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ORIGINAL RESEARCH

Arbuscular mycorrhizal fungi and biochar influence simazine decomposition and leaching

Hongguang Cheng[1,2] | **Jinyang Wang**[2] | **Chenglong Tu**[3,4] | **Shan Lin**[5] | **Dan Xing**[6] | **Paul Hill**[2] | **Dave Chadwick**[2] | **Davey L. Jones**[2,7]

[1]State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, Guizhou, China

[2]School of Natural Sciences, Bangor University, Bangor, UK

[3]Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University, Guiyang, China

[4]Toxicity Testing Center of Guizhou Medical University, Guiyang, China

[5]Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze River), College of Resources and Environment, Ministry of Agriculture, Huazhong Agricultural University, Wuhan, China

[6]Guizhou Academy of Agricultural Science, Institute of Pepper Guiyang, Guiyang, China

[7]UWA School of Agriculture and Environment, University of Western Australia, Perth, WA, Australia

Correspondence
Hongguang Cheng, State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, Guizhou 550081, China.
Email: chenghongguang@vip.gyig.ac.cn

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Abstract

The application of biochar to land has been promoted as a strategy for sequestering carbon in soils, for improving soil fertility and remediating soil pollution. However, the implications of biochar amendments on mycorrhizal associations and pesticide decomposition in agricultural soils are poorly understood. In this study, we compared the effects of four treatments; control (no biochar and no arbuscular mycorrhizal fungi (AMF), biochar (biochar without AMF), AMF (AMF without biochar) and biochar + AMF (AMF and biochar) on the fate of simazine. We specifically focused on the sorption, leaching and biodegradation behaviour of simazine. Our results showed that when symbiosis existed between plants and AMF, biochar inhibited simazine decomposition and AMF inoculation alleviated this inhibition. In contrast, this alleviation was not observed when the plant was removed. In addition, AMF inoculated into the biochar amended soil significantly decreased simazine concentration in the leachate; however, in the AMF-only treatment, no effect on simazine leaching was observed. These phenomena were attributed to variation in the soil's sorption capacity due to biochar application or AMF inoculation. Overall, biochar application combined with AMF inoculation has the potential to mitigate simazine accumulation in the topsoil and reduce its availability.

KEYWORDS
adsorption, decomposition, leaching, simazine, symbiosis

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1 | INTRODUCTION

Simazine (1-Chloro-3,5-bisethylamino-2,4,6-triazine) is a commonly used pesticide in agriculture and forestry for controlling broadleaf and grassy weeds. Because of its longer half-life (Jones et al., 2011; Wauchope et al., 1992), simazine can readily accumulate in soil where it is at risk of leaching and runoff (Jiang et al., 2011). For example, simazine is the second most commonly detected pesticide in surface water and groundwater in the United States, Europe and Australia, where it can be present at concentrations of up to several hundred micrograms per litre (Cox et al., 2000; Troiano et al., 2001). It therefore represents a risk to human and ecosystems health (Rico et al., 2012) via exposure to simazine through drinking water and the food chain, as well as direct uptake, and is known to induce some mutagenic or carcinogenic activity (Birnbaum & Fenton, 2003; Bogdanffy et al., 2000; Hayes et al., 2006). Therefore, there is a need to adopt strategies to prevent accumulation of pesticides in soil and decrease pesticide losses via leaching to safeguard human and ecosystem health.

Biochar is a carbon rich by-product produced during the pyrolysis of organic residues (Lehmann & Joseph, 2009) in an oxygen-depleted environment (Lehmann et al., 2011a). It is considered an emerging technology for carbon sequestration, mitigation of climate change, soil improvement, crop productivity enhancement and environmental remediation (Atkinson et al., 2010; Ippolito et al., 2012; Lehmann & Joseph, 2009; Maraseni, 2010; Spokas et al., 2012). Once incorporated into soil, biochar has been shown to alter soil properties (Kuppusamy et al., 2016), improve nutrient retention (Camps Arbestain et al., 2014), decrease greenhouse emissions (Chang et al., 2016) and increase sorption of contaminants (Cheng et al., 2017; Williams et al., 2015). It is well known that biochar application can increase the sorption of simazine in soil, thus decreasing its risk of leaching and direct uptake by plants. The high surface area and stronger cation exchange capacity of soil amended with biochar results in this greater adsorption of pesticides (Eibisch et al., 2015; Yu et al., 2010, 2011), resulting in lower concentration of pesticides in leachate (Larsbo et al., 2013; Tatarková et al., 2013) and low residues in the crop (Yang et al., 2010).

