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Biochar Decreases Atrazine and Pendimethalin Preemergence Herbicidal Activity

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Biochar and vinasse are by-products of biofuel production that can be used as soil amendments. However, their addition to the soil might affect PRE herbicide activity. Although studies have shown that biochar has a high herbicide adsorption capacity, there is little information available about biochar effect on weed control especially under field conditions. Therefore, the objective of this study was to determine the influence of biochar and vinasse application on atrazine and pendimethalin availability and herbicide activity under *in vitro* and field conditions. *In vitro* atrazine and pendimethalin herbicidal activities were not influenced by vinasse addition, but biochar application reduced atrazine and pendimethalin injury for all evaluated species. A sorption experiment confirmed high affinity of biochar for atrazine and pendimethalin. Linear regression analysis showed that the slope for atrazine and pendimethalin adsorption was 16 and 4 times higher in soil with biochar than in soil alone. Under field conditions, biochar at 0.5 kg m^{-2} reduced atrazine and pendimethalin weed control 75% and 60%, respectively. These results suggested that the use of biochar as a soil amendment in cropping system could decrease PRE herbicide efficacy. Therefore, mitigating practices such as the use of higher rates or reliance on POST herbicides and cultivation might be necessary to ensure proper weed control.

Nomenclature: Atrazine; pendimethalin; biochar; vinasse.
Key words: Charcoal, injury, soil amendments, stillage, weed control.

El biochar y la vinaza son subproductos de la producción de biocombustibles que pueden ser usados como enmiendas de suelo. Sin embargo, su adición al suelo podría afectar la actividad de herbicidas PRE. Aunque estudios han mostrado que el biochar tiene una alta capacidad de adsorción de herbicidas, hay poca información disponible acerca del efecto del biochar sobre el control de malezas, especialmente bajo condiciones de campo. Por esta razón, el objetivo de este estudio fue determinar la influencia de la aplicación de biochar y de vinaza sobre la disponibilidad y actividad herbicida de atrazine y pendimethalin *in vitro* y en condiciones de campo. *In vitro*, la actividad herbicida de atrazine y pendimethalin no fue influenciada por la adición de vinaza, pero la aplicación de biochar redujo el daño causado por atrazine y pendimethalin en todas las especies evaluadas. Un experimento de sorción confirmó la alta afinidad del biochar por atrazine y pendimethalin. Análisis de regresión lineal mostraron que las pendientes de las curvas de adsorción de atrazine y pendimethalin fueron 16 y 4 veces mayores en suelo con biochar que en suelo solo. Bajo condiciones de campo, el biochar a 0.5 kg m^{-2} redujo el control de malezas de atrazine y pendimethalin en 75% y 60%, respectivamente. Estos resultados sugirieron que el uso de biochar como enmienda de suelo en sistemas de cultivos podría disminuir la eficacia de herbicidas PRE. Por esto, prácticas de mitigación tales como el uso de mayores dosis o una mayor dependencia en herbicidas POST y labranza podrían ser necesarios para asegurar un control adecuado de malezas.

Pyrolysis and fermentation of plant biomass are promising methods to produce bioenergy (McKendry 2002). However, those processes can generate by-products such as biochar and vinasse (Mohan et al. 2006; Wilkie et al. 2000). Pyrolysis is the thermal decomposition of organic materials (e.g. plant biomass, organic waste) in the absence or at low oxygen conditions and high temperatures where oil, gas and charcoal (i.e. biochar) are produced (Mohan et al. 2006). Biochar is a general term referring to a diverse range of solid residues (e.g. charcoal, char, soot) containing black carbon (Mesa and Spokas 2011). Vinasse is a by-product generated during the fermentation and distillation of sugar, starch, or lignocellulosic materials for ethanol production (Wilkie et al. 2000). Both

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biochar and vinasse can be used as amendments to improve soil chemical and physical properties such as nutrient supply, bulk density, and water holding capacity (Lehmann 2007; Sheehan and Greenfield 1980), consequently promoting soil quality and crop yields.

