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Biochar and *Glomus caledonium* Influence Cd Accumulation of Upland Kangkong (*Ipomoea aquatica* Forsk.) Intercropped with Alfred Stonecrop (*Sedum alfredii* Hance)

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Both biochar application and mycorrhizal inoculation have been proposed to improve plant growth and alter bioaccumulation of toxic metals. A greenhouse pot trial was conducted to investigate growth and Cd accumulation of upland kangkong (*Ipomoea aquatica* Forsk.) intercropped with Alfred stonecrop (*Sedum alfredii* Hance) in a Cd-contaminated soil inoculated with *Glomus caledonium* and/or applied with biochar. Compared with the monocultural control, intercropping with stonecrop (IS) decreased kangkong Cd acquisition via rhizosphere competition, and also decreased kangkong yield. *Gc* inoculation (+M) accelerated growth and Cd acquisition of stonecrop, and hence resulted in further decreases in kangkong Cd acquisition. Regardless of IS and +M, biochar addition (+B) increased kangkong yield via elevating soil available P, and decreased soil Cd phytoavailability and kangkong Cd concentration via increasing soil pH. Compared with the control, the treatment of IS + M + B had a substantially higher kangkong yield (+25.5%) with a lower Cd concentration (−62.7%). *Gc* generated additive effects on soil alkalinization and Cd stabilization to biochar, causing lower DTPA-extractable (phytoavailable) Cd concentrations and post-harvest transfer risks.

Cadmium (Cd) is a non-essential metal element which may cause damage even at very low levels (the health criteria recommendation value is 7 μg/kg per body weight per week)[1], and can enter into food chains easily via plant uptake from contaminated soils[2,3]. Garden vegetables, such as upland kangkong (*Ipomoea aquatica* Forsk.), are capable of accumulating relatively high levels of Cd from contaminated soils[4,5]. It is known that metal accumulating plants, such as Cd-hyperaccumulator Alfred stonecrop (*Sedum alfredii* Hance)[6], are able to extract a large amount of metals thereby removing them from contaminated sites[7]. However, phytoextraction of Cd using hyperaccumulators would require a long time before low-Cd crops could be subsequently produced from the contaminated sites[8]. Alternatively, intercropping of edible crops with metal-hyperaccumulators may improve conditions in the shared rhizosphere and thereby affect metal accessibility to neighboring crops. It is therefore possible that under-sowing crops with small-biomass metal-accumulators may offer an alternative management strategy for marginally contaminated soils[9]. In addition, arbuscular mycorrhizal (AM) fungi usually provide beneficial effects to host plants growing on contaminated soils[10], and may improve essential nutritional status, notably phosphorus (P), to increase shoot biomass[11]. More importantly, AM fungi can elevate metal uptake/concentration of metal-accumulating plants[12], which subsequently decrease metal accumulation by neighboring edible crops[13]. Furthermore, mycorrhization may also reduce metal phytoavailability via elevating soil pH, resulting in lower transfer risks of toxic metals by post-harvest crops[14].

Plant Cd accumulation is usually influenced by Cd availability in the soi[15,16], which is dependent not only on total Cd concentration, but also upon soil conditions[17]. Therefore, physico-chemical countermeasures for reducing Cd phytoavailability have also been recommended for preventing potential accumulation risks by crops[18,19]. As mentioned earlier, soil Cd availability is negatively affected by soil pH[20], and its plant uptake becomes severe in acid soils. Thus, alkaline amendments serving as stabilizing agents may contribute significantly on reducing metal mobility by elevating soil pH and enhancing metal binding to soil particles[21]. Recently, application of biochar was proven a viable option for enhancing soil carbon sequestration and mitigating greenhouse gas emission from world cropland[22]. On the other hand, biochar contains a large amount of highly recalcitrant organic materials which are more alkaline[23,24], which would lower metal mobility and leachability in soils[25–27]. Therefore, this may be a potential of using biochar to reduce Cd phytoavailability, notably in acid soils[21].

With both the above information concerning biochar addition and mycorrhizal inoculation, there clearly are opportunities for exploiting a potential synergism that could positively affect soil quality of metal-contaminated sites[28]. It was hypothesized that during intercropping of edible crops with Cd-hyperaccumulating plants, the application of biochar would decrease phytoavailability of Cd in the soil, while inoculation of AM fungi enhance Cd acquisition by the hyperaccumulators, and thereby decrease Cd uptake by neighboring crops. Due to the fact that information regarding cooperative contribution of biochar and AM fungi to non-mycorrhizal vegetable products is limited or fragmented, the present study was conducted to investigate plant yield and Cd and P accumulation of upland kangkong intercropped with Alfred stonecrop in a Cd-contaminated acidic soil in response to AM fungal inoculation and biochar application, either solely or in combination, based on a greenhouse pot trial. The major purpose of this study was to address the additive efforts of AM fungi and biochar on Cd reductions in edible vegetables growing on Cd-contaminated soils. This work may contribute to developing application strategies of intercropping system with AM fungi and biochar for dealing with Cd-contaminated vegetable fields.

