






## Article

# Biochar Applied with Inorganic Nitrogen Improves Soil Carbon, Nitrate and Ammonium Content of a Sandy Loam Temperate Soil

Peter Omara <sup>1,2,\*</sup> , Lawrence Aula <sup>1</sup> , Fred Otim <sup>2</sup>, Alfred Obia <sup>2</sup> , Joao Luis Bigatao Souza <sup>1</sup>   
and Daryl Brain Arnall <sup>1</sup> 

<sup>1</sup> Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK 74078, USA; aula@okstate.edu (L.A.); bigatao@okstate.edu (J.L.B.S.); b.arnall@okstate.edu (D.B.A.)

<sup>2</sup> Department of Agronomy, Gulu University, Gulu P.O. Box 166, Uganda; otimakio@gmail.com (F.O.); a.obia@gu.ac.ug (A.O.)

\* Correspondence: peter.omara@okstate.edu

**Abstract:** Biochar is suggested to improve soil properties. However, its combination with inorganic nitrogen (N) fertilizer in temperate soils is not well understood. This study compared the effect of fertilizer N-biochar-combinations (NBC) and fertilizer-N (FN) on total soil N (TSN), soil organic carbon (SOC), soil nitrate ( $\text{NO}_3^-$ -N), and ammonium ( $\text{NH}_4^+$ -N). Soil samples were taken from experiments at Efaw and Lake Carl Blackwell (LCB), Oklahoma, USA with ten treatments consisting of three N rates (50, 100, and 150 kg N ha<sup>-1</sup>) and three biochar rates (5, 10, and 15 t ha<sup>-1</sup>). Results at Efaw showed greater TSN and SOC under NBC compared to FN by 3 and 21%, respectively. No percentage difference was observed for  $\text{NH}_4^+$ -N while  $\text{NO}_3^-$ -N was lower by 7%. At LCB, TSN, SOC,  $\text{NO}_3^-$ -N, and  $\text{NH}_4^+$ -N were higher under NBC by 5, 18, 24, and 10%, respectively, compared to FN. Whereas application of biochar improved SOC at both sites,  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N were only significant at LCB site with a sandy loam soil but not at Efaw with silty clay loam. Therefore, biochar applied in combination with inorganic N can improve N availability with potential to increase crop N uptake on coarse textured soils.

**Keywords:** biochar; total nitrogen; nitrate; ammonium; soil organic carbon



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## 1. Introduction

Biochar is a stable carbon (C) rich material formed through pyrolysis of organic materials [1,2]. Application of biochar to the soil is suggested to improve soil properties in addition to C sequestration [3–6]. It is reported to be beneficial in improving soil physical, biological, and chemical properties which include, among others, soil organic carbon (SOC), water retention capacity, cation exchange capacity (CEC), total soil nitrogen (TSN), and soil pH, hence contributing to soil fertility [7,8].

Soil organic C, one of the most important biological properties that determines quality of soil, is believed to be improved through application of biochar. Some research reports have documented the contribution of fertilizer-N (FN) in increasing SOC stock. They argue that FN increases quantity of crop residues added to the soil as a result of improved biomass production. Generally, high rates of FN inconsistently affect SOC where increases are observed in some cases while manure application more frequently increases surface soil SOC [9]. Biochar application with >90% of C in recalcitrant forms more consistently increases SOC. However, there are contradictory conclusions on the role of biochar in enhancing SOC storage. Some researchers have reported negative priming effect of biochar to the native SOC as a result of increasing the rate of evolution of carbon dioxide hence less storage [10,11]. This could be due to short term oxidation of the labile biochar compounds [12]. If the soil is inherently poor in SOC, application of biochar will reduce the

evolution of CO<sub>2</sub> while the opposite would be observed in soils rich in organic C [13]. Besides, C loss is always very small relative to the amount of C stored within the biochar itself [14]. In contrast, Cross and Sohi [12] reported that application of biochar did not, for the most part, indicate negative priming of the native SOC and that application of biochar could stabilize native SOC in grassland soils. Applying a combination of N and biochar could contribute to the increase in the SOC storage.

With evidence of increased SOC following biochar application, soil N is likely to increase. Soil N is present mostly in organic compounds which consist of both particulate organic N and dissolved organic N. The particulate organic N include the N in living organisms and detritus. On the other hand, dissolved organic N consists of a wide range of organic substances, such as free amino acids, and proteins, among others [15]. Biochar soil incorporation is suggested to increase the buildup of organic N. Prommer et al. [16] reported that application of inorganic N in combination with biochar had a synergistic effect by activating the belowground build-up of soil organic N. They explained that biochar reduces the transformation rates of the native soil organic N as plants and microbes draw from the inorganic fertilizer N. Bai et al. [17] added that changes in microbial processes and activities on soil organic N following biochar soil application are mediated primarily by abiotic factors such as rainfall and temperature. Therefore, biochar has a great potential in building soil organic N.

