

Multi-year and multi-location soil quality and crop biomass yield responses to hardwood fast pyrolysis biochar



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ABSTRACT

Biochar can remediate degraded soils and maintain or improve soil health, but specific and predictable effects on soil properties and crop productivity are unknown because of complex interactions associated with climate patterns, inherent soil characteristics, site-specific crop and soil management practices, and the source, production characteristics, and amount of biochar applied. This multi-location field study was designed and conducted to determine if consistent response patterns could be elucidated by controlling the type and amount of biochar applied, depth of incorporation, and soil/crop management practices as much as possible for six U.S. locations. When averaged for five reporting locations, biochar or biochar plus manure (bio + man) treatments significantly ($P < 0.001$) increased surface (0–15 cm) soil organic carbon (SOC) levels by 48 or 47%, respectively, relative to control treatments. The SOC levels for the manure only treatment were not significantly different from the control. No other measured soil properties showed significant biochar or biochar × manure interactions, even though applying manure significantly increased extractable K, Mg, Na, and P levels. Analysis of three or four years of pooled biomass yield data from the six locations showed a significant location effect ($P < 0.001$), but treatment effects were not significant. However, dividing annual plot yields by the average for all control plots at each location created a dataset of relative yields that showed a significant location × treatment interaction and higher normalized yields (36%) due to biochar ($P = 0.017$) at one of the six locations. Overall, we conclude that hardwood biochar produced by fast pyrolysis can be an effective soil amendment for increasing SOC levels within a broad range of temperate soils, but crop yield responses should be anticipated only when specific soil quality problems limit productivity.

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1. Introduction

Biochar, the solid co-product of thermochemical bioenergy production, has recently received considerable attention as a soil amendment because it has the potential to simultaneously sequester C, improve soil quality, and increase crop productivity (Lehmann, 2007; Laird, 2008 and Laird et al., 2009). These positive agronomic and environmental outcomes, however, are often not fully realized especially for temperate region soils.

The C in biochar is generally regarded as highly recalcitrant to microbial degradation in soils and is anticipated to have a half-life ranging from 100 s to 1000s of years in soil environments (Lehmann et al., 2009 and Lehmann et al., 2015). The long half-life of biochar C is supported by substantial although largely anecdotal evidence of ancient (>1000 years old) biochar C in soils that was produced by natural vegetation fires or deliberately incorporated into agricultural soils by indigenous agricultural societies (Glaser et al., 2001, Skjemstad et al., 2002 and Laird et al., 2008). Less clear is the impact of biochar amendments on the rate of biogenic soil organic matter mineralization and the stabilization (humification) of fresh residue biomass (Ameloot et al., 2013). Several studies have reported evidence that biochar may stimulate mineralization of biogenic soil organic matter (Hamer et al., 2004, Wardle et

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al., 2008 and Zimmerman et al., 2011). By contrast, other studies have reported synergistic interactions whereby biochar apparently enhances stabilization of fresh manure or crop residue C (Fang et al., 2015, Weng et al., 2015 and Chen et al., 2015). Both of these processes may occur simultaneously; for example, in a soil microcosm study biochar additions increased CO₂ emissions suggesting enhanced microbial activity and faster SOC mineralization, but when both biochar and manure were added to the soil the rate of mineralization of the manure C was reduced (Rogovska et al., 2011). A meta-analysis of literature data (Ameloot et al., 2013) indicated that increased CO₂ emissions from soils after biochar addition may result from priming of native SOC, biodegradation of labile fractions of the biochar C, and/or the abiotic release of CO₂ from carbonates and chemisorbed CO₂. The meta-analysis also found that the stability of biochar C in soils increased with the peak pyrolysis temperature and C content of the resulting biochar. The viability of using soil biochar amendments for C sequestration to help mitigate climate change depends on the net long-term impact of the amendments on SOC. Multi-year coordinated field trials using the same biochar and management protocols are needed to determine whether soil by climate interactions influence the impact of biochar amendments on SOC under field conditions.

In the absence of a C credit market or other programs to incentivize C sequestration, the economic viability of the emerging biochar industry depends on the ability of biochar to increase crop yields. A meta-analysis (Jeffery et al., 2011) of 17 studies found considerable variability (range from –28% to +39%), but an overall small mean positive (10%) crop yield response to biochar applications. Jeffery et al. (2011) reported the greatest positive responses for trials conducted on acidic and coarse textured soils, suggesting that the ability of biochar to function as a liming agent and to increase retention of plant available water by soils are important, if not the dominant, mechanisms by which biochar amendments increase crop yields. Another comprehensive study integrating data from 84 studies (Crane-Droesch et al., 2013) found a positive crop yield response to biochar applications for soils with low cation exchange capacity (CEC) and low soil organic C (SOC) levels; however, no relationship between biochar properties and crop yield responses was detected. Crane-Droesch et al. (2013) concluded that biochar has potential to increase crop yields in highly weathered soils of the humid tropics but is less likely to increase yields in nutrient rich temperate region soils. A third meta-analysis of data from 114 studies also found considerable variability in soil and crop response to biochar applications, but significant increases in mean SOC, pH, microbial biomass, total N, plant available P and K, and a small mean increase in above ground biomass production (Biederman and Harpole, 2013).

