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Plant coexistence can enhance phytoextraction of cadmium by tobacco (*Nicotiana tabacum* L.) in contaminated soil

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Abstract

A mesocosm experiment was conducted to investigate whether plant coexistence affects cadmium (Cd) uptake by plant in contaminated soil. Tobacco (*Nicotiana tabacum* L. var. K326) and Japanese clover (*Kummerowia striata* (Thunb.) Schindl.) were used. Cadmium was applied as $3CdSO_4 \cdot 8H_2O$ in solution at three levels (0, 1, and 3 mg/kg soil) to simulate an unpolluted soil and soils that were slightly and moderately polluted with Cd. Tobacco (crop), Japanese clover (non-crop), and their combination were grown under each Cd treatment. Compared to monoculture and under all Cd treatments, co-planting with Japanese clover did not affect tobacco biomass but significantly increased Cd concentration in all tobacco tissues and enhanced Cd accumulation in tobacco shoots and roots. Compared to monoculture, co-planting reduced soil pH and increased Cd bioavailability. For tobacco, co-planting with Japanese clover increased the Cd bioconcentration factor (BCF) in Cd contaminated soil. Japanese clover also accumulated substantial quantities of Cd in shoots and roots. Thus, total Cd uptake by the plants was much greater with co-planting than with monoculture. The results suggested that phytoextraction can be effectively increased through tobacco co-planting with Japanese clover in mildly Cd-contaminated soil.

Key words: plant coexistence; phytoextraction; cadmium; tobacco; Japanese clover

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Introduction

Long-term application of heavy metal-containing fertilizers, pesticides, sewage sludge, and municipal wastes, as well as emissions from industrial activities have contaminated agricultural topsoil worldwide (Marques et al., 2009; McGrath et al., 2001). In response to a growing need to reduce environmental contamination, many technologies have been developed to remediate contaminated soils. As a promising, environmentally friendly and cost-effective remediation technology, phytoextraction is receiving increased attention (Chaney et al., 1997; Pilon-Smits, 2005). Phytoextraction uses plant roots to take up contaminants and stores them in harvestable parts that can then be removed from contaminated sites (Marques et al., 2009).

Two different strategies are employed for phytoextraction (McGrath et al., 2001). One strategy is the use of metal hyperaccumulator species, which are capable of accumulating unusually high quantities of metal in their aboveground tissues without showing metal toxicity symptoms (Prasad and Freitas, 2003). Most hyperaccumulator species, however, grow slowly and have low biomass, and thus the use of hyperaccumulators for phytoextraction is limited (Arthur et al., 2005; Prasad and Freitas, 2003). The other strategy involves the use of fast-growing and high-biomass plants that contain low to moderate concentrations of heavy metals (McGrath et al., 2001; Raskin et al., 1997). Because high biomass can compensate for a relatively low capacity for metal accumulation in aboveground parts, selecting plant species that produce high biomass and are tolerant to metals has been the focus of recent phytoextraction research. Some species, such as maize (*Zea mays*), sunflower (*Helianthus annuus*), and tobacco (*Nicotiana tabacum*), have been determined to have substantial potential for extracting metal contaminants from soil (Murakami et al., 2007; Nehnevajova et al., 2005).

The success of phytoextraction depends on metal bioavailability in soil (Ernst, 1996; Everhart et al., 2006), and it is the soluble form of metals that is easily acquired by plants. The soluble form, however, often represents only a small part of the total metal content in soil (Marques et al., 2009). To enhance bioavailability of metals in soil and to maximize phytoextraction, researchers have studied several approaches involving manipulation of the rhizosphere. For example, application of chemical chelators or acidifying amendments to metal-polluted soil increases

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metal uptake by plants; however, chemical chelators are relatively expensive and can lead to secondary pollution of soil and groundwater (Chen and Cutright, 2001; Meers et al., 2005). In addition, even very low concentrations of these chemicals can decrease plant growth (Chen and Cutright, 2001). Metal bioavailability can also be increased by the plants themselves. Experiments have shown that some plant species can exude H^+ or low molecular weight organic acids (e.g., acetic, oxalic, fumaric, citric, and tartaric acids) into soil, which can increase metal mobility either directly or indirectly by affecting microbial activity (Chen et al., 2003; Chiang et al., 2006; Duarte et al., 2007; Evangelou et al., 2006; Liao and Xie, 2004). Moreover, H^+ can replace cations and make metal cations more bioavailable (Marques et al., 2009).