Plants take up N in the inorganic form; NO<sub>3</sub><sup>−</sup> and NH<sub>4</sub><sup>+</sup> which are susceptible to losses such as volatilization, denitrification, runoff, and leaching [18–20]. Biochar application may improve inorganic N retention through alteration of CEC and anion exchange capacity (AEC) with the greatest benefit on sandy soils and this has been demonstrated by many studies [21–25]. The increased AEC of biochar reduces leaching of NO<sub>3</sub><sup>−</sup>-N while the CEC increases the adsorption of NH<sub>4</sub><sup>+</sup>-N. Therefore, the application of inorganic N with biochar may reduce loss and increase uptake of both NO<sub>3</sub><sup>−</sup>-N and NH<sub>4</sub><sup>+</sup>-N. The objective of this study was to compare the effect of fertilizer N-biochar-combinations (NBC) and FN on soil NO<sub>3</sub><sup>−</sup>-N, NH<sub>4</sub><sup>+</sup>-N, SOC, and TSN. We hypothesized greater soil N content and improved SOC under NBC compared to NF following harvest of maize.

## 2. Materials and Methods

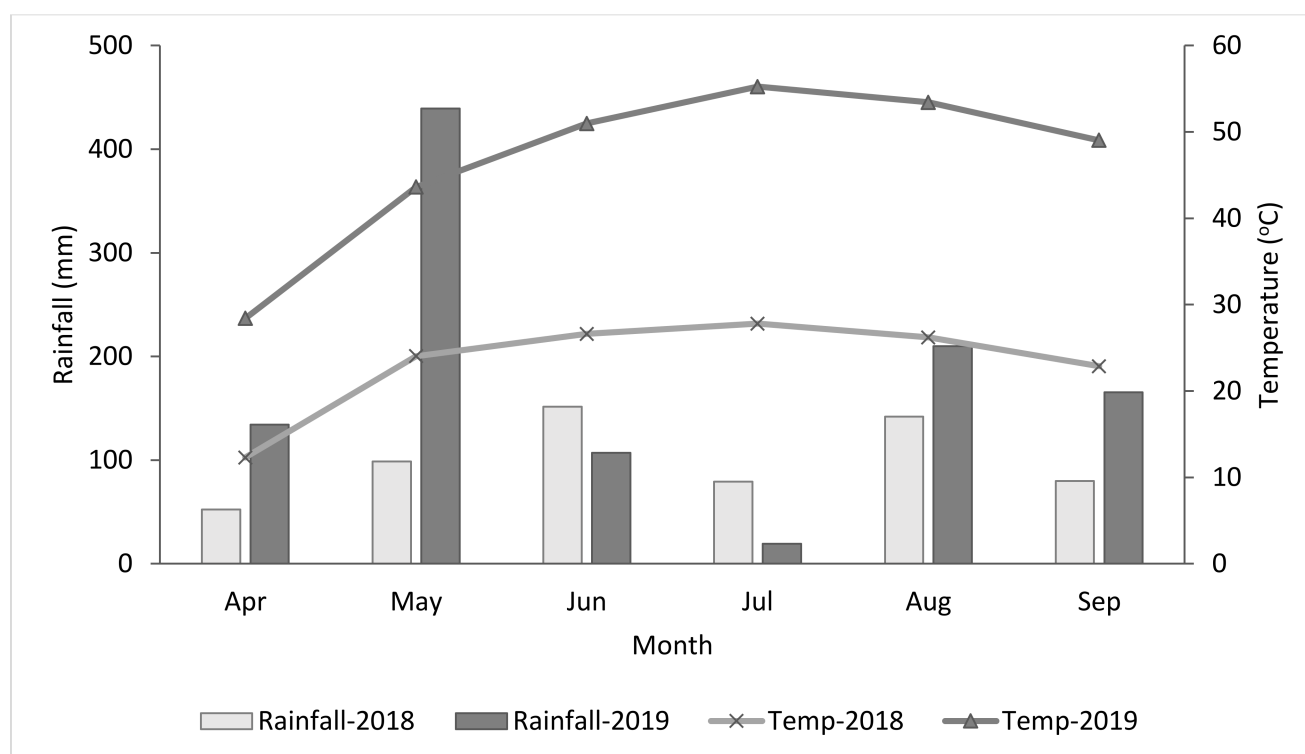
### 2.1. Experimental Sites and Design

Field trials were conducted for two years in the summer cropping season of 2018 and 2019 at Efaw Agronomy Research Station (36°08′12.6″ N 97°06′25.8″ W) and Lake Carl Blackwell research farm (36°08′58.0″ N 97°17′19.3″ W), near Stillwater, OK, USA. Efaw Agronomy Research Station had Ashport silty clay loam (fine-silty, mixed, superactive, thermic Fluventic Haplustoll) soil. Lake Carl Blackwell had Pulaski fine-sandy loam (coarse/loamy, mixed nonacid, thermic Udic Ustifluvent) soil [26]. The treatments included; 0, 50, 100, and 150 kg ha<sup>−1</sup> of FN with no biochar; and 5, 10 and 15 t ha<sup>−1</sup> of biochar with no FN. The three NBC treatments were 50 kg N plus 5 t ha<sup>−1</sup> biochar, 100 kg N plus 10 t ha<sup>−1</sup> biochar, and 150 kg N plus 15 t ha<sup>−1</sup> biochar. In the second year, treatments were applied to the same exact plots used in the first year. Biochar was obtained from Wakefield Agricultural Carbon (Columbia, MO, USA), a USDA certified biochar producing company. Physical and chemical properties of Southern Yellow Pine biochar pyrolyzed at 500 °C, and the initial soil conditions are included in Table 1. All the N and biochar treatments were applied prior to planting of maize. Total rainfall and average air temperature (April to September) in 2018 and 2019 at Stillwater, OK, USA were obtained from Oklahoma Mesonet (Figure 1).