Although these benefits of biochar have been confirmed in recent studies, there are several potential negative implications, hazards and even risks to soil and water quality that other researchers have highlighted (Graber et al., 2012; Kookana, 2010; Kookana et al., 2011; Kuppusamy et al., 2016). For example, some studies have reported that biochar inhibits simazine biodegradation (Cheng et al., 2017; Jones et al., 2011) and reduces simazine efficacy for controlling weeds or killing pests (Graber et al., 2012; Kookana, 2010; Kuppusamy et al., 2016; Nag et al., 2011; Safaei Khorram et al., 2018; Yang et al., 2006). If biochar results in a longer half-life of pesticides in the soil, then greater concentrations will accumulate in the topsoil where it is at risk of losses to water via leaching and overland flow. With growing interest in the use of modified biochar with greater sorption capacity for remediating heavy metal and organic contamination of soil (Mandal et al., 2017; Trakal et al., 2016), pesticide decomposition could be reduced further resulting in increased risk of pesticide accumulation in the soil. These potential negative implications associated with biochar addition are not yet fully understood (Graber et al., 2012; Kookana, 2010; Nag et al., 2011).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil organisms, forming symbiotic associations with the roots of c. 80% of all plants species (Bender et al., 2014). The hyphal network of AMF scavenge nutrients from soils and transfer a proportion to their host plant in return for labile plant carbon (Smith & Read, 2008). Even though AMF have no known saprotrophic capability and cannot directly break down organic nutrients (Herman et al., 2012; Smith & Read, 2008), past studies have reported that AMF can enhance decomposition of organic matter (OM; Hodge et al., 2001; Koller et al., 2013). For example, Cheng et al. (2012) showed that plant litter decomposed faster in the presence of AMF, especially under conditions of elevated CO_2 and nitrogen (N) concentrations. Likewise, Gui et al. (2017) measured increased OM decomposition while inhibiting soil microbial community development, in the presence of AMF, and hypothesized that AMF promoted OM decomposition by influencing the soil decomposer community. These studies highlighted the potential role of AMF in OM decomposition.

From this knowledge of the effects of biochar and AMF on the fate of pesticides in soil, we can hypothesize that combined amendment of biochar and AMF will positively affect simazine decomposition, adsorption and leaching. Therefore, in this study, we hypothesized that (a) higher simazine decomposition would occur when AMF was inoculated into soil amended with biochar because AMF would accelerate the decomposition of OM and (b) the sorption capacity of soil would increase when AMF was inoculated into the soil. The main aims of this study were to (1) observe the fate of simazine, including decomposition, adsorption and leaching in the soil amended with biochar; (2) investigate the fate of simazine in soil with AMF inoculation and (3) evaluate the influence of AMF inoculation combined with biochar application on the fate of simazine in soil.

2 | MATERIALS AND METHODS

2.1 | Biochar, soil and AMF

Biochar was produced from wheat (*Triticum aestivum* L.) straw, which was collected from the Henfaes Research

Centre Wales, North Wales, UK (53°140N; 4°100W). The wheat straw was oven-dried (80°C, 24 h) and then cut into 10 cm pieces before being placement into a glass pyrolysis vessel. The vessel was then placed in a muffle furnace for pyrolysis. The heating rate was 20°C min^{-1}, and the thermal treatment time was 0.5 h with peak pyrolysis temperatures 550°C. The properties of biochar are shown in Table 1.

Soil was collected from the Ah horizon (0–15 cm, sandy loam) of a freely draining, grassland soil (Eutric Cambsiol soil type), which receives regular fertilization (120 kg ha^{-1} N, 60 kg ha^{-1} K and 10 kg ha^{-1} P annually) and is located at the Henfaes Research Centre. The site is used for both grassland and arable production and has a mean annual temperature of 11°C (range −5°C to 25°C) and mean annual rainfall of 1060 mm (temperate climate regime). The soil was sieved to pass 2 mm to remove plant residues and stones and then air-dried at 20°C for 1 week prior to use. Subsequently, the soil was autoclaved (121°C, 1 h) to remove and AMF in the soil. After autoclaving, soil was placed in a greenhouse for 1 month to allow the microbial community to recover. The major properties of the soil are shown in Table 1, with additional properties shown in Jones et al. (2011, 2012) and Farrar et al. (2012).

The mycorrhizal and rhizobial symbiotic inoculants were obtained from a commercial supplier (Plantworks Ltd). We choose an AMF which associates widely with cereals, namely *Funneliformis mosseae* (formerly *Glomus mosseae*) and which is known to be present at the site where the soil was collected. The inoculum consisted of a mixture of substrate, hyphae, spores and infected root fragments.

