Effects of field-aging on the impact of biochar on herbicide fate and microbial community structure in the soil environment

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Chemosphere

DOI:
10.1016/j.chemosphere.2023.140682

Published: 01/01/2024

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
Cheng, H., Xing, D., Twagirayezu, G., Lin, S., Gu, S., Tu, C., Hill, P. W., Chadwick, D. R., & Jones, D. L. (2024). Effects of field-aging on the impact of biochar on herbicide fate and microbial community structure in the soil environment. *Chemosphere*, *348*, Article 140682. https://doi.org/10.1016/j.chemosphere.2023.140682

Soil & Tillage Research

Field-aging reduces the impact of biochar on herbicide behavior and microbial community structure in soil

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research paper
Section/Category:	Soil Management
Keywords:	Aging, Pesticide movement, Transport, Degradation, Microbial diversity
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Abstract:	Biochar addition is often promoted as a mechanism to enhance soil carbon (C) storage, however, it may also have secondary benefits by reducing the bioavailability and loss of pesticides to the wider environment. Most experiments studying biochar-pesticide interactions have focused on fresh biochar, however, it is well established that the reactivity of biochar can change after burial in agricultural soils (i.e. chemical and physical aging). From an agrochemical management perspective, it is important to study how this aging process influences pesticide behavior in soil. In this study, we compared the reactions of a common herbicide (simazine) with either fresh biochar or the same biochar recovered from a long-term field trial after 9 years. Using ^{14}C-labelled simazine, we showed that field-aged biochar increased simazine sorption in soil and reduced simazine leaching and biodegradation. Importantly, however, these effects were greatly attenuated in comparison to fresh biochar confirming that field-aging reduces the ability of biochar to mitigate herbicide bioavailability and retention in soil. In addition, field-aging lessened the effect of biochar on the structure of the soil microbial community. We conclude that long-term burial lessens the ability of biochar to interact with pesticides. It also suggests that the reported negative effects of biochar on the efficacy of pesticides targeted at soil-borne plant pests may be partially reversible.
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Type of contribution: Short communication

Date of preparation: 2 Jul 2021

Number of text pages: 16

Number of figures: 4

Title: Field-aging reduces the impact of biochar on herbicide behavior and microbial community structure in soil

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Dear Editors：

Enclosed for your consideration is a manuscript entitled “*Field-aging reduces the impact of biochar on herbicide behavior and microbial community structure in soil*” authored by *Hongguang Cheng, Dan Xing, Shan Lin,Chenglong Tu, Paul Hill, Dave*

Chadwick, Davey L Jones to be considered for publication in the journal of *Biology and Fertility of Soils*.

Application of biochar into soils has commonly been used as a strategy for sequestering carbon in soils, for improving soil fertility and remediating soil pollution. However, the implications of biochar properties variation due to aging on pesticide fate remains poorly understood. Our results showed that the variation of microbial community and adsorption capacity regulated simazine decomposition.

This is **the first report** that the influence of the natural aging in biochar on pesticide behaviors. After natural aging, the functionality of biochar application was altered due to the characters of biochar change. Therefore, due to aging, the adsorption capacity of biochar on simazine was decreased, and simazine decomposition was increased after biochar application. In addition, microbial community was significant different between the fresh biochar treatment and the aged biochar treatment. Our study demonstrated that biochar aging leaded to the variation of adsorption capacity and microbial community regulated simazine decomposition.

We believe that findings from this study have significantly advanced our understanding of the biochar application for remediation of pesticide pollution in the agricultural environment. We are also confident that the results presented in this manuscript will be of interest to readers of *Biology and Fertility of Soils.*

As the corresponding author, I confirm that none of the materials presented in this manuscript has been previously published, nor is it under consideration for publication elsewhere.

This manuscript has been prepared according to the format of *Soil Biology and Biochemistry* as a short communication. There are 214 words in the abstract, 3780 words for the main text, 4 figures, and a list of 38 references in the main text.

