Assessing Nitrogen Cycling in Corncob Biochar Amended Soil Columns for Application in Agricultural Treatment Systems

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Abstract: Biochar soil amendment to agricultural systems can reduce nitrogen (N) leaching; however, application to agricultural nitrogen treatment systems has not been extensively explored. The objective of this study was to assess the impact on N leaching in soils receiving repeated N applications which may be observed in agricultural treatment systems. In this study, 400 °C, 700 °C, and oxidized 700 °C corncob biochar was amended to sandy loam soil columns at 5% (wt/wt) to assess the impacts to N cycling following repeated synthetic N applications. Columns received weekly applications of either organic N (ORG-N), ammonium (NH$_4^+$-N), or nitrate (NO$_3^-$-N) and the N effluent, gaseous emissions, and soil N retention was measured. Biochar produced at 400 °C significantly reduced N leaching compared to control columns by 19% and 15% for ORG-N and NH$_4^+$-N, respectively, with application concentrations similar to silage bunker runoff. For NO$_3^-$-N applications, 700 °C biochar significantly reduced leaching by 25% compared to the controls. The primary mechanism reducing N effluent for biochar amended columns was enhanced soil retention of ORG-N and NO$_3^-$-N. Biochar surface chemistry analysis measured an increase in oxygenated functional groups and cationic minerals on the biochar surface, which likely enhanced retention through cationic bridging or the development of an organomineral layer on the biochar surface. Results indicated biochar amendment to agricultural treatment systems receiving N runoff may reduce the risk of N leaching.

Keywords: biochar; agriculture runoff; nitrate leaching

1. Introduction

Biochar is a carbon (C)-rich byproduct of pyrolysis, in which biomass is heated in the absence of oxygen. Biochar generally has a large surface area, alkaline pH, high water holding capacity, and a high cation exchange capacity [1,2], but properties can vary greatly depending on pyrolysis conditions. Biochar has typically been used as a soil amendment to improve soil nutrient availability, water holding capacity, enhance plant-microbe interactions, reduce nutrient leaching, and sequester carbon [3].

Multiple studies have shown that biochar amendment to agricultural soils can reduce nitrogen (N) leaching [4–12] and gaseous emissions [13,14]. Laboratory column studies have found biochar can reduce nitrate (NO$_3^-$) and ammonium (NH$_4^+$) subsurface leaching following synthetic N fertilizer application from 5% to 37% and 15% to 35%, respectively [4–10]. Additionally, biochar amended soils receiving manure applications in column studies have reported reductions in total N (TN) ranging from 11% to 59% [11,12]. Similar results have been reported in field studies [8,15–21], highlighting the potential use of biochar to better manage N leaching in agricultural soil systems.

The mechanisms for the reduction in soil N leaching following biochar amendment is not well established. Studies have suggested that biochar amendment may reduce mineralization of ORG-N [22],
possibly through ORG-N binding [23], preventing conversion to mobile inorganic N species, which are prone to leaching. However, others have suggested the additional C from biochar can enhance mineralization rates, with increases ranging from 40% to 220% [9,24]. Following mineralization, N leaching may be reduced through NH$_4^+$ binding to biochar as a result of the sorption [10,25,26]. Biochar’s ability to bind and immobilize NH$_4^+$ may also prevent nitrification, thereby reducing NO$_3^-$ concentrations and leaching [27]. However, some incubation studies have found biochar may increase nitrification rates [24,28] potentially due to the increase in soil pH. Additionally, multiple field studies have contradicted NH$_4^+$ binding as the primary mechanism for reduction in N leaching, as soil extractions have identified NO$_3^-$ to be the primary inorganic N species in biochar-amended soils [16,18–20,29,30]. Laboratory studies have found low NO$_3^-$ sorption capacities for unprocessed biochar [31–34]. Thus, NO$_3^-$ bound to biochar amended soil suggests that biochar properties are altered following soil amendment. Proposed mechanisms for this phenomenon include abiotic and biotic oxidation altering surface functional groups, development of complex organomineral layers, or unconventional hydrogen bonding [29,35,36]. Additionally, biochar has been shown to impact gaseous emissions of nitrous oxide (N$_2$O), which may be related to NO$_3^-$ binding in the soil, or an increase in soil pH due to biochar buffering capacity [13,14,16,37,38].

While most studies focus efforts on examining impacts of biochar amendment to agricultural cropping systems, there is potential to integrate biochar into agricultural treatment systems. Farmstead runoff from silage bunker pads or feedlots have high N concentrations [39–41] posing risks to water quality. Producers often use treatment systems to reduce N losses to surface water, such as vegetative treatment areas, which promote infiltration [42]. However, there is growing evidence that many agricultural treatment systems aimed at reducing runoff losses may not adequately address issues related to groundwater contamination, particularly from NO$_3^-$ leaching [19,43–45]. Biochar may be a suitable amendment to reduce NO$_3^-$ leaching through these vegetative treatment areas to reduce groundwater impacts. However, more information on the impact of N leaching when biochar is integrated in soil based treatment systems is needed to improve integration strategies.

2. Materials and Methods

The objectives of this study are to assess the impact of repeated N applications on N soil retention, leaching and gaseous emissions from biochar amended soil columns. To better understand these impacts, different N forms were applied to soil columns at varying rates. The N retained in biochar-amended soils, N leached through the columns, and the N$_2$O and ammonia (NH$_3$) emissions were quantified over ten weeks.

2.1. Soil Characteristics

An engineered soil was obtained from a local landscaping company in Madison, WI, USA. The soil was a sandy loam with 68% sand, 21% silt, and 11% clay [46]. The soil had a pH of 6.3 and a N, phosphorus (P), and potassium (K) content of 0.66, 0.20, and 0.33 g kg$^{-1}$, respectively.

