

# Biochar and Manure Effects on Net Nitrogen Mineralization and Greenhouse Gas Emissions from Calcareous Soil under Corn

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Few multiyear field studies have examined the impacts of a one-time biochar application on net N mineralization and greenhouse gas emissions in an irrigated, calcareous soil; yet this use of biochar is hypothesized as a means of sequestering atmospheric CO<sub>2</sub> and improving soil quality. We fall-applied four treatments: stockpiled dairy manure (42 Mg ha<sup>-1</sup> dry wt.), hardwood-derived biochar (22.4 Mg ha<sup>-1</sup>), combined biochar and manure, and no amendments (control). Nitrogen fertilizer was applied in all plots and years based on treatment's preseason soil test N and crop requirements and accounting for estimated N mineralized from added manure. From 2009 to 2011, we measured greenhouse gas fluxes using vented chambers, net N mineralization using buried bags, corn (*Zea mays* L.) yield, and N uptake, and in a succeeding year, root and shoot biomass and biomass C and N concentrations. Both amendments produced persistent soil effects. Manure increased seasonal and 3-yr cumulative net N mineralization, root biomass, and root/shoot ratio 1.6-fold, CO<sub>2</sub>-C gas flux 1.2-fold, and reduced the soil NH<sub>4</sub>/NO<sub>3</sub> ratio 58% relative to no-manure treatments. When compared with a class comprising all other treatments, biochar-only produced 33% less cumulative net N mineralization, 20% less CO<sub>2</sub>-C, and 50% less N<sub>2</sub>O-N gas emissions, and increased the soil NH<sub>4</sub>/NO<sub>3</sub> ratio 1.8-fold, indicating that biochar impaired nitrification and N immobilization processes. The multi-year nature of biochar's influence implies that a long-term driver is involved, possibly related to biochar's enduring porosity and surface chemistry characteristics. While the biochar-only treatment demonstrated a potential to increase corn yields and minimize CO<sub>2</sub>-C and N<sub>2</sub>O-N gas emissions in these calcareous soils, biochar also caused decreased corn yields under conditions in which NH<sub>4</sub>-N dominated the soil inorganic N pool. Combining biochar with manure more effectively utilized the two soil amendments, as it eliminated potential yield reductions caused by biochar and maximized manure net N mineralization potential.

**Abbreviations:** DOY, day of year; EC, electrical conductivity; GC, gas chromatography; Mmge, mean monthly gas emission flux; Mmat, mean monthly air temperature.

If performed on a large scale, amending soils with biochar (biomass-derived black C) potentially can reduce atmospheric CO<sub>2</sub> concentrations and mitigate climate change (Laird, 2008; Woolf et al., 2010; Matovic, 2011). However, research is needed to better understand biochar's influence on N cycling and the emission of greenhouse gases in soils, particularly for calcareous soils with low organic C concentrations.

Research has examined greenhouse gas emissions from soils amended with biochar derived from conifer wood (Clough and Condon, 2010; Anderson et al., 2011; Taghizadeh-Toosi et al., 2011; Mukome et al., 2013; Troy et al., 2013), hard-

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wood (Singh et al., 2010; Rogovska et al., 2011), animal manure (van Zwieten et al., 2010; Singh et al., 2010), and other sources (Spokas and Reicosky, 2009; Zhang et al., 2012; Mukome et al., 2013). A number of studies have also evaluated the influence of various biochars on N mineralization and respiration in soil (DeLuca et al., 2006; Deenik et al., 2010; Jones et al., 2011; Luo et al., 2011; Bruun et al., 2012; Dempster et al., 2012; Jones et al., 2012; Nelissen et al., 2012). Most of the above biochar research has been conducted with incubated soils under fallow soil conditions, and few studies have examined the effect of biochar on net N mineralization or greenhouse gas emissions in cropped fields over multiyear periods. In general, the influence of biochar additions on agricultural soils in temperate regions is not well understood (Atkinson et al., 2010). A similar knowledge gap exists concerning biochar's effect on irrigated, calcareous soils in the semiarid to arid climates.

A few studies have evaluated biochar effects on soils with  $\text{pH} > 7$ , but the examined soils developed in wetter climates and contained little if any free lime (Smith et al., 2010; Zimmerman et al., 2011). Artiola et al. (2012) examined the effect of strongly alkaline ( $\text{pH} 9.8$ ) pine biochar on  $\text{CO}_2$  emissions from a calcareous, low-organic-C, loamy sand with  $\text{pH} 8.15$ . The biochar reduced soil  $\text{CO}_2$  emissions during the first 60 d of the incubation but had no effect in the second 60-d period (Artiola et al., 2012). Additionally, Zhang et al. (2012) added strongly alkaline ( $\text{pH} 10.4$ ) wheat (*Triticum aestivum* L.) straw biochar to a calcareous, low organic-C containing loam under fertilized corn, observing no effect on season-long  $\text{CO}_2$  emissions.

A number of studies across North America have employed the same biochar to better understand how biochar effects vary for different soils (Spokas et al., 2009; Husk and Major, 2011; Dumroese et al., 2011; Elmer and Pignatello, 2011; Lentz and Ippolito, 2012). The use of only one biochar avoids obfuscation caused by variation in biochar properties. The biochar was derived from hardwood waste biomass, CQuest (Dynamotive Energy Systems, West Lorne, Ontario, Canada) and had a neutral to slightly acid  $\text{pH}$ . A Minnesota study found that addition of 10% (w/w) CQuest biochar to an acidic silt loam soil ( $\text{pH} = 6.5$ ) generally suppressed  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$  production rate during a 100-d incubation (Spokas et al., 2009). This result suggested that the biochar stabilized soil organic C, which has implications for N and S availability since they are substantially derived from organic sources. In an earlier paper, Lentz and Ippolito (2012) reported the effect of CQuest biochar and dairy manure amendments, and their interaction on soil chemical properties, crop nutrient uptake, and corn yields for the same plots employed in this current study. Lentz and Ippolito (2012) found that biochar decreased corn yield and N uptake in the second year after biochar was applied but had little influence on nutrient levels determined in annual soil samples. We hypothesized that biochar may have influenced corn yield and N uptake via effects on soil N cycling; if so, this would imply that biochar influences on N cycling are persistent and not necessarily the result of transient processes such as the release of ethylene (Spokas

et al., 2009), other organic compounds (Deenik et al., 2010), or immobilization of labile portions of the biochar (Luo et al., 2011; Bruun et al., 2012). The objective of the current study was to field evaluate the effect of CQuest biochar and dairy manure amendments and their interaction on net N mineralization, greenhouse gas emissions, and biomass distribution in a calcareous soil in relation to crop yields.

## MATERIALS AND METHODS

### Site, Soils, and Amendments

Experimental plots were established in fall 2008 on sprinkler irrigated Portneuf silt loam (coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcids) with 1.4% slopes near Kimberly, ID ( $42^\circ 31' \text{ N}$  lat;  $114^\circ 22' \text{ W}$  long; elevation of 1190 m). The surface soil contained  $200 \text{ g kg}^{-1}$  clay,  $560 \text{ g kg}^{-1}$  silt,  $12 \text{ g kg}^{-1}$  organic C, and 8.8%  $\text{CaCO}_3$  equivalent. The soil had a saturated-paste-extract electrical conductivity (EC) of  $0.04 \text{ S m}^{-1}$ , exchangeable Na percentage (ESP) of 1.5,  $\text{pH}$  of 7.6 (saturated paste), and a cation exchange capacity of  $19 \text{ cmol}_c \text{ kg}^{-1}$ . Soils on the site have been cropped to an alfalfa (*Medicago sativa* L.)–corn–bean (*Phaseolus vulgaris* L.)–grain (*Hordeum vulgare* L. or *Triticum aestivum* L.) rotation for the previous 33 yr. No manure had been applied to the soils since 1986.

Solid manure from dairy cattle (*Bos* species) was retrieved from an open pen at a local dairy, where it had been stockpiled through summer 2008 in 1.7-m-high, unconfined piles. The material contained little or no straw bedding and, at the time of application, comprised 55.3% solids. Total C and total N of the organic amendments were determined on a freeze-dried sample with a Thermo-Finnigan FlashEA1112 CN analyzer (CE Elantech Inc., Lakewood, NJ). Total elements were determined by  $\text{HClO}_4$ – $\text{HNO}_3$ – $\text{HF}$ – $\text{HCl}$  digestion (Soltanpour et al., 1996) followed by analysis using inductively coupled plasma atomic emission spectrometry (ICP–AES). Manure  $\text{NO}_3$ –N and  $\text{NH}_4$ –N were determined using a 2 M KCl extract (Mulvaney, 1996). Manure volatile solids were determined gravimetrically by combusting a sample in a crucible for 4 to 12 h at  $550^\circ\text{C}$ , and  $\text{pH}$  and EC were determined on a saturated paste extract.

Dry CQuest biochar (Dynamotive Energy Systems Inc., McLean, Virginia) with a  $<0.5$ -mm particle size was shipped to the laboratory and stored in sealed steel barrels. The charcoal was manufactured from oak and hickory hardwood sawdust using fast pyrolysis at  $500^\circ\text{C}$  with a 5-s residence time. It had a 14% ash content, O/C ratio of 0.22, surface area of  $0.75 \text{ m}^2 \text{ g}^{-1}$ , 71% volatile C, and near neutral  $\text{pH}$ . Ash content of the biochar was determined using ASTM methods for wood charcoal ( $600^\circ\text{C}$ ), and other chemical characteristics were determined as previously described for manure. Soil, manure, and biochar chemical characteristics are presented in Table 1.