Results

Mycorrhizal colonization, plant dry biomass, and Cd/P concentration and acquisition of Alfred stonecrop. Regardless of biochar application, the mycorrhizal colonization in stonecrop roots was significantly higher ($P<0.05$) in the IS + M (intercropping of upland kangkong with Alfred stonecrop plus inoculation with *Glomus caledonium*) treatment than in the IS treatment (Fig. 1a). In accordance with the increased root colonization, shoot biomass (Fig. 1b), Cd concentration (Fig. 1c) and Cd acquisition (Fig. 1e) of stonecrop were also significantly elevated ($P<0.05$) by the inoculation of *Gc* (+M), while plant P concentration (Fig. 1d) was not significantly influenced, and only plant P acquisition (Fig. 1f) tended to increase due to the higher shoot biomass. Compared with the corresponding –B (without the application of biochar) treatments, the application of biochar (+B) had no significant effects on mycorrhizal colonization, plant biomass, Cd concentration and Cd acquisition of stonecrop, but tended to decrease ($P>0.05$) plant P acquisition, causing a trend towards lower ($P>0.05$) tissue P concentration with the IS (intercropping of kangkong with stonecrop) treatment, as well as a significantly decreased ($P<0.05$) tissue P concentration with the IS + M treatment.

Shoot and root fresh biomass and Cd/P concentration, and total Cd/P acquisition of upland kangkong. Without the application of biochar (–B), kangkong shoot biomass (Fig. 2a), but not root biomass (Fig. 2b), was significantly lower ($P<0.05$) in the IS treatment than the control. However, IS also significantly decreased ($P<0.05$) kangkong Cd acquisition (Fig. 2g), causing a significant decrease ($P<0.05$) of root Cd concentration (Fig. 2d) and a trend towards lower ($P>0.05$) shoot Cd concentration (Fig. 2c). IS had no significant effects on kangkong P acquisition (Fig. 2h) and tissue P concentrations (Fig. 2e, 2f). Compared with IS, IS + M had no significant effects on plant biomass, P acquisition and P concentration of kangkong, but tended to decrease ($P>0.05$) plant Cd acquisition, and significantly decreased ($P<0.05$) Cd concentrations in both shoot and root.

Compared with –B treatments, biochar addition (+B) significantly elevated ($P<0.05$) P acquisitions and plant biomasses of kangkong regardless of the intercropping of stonecrop (IS), but had no significant effects on tissue P concentrations. +B also significantly decreased ($P<0.05$) Cd concentrations in both shoot and root of kangkong, with the exception of a trend towards lower ($P>0.05$) root Cd concentration with the IS + B treatment. +B also tended to increase ($P>0.05$) kangkong Cd acquisitions with the control and IS, but not with the IS + M.

Amongst the three +B treatments, IS had no significant effects on plant biomass, P concentration and P acquisition of kangkong compared to the control, but significantly decreased ($P<0.05$) kangkong Cd acquisition, causing a trend towards lower ($P>0.05$) Cd concentration in both shoot and root. Compared with IS, IS + M had no significant effects on plant biomass, P concentration and P acquisition of kangkong, but significantly decreased ($P<0.05$) kangkong Cd acquisition and subsequent Cd concentrations in both shoot and root.

Rhizosphere competition in Cd and P acquisition between upland kangkong and Alfred stonecrop. Without AM fungal inoculation and biochar application, the average ratios of Cd and P amounts acquired by kangkong to the total acquisitions by the two plant species in the intercropping system were 17% and 59%, respectively (Fig. 3). It can be deduced that the ratio of Cd acquired by intercropped stonecrop (83%) was 2 times that of P (41%). Regardless of biochar application, *Gc* inoculation (+M) significantly decreased ($P<0.05$) the ratio of Cd amount acquired by kangkong to the total acquisition, but had no similar effect on the ratio of P. Regardless of *Gc* inoculation, application of biochar (+B) significantly increased ($P<0.05$) the ratio of P amount acquired by kangkong to the total acquisition, but had no similar effect on the ratio of Cd.

Compared with the control, the average shoot yields of kangkong decreased by 17.9% and 14.0% for IS and IS + M, respectively, but increased by 37.7%, 30.9% and 25.5% for +B, IS + B and IS + M + B, respectively (Table 1). On the other hand, the average Cd concentrations in kangkong shoots decreased by 16.3% and 24.7% for IS and +B, respectively, and further decreased by 40.7%, 43.2% 62.7% for IS + M, IS + B and IS + M + B, respectively.