2.2. Biochar Production and Properties

Corncob biochar was produced by Biochar Options, LLC at a production temperature of 400 °C (BC$_1$) and 700 °C (BC$_2$) for a holding time of one hour under oxygen limiting conditions. Following production, biochar was crushed and sieved through a number ten mesh sieve (pore size 2 mm). Following production, a portion of the 700 °C biochar was then oxidized (BC$_3$), as oxidized biochar has shown enhanced NO$_3^-$ sorption. Biochar was oxidized by suspending a 2.5% biochar to a liquid solution of 6 mmol g$^{-1}$ sodium bromide (NaBr) and 50 mmol g$^{-1}$ sodium hypochlorite (NaClO). The pH was adjusted to 11 using hydrochloric acid (HCl) and agitated for six hours at room temperature [36]. The solution was then filtered, and biochar was washed five times by submerging in deionized water (DI) for 10 min followed by vacuum filtration. BC$_3$ was then sieved through a number ten mesh sieve (pore size 2 mm).
Biochar was characterized for elemental content and pH (Table S1). Carbon, hydrogen (H), and N was determined following ASTM D5373 using a PerkinElmer Elemental Analyzer (PerkinElmer, Waltham, MA). Oxygen content was determined by subtracting C, H, N, ash (via ASTM D1102), and moisture (via ASTM E871). The pH was measured using a 1:20 ratio of biochar to DI suspension and measuring pH with a HACH HQ440d Benchtop Multi Meter.

2.3. Experimental Design

Columns were constructed out of 3.8 cm diameter PVC pipe with a height of 36 cm. A fiberglass screen filter was attached to the bottom of the column. On top of the screen, 100 g of acid washed pea gravel (2–4 mm) was added. Columns were then packed with 300 g of soil, or for those columns containing biochar treatments, a 5% (wt/wt) biochar to soil mixture, which was incorporated into the entire depth of the soil column. Biochar and soil was mixed in 50 g increments by adding soil and biochar to plastic bottle and inverting several times. Columns were filled by adding 50 g of soil and then compacting with a 125 g weight dropped from a height of 15 cm two times and repeated until 300 g of soil mixture was added. Four column treatments included the control (sandy loam soil), 5% BC\textsubscript{1} amended, 5% BC\textsubscript{2} amended, and 5% BC\textsubscript{3} amended (Table 1). Columns were placed in a climate-controlled room at a temperature of 20 °C and relative humidity of 50%.

Table 1. Study experimental design setup, weekly application concentrations, weekly application rates, total mass loading, and average column soil bulk density.

| Treatment Group | N Applied | Concentration (mg N L\textsuperscript{-1}) | Rate (kg N ha\textsuperscript{-1}) | Total Mass Loading (mg N) | Treatment | Bulk Density (g cm\textsuperscript{-3}) |
|----------------|-----------|------------------------------------------|---------------------------------|--------------------------|-----------|-------------------------------------|
| 1              | ORG-N     | 150                                      | 198                             | 225                      | Control   | 1.70 ± 0.01                          |
|                | Low       |                                          |                                 |                          | 2.5% BC\textsubscript{1} | 1.39 ± 0.04 |
| 2              | ORG-N     | 2500                                     | 3306                            | 3750                     | Control   | 1.71 ± 0.03                          |
|                | High      |                                          |                                 |                          | 2.5% BC\textsubscript{1} | 1.39 ± 0.03 |
| 3              | NH\textsubscript{4}\textsuperscript{+}     | 50                                       | 66                              | 75                       | Control   | 1.64 ± 0.08                          |
|                | Low       |                                          |                                 |                          | 2.5% BC\textsubscript{1} | 1.42 ± 0.02 |
| 4              | NH\textsubscript{4}\textsuperscript{+}     | 1250                                     | 1653                            | 1875                     | Control   | 1.72 ± 0.05                          |
|                | High      |                                          |                                 |                          | 2.5% BC\textsubscript{1} | 1.41 ± 0.01 |
| 5              | NO\textsubscript{3}\textsuperscript{-}     | 20                                       | 26                              | 30                       | Control   | 1.69 ± 0.03                          |
|                |           |                                          |                                 |                          | 2.5% BC\textsubscript{1} | 1.38 ± 0.01 |
|                |           |                                          |                                 |                          | 2.5% BC\textsubscript{2} | 1.39 ± 0.01 |
|                |           |                                          |                                 |                          | 2.5% BC\textsubscript{3} | 1.53 ± 0.11 |

