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ENVIRONMENTAL BENEFITS OF BIOCHAR

Germination Tests for Assessing Biochar Quality

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Definition, analysis, and certification of biochar quality are crucial to the agronomic acceptance of biochar. While most biochars have a positive impact on plant growth, some may have adverse effects due to the presence of phytotoxic compounds. Conversely, some biochars may have the ability to adsorb and neutralize natural phytotoxic compounds found in soil. We evaluated the effects of biochars on seedling growth and absorption of allelochemicals present in corn (*Zea mays* L.) residues. Corn seeds were germinated in aqueous extracts of six biochars produced from varied feedstocks, thermochemical processes, and temperatures. Percent germination and shoot and radicle lengths were evaluated at the end of the germination period. Extracts from the six biochars had no effect on percent germination; however, extracts from three biochars produced at high conversion temperatures significantly inhibited shoot growth by an average of 16% relative to deionized (DI) water. Polycyclic aromatic hydrocarbons detected in the aqueous extracts are believed to be at least partly responsible for the reduction in seedling growth. Repeated leaching of biochars before extract preparation eliminated the negative effects on seedling growth. Biochars differ significantly in their capacity to adsorb allelochemicals present in corn residues. Germination of corn seeds in extracts of corn residue showed 94% suppression of radicle growth compared to those exposed to DI water; however, incubation of corn residue extracts with leached biochar for 24 h before initiating the germination test increased radicle length 6 to 12 times compared to the corn residue extract treatments. Germination tests appear to be a reliable procedure to differentiate between effects of different types of biochar on corn seedling growth.

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Biochar, one of the coproducts of pyrolysis, consists of up to 90% carbon and is a potential soil amendment for increasing productivity while simultaneously sequestering large amounts of carbon (Lehmann, 2007; Laird, 2008; Rogovska et al., 2011). Application of biochar to soils has been shown to reduce compaction, increase water and nutrient holding capacity, enhance biological activity, increase crop yields, and reduce nitrous oxide emissions (Chan et al., 2007; Lehmann and Joseph, 2009; Spokas and Reicosky, 2009; Laird et al., 2010a,b; Rogovska et al., 2011). Biochar has also been shown to increase sorption of agrochemicals and thus reduce their leaching potential (Cao et al., 2009; Laird et al., 2010b). Various residual forms of organic material including manure, urban yard waste, and crop residues, as well as dedicated energy crops such as miscanthus (*Miscanthus giganteus* J.M. Greef & Deuter ex Hodkinson & Renvoize) and switchgrass (*Panicum virgatum* L.), can be utilized as a feedstock for the pyrolysis process (Roberts et. al., 2010). Characteristics and composition of the feedstock have an impact on the physical and chemical properties of the biochar coproduct; moreover, pyrolysis conditions—temperature, heating rate, residency time, oxygen ratio, and type of reactor—influence biochar characteristics and quality as well (Goyal et al., 2008; Gaskin et al., 2008, 2010).

Biochars thus vary in physical composition, carbon, ash, and volatile matter contents, pH, elemental composition, and chemical structure (Joseph et al., 2010; Spokas, 2010). Differences in these variables can have profound influence on the behavior of biochars in soils, their stability, and effect on soil microbiological, chemical, and physical properties (Spokas and Reicosky, 2009; Spokas et al., 2010). To date, proximate analysis, which partitions biochar into fixed carbon, volatile matter, ash, and water, has been the dominant analytical method used for assessing biochar quality. Despite great variability in biochar characteristics, generally accepted methods of evaluating biochar quality for use as a soil amendment are lacking.

There are many uncertainties in defining "biochar quality." For example, biochar used for carbon sequestration purposes would be evaluated based on its decomposition rates and its effect on the native soil organic matter pool, whereas biochar used as a soil conditioner would be evaluated on its effect on soil physical properties such as bulk density, water holding capacity,

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Abbreviations: DI, deionized water; GC-MS, gas chromatography–mass spectroscopy; PAH, polycyclic aromatic hydrocarbon; TDS, thermal desorption.

and porosity. High-quality biochar for the purpose of carbon sequestration may be of little or no value as a soil conditioning agent. Effect on crop productivity is yet another consideration in defining biochar quality. The effects of biochar quality on plant growth were demonstrated by Deenik et al. (2008), who showed significantly lower soybean [*Glycine max* (L.) Merr.] plant growth from soils amended with high-volatile-matter (35%) biochar compared to no-biochar controls, whereas soils amended with low-volatile-matter (11%) biochar had no effect on plant growth. Nitrogen uptake by soybeans grown in soils amended with biochar high in volatile matter was significantly lower compared with no-biochar controls, whereas N uptake increased for soybeans grown in soil amended with biochar low in volatile matter (Deenik et al., 2008).

Another important characteristic of biochar, sorption capacity for organic compounds, can potentially affect plant growth. Sorption capacity is attributed to a combination of electrostatic, complexation, and capillary forces on biochar surfaces and in pores (Major et al., 2009; Moreno-Castilla, 2004). Adsorption governs bioavailability; thus, biochar mediates the positive or negative effects and the environmental fate and performance of agricultural chemicals (Lehmann et al., 2003; Laird et al., 2010b; Wang et al., 2010). For example, biochar was found to be a highly effective sorbent for soil-applied pesticides (Sheng et al., 2005), thus reducing leaching to groundwater but potentially increasing persistence in soil (Spokas et al., 2009; Yang et al., 2006).