**Table 1.** Physical and chemical properties of soft wood (Southern Yellow Pine) biochar supplied by Wakefield Biochar, Columbia, Missouri; the initial soil chemical properties at Lake Carl Blackwell (LCB) and Efaw research sites, Stillwater, OK, USA.

Biochar/ Site	pH	K	Ca	Mg	Mn	Fe	BD	TP	TN	TOC
	Unit	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	g cm <sup>-1</sup>	mg kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>
Biochar	7.4	612	4128	1225	234	595	0.48	4.53	5.9	876.7
LCB	5.7	349	804	207	x	x	x	12	0.8	9.1
Efaw	5.6	153	1466	354	x	x	x	13	0.7	6.8

TP, total phosphate; TN, total nitrogen; TOC, total organic carbon; BD, bulk density; x, values not determined. Initial soil properties were determined before the first year of biochar application.



**Figure 1.** Total rainfall and average air temperature (April to September) in 2018 and 2019 at Stillwater, OK, USA.

Fertilizer-N was applied as urea ammonium nitrate—UAN (28:0:0). Fertilizer-N, biochar and NBC treatments were surface applied. Biochar was broadcast and incorporated at a 15 cm soil depth using a 2720 John Deere Disk Ripper (John Deere, Moline, IL, USA). This incorporation ensured an in-depth mixing of the biochar-N fertilizer complex with soil materials for the respective treatments.

## 2.2. Data Collection and Analysis

Composite soil samples, 15–20 cores per plot at 0–15 cm, were collected five months after biochar application following harvest of maize in 2018 and 2019. Soil samples were oven-dried for 48 h at 65 °C, and ground to pass through a 1 mm sieve size to remove larger aggregates and plant roots. The extraction of inorganic N ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) was carried out from 5 g of soil with 25 mL 1 M KCl after shaking for 30 min on a rotary shaker at 200 rpm. The extracts were filtered with 0.45  $\mu\text{m}$  Whatman filter paper and then analyzed using automated Lachat QuickChem 8500 Series 2 Flow Injection Analyzer (Hach Co., Loveland, CO, USA). The SOC and TSN contents were determined from 200 mg of

soil using dry combustion [27] at 950 °C with LECO Truspec CN dry combustion analyzer LECO CN628 (LECO Inc., St. Joseph, MI, USA).

### 2.3. Statistical Analysis

In this study, the independent variables were contrasts, treatments, and replications while the dependent variables were  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, TSN, and SOC. Data were analyzed separately for each year and separated by location. The GLM procedure of the SAS statistical package was used in the analysis of variance (ANOVA) [28]. The combined ANOVA tested for the effect of the independent variables as well as key interactions on the response variables. For all the response variables, the difference between treatment means from NBC and FN were compared using single-degree-of-freedom orthogonal contrasts [29,30]. In addition to the level of statistical significance from ANOVA, the standard error (S.E) of means for each treatment and the coefficient of variation (CV) were used to indicate the precision of measurement and the extent of variability within and between groups, respectively. For each response variable, treatment means and the corresponding S.E were presented in a table that combined experimental sites and years. Additionally, contrasts that compared specific treatments of interests were presented in the bottom half of the table with corresponding F and *p*-values for each site and year.

## 3. Results

### 3.1. Soil Nitrate N

The results at Efaw location in 2018 did not show any significant difference ( $p = 0.0534$ ) in soil  $\text{NO}_3^-$ -N content between treatments (Table 2). At each fertilizer rate, soil  $\text{NO}_3^-$ -N was higher under NBC compared to FN by 5 and 7% at 50 and 100 kg N ha<sup>-1</sup>, respectively. At 150 kg N ha<sup>-1</sup>, orthogonal contrast showed that NBC was significantly ( $p = 0.0259$ ) lower than FN by 31%. The highest soil  $\text{NO}_3^-$ -N of 6.4 mg kg<sup>-1</sup> was observed under FN at 150 kg N ha<sup>-1</sup> while the lowest (3.8 mg kg<sup>-1</sup>) was observed at 10 t ha<sup>-1</sup> biochar with no N applied. In 2019, results showed significant differences in soil  $\text{NO}_3^-$ -N content ( $p < 0.0001$ ) between treatments (Table 2). However, contrasts between NBC and FN did not show significant difference in soil  $\text{NO}_3^-$ -N at all N rates. Soil  $\text{NO}_3^-$ -N was lower under NBC than observed FN by 5, 9, and 5% at 50, 100, and 150 kg N ha<sup>-1</sup>, respectively. The highest soil  $\text{NO}_3^-$ -N (5.9 mg kg<sup>-1</sup>) was observed under FN at 150 kg N ha<sup>-1</sup> while the lowest (4.1 mg kg<sup>-1</sup>) was observed under 5 t ha<sup>-1</sup> of biochar with no N applied.

