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# Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth, cadmium uptake and soil cadmium speciation in Cd-contaminated soil

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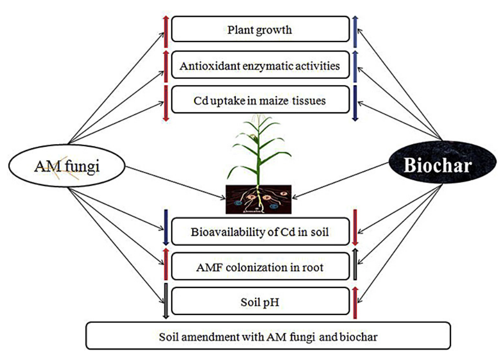
## HIGHLIGHTS

- AM inoculation or biochar alone can increase maize growth and reduce Cd uptake.
- AM inoculation was more fully at alleviating Cd stress and facilitating maize growth.
- Biochar was more effective at inducing soil alkalinization and Cd immobilization.
- AM inoculation and biochar together had a synergistic effect on decreasing Cd phytotoxicity.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Experiments conducted to understand how arbuscular mycorrhizal (AM) inoculation or biochar application affect plant growth and heavy metal uptake have thus far looked at single applications of either soil amendment. There is little evidence of their synergistic effects, in particular for plants grown in cadmium (Cd) contaminated soil. We conducted a mesocosm experiment to investigate the effect of AM inoculation (*Glomus intraradices* BEG 141) and/or wheat-straw biochar amendment on maize (*Zea mays* L. cv. Hongdan No. 897) growth, antioxidant enzymatic activities, and Cd uptake, as well as soil Cd speciation under applications of 0, 3, 6 mg Cd per kg soil. Applying either AM inoculant or biochar alone significantly increased maize growth and reduced Cd uptake. Furthermore, solo AM inoculation alleviating Cd stress more fully than biochar, in turn facilitating maize growth and decreasing soil Cd translocation into plant tissue. Still, solo biochar amendment was more effective at inducing soil alkalinization and contributing to Cd immobilization. Adding biochar together with AM inoculant significantly promoted fungal populations compared to a control. Amending soil with AM inoculant and biochar together produced the largest increase in maize growth and decrease in tissue Cd concentrations. This effect was additive, with 79.1% greater biomass, 51.42%, 82.91%, 43.96% higher activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and 50.06%, 67.19%, 58.04% and 76.19% lower Cd concentrations

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in roots, stems, leaves, and ears, respectively, at a 6 mg $kg^{-1}$ Cd contamination rate. The combined treatment also had a synergistic effect on inducing soil alkalinization and causing Cd immobilization, and decreasing Cd phytoavailability and post-harvest transfer risks. These results suggest that AM inoculation in combination with biochar application may be applicable not only for maize production but also for phytostabilization of Cd-contaminated soil.



## 1. Introduction

Environmental pollution by toxic heavy metals has become a common concern in both developing and developed countries (Fu and Wang, 2011). Excessive accumulation of heavy metals in agricultural soils can not only impair soil quality and crop growth, but also pose a threat to human, animal and plant health when accumulation occurs in agricultural products. These problems necessitate development and use of soil remediation practices (Liu et al., 2012a; Molina et al., 2013; Yi et al., 2011). Soil remediation technologies based on in situ stabilization or immobilization are simple, inexpensive and environmentally friendly and are considered to be some of the best techniques for the remediation of heavy metal contaminated soils (Marques et al., 2009). Several approaches to decrease the mobility of soluble heavy metals in soil have been suggested, such as the use of rhizosphere microbes and adjusting field management practices (Rajkumar et al., 2009, 2012).

Among soil microorganisms, arbuscular mycorrhizal (AM) fungi provide plant roots with more intimate access to nutrients and water, and are well known to be able to improve plant growth, reduce heavy metal toxicity, and influence plant uptake and transport of heavy metals (Solísdomínguez et al., 2011). Arbuscular mycorrhizal fungi play roles in phytostabilization by precipitating polyphosphate complexes, retaining heavy metals in roots and fungal structures such as extra radical mycelium, and improving plant adaptation to environmental stresses in general (Wang et al., 2005; Wu et al., 2016b). Arbuscular mycorrhizal fungi may also help drive the speciation of heavy metals and reduce metal phytoavailability by changing the microbial community structure and physical, chemical properties of rhizosphere soil (Ogar et al., 2015). The extent of these organisms' effects on remediation can depend on AM fungal species, plant tolerance to contaminants, heavy metal concentrations, and bioavailability (Yang et al., 2015).