TABLE 1 The properties of soil and biochar (data expressed on a dry weight basis)

	Soil	Biochar
pH	5.94 ± 0.15	9.70 ± 0.10
Electrical conductivity (μS cm^{-1})	87.73 ± 4.18	4560 ± 290
Water holding capacity (%)	74.97 ± 1.00	659.77 ± 9.14
Total carbon (%)	3.10 ± 0.05	71.60 ± 0.25
Total nitrogen (%)	0.34 ± 0.01	1.18 ± 0.02
Dissolved organic carbon (g kg^{-1})	15.25 ± 0.64	14.27 ± 1.27
Dissolved nitrogen (g kg^{-1})	4.63 ± 0.19	0.17 ± 0.03
Available NO_3^- (mg kg^{-1})	13.63 ± 0.58	0.00 ± 0.00
Available NH_4^+ (mg kg^{-1})	1.39 ± 0.05	2.38 ± 0.14
Available P (mg kg^{-1})	0.60 ± 0.04	206.61 ± 27.17

Note: All values represent means ± SEM ($n = 3$).

2.2 | Experimental design

Four treatments were used in the experiment: (i) control (soil only, with no biochar or AMF inoculation); (ii) soil amended with biochar (dry soil-to-dry biochar ratio of 20:1 w/w); (iii) soil inoculated with AMF; and (iv) soil amended with biochar inoculated with AMF. To create the amended soils, replicate batches of biochar (300 g, ground and sieved to pass 2 mm) were added to replicate batches (6 kg) of air-dry soil and manually mixed to ensure homogeneity. For the treatment inoculated with AMF, 3 g of commercial mycorrhizal inoculum was mixed with either the soil alone (treatment ii), or the biochar-amended soil (treatment iv). To account for the effects of the AMF carrier, 3 g of substrate containing no AMF was added to the no AMF treatments. All the soil treatments were conducted in triplicate.

2.3 | Simazine decomposition

To distinguish between the influence of AMF symbiosis and the presence of the host plant on the simazine decomposition, we divided our experiments into two parts, namely a pot experiment and a laboratory incubation experiment.

The pot experiment was performed in the isotope labelling facility at Environment Centre Wales, Bangor University. The mesocosms consisted of a cylindrical mesocosms (Figure 1) containing 1 kg of soil into which two pre-germinated maize

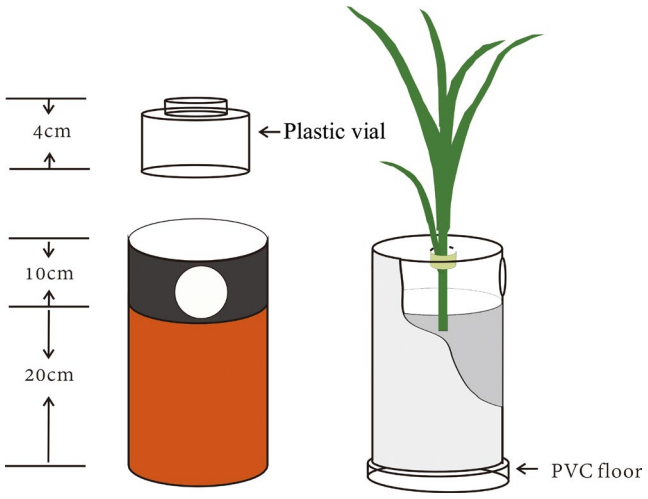

FIGURE 1 Pot equipment for exploring the effect of arbuscular mycorrhizal fungi symbiosis in biochar-amended soil on simazine decomposition

(*Zea mays* L.) seeds were placed. The mesocosms were watered regularly to maintain field capacity. After 2 weeks, the maize seedlings were thinned to one plant and a plastic vial with a hole cut in the bottom placed around the stem of the maize seedling. At the same time, 30 ml of Hoagland's modified basal salt mixture was added into each mesocosm to promote plant growth (Hoagland & Arnon, 1950). In the sixth week, the stem was sealed into the top of the vial using one-component room-temperature-vulcanizing silicone (Figure 1). The surface of the soil column was then enclosed in transparent polypropylene which was sealed with tape to the vial. Subsequently, 60 ml of ^{14}C-ring-uniformly labelled simazine (6-chloro-N,N′-diethyl-1,3,5-triazine-2,4-diamine; 5 mCi mmol^{-1}; Sigma Chemical Co.) in distilled water was added to the soil at a rate of 5 mg kg^{-1} (final simazine concentration, 8.34 mg L^{-1}; 0.10 kBq ml^{-1}). A 2 M NaOH trap (2 ml) was then placed inside the mesocosm to capture any $^{14}CO_2$ evolved and the mesocosms hermetically sealed and placed in a climate-controlled greenhouse at 25°C. The NaOH traps were replaced after 1, 3, 6, 9, 12, 15, 18 and 21 days. The $^{14}CO_2$ capture efficiency of the NaOH traps was >98% (Jones et al., 2012). The $^{14}CO_2$ content in the NaOH traps was determined by liquid scintillation counting using Optiphase 3 scintillation fluid (PerkinElmer Corp.) and a Wallac 1404 liquid scintillation counter (PerkinElmer Corp.). Three weeks after labelling, the maize plant was harvested to determine dry weight and the plant and soil retained to determine their ^{14}C content.