At LCB, the 2018 ANOVA results showed significant differences in soil  $\text{NO}_3^-$ -N ( $p < 0.0001$ ) between treatments (Table 2). For each fertilizer rate, contrasts between NBC and FN did not show significant difference in soil  $\text{NO}_3^-$ -N at 50 kg N ha<sup>-1</sup> ( $p = 0.1702$ ). However, significant differences were seen at 100 kg N ha<sup>-1</sup> ( $p = 0.0003$ ) and 150 kg N ha<sup>-1</sup> ( $p < 0.0001$ ). Nitrate under NBC was higher than that observed under FN by 11, 29, and 40% at 50, 100, and 150 kg N ha<sup>-1</sup>, respectively. The highest  $\text{NO}_3^-$ -N (3.9 mg kg<sup>-1</sup>) was seen under NBC at 100 kg N ha<sup>-1</sup> while the lowest (2.0 mg kg<sup>-1</sup>) was observed at 150 kg N ha<sup>-1</sup> under FN. In 2019, results were similar to that of 2018 where significant differences in  $\text{NO}_3^-$ -N ( $p = 0.001$ ) were observed between treatments (Table 2). Contrasts between NBC and FN did not show a significant difference in  $\text{NO}_3^-$ -N at 50 kg N ha<sup>-1</sup> ( $p = 0.3134$ ) and 100 kg N ha<sup>-1</sup> ( $p = 0.0891$ ), while significant difference was observed at 150 kg N ha<sup>-1</sup> ( $p = 0.02$ ). The observed differences showed higher  $\text{NO}_3^-$ -N under NBC than FN by 16, 23, and 27% at 50, 100, and 150 kg N ha<sup>-1</sup>, respectively. The highest soil  $\text{NO}_3^-$ -N (7.0 mg kg<sup>-1</sup>) was observed under NBC at 150 kg N ha<sup>-1</sup> while the lowest (3.7 mg kg<sup>-1</sup>) was observed at the check plot.

**Table 2.** Mean nitrate N for treatments plus the associated contrasts between N fertilizer and biochar-N combinations at Efaw and Lake Carl Blackwell, Stillwater, Oklahoma. 2018 and 2019.

Treatment	N Rate kg ha <sup>-1</sup>	Biochar t ha <sup>-1</sup>	NO <sub>3</sub> <sup>-</sup> -N at Efaw				NO <sub>3</sub> <sup>-</sup> -N at LCB			
			mg kg <sup>-1</sup>				mg kg <sup>-1</sup>			
			2018		2019		2018		2019	
			mean	±S.E	Mean	±S.E	mean	±S.E	mean	±S.E
1	0	0	4.24	0.42	4.05	0.24	2.13	0.24	3.71	0.71
2	50	0	4.76	0.14	4.81	0.54	2.66	0.17	3.94	0.21
3	100	0	4.94	0.23	5.33	0.11	2.76	0.18	4.39	0.17
4	150	0	6.38	0.22	5.88	0.18	1.96	0.06	5.11	0.28
5	0	5	4.45	0.70	4.05	0.02	2.15	0.17	3.95	0.09
6	0	10	3.80	0.39	4.22	0.03	2.14	0.06	4.00	0.11
7	0	15	4.02	0.63	4.18	0.12	2.24	0.26	4.09	0.07
8	50	5	5.01	0.35	4.58	0.04	2.98	0.03	4.67	0.70
9	100	10	5.29	0.32	4.88	0.17	3.87	0.26	5.68	0.21
10	150	15	4.86	0.85	5.58	0.01	3.27	0.12	6.98	0.88
Pr > F			0.0534		<0.0001		<0.0001		0.001	
C.V, %			17.3		7.7		11.5		16.6	
Contrasts			F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
2 vs. 8			0.17	0.6840	0.43	0.5222	2.13	0.1702	1.11	0.3134
3 vs. 9			0.35	0.5640	1.63	0.2262	25.02	0.0003	3.42	0.0891
4 vs. 10			6.45	0.0259	0.74	0.4067	34.60	<0.0001	7.18	0.0200
2, 3 & 4 vs. 8, 9 & 10			0.78	0.3944	2.60	0.1326	50.79	<0.0001	10.39	0.0073

C.V, coefficient of variation between treatments; S.E, standard error for replicated means ( $\pm$ SE,  $n = 3$ ). Nitrogen fertilizer was applied as urea ammonium nitrate—UAN (28:0:0). Biochar was applied immediately following UAN and incorporated to a depth of 15 cm.

### 3.2. Soil Ammonium N

The ANOVA at the Efaw location in 2018 did not show significant difference in soil NH<sub>4</sub><sup>+</sup>-N content ( $p = 0.9268$ ) between treatments (Table 3). At each fertilizer rate, soil NH<sub>4</sub><sup>+</sup>-N content was higher under NBC compared to FN by 8 and 9% at 50 and 100 kg N ha<sup>-1</sup>, respectively, while a decrease under NBC by 3% was observed at 150 kg N ha<sup>-1</sup> compared to FN. The highest NH<sub>4</sub><sup>+</sup>-N of 21.2 mg kg<sup>-1</sup> was seen under NBC at 100 kg N ha<sup>-1</sup> while the lowest (17.6 mg kg<sup>-1</sup>) was observed at the check plot. In 2019, results showed significant differences in NH<sub>4</sub><sup>+</sup>-N content ( $p = 0.0009$ ) between treatments (Table 3). However, contrasts between NBC and FN did not show significant difference in NH<sub>4</sub><sup>+</sup>-N at all contrasted N rates. Soil NH<sub>4</sub><sup>+</sup>-N content decreased under NBC by 13% at 150 kg N ha<sup>-1</sup> compared to FN. The highest NH<sub>4</sub><sup>+</sup>-N (5.5 mg kg<sup>-1</sup>) was observed under FN at 150 kg N ha<sup>-1</sup> while the lowest (3.9 mg kg<sup>-1</sup>) was observed under 5 t ha<sup>-1</sup> of biochar with no N applied.

