2020

Soil carbon increased by twice the amount of biochar carbon applied after 6 years: Field evidence of negative priming

Humberto Blanco-Canqui

David A. Laird

Emily A. Heaton

Samuel Rathke

Bharat Sharma Acharya

Received: 14 October 2019 | Accepted: 12 December 2019

DOI: 10.1111/gcbb.12665

ORIGINAL RESEARCH

GLOBAL CHANGE BIOLOGY BIOENERGY WILEY

Soil carbon increased by twice the amount of biochar carbon applied after 6 years: Field evidence of negative priming

Humberto Blanco-Canqui[1] | David A. Laird[2] | Emily A. Heaton[2] | Samuel Rathke[3] | Bharat Sharma Acharya[1]

[1]Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE, USA

[2]Agronomy Department, Iowa State University, Ames, IA, USA

[3]Department of Environmental Science, University of Arizona, Tucson, AZ, USA

Correspondence
Humberto Blanco-Canqui, Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583, USA.
Email: hblanco2@unl.edu

Funding information
USDA National Institute of Food and Agriculture, Grant/Award Number: 2011-68005-30411

Abstract

Applying biochar to agricultural soils has been proposed as a means of sequestering carbon (C) while simultaneously enhancing soil health and agricultural sustainability. However, our understanding of the long-term effects of biochar and annual versus perennial cropping systems and their interactions on soil properties under field conditions is limited. We quantified changes in soil C concentration and stocks, and other soil properties 6 years after biochar applications to corn (*Zea mays* L.) and dedicated bioenergy crops on a Midwestern US soil. Treatments were as follows: no-till continuous corn, Liberty switchgrass (*Panicum virgatum* L.), and low-diversity prairie grasses, 45% big bluestem (*Andropogon gerardii*), 45% Indiangrass (*Sorghastrum nutans*), and 10% sideoats grama (*Bouteloua curtipendula*), as main plots, and wood biochar (9.3 Mg/ha with 63% total C) and no biochar applications as subplots. Biochar-amended plots accumulated more C (14.07 Mg soil C/ha vs. 2.25 Mg soil C/ha) than non-biochar-amended plots in the 0–30 cm soil depth but other soil properties were not significantly affected by the biochar amendments. The total increase in C stocks in the biochar-amended plots was nearly twice (14.07 Mg soil C/ha) the amount of C added with biochar 6 years earlier (7.25 Mg biochar C/ha), suggesting a negative priming effect of biochar on formation and/or mineralization of native soil organic C. Dedicated bioenergy crops increased soil C concentration by 79% and improved both aggregation and plant available water in the 0–5 cm soil depth. Biochar did not interact with the cropping systems. Overall, biochar has the potential to increase soil C stocks both directly and through negative priming, but, in this study, it had limited effects on other soil properties after 6 years.

KEYWORDS

biochar, carbon sequestration, dedicated bioenergy crops, soil physical properties, switchgrass

1 | INTRODUCTION

Amending soil with C-enriched materials such as biochar is one strategy to enhance the numerous ecosystem services that soils provide. Such soil services include producing food, fuel, feed, and fiber as well as sequestering C, recycling water and nutrients, and regulating climate (MEA, 2005). Applying biochar to soils used for food (i.e., corn) and dedicated bioenergy crops (i.e., switchgrass) could enhance services from these agroecosystems (Kauffman, Dumortier, Hayes, Brown, & Laird, 2014; Laird, 2008; MEA, 2005). Furthermore, because of its high concentration of stable C (60%–80% C), biochar is considered the leading soil amendment to rapidly increase soil C sequestration and thereby help mitigate global climate change (Ventura et al., 2019).

Recent studies have indicated that biochar applications can increase soil C through both direct and indirect effects. The direct effect is the amount of stable biochar C added, while the indirect effect is the potential reduction in mineralization of native soil organic matter and/or fresh crop residues, a process known as negative priming (Ding et al., 2018; Wang, Xiong, & Kuzyakov, 2016). Understanding this negative priming effect is critical to assessing the long-term potential of biochar for soil C sequestration. In general, biochar may have negative, positive, or no priming effect. Positive priming occurs when biochar accelerates the decomposition of native organic matter, thereby reducing long-term C accumulation (Ding et al., 2018; Maestrini, Nannipieri, & Abiven, 2015; Wang et al., 2016). While factors and mechanisms responsible for the priming effect of biochar are not fully understood, biochar and soil characteristics along with management may influence the biochar priming effects (MEA, 2005).

Most studies of biochar priming effects have been short-term (<1 year) and mostly conducted in laboratory or greenhouse settings. Field evidence of the biochar priming effect is limited and mixed (Dong, Singh, Li, Lin, & Zhao, 2018; Reed, Chadwick, Hill, & Jones, 2017; Ventura et al., 2019). Most laboratory studies have been conducted in incubation media without plant roots; however, the presence of plant roots under field conditions may positively or negatively impact the priming effect of biochar relative to laboratory incubations (Ventura et al., 2019). Long-term field studies on biochar are needed to better understand the potential priming effects of biochar on soil C stocks as well as its interactions with available N in the soils (Chen et al., 2014).

Applying biochar could enhance C accumulation and sustainability of annual cropping systems such as corn, which is a multi-purpose crop that produces food, fiber, fuel, and feed. Corn residues are increasingly being removed for livestock feeding and bedding, and for biofuel production. High rates of corn residue removal can increase the risks of soil erosion and reduce soil C storage (Acharya & Blanco-Canqui, 2018; Blanco-Canqui, Stalker, et al., 2016; Blanco-Canqui, Tatarko, Stalker, Shaver, & Van Donk, 2016; Laird & Chang, 2013; Ruis et al., 2018). Adding biochar to corn fields is a potential strategy to mitigate the negative effects of residue harvesting on soil quality and C stocks (Backer, Schwinghamer, Whalen, Seguin, & Smith, 2016; Laird & Chang, 2013; Ventura et al., 2019). Biochar application can rapidly increase soil C levels relative to other practices such as cover crops and diversified crop rotations, which commonly take extended periods of time to significantly change soil C stocks (Poeplau & Don, 2015). While, at present, biochar usage at large scales in grain cropping systems is limited, it may become more widely practiced in the future as a co-product of thermochemical biorefineries that use crop and forestry residues to produce bioenergy products.

Similarly, applying biochar to dedicated bioenergy crops such as perennial warm-season grasses (i.e., switchgrass) where cellulosic biomass is harvested could improve soil properties and may offset potential negative effects of long-term biomass harvesting on soil C storage and soil quality (Shanta et al., 2016). Perennial bioenergy cropping systems without biochar can have limited potential to increase soil C stocks and enhance other soil properties. For example, 5 years after establishment in eastern Kansas, switchgrass, big bluestem, and miscanthus (*Miscanthus* × *giganteus*) had not increased soil C concentrations relative to corn but had increased dry soil aggregate mean size (Evers, Blanco-Canqui, Staggenborg, & Tatarko, 2013). Similarly, 2 and 3 years after establishment in eastern Nebraska, switchgrass, low-diversity grass mixture, big bluestem, and Indiangrass had not increased water infiltration or soil organic C concentrations but had increased dry and wet soil aggregate stability compared with corn (Blanco-Canqui, Mitchell, Jin, Schmer, & Eskridge, 2017).

Thus, adding biochar to such systems can directly increase soil C stocks and may enhance other soil properties, thereby allowing more frequent biomass harvesting and/or removal of greater amounts of biomass compared with biomass cropping systems not receiving biochar. A few field studies reported that biochar application at 10 and 20 Mg/ha to soils used for switchgrass production can increase soil C concentrations (Allaire et al., 2015; Backer et al., 2016), reduce soil bulk density (Aller et al., 2017), and increase water infiltration and retention (Sandhu & Kumar, 2017). Therefore, biochar applications could be effective for increasing soil productivity and enhancing the sustainability of dedicated bioenergy production systems in degraded or otherwise low-quality agricultural soils (El-Naggar et al., 2019).