Crop co-planting, which is a common agronomical practice in many countries (Olowe and Adeyemo, 2009; Temperton et al., 2007), may affect phytoextraction of metals from soil because coexistence of multiple plant species may change rhizosphere microorganisms, soil enzymes activities and abiotic micro-environment, and thus may affect the metal bioavailable in rhizosphere soil (Khan, 2005; Yang et al., 2007). In cropping systems, legumes are often included in the rotation because of their ability to fix N_2 (Karpenstein-Machan and Stuelpnagel, 2000; van Kessel and Hartley, 2000). Legumes are also excellent partner crops in intercropping systems because legume plants can provide N and induce soil acidification and thus increase the bioavailability of P and other immobile elements (Jensen and Hauggaard-Nielsen, 2003; Noble et al., 2008).

We hypothesize that the co-planting of a legume with a main plant species that produces substantial biomass will enhance phytoextraction of Cd from a contaminated soil because the legume will acidify the soil and thereby increase metal mobility. We used tobacco (*N. tabacum* L. var. K326) as the main plant species. Tobacco is metal tolerant and then considered a potential phytoremediator for its high biomass, (Chiang et al., 2006; Evangelou et al., 2007; Janouskova et al., 2005). Japanese clover (*K. striata* (Tunb.) Schindl.), a common legume cover plant which has showed to enhance the growth and mycorrhizae of the coexisting neighbors (Chen et al., 2005), was used to co-plant with tobacco. We focused on Cd because it is highly toxic to humans (Ghosh and Singh, 2005; Kirkham, 2006) and contaminates many agricultural soils in the world (Cheng, 2003; Jin et al., 2004). Our main objectives were: (1) to investigate whether co-planting with the legume (Japanese clover) affects the growth of the phytoextractor (tobacco), and (2) to investigate whether phytoextraction of Cd can be enhanced through co-planting a legume in a soil slightly or moderately contaminated with Cd.

1 Materials and methods

1.1 Soil and plants

The experimental soil was collected from the surface layer (0–20 cm) of an unpolluted paddy field in Hangzhou City (28°54′N, 111°30′E), Zhejiang Province, in south-eastern China. About 1000 kg soil was collected, air dried, mixed, and crushed to pass through a 2-cm sieve. A sample for laboratory analysis was taken from the bulk soil. The soil sample was milled to pass a 40-mesh sieve (0.35 mm) for pH determination, and further passed through a 100-mesh sieve (0.16 mm) for analysis of other chemical properties. The basic properties of the soil were as follows: pH 5.9 (2.5:1, KCl aqueous solution:soil), organic mater content 38.6 g/kg, total nitrogen (N) 2.49 g/kg, available phosphorus (P) 98.03 mg/kg, available potassium (K) 264.72 mg/kg, and total Cd 0.047 mg/kg. The contents of soil organic mater, total N, and available P and K were analyzed according to the routine analytical methods of agricultural chemistry in soil (Lu, 1999). The soil sample was wet digested with $HF + HNO_3 + HClO_4$, and total Cd in the soil was analyzed by flame atomic absorption spectroscopy (FAAS) method (AA6650, Shimadzu, Japan).

The seeds of tobacco (*N. tabacum* L. var. K326) were provided by Henan Agricultural University, China. The seeds of Japanese clover were collected from a natural population in an unpolluted field in Hangzhou City.

1.2 Experimental design and treatments

The experiment was a two-factorial design with three Cd treatments and three types of culture and replicated four times in a randomized complete block design using mesocosms. The three Cd treatments were control (CK, without Cd addition), 1 mg/kg soil (Cd1), and 3 mg/kg soil (Cd3). Treatments of Cd1 and Cd3 were designed to simulate slight and moderate Cd contamination of soil. The Cd treatments were applied by mixing $3CdSO_4{\cdot}8H_2O$ into the soil. The three types of culture were a monoculture of tobacco, a monoculture of Japanese clover, and an inter-planting of tobacco and Japanese clover.