At LCB, the 2018 results showed significant differences in soil NH<sub>4</sub><sup>+</sup>-N ( $p = 0.016$ ) between treatments (Table 3). For each fertilizer rate, contrasts between NBC and FN did not show significant difference in soil NH<sub>4</sub><sup>+</sup>-N at 50 kg N ha<sup>-1</sup> ( $p = 0.3546$ ) while significant differences were seen at 100 kg N ha<sup>-1</sup> ( $p = 0.026$ ) and 150 kg N ha<sup>-1</sup> ( $p = 0.0182$ ). Soil NH<sub>4</sub><sup>+</sup>-N was higher under NBC than FN by 6, 14, and 14% at 50, 100, and 150 kg N ha<sup>-1</sup>, respectively. The highest NH<sub>4</sub><sup>+</sup>-N (31 mg kg<sup>-1</sup>) was observed at 150 kg N ha<sup>-1</sup> under NBC while the lowest (23 mg kg<sup>-1</sup>) was observed at 10 t ha<sup>-1</sup> of biochar with no N fertilizer applied. In 2019, results were similar to that of 2018 where significant difference in NH<sub>4</sub><sup>+</sup>-N ( $p < 0.0001$ ) was observed between treatments (Table 3). Contrasts between NBC and FN did not show any significant difference in soil NH<sub>4</sub><sup>+</sup>-N at 50 kg N ha<sup>-1</sup> ( $p = 0.8881$ ) and 100 kg N ha<sup>-1</sup> ( $p = 0.1078$ ) while significant differences were seen at 150 kg N ha<sup>-1</sup> ( $p = 0.0026$ ). Soil NH<sub>4</sub><sup>+</sup>-N was higher under NBC than FN by 1, 8, and 15% at 50, 100, and 150 kg N ha<sup>-1</sup>, respectively. The highest soil NH<sub>4</sub><sup>+</sup>-N (5.2 mg kg<sup>-1</sup>) was observed under NBC at 150 kg N ha<sup>-1</sup> while the lowest (4.1 mg kg<sup>-1</sup>) was observed at the check plot.



**Table 3.** Mean ammonium N for treatments plus the associated contrasts between N fertilizer and biochar-N combinations at Efaw and Lake Carl Blackwell, Stillwater, Oklahoma. 2018 and 2019.

Treatment	N Rate kg ha <sup>-1</sup>	Biochar t ha <sup>-1</sup>	NH <sub>4</sub> <sup>+</sup> -N at Efaw				NH <sub>4</sub> <sup>+</sup> -N at LCB			
			mg kg <sup>-1</sup>				mg kg <sup>-1</sup>			
			2018		2019		2018		2019	
			mean	±S.E	Mean	±S.E	mean	±S.E	mean	±S.E
1	0	0	17.63	0.59	4.21	0.07	24.43	1.23	4.06	0.03
2	50	0	18.53	1.23	5.01	0.27	24.42	1.13	4.19	0.16
3	100	0	19.33	1.22	5.15	0.29	24.25	0.78	4.24	0.14
4	150	0	20.72	1.25	5.51	0.38	26.32	0.86	4.43	0.09
5	0	5	19.70	2.47	3.87	0.32	24.76	2.27	4.10	0.01
6	0	10	19.03	0.81	4.26	0.10	23.60	0.61	4.11	0.05
7	0	15	19.26	3.53	4.27	0.06	23.98	1.11	4.09	0.06
8	50	5	20.17	1.53	4.40	0.11	25.90	1.44	4.22	0.16
9	100	10	21.15	2.08	4.49	0.15	28.15	0.67	4.59	0.17
10	150	15	20.07	2.54	4.82	0.18	30.52	1.38	5.21	0.15
Pr > F			0.9268		0.0009		0.016		<0.0001	
C.V, %			14.9		8.3		8.4		4.6	
Contrasts			F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
2 vs. 8			0.46	0.5121	3.09	0.1044	0.93	0.3546	0.02	0.8881
3 vs. 9			0.56	0.4676	3.51	0.0854	6.45	0.0260	3.02	0.1078
4 vs. 10			0.07	0.7953	3.95	0.0702	7.47	0.0182	14.31	0.0026
2, 3 & 4 vs. 8, 9 & 10			0.45	0.5156	10.52	0.0070	12.96	0.0036	10.70	0.0067

C.V, coefficient of variation between treatments; S.E, standard error for replicated means ( $\pm$ SE,  $n = 3$ ). Nitrogen fertilizer was applied as urea ammonium nitrate—UAN (28:0:0). Biochar was applied immediately following UAN and incorporated to a depth of 15 cm.

### 3.3. Soil Organic C

At Efaw, the 2018 results indicated an overall significant difference ( $p = 0.0016$ ) in SOC between treatments (Table 4). Contrast comparing NBC and FN at 50 kg N ha<sup>-1</sup> did not indicate significance difference ( $p = 0.6542$ ) while significance differences in SOC were seen at 100 kg N ha<sup>-1</sup> ( $p = 0.0064$ ) and 150 kg N ha<sup>-1</sup> ( $p = 0.0018$ ). Higher SOC observed under NBC than FN correspond to 5, 27, and 31% at 50, 100, and 150 kg N ha<sup>-1</sup>, respectively. The highest SOC of 9.6 g kg<sup>-1</sup> was observed under NBC at 150 kg N ha<sup>-1</sup> while the lowest of 6.6 g kg<sup>-1</sup> was obtained at 150 kg N ha<sup>-1</sup> under FN. Similar observations were made in 2019 where significant differences ( $p = 0.0007$ ) in SOC were seen between treatments (Table 4). Contrasts revealed significant differences between NBC and FN at 100 kg N ha<sup>-1</sup> ( $p = 0.018$ ) and 150 kg N ha<sup>-1</sup> ( $p = 0.0007$ ). The NBC registered higher SOC than FN by 22 and 35% at 100 and 150 kg N ha<sup>-1</sup>, respectively. The highest SOC of 11 g kg<sup>-1</sup> was observed under NBC at 150 kg N ha<sup>-1</sup> while the lowest of 6.86 g kg<sup>-1</sup> was obtained at the control plot with no biochar and N applied.