Biochar could interact with row crops and dedicated bioenergy crops to sequester C in the soil and improve soil properties, but such potential interactions have not been documented using long-term field studies. Biochar may differently interact with perennial warm-season grasses relative to row crops (i.e., corn) due to differences in biomass quantity and quality. Long-term field experiments of biochar and cropping systems can be ideal laboratories to study potential biochar priming effects and crop–biochar interactions. The objectives of this field study were to: (a) quantify the impacts of biochar application to corn and perennial bioenergy crops on soil physical, chemical, and biological properties as indicators of soil quality after 6 years of management on sloping silty clay loam soils in southwestern Iowa; (b) investigate potential biochar and bioenergy crop interactions; and (c) compare the impact of perennial bioenergy crops with no-till continuous corn on soil quality indicators. We hypothesized that: (a) application of biochar would increase soil C stocks and improve soil properties, thereby enhancing the sustainability of corn production and dedicated perennial bioenergy crops; (b) perennial biomass cropping systems would improve soil properties compared with no-till continuous corn; and (c) biochar would interact with the cropping systems.

2 | MATERIALS AND METHODS

2.1 | Description of the study site and treatments

This study was conducted in a biochar experiment established in fall 2011 in southwestern Iowa at the Iowa State University Armstrong Research and Demonstration Farm near Atlantic, IA (41°18′29″N, 95°10′19″W). The site has mean annual precipitation of 939 mm and mean annual temperature of 9.3°C. The dominant soil series were Exira silty clay loam (fine-silty, mixed, superactive, mesic Typic Hapludolls; <7% slope) and Marshall silty clay loam (fine-silty, mixed, superactive, mesic Typic Hapludolls; 1% slope). A completely randomized split-plot design was implemented with four replications in 2011. Main plots were Liberty switchgrass, a low-diversity perennial grass mixture, a high-diversity prairie polyculture, and no-till continuous corn. Main plots were divided into two subplots with and without biochar. Biochar was applied in late October 2011 at an average rate of 9.3 Mg/ha on a dry-weight basis. It was incorporated to 15 cm soil depth by chiseling followed by disking. Subplots without biochar received the same tillage treatment. The biochar was derived from mixed wood (*Quercus*, *Ulmus*, and *Carya* spp. woodchips with particle sizes 0.1–2,000 mm) and produced using an augur bed gasification process at 600°C (ICM, Inc.). The biochar had a pH of 8.8, and consisted of 29% ash, 16% volatile matter, 55% fixed C, 63% total C, 2.7% total H, 0.6% total N, 0.06% total P, and 0.86% total K on a dry-weight basis (Bonin et al., 2018; Fidel, Laird, Thompson, & Lawrinenko, 2017).

Our study considered a total of 12 main plots (24 subplots), each main plot was 44 × 68 m in size. Liberty switchgrass seeds were no-till drilled in May 2012 using a Great Plains Drill 1006NT drill (Great Plains Manufacturing) in 19-cm width rows at a rate of ~323 pure live seeds/m^2 but due to poor survival during the 2012 drought, the switchgrass was reseeded in May, 2013. The low-diversity polyculture plots were seeded in May 2012 with a mixture of high-yielding native grasses consisted of 45% big bluestem, 45% Indiangrass, and 10% sideoats grama. The low-diversity perennial grass seeds were broadcast and cultipacked using a Vicon seeder (Kverneland 128 Group) and a Brillion cultipacker (Landoll Corp.) in May 2012 at a rate of ~323 pure live seeds/m^2. The high-diversity polyculture included 44 species of prairie grasses, sedges, forbs, and legumes, and were also seeded in May 2012. Results from the high-diversity polyculture plots are excluded from this report as performance of the prairie species was limited by high weed pressure and, therefore, the high-diversity polyculture was not considered a viable bioenergy cropping system. Additional field management activities during 2012–2017 included planting golden harvest 89-69 or Agrigold 63-95 corn, and spraying herbicides, applying fertilizers, mowing switchgrass and diversity plots, and harvesting biomass and corn grain. Corn plots received 224 kg/ha N as urea and ammonium nitrate at planting. Switchgrass and low-diversity grass plots received 56 kg/ha N as urea, which was surface applied in the spring. Further details of management of this experiment are found in three previous reports (Acharya, Blanco-Canqui, Mitchell, Cruse, & Laird, 2019; Bonin et al., 2018; Fidel et al., 2017). In this study, we focused primarily on soil properties. Crop yields and perennial grass productivity for this experiment were reported by Bonin et al. (2018).

2.2 | Soil sampling and measurements

Time-zero soil samples were collected in fall 2011 before application of biochar and again in November 2017, which was 6 years after experiment establishment. Soil physical properties that include soil bulk density, wet aggregate stability, water infiltration, and water retention, and soil chemical properties that include pH and cation exchange capacity (CEC), and concentration of total N were measured. Water infiltration was measured in the field, while, the rest of soil properties were measured on samples collected from the 0 to 60 cm depth.

We measured soil water infiltration at representative locations in each of the 24 subplots using the double-ring infiltrometer method (Reynolds, Elrick, & Youngs, 2002). The infiltrometer consists of an inner ring with 20 cm diameter and an outer ring with 40 cm diameter. The rings were slowly driven into the soil to about 10 cm depth, and water was added to both rings. Water level in both the outer and inner rings was maintained at the same height throughout the experiment. Change in water level in the inner ring was recorded at 1, 2, 3, 4, 5, 10, 20, 40, 60, 90, 120, 150, and 180 min. Water infiltration was carried out for 3 hr in each plot to compute cumulative infiltration.

Soil cores (60 cm long and 4 cm in diameter) were collected using a truck-mounted hydraulic probe (Giddings Machine Co.) from four locations within each plot. The four soil cores were carefully sliced at the following depth intervals: 0–5, 5–15, 15–30, and 30–60 cm, and then composited by depth. The field moist weights for each soil sample were measured. Subsequently, a subsample of each soil sample was oven-dried at 105°C for 24 hr to determine the gravimetric water content, and then bulk density was determined by the core method (Grossman & Reinsch, 2002). The rest of each soil sample was air-dried for 72 hr. A representative subsample of each air-dried soil sample was sieved through a 2 mm sieve and used to determine pH and CEC. A portion of the air-dried soil was crushed, ground in a roller mill for 24 hr, and analyzed for total C and N by the dry combustion method for the 2011 samples using a Vario Microcube (Elementar) and for the 2017 samples using a Flash 2000 C and N analyzer (CE Elantech; Nelson & Sommers, 1996). Both instruments use the same principle and were calibrated against primary standards and thus yield comparable results. We estimated differences in soil organic C and total N concentrations, CEC, pH, and bulk density between the start of the experiment (2011) and after 6 years (2017) for the 0–5, 5–15, 15–30, and 30–60 cm depths. We also estimated differences in total C stocks, but the estimation was done for 0–30 cm only because changes in soil organic C concentrations were significant only in the upper 30 cm of the soil.

Wet soil aggregate stability was analyzed on a fraction of the air-dried and sieved sample by the wet-sieving method (Nimmo & Perkins, 2002). Approximately 50 g of soil sample sieved through an 8 mm sieve were placed on a filter paper on top of a stack of sieves of different diameters (4.5, 2.0, 1.0, 0.5, 0.25 mm) and saturated with tap water for 10 min by capillarity. Then, the filter paper was carefully removed, and the soil was sieved in tap water for 10 min using custom mechanical wet-sieving equipment, which generates thirty 3-cm up-down strokes/min. Next, soil retained on each sieve was transferred to glass beakers and oven-dried at 105°C. The oven-dried samples were weighed, treated with 100 ml of 0.5% Na hexametaphosphate, left overnight to disperse soil aggregates, and passed through a 0.053 mm sieve. The samples were then oven-dried at 105°C, and weighed again to correct for sand in each aggregate size class. The mass fraction of aggregates in each size class was used to determine the mean weight diameter (MWD) of water-stable aggregates, which is a measure of wet aggregate stability (Nimmo & Perkins, 2002).