Soil pH, electrical conductivity (EC), diethylenetriaminepentaacetic acid (DTPA)- extractable Cd, available P, and acid phosphatase activity. Without the application of biochar (–B), IS had no significant effects on soil pH, EC and available P concentration (Table 2), but had a trend towards lower ($P>0.05$) DTPA-extractable Cd concentration and acid phosphatase activity than the control. Compared with IS, IS + M had no significant effects on EC and available P concentration, but significantly elevated ($P<0.05$) soil pH and acid phosphatase activity, and decreased ($P<0.05$) DTPA-extractable Cd concentration. Compared with –B treatments, the application of biochar (+B) significantly elevated ($P<0.05$) soil pH, EC and available P concentration, and decreased ($P<0.05$) DTPA-extractable Cd concentration. In addition, +B also significantly decreased ($P<0.05$) soil acid phosphatase activity with both control and IS + M, while there was only a trend towards lower ($P>0.05$) one with the IS + B treatment.

Amongst the three +B treatments, IS had no significant effects on soil pH, available P concentration and acid phosphatase activity

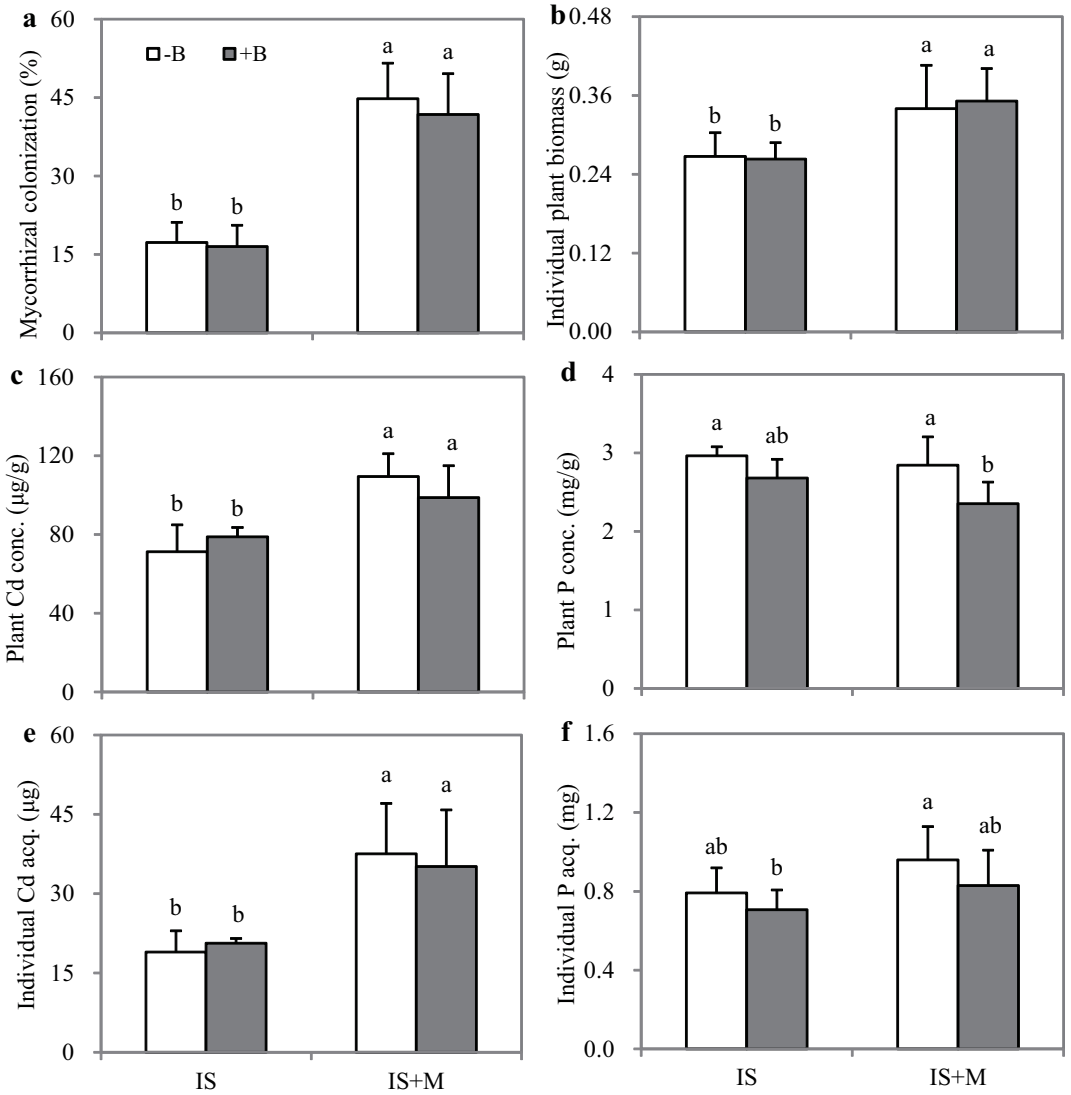

Figure 1 | Mycorrhizal colonization (a), plant dry biomass (b), Cd/P concentration (c, d), and Cd/P acquisition (e, f) of Alfred stonecrop calculated on a per plant basis. IS, intercropping with Alfred stonecrop; +M, inoculation with *Glomus caledonium* 90036; +B, application with biochar; −B, non-application of biochar. *Vertical T bars* indicate standard deviations. *Bars* not topped by the same letter indicate a significant difference in values ($P<0.05$).

compared to the control, but significantly increased ($P<0.05$) EC, and tended to decrease ($P>0.05$) DTPA-extractable Cd concentration. Compared with IS, IS + M had no significant effects on soil EC, available P concentration and acid phosphatase activity, but significantly increased ($P<0.05$) soil pH and decreased ($P<0.05$) DTPA-extractable Cd concentration.