Sincerely yours,

Hongguang Cheng

Field-aging reduces the impact of biochar on herbicide behavior and microbial community structure in soil

By Cheng et al

Highlight

- The aging effect of biochar decrease the adsorption to simazine.
- The change of biochar properties due to aging lead to the variation of microbial community.
- The variation of microbial community and the adsorption capacity regulate simazine decomposition

Field-aging reduces the impact of biochar on herbicide behavior and microbial community structure in soil

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ABSTRACT

Biochar addition is often promoted as a mechanism to enhance soil carbon (C) storage, however, it may also have secondary benefits by reducing the bioavailability and loss of pesticides to the wider environment. Most experiments studying biochar-pesticide interactions have focused on fresh biochar, however, it is well established that the reactivity of biochar can change after burial in agricultural soils (i.e. chemical and physical aging). From an agrochemical management perspective, it is important to study how this aging process influences pesticide behavior in soil. In this study, we compared the reactions of a common herbicide (simazine) with either fresh biochar or the same biochar recovered from a long-term field trial after 9 years. Using ^{14}C-labelled simazine, we showed that field-aged biochar increased simazine sorption in soil and reduced simazine leaching and biodegradation. Importantly, however, these effects were greatly attenuated in comparison to fresh biochar confirming that field-aging reduces the ability of biochar to mitigate herbicide bioavailability and retention in soil. In addition, field-aging lessened the effect of biochar on the structure of the soil microbial community. We conclude that long-term burial lessens the ability of biochar to interact with pesticides. It also suggests that the reported negative effects of biochar on the efficacy of pesticides targeted at soil-borne plant pests may be partially reversible.

Keywords: Aging, Pesticide movement, Transport, Degradation, Microbial diversity

Introduction

The extensive and inefficient use of pesticides for controlling crop pests and diseases has led to the widespread contamination of agricultural soils, drinking water and food destined for human consumption (Chiari et al., 2017; Jallow et al., 2017; Onwona-Kwakye et al., 2020). Exposure to pesticides have been implicated in a range of human health issues including skin irritation, peripheral neuropathies, allergic reactions and bone cancer (Jallow et al., 2017; Mostafalou and Abdollahi, 2017). It is now estimated that 1 in 5000 agricultural workers suffer from pesticide poisoning each year with 200,000 of these dying as a result of excessive exposure (Thundiyil et al., 2008; Mostafalou and Abdollahi, 2017). Among these, triazine herbicides (e.g. atrazine, simazine) represent one of the most commonly used pesticides in agriculture with their residues routinely found in high concentrations in soil and water (Cox et al., 2000; Troiano et al., 2001; Barber and Parkin, 2003; Jiang et al., 2011; Barizon et al., 2020). This partly reflects their resistance to microbial attack which leads to their persistence in soil and leaves them susceptible to leaching (Wauchope et al., 1992; Jones et al., 2011). Where possible, it is therefore important to identify strategies to minimize triazine release into the wider environment.

Due to its large surface area, high affinity for cations and large cation exchange capacity (CEC), waste-derived biochar has attracted increasing attention as a way to improve soil quality in degraded agricultural systems (Harter et al., 2014; Cheng et al., 2017; Weng et al., 2017). It has been shown previously that biochar application increases the soil’s ability to retain simazine, reducing its leaching and biodegradation but also repressing its herbicidal effect (Jones et al., 2011; Williams et al., 2015; Cheng et al., 2017). This suggests that it may help ameliorate the loss of simazine from soil. Although the ability of biochar to regulate simazine behavior in soil is highly dependent on the type of feedstock and pyrolysis conditions (Itoh et al., 2020; Konczak et al., 2020; Pariyar et al., 2020; Saffari et al., 2020), it is clear that biochar plays an important role in regulating the transport and fate of simazine (Jones et al., 2011; Cheng et al., 2017).