The pattern emerging from the literature is that positive crop yield responses to biochar applications are commonly observed on sandy, acidic, and highly weathered soils, while little or no yield response is often observed for high quality temperate region soils (Spokas et al., 2012). There are, however, exceptions to this rule: In two separate studies, Rogovska et al. (2014, 2016) reported maize grain yield increases in response to biochar applications on Iowa Mollisols when high levels of surface residue were present, but no response when crop residues were reduced or removed. The most plausible explanation was that biochar mitigated an allopathic response to high residue levels by adsorbing phytotoxic compounds released during residue decomposition. By contrast, Lentz and Ippolito (2012) found no maize silage yield response to biochar applications on irrigated calcareous soils in Idaho the first year after application and a 36% yield decrease relative to controls the second year of the study. Subsequent analysis (Lentz et al., 2014) showed that biochar reduced net N mineralization and soil CO₂ emissions, indicating that biochar reduced gross N mineralization and increased immobilization. The yield reduction in year 2 was associated with unusually high soil ammonium-N concentrations (relative to nitrate-N). This suggests that biochar inhibited ammonium-N uptake by the corn plants, perhaps by sequestering soil NH₄-N. We are beginning to understand the mechanisms and processes by which biochar

influences crop yields; however, all of the studies reviewed above are 'one-off' studies in which different types of biochar, soils, climates, crops, and management protocols were employed. There are no coordinated multi-location studies that assess the differential soil quality and crop yield response to biochar under different climates and on different soils.

Our goal was to assess soil property and crop biomass yield responses to common biochar and manure application protocols across multiple locations with diverse soils, climates, but common fertilizer (inorganic and manure) and cropping system management protocols. We hypothesized; 1) synergistic interactions between manure and biochar would enhance soil quality and boost crop biomass yields, 2) complex soil-climate-biochar interactions would result in different soil quality and crop yield responses in different locations, and 3) the common biochar used in this study would be similarly effective for C sequestration across multiple soils and climates.

2. Materials and methods

2.1. Experimental site description

This experiment was conducted in field plots located at six USDA-ARS locations across the USA (Table S1). Individual plot size ranged from 16 to 34 m², had management histories of either no-till or conservation tillage (Table S1) and either continuous corn (*Zea mays* L.) (5 sites) or sorghum (*Sorghum bicolor* L.) (1 site). The field plots were established in the fall of 2008 at three locations (Ames IA, Kimberly ID, and St. Paul MN), Fall of 2009 for two locations (Prosser WA and Big Springs TX), and one location in 2010 (Bowling Green KY; Table S2). The four treatments at each location were: (1) *control* (no amendments); (2) *manure* (a manure application based on local soil test values); (3) *biochar* (a 20 Mg ha⁻¹ biochar application); and (4) *bio + man* (a combined manure + biochar application using the same rates as for the manure and biochar treatments). The biochar and manure were manually applied and then incorporated with rotary tillage (0.15 m deep). The treatments were applied once at the beginning of the experiment with three replications of each treatment at each location.

2.2. Hardwood biochar production and characterization

A hardwood biochar was produced using fast pyrolysis (500–600 °C) by Dynamotive Technologies Corp. (West Lorne, Ontario, Canada) using sawdust generated during the production of wood flooring. Thus, the feedstock was mixed species of hardwood. The chemical and physical properties of the biochar are given in Table S3. The biochar was shipped to the individual sites in sealed drums.

2.3. Agronomic management

Corn was planted at five locations and sorghum was planted at the sixth location; the plots were managed and crop yield data collected for either three or four crop years at each location (Table S2). All plots received N-P-K fertilization following best management practices at each location (Table S2). Each location chemically managed weeds and insect pressure according to local best management practices, again universally across all treatments. Three locations (IA, MN, and KY) relied on natural precipitation, and two locations (ID and WA) required supplemental irrigation to ensure crop production (Fig. S1).

Above ground biomass (grain + stover) yields were estimated for each plot by hand harvesting (stalks cut ~25-mm above the soil surface) a known area (varied by location) selected randomly from the central portion of each plot. We calculated the total above-ground crop productivity (kg ha⁻¹) as the sum of the dry grain yield and above ground stalks & leaves. At the ID location, the grain yield was not collected separately (silage processing).

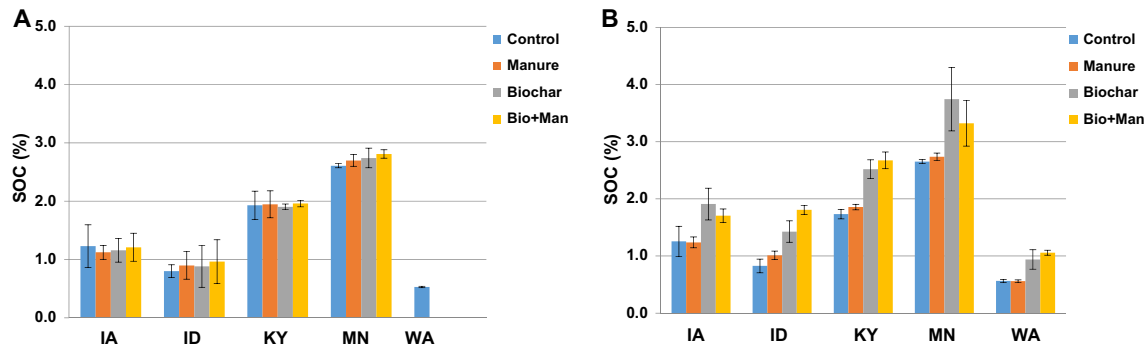


Fig. 1. Average surface (0–15 cm) soil organic C content (A) for samples collected before treatments were imposed and (B) for samples collected after treatments were imposed. Error bars are standard deviations.