The addition of a strong adsorbent such as biochar may also disrupt function of allelochemicals in soils. Yields in continuous corn cropping systems are consistently lower than corn yields in corn–soybean rotations (Stanger and Lauer, 2008). Among other factors, the leaching of allelopathic compounds from corn residue has been hypothesized to contribute to the observed yield loss in continuous corn cropping systems (Guenzi and McCalla, 1962). Allelopathic stress on a plant may also favor pathogens, resulting in more severe disease than if allelochemicals are absent (Elmer, 2004; Narval, 1999).

Biochar has been shown to enhance sorption and reduce bioavailability of polycyclic aromatic hydrocarbons (PAHs) and is viewed as a remediation agent for contaminated land (Rhodes et al., 2008; Beesley et al., 2010; Chen and Yuan, 2011). Polycyclic aromatic hydrocarbons are of particular concern due to their hydrophobic, recalcitrant, persistent, potentially carcinogenic, mutagenic, and phytotoxic properties. Plants grown in PAH-contaminated soils or water can become contaminated with PAHs, although the mechanism of uptake is poorly understood. Hundreds of individual PAHs may be produced during the incomplete combustion of organic material associated with both natural events (forest fires and volcanic emissions) and anthropologic activities (industrial processes, fuel combustion, etc.) (IPCS, 1998). Polycyclic aromatic hydrocarbons can also be produced during the pyrolysis process itself and adsorbed onto biochar surfaces. The formation of PAHs during pyrolysis is believed to be a multistep process with conversion of biomass to primary products such as tar and char followed by secondary reactions forming PAHs (Sharma and Hajaligol, 2003). Both substrate composition and temperature during pyrolysis play an important role in PAH formation (Sharma and Hajaligol, 2003). The presence of PAHs and other phytotoxic compounds in biochar could introduce unacceptable agronomic and even human health risks into its widespread use as a soil amendment. Testing for PAH presence, therefore, should be a component of biochar quality assessment.

Because of these potentially adverse effects, there is a need to develop a fast and reliable procedure to screen biochars for potential negative effects on plant growth before large-scale application. In response to this need, the International Biochar Initiative (IBI, 2011) is developing a classification system for biochar quality. Specific analytical methods and property ranges for separating biochars into different classes depending on their effect on soil quality and plant growth are not yet established. Clearly, the most definitive screening would be small-plot experiments; however, these take 4 to 8 mo to produce meaningful results and require relatively large quantities of biochar and other resources. Greenhouse studies are a cheaper and faster alternative to small-plot experiments, but still require considerable time and resources that limit the number of samples that can be screened. We propose to test a relatively inexpensive and fast method for screening biochar for potential toxic effects through adaptation of the standard germination test (AOSA, 1996).

The goal of this project was to evaluate the use of standard germination tests as an indicator of biochar quality. We tested the following hypotheses: (i) some biochars contain water-soluble organic compounds that inhibit germination and early growth of corn seedlings; (ii) leaching biochar to remove water-soluble organic compounds will eliminate any negative effects of biochar extracts on early growth of corn seedlings; and (iii) leached biochar has the capacity to adsorb allelochemicals present in corn residue extracts and thus reduce the inhibitory effect of corn residue extracts on early growth of corn seedlings.

Materials and Methods

Six different biochars (C1 through C6) that varied in feedstock type, conversion process, and temperature were evaluated in this study (Table 1). Biochar C1 was produced as lump charcoal in traditional steel kilns by Streumph Charcoal Co., Belle, MO. Biochar C2 was produced in an industrial-scale bubbling fluid-bed reactor by Dynamotive Energy Systems (Vancouver, CA). Biochars C3–C4 and C5–C6 were produced by fast pyrolysis and gasification, respectively, at Iowa State University Center for Sustainable Environment Technology in Nevada, IA. All biochars were obtained and evaluated as received from the suppliers except C1 (lump charcoal), which was ground in a mortar and pestle to pass through a 0.5-mm-screen sieve; this was done so that all biochars would have similar particle size.

Biochar Characterization

Proximate analysis of solid biochar samples (ASTM D3172) was performed by Hazen Research (Golden, CO). Aromatic and PAH content of biochar was quantified using thermal desorption (TDS) coupled with gas chromatography–mass spectroscopy (GC-MS) analysis (Lavrich and Hays 2007). In brief, 1 to 2 mg of biochar was loaded into empty glass liners with quartz wool packing and analyzed on a TDS unit (Gerstel, Inc., Baltimore, MD) interfaced with an Agilent 6890N GC (Agilent Technologies, Inc., Wilmington, DE) and a 5975 Inert MSD (Agilent Technologies, Inc.). The GC-MS system was equipped with a programmed temperature vaporizer (PTV) inlet (CIS 4, Gerstel, Inc.) and separated

compounds on a 60-m by 0.32-mm by 1.0-μm DB-5MS column (J&W Scientific, Inc., Wilmington, DE) using a carrier helium gas set at a 220-kPa constant pressure. The TDS and PTV inlet parameters were as outlined in Trabue et al. (2010). The GC oven temperature program was the following: (i) initial temp, 45°C hold 0.05 min; (ii) ramp 10°C min^{-1} to 220°C; and (iii) ramp 40°C min^{-1} to 325°C and hold 10 min.