Soil columns were grouped into five trials where each group received different N applications (Table 1). Treatment group one consisted of a control and 5% BC\textsubscript{1} receiving repeated applications of low ORG-N blend (150 mg ORG-N L\textsuperscript{-1}), similar to concentrations in silage bunker runoff [41]. Treatment group two consisted of control and 5% BC\textsubscript{1} receiving repeated high ORG-N applications (2500 mg ORG-N L\textsuperscript{-1}), similar to concentrations in manure [11]. The ORG-N solution in treatment groups one and two were made up of 50% arginine, 40% glycine, and 10% alanine, which are abundant ORG-N forms in manure [47]. Group three consisted of a control and 5% BC\textsubscript{1} receiving repeated low NH\textsubscript{4}\textsuperscript{+}-N applications (50 mg NH\textsubscript{4}\textsuperscript{+}-N L\textsuperscript{-1}), similar to concentrations in silage bunker runoff [41]. Treatment group four consisted of a control and 5% BC\textsubscript{1} receiving repeated high NH\textsubscript{4}\textsuperscript{+}-N applications (1,250 mg NH\textsubscript{4}\textsuperscript{+}-N L\textsuperscript{-1}), similar to concentrations in manure [11]. Group five consisted of control, 5% BC\textsubscript{1}, 5% BC\textsubscript{2}, and 5% BC\textsubscript{3} treatments receiving repeated NO\textsubscript{3}\textsuperscript{-}-N applications (20 mg NO\textsubscript{3}\textsuperscript{-}-N L\textsuperscript{-1}), similar to tile drainage effluent [48]. More treatments were present in group five to identify if production temperature or oxidation impacted NO\textsubscript{3}\textsuperscript{-} leaching. The NH\textsubscript{4}\textsuperscript{+}-N and NO\textsubscript{3}\textsuperscript{-}-N solutions were prepared using ammonium chloride (NH\textsubscript{4}Cl) and sodium nitrate (NaNO\textsubscript{3}), respectively. Solutions ionic strength was adjusted to be representative of manure, silage bunker runoff, or tile drainage effluent by adding monopotassium phosphate (KH\textsubscript{2}PO\textsubscript{4}). Target ionic strengths were estimated by using electrical
conductivity values reported for manure (17.00 ms cm\(^{-1}\)) [11], silage bunker runoff (1.24 ms cm\(^{-1}\)) [41], and tile drainage effluent (0.38 ms cm\(^{-1}\)) [48] and applying the Snoeyink and Jenkins [49] relationship to determine ionic strength. The amount of KH\(_2\)PO\(_4\) added to the solution was then determined using Equation (1):

\[
I = \frac{1}{2} \sum C_i Z_i^2
\]  

where \(I\) is the ionic strength, \(C_i\) is the molar concentration (M) of an ion, and \(Z_i\) is the charge number.

Solutions were applied at a volume of 150 mL or 13.2 cm, which corresponds to a N application rate for groups one, two, three, four, and five of 198, 3306, 66, 1653, and 26 kg N ha\(^{-1}\), respectively (Table 1). Applications occurred once a week (seven days between applications) for ten weeks. During application, columns were allowed to drain completely which typically took between one to two hours. Following EPA guidelines, samples were immediately analyzed for pH and preserved and stored at 4 °C (no longer than 28 days). Samples were analyzed for total phosphorus (TP), total Kjeldahl nitrogen (TKN), ammoniacal nitrogen (TAN), nitrates (NO\(_3^−\)+NO\(_2^−\)-N), and nitrite (NO\(_2^−\)-N) following USEPA approved methodology (Table S2).

A dynamic flow through chamber was used to measure NH\(_3\) emissions from the soil. The column walls served as the chamber anchor and a PVC cap with inlet and outlet ports served as the lid. The lid was sealed to the column using rubber stripping and hose clamps. A SKC PCXR4 Universal Sample Pump (SKC, Eighty Four, PA) pumped ambient air into the chamber at a flow rate of 250 mL min\(^{-1}\). Emissions were then recorded every 15 s for 5 min using a MAS 5X gas monitor (Mine Safety Appliances, Cranberry Township, PA). All connection tubing was Teflon to minimize chemical reactions. Measurements were taken 1, 6, 24, and 72 h after weekly applications. Flux of NH\(_3\) [50] was determined following Equation (2):

\[
J = \frac{Q(C_{eq} - C_i)}{A}
\]

where \(J\) is the NH\(_3\) flux (mg NH\(_3\)-N m\(^{-2}\) s\(^{-1}\)), \(Q\) is the dynamic chamber flow rate (m\(^3\) s\(^{-1}\)), \(C_{eq}\) is the equilibrium concentration (mg NH\(_3\) m\(^{-3}\)), \(C_i\) is the initial NH\(_3\) concentration (mg NH\(_3\) m\(^{-3}\)), and \(A\) is the soil surface area (m\(^2\)). Total loss gas (loss due to chamber or sample lines) was determined to be negligible. Cumulative emissions were determined using fluxes and multiplying by the time between sampling points.

A static chamber was used for N\(_2\)O emissions following GRACEnet protocol [51]. The column walls served as the anchor and the lid was a PVC cap with a septum installed. The PVC lid was sealed using the same method as the dynamic chamber. Sample collection took place once a week 24 h after nutrient application. Gas was measured by sampling headspace gas at 12-min intervals for 36 min. Glass 5.9 mL Exetainer vials (Labco Limited, Buckinghamshire, UK) were overcharged with 10 mL gas samples. Gas concentrations were measured by gas chromatography with a 15 mCi 63Ni electron capture detector for N\(_2\)O (Agilent 7890A GC system). Flux of N\(_2\)O was determined following Equation (3) [51]:

\[
F = \frac{(S - V_c)}{A}
\]

where \(F\) is the N\(_2\)O flux (mg N\(_2\)O-N m\(^{-2}\) h\(^{-1}\)), \(V_c\) is the chamber volume (m\(^3\)), \(A\) is the soil surface area (m\(^2\)), and \(S\) is the slope of change in concentration over time (mg N\(_2\)O m\(^{-2}\) h\(^{-1}\)), which was calculated based on the concentration linear regression. Cumulative emissions were determined using fluxes and multiplying them by the time between sampling points. Using a mass balance approach, any unaccounted influent N (i.e., influent N subtracted by effluent N, retained N, N\(_2\)O, and NH\(_3\)) was assumed to be lost as N\(_2\) through denitrification.
2.4. Soil and Biochar Analysis

Following ten weeks of nutrient applications, soil was removed from each column. Soil was dried at 50 °C for 48 h [52], ground to pass through a 2mm sieve, and stored at 4 °C until analysis. Soil pH was determined with a HACH HQ440d Benchtop Multi Meter pH probe after diluting soil 1:2.5 by mass with DI [53]. Extractable NO$_3^-$-N and NH$_4^+$-N were determined using a modified KCl extraction method, as past studies have reported standard KCl soil extractions underestimate biochar NO$_3^-$-N content [30,35,54]. It has been suggested that KCl extractions for biochar amended soils be conducted at higher temperatures (>50 °C) for a long duration to ensure extraction efficiency [35]. Methods outlined in Walsh et al. [55] were followed for inorganic N extractions. Soil samples were suspended with 2 M KCl at a ratio of 1:10 (wt/vol) and placed on a temperature-controlled shaker at 50 °C and 100 rpm for 24 h. Suspensions were then vacuum filtered through 1.5 µm filter paper and collected. The soil was rinsed twice by pouring 25 mL of DI over the biochar and vacuum filtering. Filtrate was preserved and stored at 4 °C (no longer than 28 days) until analyzed for NO$_3^-$NO$_2^-$-N and TAN following EPA approved methods (Table S2). Additionally, soil samples were analyzed for TKN using a copper catalyst digestion method [56].