2.4. Soil sampling

Soil samples were collected prior to application of the treatments and at least twice after the treatments had been in place. The total number of soil sampling times varied by location with KY sampling a total of 12 times, ID sampling 4 times, and IA, MN, and WA each sampling a total of three times. Soils data was not collected at the TX location. The time after treatment for the last soil sampling ranged from 730 days at the MN site to 1520 days at the WA site. Composite samples were collected from the 0–15 cm and 15–30 cm depths at all locations with the exception of MN where samples were collected from the 0–5 cm, 5–15 cm, and 15–30 cm depths. For purposes of this study, weighted average 0–15 cm soil property values for the MN samples were calculated and used for the statistical analysis ($X_{0-15\text{ cm}} = (X_{0-5\text{ cm}} + 2X_{5-15\text{ cm}}) / 3$; where X is a soil property value).

2.5. Soil analysis

Soil samples were analyzed independently at each location, thus resulting in the use of different analytical methods and instruments. Total C and N were determined by thermal combustion for all locations. Organic C was equal to total C for all locations except ID, where it was determined as the difference between total C and inorganic C, because the ID soils contained substantial amounts of CaCO_3 . Inorganic C in the ID soils was determined using the pressure-calcimeter method (Sherrod et al., 2002). Soil pH was measured with a pH meter and a glass electrode using 1:1 soil to water paste for all locations except KY where 1:1 soil to 0.01 M CaCl_2 was used. Extractable soil nutrients were determined using the Mehlich 3 method (Mehlich, 1984) in IA and KY, using DTPA extraction according to Method S-6.10 (Gavlak et al., 2003) in ID, and using 1 M ammonium acetate extraction in MN. Extractable P was determined separately in MN using a dilute acid and ammonium fluoride extraction (weak Bray) and in ID using the Olsen-P

method (Method S-4.10 and Gavlak et al., 2003). Cation exchange capacity was measured using the pH 7 NH_4OAc method (US Department of Agriculture, 1996) in IA and MN, and CEC of the calcareous ID soils was determined by a modified EPA 9081 method.

2.6. Statistics

Management and intrinsic soil properties were different at each location, and both the number of soil samples collected after the treatments were imposed and the timing of soil sample collection differed for each location. To assess treatment effects, we weighted each location equally by comparing pre-treatment soil property values with average post-treatment soil property values (average values for all samples collected after the treatments were imposed). This approach allowed us to statistically isolate the “treatment effect” but lumped effects due to differences in soil type, climate, management, and sampling times in the “location effect”. The experiment was a randomized complete block design with four treatments, five locations, and three replications. Data sets for average post-treatment soil property values were complete and balanced allowing analysis of variance (ANOVA). By contrast, the pre-treatment soil property data sets were not complete as the WA location collected only 3 pre-treatment soil samples to represent the entire plot area while the other four locations collected pre-treatment soil samples from each of the 12 plots. Because of the unbalanced design, the pre-treatment soil organic C and total N values were evaluated using PROC GLM (SAS Institute). Soil pH values for the WA location were excluded from the statistical analysis because of missing data and a large unexplained discrepancy in the pre-treatment and post-treatment pH values. Because different nutrient extraction methods were used at different locations, we used post-treatment to pre-treatment extractable nutrients ratios for the statistical analysis. Differences between average post-treatment and pre-treatment soil property values and ratios of average post-treatment to pre-treatment

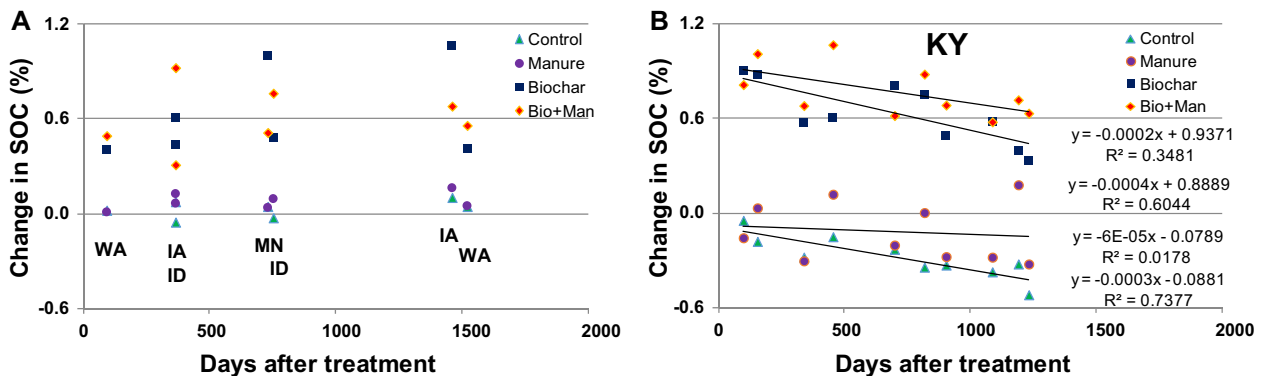


Fig. 2. Trends with time after treatments were imposed in differences between post-treatment and pre-treatment surface (0–15 cm) SOC values for the (A) Ames Iowa (IA), Kimberly Idaho (ID), St. Paul Minnesota (MN), and Prosser Washington (WA) locations and (B) for the Bowling Green Kentucky (KY) location.