Until now, most studies on biochar-pesticide interactions have focused on fresh biochar, however, it is known that many of biochar's intrinsic properties change over time after burial in soil (Wang et al., 2020). This weathering (or 'aging') process is regulated by a range of biotic and abiotic factors including microbial activity, crop and tillage regime, UV exposure, moisture and temperature fluctuations (Sorrenti et al., 2016). Typically, as the biochar ages its pores become blocked with mineral particles, roots, organic matter or microbes, theoretically leading to a reduction in the area of surfaces available to undertake chemical reactions (Ren et al., 2018). Understanding how biochar aging affects pesticide behavior in soil is important for predicting the long-term benefits of biochar in agroecosystems. Therefore, in this study we directly compared the sorption and degradation of simazine in soil either in the presence or absence of fresh or field-aged biochar.

Materials and methods

Fresh and aged biochar

Soil was collected from the Ah horizon (0-15 cm depth) of a freely draining, sandy clay loam textured, *Lolium perenne* L. dominated grassland soil (Eutric Cambsiol soil type) adjacent to a long-term biochar field trial located at Abergwyngregyn, Wales, UK (53°14'19.176"N, 4°0'45.72"W). The site has a temperate oceanic climate regime (1066 mm rainfall, 10°C mean annual temperature) and is fertilized regularly with 120 kg N, 60 kg K and 10 kg P per annum. Prior to use, the soil was air-dried and sieved to pass 2 mm to remove stones and roots. The properties of the soil are shown in Table S1, with additional properties presented in Jones et al. (2012). Field-aged biochar was recovered from the adjacent replicated, long-term field trial in which wood-derived biochar was added 9 years previously at a rate of 100 t ha^{-1} and incorporated into the 0-20 cm soil layer (Jones et al., 2012). The corresponding unused biochar from the trial (fresh biochar) had been stored in a vacuum sealed container during the same period (see Supplementary Information for further details). The properties of the fresh and aged biochar are shown in S1. Both fresh and aged biochar were then sieved (100 mesh sieve) to ensure a common particle size was used in the subsequent experiments. The experiment consisted of three treatments: (i) soil without biochar (control), (ii) soil amended with fresh biochar, and (iii) soil amended with aged biochar. Fresh and aged biochar were added to soil at a rate of 1:25 (w/w) and homogenized by hand. This rate was designed to reflect a hotspot of biochar in soil, reflecting its uneven distribution in the field (i.e. 200 t ha^{-1} in the top 0-5 cm layer). All samples were prepared in quadruplicate.

Incubation experiment

At the start of the experiments, the prepared sample (300 g) including soil and biochar was placed into individual polypropylene containers and distilled water added to raise the soil to 70% of its water holding capacity. The mesocosms were then placed at 10°C in the dark for 14 d to allow the microbial community to recover, after which they were transferred to 20°C for 30 d. To determine changes in microbial community structure at the end of the 30 d incubation period, 10 g of soil was recovered and subjected to phospholipid fatty acid (PLFA) analysis according to the high-throughput method of Buyer and Sasser (2012) (see S2). A further 6.5 g of soil (eq dry soil 5.0 g) was placed in a sterile polypropylene tube to which 0.5 ml of ^{14}C-labeled simazine (0.60 mg l^{-1}, 0.54 kBq ml^{-1}) was added. A trap containing 1 M NaOH (1 ml) was then suspended above the soil surface to capture any $^{14}CO_2$ evolved and the tubes sealed. The traps were replaced after 1, 3, 5, 7, 10, 14, 17 and 21 d after simazine addition. The $^{14}HCO_3^-$ content of the NaOH traps was determined using Optiphase HiSafe 3 scintillation fluid (PerkinElmer Inc., Waltham, MA) and a Wallac 1400 liquid scintillation counter (PerkinElmer Inc.) with automated quench correction.