A portion of biochar was removed from the soil and analyzed for changes in surface functional groups. Fourier-transform infrared spectroscopy (FTIR) spectra were analyzed with a PerkinElmer Spectrum 100 FT-IR Spectrometer (PerkinElmer, Waltham, MA) using potassium bromide (KBr) pellets containing 0.3% of finely ground biochar [57]. Spectra were obtained from 550 to 4000 cm$^{-1}$ over 100 scans with a resolution of 4 cm$^{-1}$. To illicit potential nitrogen binding mechanisms X-ray photo-electron spectroscopy (XPS) analyses using a Thermo K-Alpha X-ray Photoelectron Spectrometer (Thermo Scientific, Waltham, MA) was used. Survey spectra were scanned from 1350 to 0 eV binding energy range and recorded with a pass energy of 50 eV over 20 scans. Thermo Avantage version 5.9 was used for peak identification and fitting.

2.5. Data Analysis

Analysis was performed in SAS [58]. Repeated measures (Proc Mixed) using first-order auto-regressive variance-covariance structure was used to determine statistical differences of cumulative effluent nutrient content between treatments. One-way analysis of variance (ANOVA) with Fisher’s least significant difference test (LSD) was conducted to compare influent and effluent nutrient concentrations for each event for water quality parameters and soil N content following applications.

3. Results

3.1. Organic Nitrogen Application (Treatment Groups 1 and 2)

For weekly applications of ORG-N low (treatment group 1), the biochar amended columns significantly reduced cumulative TN leaching by 15.8% and NO$_3^-$-N leaching by 16.5% compared to the control (Figure 1A and Figure S1A). Most of the leachate was in the form of NO$_3^-$-N. The reduced leaching was because the biochar amendment significantly increased the TN retained in the soil (Figure 2A) where most of the N retained was ORG-N. The NO$_3^-$-N bound in the soil/biochar matrix was more than double the control, but was only a small fraction of the overall TN retained (Table 2). Gaseous emission losses of NH$_3$ was negligible, and N$_2$O accounted for 2.3% and 1.2% of the influent TN applied for control and biochar columns (Figure 2A), respectively, and were not statistically different. For the control and biochar-amended columns 36.2% and 24.5% of the total N applied was calculated to be lost as N emissions other than NH$_3$ or N$_2$O (Figure 2A).
Figure 1. Cumulative mass of influent and effluent nitrogen (N) (mg N) for control and biochar columns receiving (A) organic nitrogen (ORG-N) low (treatment group 1) and (B) ORG-N high (treatment group 2) application rates. Entire bar (with no fill) represents influent total nitrogen (TN), where entire solid bar represents effluent TN. Individual N species in the effluent are denoted based on color (blue, red, or green). Error bars represent standard deviation of effluent TN between triplicate columns for treatment. Letters signify statistically significant difference between treatments.

Figure 2. Nitrogen mass balance for influent TN for columns receiving (A) ORG-N low (treatment group 1) and (B) ORG-N high (treatment group 2) application rates. *Assumed that influent TN unaccounted for in effluent N, retained N, N₂O, or NH₃ was assumed to be lost as N₂.
Table 2. Change in soil N for ORG-N low (treatment group 1), ORG-N high (treatment group 2), NH₄⁺-N low (treatment group 3), NH₄⁺-N high (treatment group 4) and NO₃⁻-N (treatment group 5) after 10 weeks of nutrient application. Values are means (standard deviation) of triplicates. Different letters represent significant difference (p < 0.05) between soil N within application group.

| Application + Treatment | NH₄⁺-N (mg N) | NO₃⁻-N (mg N) | TKN (mg N) | ORG-N † (mg N) | TN (mg N) |
|-------------------------|---------------|----------------|------------|----------------|----------|
| ORG-N Low Control       | 10.52 (1.28)  | 0.91 (1.10)    | 0.60 (8.41) | -9.91 (7.75)   | 1.51 (9.50) |
| BC1                     | 10.52 (2.19)  | 6.76 (1.00)    | 63.64 (27.00) | 53.13 (26.24)  | 55.21 (26.39) |
| ORG-N High Control      | 180.61 (9.60) | -3.65 (0.07)   | 294.32 (3.59) | 113.70 (6.20)  | 290.66 (3.54) |
| BC1                     | 217.15 (17.68) | -4.17 (1.04)  | 360.04 (46.66) | 142.89 (60.80) | 326.47 (16.26) |
| NH₄⁺-N Low Control      | 2.42 (1.51)   | -0.40 (0.71)   | 0.50 (0.83)  | -2.89 (3.09)   | 0.10 (0.26)  |
| BC1                     | 8.92 (3.22)   | 3.58 (0.27)    | 8.52 (2.21)  | -0.41 (1.08)   | 12.09 (2.44) |
| NH₄⁺-N High Control     | 150.76 (13.98) | 17.54 (5.89)   | 107.40 (3.09) | -43.36 (11.40) | 124.94 (7.95) |
| BC1                     | 132.51 (6.49) | 91.85 (8.47)   | 116.82 (8.98) | -15.69 (11.70) | 208.68 (17.36) |
| NO₃⁻-N Control          | 1.98 (0.72)   | -2.25 (0.12)   | -6.99 (4.24) | -8.96 (3.56)   | -9.24 (4.13) |
| BC1                     | 9.98 (3.99)   | -2.79 (0.08)   | -4.73 (3.79) | -14.71 (5.07)  | -7.52 (3.76) |
| BC2                     | 5.56 (1.45)   | 6.73 (0.94)    | 2.61 (1.55)  | -2.95 (3.00)   | 9.34 (2.04)  |
| BC3                     | 7.62 (0.74)   | 0.26 (0.76)    | -11.74 (2.12) | -19.35 (2.69)  | -11.48 (1.79) |