Identification and quantification of PAH compounds was based on retention time and ion ratio match of the target compounds. External standard curves were used for quantification of samples. Reference chemicals were purchased from either Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Thermo Fisher Scientific, Waltham, MA), or Cole Palmer (Cole-Parmer Instrument Company, Vernon Hills, IL) with a minimum purity of 97% or greater (GC grade). Methanol used to dilute reference standards was high-performance liquid chromatography (HPLC) grade and purchased from Sigma-Aldrich.

Germination and early growth of corn seedlings was used as a bioassay for testing biochar quality. The standard germination test procedure for corn seeds (AOSA, 1996) was adopted by germinating corn seeds in aqueous extracts of biochars. A preliminary study was conducted to assess the optimum extraction time for preparation of biochar extracts.

Preliminary Study

This experiment on biochar evaluated the length of water extraction time to achieve the greatest concentrations of organic compounds in solution as evidenced by absorbance of light in the 190- to 500-nm wavelength range. Ten grams of each biochar (C1 through C6) were mixed with 30 mL of DI water in 50-mL plastic bottles and shaken for 5 min. Bottles containing the biochar suspensions were either filtered (paper filter No. 2 then 0.45-μm filter) immediately (T0), or equilibrated on the lab bench for 5 h (T5), 24 h (T24), 48 h (T48), 72 h (T72), 96 h (T96), 120 h (T120), and 144 h (T144) before filtering. Each combination of biochar type and equilibration time was replicated three times for a total of 126 samples. Immediately after filtration, biochar extracts were analyzed for absorbance in the 190- to 500-nm wavelength range using ultraviolet–visible (UV/Vis) spectroscopy. Most organic compounds adsorb light in the ultraviolet or visible region of the electromagnetic spectrum; therefore, the absorbance spectra demonstrate differences in the amounts of organic compounds extracted from the various biochar samples. Additionally, extracts were analyzed for pH and for inorganic element composition and concentrations by inductively coupled plasma–atomic (ICP) emission spectroscopy (Thermo Jarrell Ash ICAP 61E). Nitrate N was extracted with 2 M KCl and N concentrations were measured by steam distillation as described by Keeney and Nelson (1982). For most biochars, there was no significant difference in absorbance spectra for all extraction times with the exception of T0. For practical reasons, extraction time of 24 h was selected for the bioassay. Water extracts of biochars (T24) were analyzed for presence of PAHs by stir-bar sorptive extraction (Magi et al., 2010) followed by TDS and GC-MS analysis (Lavrich and Hays, 2007). This technique is based on sorption of the investigated analytes onto a thick film of sorptive material coated onto a magnetic stir bar. In brief, aqueous biochar exacts (approximately 10 mL) were placed in 20-mL vials along with 0.3 g of sodium chloride and a coated stir bar and incubated for 1 h at room temperature. Stir bars were removed from the extracts, washed with HPLC-grade water, and analyzed by TDS GC-MS as described previously.

Experiment I

Experiment I evaluated the effect of different biochars on germination and early growth of corn seedlings. Bulk quantities of biochar extracts prepared using a 1:30 (solid:liquid) ratio were used for the germination tests. Six nutrient solutions were prepared to mimic the nutrient composition and pH of the six biochar extracts (T24). This was done to distinguish the effects of pH and inorganic composition on germination and early growth of corn seedlings from the effects of organic compounds in the biochar extracts.

Aqueous extracts of six biochars (T24), their corresponding nutrient solutions, and DI water as a control were used to moisten 30- by 30-cm germination paper (Ancor Paper Co.) with approximately 2.6 mL of liquid g^{-1} paper. Twelve corn seeds were placed on two moistened sheets of germination paper, covered with a third moistened sheet, and rolled into a cylinder. Each treatment was replicated four times for a total of 52 cylinders. The cylinders were placed in buckets, which were covered with clear plastic bags to minimize moisture loss and placed in a dark, 25°C temperature chamber for 7 d. Every 2 d additional liquid (biochar extracts, nutrient solutions, or DI water) was sprayed on the cylinders to replenish moisture loss due to evaporation and/or seedlings uptake. After incubation, seeds were evaluated for percent germination and early seedling growth by measuring shoot and radicle lengths. A seed was considered germinated if the length of shoot and radicle was at least 5 mm each.

Experiment II

This experiment tested whether repeated leaching of biochars to remove water-soluble compounds would eliminate the negative effects of biochar extracts on early growth of corn seedlings. Bulk

Table 1. Biochars selected for the study.

Biochar	Feedstock	Conversion process†	Conversion temp.	Source
			°C	
C1	Hardwood	SP	~500	Streumph Charcoal, Belle, MO
C2	Hardwood	FP	450–500	Dinamotive Energy Syst., Vancouver, CA
C3	Corn	FP	500	CSET‡
C4	Corn	FP	732	CSET
C5	Switchgrass	GS	850	CSET
C6	Corn	GS	845	CSET

† SP, slow pyrolysis; FP, fast pyrolysis; GS, gasification.

‡ Center for Sustainable Environment Technologies, Iowa State Univ., Ames.

quantities of each of the six biochars (~80 g) were mixed with DI water, shaken for 5 min, equilibrated for 24 h, and the aqueous phase removed by filtration. Absorbance spectra of the filtrates in the range of 190 to 500 nm were measured by UV/Vis spectroscopy. This procedure was repeated until no change in absorbance spectra was observed between the last two filtrates. All of the biochars required six leachings, except Biochar C3 which required 11 leachings before no change in absorbance spectra was observed.