Sorption experiment

Sorption isotherms of simazine were obtained using a batch equilibration technique. Briefly, 2.6 g soil (eq dry soil 2.0 g) was placed in individual polypropylene tubes. The tubes were then heated (80°C, 30 min) to minimize microbial activity (Kuzyakov and Jones, 2006). Subsequently, different concentrations ^{14}C-labelled simazine (6.25, 12.5, 25, 50 and 100 µg l^{-1}; 20 ml; 0.05 kBq ml^{-1}) in a background of 0.01 M $CaCl_2$ were added to each tube and the samples shaken (200 rev min^{-1}) for 24 h at 20°C. After shaking, the tubes were centrifuged (3850 *g*, 10 min) and the amount of ^{14}C-simazine remaining in the supernatant determined by liquid scintillation counting as described above. The distribution coefficient (K_d) and the isotherm adsorption curves were determined as described in the S3.

Leaching experiment

To evaluate ^{14}C-simazine leaching, 6.5 g soil (eq dry soil 5 g) was placed in a 25-ml inverted syringe (20 mm diameter) with a 1-mm polypropylene mesh placed at the bottom to prevent soil loss (Cheng et al., 2017). Subsequently, 1 ml of ^{14}C-labeled simazine (2.50 mg l^{-1}, 0.05 kBq ml^{-1}) was added to the soil surface and the herbicide allowed to equilibrate with the soil for 1 h. Another 1-mm polypropylene mesh was then placed on the soil surface and a syringe-pump used to add distilled water to the top of the column at a rate of 0.2 ml min^{-1}. The resulting leachate was sequentially collected (equivalent to 1, 2, 3, 4, 5, 6 soil pore volumes) and its ^{14}C activity determined as described above.

Statistical analysis

Variables were first tested for normality and homogeneity of variance. Variables with normal distributions and equality of variance were analyzed using a one-way ANOVA with Fisher's least significant difference (LSD). Variables with non-normal distributions or unequal variance (decomposition and leaching) were analyzed non-parametrically using a Wilcoxon paired signed-rank test. All differences were considered significant at the $P < 0.05$ level. Linear regression was undertaken in Origin 2019b.0 (OriginLab Corp, Northampton, MA).

Results

Effect of field ageing on simazine adsorption

Figure 1 shows the variation of simazine sorption in soil amended with fresh and aged biochar. Compare to the control, the sorption of simazine was significantly greater in the biochar amended soils ($P < 0.001$). At low concentrations, there was slightly greater sorption in the soil amended with fresh biochar relative to the aged biochar ($P = 0.005$). Overall, the solid phase-to-soil solution partition coefficient (K_d) was much higher in the biochar treatments (0.022 ± 0.001) in comparison to the control soil (0.007 ± 0.000). The different parameters calculated for Freundlich and Langmuir isotherm models are presented in Table S3.

Effect of field ageing on simazine leaching

Fig 2 shows simazine leaching results in the soils with or without biochar. In the control, on average, 63.4 ± 1.3% of the simazine was leached from soil over the course of the experiment (Fig. 2). In contrast, the presence of both aged and fresh biochar significantly reduced this with only 27.7 ± 1.1% and 22.9 ± 3.2%, respectively, leached over the same period. The amount lost by leaching was slightly greater in the aged biochar treatment relative to the fresh biochar treatment ($P = 0.046$).

Effect of field ageing on simazine mineralization

Fig 3 shows simazine mineralization in soils with or without biochar. Compared to the unamended control, simazine mineralization was significantly reduced in the soils amended with either fresh biochar or aged biochar ($P < 0.05$; Fig. 3). The suppression in simazine turnover was greatest in the soil amended by fresh biochar (by 56.9%; $P < 0.001$) in comparison to that containing aged biochar (by 33.6%; $P = 0.046$).