An identical experiment to that described above was also established. In this experiment, however, immediately after the application of unlabelled simazine, the plants in the mesocosms were removed and soil was collected for biodedgradation and sorption experiments. Soil from each treatment (5 g) was weighed into 50 ml polypropylene tube and adjusted to 60% of the water holding capacity (WHC). Subsequently, 0.5 ml of ^{14}C-labelled simazine (1.0 mg ml^{-1} and 1.08 kBq ml^{-1}) was added to each sample. A 1 ml NaOH trap (1 M) was placed above the soil to capture CO_2 released from the soil. The NaOH traps were replaced after 1, 3, 6, 9, 12, 15, 18 and 21 days. The $^{14}CO_2$ content of the NaOH was measured as described above.

2.4 | Simazine adsorption

Soil from each treatment was placed in individual 50 ml polypropylene tubes. The tubes were then heat sterilized (80°C, 30 min) to minimize microbial activity (Kuzyakov & Jones, 2006). A ^{14}C-labelled simazine solution in a background electrolyte of 0.01 M $CaCl_2$ (20 ml) was then applied to the soil (total activity 0.1 kBq). A total of six concentrations of ^{14}C simazine were used, including 0, 6.25, 12.5, 25, 50 and 100 μg L^{-1}. The soil suspensions were shaken (200 rev min^{-1}, 24 h), which represented quasi-equilibrium conditions (Kookana et al., 1993). Subsequently, an aliquot (1.0 ml) of the soil suspension was centrifuged (10,000 *g*; 5 min) and the ^{14}C activity in the supernatant determined by liquid scintillation counting as described above. The partition coefficient (K_d) of simazine between the soil and the solution phase was calculated as follows:

$$K_d = C_{ads}/C_{sol}, \quad (1)$$

where C_{ads} (μg g^{-1}) is the concentration sorbed to the soil solid phase at equilibrium and C_{sol} (mg L^{-1}) is the equilibrium solution concentration.

2.5 | Simazine leaching

The effect of biochar and AMF on simazine leaching was determined according to the method of Jones et al. (2011) and Cheng et al. (2017). Briefly, approximately 5.0 g from each replicate treatment was placed into the barrel of individual 25 ml syringe (20 mm diameter). A 1 mm polypropylene mesh was placed at the base of the column to prevent soil loss. Subsequently, distilled water was added to each column to saturate the soil. ^{14}C-labelled simazine (1 ml, 2.5 mg L^{-1} and 0.05 kBq ml^{-1}) was then added to the soil surface and the sample left to equilibrate at 20°C for 1 h. Before the start of leaching, another 1 mm polypropylene mesh was placed onto the soil surface to minimize droplet impact on the soil surface. A syringe-pump was then used to add distilled water at a rate of 0.2 ml min^{-1} to the soil surface and the leachate collected from the base of the columns after the passage of 1, 2, 3, 4 and 5 pore volumes. The ^{14}C content of the leachate was determined as described above.

2.6 | Soil, biochar properties and biomass analysis

The pH and electrical conductivity (1:5 w/v with distilled water for biochar and 1: 2.5 w/v for soil) of the biochar and soil were determined with standard electrodes. The WHC was measured using the international standard method, ISO16378 (Ref). Briefly, 2.0 g of biochar, soil or straw was saturated in distilled water for 4 h, and then placed on moist sand for 2 h. The sample was then oven-dried (105°C, 24 h) to determine their water content. Available NH_4^+ and NO_3^- were determined in 0.5 M K_2SO_4 extracts (1:5 w/v) using the colorimetric methods of Mulvaney (1996) and Miranda et al. (2001), respectively. Available P was extracted from the soil and biochar using 0.5 M acetic acid (1:5 w/v) and P determined colorimetrically using the molybdate blue method of Murphy and Riley (1962).

2.7 | Statistical analyses

A Langmuir (Equation 2) or Freundlich (Equation 3) isotherm equation was fitted to the simazine adsorption data as shown below:

$$\frac{C_e}{q_e} = \frac{1}{q_{max}K_L} + \frac{C_e}{q_{max}}, \quad (2)$$

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e, \quad (3)$$

where q_e and q_{max} are the equilibrium and maximum adsorption capacities (mg g^{-1}), respectively; K_L, the Langmuir constant related to the affinity of the binding sites (L mg^{-1}) and C_e, the equilibrium concentration of adsorbate in an aqueous phases (mg L^{-1}). K_F is a constant representing adsorption capacity, and n is a constant reflecting the adsorption intensity.