Leached biochars were air-dried and then extracted with DI water for 24 h using solid-to-solution ratios of 1:30. These aqueous extracts of the leached biochars were used for germination testing as described above. Seeds were evaluated for percent germination and early seedling growth by measuring shoot and radicle lengths.

Experiment III

Experiment III evaluated the potential of different biochars to adsorb allelochemicals present in corn residue extracts. Fresh corn residues, consisting mainly of stalks and leaves, were collected from an agricultural field shortly after grain harvest in Central Iowa. The residues were dried and ground to pass through a 1-mm sieve. Residue extract for corn germination studies was prepared by thoroughly mixing corn residues with DI water in 1:10 (solid:liquid) ratio. The 1:10 ratio has been used by other researchers in this area (Yakle and Cruse, 1984; Martin et al., 1990) and shown to induce phytotoxic effects. The mixture was left undisturbed for 24 h at room temperature (22°C), after which the extract was separated from the residues by pouring through cheesecloth to remove coarse material and then vacuum filtered through No. 2 filter paper to remove fine particulates.

Freshly prepared corn residue extracts were mixed with the six leached biochars (1:30 solid-to-liquid ratio), equilibrated for 24 h, and filtered. The germination test utilized corn residue extract, filtered corn residue extract that had been equilibrated with the six leached biochars, and DI water as a control. Each treatment was replicated four times for a total of 32 assays. Seeds were evaluated for percent germination and early seedling growth by measuring shoot and radicle lengths.

Statistical Analyses

Differences in plant growth between different variables, as well as comparisons to DI water were evaluated by contrast statements in PROC GLM (SAS Inst., Cary, NC). Tests were based on a 0.05 significance level.

Results and Discussion

Biochar Characterization

Proximate analysis of the six biochar samples revealed a wide range in composition (Table 2). Percent ash, volatile matter, and fixed C content ranged from 5 to 78%, 8 to 30%, and 6 to 60%, respectively (Table 2). Thermally extractable aromatic and polyaromatic organic compounds adsorbed on the surfaces of biochar are presented in Table 3.

Nineteen compounds, representative of an extensive group of PAHs, were identified and quantified (Table 3). The concentrations of PAHs were related to the pyrolysis temperature, which is consistent with previous observations of Sharma and Hajaligol (2003) where PAH content in biochars increased with temperature. The correlation coefficients of the relationship between individual PAH and pyrolitic temperature ranged from 50 to 68, with an average $r = 0.60$ (data not shown), indicating that in addition to pyrolitic temperature, other factors played an important role in PAH formation and adsorption onto biochar surfaces. In general, biochars produced at relatively low temperatures (<500°C) had the lowest total concentrations of PAHs (Table 3) and might be more suitable for agronomic use.

As noted by Spokas (2010), factors such as feedstock type and composition, pyrolitic temperature, and residence time in the reactor significantly influence composition of sorbed volatiles. There are indications that longer (~30 min) residence times decrease concentrations of volatiles sorbed on biochar surfaces. There was no difference in reactor residence times for Biochars C3 through C6, which were in the range of 1 to 2 sec. Specific residence times for Biochars C1 and C2 are unknown; however, residence times for slow pyrolysis in traditional kilns are typically measured in days (C1), whereas residence times for the fast-pyrolysis process employed by Dynamotive Energy Systems, Inc. is probably only a few seconds (C2). Total PAH concentrations were lowest for Biochar C3 (0.8 μg g^{-1}; fast-pyrolysis corn stover at 500°C), and highest for Biochar C6 (256 μg g^{-1}; gasification of corn fiber at 845°C).

The main risk associated with certain PAHs is their carcinogenic properties. The USEPA (1993) currently identifies seven PAHs as "probable human (B2) carcinogens": benzo(*a*)pyrene, benzo(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, chrysene, dibenz(*a,h*)anthracene, and indeno(1,2,3-*cd*)pyrene (Bradley et al., 1994). High-temperature gasification chars (C5 and C6) had the greatest concentrations of those types of PAHs posing a risk of soil contaminations if used as a soil amendment. In the soil, PAHs may enter plant roots through passive (driven by diffusion or mass flow) or active (metabolically driven) uptake or combination of both (Zhan et al., 2010).

Soil is often considered for remediation if concentrations of individual carcinogenic PAHs are >0.33 mg kg^{-1} soil (MDNR, 1993). Biochars, however, can be specifically engineered for agronomic purposes, limiting production and adsorption of PAHs and other volatile compounds to biochar surfaces (Spokas, 2010).

Table 2. Proximate analysis of six biochars before and after leaching events.

Biochar	Moisture	Ash	Volatiles	Fixed C†
	% mass			
		Before		
C1	5.3	15.6	19.3	59.9
C2	4.9	5.4	29.9	59.9
C3	4.8	49.5	18.8	27.1
C4	2.8	60.9	8.5	27.8
C5	2.6	78.1	13.7	5.6
C6	6	40.9	20.9	32.2
		After		
C1	2.6	11.1	36.5	49.8
C2	3	4	36.4	56.7
C3	3	49	17.8	30.3
C4	1.4	66.6	9.8	22.2
C5	2	74.3	8.7	15.1
C6	2.9	33.5	8.4	55.2

† Fixed C is the difference between 100% and the sum of the percentages of moisture, ash, and volatile matter.