To determine soil water retention characteristics, a total of 192 intact soil cores (5 × 5 cm; 4 per subplot) were collected from the 0–5 and 5–10 cm depths. The intact cores were trimmed at both ends, weighed, and saturated with water from the bottom-up using a Mariotte bottle for 24 hr. The saturated soil cores were weighed, transferred to low-pressure extractors, drained for about 10 days until drainage ceased, and weighed to determine volumetric water content at −33 kPa matric potential (Dane & Hopmans, 2002). Water content at −1,500 kPa matric potential was determined using high-pressure extractors (Dane & Hopmans, 2002). Cores drained at −33 kPa potential were air-dried, broken, sieved through 2 mm sieves, and transferred to sample retaining rings placed on the −1,500 kPa matric potential ceramic plates. Soil samples were saturated for 24 hr and then equilibrated at −1,500 kPa in the extractors for about 7 days until drainage stopped. At the end of measurement, soil samples were removed from the extractors, weighed, oven-dried at 105°C for 24 hr, and reweighed to determine gravimetric water content and then volumetric water content based on the bulk density as determined for each intact core (Grossman & Reinsch, 2002). Plant available water was computed as the difference in volumetric water content between −33 kPa (field capacity) and −1,500 kPa (permanent wilting point) potential.

2.3 | Statistical analyses

Data analyses were conducted using PROC MIXED in SAS v. 9.4 for randomized complete split-plot design (SAS, 2019). Data were sorted by depth and analyzed to determine the effect of crop, biochar, and their interactions on soil quality parameters by soil depth. The fixed factors were switchgrass, low-diversity grass, and corn as main plot treatments, and biochar as subplot treatments. Replication was the random factor. Prior to analysis of treatment effects, data were examined for normal distribution using the Shapiro–Wilk test in PROC UNIVARIATE in SAS. Statistical differences were reported at $\alpha = .05$. Statistical analysis on soil organic C concentration and stocks, total N concentration, CEC, pH, and bulk density was conducted on the differences between the start of the experiment (2011) and after 6 years (2017). Because interactions between biochar and bioenergy crops were not significant for any soil property, data were analyzed across either biochar or cropping system treatments.

3 | RESULTS

3.1 | Soil organic carbon accumulation

Changes in soil organic C concentration and stocks between 2011 and 2017 due to biochar application and bioenergy crop adoption were studied. Table 1 shows the data on soil organic C for 2011 and 2017. Both biochar (Figure 1) and bioenergy crops (Tables 2 and 3) had significant effects on changes in soil organic C concentration. However, only biochar significantly changed soil C stocks (Figure 2A). The biochar × bioenergy crop interaction for C concentration for all depth intervals was not significant (Table 2). Similarly,

TABLE 1 Select soil properties measured in 2011 (experiment start) and 2017 (this study) under dedicated bioenergy crops, no-till continuous corn, and biochar application (9.3 Mg/ha) for an experiment on silty clay loams in southwestern Iowa

		Soil depth	Bulk density	Soil organic C	Total N	Bulk density	Soil organic C	Total N
			2011			2017		
Crop	Biochar	cm	Mg/m^3	g/kg	g/kg	Mg/m^3	g/kg	g/kg
Corn	Yes	0–5	1.15	24.00	2.65	1.43	29.68	2.21
Corn	No		1.12	25.22	2.67	1.49	23.43	2.16
Switchgrass	Yes		1.12	23.12	2.29	1.40	31.56	2.48
Switchgrass	No		1.13	23.90	2.30	1.34	28.85	2.46
Low-diversity grass	Yes		1.08	26.09	2.65	1.48	38.76	2.96
Low-diversity grass	No		1.19	23.83	2.52	1.45	29.31	3.18
Corn	Yes	5–15	1.22	12.90	1.44	1.15	18.32	1.55
Corn	No		1.21	15.15	1.60	1.16	14.48	1.43
Switchgrass	Yes		1.19	19.50	1.92	1.11	20.25	1.81
Switchgrass	No		1.20	18.93	1.80	1.14	18.51	1.72
Low-diversity grass	Yes		1.18	16.90	1.73	1.12	21.48	1.83
Low-diversity grass	No		1.28	14.91	1.63	1.17	15.80	1.51
Corn	Yes	15–30	1.21	8.24	0.87	1.13	9.19	0.89
Corn	No		1.19	9.13	0.97	1.12	8.46	0.87
Switchgrass	Yes		1.12	15.56	1.50	1.10	17.72	1.61
Switchgrass	No		1.22	15.01	1.28	1.10	14.77	1.29
Low-diversity grass	Yes		1.17	14.63	1.34	1.12	15.61	1.43
Low-diversity grass	No		1.19	12.22	1.21	1.02	11.93	1.18
Corn	Yes	30–60	1.30	6.21	0.35	1.17	6.23	0.38
Corn	No		1.24	5.89	0.57	1.15	6.08	0.59
Switchgrass	Yes		1.26	14.42	1.31	1.44	14.30	1.57
Switchgrass	No		1.28	10.69	0.87	1.09	10.55	0.71
Low-diversity grass	Yes		1.22	11.60	1.00	1.11	10.42	1.12
Low-diversity grass	No		1.31	10.34	1.01	1.35	10.03	1.61

the biochar × bioenergy crop interaction for C stocks for the 0–30 cm depth was not significant ($p = .93$). The change in soil organic C concentration was significantly higher in biochar-amended than in non-biochar-amended plots in the upper 30 cm of soil (Figure 1). As expected, the change in soil organic C concentration was the largest in the 0–5 cm depth and lowest in the 15–30 cm depth.

Biochar application had a large and significant effect on increasing soil C stocks (Figure 2A). After 6 years, on average soil C stocks increased by 14.07 Mg/ha in the biochar-amended plots, while it increased by 2.25 Mg/ha in the non-biochar-amended plots. This indicates that soil C stocks increased more (11.82 Mg/ha) when biochar was added than when no biochar was added. Most importantly, total C stocks in biochar-amended plots in 2017 were nearly double (14.07 Mg/ha) the amount of C added with the biochar (7.25 Mg/ha).

Dedicated bioenergy crops significantly increased total C concentration between 2011 and 2017, but the difference in changes were significant only for the 0–5 cm soil depth (Tables 2 and 3). Soil C concentration under the low-diversity grass mix increased by about five times compared with corn, but C concentration under switchgrass did not differ from corn and low-diversity grass mixture (Table 3). Soil C stocks among corn, switchgrass, and low-diversity grass mix were not significantly different at any soil depth (Figure 2B).

3.2 | Soil physical and chemical properties

Biochar application at 9.3 Mg/ha did not affect soil properties including bulk density, MWD of water-stable aggregates, volumetric water content at −0.33 and −1,500 kPa water potentials, and plant available water for any depth interval. Similarly, biochar application did not affect water infiltration. Biochar application and perennial grass bioenergy crops did

TABLE 2 Statistical analysis of data on soil bulk density, soil organic C concentration, and soil N concentration for four depth intervals as affected by dedicated bioenergy crops, no-till continuous corn, and biochar application (9.3 Mg/ha) after 6 years for an experiment on silty clay loams in southwestern Iowa

	$p > F$		
Treatments	**Bulk density**	**Soil organic C**	**Soil N**
0–5 cm soil depth			
Crop	ns	.06†	ns
Biochar	ns	*	ns
Crop × Biochar	ns	ns	ns
5–15 cm soil depth			
Crop	ns	ns	ns
Biochar	ns	**	ns
Crop × Biochar	ns	ns	ns
15–30 cm soil depth			
Crop	ns	ns	ns
Biochar	ns	**	ns
Crop × Biochar	ns	ns	ns
30–60 cm soil depth			
Crop	ns	ns	ns
Biochar	ns	ns	ns
Crop × Biochar	ns	ns	ns

Abbreviation: ns, non-significant.