Different letters signify significant difference (p < 0.05) between control and biochar treatments for a specific parameter within a treatment group. † Data presented in tables is the change in soil N content or the final soil N content subtracted by the initial soil N content. Negative values indicate that species of N in the soil was converted or lost over the study period. ‡ Calculated from ORG-N = TKN – NH₄⁺-N.

For ORG-N high applications (treatment group 2), that were designed to be similar to manure concentrations, there was no significant difference between the two treatments for cumulative TN leaching (Figure 1B). The soil TN increased for both treatments, primarily in the form of NH₄⁺-N and ORG-N, but treatments were not significantly different (Table 2). Emissions of N₂O were negligible; however cumulative NH₃ emissions accounted for 2.5% and 1.1% of the influent TN for the control and biochar columns, respectively (Figure 2B). Total influent N lost as emissions other than N₂O or NH₃ for control and biochar columns was 19.6% and 22.4%, respectively (Figure 2B).

3.2. Ammonium Applications (Treatment Groups 3 and 4)

Following weekly applications of NH₄⁺-N low application (treatment group 3), that were designed to be similar to silage bunker runoff concentrations, cumulative TN in leachate was significantly lower for the biochar amended columns (Figure 3A). Most of the leachate was in the form of NO₃⁻-N. Biochar amendment significantly reduced NO₃⁻-N leaching by 14.6% compared to the control (Figure 3B). Biochar amendment resulted in a significant increase in soil TN retention (Figure 4A). BC₁ amended soils had significantly greater NO₃⁻-N retention than the control soil (Table 2). Emissions as N₂O accounted for 1.7% and 2.0% of the losses of the influent TN applied for control and biochar columns (Figure 4A). Nitrogen calculated to be lost as emissions other than N₂O or NH₃ for control and biochar columns was 11.9% and 10.4%, respectively.

Following NH₄⁺-N high application (treatment group 4), designed to be similar to manure land applications, biochar columns significantly decreased TN leaching compared to the control (Figure 3B). There was not a significant difference between TAN in leachate between treatments. Biochar columns reduced NO₃⁻-N leaching significantly by 25% (Figure 3C). Biochar amendment significantly increased the TN retained in the soil compared to the control (Figure 4B). There was not a significant difference in soil NH₄⁺-N retention between treatments, but biochar amended soils significantly increased the retention of NO₃⁻-N and reduced the loss of the ORG-N originally contained within the soil (Table 2). Emissions from the columns as NH₃ were negligible. N₂O emissions accounted for 0.7% and 0.4% of the cumulative influent N for control and biochar columns, respectively. Based on the TN applied to the columns over the study, N lost as emissions other than N₂O and NH₃ was calculated to be 6.8% and 9.4% for control and biochar columns, respectively.
Figure 3. Cumulative mass of influent and effluent N (mg N) for control and biochar columns receiving (A) NH₄⁺-N low (treatment group 3), and (B) NH₄⁺-N high (treatment group 4) application rates. Error bars represent standard deviation of effluent TN between triplicate columns for treatment. Letters signify statistically significant difference between treatments. Note: Entire bar (with no fill) represents total influent TN, where entire solid bar represents TN in effluent. Individual N species in the effluent are denoted based on color (blue, red, green).

Figure 4. Nitrogen mass balance for influent N for columns receiving (A) NH₄⁺-N low (treatment group (3) and (B) NH₄⁺-N high (treatment group (4) application rates. * Assumed that influent TN unaccounted for in effluent N, retention N, N₂O, or NH₃ was assumed to be lost as N₂.
3.3. Nitrate Application (Treatment Group 5)

For columns receiving weekly applications of NO$_3^-$-N (treatment group 5), BC$_2$ and BC$_3$ reduced the NO$_3^-$-N leaching by 24.8% and 8.8% compared to the control column, respectively. There was no significant difference between control and BC$_1$ (Figure 5A). The soil NO$_3^-$-N concentrations significantly increased for BC$_2$ compared to other treatments (Table 2), and the mass retained accounted for the entire difference between N leached through BC$_2$ versus the influent. For BC$_2$, 7.9% of the total influent N applied was lost as N emission other than NH$_3$ and N$_2$O. All biochar treatments significantly reduced N$_2$O emissions compared to control columns, but there was no significant difference between biochar treatments (Figure 5B).

![Figure 5](image)

Figure 5. Cumulative (A) NO$_3^-$-N leached and (B) N$_2$O emitted from columns receiving NO$_3^-$-N applications. Different letters signify significant difference ($p < 0.05$) between control and biochar treatments. Error bars represent standard deviation between treatment triplicates.