Biochar is a carbon-rich byproduct of pyrolysis of organic residues, where materials are partially burned under low oxygen condition (Nguyen et al., 2008; Wu et al., 2016a). Amending soil with biochar soil amendments is receiving increased attention because of this strategy's ability to improve soil quality and crop productivity (Ahmad et al., 2014; Liu et al., 2017; Wu et al., 2017). In soils contaminated by heavy metals, it has been reported that biochar can adsorb and immobilize metal ions due to its porous structure, large surface area, and high surface charge density and pH values (Hossain et al., 2010; Van Zwieten et al., 2010; Zeng et al., 2015). The role and effectiveness of biochar in soil remediation may depend on plant type, soil texture, biochar production temperature, and application rate (Rehman et al., 2016; Rizwan et al., 2015). A few studies have shown biochar addition to benefit AM fungi, likely by modifying soil physiochemical properties and facilitating spore germination and AM fungi hyphal branching and growth (Hammer et al., 2014, 2015). Biochar and AM fungi in combination affect nutrient cycling and may therefore also influence the speciation of heavy metals and reduce metal phytoavailability by changing soil microbial activity and community structure (Hammer et al., 2014).

Many studies have demonstrated synergistic effects of AM inoculation and organic amendments on improving soil quality and plant performance under stress conditions, such as in degraded or polluted soil (Alguacil et al., 2011; Medina et al., 2010). Biochar is one of the most widely used organic amendments, but there have been very few factorial studies about AM fungi inoculation and biochar amendment in general, and it remains unknown whether this combination can also modulate heavy-metal uptake by plants. Cadmium (Cd), which is harmful to both human and plant health, is among the most common heavy metal contaminants in farmland soils as a result of industrial activity, over-fertilization, wastewater irrigation, and improper waste disposal (Van der Ent et al., 2013; Yi et al., 2011). It readily accumulates in soil because, unlike organic contaminants, it cannot be biodegraded. It can be taken up by plants grown in contaminated soil and enter human and animal bodies via the food chain (Liu et al., 2012b). Maize is grown on more acres than any agronomic crop except one of the most important food in the world and is readily colonized by AM fungi (Cao et al., 2017). Here, we hypothesized that AM inoculation and biochar application would improve plant growth, stimulate soil microbial activity to reduce Cd phytoavailability under elevated soil Cd concentrations, and have a synergistic effect on lowering Cd uptake by maize. We conducted a pot culture experiment to investigate these treatments' impacts on maize growth, antioxidant enzyme activities, and Cd concentrations, as well as soil parameters at different levels of Cd. Our ultimate aim was to develop an environmentally friendly phytoremediation approach, in order to properly manage Cd-contaminated soil.

## 2. Materials and methods

### 2.1. Mycorrhizal inoculum

The AM fungal inoculum, *Glomus intraradices* BEG 141, kindly provided by China Agricultural University, was propagated on Sudangrass [*Sorghum sudanese* (Piper) Stapf.] grown in autoclaved (121 °C for 1 h on three successive days) sand for three successive propagation cycles (4 months each). The non-mycorrhizal inoculum was also prepared with the same sterilized substratum with the host plant cultivated under the same conditions. These media were air-dried and sieved through a 2 mm sieve before inoculation.

### 2.2. Biochar preparation

The biochar was provided by the Sanli New Energy Company, Henan province, China. It was derived from wheat straw pyrolysed at 450 °C under an oxygen-free environment for 2 h. The biochar was ground to pass through a 2 mm sieve and mixed thoroughly before application. The biochar was analyzed according to Lu (2000). Total organic C and N were determined using a Perkin-Elmer 2400 CHNS/O elemental analyser (Norwalk, CT, USA). Total K content was determined by acid digestion followed by elemental analysis by atomic adsorption spectroscopy. Available phosphorus (P) was extracted by 0.5 M $NaHCO_3$ and measured colorimetrically. Dissolved organic carbon (DOC) was extracted from 5 g of biochar