†Differences among crops within this depth were significant only at the .10 probability level.

*Significant at the .05 probability level.

**Significant at the .01 probability level.

TABLE 3 Difference in soil bulk density (mean ± *SD*) and concentrations of soil organic C and total N by soil depth between 2011 (experiment start) and 2017 (this study) under dedicated bioenergy crops and no-till continuous corn averaged across two biochar levels (0 and 9.3 Mg/ha) for an experiment on silty clay loams in southwestern Iowa

	Bulk density	Soil organic C	Total N
Crop	**Mg/m^3**	**g/kg**	**g/kg**
0–5 cm soil depth			
Corn	0.33 ± 0.10	1.94 ± 4.70b	−0.47 ± 0.32
Switchgrass	0.25 ± 0.17	6.70 ± 4.77ab	0.17 ± 0.51
Low-diversity grass	0.33 ± 0.18	9.07 ± 6.98a	0.48 ± 0.47
5–15 cm soil depth			
Corn	−0.11 ± 0.11	2.37 ± 4.04	−0.03 ± 0.26
Switchgrass	0.00 ± 0.39	0.17 ± 2.74	−0.10 ± 0.24
Low-diversity grass	−0.04 ± 0.31	2.73 ± 2.87	−0.01 ± 0.19
15–30 cm soil depth			
Corn	−0.06 ± 0.05	0.14 ± 1.36	−0.04 ± 0.13
Switchgrass	−0.07 ± 0.07	0.96 ± 1.84	0.06 ± 0.18
Low-diversity grass	−0.09 ± 0.10	0.35 ± 1.54	0.03 ± 0.18
30–60 cm soil depth			
Corn	−0.08 ± 0.04	0.11 ± 1.34	0.03 ± 0.15
Switchgrass	−0.07 ± 0.11	−0.13 ± −0.13	0.05 ± 0.29
Low-diversity grass	−0.11 ± 0.23	−0.75 ± −0.75	0.36 ± 0.57

Note: Means with different lowercase letters within crops and biochar treatments are significantly different at the .05 probability level.

not affect changes in total N concentration (Tables 2 and 3) nor soil pH and CEC. Averaged across all treatments, soil pH was 6.9 ± 0.47 (mean ± SD) and CEC was 19.8 ± 2.7 cmol/kg for the 0 to 30 cm depth. The biochar × bioenergy crop interaction for soil physical properties was not significant (p > .10). Thus, data were averaged across either crops or biochar treatments. The perennial bioenergy crops, however, had a significant effect on soil physical properties such as soil aggregate stability, water retention, and plant available water but no effect on bulk density and water infiltration compared with no-till continuous corn. Additionally, neither biochar nor bioenergy crops affected changes in soil bulk density between 2011 and 2017 (Tables 2 and 3).

The switchgrass and low-diversity prairie grass treatments increased MWD of water-stable aggregates by 23% (1.7 mm vs. 2.2 mm) compared with no-till continuous corn in the 0–5 cm depth. In the 5–15 cm depth, low-diversity grass treatment increased MWD of water-stable aggregates by 29% (1.41 mm vs. 1.99 mm) compared with switchgrass and corn treatments. Below 15 cm soil depth, differences in MWD of water-stable aggregates among corn, switchgrass, and low-diversity grass mixture were not significant (data not shown).

Effect of the perennial bioenergy crops on volumetric water content at −33 and −1,500 kPa matric potentials and plant available water was significant but it depended on soil depth (Table 4). The perennial grasses increased volumetric water content at −33 kPa matric potential by 14% compared with corn for the 0–5 cm, but the effects were not significant for the 5–10 cm soil depth. Effect of perennial grass bioenergy crops on volumetric water content at −1,500 kPa matric potential was not significant for the 0–5 cm depth, but, for the 5–10 cm depth, corn and low-diversity grass mixture increased water content compared with switchgrass. Most notably, perennial grass bioenergy crops consistently increased available water in the surface soil. They increased available water by 30% for the 0–5 cm depth and by 29% for the 5–10 cm depth, indicating that effects of perennial grasses on available water were large and significant.

TABLE 4 Mean (± *SD*) wet aggregate stability expressed as mean weight diameter of water-stable aggregates, volumetric water content at −33 and −1,500 kPa matric potentials, and plant available water as affected by dedicated bioenergy crops, no-till continuous corn, and biochar application (9.3 Mg/ha) after 6 years for an experiment on silty clay loams in southwestern Iowa

Treatments	Mean weight diameter of water-stable aggregates (mm)	Water content at −33 kPa (cm^3/cm^3)	Water content at −1,500 kPa (cm^3/cm^3)	Plant available water (cm^3/cm^3)
0–5 cm soil depth				
Corn	1.74 ± 0.38b	0.39 ± 0.03b	0.19 ± 0.02	0.20 ± 0.04b
Switchgrass	2.21 ± 0.58a	0.44 ± 0.03a	0.18 ± 0.01	0.26 ± 0.03a
Low-diversity grass	2.23 ± 0.30a	0.45 ± 0.02a	0.19 ± 0.03	0.26 ± 0.06a
Biochar	1.99 ± 0.53	0.42 ± 0.04	0.19 ± 0.03	0.23 ± 0.06
No biochar	2.13 ± 0.40	0.43 ± 0.03	0.18 ± 0.02	0.24 ± 0.03
5–10 cm soil depth				
Corn	1.41 ± 0.23b	0.38 ± 0.01	0.25 ± 0.02a	0.13 ± 0.01b
Switchgrass	1.45 ± 0.22b	0.39 ± 0.02	0.21 ± 0.01b	0.18 ± 0.03a
Low-diversity grass	1.99 ± 0.37a	0.40 ± 0.02	0.25 ± 0.02a	0.15 ± 0.03b
Biochar	1.58 ± 0.40	0.39 ± 0.02	0.21 ± 0.03	0.17 ± 0.03
No biochar	1.85 ± 0.42	0.39 ± 0.02	0.21 ± 0.02	0.19 ± 0.03
$p > F$				
0–5 cm soil depth				
Crop	.06†	**	ns	*
Biochar	ns	ns	ns	ns
Crop × Biochar	ns	ns	ns	ns
5–10 cm soil depth				
Crop	**	ns	ns	ns
Biochar	ns	ns	ns	ns
Crop × Biochar	ns	ns	ns	ns

Note: Means with different lowercase letters within crops differ at the .05 probability level.

†Differences among crops within this depth were significant only at the .10 probability level.

*Significant at the .05 probability level.

**Significant at the .01 probability level.

4 | DISCUSSION

4.1 | Soil carbon accumulations

Significant increases in soil C concentrations and stocks were measured in this study 6 years after biochar application (Figures 1 and 2), which supports the widely proposed strategy of using biochar applications to store C in soils (Kauffman et al., 2014; Laird, 2008; Matovic, 2011). Plots that received 9.3 Mg/ha of biochar in 2011 stored 11.82 Mg/ha more soil C in the upper 30 cm of the soil profile in 2017 compared with plots without biochar (Figure 2A). Most notably, C stocks in plots with biochar increased by nearly double the amount of C that was added with the biochar (14.07 Mg soil C/ha vs. 7.25 Mg biochar C/ha; Figure 2A) in the 0–30 cm soil depth. This finding indicates biochar either decreased the rate of soil organic C mineralization and/or increased the efficiency of crop residue C stabilization (reduced mineralization of crop residue C), processes collectively known as "negative priming."