Results at LCB for 2018 did not show an overall significant difference ( $p = 0.0758$ ) in SOC between treatments (Table 4). For each fertilizer rate, contrast comparing NBC with FN did not show significant difference at 50 kg N ha<sup>-1</sup> ( $p = 0.0858$ ) but differences were seen at 100 kg N ha<sup>-1</sup> ( $p = 0.0058$ ) and 150 kg N ha<sup>-1</sup> ( $p = 0.0006$ ). The SOC under NBC was higher than that observed under FN by 17, 21, and 28% at 50, 100, and 150 kg N ha<sup>-1</sup>, respectively. The highest SOC of 12 g kg<sup>-1</sup> was observed at 150 kg N ha<sup>-1</sup> under NBC while the lowest of 8.2 g kg<sup>-1</sup> was observed at 50 kg N ha<sup>-1</sup> under FN. In 2019, overall ANOVA showed significant differences ( $p = 0.0015$ ) in SOC between treatments (Table 4). Contrasts between NBC and FN showed significant differences at 50 kg N ha<sup>-1</sup> ( $p = 0.0415$ ), 100 kg N ha<sup>-1</sup> ( $p = 0.0241$ ) and 150 kg N ha<sup>-1</sup> ( $p = 0.0335$ ). The observed differences showed higher SOC under NBC more than under FN by 14, 15, and 12% at 50, 100, and 150 kg N ha<sup>-1</sup>, respectively. The highest SOC of 13 g kg<sup>-1</sup> was observed under NBC at 150 kg N ha<sup>-1</sup> while the lowest of 8.2 g kg<sup>-1</sup> was seen at 50 kg N ha<sup>-1</sup> under FN.

**Table 4.** Mean soil organic C for treatments plus the associated contrasts between N fertilizer and biochar-N combinations at Efaw and Lake Carl Blackwell, Stillwater, Oklahoma. 2018 and 2019.

Treatment	N Rate kg ha <sup>-1</sup>	Biochar t ha <sup>-1</sup>	Soil Organic C at Efaw				Soil Organic C at LCB			
			g kg <sup>-1</sup>				g kg <sup>-1</sup>			
			2018		2019		2018		2019	
			mean	±S.E	Mean	±S.E	Mean	±S.E	mean	±S.E
1	0	0	6.76	0.35	6.86	0.43	9.14	0.98	8.53	0.19
2	50	0	7.05	0.10	7.25	0.44	8.24	0.21	8.23	0.21
3	100	0	6.76	0.37	7.13	0.13	8.40	0.16	8.58	0.16
4	150	0	6.61	0.25	7.34	0.31	8.76	0.12	10.39	0.37
5	0	5	7.79	0.06	8.48	1.13	10.23	1.60	10.48	0.46
6	0	10	8.69	1.03	10.09	0.55	9.79	0.19	9.32	0.73
7	0	15	9.37	0.20	7.03	0.36	10.46	1.38	11.18	1.40
8	50	5	7.39	0.38	7.55	0.63	9.64	0.09	9.58	0.57
9	100	10	9.22	0.96	9.37	0.58	10.91	0.71	10.11	0.25
10	150	15	9.58	0.64	11.01	0.99	12.23	1.04	12.72	0.72
Pr > F			0.0016		0.0007		0.0758		0.0015	
C.V, %			11.8		13.2		14.9		10.9	
Contrasts			F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
2 vs. 8			0.21	0.6542	0.14	0.7147	3.50	0.0858	5.21	0.0415
3 vs. 9			10.87	0.0064	7.50	0.0180	11.22	0.0058	6.65	0.0241
4 vs. 10			15.79	0.0018	20.11	0.0007	21.51	0.0006	5.76	0.0335
2, 3 & 4 vs. 8, 9 & 10			19.92	0.0008	19.24	0.0009	28.27	0.0002	17.58	0.0012

C.V, coefficient of variation between treatments; S.E, standard error for replicated means ( $\pm$ SE,  $n = 3$ ). Nitrogen fertilizer was applied as urea ammonium nitrate—UAN (28:0:0). Biochar was applied immediately following UAN and incorporated to a depth of 15 cm.

### 3.4. Total Soil N

The ANOVA at Efaw, did not show any significant difference ( $p = 0.3316$ ) in TSN between treatments in 2018 (Table 5). Total soil N was 14% lower under NBC than observed under FN at 50 kg N ha<sup>-1</sup>. At 100 and 150 kg N ha<sup>-1</sup>, TSN was higher under NBC than FN by 6 and 5%, respectively. In 2019, similar observations were made where no significant difference ( $p = 0.6854$ ) in TSN among treatments (Table 5). At 50 kg N ha<sup>-1</sup>, TSN was higher under NBC than FN by 10%.