Discussion

In the present study, the intercropping system of Cd-hyperaccumulator (Alfred stonecrop) and edible vegetable (upland kangkong) was conducted for the purpose of producing vegetable with an acceptable level of Cd in farm soils enriched with Cd. Regardless of biochar application, the significantly lower Cd acquisitions and the trend towards lower Cd concentrations in both shoot and root of kangkong intercropped with stonecrop relative to the monocultural control (Fig. 2g) indicated that intercropping with Cd-hyperaccumulator is a feasible practice to decrease the instantaneous accessibility of Cd to neighboring vegetables, likely through competition for phytoavailable Cd in their shared rhizosphere (Fig. 3a). Similarly, Zn uptake by another crop *Hordeum vulgare* was significantly decreased when intercropped with Zn-hyperaccumulator *Thlaspi caerulescens*, probably through Zn depletion within the zone of their rhizosphere[9]. Fulfilling the objective of enhancing the competency of hyperaccumulator in acquiring Cd, inoculation of *Glomus caledonium* (*Gc*) significantly increased plant biomass, Cd concentration, and total Cd acquisition of stonecrop (Fig. 1), similar to the inoculation of *Gc* which elevated Cu extraction by Cu-accumulator *Elsholtzia splendens*[29], and inoculation of *G. intraradices* which elevated Cd absorption by another Cd-hyperaccumulator *Helianthus annuus*[30]. As a result, *Gc*-inoculated stonecrop further decreased Cd acquisitions and subsequent Cd concentrations in both shoot and root of neighboring kangkong when compared to the non-inoculation IS treatment (Fig. 2; Table 1).

Without biochar addition, intercropping with stonecrop (IS) also significantly decreased kangkong shoot yield compared to the monocultural control (Fig. 2a). The mechanisms causing such reduction are not fully understood but seem to be due to nutrient competition, such as P (Fig. 3b). Soil phosphatase is closely related to plant P nutrition[31]. There was also a trend towards lower acid phosphatase activity with the IS treatment (Table 2). Thus, the trend towards higher P acquisition by stonecrop upon *Gc* inoculation (Fig. 1f) seemed to be due to the elevation of soil acid phosphatase activity (Table 2). It may involve AM fungi indirectly: mycorrhizal roots may release more root exudates containing soil enzymes because of the