### Experimental Design

The experimental design was a randomized complete block with three replicates. Four amendment treatments included a (i) control (no manure or biochar application); (ii) manure,

**Table 1. Chemical properties, total element, and extractable inorganic N concentrations (dry wt. basis) in amendments and soil.**

Material	Volatile solids	EC	pH	C/N	C	N	NO <sub>3</sub> -N†	NH <sub>4</sub> -N†	Ca	K	P	Na
	g kg <sup>-1</sup>	dS m <sup>-1</sup>	g kg <sup>-1</sup>									
Manure	521	13.4	8.8	11.8	264	22.4	<0.01	<0.01	22.0	13.5	4.1	3.8
Biochar	707	0.7	6.8	208.2	662	3.2	0.2	0.1	3.7	3.4	0.3	0.2
Soil†	–	0.4	7.7	16.4	18	1.1	<0.01	<0.01	33.3	26.8	4.2	10.4

† Values for soil electrical conductivity (EC), pH, C, and N components are averages for the control treatment, 0 to 30 cm.

42 Mg ha<sup>-1</sup> (dry wt.) application of stockpiled dairy manure; (iii) biochar, 22.4 Mg ha<sup>-1</sup> (dry wt.) application; and (iv) manure + biochar, combined application using rates identical to manure-only and biochar-only treatments. The chosen manure rate supplied sufficient mineralized N to meet the 2009 corn yield target. Manure and biochar amendments were applied only once in fall 2008, and corn was grown on the plots in the succeeding 3 yr. Spring soil sampling each year indicated that soils contained adequate P and K for the crops. Thus, only inorganic N was added to plots in 2009, 2010, and 2011 to meet yield targets. The amount of inorganic N fertilizer supplied to each treatment each growing season (listed in Table 2) was determined using a local corn fertilizer guide table (Brown et al., 2010), with recommendations based on yield target and a preplant soil N test. For manure and biochar + manure plots, we adjusted the preplant soil N test values to account for N mineralized from manure during the growing season, which was estimated using N mineralization data obtained from an earlier study on an adjacent field (unpublished data, 2012). Specific inferences included (i) of the total manure N added, 21% would become available in the first growing season, 12% in the second, and 10% in the third, and (ii) biochar supplied little N to soils in any year. We employed a silage yield target of 18.5 Mg ha<sup>-1</sup> (dry wt.) in 2009, which was increased to 22.2 Mg ha<sup>-1</sup> for 2010 and 2011. Plots were 4.6 m wide, 5.2 m long, and included eight planted rows. Limited biochar availability precluded larger plot sizes and additional experimental blocks. Plots were separated by a 1.5-m wide planted buffer and a planted border strip 4 m wide comprised the perimeter.

## Field Operations

Spring barley (*Hordeum vulgare* L.) was grown on the plots in 2008. After the grain harvest, plot soils were moldboard

plowed to a 0.20-m depth. On 21 Nov. 2008 solid manure from a local dairy was collected and hand-applied to designated plots. The manure was subsampled during application and the composite volume stored at 4°C for later analysis. Biochar was hand-applied to designated plots on 24 Nov. 2008, and immediately thereafter all plots were rototilled to a 0.15-m depth. Plots were roller harrowed on 21 Apr. 2009 and Round-Up ready silage/grain corn variety was planted on 12 May 2009 in 0.76-m spaced rows. On 8 June 2009, ammonium sulfate was applied by hand to plots, as needed (Table 2) followed by a sprinkler-applied, 21-mm irrigation. The initial soil N levels and N from mineralization in manure and biochar + manure plots was determined to be adequate for the 2009 corn crop.

Two postemergence applications of 2,4-D amine and glyphosate were used in June 2009 to control weeds. Irrigation through the growing season was supplied via sprinkler every 7 to 14 d to meet crop evapotranspiration (ET) requirements. A total of nine irrigations were applied during the growing season. Irrigation water had an average electrical conductivity of 0.05 S m<sup>-1</sup> and Na adsorption ratio of 0.5. Corn yield was sampled on 18 Sept. 2009 (described later), the remaining crop was harvested on 9 Oct. 2009, and residual corn stover (15- to 30-cm-tall stems with leaves) flail chopped in preparation for a no-till planting in the spring 2010. Round-Up ready (Monsanto) corn was planted into the row spaces of the previous corn crop on 19 May 2010. Planting into the low lying inter-row spaces proved inconsistent across all plots, thus any skips in emerged seedlings observed within plots were replanted by hand 5 d after the original seeding had emerged. On 25 June 2010, urea was applied to plots by hand as needed (Table 2), immediately followed by a 57-mm irrigation. An application of 2,4-Dichlorophenoxyacetic acid and dicamba and Diflufenzopyr (Distinct, BASF) was applied on 14 July 2010 to control weeds. Irrigation was applied

**Table 2. Inorganic fertilizer N amounts added to treatment plots each year to ensure that total available N, including that mineralized from manure, was adequate to meet yield targets.**

Treatment	21 Nov. 2008	8 June 2009	25 June 2010	28 June 2011
	Organic amendment† (dry wt.) Mg ha <sup>-1</sup>	Fertilizer N applied as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> for yield target of 18.5 Mg ha <sup>-1</sup> ‡	Fertilizer N applied as urea ‡	Fertilizer N Applied as urea ‡
Control	0	200	224	242
Manure	42.0	0	67	197
Biochar	22.4	200	224	242
Biochar + manure	Manure – 42.0 Biochar – 22.4	0	67	197

† Manure and biochar was applied only once in the fall of 2008.

‡ The fertilizer N rate was obtained from fertilizer guide (Brown et al., 2010) recommendations, based on yield target and early-season soil N values adjusted for estimated N mineralized from manure during the growing season. Mineralized manure N was estimated using N mineralization data obtained from an earlier study on Portneuf soils on an adjacent field (unpublished data, 2012). The silage yield targets in years 2009, 2010, and 2011 were 18.5, 22.2, and 22.2 Mg ha<sup>-1</sup> (dry wt.), respectively.

using the same method as in 2009, with 10 irrigations applied. The corn yield was sampled on 15 Oct. 2010. The remaining crop was harvested for grain on 16 Feb. 2011, and the remaining corn stover was flail chopped and raked to remove excess residue. On 2 May 2011, the plots were rototilled twice, treated with Triazine herbicide (Atrazine, Syngenta), and roller harrowed. Corn was planted on 12 May 2011. On 28 June 2011, urea was applied to plots by hand as needed (Table 2), immediately followed by a 76-mm irrigation. Irrigation was provided as in 2009, with nine irrigations applied. Corn yield was sampled on 30 Sept. 2011, the remaining crop was harvested for grain on 7 Oct. 2011, and the residual corn stover flail chopped and raked before the field was rototilled on 29 Nov. 2011.

## Greenhouse Gas Fluxes

Soil greenhouse gas fluxes during the late spring to fall period were estimated from in situ  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$  emissions monitored from July 2009 through October 2011. Measurements were made between 0830 and 1130 h every week, or every other week. Gas fluxes were measured using three 29-cm-wide by 72-cm-long by 28-cm-tall, two-piece (base and cover), static, vented, Teflon lined, steel chambers (Hutchinson and Livingston, 2002).

Each chamber base was placed diagonally in between two adjacent corn rows so that opposite corners lay between corn plants in each row. Three chamber bases were placed across four adjacent corn rows in each plot and were connected together via Teflon tubes, which led from the central space of each chamber cover volume to a fourway connector fitting. The headspace in the three chambers was sampled simultaneously via the fourth branch of the fourway connector at 0, 10, 20, and 30 min after chamber placement. In 2009, gas samples from the chamber headspace were collected straight from the fourway connector and concentrations were measured directly in the field using a Model 1412 photoacoustic field gas monitor (Innova Air Tech Instruments, Ballerup, Denmark). In 2010 and 2011, 5 mL of headspace gas was collected from the chamber at known time intervals and transferred by syringe into a previously He flush headspace vial (10 mL; Agilent). Samples were then returned to the laboratory and analyzed by a customized gas chromatography (GC) system that has been previously described (Spokas et al., 2009). The GC system used a headspace auto sampler (HP 7694) to transfer three independent samples to three separate columns in two GC units (HP 5890) for the analysis of  $\text{O}_2$ ,  $\text{N}_2$ ,  $\text{CO}_2$  (CTR-1, Grace, TCD),  $\text{CH}_4$  (Porapak T; FID), and  $\text{N}_2\text{O}$  (Porapak Q; ECD with a naffion dryer [Perma Pure]). The system was checked daily for accuracy with NIST traceable gas standards (Minneapolis Oxygen, Minneapolis, MN).

Gas concentrations in ppmv units were converted to  $\text{mol vol}^{-1}$  via the gas law, using local air pressure and temperature at sampling. Gas flux (e.g.,  $\mu\text{g CO}_2\text{-C m}^{-2} \text{d}^{-1}$ ) was calculated from measured changes in headspace gas concentrations with time using a best-fitting nonlinear model. These fluxes were determined using HMR, a program package written in the R

statistical language (Pedersen et al., 2010). Cumulative gas emissions across sampling dates were calculated using trapezoidal integration, assuming that measured fluxes represented average daily fluxes.