Testing biochars for PAH presence should be a critical component of overall evaluation of biochar quality for agricultural use. Unfortunately, the relatively fast and inexpensive procedure of measuring volatile matter content (e.g., proximate analysis) does not provide sufficient information about concentrations of specific PAH compounds in biochar. Moreover, in our study there was no relationship between total PAH concentration and percent volatile matter of biochars.

Characterization of Biochar Extracts and Nutrient Solutions

Inorganic nutrient composition and pH of the six aqueous biochar extracts are presented in Table 4. The most common elements identified in biochar extracts were K, P, Na, Ca, Mg, and in smaller amounts Al, Fe, Mn, Si, N, and Mo. High-temperature gasification biochars (C5 and C6) had substantially greater concentrations of K. As noted by Chan and Xu (2009), nutrient availability and composition of biochars vary with the feedstock and pyrolitic conditions under which biochar was produced. Some of the nutrients such as N, P, K, and S present in biomass are partially lost as volatiles during thermochemical conversion. It was documented, for example, that about half of the K and Na content was lost by vaporization at 472 and 673°C conversion temperatures (Yu et al., 2005). With increase in temperature, nutrients remaining in the biochar may undergo changes in their chemical structure such that a greater proportion of nutrients remaining in the biochars is not soluble in water (Yu et al., 2005).

With the exception of Biochar C2, the pH of biochars was neutral to highly alkaline (Table 4). Aqueous biochar extracts contained PAHs; however, not all compounds identified in solid biochars were present in water extracts (Table 5). The most abundant compounds identified were two- or three-ringed PAHs that have relatively greater solubility in water. It is also possible that other unidentified organic compounds in the biochar extracts acted as surfactants enhancing solubility and bioavailability of PAHs (Li et al., 2001). Aqueous extracts of Biochar C6 had the greatest relative abundance of PAHs, and extracts of C1 had the lowest levels of PAHs (Table 5).

Experiment I

Table 4 shows elemental composition of the six aqueous biochar extracts measured by ICP. Percent germination of corn seedlings grown in aqueous extracts of biochar and corresponding nutrient solutions ranged from 94 to 100%, and was not statistically different from DI water (96%). There were significant differences in shoot lengths of corn seedlings germinated in biochar extracts, nutrient solutions, and DI water (Fig. 1). Shoot lengths of seedlings germinated in biochar extracts C4, C5, and C6 were significantly shorter in comparison to those germinated in DI water, suggesting that those biochar extracts contained water-soluble phytotoxic compounds. These three biochars are characterized by relatively high temperature during the conversion process, ranging from 732 to 850°C, and high concentrations of PAHs in both solid biochars and water extracts of biochars.

Polycyclic aromatic hydrocarbon compounds with the highest water solubility showed the greatest phytotoxicity due mainly to increased bioavailability (Thygesen and Trapp, 2002; Hamdi et al., 2007; Juhasz et al., 2010). Polycyclic aromatic hydrocarbon compounds with fewer than three phenyl rings may have the greatest impact on plants in PAH-contaminated soils and

Table 3. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in six solid biochars.

PAH†	Solubility in water‡	Biochar					
		C1	C2	C3	C4	C5	C6
	μmol L^{-1}	μg g^{-1}					
Naphthalene	249	7.8	1.5	0.4	13.3	13.1	65.1
Quinoline	49,000	0.3	0.1	ND§	0.4	0.6	13.4
2-Methyl naphthalene	NA¶	1	ND	ND	1.1	0.3	2.1
Biphenyl	46	ND	ND	ND	0.6	0.9	9.4
Biphenylene	NA	ND	ND	ND	0.1	0.1	3.3
Acenaphthylene	29	0.9	ND	ND	5.2	3.7	28.1
Dibenzofuran	39	1.5	0.4	0.1	0.4	0.9	6.4
Isocyano naphthlene	NA	ND	0.2	0.1	ND	ND	4.1
2,3-Diazaphenathrene	NA	ND	0.3	0.1	0.8	1.1	9.3
Phenanthrene	7.2	3.2	0.8	ND	3.2	4.1	16.1
Anthracene	0.37	0.7	0.2	0.1	1.2	0.9	16.1
Fluoranthene	1.2	0.9	0.1	ND	1.1	1.4	30.7
Pyrene	0.72	ND	ND	ND	0.3	0.2	3.1
Triphenylene	0.18	ND	ND	ND	ND	1.5	0.9
Chrysene	0.013	ND	ND	ND	ND	0.5	7.6
Benzo(a)*anthracene*	0.048	ND	ND	ND	ND	ND	17.7
Benzo(k)*fluoranthene*	0.003	0.2	0.1	ND	0.6	0.3	9.2
Perylene	0.002	0.2	0.1	ND	0.6	0.3	9
Benzo(a)*pyrene*	0.05–0.006	ND	ND	ND	0.2	0.3	3.7
∑PAH		16.7	3.8	0.8	29.1	30.2	255.3

† Compounds in italics are probable human carcinogens (Bradley et al., 1994).

‡ Pearlman et al. (1984).

§ ND, not detected.