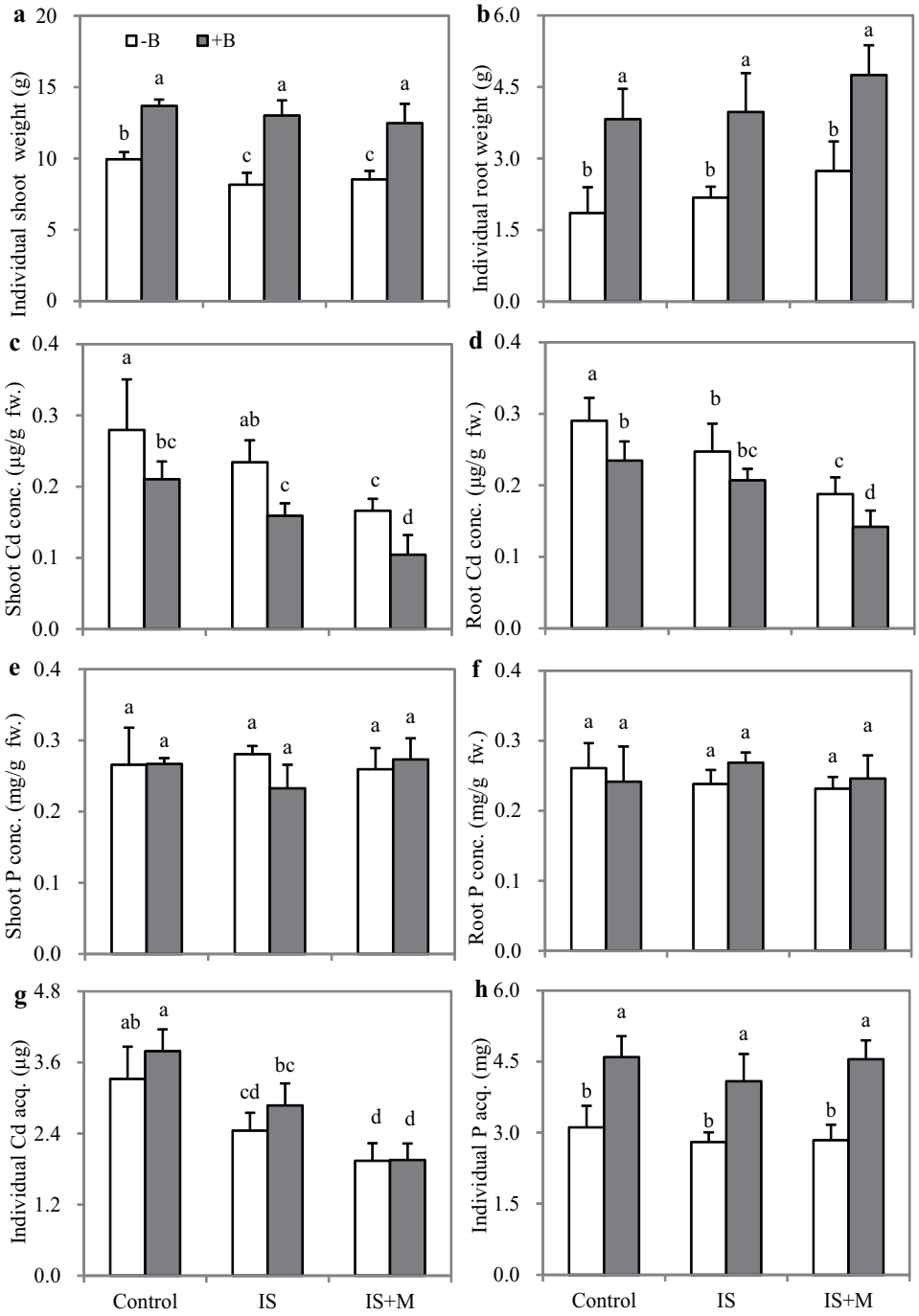

Figure 2 | Shoot and root fresh weight (a, b), Cd/P concentration (c–f), and total Cd/P acquisition (g, h) of upland kangkong calculated on a per plant basis. Control, monoculture of upland kangkong; IS, intercropping with Alfred stonecrop; +M, inoculation with *Glomus caledonium* 90036; +B, application with biochar; −B, non-application of biochar. *Vertical T bars* indicate standard deviations. *Bars* not topped by the same letter indicate a significant difference in values ($P<0.05$).

larger root system and/or improved nutrition[32]. Nevertheless, a mutualistic association with AM fungi is of particular importance in improving P uptake of the host plant[33,34], but not of the neighbors. Therefore, *Gc* inoculation had no significant influences on P acquisition and yield of kangkong compared to the non-inoculated IS treatment. Unlike such practice, application of biochar (+B) containing high amounts of easily soluble P directly increased soil available P concentration (Table 2), and greatly increased P acquisition and yield of kangkong (Fig. 2a). It also increased the competency of kangkong in acquiring P when intercropping with stonecrop (Fig. 3b). However, biochar may be detrimental to phosphatase and limit its benefits because of the large amounts of associated P which are readily soluble. Therefore, soil acid phosphatase activity decreased significantly or appulsively with all +B treatments (Table 2).

More importantly, this experiment showed positive effects of biochar on reducing Cd concentrations in kangkong shoots growing in this Cd-contaminated acidic soil (Fig. 2a; Table 1). As mentioned earlier, plant Cd accumulation is generally controlled by Cd mobility in soil, which is in turn highly dependent on soil pH[20]. As in the cases of red mud and cyclonic ashes[35,36], the effects of such amendments on reducing metal mobility and plant uptake were mainly attributed to the increased soil pH as result of alkaline reaction of the material

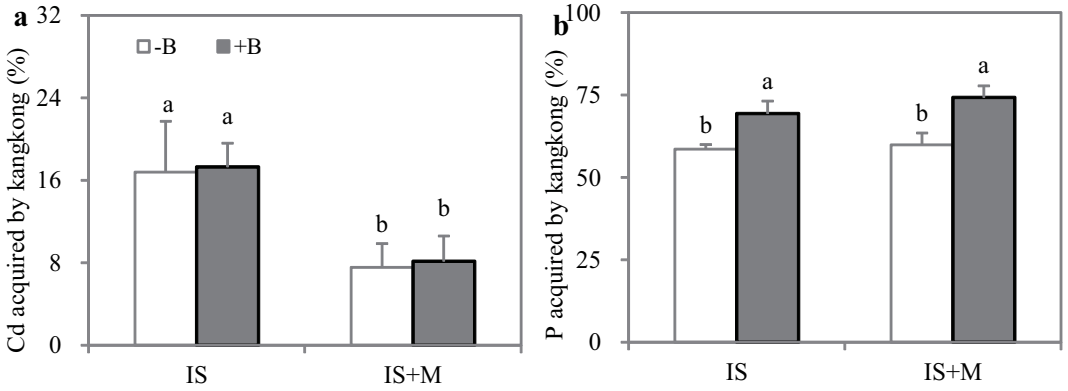

Figure 3 | The ratios of the amounts of Cd (a) and P (b) acquired by upland kangkong to the total acquisitions of Cd and P by Alfred stonecrop and upland kangkong. IS, intercropping with Alfred stonecrop; +M, inoculation with *Glomus caledonium* 90036; +B, application with biochar; −B, non-application of biochar. *Vertical T bars* indicate standard deviations. *Bars* not topped by the same letter indicate a significant difference in values ($P<0.05$).