3.4. Changes in Biochar Characteristics

The FTIR spectra measured an increase in oxygenated functional groups on the biochar surface following applications of all N forms (Figure 6). The small shoulder present in all spectra between 3500 and 3200 cm$^{-1}$ is indicative of hydroxyl bands (-OH) associated with carboxylic acids, alcohols or phenols [29,59–61]. Peaks at 2850 cm$^{-1}$ is indicative of addition of methoxyl (O-CH$_3$) to biochar. Carboxylic groups are represented by 2850, 1712, and 1437 cm$^{-1}$ [60,62]. Carbonates are represented
at 1436 and 875 cm\(^{-1}\) [63]. Carbonyl groups (C=O) are represented at 2150 and 1580 cm\(^{-1}\) [59,61]. Increase in phenolic groups are represented by peak increases at 1262 and 1380 cm\(^{-1}\) [62]. Small peaks at 2165 and 1384 cm\(^{-1}\) indicate N on the biochar. A small peak for triple bonded N at 2165 cm\(^{-1}\) is observed [64]. Nitrite bound to the biochar can be observed at 1384 cm\(^{-1}\) [64–66].

![Figure 6](image)

**Figure 6.** Fourier-transform infrared spectroscopy (FTIR) spectra of (A) BC\(_1\) initial and final for treatments 1 through 4, and (B) BC\(_1\), BC\(_2\), and BC\(_3\) initial and final for treatment group 5.

The XPS data confirmed chemical changes to biochar following soil amendment and application of N solutions. High resolution C 1s scan found a decrease in C-C and C-H bounds and an increase in oxygenated functional groups (Table 3 and Table S3). Oxygen content was roughly 1.5 times higher for
all treatments following soil amendment. Additionally, other major elements including aluminum (Al), calcium (Ca), iron (Fe), magnesium (Mg), silicon (Si), and tin (Sn) were detected on the biochar surface following soil amendment.

Table 3. X-ray photo-electron spectroscopy (XPS) scan of major elements and functional groups on the surface of BC before and after soil amendment for columns receiving ORG-N and NH\textsuperscript{4}-N low and high application rates. Value is mean (standard deviation) from triplicate columns. Significant difference (\(p = 0.05\)) between BC\textsubscript{1} initial and BC\textsubscript{1} final for treatments one through four are denoted with an asterisk (*). Elemental peaks that were not detected are signified by n.d.

| Atomic Composition (%) | BC\textsubscript{1} Initial | BC\textsubscript{1} after ORG-N Low Application (Treatment Group 1) | BC\textsubscript{1} after ORG-N High Application (Treatment Group 2) | BC\textsubscript{1} after NH\textsuperscript{4}-N Low Application (Treatment Group 3) | BC\textsubscript{1} after NH\textsuperscript{4}-N High Application (Treatment Group 4) |
|------------------------|-----------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|
| C                      | 84.79 (1.53)                | 68.13 (1.79) *                                              | 69.65 (2.17) *                                              | 72.17 (2.79) *                                              | 71.12 (3.03) *                                              |
| C-C/C-H                | 67.25 (0.72)                | 48.83 (0.49) *                                              | 47.90 (2.19) *                                              | 49.63 (3.27) *                                              | 48.03 (2.70) *                                              |
| C-O/C-OC              | 9.38 (0.43)                | 10.19 (0.66)                                                | 11.44 (0.78) *                                              | 10.88 (0.65)                                                | 11.58 (0.93) *                                              |
| C=O                   | 3.63 (0.22)                | 4.10 (0.27) **                                               | 4.70 (0.50) *                                              | 4.45 (0.30) *                                              | 5.39 (0.79) *                                              |
| O=C-C                 | 2.24 (0.15)                | 2.80 (0.70) **                                               | 3.41 (0.27) *                                              | 3.46 (0.62) *                                              | 2.91 (0.54) *                                              |
| Shake up              | 1.44 (0.36)                | 1.28 (0.52)                                                 | 1.04 (0.31)                                                | 2.53 (0.79)                                                 | 2.01 (0.14)                                                 |
| Quinone               | 0.86 (0.07)                | 0.94 (0.07)                                                  | 1.16 (0.25)                                                 | 1.21 (0.35)                                                 | 1.19 (0.30)                                                 |
| O                      | 13.49 (0.73)               | 20.89 (0.29) *                                              | 20.95 (1.60) *                                              | 19.10 (1.82) *                                              | 19.96 (1.62) *                                              |
| N                      | 1.21 (0.04)                | 1.70 (0.37) *                                               | 3.15 (0.25) *                                              | 1.66 (0.28) *                                              | 1.86 (0.11) *                                              |
| P                      | n.d.                       | 0.88 (0.19) *                                               | 1.26 (0.24)                                                | 0.21 (0.07)                                                | 0.42 (0.03) *                                              |
| Al                     | n.d.                       | 2.11 (0.35) *                                               | 0.99 (0.08)                                                | 1.74 (0.29)                                                | 1.51 (0.06)                                                 |
| Ca                     | n.d.                       | 0.63 (0.12) *                                               | 0.65 (0.07)                                                | 0.57 (0.03)                                                | 0.66 (0.04)                                                 |
| Cl                     | n.d.                       | n.d.                                                        | n.d.                                                       | 0.14 (0.04)                                                | 0.32 (0.12)                                                 |
| Fe                     | n.d.                       | 0.62 (0.20) *                                               | 0.65 (0.11)                                                | 0.40 (0.07)                                                | 0.12 (0.01)                                                 |
| Mg                     | 0.11 (0.01)                | 0.41 (0.04) *                                               | 0.27 (0.05)                                                | 0.37 (0.03)                                                | 0.51 (0.11)                                                 |
| Si                     | 0.40 (0.01)                | 4.31 (0.74) *                                               | 2.09 (0.20)                                                 | 3.35 (0.86)                                                 | 3.18 (1.13)                                                 |
| Sn                     | n.d.                       | 0.32 (0.03) *                                               | 0.35 (0.05)                                                 | 0.30 (0.06)                                                 | 0.33 (0.07)                                                 |