¶ NA, not available.

solutions (Henner et al., 1999). In our experiment, all of the PAHs present in aqueous extracts of biochars were two- and three-ringed compounds. From our study it is unclear whether identified PAHs or other unidentified organic compounds suppressed seedling growth, although similar reports show reduction in shoot and root length due to contamination with individual PAHs such as naphthalene and pyrene (Baek et al., 2004).

Shoot lengths of seedlings germinated in nutrient solutions corresponding to Biochars C3, C4, and C6 were significantly longer than those germinated in biochar extracts. Given that there were no differences in inorganic composition and pH between biochar extracts and corresponding nutrient solutions, differences in shoot lengths were attributed to the inhibiting effect of PAHs and/or other organic compounds present in water extracts of biochars. The identification of all organic compounds present in water extracts of biochars was beyond the scope of this project.

Radicle length of the corn seeds germinated in biochar extract C1 was significantly greater than those germinated in DI water (Fig. 1). This might be explained by additional nutrients found in the biochar extract compared to DI water. Similar to shoot length, there were significant differences in radicle length between seeds germinated in biochar extracts C3 and C6 and the corresponding nutrient solutions.

Table 4. Nutrient composition and pH of six aqueous biochar extracts before and after leaching events.

Parameter	Biochar					
	C1	C2	C3	C4	C5	C6
			Before			
pH	7.1	6.6	8.0	11.6	11.3	10.2
Al	trace†	trace	trace	trace	trace	ND‡
Ca	31.9 (0.7)§	10.5 (0.2)	35.0 (2.4)	168 (25)	141 (51)	1.9 (0.1)
K	36.9 (2.6)	61.9 (2.8)	284 (22)	139 (15)	1508 (258)	1150 (74)
Mg	4.9 (0.2)	1.9 (0.1)	31.9 (3.1)	ND	trace	11.0 (0.9)
Mn	trace	trace	trace	ND	ND	ND
Na	4.9 (0.2)	6.7 (0.1)	7.5 (0.2)	9.0 (1.0)	37.6 (4.2)	52.7 (3.2)
P	trace	1.4 (0.1)	8.9 (0.2)	trace	trace	81.3 (6.2)
S	3.0 (0.2)	1.1 (0.1)	8.2 (0.0)	4.5 (0.0)	20.0 (6.1)	10.9 (0.4)
Fe	ND	2.2 (1.5)	2.5 (2.6)	ND	ND	trace
N	trace	trace	trace	trace	trace	trace
Mo	ND	ND	ND	ND	6.0 (1.5)	3.3 (0.3)
			After			
pH	7.0	6.0	6.2	7.9	NA¶	9.1
Al	trace	trace	trace	trace	trace	ND
Ca	19.3 (0.1)	3.8 (0.0)	15.6 (0.0)	11.8 (0.2)	15.2 (0.1)	1.8 (0.0)
K	6.4 (0.6)	10.2 (0.8)	10.7 (0.3)	21.0 (0.3)	59.28 (0.2)	104.0 (1.2)
Mg	trace	trace	7.8 (0.1)	ND	trace	10.4 (0.1)
Mn	trace	trace	trace	ND	ND	ND
Na	trace	trace	trace	trace	2.6 (0.0)	1.7 (0.0)
P	trace	trace	4.7 (0.1)	2.4 (0.1)	2.6 (0.1)	35.6 (0.1)
S	trace	trace	trace	trace	3.2 (0.3)	7.6 (0.2)
Fe	ND	trace	trace	ND	ND	trace
N	trace	trace	trace	trace	trace	trace
Mo	ND	ND	ND	ND	trace	trace

† Trace: concentration < 1 mg kg^{-1}.

‡ ND, not detectable.

§ Average of three replications followed by standard deviation in parenthesis.

¶ NA, not available.

Table 5. Presence and relative abundance of polycyclic aromatic hydrocarbons (PAHs) in six aqueous biochar extracts.

PAH	Biochar					
	C1	C2	C3	C4	C5	C6
	area counts in thousands					
Naphthalene	112	281	421	506	368	52,406
Quinoline	4	19	143	64	41	18,444
Isoquinoline	104	43	507	18	18	396
2-Methyl naphthalene	12	698	59	44	25	51
Acenaphthylene	9	325	56	52	14	2,490
Dibenzofuran	68	168	111	97	115	640
Fluoren-9-one	9	130	11	9	12,368	396

It appears that utilization of percent germination alone as an indicator of biochar quality is not sufficient and additional measurements are needed. Among the three indicators used (percent germination, shoot and radicle lengths), shoot length appeared to be the most sensitive measure of the plant response to phytotoxic compounds present in aqueous biochar extracts. Our observations are in agreement with results of similar studies that utilized shoot and radicle length measurements as reliable indicators (Stephenson et al., 1997).

Experiment II

This experiment tested if repeated leaching of biochar could remove potentially phytotoxic water-soluble compounds and eliminate negative effects of biochar extracts on early growth of corn seedlings. Proximate analysis of solid biochars and nutrient composition of water extracts of leached biochars are presented in Tables 2 and 4, respectively. In general, repeated leaching resulted in pH decrease and reduction in concentrations of inorganic elements present in biochar extracts and in removal of water-soluble organic compounds as indicated by the decrease in absorbance values in 190- to 500-nm wavelength range (Fig. 2). Most organic compounds absorb light in the ultraviolet or visible region of the electromagnetic spectrum; therefore, absorbance spectra demonstrate differences in the amounts of organic compounds present in the filtrate as a function of leaching events.