The mechanisms responsible for the negative priming observed in this study are not clear. Bonin et al. (2018) reported that biochar application had no effect on corn and perennial grass yields for this study. Thus, the increase in biogenic soil organic C cannot be attributed to an increase in aboveground residue C inputs. Furthermore, Fidel et al. (2017) found that soil CO_2 fluxes did not significantly differ between plots with and without biochar 3 years after biochar application. Not investigated, however, is the possibility that biochar increased belowground C inputs through root proliferation, which has been observed in other studies (Olmo, Villar, Salazar, & Alburquerque, 2016).

While mechanisms responsible for the accumulation of biogenic soil organic C in the biochar-amended plots are not yet fully understood, we suggest, based on literature, that time after biochar application, soil textural class, soil water content, biochar pyrolysis temperature, and quality and

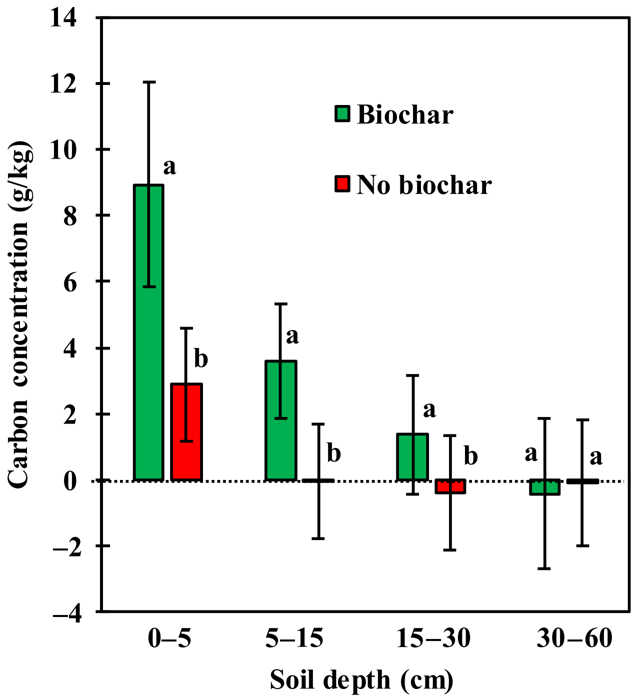

FIGURE 1 Differences in soil organic C concentrations between 2011 and 2017 by soil depth due to biochar application (9.3 Mg/ha) averaged across dedicated bioenergy crops and no-till continuous corn, for an experiment on silty clay loams in southwestern Iowa. Different lowercase letters indicate significant differences between biochar treatments at the .05 probability level for each soil depth. The error bars indicate standard deviation of the mean

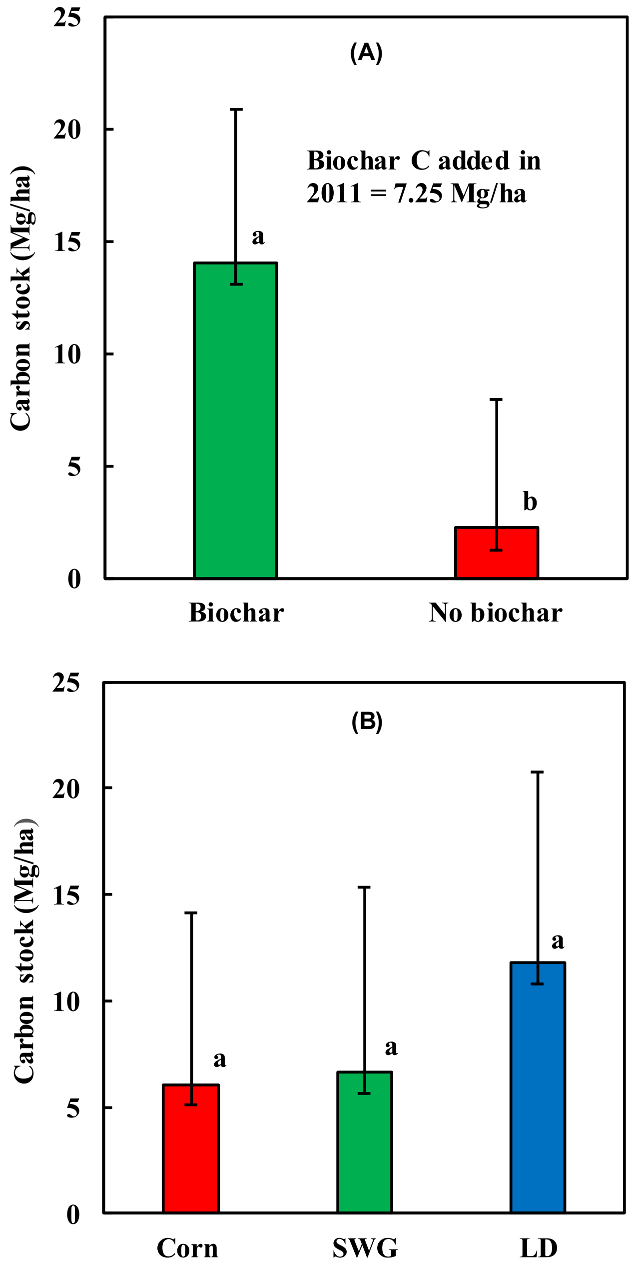

FIGURE 2 Difference in soil organic C stocks between 2011 and 2017 in the 0–30 cm depth for: (A) biochar treatments (0 and 9.3 Mg/ha) averaged across cropping systems and (B) dedicated bioenergy crops and no-till continuous corn averaged across biochar levels after 6 years for an experiment on silty clay loams in southwestern Iowa. Different lowercase letters indicate significant differences between and treatments at the .05 probability level. The error bars indicate standard deviation of the mean. LD, low-diversity grass; SWG, switchgrass

quantity of initial soil C are potential factors affecting priming (Ding et al., 2018; Maestrini et al., 2015; Wang et al., 2016). For instance, the negative priming effect can increase with an increase in soil water content, biochar pyrolysis temperature, and soil clay content (Ding et al., 2018). A review of 21 studies, although mostly laboratory studies, concluded that biochar retarded the mineralization of soil organic matter by 3.8% in fine-textured soils, but it accelerated mineralization of soil organic matter by 20.8% in sandy and low-fertility soils (Wang et al., 2016). Several studies indicated that biochar application could induce a positive priming effect in the short term (<2 years) but induce a negative priming effect in the long term (Ding et al., 2018; Maestrini et al., 2015). Our study was clearly long enough (6 years) for negative priming effect of the applied biochar to be apparent. In the long term, the biochar could promote C accumulation through adsorption and physical protection of dissolved organic C from the soil solution, inducing the negative priming effect (Maestrini et al., 2015).