Discussion

Effect of field ageing on simazine sorption and leaching

Current evidence suggests that biochar aging may affect pesticide sorption by a range of competing mechanisms including: (1) an increase in surface negative charge which promotes electrostatic sorption (Cheng et al., 2014); (2) an increase of oxygen-containing functional groups on the biochar surface providing more complexation sites for simazine (Ren et al., 2018); (3) a decrease in specific surface area (SAA) and pH which reduces simazine sorption (Dong et al., 2017), (4) both increases and reductions in biochar CEC (Guo et al., 2014; Shi et al., 2015; Mia et al., 2017), and (5) occupation of sorption sites by humic substances (Uttran et al., 2018). In this study, changes in biochar properties with field ageing are presented in S2. Briefly, field-aging decreased the pH of the biochar from 9.61 to 7.65, decreased its CEC from 43.5 to 24.3 cmol kg^{-1}, reduced the SSA from 46.0 to 38.2 m^{2} g^{-1} and increased the Zeta potential from -38.6 mV to -40.7 mV. Lastly, changes in the amount of oxygen functional groups present were also altered by burial in soil. These alterations in the properties of the biochar suggest that mechanisms 1-5 may all have taken place simultaneously in the field. Taken together, however, our results show that field ageing slightly reduces its sorption potential for simazine, which in turn increases its soil solution concentration and rate of leaching. It also suggests that simazine was not held as tightly on the biochar leading to greater desorption and making it more susceptible to microbial biodegradation. The lower reactivity of field-aged biochar in comparison to fresh biochar when added to soil is also supported to some extent by its reduced effect on soil microbial community structure. This suggests that the widely reported ability of biochar to retain pesticides in soil (Kookana, 2010) may reduce on a decadal timescale. This greatly extends other studies on the effects of biochar ageing which have reported reductions in herbicide sorption after much shorter burial time periods in soil (e.g. 60 d; Gamiz et al., 2019; Wu et al., 2019).

Effect of field ageing on simazine mineralization

Nowadays, the effect of biochar as an amendment in agriculture soil on pesticide mineralization have different understandings (Khalid et al., 2020). One is that the application of biochar in the soil reduce the biodegradation of pesticides by enhancing its adsorption to the soil (Jones et al., 2011; Cheng et al., 2017). The other is that biochar assisted microbial activity in the soil, thereby increasing the pesticides mineralization (McCormack et al., 2013). In this study, the results show biochar application decreased simazine mineralization which is consistent with the previous studies (Cheng et al., 2017). Furthermore, after field ageing, the inhabit of biochar application on simazine mineralization was lessened. Apparently, biochar ageing altered the properties of biochar, which reduces the adsorption capacity of the soil (Mia et al., 2017), thus increases the contact of microbial with simazine, and promotes the simazine decomposition. This viewpoint is confirmed by the results of adsorption experiment. In addition, PLFA profiling indicated that neither type of biochar had an appreciable effect on the size of the microbial biomass ($P = 0.467$), it did alter the structure of the microbial community (Fig. 4). Relative to the control, this change was greater in the fresh biochar in comparison to the aged biochar treatment. It is possible that field ageing leads the change of microbial community regulating simazine mineralization. However, this inference needs further research.

Conclusion

This study has demonstrated that field-aging reduces the impact of biochar on herbicide behavior and microbial community structure in soil. However, biochar application to an agricultural soil still has the capacity to greatly influence simazine sorption and degradation after a decade, albeit to a lesser extent than fresh biochar. This reduction in reactivity may also support the repeated application of biochar over long timescales for promoting greater C storage in agricultural soils.

Acknowledgements. This research was supported by the Strategic Priority Research Program of Chinese Academy of Sciences (No. XDB40020402), the National Key Research and Development Program of China (2018YFC1802601), Science and Technology Plan Project of Guizhou Province (No [2018]2329), Opening Fund of the State Key Laboratory of Environmental Geochemistry (SKLEG 2020205).