## Soil and Plant Sampling and Analyses

We employed a buried bag method (Westermann and Crothers, 1980; Meek et al., 1994; Lentz et al., 2011), described previously by Lentz and Lehrsch (2012), to measure net N mineralization in plot soils. In each plot, three or four 5.7-cm-diam. soil cores, 0 to 30 cm deep were collected, composited, and passed through a 0.4-cm screen. If the soil's water content was estimated to be less than 17 to 20  $\text{kg kg}^{-1}$ , reverse osmosis water was added to achieve this water content. A subsample of the composited soil was collected to determine inorganic N and the final soil water content. The soil's inorganic N concentration was calculated as the sum of the extractable  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations as  $\text{mg N kg}^{-1}$  of dry soil (analysis described below). Part of the remaining soil was placed in 10- $\mu\text{m}$ -thick, 5-cm-diam. polyethylene tubes that were sealed on one end. The soil was settled using a vertical shaking action, and the open end was then sealed, resulting in a 30-cm-long soil column that was inserted into one of the sample holes created previously. Surface soil was placed in the hole around the bag as needed to fill the cavity, thus ensuring representative soil temperatures in the bagged soil. Soil was also mounded on the soil surface atop the bag to eliminate water flow along the bag's sidewalls.

In 2010 and 2011, buried bags were also installed at 30- to 60-cm depths. The procedure was the same as that used for 0 to 30 cm, except that two to three 30- to 60-cm soil cores were collected per plot, and the buried bags were placed at 30- to 60-cm depths. A bag was installed on the April 21st ( $\pm 3$  d) each year (except 2011, when an unusually cold and wet spring delayed installation until 5 May 2011). We installed one 0- to 30-cm buried bag in each plot and beginning in 2010 one 30- to 60-cm buried bag below the 0- to 30-cm bag. The buried bag at each depth was retrieved on June 15th, at which time two additional sets of buried bags were installed in the same plots (with the same depth arrangement) using the procedure above. In 2009 one of these two sets was retrieved on 3 Aug., and the other on 1 Oct. In 2010 and 2011, the bags installed on 15 June were retrieved on July 15th and August 15th each year. Then two more bags were installed, to be retrieved on September 15th and October 15. We determined inorganic N concentrations and soil water content in soils from the initial sample and retrieved buried bags. After being air-dried at 35°C and crushed to pass a 2-mm screen, soil N in samples was extracted using a 2 M KCl solution (Mulvaney, 1996). The  $\text{NO}_3\text{-N}$  concentration in each extract was determined within 6 h of extraction using an automated flow injection analyzer (Lachat Instruments, Loveland, CO) after Cd reduction (Method 12-107-04-1-B) while  $\text{NH}_4\text{-N}$  concentration was determined simultaneously using a salicylate-hypochlorite method (Method 12-107-06-2-A).

The net N mineralization during the period between burial and retrieval was calculated by subtracting the inorganic N concentration of the initial soil from that of the soil in the retrieved bag. A positive difference indicated net N mineralization, while a negative value indicated net N immobilization during the period. We compared the water content of the retrieved bag soil with that measured at the time of burial to verify bag integrity. We reported net N mineralization for the *spring* (April or May to June 15th); *early summer* (June to August) and *late summer* (August to October) periods. When two bags installed together were retrieved on staggered dates, values for the second bag pulled was computed by difference relative to the first retrieved buried bag sample. The net N mineralized (termed minN) was reported directly as mg N kg<sup>-1</sup> soil. We used the same procedure to identify the net gains realized by the two inorganic N component concentrations (NO<sub>3</sub>-N or NH<sub>4</sub>-N) in buried bag soils during monitoring periods.

Corn silage yields were measured by hand clipping corn plants (3 cm above soil surface) from 3-m lengths of two adjacent rows within each plot. The sample was weighed and chopped, and a subsample was collected, dried at 65°C, and ground in a Thomas Wiley mill (Swedesboro, NJ) to pass an 865- $\mu$ m screen. We determined corn biomass total N concentration by combustion using a Thermo-Finnigan FlashEA1112 CN analyzer (Thermo-Finnigan, Waltham, MA).

Corn was also grown on these plots in 2012 and 2013 (data not presented). At the end of the 2013 growing season, we measured shoot and root biomass, harvested (aboveground biomass taken during mechanized harvest), and residual biomass (above- and below-ground biomass remaining after mechanized harvest) to evaluate the potential effect of treatment on corn root/shoot and residual/harvested ratios for biomass, C, and N. Roots were measured using soil cores (Laboski et al., 1998). After harvest, three 45-mm-diam., 1.2-m-deep soil cores were collected from each plot at three positions relative to the plant: 0 m (between corn plants in the row); 0.13 m from plant, in the nontrafficked area between plant and midrow; and 0.38 m from plant, at midrow. The soil core samples in 30-cm increments were placed in nested sieves, and roots were separated from the soil by repeated water immersion, flotation, and hand picking. Root samples were dried, weighed, and analyzed similar to silage biomass. Two randomly selected postharvest corn crowns and roots from a 15-cm-diam., 15-cm-deep, soil volume centered on the stem were collected from each plot, washed, dried, separated into above- and below-ground portions, and weighed. The mass of the residual stem projecting >3-cm above the soil was subtracted from the corn silage yield to obtain the harvested biomass.

## Calculations and Statistical Analysis

The statistical analysis employed orthogonal contrasts to investigate how the two factors, biochar and manure, and their interaction influenced measured parameters. We examined net N-mineralization data for given reporting intervals separately via analysis of variance (ANOVA), using PROC Mixed in SAS

version 9.2 (SAS Institute, 2009). The statistical model included treatment as the fixed effect and block as the random effect, and included the three orthogonal class comparisons (biochar vs. no biochar; manure vs. no manure; and the biochar  $\times$  manure interaction), in addition to class contrasts (i) manure + biochar vs. all other treatments (control, biochar, manure), and (ii) biochar only vs. all other treatments (control, manure, manure + biochar). Pairwise class comparisons of means were performed using the Tukey option (SAS Institute, 2009).

Season-long cumulative gas emission values were analyzed by ANOVA using PROC Mixed. The model included treatment, year, and their interaction as fixed effects with block as the random effect. In addition, orthogonal and class comparisons and mean separations were conducted as described previously. The cumulative CO<sub>2</sub> gas values were transformed using the reciprocal square root to stabilize variances and improve normality. The LSmeans were back transformed to original units for reporting. Data for N<sub>2</sub>O and CH<sub>4</sub> gases did not require transformation.

A graphical display of cumulative gas flux data for each year would show 2009 data shifted downward and to the right compared to years 2010 and 2011, an artifact caused by the late start of the 2009 monitoring. To establish a more realistic positioning of the graphed 2009 data, we added the cumulative gas flux values for the spring and early summer period of 2010 (16 Apr. through 29 July 2010) to the 2009 values. Both the original and approximated 2009 data were presented.

Mean monthly gas emission fluxes for each gas, experimental unit, and year were computed using the nonadjusted 2009, 2010, and 2011 data. We evaluated the relationship between mean monthly flux and mean monthly air temperature via linear regression using PROC Reg (SAS Institute, 2009).

The single-year corn root, root/shoot, and residual/harvested biomass data were analyzed as for net N-mineralization except that values were transformed ( $\log_{10}$ ) before processing. Analogous ratios for biomass C and N were similarly analyzed, except no transformations were required. Standard errors were calculated from nontransformed data. All analyses were conducted at an  $\alpha = 0.1$  significance level.

## RESULTS

### Climatic Conditions

Spring (March through June) air temperatures were relatively cool during the study and became progressively cooler from year 2009 through 2011 (Fig. 1). The 2009 spring air temperature was 0.8°C cooler, 2010 was 1.7°C cooler, and 2011 was 1.9°C cooler than average (mean of previous 14-yr). The 7.5-cm soil temperatures during the growing season varied substantially among years: (i) 2009 and 2011 were similar except the 2009 mid-May to mid-June period was 2 to 7°C warmer than for either 2010 or 2011; and (ii) 2010 differed from both 2009 and 2011 in that summer soil temperatures peaked 2 to 3 wk earlier, and post-June soil temperatures were 2.5 to 5°C cooler than for the other years (Fig. 2). The cool spring conditions during the study