While negative priming attributed to biochar has been previously observed in laboratory incubation studies; the increase in soil C stocks by twice the amount of biochar C applied in this study is, to our knowledge, the first field evidence for the negative priming effect and highlights the potential use of biochar as a C sequestration agent. The doubling of C stocks with biochar application can have large implications for soil C management in croplands. This potential of biochar to increase soil C both directly through addition of recalcitrant biochar C and indirectly through

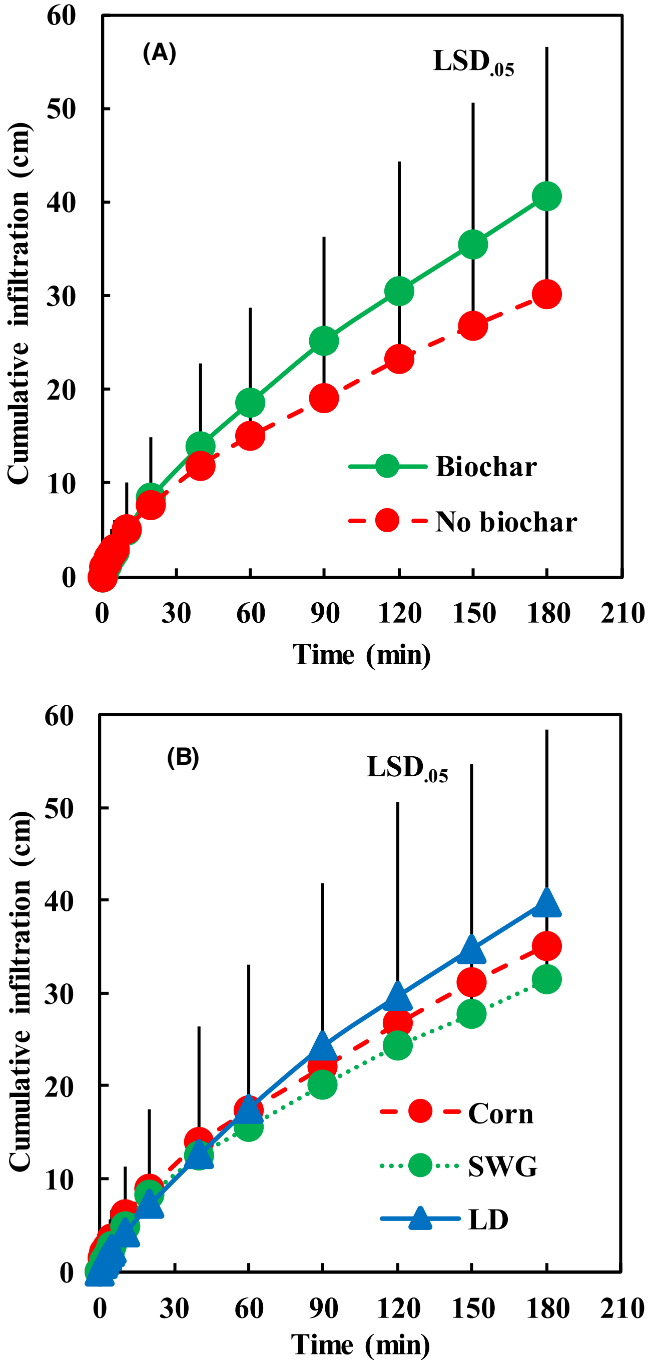

FIGURE 3 Cumulative water infiltration for (A) biochar application levels (0 and 9.3 Mg/ha) averaged across cropping systems and (B) dedicated bioenergy crops and no-till continuous corn averaged across biochar levels after 6 years for an experiment on silty clay loam soils in southwestern Iowa. The error bars are the least significant differences at the .05 probability level. LD, low-diversity grass; SWG, switchgrass

negative priming deserves further investigation to optimize the management practices for C sequestration. Specifically, mechanistic models that can predict the magnitude and direction of the priming effect for different soil textural classes, soil organic matter levels, cropping systems, climates, and management systems under various biochar amendment scenarios are needed.

The increase in soil C concentration near the soil surface under low-diversity grass mixture (p = .07) but not under switchgrass (p = .20) suggests that perennial grasses can have variable effects on soil C concentrations. Previous studies on warm-season grasses from the region found no effect of perennial grasses on soil organic C concentrations in short-term (<5 years) field studies. In Kansas, after 5 years, soil C concentrations under switchgrass, big bluestem, miscanthus, and corn did not significantly differ (Evers et al., 2013). In Nebraska, after 2 and 3 years, switchgrass, big bluestem (*Andropogon gerardii* Vitman), and low-diversity grass mixture [big bluestem, Indiangrass (*Sorghastrum nutans* (L.)] did not affect soil C concentrations when compared with corn (Blanco-Canqui et al., 2017). The significant increase in soil organic C concentrations with low-diversity grass mixture in this study, unlike in previous studies, may be due to the relatively longer duration of the present study (6 years).

The lack of effect of both perennial grasses (switchgrass and low-diversity grass mixture) on C stocks for the 0–30 cm depth compared with corn indicates that 6 years may not be enough time for perennials to increase C stocks. We expect that perennial grasses will increase C stocks in the long term (>6 years). For example, in eastern Nebraska, switchgrass when used as conservation buffers accumulated about 0.85 Mg ha^{-1} year^{-1} of soil C in the 0–15 cm soil depth after 15 years compared to adjacent row crops (Blanco-Canqui, Gilley, Eisenhauer, Jasa, & Boldt, 2014).

Our critical question in this study was whether there would be a synergistic interaction between biochar and bioenergy crops for sequestering C and improving soil properties. Our analysis indicated that biochar × bioenergy crop interaction for C concentrations and stocks was, however, not significant (p > .10; Table 2; Figure 1). The lack of significant interaction between biochar and bioenergy crops was somewhat surprising. We expected that biochar would increase soil C stocks under perennial grasses more than under corn due to differences in quality, quantity, and timing of residue C input to the soils. The lack of a significant interaction could be due to the relatively high variability in soil organic C within plots. Alternatively, the negative biochar priming effects on soil organic C as well as the extent to which biochar sequesters C may not depend on the cropping system (annual row crops vs. perennial bioenergy crops). Clearly, additional studies are needed to further explore the potential biochar × cropping system interactions. However, results indicate that perennial grasses can increase soil C concentration relative to corn regardless of biochar application. The increased soil C concentration under perennial grasses is likely due to the increased root biomass and root exudate C, and increased wet soil aggregate stability under perennial grasses relative to corn. Indeed, soil C concentration was positively correlated with wet aggregate stability across all treatments. Previous studies suggested that perennial grass can protect soil C by enhancing soil aggregate formation and stability (Kravchenko et al., 2019; Tiemann & Grandy, 2015).

4.2 | Soil physical and chemical properties

The lack of differences in soil physical properties such as bulk density, wet soil aggregate stability, infiltration, water retention, available water, soil pH, and CEC between the biochar and no-biochar plots after 6 years indicates that application of 9.3 Mg/ha biochar had limited or no effects on soil chemical and physical properties for these silty clay loam soils (Tables 2 and 3; Figures 1–3). The results are not entirely surprising. Several previous studies found that biochar does not always rapidly improve soil properties, particularly physical properties, depending on management (Blanco-Canqui, 2017; Prober, Stol, Piper, Gupta, & Cunningham, 2014; Rogovska, Laird, & Karlen, 2016). In this study, the lack of effects of biochar on soil properties can be due to various factors including: (a) amount of biochar applied; (b) experiment duration; and (c) soil textural class, among others (Blanco-Canqui, 2017; Glab, Palmowska, Zaleski, & Gondek, 2016; Kameyama, Miyamoto, Iwata, & Shiono, 2016):

- The amount of biochar used in this study may not have been high enough to alter soil physical properties. For example, previous studies suggested that biochar application at rates <10 Mg/ha may not affect soil hydraulic properties (Glab et al., 2016; Kameyama et al., 2016), but application rates >10 Mg/ha can increase available water (Blanco-Canqui, 2017).
- Time (6 years) after biochar application may not have been long enough for biochar to change soil physical properties, which can be slower to respond to management than chemical and biological properties. Effects could develop in the longer term (>6 years) as biochar ages and reacts with other soil constituents. For example, old wood biochar can absorb more water or repel less water than fresh wood biochar (Aller et al., 2017; Briggs, Breiner, & Graham, 2012), which suggests that water retention capacity of soil–biochar mixtures may increase with time after application.
- The soils at our study site were silty clay loams, which may be slower to respond to biochar application due to the high clay content relative to coarse-textured soils. Previous studies suggested that sandy soils are more responsive to biochar application than fine-textured soils (Blanco-Canqui, 2017).