4. Discussion

Biochar application (BC\textsubscript{1}) had a significant reduction on N leaching for all treatments compared to the control, except for the ORG-N high (treatment group 2) and NO\textsubscript{3}-N application. For BC\textsubscript{1} amended columns with ORG-N low application (treatment group 1) TN leaching was reduced by 18.7% compared to control columns. Almost all effluent (greater than 95%) was in the form of NO\textsubscript{3}-N, resulting in 58.7% and 47.3% of the influent TN leaching through the control and BC\textsubscript{1} columns as NO\textsubscript{3}-N, respectively, suggesting that a majority of the influent N underwent mineralization and/or nitrification. Similarly, in Xu et al. [9], where urea was applied at 250 kg N ha\textsuperscript{-1} to soil amended with biochar made from corn straw, the cumulative TN leaching was reduced up to 20% by biochar amended soils compared to controls, and more than 90% of the effluent was in the form of NO\textsubscript{3}-N. At lower application rates of ORG-N, biochar is able to reduce the NO\textsubscript{3}-N lost as leachate. Weekly TN reduction efficiency averaged 17 ± 5% through the ten weeks where removal performance did not change over time. This suggests that low-temperature biochar amendment could effectively be implemented into agricultural treatment systems receiving regular application doses of ORG-N, similar to silage bunker pad runoff, to reduce N leaching to groundwater sources. When higher ORG-N applications were applied almost all of the leached N remained in the form of ORG-N, where 71.0% and 68.2% of the influent from control and biochar columns was lost as ORG-N in the effluent. In past manure leaching studies reductions in TN leaching up to 60% were measured with additions of biochar [11,12]. In this study, the applications of N at manure concentrations were applied repeatedly, resulting in much higher N applications than other studies. It is hypothesized that this likely reduced
the mineralization to inorganic N forms due to an imbalance of the C to N ratio as no additional C sources were applied. This may also suggests that the C added via biochar is not bioavailable in the soil in this timeframe.

At lower NH$_4^+$-N application concentrations (treatment group 3) TN leaching was reduced by 14.6% for biochar amended columns compared to control columns. As with the ORG-N applications, a majority of the leachate was in the form of NO$_3^-$-N (99%), suggesting a majority of the influent N underwent nitrification. Weekly repeated applications of NH$_4^+$-N were consistent in TN retention similar to the ORG-N column, where the NH$_4^+$-N low columns averaged a 13 ± 5% reduction of TN leaching from the control over the ten weeks of application. For NH$_4^+$-N high application rate (treatment group 4), TN leaching was significantly reduced by 8% when biochar was amended to the soil. Unlike the lower application rate, the effluent was a mix of both NH$_4^+$-N (84%–87%) and NO$_3^-$-N (13%–15%), which could be due to the imbalance of C to N in the soil. Other studies that have applied synthetic NH$_4^+$-N fertilizers to biochar amended soils at high application rates have found NH$_4^+$-N to make up a majority of the effluent TN [67].

In the columns receiving ORG-N and NH$_4^+$-N applications (treatment groups 1 through 4), soil N retention is the primary mechanism for reducing N leaching in biochar amended soils. In all cases where N leaching was significantly reduced, the change in TN retained in the soil was significantly higher for those amended with BC$_1$. Many studies have theorized that biochar primarily retains N though binding of NH$_4^+$ due to the increased CEC content [28,68]. However, in this study there was no significant difference in soil NH$_4^+$-N content between biochar and control columns for any of the applications. Similar results have been observed in field studies performing soil extractions following amendment, which have found that NO$_3^-$-N was more abundant than NH$_4^+$-N [16,18–20,29,30]. While there was a significant increase in soil NO$_3^-$-N between treatments when N leaching was reduced, the binding of NO$_3^-$-N in the soil did not account for the majority of the difference in leached N between control and biochar columns. The retention of ORG-N within the columns accounted for most of the difference in the N leaching suggesting it is the primary mechanism for leaching reduction. For columns receiving low ORG-N applications (treatment group 1), the BC$_1$ amended columns had a significantly higher change in soil ORG-N following applications, which accounted for most of the difference in N leaching between control and biochar columns. For the columns receiving NH$_4^+$-N applications (treatment groups 3 and 4), NO$_3^-$-N binding played the major role in retaining N within the column, but the biochar columns also retained more of the ORG-N originally in the soil. Thus, biochar may have inhibited some of the soils original ORG-N from undergoing mineralization.

Biochar increased retention of NO$_3^-$-N in the all columns receiving ORG-N and NH$_4^+$-N applications even though biochar has been reported to have a low sorption capacity for NO$_3^-$-N [31,34]. Biochar is known to undergo changes when aged in soil or composted [29,30]. Biochar properties, such as oxidation, are altered following amendment to soil that results in NO$_3^-$-N binding. The FTIR spectra in this study measured an increase in peaks related to oxygenated functional groups (Figure 6). The XPS data also measured an increase in oxygenated functional groups on the biochar surface and an increase in positively-charged minerals (particularly Al, Ca, Fe, Mg, Si, and Sn) bound to the biochar surface (Table 2). The increase in minerals and oxygenated functional groups may have resulted in cationic bridging between NO$_3^-$-N and biochar, as previously suggested [36]. However, changes in oxygenated functional groups and minerals were similar to results observed in Joseph et al. [29], which concluded that the formation of an organomineral layer on biochar surface resulted in the uptake of NO$_3^-$-N. In that study, the formation of the organomineral layer occurred on biochar that underwent composting after six weeks, thus it is plausible that the formation of organomineral layer in this ten-week study could have occurred. It is likely that both these processes are contributing to the increase in NO$_3^-$-N retention.

