{pH } 8$) [34]. The original soil pH of the three soils was <8.0 , and the pH values were 6.1, 7.5 and 6.5 (soil:water = 1:1) for the SAO, MAI and SAI soils, respectively [15]. After biochar treatment, therefore, more N immobilization was observed in the first week (Table 3), because the pH showed a significant increase, confirming that the biochar-induced pH increase was responsible for the N immobilization, and especially so for the higher rate (5%). These results were also supported by the significant negative correlation between soil pH and $\text{NH}_4^+ - \text{N}$, and the TIN content ($r = -0.77$ and -0.73 , respectively, $p < 0.0001$) (Table S1), in the first week. At 57 weeks, our results showed relatively insignificant, and lower, changes (Table 3). Furthermore, the significant negative correlation between soil pH and $\text{NO}_3^- - \text{N}$, $\text{NH}_4^+ - \text{N}$, and TIN content ($r = -0.78$, -0.95 and -0.85 , respectively, $p < 0.0001$) confirmed that the biochar-induced pH increase was responsible for the N immobilization. However, the nitrate content of the ash biochar treatment (A2 and A5) significantly increased in the first week, and it also increased in the bamboo and lead tree biochar treatments, but less so, suggesting an increase in nitrification at that time. Table S1 shows that the nitrate content was significantly positively correlated ($r = 0.47$, $p < 0.0001$) with soil pH, which indicates the increase in nitrification rate with the increasing soil pH (caused by alkaline biochar addition) in the first week. On the contrary, after the incubation (at 57 weeks), the nitrate content showed a significant negative correlation ($r = -0.78$, $p < 0.0001$) with soil pH. The increased utilization and immobilization of soil nitrate could be attributed to the suitability of the pH environment, resulting from the increment in soil pH after biochar addition [9]. At both the 1st week and the 57th week, the ammonium content showed a significant negative correlation with soil pH ($r = -0.77$ and -0.95 ($p < 0.0001$), respectively).

In addition to biochar, Ippolito et al. [35] indicated that even at a high biochar application rate (10%, *w/w*), the soil's buffering capacity—the ability to resist changes in pH—might prevent major changes in soil pH. The soil's organic and mineral content and the soil's physical characteristics would affect soil buffering capacity [36]. Tsai and Change [28] indicated that major changes in soil pH have been prevented by the soil's buffering capacity, and the acidification and nitrification of 2% compost decomposition may offset the neutralizing effect of rice husk biochar. In the current study, a higher compost rate (5%) may have greater potential for offsetting the neutralizing effects of the studied biochars, as confirmed by the soil pH increasing by a maximum of 0.24, 0.18, and 0.26 pH units for the SAO, MAI, and SAI soils, respectively, and by a maximum of 0.16, 0.13, and 0.26 pH units for the A, B, and L biochars, respectively. The soil pH showed a relatively lower change, and some insignificance between the biochar treatments and the control was shown in the slightly alkaline MAI soil. Upon the addition of a mix of pine and spruce wood biochar (3 Mg ha^{-1}) to Luvisol (pH 7.2) in southern Ontario, no significant changes in soil pH, SOC, or TN could be found [37]. After a 70-d incubation, the application of pinewood biochar (3 Mg ha^{-1}) alone to two Mollisols with soil pH values of 6.62 and 7.86 did not significantly alter the pH, EC, TN, SOC or TP relative to the control [38]. Furthermore, the minimal effect of the pinewood biochar is to be expected in the studied

Mollisols, of a slightly alkaline calcareous nature, owing to their highly active clays and strong buffering potential. Because of the MAI soil's clay content being similar to that of the SAI soil (39% in MAI soil and 34% in SAI soil) and lower than that of the SAO soil (59%), the higher carbonate content (0.81%) of the MAI soil may give it a strong buffering potential, thus limiting the change in the biochar-amended soil. For the SAO and SAI soils, the soil pH was similar, but there was a higher clay content in the SAO soil than in the SAI soil, and this could provide the SAI soil's stronger buffering potential.

4.3. Effects of C:N Ratio on Soil N Mineralization

As attributed to the loss of gaseous N during pyrolysis [39], biochars frequently have low concentrations of extractable N (as NO_3^- , NH_4^+ and NO_2^-) [40]. According to the reports of Ippolito et al. [8], in all cases (biochars), the percentage of available N in the form of nitrate is <0.01% of the total. In this study, for the ash biochar, the bamboo biochar and the lead tree biochar, the cumulative contents of total water extractable organic nitrogen ($\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$) as compared to total N were about 0.10% and 0.04%, 0.08% and 0.09%, and 0.12% and 0.01%, respectively. Thus, the three biochars we studied contain more forms of bioavailable N than those in the report [8]. However, while biochar may contain bioavailable N forms, its mineralization and release will depend on how recalcitrant the biochar and soil N and C pools are, the soil and biochar C:N ratio, the relative magnitude of the soil and biochar C and N pools, and the studied ecosystems [12].