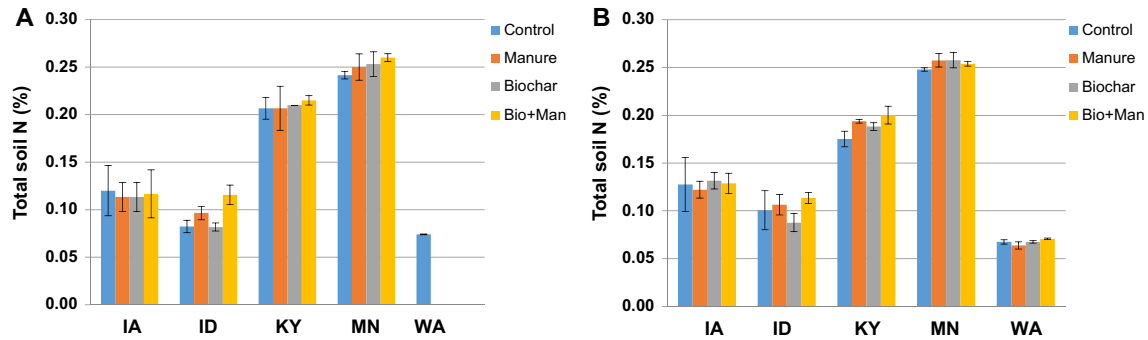


Fig. 3. Average surface (0–15 cm) total soil N content (A) for samples collected before treatments were imposed and (B) for samples collected after treatments were imposed. Error bars are standard deviations.

extractable nutrient values were calculated independently for each plot for four locations (IA, ID, MN, and KY) and using average pre-treatment values for the WA plots. Statistical analysis were conducted using SAS 9.2 for Windows (SAS Institute), and significant differences ($\alpha = 0.05$) between treatment means and locations were assessed using Tukey's post-hoc test.

The total above ground crop biomass data passed the Levene's test for homogeneity of variances, but failed the Shapiro-Wilk normality test ($W = 0.88$, $P = 1.19 \times 10^{-5}$). Therefore, above ground yield data was natural log transformed. The R (R Core Team, 2014) statistical software was used with the corresponding packages lme4 (Bates et al., 2014) and lmerTest (Kuznetsova et al., 2014) to perform a linear mixed effects model analysis to assess the interactions between treatment and location on yield using total above ground biomass yield data (dependent variable) with treatment and location as fixed effects and year as the random effect. In order to better assess if there was any statistically significant impact of the biochar and bio + man treatments across all locations, the raw yields were normalized annually by the control yields at each location and then evaluated using a one-way ANOVA with years as replications. Significant treatment differences ($\alpha = 0.05$) were evaluated using Tukey's post-hoc test.

3. Results

3.1. Agronomic management and climate description

Annual precipitation or precipitation + supplemental irrigation totals varied considerably between locations and within the time frame of the study (Fig. S1). The IA, KY and MN locations had sufficient precipitation to support maize production; whereas, supplemental irrigation (312 and 762 mm, respectively) was required for maize production at the ID and WA locations. Annual precipitation at the TX location was

adequate to produce sorghum crops in 2009 and 2010, but an extensive drought in 2011 and 2012 prevented any measurable yields. No supplemental irrigation was available at the TX location.

3.2. Biochar characteristics

Properties of the biochar used in this study varied from barrel to barrel (Table S3), but on average ash content = 156 g kg^{-1} , volatile matter = 269 g kg^{-1} , and fixed carbon = 575 g kg^{-1} by proximate analysis, and total C = 696 g kg^{-1} , H = 28.7 g kg^{-1} , N = 4.63 g kg^{-1} , and O = 135 g kg^{-1} (O was determined by difference) by ultimate analysis. Plant nutrient contents (Ca, K and P) of the biochar were low ($<7 \text{ mg kg}^{-1}$); and the pH of the biochar in a 1:1 water suspension was 5.59.

3.3. Soil properties

Surface (0–15 cm) soil organic C (SOC) values for samples collected before the treatments were imposed varied substantially by location (Fig. 1A), averaging 1.18%, 0.89%, 1.81%, 2.40% and 0.53% for the IA, ID, KY, MN and WA sites, respectively. Across locations, SOC values for soil samples collected before the treatments were imposed averaged 1.42, 1.44, 1.44 and 1.49% C for the control, manure, biochar and bio + man treatments, respectively; by contrast, SOC values for all surface samples collected after the treatments were imposed averaged 1.41, 1.48, 2.11 and 2.11% C for the control, manure, biochar and bio + man treatments, respectively (Fig. 1B). The biochar and bio + man treatments significantly ($P < 0.001$) increased average surface SOC values by 46 and 41% relative to the control treatment; however, SOC values for the manure treatment were not significantly different from those of the control treatment and SOC values for the bio + man treatment were not significantly different from those of the biochar