Results from this study indicate, however, that perennial bioenergy crops such as switchgrass and low-diversity prairie grass had larger effects on soil physical properties than biochar application (Tables 2 and 3). The increase in wet soil aggregate stability, water retention, and available water with perennial bioenergy crops near the soil surface suggest that planting perennial warm-season grasses on sloping croplands and fine-textured soils can improve soil physical properties. Specifically, based on our results, growing perennial bioenergy crops could improve soil structural stability and the ability of the soil to retain plant available water relative to corn (Table 4). These results agree with previous studies in the region, which found that switchgrass can increase wet aggregate stability and water retention in the long term (>10 years; Blanco-Canqui, 2017; Rachman, Anderson, Gantzer, & Alberts, 2004). Indeed, the significant increase in plant available water under perennial grasses for the whole soil profile (60 cm depth; Table 4) indicates that growing perennial grasses can enhance the ability of the soil to absorb and retain water relative to corn production. The increase in soil organic C concentration with perennial bioenergy crops was partly responsible for the increase in wet aggregate stability. The increase in soil organic C concentration increased wet aggregate stability ($r = .41$; $p = .04$) but the correlation with available water ($r = .32$; $p > .10$) was not significant for the 0–5 cm depth.

The lack of significant effects of perennial bioenergy crops on water infiltration can be primarily due to the short duration of the experiment (Figure 3). A previous study found higher water infiltration under switchgrass than under corn in the longer term (10 years; Rachman et al., 2004). Thus, we suggest that perennial bioenergy crops may require more than 6 years before the increase in water infiltration can be detected relative to corn. Full potential of switchgrass to alter soil quality properties may manifest 10–15 years after establishment (Bharati, Lee, Isenhart, & Schultz, 2002; Corre, Schnabel, & Shaffer, 1999; Rachman et al., 2004).

In summary, biochar application to soil used for corn and dedicated bioenergy crops increased soil C stocks more than the amount of C added with the biochar, indicating a negative priming effect of biochar after 6 years under field conditions, whereas the perennial bioenergy crops improved several soil quality parameters but had only a small effect on soil C after 6 years in these Midwestern US soils. Surprisingly, the interaction between biochar and cropping systems was not significant ($p > .10$), suggesting that the negative priming effect and C sequestration potential of biochar did not differ for annual corn and perennial bioenergy crops.

ACKNOWLEDGEMENTS

This project was supported by the Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30411 from the USDA National Institute of Food and Agriculture. The authors also thank Dr. Kent Eskridge for his assistance with the statistical analysis of the data, and ICM Corporation for providing the biochar.

ORCID

Humberto Blanco-Canqui https://orcid.org/0000-0002-9286-8194

REFERENCES

Acharya, B. S., & Blanco-Canqui, H. (2018). Lignocellulosic-based bioenergy and water quality parameters: A review. *GCB Bioenergy*, *10*, 504–533. https://doi.org/10.1111/gcbb.12508

Acharya, B. S., Blanco-Canqui, H., Mitchell, R. B., Cruse, R., & Laird, D. (2019). Dedicated bioenergy crops and water erosion. *Journal of Environmental Quality*, *48*, 485–492. https://doi.org/10.2134/jeq2018.10.0380

Allaire, S. E., Baril, B., Vanasse, A., Lange, S. F., MacKay, J., & Smith, D. L. (2015). Carbon dynamics in a biochar-amended loamy soil under switchgrass. *Canadian Journal of Soil Science*, *95*, 1–13. https://doi.org/10.4141/cjss-2014-042

Aller, D., Mazur, R., Moore, K., Hintz, R., Laird, D., & Horton, R. (2017). Biochar age and crop rotation impacts on soil quality. *Soil Science Society of America Journal*, *81*, 1157–1167. https://doi.org/10.2136/sssaj2017.01.0010

Backer, R. G., Schwinghamer, T. D., Whalen, J. K., Seguin, P., & Smith, D. L. (2016). Crop yield and SOC responses to biochar application were dependent on soil texture and crop type in southern Quebec, Canada. *Journal of Plant Nutrition and Soil Science*, *179*, 399–408. https://doi.org/10.1002/jpln.201500520

Bharati, L., Lee, K. H., Isenhart, T. M., & Schultz, R. C. (2002). Soil-water infiltration under crops, pasture, and established riparian buffer in Midwestern USA. *Agroforestry Systems*, *56*, 249–257. https://doi.org/10.1023/A:1021344807285

Blanco-Canqui, H. (2017). Biochar and soil physical properties. *Soil Science Society of America Journal*, *81*, 687–711. https://doi.org/10.2136/sssaj2017.01.0017

Blanco-Canqui, H., Gilley, J., Eisenhauer, D. E., Jasa, P. J., & Boldt, A. (2014). Soil carbon accumulation under switchgrass barriers. *Agronomy Journal*, *106*, 2185–2192. https://doi.org/10.2134/agronj14.0227

Blanco-Canqui, H., Mitchell, R. B., Jin, V. L., Schmer, M. R., & Eskridge, K. M. (2017). Perennial warm-season grasses for producing biofuel and enhancing soil properties: An alternative to corn residue removal. *GCB Bioenergy*, *9*, 1510–1521. https://doi.org/10.1111/gcbb.12436

Blanco-Canqui, H., Stalker, A. L., Rasby, R., Shaver, T. M., van Drewnoski, M. E., Donk, S., & Kibet, L. (2016). Does cattle grazing and baling of corn residue increase water erosion? *Soil Science Society of America Journal*, *80*, 168–177. https://doi:10.2136/sssaj2015.07.0254

Blanco-Canqui, H., Tatarko, J., Stalker, A., Shaver, T., & Van Donk, S. (2016). Impacts of corn residue grazing and baling on wind erosion potential in a semiarid environment. *Soil Science Society of America Journal*, *80*, 1027–1037. https://doi.org/10.2136/sssaj2016.03.0073

Bonin, C. L., Fidel, R. B., Banik, C., Laird, D. A., Mitchell, R., & Heaton, E. A. (2018). Perennial biomass crop establishment, community characteristics, and productivity in the upper US Midwest: Effects of cropping systems seed mixtures and biochar applications. *European Journal of Agronomy*, *101*, 121–128. https://doi.org/10.1016/j.eja.2018.08.009

Briggs, C., Breiner, J. M., & Graham, R. C. (2012). Physical and chemical properties of *Pinus ponderosa* charcoal: Implications for soil modification. *Soil Science*, *177*, 263–268. https://doi.org/10.1097/SS.0b013e3182482784

Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., … Kuzyakov, Y. (2014). Soil C and N availability determine the priming effect: Microbial N mining and stoichiometric decomposition theories. *Global Change Biology*, *20*, 2356–2367. https://doi.org/10.1111/gcb.12475

Corre, M. D., Schnabel, R. R., & Shaffer, J. A. (1999). Evaluation of soil organic carbon under forests, cool-season and warm-season grasses in the northeastern US. *Soil Biology and Biochemistry*, *31*, 1531–1539. https://doi.org/10.1016/S0038-0717(99)00074-7

Dane, J. H., & Hopmans, J. H. (2002). Water retention and storage. In J. H. Dane & G. C. Topp (Eds.), *Methods of soil analysis: Part 4, SSSA book series, 5* (pp. 671–717). Madison, WI: SSSA.