Germination of corn seedlings in water extracts of leached biochars resulted in improved seedling growth as measured by shoot and radicle lengths (Fig. 3). Shoot length of seeds germinated in water extracts of leached biochars was significantly greater than those germinated in unleached Biochars C3, C5, and C6. Significant improvement in radicle length was observed only for Biochar C3.

These observed improvements in early seedling growth after biochar leaching could be explained by the removal of water-soluble organic compounds that inhibited seedling growth. The results suggest that leaching of biochar may be an effective means of removing phytotoxic compounds from fresh biochar. However, leaching of biochar as a management option to remove phytotoxic compounds on the industrial scale may not be practical. Composting or otherwise aging biochars that contain phytotoxic compounds may prove more practical; however, the efficacy of such treatments has yet to be established.

Experiment III

This experiment evaluated the potential of different biochars to adsorb allelochemicals present in corn residue extracts. As noted by Uchimiya et al. (2010), different biochars can vary in sorption capacity, a possible indicator of biochar quality. Germination of corn seeds in water extracts of corn residues (2%) was significantly inhibited compared to germination in DI water (96%). Equilibrating water extracts of corn residue with leached solid biochars for 24 h probably resulted in adsorption of allelochemicals present in residue extracts onto surfaces of the biochars as evident by improved germination rates. Percent germination of corn seedlings grown in residue extract equilibrated with the six biochars ranged from 92 to 98% and were significantly greater than those grown in residue extract alone (2%), but not different from those grown in DI water (96%).

In addition, seeds germinated in residue extracts equilibrated with leached biochars exhibited improved early seedlings growth as indicated by greater length of shoot and radicle compared to seeds germinated in residue extract alone (Fig. 4). Among six biochars studied, hardwood slow-pyrolysis biochar (C1) appears to have the greatest capacity to adsorb allelochemicals present in corn residue extract. Shoot and radicle lengths of seeds germinated in residue extract equilibrated with Biochar C1 were significantly greater than those germinated in residue extracts equilibrated with other biochars (Fig. 4).

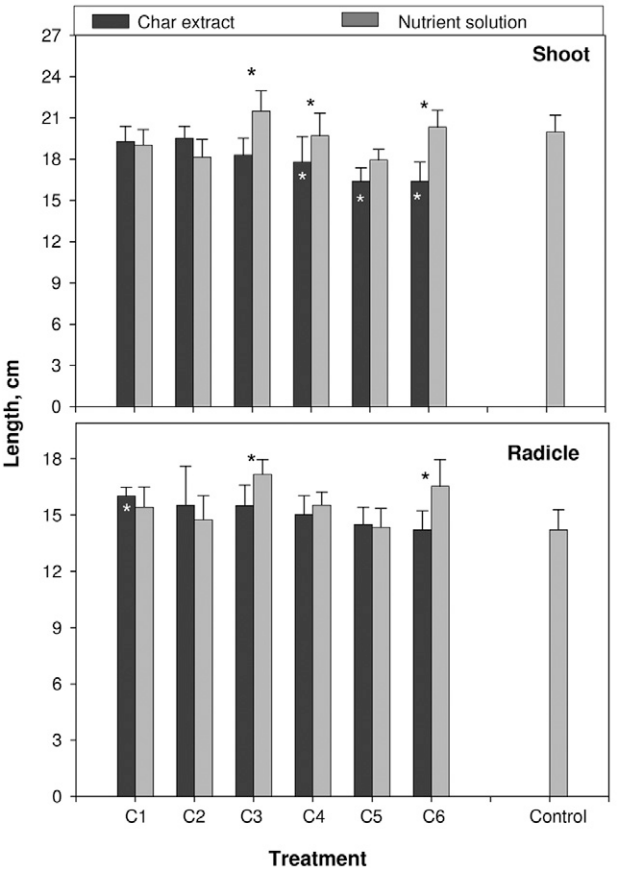

Fig. 1. Length of shoot and radicle of corn seedlings germinated in aqueous extracts of six biochars (C1 through C6), corresponding nutrient solutions, and deionized (DI) water as a control. Asterisks within the bar indicate significant difference between biochar extract and DI water. Asterisks between the bars indicate significant difference between biochar extract and corresponding nutrient solution.

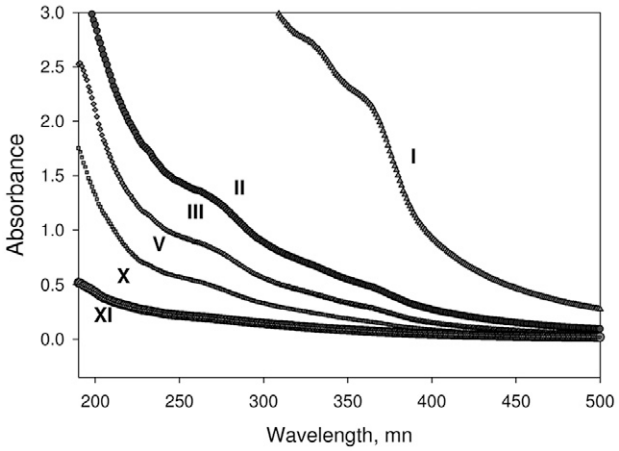

Fig. 2 Plot of absorbance vs. wavelength for Biochar C3. Roman numerals represent consecutive leaching events.