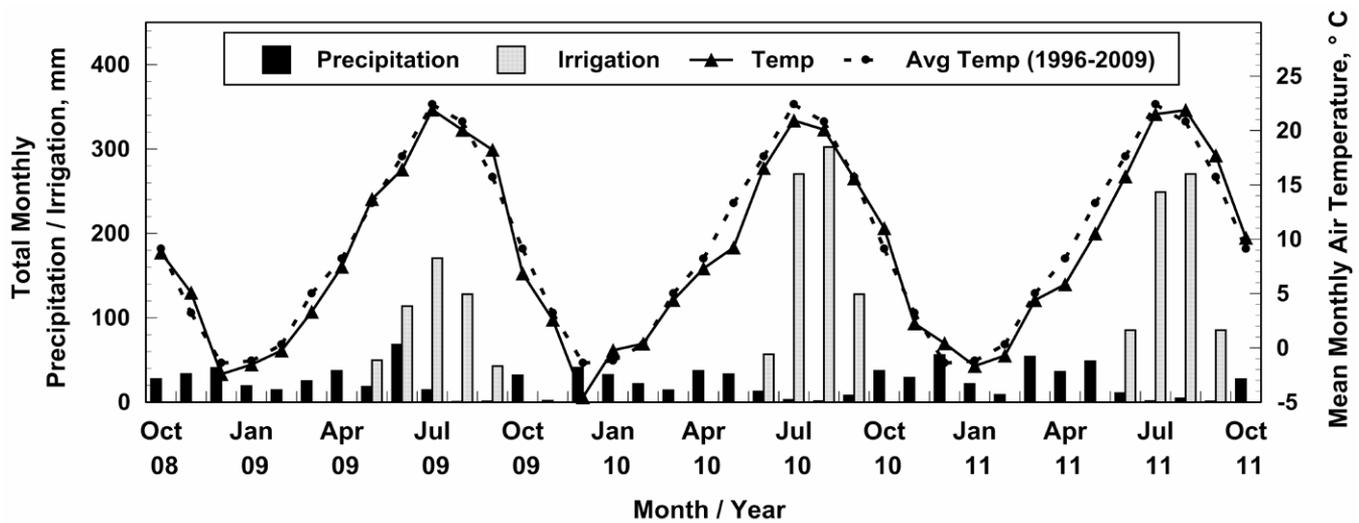


Fig. 1. Total monthly precipitation and irrigation and mean monthly air temperature at the study site from fall 2008, when the biochar and manure amendments were applied, through October 2011, the last year net N mineralization was reported.

delayed corn emergence and seedling establishment by 1 to 2 wk relative to more typical growing seasons.

### Net Nitrogen Mineralization

A biochar × manure interaction influenced the 0- to 30-cm, net N mineralization as measured by the season-long mean and the 3-yr cumulative total, even though the interaction effect was obvious in only two of nine individual periods (Table 3, Fig. 3). The pattern of the interaction was similar in each case, indicating that biochar decreased net mineralization when added alone but increased net N mineralization when added with manure (Fig. 4a). Similar results were found for the 30- to 60-cm soil layer (Fig. 4b). Manure additions increased net N mineralization in most of the individual measurement periods, over the entire season each

year, and for the 3-yr cumulative total. As a class, manure treatments produced an average 1.6-fold greater season-long and 3-yr cumulative net mineralization than the two no-manure treatments (Table 4). Manure similarly influenced net N mineralization at the 30- to 60-cm depth, except that the increase produced by manure was 1.3-fold times that in no-manure plots (Table 4).

At the 0- to 30-cm depth, the biochar-only treatment produced the lesser (33% reduction), and manure + biochar produced the greater (1.5× increase) net N mineralization relative to their respective all-other treatment classes (Table 3, class comparisons). This was demonstrated in six of nine individual-period values (data not shown), and for the overall seasonal mean, and the cumulative total values. The magnitude of these differences were consistent across the 3 yr for a given soil layer (Table 4). These relationships are a reflection of, and reinforce conclusions drawn from, the biochar × manure interaction results. A similar pattern was observed in the 30-to 60-cm soil, except that differences were smaller and sometimes not significant (Table 4, Fig. 5).

The net  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  gains in 0- to 30-cm buried bag soils differed depending on the monitoring period and year (Fig. 6). When significant treatment differences occurred, the responses from the two manure treatments tended to be similar to one another and different from the two no-manure treatments. In 2009 through early summer 2010, the patterns showed fertilizer- $\text{NH}_4\text{-N}$  undergoing relatively rapid nitrification to  $\text{NO}_3\text{-N}$  and manure treatments producing gains in  $\text{NO}_3\text{-N}$ . However, by late summer 2010, that is, day of year (DOY) 260, this pattern had reversed, with manure and no-manure attaining a peak in  $\text{NH}_4\text{-N}$  gains with no associated change in  $\text{NO}_3\text{-N}$  (Fig. 6). The large  $\text{NO}_3\text{-N}$  gain in early summer 2009 and in spring 2010 indicated that both ammonification and nitrification processes were active in all soils, while the substantial  $\text{NH}_4\text{-N}$  gains, and lack of  $\text{NO}_3\text{-N}$  increases, during late summer 2010 indicated that the ammonification rate was greater than that of nitrification.

The differences in relative nitrate and ammonium production influenced the resulting concentration ratio of  $\text{NH}_4\text{-N}$  to

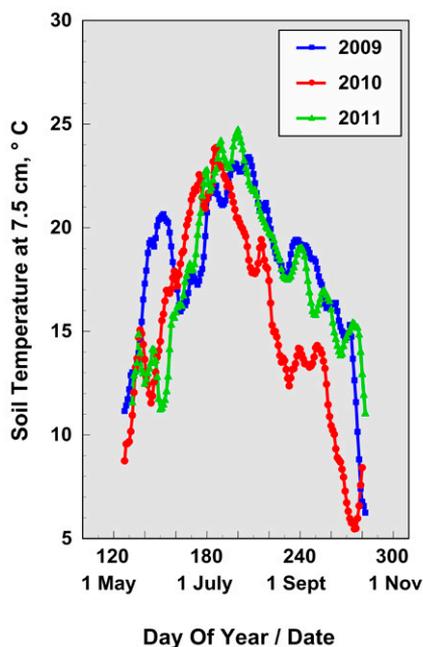


Fig. 2. Soil temperature at a 7.5-cm depth during the 2009, 2010, and 2011 growing seasons. The data for each year are plotted as a 7-d moving average.

**Table 3. Biochar and manure effects on annual seasonal, average seasonal, and total cumulative net N mineralization at 0- to 30-cm and 30- to 60-cm depths for 2009 to 2011. Table includes *P*-values from an analysis of variance for factor effects and contrast comparisons.**

Source of variation	0 to 30 cm					30 to 60 cm				
	Spring-summer			Season-long mean 2009-11	3-yr Total 2009-11	Spring-summer		Season-long mean 2010-11	2-yr Total 2010-11	
	2009	2010	2011			2010	2011			
Biochar	ns	ns	ns	ns	ns	ns	0.1	ns	ns	
Manure	**	**	***	***	***	0.06	ns	***	***	
Biochar × manure	ns	*	ns	0.06	0.06	ns	0.07	0.06	0.06	
<i>Contrasts †</i>										
Biochar only vs. All Other-1	*	**	**	**	**	0.07	ns	**	**	
Biochar + manure vs. all Other-2	*	**	**	**	**	ns	*	**	**	

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

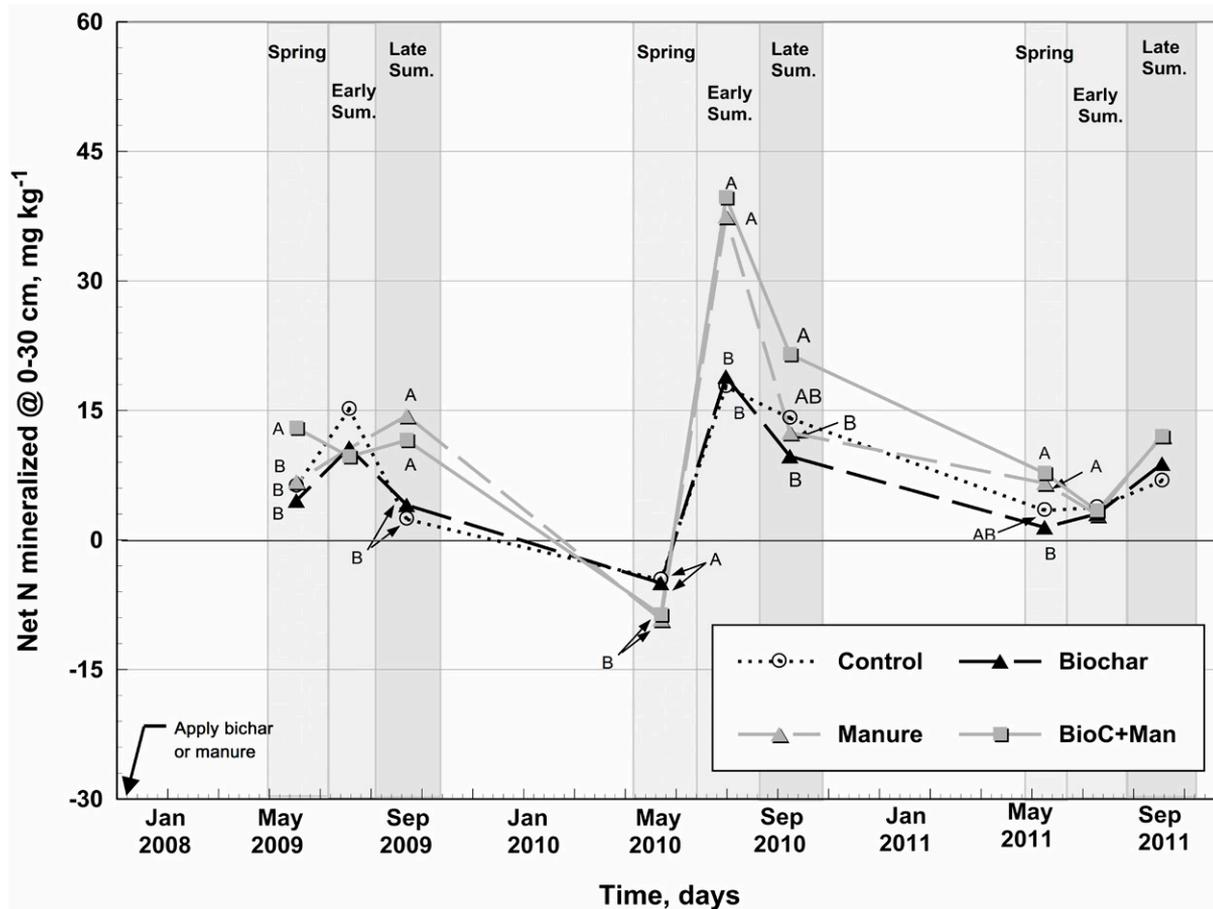
† All Other-1 = control, manure, and biochar + manure treatments; All Other-2 = control, manure, and biochar-only treatments.