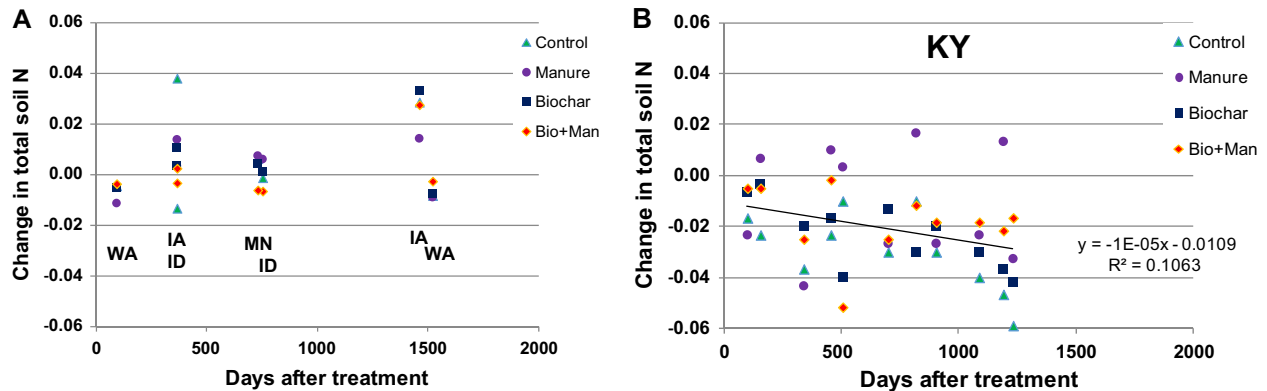


Fig. 4. Trends with time after the treatments were imposed in differences between post-treatment and pre-treatment surface (0–15 cm) total soil N values for the (A) Ames Iowa (IA), Kimberly Idaho (ID), St. Paul Minnesota (MN), and Prosser Washington (WA) locations and (B) for the Bowling Green Kentucky (KY) location.

Table 1
Influence of treatments and location on surface soil CEC and pH values for samples collected before and after the treatments were imposed. Different letters in Tukey Groups indicate significant differences ($\alpha = 0.05$) within columns.

Pre-treatment CEC (cmol kg ⁻¹)			Post-treatment CEC (cmol kg ⁻¹)			Pre-treatment pH			Post-treatment pH		
Treatment	Mean	Tukey Group	Treatment	Mean	Tukey Group	Treatment	Mean	Tukey Group	Treatment	Mean	Tukey Group
Control	17.08	A	Control	16.24	A	Control	5.92	A	Control	5.98	A
Manure	17.48	A	Manure	16.73	A	Manure	5.85	A	Manure	6.08	A
Biochar	17.67	A	Biochar	16.13	A	Biochar	5.92	A	Biochar	6.00	A
Bio + man	17.82	A	Bio + man	16.60	A	Bio + man	5.88	A	Bio + man	6.06	A
Location	Mean	Tukey Group	Location	Mean	Tukey Group	Location	Mean	Tukey Group	Location	Mean	Tukey Group
IA	14.34	C	IA	12.46	C	IA	5.85	B	IA	5.92	B
ID	20.85	A	ID	20.08	A	ID	7.54	A	ID	7.67	A
MN	17.35	B	MN	16.74	B	MN	5.65	C	MN	5.76	C
						KY	4.53	D	KY	4.79	D
						WA*	6.65		WA	7.78	

* WA sample set was incomplete; WA samples were not included in statistical analysis.

treatment. For sub-soil samples (15–30 cm), no treatment effects on SOC values were evident (IA, ID, MN and KY samples); the sub-soil samples were collected below the depth of biochar and manure incorporation (data not presented).

We evaluated differences between pre-treatment and post-treatment samples to assess temporal effects on surface soil SOC values (Fig. 2A and B). The SOC difference values (average post-treatment SOC values minus the average pre-treatment SOC values) were consistently greater for the biochar and bio + man treatments than for the manure and control treatments up to 1500 days after the treatments were imposed, and no trends with time were evident for any of the treatments at the IA, ID, MN or WA locations (Fig. 2A). When considered separately, the SOC difference values for the KY location showed a small increase for the manure and bio + man treatments relative to the control and biochar treatments, respectively, and a significant decrease with time for the control, biochar, and bio + man treatments (Fig. 2B). The decrease with time in the KY SOC difference values for the manure treatment was not significant.

Total N values for surface soil samples collected both before and after the treatments were imposed varied substantially by location; however, no significant treatment effects were observed (Fig. 3A and B). No temporal trends in total N difference values (post-treatment total N values minus pre-treatment total N values) were evident for samples collected from the IA, ID, MN and WA locations (Fig. 4A). Considered separately, the total N difference values for the KY location showed no treatment effects, but did show a significant decrease with time (Fig. 4B).

Pre-treatment and average post-treatment surface soil CEC values were available for only 3 locations (IA, ID and MN), while pre-treatment

and average post-treatment surface soil pH values were available for 5 locations (IA, ID, MN, KY and WA). Location differences were significant, but no treatment effects were evident for either the pH or the CEC values (Table 1). We also evaluated average post-treatment/pre-treatment ratios and differences between average post-treatment and pre-treatment pH and CEC values (data not shown); neither of these results showed significant treatment effects. Interestingly, there was a decrease in average CEC values and an increase in average pH values for samples collected after the treatments were imposed relative to samples collected before the treatments were imposed; however, these trends were independent of treatment. The cause of these differences is not clear.