Ding, F., Van Zwieten, L., Zhang, W., Weng, Z., Shi, S., Wang, J., & Meng, J. (2018). A meta-analysis and critical evaluation of influencing factors on soil carbon priming following biochar amendment. *Journal of Soils and Sediments*, *18*, 1507–1517. https://doi.org/10.1007/s11368-017-1899-6

Dong, X., Singh, B. P., Li, G., Lin, Q., & Zhao, X. (2018). Biochar application constrained native soil organic carbon accumulation from wheat residue inputs in a long-term wheat-corn cropping system. *Agriculture Ecosystems & Environment*, *252*, 200–207. https://doi.org/10.1016/j.agee.2017.08.026

El-Naggar, A., Lee, S. S., Rinklebe, J., Farooq, M., Song, H., Sarmah, A. K., … Ok, Y. S. (2019). Application of biochar to low fertility soils: A review of current status, and future prospects. *Geoderma*, *337*, 536–554. https://doi.org/10.1016/j.geoderma.2018.09.034

Evers, B. J., Blanco-Canqui, H., Staggenborg, S. A., & Tatarko, J. (2013). Dedicated bioenergy crop impacts on soil wind erodibility and organic carbon in Kansas. *Agronomy Journal*, *105*, 1271–1276. https://doi.org/10.2134/agronj2013.0072

Fidel, R. B., Laird, D. A., Thompson, M. L., & Lawrinenko, M. (2017). Characterization and quantification of biochar alkalinity. *Chemosphere*, *167*, 367–373. https://doi.org/10.1016/j.chemosphere.2016.09.151

Glab, T., Palmowska, J., Zaleski, T., & Gondek, K. (2016). Effect of biochar application on soil hydrological properties and physical quality of sandy soil. *Geoderma*, *281*, 11–20. https://doi.org/10.1016/j.geoderma.2016.06.028

Grossman, R. B., & Reinsch, T. G. (2002). Bulk density and linear extensibility. In J. H. Dane & G. C. Topp (Eds.), *Methods of soil analysis: Part 4, SSSA book series, 5* (pp. 201–225). Madison, WI: SSSA.

Kameyama, K., Miyamoto, T., Iwata, Y., & Shiono, T. (2016). Effects of biochar produced from sugarcane bagasse at different pyrolysis temperatures on water retention of a calcaric dark red soil. *Soil Science*, *181*, 20–28. https://doi.org/10.1097/SS.0000000000000123

Kauffman, N., Dumortier, J., Hayes, D. J., Brown, R. C., & Laird, D. A. (2014). Producing energy while sequestering carbon? The relationship between biochar and agricultural productivity. *Biomass and Bioenergy*, *63*, 167–176. https://doi.org/10.1016/j.biombioe.2014.01.049

Kravchenko, A. N., Guber, A. K., Razavi, S., Koestel, J., Quigley, M. Y., Robertson, G. P., & Kuzyakov, Y. (2019). Microbial spatial footprint as a driver of soil carbon stabilization. *Nature Communications*, *10*, 3121. https://doi.org/10.1038/s41467-019-11057-4

Laird, D. A. (2008). The charcoal vision: A win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. *Agronomy Journal*, *100*, 178–181. https://doi.org/10.2134/agronj2007.0161

Laird, D. A., & Chang, C. W. (2013). Long-term impacts of residue harvesting on soil quality. *Soil and Tillage Research*, *134*, 33–40. https://doi.org/10.1016/j.still.2013.07.001

Maestrini, B., Nannipieri, P., & Abiven, S. (2015). A meta-analysis on pyrogenic organic matter induced priming effect. *GCB Bioenergy*, *7*, 577–590. https://doi.org/10.1111/gcbb.12194

Matovic, D. (2011). Biochar as a viable carbon sequestration option: Global and Canadian perspective. *Energy*, *36*, 2011–2016. https://doi.org/10.1016/j.energy.2010.09.031

MEA (Millennium Ecosystem Assessment). (2005). *Ecosystems and human well-being*. Washington, DC: Island Press.

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter: Laboratory methods. In D. L. Sparks (Ed.), *Methods of soil analysis: Part 3, SSSA book series, 5* (pp. 961–1010). Madison, WI: SSSA and ASA.

Nimmo, J. R., & Perkins, K. S. (2002). Aggregate stability and size distribution. In J. H. Dane & G. C. Topp (Eds.), *Methods of soil analysis: Part 4, SSSA book series, 5* (pp. 317–327). Madison, WI: SSSA.

Olmo, M., Villar, R., Salazar, P., & Alburquerque, J. A. (2016). Changes in soil nutrient availability explain biochar's impact on wheat root development. *Plant and Soil*, *399*, 333–343. https://doi.org/10.1007/s11104-015-2700-5

Poeplau, C., & Don, A. (2015). Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. *Agriculture, Ecosystems & Environment*, *200*, 33–41. https://doi.org/10.1016/j.agee.2014.10.024

Prober, S. M., Stol, J., Piper, M., Gupta, V., & Cunningham, S. A. (2014). Enhancing soil biophysical condition for climate-resilient restoration in mesic woodlands. *Ecological Engineering*, *71*, 246–255. https://doi.org/10.1016/j.ecoleng.2014.07.019

Rachman, A., Anderson, S. H., Gantzer, C. J., & Alberts, E. E. (2004). Soil hydraulic properties influenced by stiff-stemmed grass hedge systems. *Soil Science Society of America Journal*, *68*, 1386–1393. https://doi.org/10.2136/sssaj2004.1386

Reed, E. Y., Chadwick, D. R., Hill, P. W., & Jones, D. L. (2017). Critical comparison of the impact of biochar and wood ash on soil organic matter cycling and grassland productivity. *Soil Biology and Biochemistry*, *110*, 134–142. https://doi.org/10.1016/j.soilbio.2017.03.012

Reynolds, W. D., Elrick, D. E., & Youngs, E. G. (2002). Field methods (vadose and saturated zone techniques). In J. H. Dane & G. C. Topp (Eds.), *Methods of soil analysis: Part 4, SSSA book series, 5* (pp. 817–877). Madison, WI: SSSA.

Rogovska, N., Laird, D. A., & Karlen, D. L. (2016). Corn and soil response to biochar application and stover harvest. *Field Crops Research*, *187*, 96–106. https://doi.org/10.1016/j.fcr.2015.12.013

Ruis, S., Blanco-Canqui, H., Burr, C., Olson, B., Reiman, M., Rudnick, D., … Shaver, T. (2018). Corn residue baling and grazing impacts on soil carbon stocks and other properties on a Haplustoll. *Soil Science Society of America Journal*, *82*, 202–213. https://doi.org/10.2136/sssaj2017.05.0177

Sandhu, S. S., & Kumar, S. (2017). Impact of three types of biochar on the hydrological properties of eroded and depositional landscape positions. *Soil Science Society of America Journal*, *81*, 878–888. https://doi.org/10.2136/sssaj2016.07.0230

SAS Institute. (2019). *The SAS system for windows*. V. 9.3. Cary, NC: SAS Institution.

Shanta, N., Schwinghamer, T., Backer, R., Allaire, S. E., Teshler, I., Vanasse, A., … Smith, D. L. (2016). Biochar and plant growth promoting rhizobacteria effects on switchgrass (*Panicum virgatum* cv. Cave-in-Rock) for biomass production in southern Québec depend on soil type and location. *Biomass and Bioenergy*, *95*, 167–173. https://doi.org/10.1016/j.biombioe.2016.10.005

Tiemann, L. K., & Grandy, A. S. (2015). Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *GCB Bioenergy*, *7*, 161–174. https://doi.org/10.1111/gcbb.12126

Ventura, M., Alberti, G., Panzacchi, P., Delle Vedove, G., Miglietta, F., & Tonon, G. (2019). Biochar mineralization and priming effect in a poplar short rotation coppice from a 3-year field experiment. *Biology and Fertility of Soils*, *55*, 67–78. https://doi.org/10.1007/s00374-018-1329-y

Wang, J., Xiong, Z., & Kuzyakov, Y. (2016). Biochar stability in soil: Meta-analysis of decomposition and priming effects. *GCB Bioenergy*, *8*, 512–523. https://doi.org/10.1111/gcbb.12266

How to cite this article: Blanco-Canqui H, Laird DA, Heaton EA, Rathke S, Acharya BS. Soil carbon increased by twice the amount of biochar carbon applied after 6 years: Field evidence of negative priming. *GCB Bioenergy*. 2020;12:240–251. https://doi.org/10.1111/gcbb.12665