Land application of biochar or charcoal materials influence soil's affinity to organic compounds including herbicides (Kookana 2010). Biochar-herbicide adsorption can delay herbicide loss due to leaching and degradation, but also it can reduce herbicide biological availability or delay dissipation in the soil causing carry over problems (Graber et al. 2012). Feedstock material and pyrolysis conditions (i.e., temperature and time) determine specific surface area (SSA), microporosity, organic carbon content, pH, and aromaticity of biochar (Kookana et al. 2011). Previous research has highlighted the importance of these physical and chemical biochar characteristics as critical factors modifying sorption dynamics between soil and a specific organic compound (Kookana et al. 2011). Furthermore, Nag et al. (2011) suggested that biochar sorption capacity is related to water solubility and sorption coefficient (K_{oc}) of the organic compound. Activated carbon has been used as an herbicide safener to prevent herbicide injury on crop seedlings (Coffey and Warren 1969). This safening potential of activated carbon is a result of its high sorption capacity due to a large surface area acquired during the activation process (Coffey and Warren 1969). Depending on feedstock and manufacturing temperature, biochar adsorptive characteristics can be similar or higher than activated carbon (Kearns et al. 2014).

Vinasse is a liquid characterized by high organic matter and salts content and is often reported to promote soil microbial activity (Miyamoto et al. 2013; Soni et al. 2014; Tejada et al. 2006). Vinasse applications can lead to transient changes of soil chemical properties that can affect weed germination and growth, and herbicide persistence (Christofoletti et al. 2013; Soni et al. 2014). Vinasse-amended soil at 10 and 20 L m^{-2} reduced ametryn bioavailability due to degradation by microbial activity and not by vinasse sorption (Prata et al. 2001).

Atrazine and pendimethalin are among the most widely used herbicides in the United States (Fernandez-Conejo et al. 2014). Atrazine is a chlorotriazine that inhibits photosystem II and is applied PRE or POST to control several broadleaved and grass weed species. Pendimethalin is a dinitroaniline that inhibits mitosis, which is used as a soil applied herbicide to control mainly grasses (Shaner 2014).

A better understanding of the effects of biochar and vinasse on herbicide performance is needed to properly adapt weed management programs to the potential widespread use of these amendments in agricultural fields. We hypothesized that biochar and vinasse used as soil amendments might decrease weed control by reducing atrazine and pendimethalin availability. Thus, the objective of this study was to determine the effect of biochar and vinasse on bioavailability and herbicidal activity of atrazine and pendimethalin under *in-vitro* and field conditions.

Materials and Methods

Herbicide *In Vitro* Bioavailability. A laboratory experiment was conducted to evaluate the effect of different levels of biochar and vinasse mixed with soil on herbicidal activity of atrazine and pendimethalin. The indicator species evaluated in this experiment were southern crabgrass [*Digitaria ciliaris* (Retz.) Koel.], common lambsquarter (*Chenopodium album* L.), iceberg lettuce '9285′ (*Lactuca sativa* L.), and common wheat 'Baldwin' (*Tritcum aestivum* L.). Crop species were included as controls for plants with known herbicide sensitivity. A commercial biochar (AGCARB, Standard Purification Company, Dunnellon, FL) made from pine wood chips at 800 C was used (Table 1). Vinasse was obtained from the lignocellulosic fermentation of sugarcane bagasse obtained at University of Florida Stan Mayfield Biorefinery Pilot Plant, Perry, FL (Table 2). The soil in the laboratory research was a Dothan sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiudult). The soil was spread to form a 5-cm layer, covered and steamed with water vapor to reach a maximum temperature of 93 C during 7 h (Runia 2000) to eliminate the native weed seed bank and sieved through a 1.18 mm sieve before treatments were applied.

Four rates of biochar (0, 0.5, 1, and 2 kg m^{-2}) and vinasse (0, 2.6, 5.3, and 10 L m^{-2}) were mixed with soil for southern crabgrass and common lambsquarter. In a separate experiment, wheat and

Table 1. Characteristics of biochar used in the study. Biochar was produced from pine wood chips by pyrolysis at 800 C.