Statistical analyses were performed with software SPSS v26.0 (IBM Inc.). First, each variable was tested for normality with the Shapiro–Wilk's test and homogeneity of variance test with Levene's test. The variables which were not normally distributed or had unequal variances (such as decomposition and adsorption) were tested using a non-parametric Wilcoxon paired signed-rank test. The difference in biomass, simazine residues and leachates between the different treatments was analysed using a one-way ANOVA with Fisher's least significant difference (LSD), and paired sample t-tests. All the differences were considered significant at the $p < 0.05$ level. The linear regression was undertaken in Origin 2019b (OriginLab Corp).

3 | RESULTS

3.1 | Soil and biochar properties and maize yield

The physical and chemical properties of the biochar and soil are listed in Table 1. Maize yields from the pot experiment are shown in Figure 2, and show no significant effect between the biochar and biochar + AMF treatments and the control. However, the maize yield from the AMF treatment was significantly lower than the yields in other treatments.

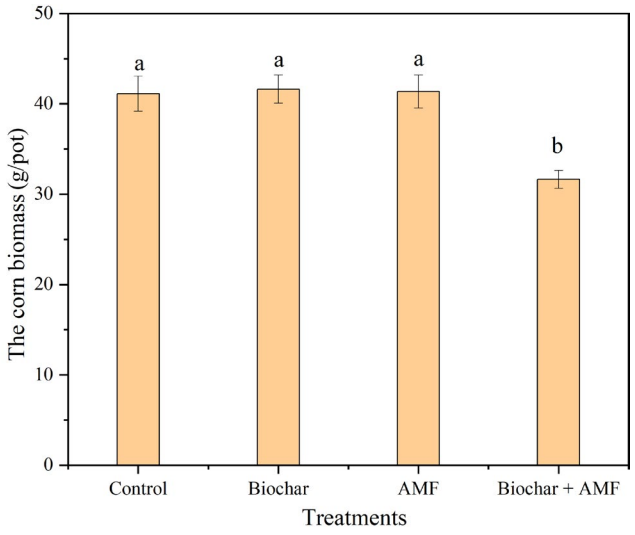

FIGURE 2 Effects of arbuscular mycorrhizal fungi (AMF) and biochar amendments on maize biomass

3.2 | Simazine decomposition

The decomposition rates of simazine are shown in Figure 3. At the end of the symbiosis experiment (in the presence of plants; Figure 3a), the cumulative decomposition of simazine in the biochar treatment was significantly lower (3%) than that in the control. Compared to biochar treatment, AMF inoculation significantly increased (0.5%) the decomposition of simazine. However, the cumulative decomposition of simazine in the AMF treatment was not significant different to the decomposition of simazine in the control, although at the mid-point of the pot experiment, the decomposition of simazine in the inoculated AMF treatment was faster than that of the control.

In the no symbiosis (absence of plants) incubation experiment (Figure 3b), AMF inoculation did not increase simazine decomposition, with the decomposition of simazine in the AMF treatment being significantly lower than in the control. Moreover, the ^{14}C activity in the plant and soil are shown in

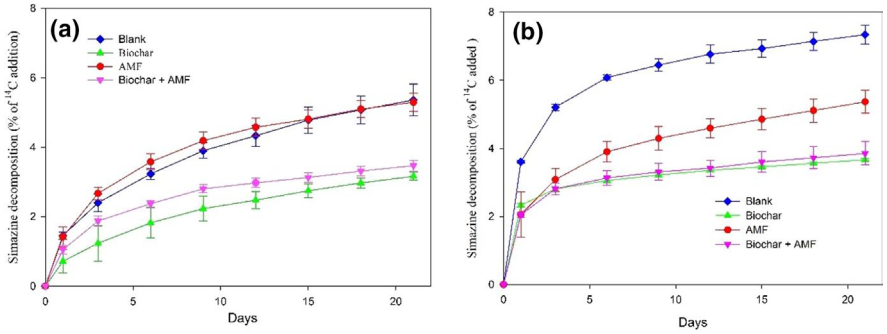

FIGURE 3 Cumulative decomposition rates of simazine (a) in the presence of plants; (b) in the absence of plants