The various locations used different methods for extracting plant available nutrients from soils. Furthermore, soil nutrient levels are strongly influenced by intrinsic soil properties, climate, and management practices. Thus direct comparison of extractable nutrient values across locations is problematic. Here we considered only ratios of average post-treatment to pre-treatment extractable nutrient levels for the 4 locations reporting extractable nutrient data (IA, ID, KY and MN). ANOVA of extractable nutrient post-treatment to pre-treatment concentration ratios showed significant location, treatment, depth, and location \times treatment interaction effects (Table 2). The manure and bio + man treatments typically had greater average post-treatment to pre-treatment extractable nutrient ratios than the biochar or control treatments and the surface soil samples typically had greater ratios than the subsoil samples. Different responses to the treatments were evident for the various nutrients. For example, some nutrients (Fe and Cu) showed no significant treatment effects, treatment effects for P were only significant in the surface soil samples, and treatment effects

Table 2
Influence of treatment, location, and sampling depth (depth 1 = 0–15 cm, depth 2 = 15–30 cm) on ratios of average extractable soil nutrients for samples collected after the treatments were imposed to values for samples collected before the treatments were imposed. Different letters indicate significant differences ($\alpha = 0.05$) within columns.

Treatment	Depth	Post-treatment to pre-treatment extractable nutrient ratios						
		Cu	Fe	K	Mg	Mn	Na	P
Control	1	1.43 A	1.24 A	0.90 C	1.02 CD	0.53 A	0.87 C	1.06 B
Manure	1	1.79 A	1.32 A	1.63 A	1.13 ABC	0.46 A	1.05 ABC	2.67 A
Biochar	1	1.37 A	1.20 A	1.07 BC	0.99 D	0.48 A	0.98 C	1.21 B
Bio + man	1	1.57 A	1.22 A	1.68 A	1.16 AB	0.44 AB	1.07 ABC	2.88 A
Control	2	1.38 A	1.14 A	0.80 C	1.15 AB	0.33 BC	1.00 BC	0.89 B
Manure	2	1.31 A	1.21 A	1.30 ABC	1.11 BC	0.27 C	1.30 AB	1.27 B
Biochar	2	1.36 A	1.18 A	1.04 BC	1.09 BCD	0.26 C	1.09 ABC	0.98 B
Bio + man	2	1.50 A	1.16 A	1.45 AB	1.23 A	0.29 C	1.34 A	1.24 B
ANOVA		Probability > F						
Location		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment		0.1196	0.1831	<0.0001	<0.0001	0.0549	0.0007	<0.0001
Depth		0.1058	0.0184	0.0443	0.0004	<0.0001	0.0003	<0.0001
Location \times treatment		0.0337	0.6025	<0.0001	<0.0001	0.0019	0.0004	<0.0001
Depth \times treatment		0.2348	0.6738	0.5602	0.0412	0.562	0.5447	<0.0001
Location \times treatment \times depth		0.0327	0.0203	0.8399	0.03	<0.0001	0.5518	<0.0001

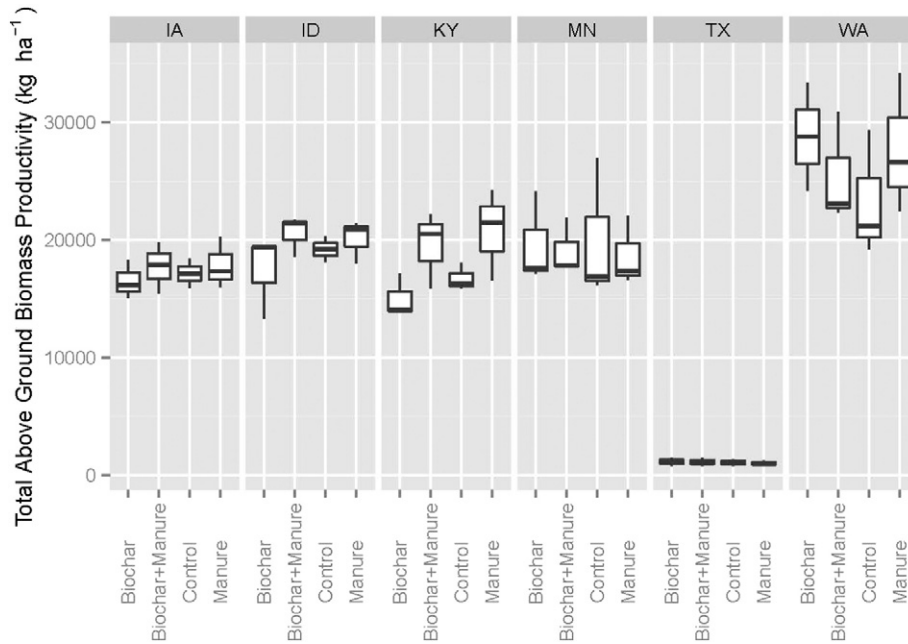


Fig. 5. Box plot showing average total above ground crop biomass yields for all treatments from individual plots (averaged across years) for the Ames Iowa (IA), Kimberly Idaho (ID), Bowling Green Kentucky (KY), St. Paul Minnesota (MN), Big Springs Texas (TX), and Prosser Washington (WA) locations.

for Na (a relatively mobile element), were only significant for the sub-soil samples. In general, extractable nutrients ratios for the bio + man treatment were slightly greater than ratios for the manure treatment and ratios for the biochar treatment were slightly greater than the ratios for the control treatments (comparisons that isolate the biochar effect); however neither the biochar nor the biochar × manure interaction effects were statistically significant.