Characteristic[a]	Value
pH	9.2
EC (µS cm^{-1})	1,775
Nitrogen (%)	0.53
Carbon (%)	62.5
P_2O_5 (%)	0.41
K_2O (%)	0.19
Sulfur (%)	0.12
Boron (%)	0.008
Calcium (%)	1.96
Magnesium (%)	0.49
Zinc (ppm)	2,400
Manganese (ppm)	200
Iron (ppm)	1,500
Copper (ppm)	40
Sodium (ppm)	1,460
Aluminum (ppm)	3,659
Molybdenum (ppm)	0.592

[a] Values were determined in aqueous solution, and concentrations are based on samples with 2% moisture content.

Table 2. Characteristics of vinasse used in the study. Vinasse was produced from lignocellulosic fermentation of sugarcane bagasse to ethanol.

Characteristic	Value
Color	dark brown
pH	5.7
EC (µS cm^{-1})	13750
Total N (mg L^{-1})	2298
P_2O_5 (mg L^{-1})	387
K_2O (mg L^{-1})	44
Mg (mg L^{-1})	39
Na (mg L^{-1})	90
Ca (mg L^{-1})	49
Biological Oxygen Demand (mg L^{-1})	170

lettuce species were exposed to biochar levels of 0 and 2 kg m^{-2} and vinasse levels of 0 and 10 L m^{-2}. Biochar and vinasse rates were based on an expected biomass yield of 16.5 Mg ha^{-1} from sweet sorghum for energy production (Erickson et al. 2011). Based on conversion efficiency, amendment rate that should be returned to the field for nutrient recycling was assumed to be 0.5 kg m^{-2} for biochar and 10 L m^{-2} for vinasse. These rates were calculated to simulate biochar and vinasse soil incorporation in the top 10 cm of soil. Solutions with deionized water of formulated atrazine (AAtrex®, Syngenta, Greensboro, NC) at 2.81 kg ai ha^{-1} and formulated pendimethalin (Prowl H_2O®, BASF, Davis Drive, NC) at 1.27 kg ai ha^{-1} were prepared, and mixed with all biochar and vinasse rates. Approximately 90 g of treated soil was added per petri dish (10 cm diam, 2.5 cm height). Four days after treatment all petri dishes were irrigated with 7 mL deionized water to ensure proper soil moisture for germination, and 50 seeds of each species were planted at 0.25-cm depth per petri dish. Petri dishes were placed in a germination chamber with a regimen of 14 h of light at 28 C and 10 h of darkness at 25 C. Injury was visually assessed after an incubation period of 7 d. Herbicide injury symptoms included stunted seedling growth, chlorosis, and morphological abnormalities. The experiment was conducted twice using completely randomized designs with 4 replications. Data from both experimental replications was subjected to analysis of variance (ANOVA) using the GLIMMIX procedure in the Statistical Analysis Software (SAS 9.3, SAS Institute Inc., Cary NC). Species, amendment (i.e. vinasse, biochar) and rate were used as fixed effects. Because experiment replication did not interact with any other factor ($P > 0.05$) it was consider a random effect in the ANOVA. Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$) was used for mean separation.

Biochar-Herbicide Sorption. An experiment was conducted to quantify herbicide sorption to soil and biochar. Atrazine and pendimethalin (ChemService, West Chester, PA) with 98.1% and 99.5% of purity respectively, were used. Atrazine and pendimethalin were dissolved first in methanol and then diluted in deionized water solutions. All herbicide solutions had a final concentration of 4% v/v methanol. Herbicide treatments were 10 mL of atrazine solution at 0, 23, 46, 70, 93, and 116 µmol L^{-1} and pendimethalin at 0, 18, 89, 178, and 355 µmol L^{-1}. Using these solutions, sorption curves were generated for each herbicide for four conditions: no soil/no biochar, no soil/ 11.5 mg biochar, 3 g soil/ no biochar, and 3 g soil/11.5 mg biochar. Samples were shaken in the dark during 12 h. After this, a 1.5 mL aliquot was passed through a 0.45 µm nylon filter and then centrifuged at 9600 *g* for 10 min. An aliquot of 700 µl from the supernatant of each centrifuged sample was used for absorbance measurements. Standard curves based on liquid solutions of the herbicides were used to determine the relation between absorbance and herbicide concentration. Absorbance was determined at 235 nm for

Table 3. Soil type, texture composition, organic matter (OM) content, and pH of field experiment sites before biochar addition.