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Figure legends

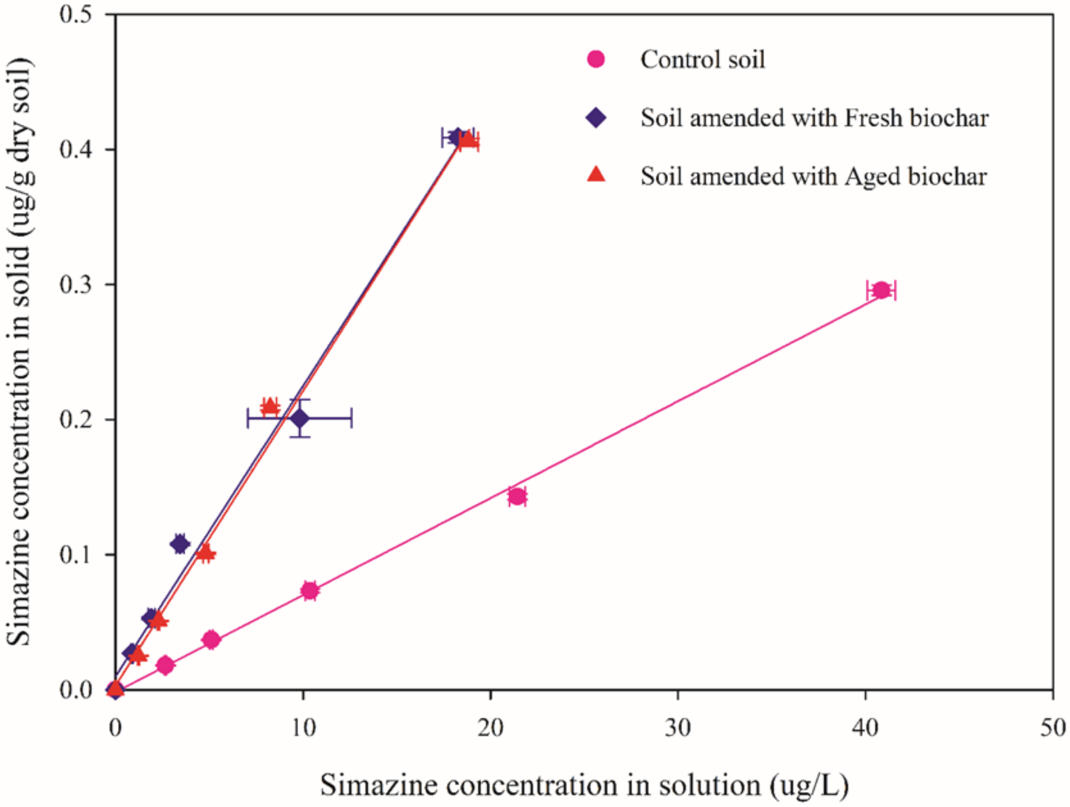
Fig. 1 Effect of fresh and field-aged biochar on simazine sorption to the solid phase in an agricultural soil. Values represent means ± SEM (*n* = 4).

Fig. 2 Effect of fresh and field-aged biochar on potential simazine leaching in an agricultural soil. Values represent means ± SEM (n = 4).

Fig. 3 Effect of fresh and field-aged biochar on simazine decomposition in an agricultural soil. Values represent means ± SEM (n = 4).

Fig. 4 Effect of fresh and field-aged biochar on soil microbial community structure in an agricultural soil. The left-hand panel shows the principal component analysis of PLFA fingerprints while the right-hand panel shows the relative abundance of each microbial group and the effect of biochar relative to the control.

Figure 1



0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
Simazine concentration in solid (ug/g dry soil)
Simazine concentration in solution (ug/L)
Control soil
Soil amended with Fresh biochar
Soil amended with Aged biochar