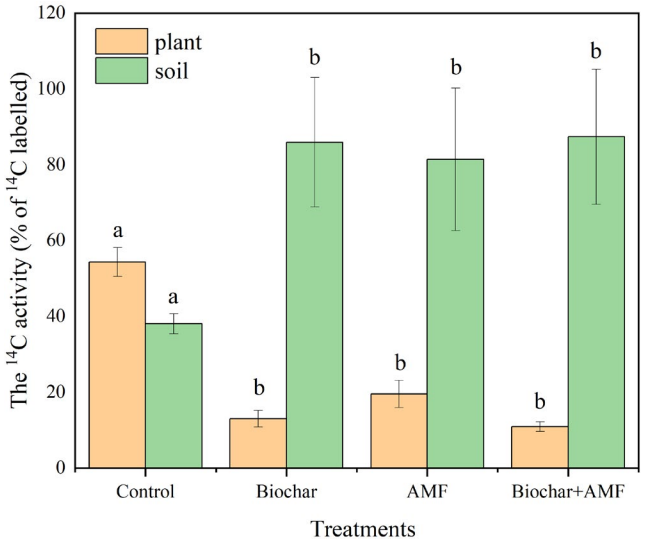

FIGURE 4 Effect of arbuscular mycorrhizal fungi (AMF) and biochar amendments on ^{14}C activity in maize shoots and soil

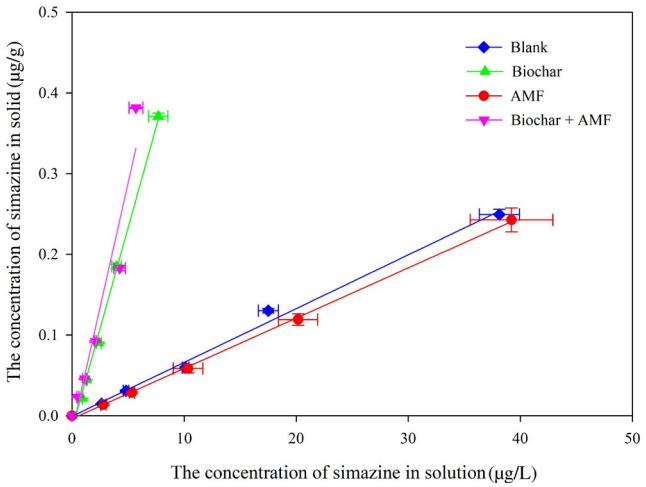

FIGURE 5 Effect of arbuscular mycorrhizal fungi (AMF) and biochar amendments on simazine sorption

Figure 4. The ^{14}C activity in plants which were collected from the biochar amended treatment and AMF inoculation treatment was low, whereas the ^{14}C activity in soil which were collected from biochar and AMF added treatments was high.

3.3 | Simazine adsorption

Adsorption isotherms of simazine for the biochar and AMF treatments were evaluated using the models of Langmuir and Freundlich. According to the R^2 values, it was clear that Freundlich equation better fitted to the experiment ($R^2 > 0.94$). The K_d was significantly higher in the soil amended with biochar (Figure 5). The adsorption capacity constant K_f were 0.005, 0.027, 0.005 and 0.043, respectively, in the control, soil amended with biochar, soil inoculated AMF, and soil amended with biochar inoculated AMF. Additionally, the adsorption intensity constant n was 0.94, 0.77, 0.93 and 0. 94, respectively, in the control, soil amended with biochar, soil inoculated AMF and soil amended with biochar inoculated AMF.

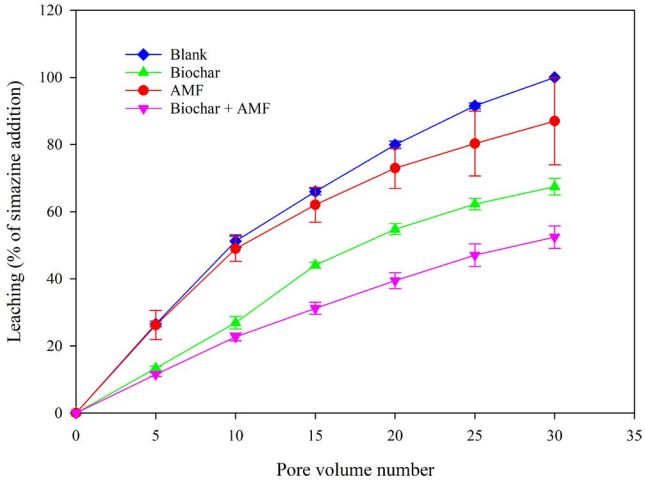

FIGURE 6 Effect of arbuscular mycorrhizal fungi (AMF) and biochar amendments on simazine leaching

3.4 | Simazine leaching

The results of simazine leaching are shown in Figure 6. Biochar amendments significantly decreased simazine concentrations (by 32.5%) in the leachate compared to control treatment. Moreover, the AMF inoculation also decreased the simazine concentration (13.0%) in the leachate. The lowest concentration of simazine in the leachate, compared with the control (47.6%), was observed in the AMF inoculation with biochar treatment ($p < 0.05$).