Site no.	Soil series	Sand	Clay	Silt	OM	pH
		%				
1	Dothan sandy loam (fine-loamy kaolinitic, thermic Plinthic Kandiudult)	36	30	34	2.3	6.5
2	Lakeland sandy (thermic, coated Typic Quartzipsamment)	70	18	12	2.0	6.2
3	Angie variant clay (fine, mixed, semiactive, thermic Aqui Paleudult)	42	28	30	0.8	6.7
4	Fuquay sandy (loamy, kaolinitic, thermic Arenic Plinthic Kandiudult)	68	26	6	2.5	6.1

atrazine and 240 nm for pendimethalin using an Evolution™ 260 bio UV-visible spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA). Limit of detection was 7 and 25 µmol L^{-1} for atrazine and pendimethalin, respectively. The biochar sorption experiment was conducted twice using completely randomized designs with 3 replications. Data from both experiments were pooled (no interaction with other factors) and analyzed using linear regression with SigmaPlot version 12.5.

Biochar Effect on Herbicide Efficacy under Field Conditions. Four field experiments were established during July 2014 to determine if soil incorporation of biochar affects atrazine and pendimethalin herbicidal activity. The four experiments were located in a radius of 25 km from the West Florida Research and Education Center in Jay, FL (30.78°N, 87.14°W). Soil texture, organic matter content, and pH were determined for each site before biochar was added (Table 3). Biochar was applied at 0 and 0.5 kg m^{-2} and incorporated at 15 cm depth in the soil using a field cultivator. Herbicide treatments were atrazine (AAtrex®, Syngenta, Greensboro, NC) at 0, 1.12, 2.24, and 4.48 kg ha^{-1} and pendimethalin (Prowl H_2O®, BASF, Davis Drive, NC) at 0, 0.55, 1.11, and 2.22 kg ha^{-1}. Those rates were equivalent to 0, 0.5X, 1X, and 2X of atrazine and pendimethalin label rates (Anonymous 2012, 2013). Timing between biochar and herbicide application was 4 to 6 d. Herbicides were applied with a CO_2 pressurized field sprayer calibrated to deliver 187 L ha^{-1} and incorporated at 5 cm with a field cultivator. Plots were 2 m by 3 m. Herbicide efficacy was assessed using the natural population of susceptible weed species for each herbicide. Thus, atrazine herbicidal activity was assessed on broadleaved species {sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby], pitted morningglory (*Ipomoea lacunosa* L.), Benghal dayflower (*Commelina benghalensis* L.), Florida pusley (*Richardia scabra* L.)}, and pendimethalin on grass weed species {southern crabgrass [*Digitaria ciliaris* (Retz.) Koel.], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], goosegrass [*Eleusine indica* (L.) Gaertn.], crowfootgrass [*Dactyloctenium aegyptium* (L.) Willd.]}. Density of combined susceptible weed species for each herbicide was > 45 plants m^{-2}. Density of broadleaf and grass weed species was evaluated 4 wk after treatment (WAT). Weed density per species was determined counting emerged seedlings in a 0.25-m^2 area. Based on these data, percent reduction compared to the nontreated control was calculated to quantify weed control. The experiments were conducted using a randomized complete block design in a split plot arrangement with biochar as the main plot and herbicide the subplot with four replications. Data from all sites were analyzed with ANOVA using the GLIMMIX procedure of SAS. Herbicide dose was considered as a fixed effect, whereas block and block by biochar interaction were considered as random effects. Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$) was used for mean separation. In addition, linear and non-linear regression analyses were conducted with SigmaPlot (version 12.5, Systat Software, San Jose, CA) to determine atrazine and pendimethalin rate effect on weed control.