$\text{NO}_3\text{-N}$  in exhumed bag soils. The  $\text{NH}_4/\text{NO}_3$  ratio peaked (1.4 for manure vs. 3.3 for no-manure treatments) during periods when buried bags showed the largest  $\text{NH}_4\text{-N}$  gains (2010-DOY 260 and 2011-DOY 166) but decreased (0.41 for manure vs. 0.66 for no-manure) for periods with small  $\text{NH}_4\text{-N}$  gains (Supplemental Table S1). Note that treatment means for  $\text{NH}_4/\text{NO}_3$  ratio followed an order similar to other response variables except that the sequence was reversed: biochar-only treatment maximized, and manure + biochar minimized, the  $\text{NH}_4/\text{NO}_3$  ratio among the four treatments on average (Supplemental Table S1).

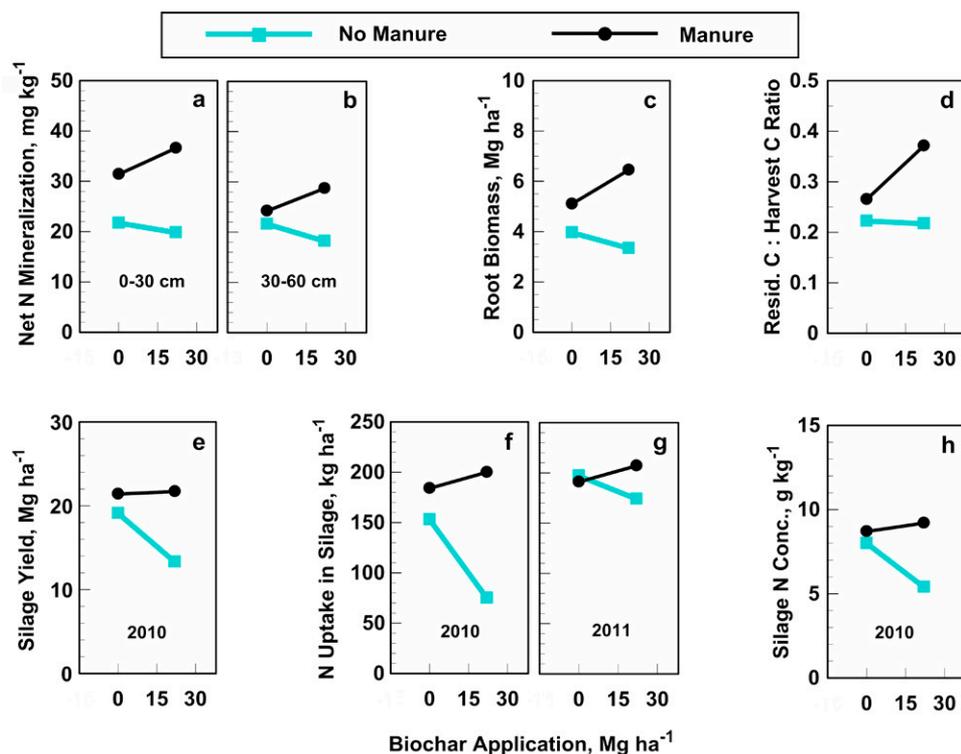
### Greenhouse Gas Emissions

Main factors, treatment and year, influenced cumulative seasonal  $\text{CO}_2\text{-C}$  fluxes, whereas year alone influenced seasonal  $\text{N}_2\text{O-N}$  and  $\text{CH}_4\text{-C}$  gas fluxes (Table 5). Manure increased cumulative seasonal  $\text{CO}_2\text{-C}$  flux 1.2-fold relative to no-manure plots. Similar to net N mineralization results, the biochar-only treatment produced the least  $\text{CO}_2\text{-C}$  flux of the four treatments, resulting in a 17% on average reduction (unadjusted data, Table 5).

Class comparisons for adjusted seasonal data (when the absent 2009 early-season data was estimated using that of the 2010



**Fig. 3. The net N mineralized at 0- to 30-cm soil depths for defined periods from April 2009 through October 2011. Means of treatment values for a given period are significantly different if labeled with different letters. Back panels in the figure identify the measurement interval used for each data point.**



**Fig. 4.** The influence of biochar with and without manure (biochar  $\times$  manure interaction) on mean season-long net N mineralization at 0 to 30 cm (a), 30 to 60 cm (b) depths, corn root parameters (c, d), silage yields (e), and silage N concentration (h) and uptake (f, g).

early-season), showed similar results to that of unadjusted for  $\text{CO}_2\text{-C}$  fluxes; however, they also showed that the biochar-only treatment produced the least  $\text{N}_2\text{O-N}$  gas flux among the four treatments on average (50% reduction). The decline in  $\text{N}_2\text{O-N}$  gas fluxes for biochar relative to other treatments is readily noticeable in Supplemental Fig. S1e, S1f, S1g, and S1h. Neither treatment nor year factors nor treatment class significantly influenced the 3-yr cumulative emissions for any of the gases.

**Table 4.** Biochar and manure effect means for annual seasonal, average seasonal, and total cumulative net N mineralization at 0- to 30-cm and 30- to 60-cm depths, and results for class comparisons (contrasts).

Treatment or class $\ddagger$	0–30 cm					30–60 cm†			
	Spring–summer			3-yr Seasonal mean	3-yr Total	Spring–summer		2-yr Seasonal mean	2-yr Total
	2009	2010	2011			2010	2011		
	mg kg <sup>-1</sup>								
Biochar	26.8	39.7	18.2	28.2	84.7	28.3	18.6	23.5	46.9
No biochar	27.8	34.0	17.9	26.6	79.6	29.9	15.9	22.9	45.8
Manure	33.0 a	46.6 a	22.4 a	34.0 a	102.0 a	34.5	18.4	26.4 a	52.9 a
No manure	21.5 b	27.1 b	13.7 b	20.8 b	62.2 b	23.8	16.0	19.9 b	39.8 b
<i>Class comparisons</i> $\S$									
Biochar only	19.3 b	27.0 b	13.3 b	19.8 a	59.5 b	20.5	15.9	18.2 b	36.4 b
All Other-1	29.9 a	40.1 a	19.6 a	29.9 b	89.6 a	32.0	17.7	24.8 a	49.7 a
Biochar + manure	34.2 a	52.5 a	23.1 a	36.6 a	109.8 a	36.1	21.3 a	28.7 a	57.4 a
All Other-2	24.9 b	31.6 b	16.3 b	24.3 b	72.9 b	26.8	15.9 b	21.3 b	42.7 b

† For the 30- to 60-cm depth, no measurements were made in 2009 or spring 2010; hence, the 2010 seasonal value excludes the spring period, and the cumulative total includes only data for 2010 and 2011.

‡ For each treatment class comparison, means followed by the same letter are not significantly different ( $P \leq 0.1$ ). Letters are not displayed if the effect was not significant in the ANOVA.

§ No Manure = control, biochar; Manure = manure, biochar + manure; All Other-1 = control, manure, and biochar + manure treatments; All Other-2 = control, manure, and biochar treatments.

Greenhouse gas fluxes varied with year, although the effect of year on  $\text{CO}_2\text{-C}$  gas fluxes was less pronounced than for  $\text{N}_2\text{O-N}$  or  $\text{CH}_4\text{-C}$  (Table 5). (See also cumulative gas emission plots comparing gas fluxes for  $\text{CO}_2\text{-C}$  [Supplemental Fig. S1a, S1b, S1c, and S1d],  $\text{N}_2\text{O-N}$  [Supplemental Fig. S1e, S1f, S1g, and S1h], and  $\text{CH}_4\text{-C}$  [Supplemental Fig. S1i, S1j, S1k, and S1l].) This suggested that  $\text{N}_2\text{O-N}$  and  $\text{CH}_4\text{-C}$  may be more sensitive to fluctuations in the soil temperature regime, such as the cooling that occurred in 2010 (Fig. 2). However, the regression analyses of mean monthly gas emission ( $\text{Mmge}$ ) fluxes (for example,  $\mu\text{g CO}_2\text{-C m}^{-2} \text{min}^{-1}$ ) on mean monthly air temperature ( $\text{Mmat}$ ,  $^\circ\text{C}$ ) indicated that while emission rate of each gas was correlated to air temperature, temperature explained a greater portion of the variability associated with  $\text{CO}_2\text{-C}$

( $R^2 = 0.48$ ;  $P < 0.0001$ ) than  $\text{N}_2\text{O-N}$  ( $R^2 = 0.21$ ;  $P < 0.0001$ ) or  $\text{CH}_4\text{-C}$  ( $R^2 = 0.07$ ;  $P = 0.0001$ ). Regression analysis also indicated that  $\text{CO}_2\text{-C}$  fluxes for the manure treatments as a class were more sensitive to changes in air temperature ( $\text{Mmge} = 74.2 \times \text{Mmat} - 166.5$ ;  $R^2 = 0.48$ ;  $P < 0.0001$ ) than non-manure treatments on average ( $\text{Mmge} = 59.4 \times \text{Mmat} - 104.8$ ;  $R^2 = 0.51$ ;  $P < 0.0001$ ).