3.4. Above-ground biomass yields

A comparison of average above ground crop biomass yields for the various treatments at each location is presented in Fig. 5. The results from the analysis of the two-factor linear model with annual variability accounted for as a random factor are shown in Table 3. Location was a significant factor influencing yield ($P < 0.001$), but the treatment and treatment × location interactions effects were not significant. At all 5 locations growing corn, there was extensive year to year variability in biomass yields. Normalizing biomass yields relative to yields for the control plots each year at each location revealed significant location and location × treatment interaction effects. Pair-wise comparisons of treatment means for each location were conducted to further assess the location × treatment interaction effects (Table 4). No consistent yield improvements across locations were observed following the biochar additions; however, the WA site did show a significant yield increase for the biochar plots compared to the control plots (Table 4, $P = 0.017$). The crop yield data shows no evidence of a synergistic interaction between

the biochar and manure treatments either across locations or at any one location.

4. Discussion

The study shows that the fast pyrolysis hardwood biochar studied here is effective for increasing SOC levels at five locations with diverse climates and soil properties across the US. This finding is consistent with numerous other studies that have previously shown that biochars

Table 3
Analysis of variance results using the raw and normalized total above ground crop biomass (grain + stover) yields[†].

Variable	Raw yields		Normalized yields	
	F value	$P \geq F$	F value	$P \geq F$
Location	48.124	<0.001***	4.288	0.003**
Treatment	0.320	0.811	1.263	0.298
Location × treatment	0.954	0.517	2.015	0.036*

[†] Where the symbols, *, **, and *** are used to show significance at the $P = 0.05, 0.01,$ and 0.001 levels, respectively.

Table 4
Pair-wise comparisons (P values from two-tailed t -test) of normalized crop biomass yields for plots at each location.

	Biochar	Bio ± man	Control
Prosser (WA)			
Bio + man	0.017		
Control	0.003	0.267	
Manure	0.094	0.310	0.052
Ames (IA)			
Bio + man	0.067		
Control	0.263	0.386	
Manure	0.047	0.829	0.288
Kimberly (ID)			
Bio + man	0.096		
Control	0.317	0.437	
Manure	0.148	0.784	0.608
Big Springs (TX)			
Bio + man	0.708		
Control	0.328	0.517	
Manure	0.093	0.148	0.340
St. Paul (MN)			
Bio + man	0.970		
Control	0.750	0.720	
Manure	0.800	0.830	0.560
Bowling Green (KY)			
Bio + man	0.083		
Control	0.472	0.256	
Manure	0.035	0.597	0.114

are effective for sequestering C in soils (Lehmann et al., 2015). For four locations (IA, ID, MN and WA), SOC levels were stable for at least 1500 days after the treatments were imposed. By contrast, the KY soils were rapidly losing SOC with time; however, the rates of loss of SOC for the control, biochar, and bio + man treatments were similar, indicating that the biochar amendments were not the cause of the SOC decline in the KY soils. Soils at the KY site had been under forage prior to this study (Table S1), it is likely that labile biogenic SOC that built up under the perennial forage system was rapidly mineralized in the warm humid climate after the KY soils were plowed and converted to annual maize production. The manure treatments, by contrast, had little effect on SOC levels. Long-term applications of manure have been shown to increase SOC in fields under intensive cultivation (Manna et al., 2005 and Gong et al., 2008); however, the manure applications used in this study were insufficient to significantly increase SOC.

The biochar, manure, and bio + man treatments had no effect on total soil N, CEC or pH. In hindsight, these results are not surprising given that the mixed hardwood fast pyrolysis biochar used in this study has both a low pH (pH = 5.6) and a low specific surface area ($<1 \text{ m}^2 \text{ g}^{-1}$) (Table S3). Because of the low pH, the biochar did not function as a liming agent and the low surface area limited the contribution of the biochar to CEC and the ability of this biochar to adsorb both organic and inorganic N compounds. The results do not support a key hypothesis being tested in this study; that a synergistic interaction between biochar and manure treatments would enhance soil quality. The results, however, are specific for the type of biochar used and the set of soil parameters measured. Most biochars are alkaline and have substantially higher specific surface areas, and therefore may increase both pH and nutrient retention by soils (Chee et al., 2015 and Ippolito et al., 2015). Furthermore, the literature indicates that most biochars decrease soil bulk density and increase soil water retention (Masiello et al., 2015), two soil quality parameters not measured in present study.