**Table 5.** Mean total soil N for treatments plus the associated contrasts between N fertilizer and biochar-N combinations at Efaw and Lake Carl Blackwell, Stillwater, Oklahoma. 2018 and 2019.

Treatment	N Rate kg ha <sup>-1</sup>	Biochar t ha <sup>-1</sup>	Total Soil N at Efaw				Total Soil N at LCB			
			g kg <sup>-1</sup>				g kg <sup>-1</sup>			
			2018		2019		2018		2019	
			mean	±S.E	Mean	±S.E	mean	±S.E	mean	±S.E
1	0	0	0.71	0.01	0.79	0.04	0.80	0.03	0.79	0.03
2	50	0	0.82	0.06	0.74	0.05	0.76	0.04	0.77	0.04
3	100	0	0.70	0.05	0.80	0.06	0.75	0.08	0.75	0.02
4	150	0	0.68	0.01	0.73	0.04	0.78	0.00	0.81	0.06
5	0	5	0.78	0.02	0.79	0.04	0.73	0.03	0.85	0.03
6	0	10	0.72	0.04	0.81	0.04	0.84	0.04	0.77	0.01
7	0	15	0.73	0.01	0.72	0.03	0.78	0.04	0.87	0.00
8	50	5	0.71	0.02	0.83	0.04	0.82	0.05	0.81	0.05
9	100	10	0.75	0.04	0.77	0.04	0.82	0.03	0.84	0.03
10	150	15	0.71	0.06	0.80	0.05	0.82	0.05	0.78	0.02
Pr > F			0.3316		0.6854		0.6466		0.2424	

Table 5. Cont.

Treatment	N Rate kg ha <sup>-1</sup>	Biochar t ha <sup>-1</sup>	Total Soil N at Efaw				Total Soil N at LCB			
			g kg <sup>-1</sup>				g kg <sup>-1</sup>			
			2018		2019		2018		2019	
C.V, %			8.8		9.7		9		7.2	
Contrasts			F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
2 vs. 8			2.77	0.1221	1.62	0.2268	0.85	0.3740	0.39	0.5462
3 vs. 9			0.49	0.4954	0.16	0.6990	1.39	0.2609	2.63	0.1309
4 vs. 10			0.30	0.5921	1.32	0.2730	0.37	0.5533	0.38	0.5512
2, 3 & 4 vs. 8, 9 & 10			0.06	0.8170	1.37	0.2647	2.45	0.1432	0.88	0.3654

C.V, coefficient of variation between treatments; S.E, standard error for replicated means ( $\pm$ SE,  $n=3$ ). Nitrogen fertilizer was applied as urea ammonium nitrate—UAN (28:0:0). Biochar was applied immediately following UAN and incorporated to a depth of 15 cm.

At LCB, results for 2018 did not show any significant difference ( $p = 0.64$ ) in TSN between treatments (Table 5). At each fertilizer rate, single degree of freedom contrast did not indicate significant difference in TSN between NBC and FN ( $p = 0.143$ ). Total soil N averaged 0.76, 0.75, and 0.78 g kg<sup>-1</sup> for FN and 0.82, 0.82, and 0.82 g kg<sup>-1</sup> for NBC at 50, 100, and 150 kg ha<sup>-1</sup>, respectively. Similar observations were made in 2019 where ANOVA did not indicate significant difference ( $p = 0.2424$ ) in TSN between treatments (Table 5). Total soil N averaged 0.77, 0.75, and 0.81 g kg<sup>-1</sup> for FN and 0.81, 0.84, and 0.78 g kg<sup>-1</sup> for NBC at 50, 100, and 150 kg ha<sup>-1</sup>, respectively.

#### 4. Discussion

##### 4.1. Soil Inorganic N

The soil NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N content were both improved with biochar application. Differences in both soil NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N content were seen between experimental years. In 2018, significantly higher soil NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N content were observed than in 2019. This could be due to high amount of rainfall received in 2019 that could have caused substantial leaching of inorganic N compared to 2018. Overall, NO<sub>3</sub><sup>-</sup>-N across sites and years increased by 8.8% while NH<sub>4</sub><sup>+</sup>-N increased by 4.8% with biochar application. Similar observations were made by Yao et al. [31] who reported significant increase in NO<sub>3</sub><sup>-</sup>-N (34%) and NH<sub>4</sub><sup>+</sup>-N (35%) following biochar application. In addition, Singh et al. [7] observed up to 94% more soil NH<sub>4</sub><sup>+</sup>-N under biochar amendment than in the untreated plot. It is important to note that most of the studies that report high proportion of retained inorganic N were soil column leaching experiment compared to the current study that was conducted under field conditions as demonstrated by Libutti et al. [32]. In the current study, positive effects of biochar application in increasing the availability of NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N were observed at 10 and 15 t ha<sup>-1</sup>. With 20 t ha<sup>-1</sup> of wood biochar, Gao et al. [33] observed NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N recovery of 33 and 53%, respectively, under field conditions. In an attempt to offer explanations, Zheng et al. [34] indicated the increase in soil water holding capacity, NH<sub>4</sub><sup>+</sup>-N adsorption, and enhanced N immobilization as the main reasons for the increase in recovery of fertilizer-N following biochar soil application. Indeed, increasing the capacity of the soil to hold water increases chances of retaining both NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N within soil solution [35]. The enhanced adsorption of NH<sub>4</sub><sup>+</sup>-N has been attributed to increase in CEC as a result of the oxidation of aromatic C and formation of carboxyl groups [36]. Lawrinenko and Laird [25] reported an increase in the anion exchange capacity (AEC) of biochar, which reduces leaching of anionic nutrients. They explained that the increased AEC is due to the formation of oxonium functional group (–O<sup>+</sup>) and non-specific proton adsorption by condensed aromatic rings. Therefore, the rate at which biochar increases the availability of NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N is largely dependent on specific biochar production conditions.