## Corn Silage Yields, Nitrogen Uptake, and Biomass Distribution

Across all treatments, corn silage yields averaged 18.5 Mg ha<sup>-1</sup> (dry wt.) in 2009, 18.9 in 2010, and 20.5 in 2011. The influence of biochar and manure on corn yields differed each year: (i) in 2009 biochar slightly increased corn yields, while manure had no effect; (ii) in 2010 a biochar × manure interaction prevailed; and (iii) in 2011 biochar had no effect, while manure slightly increased yields (Table 6). The interaction effect in 2010 revealed that adding biochar without manure reduced yields by 30% relative to the control treatment (13.3 vs. 19.1 Mg ha<sup>-1</sup>); while adding biochar with manure produced no yield reduction (Table 6, Fig. 4e). Biochar and manure also interacted to influence silage N concentration in 2010 (Fig. 4h) and silage total N uptake in 2010 and 2011 (Fig. 4f and 4g). In each case, the interaction mirrored that observed for yield and net N mineralization, such that biochar decreased silage N concentration and uptake when added alone but increased silage N concentration and uptake when added with manure (Table 6). Where an interaction occurred, and except for 2009, the biochar-only treatment produced the least silage yield and N uptake values when compared to the other three treatments together. The most substantial reductions occurred in 2010, when a 36% reduction in silage yield and 37% reduction in silage N concentration resulted in a 58% decrease in total N uptake for biochar-only compared to the other treatments.

Biochar and manure influenced corn root biomass and the biomass, C, and N distribution ratios (Table 7) much as they did for net N mineralization (Table 4): (i) Biochar decreased or had no effect on the corn root biomass and residual-C/harvested-C ratio when added alone but increased these parameter values when added with manure (Fig. 4c and 4d); (ii) Manure addition increased corn root biomass and the biomass, C, and N distribution ratios about 1.6-fold compared to no-manure plots; (iii) The biochar-only treatment produced the least, and manure + biochar produced the greatest values for the corn root biomass and biomass, C, and N distribution ratios among the four treatments on average (Table 7). Parameter values for the biochar-only averaged 31% less and manure + biochar averaged 1.6-fold greater than the all-other classes (Table 7).

## DISCUSSION

At least a 5-mo delay occurred between the November 2008 application of biochar and manure soil amendments and the start of net N mineralization and greenhouse gas measurements. Hence, our results reflect long-term impacts on soil N cy-

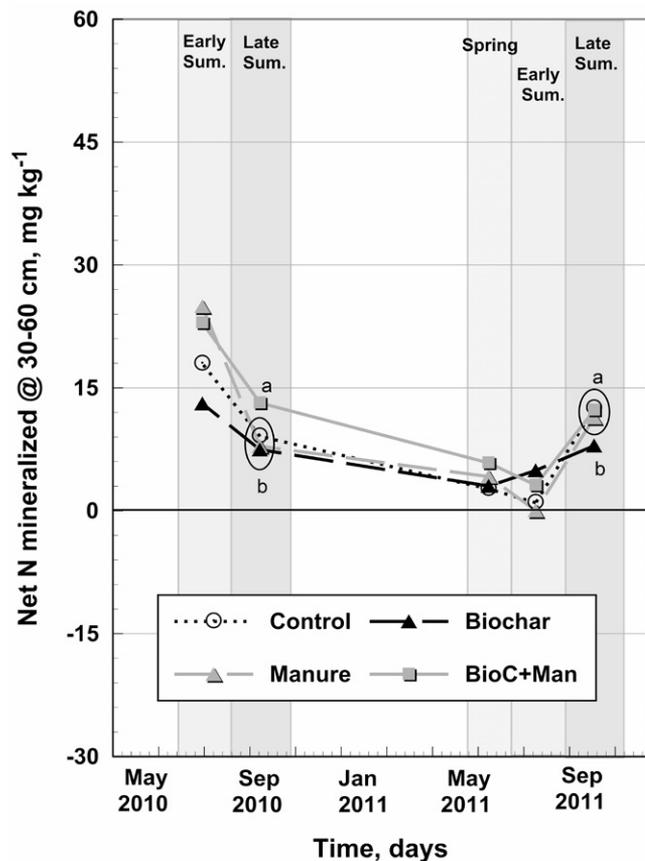


Fig. 5. The net N mineralized at 30- to 60-cm soil depths for defined periods from June 2010 through October 2011. Means of treatment classes are significantly different if labeled with different lowercase letters (contrasts). Back panels in the figure identify the measurement interval used for each data point.

cling, in contrast to most results reported in the literature, which commonly were derived from 1- to 14-wk incubation studies (DeLuca et al., 2006; Spokas et al., 2009; Novak et al., 2010;

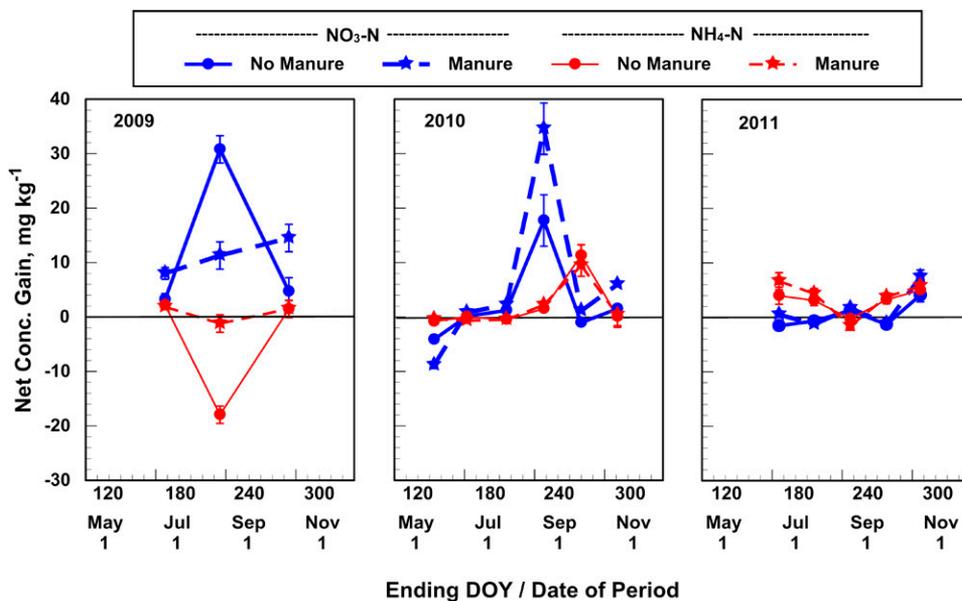


Fig. 6. The net gain in soil NO<sub>3</sub>-N and NH<sub>4</sub>-N concentrations in 0- to 30-cm buried-bags for manure and no-manure treatment classes for each measurement period. Each leg of the error bars represents one standard deviation ( $n = 6$ ).

**Table 5. The influence of biochar and manure, and year and treatment on seasonal cumulative greenhouse gas emissions for 2009 to 2011 and 3-yr cumulative gas emissions. Table includes *P*-values from an analysis of variance for factor effects and contrast comparisons.**

				Includes adjusted 2009 Data †			3-Year cumulative gas Emissions		
	CO <sub>2</sub> -C	N <sub>2</sub> O-N	CH <sub>4</sub> -C	CO <sub>2</sub> -C	N <sub>2</sub> O-N	CH <sub>4</sub> -C	CO <sub>2</sub> -C	N <sub>2</sub> O-N	CH <sub>4</sub> -C
	<i>P</i> -Values								
Biochar	ns	ns	ns	ns	ns	ns	ns	ns	ns
Manure	**	ns	ns	**	ns	ns	0.06	ns	ns
Biochar × manure	ns	ns	ns	ns	ns	ns	ns	ns	ns
Biochar vs. All Other-1‡	**	ns	ns	**	*	ns	0.07	ns	ns
Biochar + manure vs. All Other-2‡	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year	***	*	***	0.06	*	***	–	–	–
Trt × year	ns	ns	ns	ns	ns	ns	–	–	–
	g CO <sub>2</sub> -C m <sup>-2</sup>	mg N <sub>2</sub> O-N m <sup>-2</sup>	mg CH <sub>4</sub> -C m <sup>-2</sup>	g CO <sub>2</sub> -C m <sup>-2</sup>	mg N <sub>2</sub> O-N m <sup>-2</sup>	mg CH <sub>4</sub> -C m <sup>-2</sup>	g CO <sub>2</sub> -C m <sup>-2</sup>	mg N <sub>2</sub> O-N m <sup>-2</sup>	mg CH <sub>4</sub> -C m <sup>-2</sup>
Manure	169 a §	63	191	208 a	80	296	587 a	216	569
No manure	143 b	47	161	171 b	54	322	496 b	169	483
<i>Class comparisons§</i>									
Biochar only	135 b	40	120	161 b	38 b	240	465 b	146	359
All Other	163 a	60	195	200 a	76 a	332	567 a	208	581
<i>Year</i>									
2009	119 c	84 a	172 b	196 a	102 a	569 a	–	–	–
2010	164 b	32 b	404 a	164 b	32 b	404 a	–	–	–
2011	196 a	70 ab	–52 c	196 a	70 ab	–52 b	–	–	–

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

† To better estimate cumulative fluxes for 2009 when measurements were started in late July, the cumulative gas flux values for the spring and early summer period of 2010 (16 Apr. through 29 July 2010) were added to the 2009 values.