4 | DISCUSSION

4.1 | Simazine behaviour in soil amended with biochar

Due to their high specific area and micro-porous structures (Khorram et al., 2015; Srinivasan & Sarmah, 2015), biochar is considered be a good sorbent, which results in the suppression of pesticide biodegradation (Jones et al., 2011; Yu et al., 2009) and also lower potential leaching of pesticides into groundwater (Marín-Benito et al., 2013). As seen previously (Cheng et al., 2017; Jones et al., 2011), in this study, biochar addition also significantly decreased the decomposition of simazine and decreased the simazine concentration in the leachate compared to an unamended soil. In addition, biochar application significantly decreased the ^{14}C activity in the plant and increased the

TABLE 2 Parameters of the simazine sorption isotherm for biochar using the Langmuir and Freundlich model

	Freundlich			Langmuir			
Treatments	n	K_f	R^2	q_m	K_l	R^2	K_d
Control	0.940 ± 0.021^a	0.005 ± 0.0001^a	0.988 ± 0.005	15.75 ± 14.75^a	0.0037 ± 0.0018^a	0.192 ± 0.120	6.70 ± 0.42^a
Biochar	0.772 ± 0.021^b	0.027 ± 0.0018^b	0.979 ± 0.010	0.49 ± 0.13^b	0.0629 ± 0.0145^b	0.430 ± 0.169	50.73 ± 4.67^b
AMF	0.926 ± 0.034^a	0.005 ± 0.0009^a	0.995 ± 0.003	1.56 ± 0.59^a	0.0049 ± 0.0022^a	0.379 ± 0.164	6.50 ± 0.78^a
Biochar + AMF	0.943 ± 0.074^a	0.043 ± 0.0021^c	0.949 ± 0.012	1.31 ± 0.52^a	0.0439 ± 0.0203^b	0.229 ± 0.235	64.67 ± 6.63^c

Note: All values represent means ± SEM ($n = 3$). Different superscript letters represent significant differences between treatments at the $p < 0.05$ level (n represents ANOVA results and K_f represents non-parametric test: Mann–Whitney test results).

^{14}C activity in the soil (Figure 4). Two mechanisms are likely to explain these observations: (1) biochar altered the bioavailability of pesticides in soil (Jones et al., 2011). The adsorption results (Figure 5) indicated that biochar application increased the adsorption capacity of soil (Table 2), resulting in more simazine being retained in the soil and less simazine uptake by the crop. Although the ^{14}C activity (Figure 4) in the plant (or soil) was not equal to the simazine content in the plant (or soil), it indirectly showed that there is a less uptake of simazine by plant in the soil amended with biochar. The increased sorption by the biochar reduced the probability of simazine contacted with extracellular enzymes or microorganisms (Virchenko et al., 1986; Zhou et al., 2010), thus decreasing simazine biodegradation (Loganathan et al., 2009). (2) Biochar application modified the microbial community composition and activity (Lehmann et al., 2011) which subsequently affected the degradation of simazine. Biochar applications can result in increased soil pH, the priming of carbon and addition of micro-porous structures that are all important factors in regulating microbial community composition, microbial biomass and activity (Lehmann et al., 2011). For example, Cheng et al. (2019) reported that biochar application significantly influenced soil bacterial community characteristics in karst soil by affecting soil physiochemical properties, suggesting that biochar addition affected microbial population abundance, community structure and enzyme activities. Overall, these results showed that biochar application has the potential to decrease simazine decomposition and reduce the risk of groundwater and surface water pollution (Ahmad et al., 2014).

4.2 | The influence of AMF inoculation on the fate of simazine in soil

Arbuscular mycorrhizal symbiosis is a key evolutionary strategy for enhancing nutrient capture by the associated host plant, while in return providing a supply of carbon to the fungus (Smith & Read, 2008). Previous studies have demonstrated that a range of abiotic and biotic factors, including AM associations (Hodge & Millard, 1998; Hodge et al., 2007; Jones et al., 2004, 2009; Paterson et al., 1999) influence soil OM decomposition and rhizodeposition processes (Bird et al., 2011; Bottner et al., 1999; Dijkstra et al., 2009). In this study, AMF inoculated soil had no influence on simazine decomposition when the symbiosis had formed with the host plant (Figure 3a), whereas AMF inoculation decreased simazine decomposition in the absence of plants (Figure 3b). These results are in contrast with those reported previously, where arbuscular mycorrhizal symbiosis enhances OM mineralization (Hodge et al., 2007; Jones et al., 2004, 2009; Paterson et al., 1999). Because AMF has no saprotrophic capability, AMF inoculation results in the competition between AMF and other microbial decomposers for nutrition, which inhibited the development of other decomposing microorganisms (Hodge & Millard, 1998). This could be the reason for the significant decrease in simazine decomposition in the AMF inoculation treatment without plants. The plant biomass in the different treatment (Figure 2) also confirmed that nutrient competition between AMF and plants or other microorganisms may exist. The decreased of simazine decomposition due to nutrient competition and the increased of simazine decomposition because of enhancement of carbon and nutrient circulation both worked simultaneously, resulted in no difference in simazine decomposition between the AMF inoculation treatment and the control when plants were present (Figure 3a). This interpretation is supported by the measured simazine degradation rates in the absence of plants where there was no AM symbiosis.