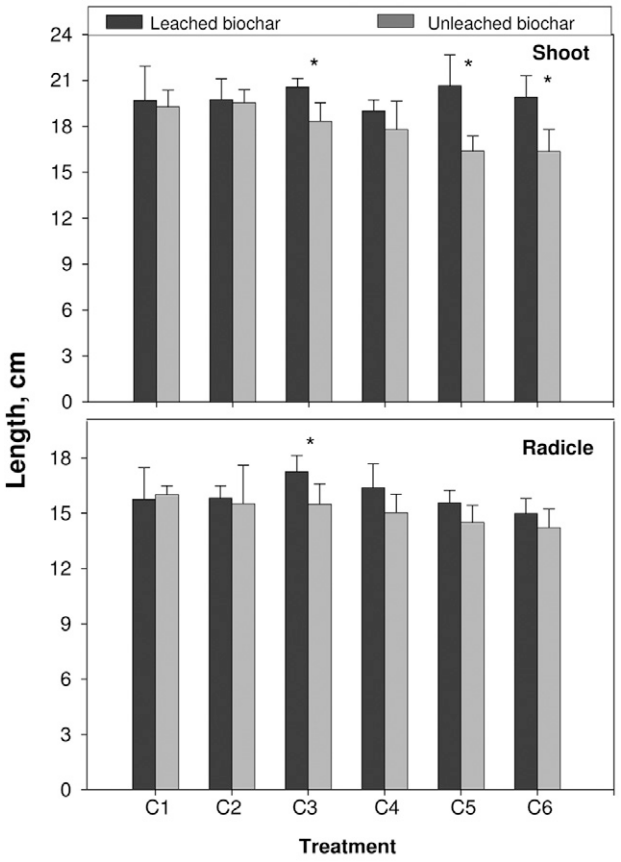

Fig. 3. Length of shoot and radicle of corn seedlings germinated in aqueous extracts of the six leached and unleached biochars (C1 through C6). Asterisks between the bars indicate significant difference between leached and unleached biochar extracts.

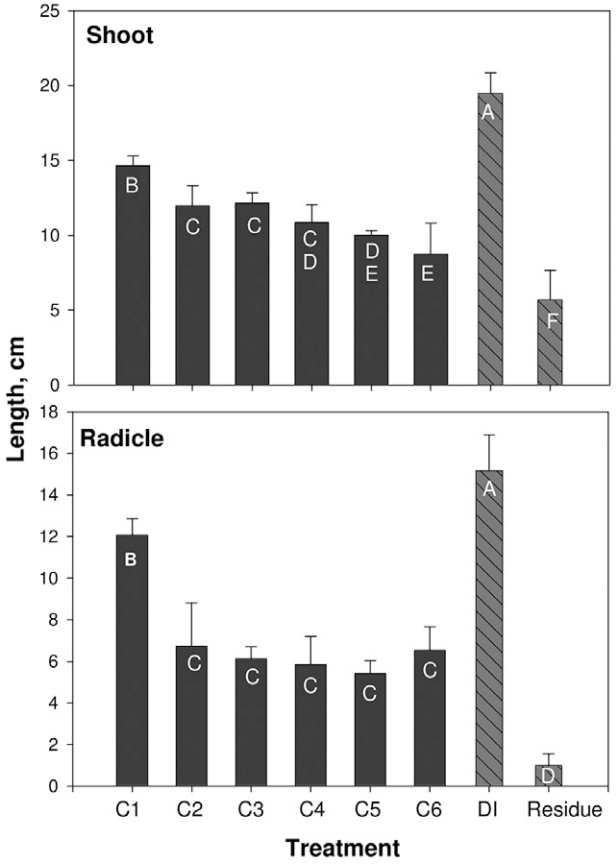

Fig. 4. Length of shoot and radicle of corn seedlings germinated in corn residue extract, corn residue extract equilibrated with leached biochars (C1 through C6), and deionized water. Different letters indicate significant differences between treatments.

Findings from this experiment indicate a potential role of biochar as a soil amendment in reducing or eliminating yield lag associated with growing continuous corn. From this experiment it is difficult to predict the potential impact of biochar on the absorption of allelochemicals under field conditions, as soils have the capacity to adsorb and degrade those compounds as well (Yakle and Cruse, 1983).

Conclusions

Germination tests using aqueous biochar extracts appear to be effective at identifying biochars that contain phytotoxic compounds. Among indicators such as percent germination, and shoot and radicle lengths, shoot length appeared to be the most sensitive measure of plant response to phytotoxic compounds present in aqueous biochar extracts. In our study, aqueous extracts of hardwood biochar produced by both fast and slow pyrolysis had similar effects on seedling growth as DI water, while aqueous extracts of biochar produced by high-temperature gasification and pyrolysis contained compounds that suppressed seedling growth. Repeated leaching of biochars significantly improved early growth of corn seedlings. Hardwood biochar produced by slow pyrolysis had the greatest capacity to neutralize allelochemicals present in corn residues. The findings of this study indicate that biochar is able to reduce or eliminate allelopathic effects associated with corn residue extracts in germination tests. Further research using greenhouse and possibly small-plot experiments are needed to determine whether soil biochar amendments will reduce the yield lag associated with continuous corn.

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