#### 4.2. Soil Organic C

The SOC was significantly increased with biochar application. The general ANOVA did not show significant difference in SOC content between experimental years. However, differences in SOC content were observed between experimental sites. Higher SOC content was observed at LCB with a coarse textured sandy loam soil compared to Efaw with silty clay loam. Results averaged across experimental sites and years indicate a 19.3% increase in SOC under biochar soil amendment. The significant impact of biochar on SOC have been well documented [21,25,37]. For instance, Liu et al. [38] observed as high as 40% increase in SOC under biochar treatment. At just 8 t ha<sup>-1</sup> of biochar derived from wheat straw, Zhang et al. [39] observed 34–80% increase in SOC. Similarly, Gao et al. [33] reported that wood biochar application increased SOC by 33% at 20 t ha<sup>-1</sup> under tropical soils. Soil organic C increased at all biochar rates used in this study. The apparent and perhaps obvious reason for the increased SOC under biochar soil amendment is the fact that biochar contains high proportion of C by weight compared to other elements. In this study, the pine wood biochar used contained 87% organic C. Indeed, application of material with such high organic C content will certainly increase the SOC of the amended soil. With other factors constant, this implies that the increase in SOC following biochar application is dependent on the rate of biochar application. This notion is consistent with observation in the current study where SOC increased with biochar application rate. In addition, biochar is also known to persist in soil for a long period of time. In the latter case, some researchers have presented evidence on the stability of biochar in the soil and suggested its application as a strategy for soil C sequestration [40,41]. Therefore, application of biochar in agricultural soil are important both from the agronomic and environmental perspectives.

#### 4.3. Total Soil N

Overall, TSN content was not significantly improved following biochar application. However, significant difference was observed between experimental years. The TSN content in 2018 across treatments was lower than observed in 2019, probably due to the cumulative effect since treatments were applied to the same exact plots as in 2018. The TSN content observed at LCB was higher than that at Efaw. This is due to differences in soil type. Biochar effect was significant at the LCB site with a sandy loam soil but not at Efaw with silty clay loam. Across sites and years, an overall observed increase in TSN under biochar soil amendment was 3.7%. This finding is similar to the observations by Agegnehu et al. [25], using waste willow wood (*Salix* spp.) as biochar feedstock. Significant differences between TSN of FN treatment and NBC were not seen. The non-significant response of TSN to biochar application in the above scenarios is probably attributed to limited N in biochar from woody sources, and that was insufficient to support TSN increase within experimental periods and rates used in these studies. Total soil N is a quantity that builds up in soil over a period of time. To illustrate this viewpoint, Omara et al. [42] observed a significant trend in buildup of TSN in a long-term experiment where fertilizer N was applied on a yearly basis. Therefore, the element of time and rate of application, alongside N content of biochar, is paramount in explaining the behavior of TSN following biochar application. Contrary to these findings, Uzoma et al. [21] observed significant increase in TSN using dry cow manure biochar at similar rates as in the current study. Using dry cow manure biochar could have resulted to this significant difference in TSN buildup as compared to biochar from woody sources within the rates used in this study. With hard-wood biochar, Prommer et al. [16] reported significant increase in TSN following biochar application in combination with inorganic N compared to inorganic N alone. They could have seen the positive results due to high rate of biochar applied with up to 72 t ha<sup>-1</sup> compared to only 15 t ha<sup>-1</sup> as the maximum application rate of biochar used in the current study. The authors alluded that application of inorganic fertilizer-N in combination with biochar compensate for the reduction in organic N mineralization. Therefore, application rate and nature of biochar seems to play an important role in determining the rate of TSN increase following biochar application.

## 5. Conclusions

The study used postharvest soil samples taken from fields following maize crop five months after biochar application to compare the effect of FN and NBC on soil  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, SOC, and TSN. It was hypothesized that greater recovery of inorganic N and SOC would be observed under NBC compared to NF following harvest of maize. Generally, results averaged over sites and years showed some advantages of applying NBC as opposed to FN where  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, SOC, and TSN increased under the combination by 4%, 20%, 9%, and 5%, respectively. Positive impact of the combination was realized with 10 and 15 t ha<sup>-1</sup> of biochar. The SOC,  $\text{NO}_3^-$ -N, and  $\text{NH}_4^+$ -N increased with increase in fertilizer rates. Whereas biochar improved SOC at both sites, significant response of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N availability to biochar application were only observed at LCB site with sandy loam soil but not at Efaw with silty clay loam. Thus, the application of biochar in combination with fertilizer-N improves N availability with the potential to increase crop N uptake on coarse textured soils compared to soils with fine texture. Therefore, given the pivotal role of C and N in soil quality, this study shows that application of a combination of biochar and inorganic N could be important in the future management of soils which are inherently poor with texture-related limitations. Since the current study did not specifically evaluate the retentive capacity of biochar, future study could estimate the actual adsorption capacity by analyzing the CEC, AEC, and base saturation of soils treated with biochar.

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