‡ No Manure = control, biochar; Manure = manure, biochar + manure; All Other-1 = control, manure, and biochar + manure treatments; All Other-2 = control, manure, and biochar-only treatments.

§ For each class or year category, means followed by the same letter are not significantly different ( $P \leq 0.1$ ). Letters are not displayed if the effect was not significant in the ANOVA.

Bruun et al., 2012; Nelissen et al., 2012; Dempster et al., 2012). Our results indicate that biochar or manure effects on net N mineralization and CO<sub>2</sub>-C or N<sub>2</sub>O-N emissions were not temporary. Conversely, a field study that evaluated high-organic C, acidic soils for 3 yr found that hardwood biochar (slow pyrolysis at 450°C) increased soil respiration and microbial growth rate in the second year after biochar application but not in the third year (Jones et al., 2012). Other research reported that biochar effects on gas emissions commonly did not continue more than 50 d after application (van Zwieten et al., 2010; Singh et al., 2010; Taghizadeh-Toosi et al., 2011; Zhang et al., 2012; Mukome et al., 2013). However, Rogovska et al. (2011) observed biochar effects on CO<sub>2</sub>-C emissions extending beyond 300 d.

### Manure Effects

Adding an organic C and N source with a relatively low C/N ratio stimulated microbial growth and N mineralization. The mean 1.6-fold increase in seasonal net N mineralization for manure-treated soils vs. no-manure was nearly identical to the 1.64-fold increase in 3-yr cumulative net N mineralization reported by Lentz et al. (2011) for a similar soil and manure application and compares favorably with the 1.54-fold increase observed in a Nebraska field study (Eghball, 2000). These results contrast with those of Ma et al. (1999), who applied manure at rates below those

required to supply needed N to the corn crop and resulted in a mean seasonal net N mineralization for manure that was less than that of the no-manure treatment (Ma et al., 1999).

Previous research indicates that manure application commonly increases soil CO<sub>2</sub>-C emissions, with the magnitude dependent on manure rate, current crop and residue return rate, and previous crop residue (Gregorich et al., 1998; Drury et al., 2008; Adviento-Borbe et al., 2010; Heller et al., 2010; Shrestha et al., 2013). In the current study, the 1.2-fold increase in CO<sub>2</sub>-C emissions for manure relative to no-manure treatments matched that observed in a corn-small-grain rotation after livestock manure (at a rate similar to ours) was applied to an Alberta, Canada field (Ellert and Janzen, 2008). In the current study, increased CO<sub>2</sub>-C emissions produced by manure may have resulted, in part, in response to increased root biomass (Table 7). Manure's ability to increase root biomass has been attributed to its capacity to decrease soil bulk density and soil strength (Laboski et al., 1998; Mosaddeghi et al., 2009). Our analysis showed no effect of manure on N<sub>2</sub>O-N emissions relative to no-manure treatments, confirming results reported by Adviento-Borbe et al. (2010), but contradicted a study reporting that manure decreased N<sub>2</sub>O-N emissions relative to fertilized soils (Ellert and Janzen, 2008).

## Biochar Effects

In this study the biochar-only treatment produced the least net N mineralization on average than all other treatments (Fig. 4a and 4b, Table 4). Bruun et al. (2012) reported a similar decrease in soil mineralized N for 65 d after the addition of flash pyrolyzed (525°C) wheat straw. This coincided with an increase in microbial biomass C, hence Bruun et al. (2012) concluded that (i) the microbes mineralized labile, incompletely pyrolyzed feedstock C in the biochar, and (ii) the resulting N immobilization was likely a transient phenomenon, lasting only a few months in the soil. A year-long laboratory incubation with the same biochar and soil used in the current study observed bio-

**Table 6. Effect of biochar and manure on corn silage yield, silage N concentration (TN), and silage total N uptake for 2009, 2010, and 2011. Table includes *P*-values from an analysis of variance for factor effects.**

Source of Variation	Silage yield			Silage N (TN)			Total N uptake		
	2009	2010	2011	2009	2010	2011	2009	2010	2011
	<i>P</i> -Values								
Biochar	0.09	0.08	ns	ns	0.09	ns	ns	ns	ns
Manure	ns	**	0.06	ns	**	ns	ns	***	ns
Biochar × manure	ns	*	ns	ns	*	ns	ns	*	0.09
Biochar vs. All Other-1†	0.08	***	0.06	ns	**	ns	ns	*	0.09
<i>Factor</i>	Mg ha <sup>-1</sup>			g kg <sup>-1</sup>			kg ha <sup>-1</sup>		
Biochar	19.0 a ‡	17.5 a	20.4	12.6	7.3 b	9.4	239	138	191
No biochar	18.1 b	20.3 b	20.6	12.5	8.4 a	9.4	225	169	194
Manure	18.3	21.6 a	21.2 a	12.7	9.0 a	9.4	231	192 a	199
No manure	18.8	16.2 b	19.9 b	12.4	6.7 b	9.4	232	114 b	186
<i>Class comparison</i>									
Biochar only	19.4 a	13.3 b	19.4 b	12.2	5.4 b	9.0	238	75 b	174 b
All Other-1	18.2 b	20.7 a	20.9 a	12.6	8.6 a	9.5	229	179 a	198 a

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

† For each class or year category, means followed by the same letter are not significantly different ( $P < 0.1$ ). Letters are not displayed if the effect was not significant in the ANOVA.

‡ No manure = control, biochar; manure = manure, biochar + manure; All Other-1 = control, manure, and biochar + manure treatments.

**Table 7. The influence of biochar and manure on corn root/shoot and residual/shoot biomass ratios, and biomass-C and biomass-N ratios in 2013. Table includes *P*-values from an analysis of variance for main effect and contrast comparisons between select treatment classes.**

Source of variation	Root biomass	Root/Shoot	Residual/Harvest	Residual-C/Harvest-C	Residual-N/Harvest-N
	<i>P</i> -Values				
Biochar	ns	ns	ns	0.1	ns
Manure	**	**	*	**	***
Biochar × manure	0.1	ns	ns	0.1	ns
Biochar vs. All Other-1 †	**	**	0.08	0.08	**
B & M vs. All Other-2 †	*	*	*	**	***
<i>Factor</i>	Mean values				
	Mg ha <sup>-1</sup>				
Biochar	4.90	0.28	0.36	0.30 a‡	0.31
No biochar	4.53	0.26	0.31	0.25 b	0.28
Manure	5.78 a	0.33 a	0.41 a	0.32 a	0.36 a
No manure	3.65 b	0.20 b	0.25 b	0.22 b	0.22 b
<i>Class comparisons †</i>					
Biochar only	3.34 b	0.19 b	0.25 b	0.22 b	0.22 b
All Other-1	5.17 a	0.29 a	0.36 a	0.29 a	0.32 a
Biochar+manure	6.45 a	0.36 a	0.46 a	0.37 a	0.40 a
All Other-2	4.13 b	0.23 b	0.29 b	0.23 b	0.26 b

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

† B&M = biochar + manure treatment; no manure = control, biochar; manure = manure, B&M; All Other-1 = control, manure, and B&M; All Other-2 = control, manure, and biochar treatments.

‡ For each treatment or class, means followed by the same letter are not significantly different ( $P \leq 0.1$ ). Letters are not displayed if the effect was not significant in the ANOVA.

char-induced N immobilization occurring only in the third and fourth months after application (Ippolito et al., 2014). Similar reductions in N mineralization or increased N immobilization have been observed during <14-wk incubations of acidic soils amended with wood-based biochar (Dempster et al., 2012; Jones et al., 2012; Ameloot et al., 2013).

The minimizing of soil CO<sub>2</sub>-C emissions by the biochar-alone treatment relative to our other treatments (Table 5) was consistent with 100-d incubation results reported by Spokas et al. (2009), for two out of three noncalcareous soils amended with the same biochar. A similar outcome was observed for other kinds of biochars (van Zwieten et al., 2010). However, our results contradict other studies, which reported that biochar had no effect (Troy et al., 2013; Mukome et al., 2013) or increased CO<sub>2</sub> emissions (Rogovska et al., 2011; Zhang et al., 2012; Ippolito et al., 2014).

Evidence suggesting that biochar-only minimized N<sub>2</sub>O-N gas emissions relative to the other three treatments on average (Table 5), confirms reports from previous research that have employed various types of biochar and soils (Spokas et al., 2009; van Zwieten et al., 2010; Singh et al., 2010; Rogovska et al., 2011; Taghizadeh-Toosi, 2011; and Zhang et al., 2012). In a few studies, biochar was reported to increase N<sub>2</sub>O emissions, but in one case the biochar was co-applied with 760 kg N ha<sup>-1</sup> as urine (Clough et al., 2010), and in the other, the increased N<sub>2</sub>O emissions were temporary, occurring only in the first 2 d after application (Mukome et al., 2013).