Results and Discussion

Herbicide *In Vitro* Bioavailability. No statistical differences were observed among the various biochar and vinasse application levels ($P = 0.48$), therefore results from herbicide injury *in vitro* conditions were pooled and analyzed only considering biochar and vinasse main effects per species. Atrazine and pendimethalin herbicidal activity in vinasse treatments showed similar injury to the soil-herbicide control treatment (Figure 1). While our results showed no vinasse effect on herbicide efficacy, other researchers have shown a decrease in residual activity due to herbicide degradation.

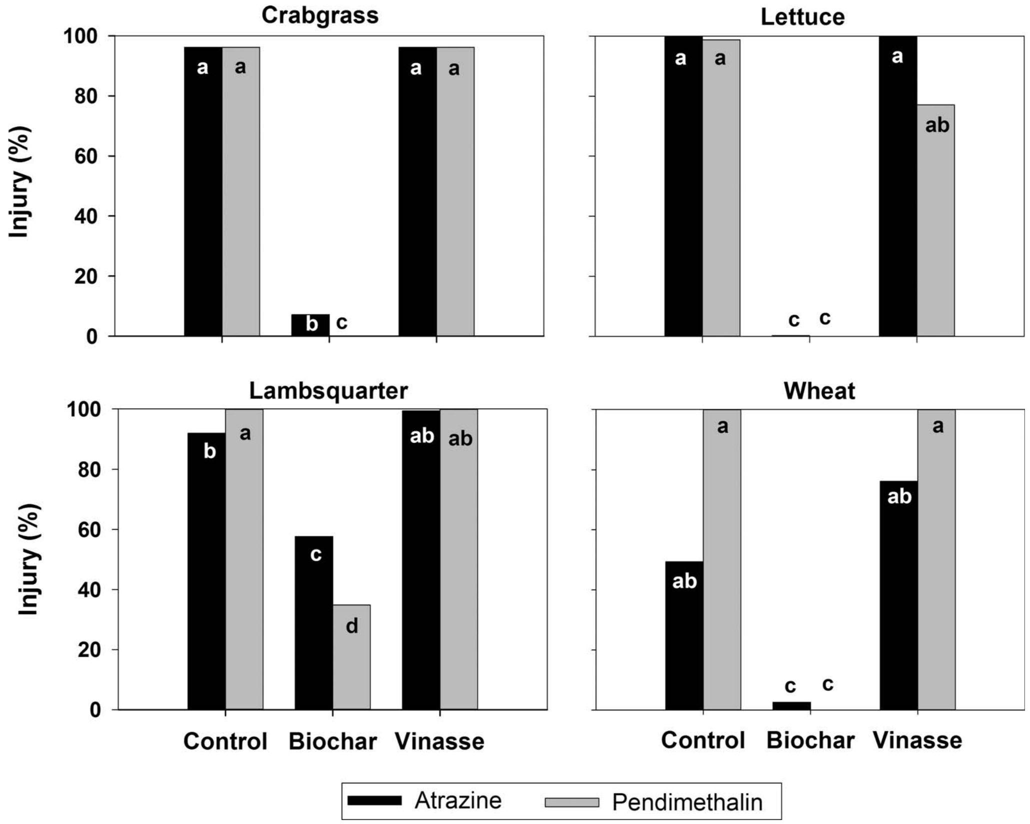

Figure 1. Atrazine at 2.81 kg ai ha^{-1} and pendimethalin at 1.27 kg ai ha^{-1} injury on southern crabgrass, common lambsquarter, lettuce, and wheat after seven days of incubation for control, biochar and vinasse treatments. Means with the same letter are not significantly different based on Tukey-Kramer Honestly Significant Difference ($\alpha = 0.05$).