Reflecting the contents of C and N that are available for organisms, the C:N ratio is a key factor controlling the balance between N mineralization and N immobilization, which could occur at the same time within relatively small volumes of soil [33]. The soil N mineralization and immobilization rates are a function of the C and N pools available to microorganisms [12], and the immobilization of N occurs with an increasing C:N ratio. The immobilization of N has been reported following the addition of high C/N ratio biochar [9,29,41,42]. The raw C/N values of the three soils and compost were 8.5 (SAO), 4.8 (MAI), 5.9 (SAI), and 10.3 (compost), respectively. However, the three biochars have a high C content (>80%) and low N content (<0.8%), and exhibit high C/N ratios of 154, 128 and 118 for ash, bamboo, and lead tree biochar, respectively. A further reason for the decline is the immobilization of N due to the high C/N ratio of our biochar, because N immobilization generally occurs when the biochar C/N ratio exceeds 20 [21,43] and/or 32 [21,44]; an initial net immobilization of N is expected [45].

In degraded coastal wetland soils, biochar co-applied with urea fertilizer initially reduced the net N mineralization, but this slightly increased later on. This biochar-induced reduction in net N mineralization was mainly attributed to the increased C:N ratio, as well as the reduced urease activity [27]. Even in nutrient-poor alkaline calcareous soil, biochar alone, as well as with wheat straw ($22.5 \text{ t} \cdot \text{ha}^{-1}$ and $45 \text{ t} \cdot \text{ha}^{-1}$), resulted in either net negative N mineralization or net N immobilization [10]. Under condition of excessive compost, the current study results suggest that the addition of biochar increases the soil C:N ratio because of its high alkalinity and C content (Figure S1d, Table 4). Similar to the results of the reports [11,30], net immobilization occurred during the first 3–7 d after biochar addition, which is attributed to the high C:N ratio of biochar. In the first week (Table 4), the soil C/N showed a significant increase as the rate of biochar addition increased. The 5% addition treatments mostly gave rise to higher C/N (close to or higher than 20), indicating the biochar in those treatments will trigger N immobilization, as evidenced by the significant reduction in ammonium concentrations (Table 3). After the incubation test, the soil C/N also showed a significant increase as the rate of biochar addition increased, but these values were relative lower than those in the first week. The C/N values of the 5% treatments were mostly lower than 20, but not by much, indicating the biochar's maintained potential for triggering N immobilization within the incubation time, as evidenced by the significant reduction in ammonium concentrations (Table 3). In the first week, the relatively lower C/N in the bamboo biochar treatments could be attributed to the higher levels of water-extractable N (Figure 1). In addition, as shown in Table S1, the application of biochar

to soils with high C/N (or low TN content) may reduce the N availability at 57 weeks, given the negative correlation between nitrate, ammonium and TIN concentration, and soil C/N ($r = -0.44$, $p < 0.0001$; $r = -0.01$, $p > 0.05$; $r = -0.36$, $p < 0.0001$, respectively). However, in the first week, the correlation between nitrate concentration and soil C/N was positive ($r = 0.21$, $p > 0.05$), and the correlation between nitrate and TIN concentration and soil C/N was negative ($r = -0.0$ and -0.01 , $p > 0.05$). In general, the C/N following biochar treatment was relatively higher in the SAI soil, followed by the MAI and SAO soils, indicating that greater N immobilization occurred in the SAI soil than in the MAI and SAO soils. As regards the three biochars, the values of soil C/N in the SAO and SAI soils were measured in the following decreasing order: $B > A \approx L$; the order of these values in the MAI soil was $A > B > L$.

5. Conclusions

Our study has indicated the significant role that a high biochar rate plays in reducing the ammonium and nitrate in soils, as well as its consistent effect on the pH and C/N ratio of soils. The immediate effects of the three studied high-temperature biochars on soil ammonium and nitrate were negative, irrespective of soil properties. In addition, over time, the amount of reduced soil ammonium and nitrate becomes soil-specific, and is primarily determined by the soil's original pH and texture. Soil with a lower pH and higher clay content (such as Oxisols) may exhibit a reduced degree of decline, whereas a low proportion of clay facilitates the reduction of ammonium and nitrate in the soil. However, the reduced effect is also biochar-specific, and is primarily determined by the biochar's original pH and C/N ratio. Biochars with a relatively lower pH and C/N ratio (such as the lead tree biochar) could facilitate the decline in ammonium and nitrate in the soil, reducing the extent of the decline, whereas biochars with a higher pH and C/N ratio reduce the extent of the decline but have inconsistent effects on the ammonium contents of soils. Based on the above conclusions, we affirmed our hypothesis that greater biochar application rates (i.e., 5 wt. %) would have a negative effect. Therefore, when 5% biochar was applied with 5% compost, the biochar reduced the concentrations of excess soil $\text{NO}_3^- - \text{N}$ accumulation, and thus may benefit producers by leading to more efficient N use. Our previous and current studies probe the interactions between a high biochar rate and excessive compost as an opportunity for C sequestration and, simultaneously, N conservation in over-fertilized soils. However, further research under field conditions is necessary for validating the laboratory findings.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4395/11/4/617/s1>, Figure S1: The temporal change of (a) ammonium ($\text{NH}_4^+ - \text{N}$) release, (b) nitrate ($\text{NO}_3^- - \text{N}$) release, (c) total inorganic nitrogen (TIN) ($\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N}$) release, and (d) pH for all treatments from SAO, MAI, and SAI soil during the incubation period. Table S1: Pearson correlation coefficients between measured parameters at 1 week and 57 weeks for all treatments.

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