### Mechanism by Which Biochar Effects Soil Processes

Net N mineralization was less for biochar on average than all other treatments (Fig. 4a and 4b, Table 4), suggesting that biochar either inhibited microbial activity, which decreased mineralization, or decreased the ratio of gross N mineralization to gross N immobilization rates in the soil (Luxhøi et al., 2006). Since soil water content of buried bags was controlled, we assume that denitrification was not an important factor (Jones et al., 2012). The attendant decrease in CO<sub>2</sub>-C emissions associated with biochar addition confirms that N immobilization decreased since N immobilization is highly correlated with respiration rate (Flavel and Murphy, 2006; Luxhøi et al., 2006). This indicates that gross N mineralization rates also declined. The evidence suggests that biochar either inhibited soil microbial activity, altered microbial community composition, or changed the partitioning of N through proteolysis, ammonification, or nitrification pathways, and subsequently reduced N assimilation by microbes (Myrold and Bottomly, 2008). A change in N partitioning associated with a decrease in soil nitrification is strongly implicated because the biochar-only treatment maximized the soil NH<sub>4</sub>/NO<sub>3</sub> concentration ratio (Supplemental Table S1) relative to other treatments on average. The overall effect of these changes would result in the apparent stabilization of soil C (Kimetu and Lehmann, 2010). The decreased root biomass of biochar-only treatment (Fig. 4c), which may have contributed to reduced CO<sub>2</sub>-C emissions, could have resulted from low net N miner-

alization and hence low inorganic N availability in soils (Giehl et al., 2014).

An inhibitory effect of biochar on microorganisms has been observed by others (Dempster et al., 2012) and attributed to water-soluble, low molecular-weight (<500 Da) organic species (Smith et al., 2012), volatile organic compounds (Clough et al., 2010), or ethylene (Spokas et al., 2010) released from the biochar. Biochar additions have also been shown to alter the composition of soil bacterial populations, increasing those of families involved with reduction of NO<sub>3</sub> to NH<sub>4</sub>, N<sub>2</sub> fixation, and denitrification but decreasing those involved in nitrification of NH<sub>4</sub> to NO<sub>2</sub> (Anderson et al., 2011; Khodadad et al., 2011; Lehmann et al., 2011; Ducey et al., 2013).

If the release of organic materials from biochar were impacting soil microbes, one may expect the effect to decline with time as degradation, volatilization, or leaching reduced their concentrations (Saadi et al., 2007; Kim et al., 2011; Tsiknia et al., 2014). The persistent multi-year effects of biochar in our study may instead be related to its physical porosity and chemical binding capacity. The CQuest biochar's small initial surface area (0.75 m<sup>2</sup> g<sup>-1</sup>) indicated that pores were filled with volatile organic materials (Mukherjee et al., 2011), which are susceptible to mineralization and volatilization over time (Zimmerman et al., 2011; Kim et al., 2011). The disappearance of these organics with soil aging likely (i) increased porosity and surface area akin to the effect that activation has on charcoal (González et al., 2009; Yang et al., 2010); and (ii) increased negatively charged sites on biochar (Cheng et al., 2008). The charged sites can bind NH<sub>4</sub>-N, making inorganic N less available for microorganisms and leaching (Lehmann et al., 2003; Steiner et al., 2008; Dempster et al., 2012). A change in pore size distribution in the biochar amended soil can also have an important influence on bacterial community composition (Sleutel et al., 2012). The biochar acts as a sink, limiting the availability of NH<sub>4</sub>-N to heterotrophic and autotrophic (nitrifiers) microbes. This suggests that an important influence of biochar on soil processes results from its capacity to sequester inorganic N, primarily NH<sub>4</sub>. This concept is supported by the observation that biochar-alone maximized the soil NH<sub>4</sub>/NO<sub>3</sub> concentration ratio, on average, relative to other treatments.

### Biochar's Effect on Corn Yield

The changing effects of biochar, shifting from a slightly positive influence on corn yield in 2009 (Table 6) to a substantial negative effect on corn yield, biomass N concentration, and uptake in 2010 (Fig. 4e, 4f, and 4h) suggested that the negative influence arose in response to particular circumstances that changed from year to year. The cooler soil temperatures in the last half of the 2010 growing season compared to 2009 and 2011 (Fig. 2) may have decreased N immobilization rates (Nicolardot et al., 1994), resulting in the increased net N mineralization (0–30 cm) we observed in 2010 relative to other years (Fig. 3). In addition, cooler soil temperatures have been shown to impede nitrification rates more than ammonification rates (Campbell et al., 1971). The lack of tillage in 2010 and change from barley

straw residue ( $C/N = 81$ ) to corn residue ( $C/N = 35$ ) may have inhibited growth and activity of soil nitrifiers (Doran, 1980; Rice and Smith, 1983; Franzluebbers et al., 1994) and increased activity of nitrate reducers (Chèneby et al., 2010). This confluence of factors may explain why  $NH_4-N$  gains were greater in late-summer 2010 buried bags than in other years, resulting in a large increase in the proportion of soil  $NH_4-N$  concentrations relative to  $NO_3-N$  (Fig. 4c, Fig. 6). We hypothesize that this predominance of  $NH_4-N$  in soil, combined with biochar's capacity to sequester  $NH_4-N$ , led to a shortage of available inorganic N for crop uptake. This nutrient limitation was further exacerbated by the minimal rooting volume of the biochar crop (Table 7), resulting in reduced biochar corn yields. Note that the accumulation of  $NH_4-N$  concentrations in these soils was observed only one other time in 10 yr of buried-bag N mineralization research conducted at this location (unpublished data, 2011), suggesting that the particular circumstances leading to the condition do not commonly occur (data not shown).

### Biochar and Manure Interaction

When applied with manure, biochar positively influenced seasonal mean and cumulative total net N mineralization (Table 3), yet the effect was less apparent during individual measurement periods (Fig. 3). This likely occurred because some of the periods included a large immobilization potential (Lentz et al., 2011). Since manure can produce large immobilization rates (Lentz et al., 2011), the manure substantially reduced net N mineralization values, in some cases below that of control and biochar plots (Fig. 3).

When added alone, biochar minimized net N mineralization,  $CO_2-C$ , and  $N_2O-N$  gas emissions, root biomass, and maximized the soil  $NH_4/NO_3$  concentration ratio, on average; for manure, the response was opposite; it increased season-long and multiyear cumulative net N mineralization,  $CO_2-C$  gas emissions, root biomass, and reduced the soil  $NH_4/NO_3$  concentration ratio (Tables 3, 4, and 6 and Supplemental Table S1). However, when biochar was added to manured soil, rather than reduce the manure response, it maximized net N mineralization and root biomass and minimized the soil  $NH_4/NO_3$  concentration ratio but did not further increase  $CO_2-C$  or alter  $N_2O-N$  gas emissions. This result is compatible with biochar effects described above. Manure addition increased N mineralization in the manure + biochar soil and presumably, biochar slightly restrained those N mineralization and immobilization rates (possibly by stabilizing organic C). This did not reduce net mineralization because less of the  $NH_4-N$  released from the mineralized manure became bound to the biochar. This was likely due to the saturation of biochar  $NH_4$ -binding sites or to the increase of manure-supplied cations (Mg, Ca, K, and Na) in the soil solution (Lentz and Ippolito, 2012), which replaced adsorbed  $NH_4-N$  at some binding sites. The excess, nonsequestered  $NH_4-N$  was then nitrified. By restraining N immobilization in the manured soil, biochar increased net N mineralization and  $NO_3-N$  accu-

mulation in the soil, leading to the minimized  $NH_4/NO_3$  concentration ratio and minimized soil respiration rate.

## CONCLUSIONS

A 3-yr field study on irrigated calcareous soils indicated that a single application ( $22.4 \text{ Mg ha}^{-1}$  in 0- to 15-cm soil) of fast pyrolyzed hardwood biochar with near neutral pH and high volatile matter and/or manure produced soil effects that persisted for 3 yr. Biochar applied alone influenced soil processes differently than when co-applied with manure. We conclude that the biochar employed in this study negatively affected nitrification and N immobilization processes in these soils, which resulted in minimized net N mineralization and  $CO_2-C$  and  $N_2O-N$  gas emissions, and maximized soil  $NH_4/NO_3$  concentration ratios relative to other treatments. This implies that biochar increased the stability of organic C in the soil. Furthermore, the long-term nature of these biochar effects suggest that changes in N-transformation processes may be caused by more enduring characteristics of the biochar such as porosity and surface chemistry, which influence N-transformation processes by increasing ammonium binding capacity of the soil and, possibly by altering microbial populations. While the biochar-only treatment demonstrated a potential to minimize  $CO_2-C$  and  $N_2O-N$  gas emissions in these calcareous soils, biochar also caused decreased corn yields under certain soil nutrient conditions. If farmers wish to apply biochar to these soils, combining it with manure appears to be an effective method of utilizing these soil amendments, as it eliminated potential yield reductions from biochar and maximized net N mineralization potential of the added manure. More research is needed to fully understand which potentially unique properties of the Dynamotive C-quest biochar drive the outcomes observed above, and how these may differ in comparison to other biochars derived from different source materials and processes.

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