4.3 | The influence of AMF inoculation on the fate of simazine in soil amended with biochar

When AM symbiosis existed, simazine decomposition was significantly increased in AMF inoculated soil amended with biochar, compared to the uninoculated AMF treatment (Figure 3a). Whereas, when there were no plants present, there was no difference in simazine degradation between the AMF + Biochar treatment and the biochar (only) treatment (Figure 3b). Compared to the control, biochar application directly supplies nutrients to the soil (Biederman & Stanley Harpole, 2013), which mitigates the competition for nutrients between AMF and plants or other microbial decomposers, and

increased simazine decomposition. The plant biomass results (Figure 2) also confirm that nutrient supplies was better in the biochar + AMF treatment than in the control. Therefore, AMF inoculation in the biochar amended soil alleviated the inhibiting effect of biochar on simazine decomposition. Furthermore, AMF inoculated into the biochar amended soil greatly increased the adsorption capacity of simazine (Table 2) compared to the biochar (only) application, with sorption capacity K_f, increasing from 0.027 (in the biochar only) to 0.043 (in the AMF + Biochar). The increase in contact area between the simazine and solid interphases, provided by the AMF mycelium, was not sufficient to explain this.

4.4 | Potential implications of combined AMF and biochar for controlling environmental contamination

Recently, biochar and AMF amendments have reviewed increasing attention as technologies for mitigating heavy metal contamination in soil (Hu et al., 2013; Vejvodová et al., 2020). For biochar, this is because of its porous structure, large surface area and dominance of micropores (Ahmad et al., 2014; Cao & Harris, 2010). For AMF, multiple studies have shown that AMF can immobilize and compartmentalize heavy metals in hyphal cells (Andrade et al., 2010; Göhre & Paszkowski, 2006), or produce metal chelation of glomalin, or fungal polyphosphates and metallothioneins to bind the heavy metal (Kaldorf et al., 1999; Vodnik et al., 2008). However, despite this increased understanding of the individual effects of AMF and biochar, there have been few studies that have explored the interactive, and potential synergistic effects (Mickan et al., 2016). In this study, AMF inoculation of soil amended with biochar decreased simazine concentrations in leachates (Figure 6) mitigated the inhibition of biochar on simazine decomposition (Figure 3), and decreased pesticide uptake by plants (Figure 4). These results indicate the synergistic effect of combining AMF and biochar amendment could be exploited as a strategy to reduce diffuse losses of pesticides like simazine to surface waters and aquifers, with implications for the protection of human health. Moreover, AMF inoculation of biochar amended soil mitigated against pesticide accumulation that is often observed in biochar only amended soil, increasing pesticide degradation. Because there have been so few studies that have explored the synergistic effects of AMF inoculation of biochar amended soil, we propose that further research should explore the way in which combined AMF and biochar affect the biotic and abiotic processes that control the fate of pesticides.

5 | CONCLUSION

The application of biochar to agricultural soils has been shown to mitigate greenhouse gas emissions, improve soil quality and reduce the losses of contaminants, for example, heavy metals and pesticides, to water. The results presented here suggested that AMF inoculation to soil amended with biochar has the potential to significantly mitigate the negative influence of biochar (alone) on simazine decomposition. We conclude that AMF inoculation to biochar amended soil will decrease the potential contamination of surface and groundwaters with pesticides such as simazine, as well as reduce human exposure via potable water sources and direct crop uptake. Consequently, biochar application combined with AMF inoculation may make a valuable contribution to the development of sustainable agricultural systems. However, a more comprehensive understanding of factors that control the synergistic effects of AMF and biochar on the biotic and abiotic processes that control the fate of pesticides is needed.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Hongguang Cheng was responsible for the manuscript writing and the experiment design, Dan Xing and Jinyang Wang analysed the data, Jinyang Wang and Shan Lin were responsible for the experimental work, and Chenglong Tu was responsible for the experimental design. Davey L. Jones, Dave Chadwick and Paul Hill were responsible for the overall planning of the experiment and the revision of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Hongguang Cheng https://orcid.org/0000-0003-4731-7374

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