Figure 2

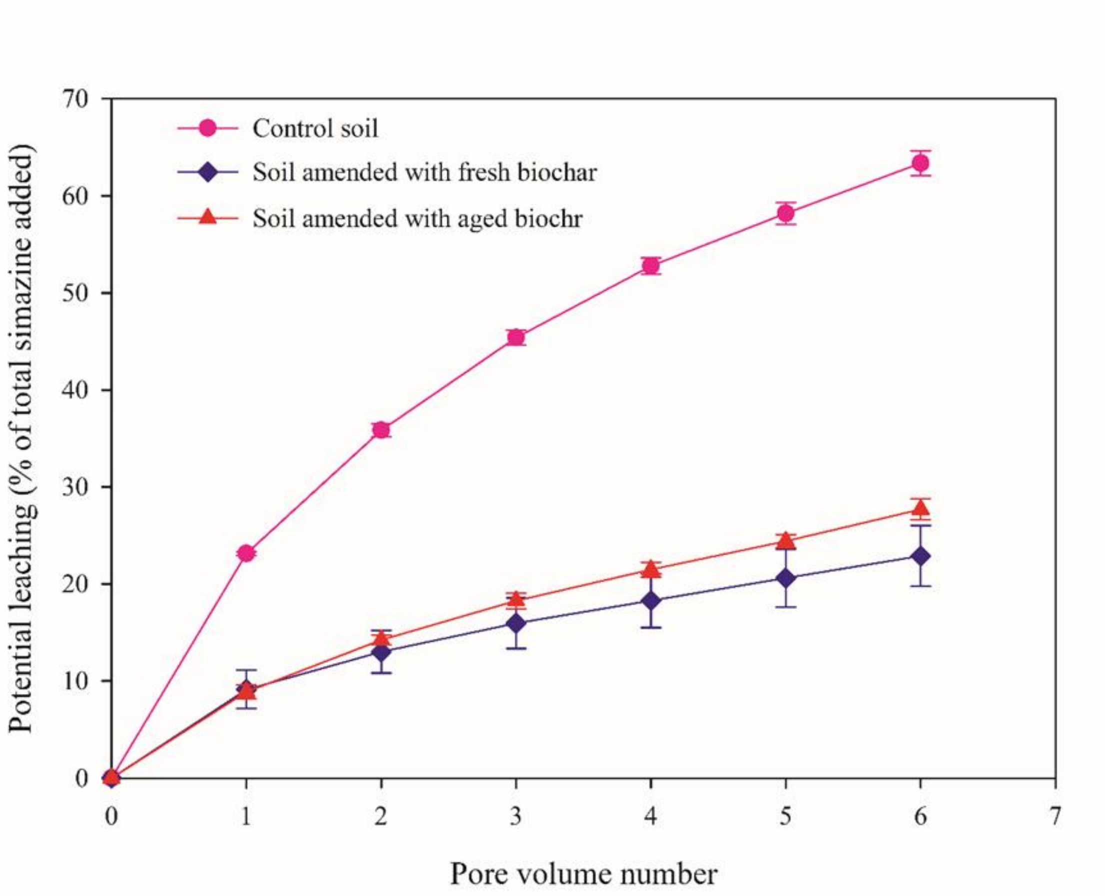

Control soil
Soil amended with fresh biochar
Soil amended with aged biochr
Potential leaching (% of total simazine added)
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Pore volume number