added in large amounts[21]. In this study, biochar addition also significantly increased soil pH and thereby decreased DTPA-extractable Cd concentration, regardless of the intercropping of stonecrop (Table 2). On the other hand, biochar could have enhanced the binding and aging capability for mobile metals[37], thus exerting a stronger control on Cd availability to kangkong. DTPA extraction was tentatively proposed to measure the pool of a metal to release from soil solid phase into solution through forming chelates, which was generally accepted as an indicator of accessibility to plant root uptake[38]. Accordingly, a lower DTPA extractability refers to a higher fraction of bound metals, and this would account for the dominant deceases in kangkong Cd acquisition and subsequent tissue Cd concentrations.

The observed decrease in kangkong shoot Cd concentration upon biochar amendment was more profound in the intercropping systems, especially the treatment inoculated with AM fungi (Table 1). Therefore, there should be additive effects of stabilization by biochar (Table 2) and competition by stonecrop (Fig. 3a) on Cd accessibility to kangkong. However, no significant effects of biochar addition were observed on mycorrhizal colonization and Cd uptake of stonecrop in this experiment (Fig. 1). Up to now, the reported results involving biochar effects on AM fungi from literatures were not consistent[28]. For example, Ezawa observed a doubled infectivity of AM fungi upon addition of biochar at an application rate of 33% by volume[39]. Similarly, Yamato *et al.* found that biochar increased root mycorrhizal colonization by 42% in the field with an application rate of 10 L/cm[40]. In contrast, Warnock observed that AM fungal abundances were unchanged or decreased with biochar amendment across multiple treatments[41]. Therefore, conditions of the growth substrate and the application rate of biochar might be influencing factors on mycorrhizal colonization. On the other hand, it is noteworthy that the decreased soil DTPA-extractable Cd concentration upon biochar amendment also did not affect Cd uptake by stonecrop (Fig. 1), which was totally different from that by kangkong. It can be deduced that Cd-hyperaccumulator might be less sensitive to changes of Cd phytoavailability in soil than common vegetables.

Table 1 | Responsiveness (%) of shoot yield and Cd concentration of upland kangkong as affected by the intercropping of Alfred stonecrop (IS), the inoculation of *Glomus caledonium* 90036 (+M), and the application of biochar (+B)

Factor	Kangkong shoot yield (fw.)	Kangkong shoot Cd concentration (fw.)
IS	−17.9	−16.3
IS + M	−14.0	−40.7
+B	+37.7	−24.7
IS + B	+30.9	−43.2
IS + M + B	+25.5	−62.7

After plant harvest, the two *Gc*-inoculated treatments also significantly decreased DTPA-extractable Cd concentrations in the soils compared to the two non-inoculated IS treatments, respectively (Table 2). Although the non-inoculated stonecrop plants acquired on average 75.9–82.5 μg of Cd from the soils per pot, it did not decrease DTPA-extractable Cd concentrations significantly compared to the monocultural controls (Table 2). Therefore, the relatively high efficiencies of *Gc*-inoculated stonecrop plants in acquiring Cd (on average 112 and 105 μg per pot for −B and +B, respectively), could be one of the causes but not the entire reason for the significantly decreased DTPA-extractable Cd concentrations. Even more importantly, soil pH was also significantly increased upon *Gc* inoculation (Table 2), likely due to the release of OH^- as a consequence of active nitrate uptake by the fungus[42]. Then, the decrease of soil DTPA-extractable Cd (Table 2) occurred because AM fungi changed Cd availability by increasing soil pH. Unlike the alkalinization effects provided by biochar which could occur at the very beginning of experiment, the alkalinization effects of AM fungi generated and cumulated during the growing period of the host plant (stonecrop). Consequently, there were additive effects of biochar and *Gc* on soil alkalinization and Cd stabilization after plant harvest (Table 2), causing a lower post-harvest transfer risk. Therefore, the results suggested the combined application of AM fungi and biochar would facilitate the intercropping systems in dealing with Cd-contaminated farm soils.

In conclusion, biochar increased kangkong yield via elevating soil available P, and decreased Cd phytoavailability and kangkong Cd concentration via elevating soil pH. Intercropping with stonecrop decreased soil Cd accessibility to neighboring kangkong through rhizosphere competition. *Gc* inoculation accelerated plant growth and Cd acquisition of stonecrop, and thus resulted in further decreases of Cd acquisition/concentration of kangkong. Compared with the control, there was a higher kangkong yield with a substantially lower Cd concentration under the combined treatment intercropped with stonecrop, inoculated with *Gc* and applied with biochar. *Gc* generated additive effects on soil alkalinization and Cd stabilization, causing lower post-harvest transfer risks.