In this study, biochar significantly increase biomass yields at only one of six locations (Table 4 and Fig. 6). This outcome is consistent with the literature, which shows variable yield responses to biochar for temperate region soils (Jeffery et al., 2011, Crane-Droesch et al., 2013 and Biederman and Harpole, 2013). Crop yield responses to biochar applications are more consistently positive for tropical soils,

which are acidic and heavily leached of nutrients, and on sandy soils, which have low nutrient and water holding capacity. Many non-soil related factors may limit crop productivity, such as weather, management, disease, and insects. Biochar is anticipated to increase crop productivity only when soil related problems such as high bulk density (restricted root growth), low nutrient and water holding capacity, and acidity are limiting and only when the type of biochar used and the application protocols are able to ameliorate those limitations.

The one location, WA, where biomass yields apparently increased in response to biochar amendments (Table 4 and Fig. 6) is dominated by poor quality sandy soils (49% sand and 8% clay; Table S1). We speculate that an increase in soil moisture retention capacity and possibly nutrient retention capacity caused the apparent yield response at the WA location. Although not measured in this study, biochar amendments are known to increase water retention in sandy soils (Basso et al., 2013, Barnes et al., 2014 and Novak et al., 2012). The TX location also has very sandy soils (80% sand and 10% clay; Table S1), however biomass yields were so low at the TX location (Fig. 5; due to lack of sufficient precipitation) that meaningful comparisons are precluded. This assessment is in agreement with the results of a meta-analysis that reported biochar amendments on sandy soils frequently have a positive impact on yield (e.g., Jeffery et al., 2011).

Biochar can also have different impacts on crop yields in years following its application. A wood-derived biochar that was applied to an acidic Oxisol had no effect on the first season maize growth, but in the subsequent three seasons there was a significant improvement in crop yields (Major et al., 2010). Our results, along with the existing literature studies, stress the importance of conducting long-term (>3 yr) field trials, since biochar characteristics change with age and degree of weathering (Joseph et al., 2010) and weather variability can provide erroneous results in short-term studies.

In this study, the hardwood biochar was also co-applied with several types of animal manures (horse, dairy, poultry, and beef). Despite the long history of co-applying biochar (charcoal) and animal manures (e.g., Crews, 1880), we observed no consistent positive yield improvement with the combination of biochar and manure (bio + man treatment) on crop productivity across the six locations ($P > 0.10$). This may be related to the one-time manure application, the low surface area of the biochar, and high stability of the biochar used in this study.

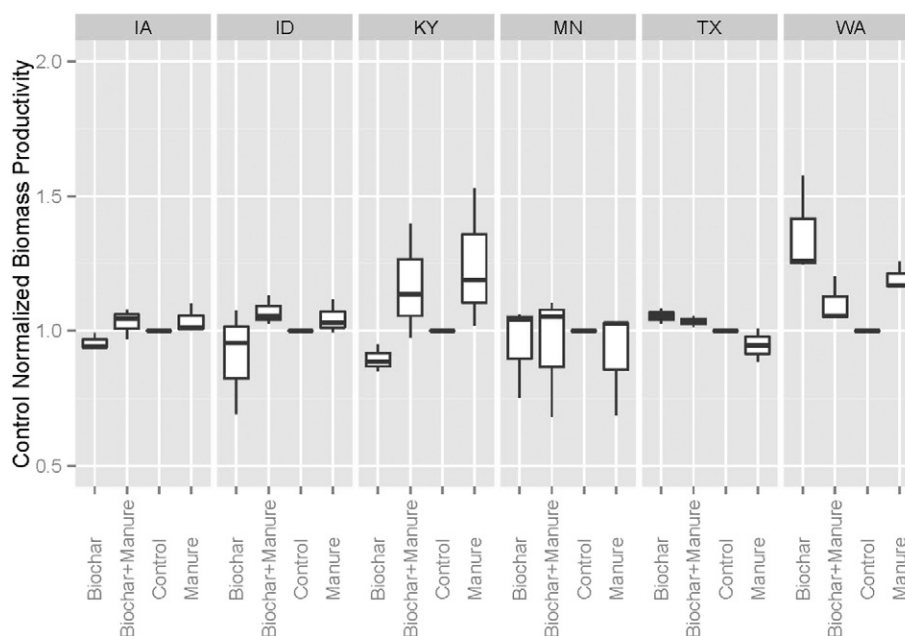


Fig. 6. Box plot showing control normalized total above ground biomass yields for all treatments for individual locations (averaging across years) for the Ames Iowa (IA), Kimberly Idaho (ID), Bowling Green Kentucky (KY), St. Paul Minnesota (MN), Big Springs Texas (TX), and Prosser Washington (WA) locations.

5. Conclusions

There was no evidence of a synergistic interaction between manure and the biochar used in this study on either soil quality or crop yields. This conclusion, however, does not preclude synergistic interactions when other types of biochar, manures, and different crops, soils, management systems and climates are considered. We also hypothesized that complex soil \times climate \times biochar interactions would result in different soil quality and crop yield responses in different locations. The results of this study generally support this hypothesis. Across all locations, biochar amendments were effective for increasing SOC levels and our results indicate that the effect of biochar on SOC persisted for at least 1500 days under field conditions. Therefore, our multi-location field trials support a growing body of evidence that biochar amendments are an effective means for soil C sequestration. Our results also suggest that positive crop yield responses to biochar applications are possible, but are only anticipated when specific soil quality problems are limiting crop productivity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.geoderma.2016.11.025>.

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