Lourencetti et al. (2012) reported a reduction in diuron and tebuthiuron dissipation time (DT_{50}) of 73 and 55 d, respectively when vinasse at 15 L m^{-2} was added to a clay soil, although DT_{50} for hexazinone did not differ from the nontreated control.

Plant injury decreased with the addition of biochar compared to the control treatment (Figure 1). Relative to the control, biochar reduced atrazine injury by 89%, 34%, 100%, and 45% for southern crabgrass, common lambsquarter, lettuce, and wheat, respectively. Similarly, biochar addition decreased pendimethalin activity for all the studied species. Southern crabgrass, lettuce and wheat presented no injury, whereas common lambsquarters had only 38% injury in biochar plus pendimethalin treatments, while the non-biochar control treatment had > 95% injury across all species. Yang et al. (2006) reported no diuron bioavailability after applying biochar at 0.5 kg m^{-2}. Similar reductions in bioavailability were demonstrated with carbofuran and chlorpyrifos when biochar was applied at 1 kg m^{-2} compared to the control (Yu et al. 2009). Nag et al. (2011) reported that trifluralin GR_{50} (herbicide dose required to reduce 50% of plant growth) increased by 1.5 times when biochar was added at 1 kg m^{-2} in a ferrosol soil. Results from this study suggested that biochar soil incorporated had a high capacity to reduce herbicide bioavailability for plant uptake (Clay and Malo 2012; Kookana et al. 2011). Therefore, biochar-treated soils may require greater herbicide rates, more applications and/or shift to POST herbicides to achieve acceptable weed control.

Biochar-Herbicide Sorption. Considering the previous results where biochar addition reduced plant injury, a sorption experiment was conducted to quantify the effect of biochar on herbicide concentration in solution (Monks and Banks 1993). Linear regression analysis showed that the slope for atrazine adsorption was 16 times higher in soil with biochar than that of soil alone (Figure 2). Burned wheat residues increased diuron sorption compared