Methods

Biochar preparation. The biochar was provided by the Kuake Science and Technology Limited Liability Company, Chinese Academy of Sciences, Nanjing, China. It was produced under no-oxygen condition by a biochar reactor, which was heated by a step-wise procedure. The starting temperature was set at 350°C, then elevated to 400°C, 450°C, 500°C, and finally to the target temperature (550°C). At each temperature (except for final temperature), the process was maintained for

Table 2 | Soil pH, EC, DTPA-extractable Cd concentration, available P concentration, and acid phosphatase activity

Treatment[a]		pH (H_2O)	EC (μS/cm)	DTPA-extractable Cd (mg/kg)	Available P (mg/kg)	Acid phosphatase activity (mg/kg/24h)
Control	−B	4.14 ± 0.11 d	65.9 ± 14.9 c	0.163 ± 0.009 a	85.5 ± 1.9 b	2.36 ± 0.10 ab
	+B	4.60 ± 0.04 bc	137.0 ± 33.0 b	0.143 ± 0.010 bc	103.1 ± 1.6 a	2.18 ± 0.08 c
IS	−B	4.17 ± 0.02 d	63.2 ± 5.6 c	0.156 ± 0.013 ab	83.0 ± 1.6 b	2.31 ± 0.04 bc
	+B	4.52 ± 0.07 c	210.1 ± 46.2 a	0.134 ± 0.011 c	104.1 ± 1.2 a	2.17 ± 0.12 c
IS + M	−B	4.68 ± 0.04 b	96.3 ± 13.4 bc	0.136 ± 0.016 c	85.0 ± 1.6 b	2.48 ± 0.11 a
	+B	4.90 ± 0.06 a	241.5 ± 41.0 a	0.109 ± 0.006 d	104.1 ± 3.5 a	2.19 ± 0.09 c

[a]Control, monoculture of upland kangkong; IS, intercropping with Alfred stonecrop; +M, inoculation with *Glomus caledonium* 90036; −B, non-application of biochar; +B, application with biochar. Values are means of four replications ± standard deviations. Values within the same column not followed by the same letter differ significantly ($P<0.05$).

1.5 h. The whole process was stopped when no further smoke came out from the gas exit pipe, and 35% of rice straw biomass was converted to biochar. It was then ground to pass 2 mm sieve. Subsamples were used for analyzing selected properties. The pH and EC (biochar : deionized water = 1 : 10) were measured with a pH meter (Beckman) and an EC meter (Orion 160), respectively. After a subsample (0.5 g) was digested by conc. nitric acid, total Cd and P concentrations in the biochar were determined using an atomic absorption (AA) spectrophotometer (SpetrAA-20, Varian, U.S.) and an UV–Vis spectrophotometer (UV-1061, Shimadzu, Kyoto) based on the molybdenum blue reaction[43], respectively. The biochar had a pH of 10.5 and an EC of 3.2 mS/cm, and contained 1.7 g/kg of total P and 1.3 mg/kg of total Cd.

Mycorrhizal inoculum. The AM fungal inoculum, *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann 90036 (*Gc*), was chosen due to its performance in enhancing plant Cd accumulating in our previous study[13]. It was isolated from a fluvo-aquic soil in Henan Province, China, and deposited at the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China. It was propagated on white clover (*Trifolium repens* L.) grown in an autoclaved (121°C for 1 h on 3 successive days) substrate (sand : soil : vermiculite : zeolite = 2 : 1 : 1 : 1) for 2 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. They were air-dried and sieved through a 2 mm sieve before inoculation.

Soil preparation. A surface soil sample (0–20 cm) was collected by a shovel from an arable and flat agricultural land at the suburb of Guangzhou, China on May 26, 2010. The soil was classified as orthic antrosols, and the previous crop grown before soil collection was upland kangkong. The air-dried soil sample was ground with a wooden pestle, and homogenized by sieving through a 5 mm sieve. A subsample was sieved through a 2 mm sieve for analysing selected soil properties. It had a pH of 3.9 (H_2O) and an EC of 275 μS/cm, and contained 1.6, 40, 85, and 140 mg/kg of total Cd, copper (Cu), lead (Pb), and zinc (Zn), as well as 90 mg/kg of available P[13]. All the total concentrations of Cu, Pb, and Zn were acceptable according to the permissible limits (50, 250, and 200 mg kg^{-1}) for agricultural soils with pH < 6.5 set by China[44]; with the exception of total Cd concentration that greatly exceeded the limit (≤0.3 mg/kg), due to the application of Cd-contaminated sediment from the Pearl River[45].