The enhanced retention of ORG-N within the soil columns amended with biochar may be the result of similar mechanisms of NO$_3^-$-N. Sorption of ORG-N to biochar has not been studied extensively, but studies have identified biochar as being able to bind dissolved organic C. Chun et al. [62] observed
that biochar could adsorb organic contaminates and linked the uptake to surface functional groups present on the biochar, with higher content of carboxyl groups increasing uptake. Güereña et al. [17] observed biochar amended soil in a field study had higher dissolved ORG-N content and suggested that the same surface functional mechanism that bound organic contaminates in Chun et al. [62] likely increase biochar uptake of ORG-N.

The reduction in N leaching following applications similar to silage bunker runoff are promising for future implementation into treatment systems. These systems have been reported to result in high leachate concentrations of NO$_3^-$ [44,45], thus a 16.5% (treatment group 1) or 14.6% (treatment group 3) reduction could have a significant impact on NO$_3^-$ leaching through treatment systems. However, it is important to note that this study was conducted in a laboratory setting, thus conditions were different than actual field conditions. Climate, soil texture, and influent runoff all could have impacts on treatment performance in the field, thus future studies should further evaluate biochar amendment to these treatment systems receiving N-rich silage bunker runoff.

Based on the findings from the ORG-N and NH$_4^+$-N application columns it would be expected that biochar would reduce N leaching when receiving NO$_3^-$-N. However, BC$_1$ did not significantly reduce leaching compared to control as it did in the other trials. A potential explanation may be due to retention time required for NO$_3^-$-N binding to biochar exchange sites. Past studies have indicated that significant retention time (>1 hr) is required to bind NO$_3^-$-N to oxidized biochar [36]. In the ORG-N and NH$_4^+$-N columns it is likely that the soils natural exchange sites retained a portion of influent ORG-N and NH$_4^+$-N, and between application events N underwent mineralization and nitrification. Therefore, between applications the NO$_3^-$-N had sufficient time to bind to the biochar exchange sites. When comparing the difference between control and biochar amended columns by week, none of the columns resulted in significant difference in NO$_3^-$-N leaching between treatments until week two (Figure S1), supporting this theory. However, in the NO$_3^-$-N application events, because NO$_3^-$-N is known to be highly mobile in soil, it is possible that the NO$_3^-$-N was flushed out during application, resulting in little N remaining to undergo binding between application events. However, higher temperature produced biochar, BC$_2$, did result in a significant reduction in N leaching by 24.8% compared to control. Other studies have found that biochar produced at high temperatures can bind NO$_3^-$-N due to the higher surface area of the biochar [34]. However, other studies have suggested enhancement of functional groups may result in greater binding of high temperature biochar [6,31]. The XPS data measured a greater abundance of oxygenated functional groups of higher temperature biochar (Table S3), which is likely to have increased sorption of NO$_3^-$-N in BC$_2$ columns. To enhance retention of NO$_3^-$-N, it was hypothesized that pre-oxidized biochar (BC$_3$) would result in a higher retention [36]. However, similar to Mia et al. [67], BC$_3$ did not result did not significantly enhance the soil retention of NO$_3^-$-N, suggesting that chemical oxidation prior to soil amendment may not be a substitute to chemical change occurring in the soil, or the dominant process may be the development of an organomineral layer.

There were no significant differences in measured N emissions from columns, with the exception of the NO$_3^-$-N application, which resulted in lower N$_2$O emissions from soil columns amended with biochar (Figure 5B). This suggest that biochar may aid in mitigating greenhouse gas emissions from agricultural treatment systems. Since BC$_1$ biochar did not result in significant soil retention of NO$_3^-$-N but did reduce N$_2$O emissions, it is likely that immobilization of NO$_3^-$-N is not the primary mechanisms for emission reductions. Emissions of N$_2$O are correlated to pH, and biochar may have resulted in buffering the soil to reduce the production of N$_2$O. However, soil pH was not significantly different with biochar amendment in this study (Table S4). Biochar may have impacted microbial communities as suggested in past studies which found increases in microbial gene abundance of nosZ with biochar amendment [13,14].
5. Conclusions

Amendment of biochar to a sandy loam soil significantly reduced the leaching of N when receiving repeated applications with N concentrations similar to farmstead runoff. Soil analysis indicated that biochar impacted N leaching through enhanced soil retention of both ORG-N and NO$_3^-$-N. Surface chemistry analysis indicated that biochar, following soil amendment, had an increase in oxygenated functional groups and cationic minerals on the biochar surface increasing soil retention of ORG-N and NO$_3^-$-N through cationic bridging or the development of an organomineral layer on the biochar surface. The repeated application of N over the course of ten weeks did not impact biochar N leaching reduction performance, although it is likely that a maximum reduction potential would be reached. However, to effectively reduce N leaching from direct application of NO$_3^-$-N, only high temperature production biochars at 700 °C resulted in a significant reduction in N leaching of 24.8%. However, it is important to note that this was a short-term laboratory study, which varies greatly from field conditions. Thus, before widespread implementation, future fields studies are necessary to confirm performance under field conditions. Natural oxidation and development of organomineral layers were more effective in retaining applied NO$_3^-$-N than those oxidized prior to soil amendment, which may indicate organomineral layer development may be more important or natural oxidation processes are more effective at developing conditions that can bind NO$_3^-$-N, but more investigation is needed to assess this.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/7/979/s1, Table S1: Biochar production temperature, treatments, elemental content, and pH; Table S2: Water quality analysis parameters, EPA approved method, and detection limits; Table S3: XPS scan of major elements and carbon functional groups on the surface of biochar before and after soil amendment for columns receiving NO3-N low and high application rates; Figure S1: Weekly cumulative NO$_3^-$-N effluent for (A) ORG-N low, (B) NH$_4^+$-N low, and (C) NH$_4^+$-N high application; Table S4: Soil pH following application for treatments.

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