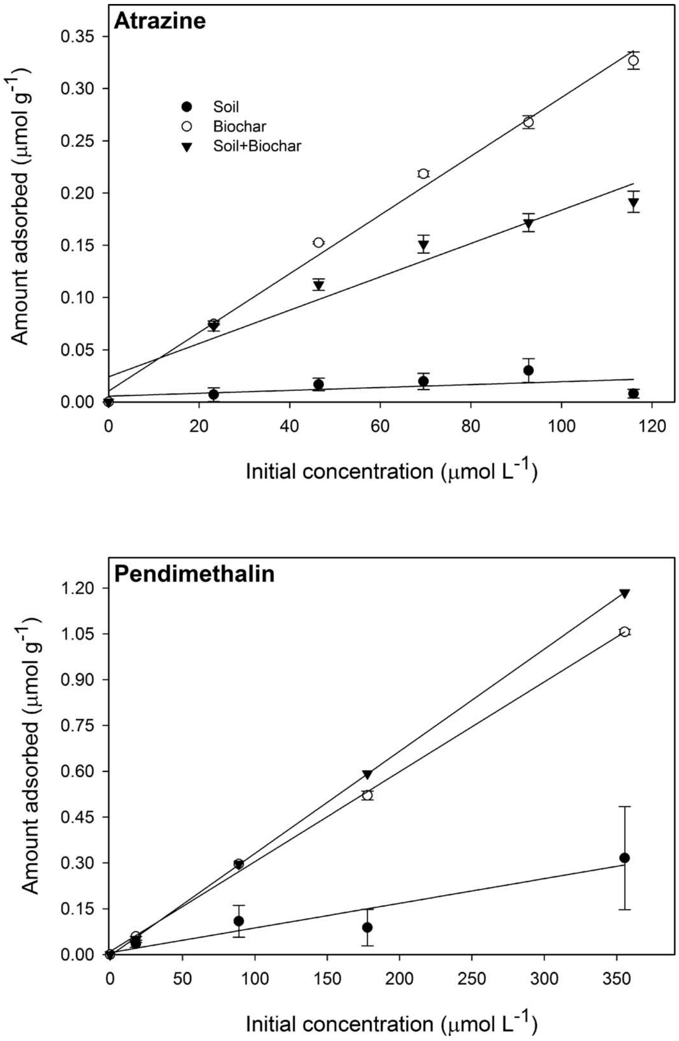

Figure 2. Atrazine and pendimethalin in vitro adsorption to soil, biochar and soil mixed with biochar. Sorption curves models for atrazine are: soil $y = 0.0056 + 0.0001x$ ($r^2 = 0.08$ and $P = 0.07$); biochar $y = 0.0105 + 0.0028x$ ($r^2 = 0.98$ and $P < 0.0001$); and soil with biochar $y = 0.024 + 0.0016x$ ($r^2 = 0.88$ and $P < 0.0001$); and for pendimethalin are: soil $y = 0.0062 + 0.0008x$ ($r^2 = 0.23$ and $P = 0.006$); biochar $y = 0.0098 + 0.0029x$ ($r^2 = 0.99$ and $P < 0.0001$); and soil with biochar $y = -0.0036 + 0.0033x$ ($r^2 = 0.99$ and $P < 0.0001$).

to soil sorption (Yang and Sheng 2003). Atrazine adsorption was higher with biochar than with soil plus biochar. This is likely due to competition for char adsorption sites between atrazine and other soil components such as dissolved soil organic matter and nutrients (Loganathan et al. 2009). The slope for pendimethalin adsorption in soil with biochar was up to 4 times higher than in soil without biochar (Figure 2). Soil plus biochar exhibited the highest pendimethalin adsorption but this was similar to biochar alone. Biochar sorption mechanisms are related to an increment in specific surface area, and hydrophobic and aromatic C structures (Hao et al. 2013). Therefore, organic molecules such as atrazine and pendimethalin have high affinity to biochar. Herbicide chemical characteristics such as water solubility and sorption coefficient (K_{oc}) influence the amount of herbicide that can be adsorbed by biochar (Nag et al. 2011). Pendimethalin has a higher affinity to bind organic matter (K_{oc} 13,000 mL g^{-1}), and it is less soluble in water (0.275 mg L^{-1}) compared to atrazine, which has a lower K_{oc} (70 mL g^{-1}), and it is more water soluble (33 mg L^{-1}) (Shaner 2014). Biochar's sorption capacity may decrease over time, but factors such as application rate, biochar type, and soil properties might influence biochar persistence potentially promoting sorption (Martin et al. 2012). Moreover, biochar aging could modify sorption capacity due to an increase in the formation of aromatic components (Kookana et al. 2011). The results indicated that the low plant injury observed in the *in vitro* bioavailability experiment was related to a reduction of herbicide in solution due to biochar sorption.

Biochar Effect on Herbicide Efficacy under Field Conditions. Interactions between site, herbicide or biochar factors were not significant for atrazine ($P = 0.90$) and pendimethalin ($P = 0.86$). Therefore, data from the four sites were pooled, and only biochar and herbicide interactions are discussed. Biochar reduced weed control with atrazine ($P = 0.0008$) and pendimethalin ($P = 0.0001$) compared to non-amended treatments. Broadleaf and grass weed control data were fitted with non-linear and linear regression to compare atrazine and pendimethalin rates with and without biochar (Figure 3). Atrazine with no biochar controlled broadleaf weeds 70% to 80% across rates. Biochar addition at 0.5 kg m^{-2} decreased broadleaf control to 5% at the highest atrazine rate. In treatments without biochar, pendimethalin caused 100% grass control. Conversely, when biochar was present the highest pendimethalin rate had less than 50% grass control. Atrazine and pendimethalin at 2X label rate (4.48 and 2.22 kg ha^{-1}, respectively) in amended soil did not provide adequate weed control. Currently, there are few studies about the impact of biochar on PRE herbicides activity under field conditions.