Figure 3

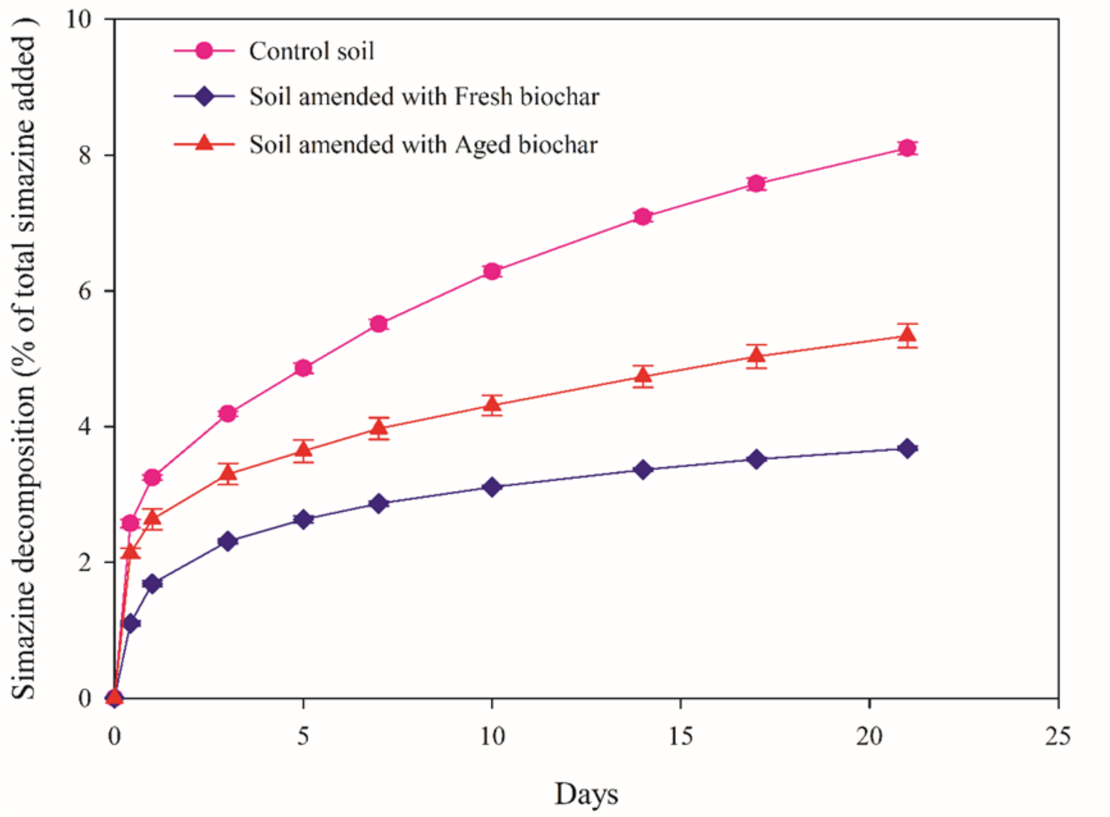
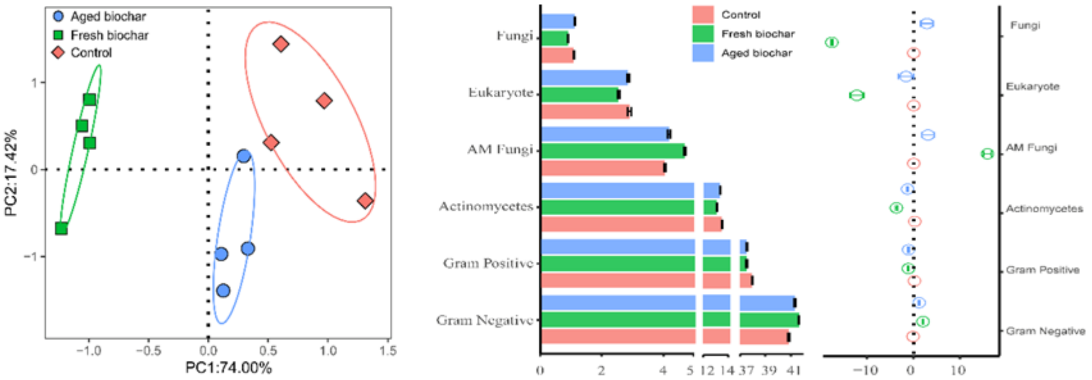

Control soil
Soil amended with Fresh biochar
Soil amended with Aged biochar
Simazine decomposition (% of total simazine added)
0
2
4
6
8
10
0
5
10
15
20
25
Days

Figure 4


Aged biochar
Fresh biochar
Control
PC1:74.00%
PC2:17.42%
1
0
−1
−1.0
−0.5
0.0
0.5
1.0
1.5
Control
Fresh biochar
Aged biochar
Fungi
Eukaryote
AM Fungi
Actinomycetes
Gram Positive
Gram Negative
0
2
4
5
12
14
37
39
41
−10
0
10
Fungi
Eukaryote
AM Fungi
Actinomycetes
Gram Positive
Gram Negative

Declaration of Interest Statement

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.

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1 Jul 2021