Pot experiment. Upland kangkong and Alfred stonecrop were used as the tested vegetable and Cd-hyperaccumulator, respectively. There were 3 treatments each under both application (+B) and non-application (−B) of biochar: (1) monoculture of kangkong (control), (2) kangkong intercropped with stonecrop (IS), (3) IS plus inoculation with *Gc* (IS + M). Each polyvinyl chloride pot (22 cm diameter × 20 cm depth) contained 3 kg of soil, which was mixed with 150 g of mycorrhizal/non-mycorrhizal inoculum, and also 75 g of biochar for each +B pot. Kangkong seeds were sterilized with 0.5% NaClO, washed with distilled water and then sowed into each pot. The kangkong seedlings were thinned to 6 per pot 3 days after germination, and 4 stem cuttings of stonecrop with similar size (an average length of 6 cm) were planted into each IS pot. Pots were randomly arranged with 4 replicates per treatment, and grown in a glasshouse with temperature control (22–25°C), supplemented with additional illumination (with a light intensity of 250 μmol/m^2/s, under a 14/10 h–light/dark cycle). Plants were watered by hand with a watering can to maintain soil moisture at about 50% of the water-holding capacity (the maximum ability of a soil to contain and retain water), which was determined using a cutting ring before the pot experiment and was calculated as (mass of water contained in the saturated soil)/(mass of the saturated soil) × 100%. After growing for 10 weeks, both kangkong and stonecrop plants were harvested, and soil samples were also collected.

Mycorrhizal colonization and plant analysis. Fresh roots of stonecrop were all cleaned by 10% KOH and stained with acid fuchsin[46]. Root mycorrhizal colonization was then assessed by the grid-line intersect method with light microscopy[47]. Stonecrop shoot was weighed after oven-drying at 70°C for 48 h. The mean individual biomass was then calculated by dividing the total value by 4. Kangkong plants were divided into shoots and roots. The mean individual fresh weights of shoot and root were then measured by dividing their total weights by 6, respectively. The dry biomasses were also obtained after oven-drying at 70°C for 48 h. Subsamples of dried and ground shoots of stonecrop (0.2 g), as well as shoots and roots of kangkong (0.5 g), were digested by conc. nitric acid, followed by AA spectrophotometry (SpetrAA-20) and molybdenum-ascorbic acid spectrophotometry (UV-1061) to measure tissue Cd and P concentrations[48], respectively. Both standard reference material (Tomato Leaves 1573a, NIST) and blank were included for quality assurance. The recovery rate of tissue Cd was 92%. For kangkong, both Cd and P concentrations were expressed on a fresh weight basis by correcting for water content in the sample.

Competency (C) of kangkong in the intercropping system, expressed as percentages of Cd or P acquired in the two plant species present in the kangkong plant, was calculated using the following equation:

$$C = K_{acp.} \times 6 \,/\, \left(K_{acp.} \times 6 + S_{acp.} \times 4\right) \times 100\%$$

where $K_{acq.}$ and $S_{acq.}$ are the total amounts of Cd/P acquired in individual kangkong and stonecrop, respectively.

Responsiveness (R) of kangkong as affected by IS, +M and +B, either alone or in combination, expressed as percentage alterations in both shoot yield and Cd concentration, was calculated using the following equation:

$$R = (K_{treatment} - K_{control}) \,/\, K_{control} \times 100\%$$

where $K_{treatment}$ and $K_{control}$ are mean shoot yield (or Cd concentration) under treatment and control, respectively.

Soil chemical and enzymatic property analysis. Soil samples were air-dried and passed through a 2 mm mesh sieve. Soil pH and EC (soil : deionized water = 1 : 5) were determined by a pH meter (Beckman) and an EC meter (Orion 160), respectively. Soil acid phosphatase activity was determined by incubation at 37°C with acetate buffer (pH 5) according to the method of Tabatabai[49], and was given in the unit of mg *p*-nitrophenol produced per g soil per 24 h. Soil available P was extracted by the Bray and Kurtz method with an acid extracting solution (0.025 M hydrochloric acid and 0.03 M ammonium fluoride)[50], to measure the P concentration in extracts based on the molybdenum blue reaction with the UV–Vis spectrophotometer (UV–1061)[43]. Soil DTPA-extractable Cd (0.005 M DTPA, 0.1 M triethanolamine and 0.01 M $CaCl_2$, pH 7.3; solution : soil = 2 : 1, extraction for 2 h)[51] was measured using the AA spectrophotometer (SpetrAA–20). All these results were expressed on an oven-dried soil weight basis (105°C, 24 h).

Statistical analysis. The means and standard deviations of 4 replicates were computed. An analysis of variance was carried out using the One-way ANOVA procedure with SPSS software, while the comparison of mean effects was based on least significant difference multiple-comparison tests. Differences were considered significant at $P < 0.05$.

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Author contributions

J.L.H. performed the experiments, carried out data analysis and wrote the manuscript. F.Y.W., S.C.W. and C.L.L participated in field sampling collection or greenhouse experimental set-up. X.G.L. and M.H.W supervised the study. All authors reviewed the manuscript.

Additional information

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