Our field results demonstrated that modifications to herbicide programs might be necessary to

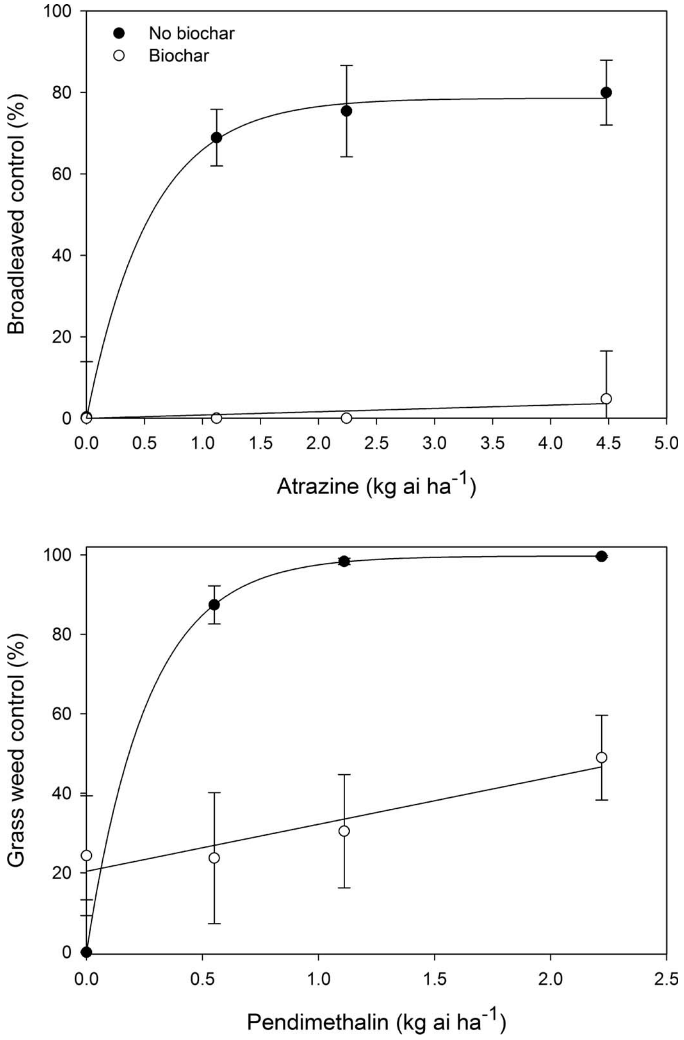

Figure 3. Control of broadleaved and grass weed species in response to increasing atrazine and pendimethalin rates, respectively. Dose-response models for atrazine without biochar was $y = 78.59$ (*1–exp* (–1.82*x*) ($r^2 = 0.42$ and $P < 0.0001$); and with biochar was $y = -21.02 + 4.95x$ ($r^2 = 0.02$ and $P = 0.28$). Pendimethalin dose-response models were: without biochar 99.68 (*1 – exp* (–3.80*x*) ($r^2 = 0.72$ and $P < 0.0001$) and with biochar $y = 15.54 + 15.22x$ ($r^2 = 0.05$ and $P = 0.07$).

compensate for biochar use in cropping systems. Biochar application implies a large addition of organic carbon to the soil, which in terms of herbicide management creates conditions similar to a high organic matter soil type. Therefore, PRE herbicide rates necessary to provide adequate weed control after biochar application might have to be similar to those used for histosols or muck soils (high organic matter content). Few studies have considered the implications on herbicide activity of long-term biochar use under field conditions. For instance, Kookana (2010) discussed the possibility that pesticides could be held by biochar micropores through time, limiting bioavailability and accumulating in the amended soil. In addition, Jones et al. (2011) reported that biochar adsorption capacity on PRE herbicides could remain even two years after the amendment application under field conditions.

Biochar reduced herbicide efficacy *in vitro* and under field conditions. Results from this study confirmed our hypothesis that biochar soil incorporation could reduce atrazine and pendimethalin herbicidal activity. Biochar use in cropping systems could modify current weed management practices because of the reduction in PRE herbicide efficacy. Increased reliance on POST herbicides, cultivation and cultural practices such as mulches and hand weeding might be necessary for ensuring proper weed control in fields amended with biochar.

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