Plastic containers used in the mesocosms experiment were 42 cm in length, 32 cm in width and 16 cm in depth. Each mesocosm was filled with 20 kg of well-mixed soil. Soil moisture content was adjusted to about 60% of water holding capacity based on weight, and the soil was placed in the mesocosm one month before planting to stabilize various sorption mechanisms and other processes. During one month, water was added for maintaining soil moisture at 60% of field water holding capacity.

All seeds were surface sterilized with 3% H_2O_2 for 10 min and then washed with tap water three times. Consistency in the number, size, and vigor of plants added to the mesocosms was obtained by using transplants rather than seeds. The seeds of tobacco and Japanese clover were germinated and grown in the plastic mesh plate containing vermiculite and peat in a greenhouse under natural light and temperature. Forty days after germination, when tobacco seedlings had five leaves and were about 8 cm tall, tobacco and Japanese clover seedlings were transplanted into the mesocosms. For the monoculture of tobacco, four plants were planted in each mesocosm. For inter-planting of tobacco with Japanese clover, four plants of tobacco and six plants of Japanese clover were inter-planted in each mesocosm. For the monoculture of

Japanese clover, 12 plants were planted in each mesocosm.

Mesocosms were arranged in greenhouse in a complete randomized block design. Plants were maintained under natural light and temperature conditions. Plants were watered daily to maintain soil moisture at 70%–90% of field water-holding capacity. No additional nutrients were applied during the experiment.

1.3 Sampling and measurement

Plants were harvested 90 days after transplanting by cutting the shoots at the soil surface and carefully separating the roots from the soil. Tobacco tissue was divided into root, stem, lower leaves (1st–6th leaves from the bottom of the main stem), cutter leaves (7th–15th), and upper leaves (16th–20th), and Japanese clover tissue was divided into shoot and root. Subsamples of fresh roots of tobacco and Japanese clover from each mesocosm were fixed in FAA (37% formaldehyde, glacial acetic acid, 95% ethanol, 9:0.5:0.5, *V*/*V*/*V*) for quantification of colonization by arbuscular mycorrhizal fungal (AMF). The fresh weight of total roots and of subsamples was measured. After shoots and remaining roots were washed briefly with tap water and then with deionized water to remove surface dust and soil, they were dried at 80°C for 48 hr and then weighed. The water content (%) of remaining roots and total root fresh weight were used to estimate the total root dry weight.

Plant samples for testing Cd concentration were ground to < 0.25 mm in a stainless steel mill. The milled sample (4 g) was reduced to ash at 600°C for 6 hr in a muffle furnace and then cooled and dissolved in 1:1 nitric acid. The suspension was passed through a quantitative filter paper (Chen et al., 2005). The filtrate was adjusted to 50 mL with deionized water.

After plants were harvested, soil samples were collected and were air dried, crushed, and passed through a 100-mesh screen. The soil exchangeable Cd was quantified as described before (Tessier et al., 1979). Briefly, soil (1 g) was extracted with 8 mL of 1 mol/L $MgCl_2$ at pH 7.0 in a 50-mL plastic centrifuge tube for 20 min with continuous agitation at room temperature.

Cadmium concentrations in the solutions extracted from plant and soil samples were analyzed by FAAS. Standards for the FAAS calibration were prepared in the extraction solution by the addition of appropriate quantities of Cd.

AMF colonization of roots was quantified using a microscope (100×) after the roots had been cleared in 10% KOH (*W*/*V*) and stained in acid fuchsin. The gridline intersection method (Giovannetti and Mosse, 1980) was used to determine the proportion of root intersections in which arbuscules, vesicles, or hyphae occurred. Soil pH was measured by suspending 10.0 g air-dried soil in 50 mL deionized water; a pH meter with a combined glass electrode was used.

1.4 Calculation of bioconcentration factor

Bioconcentration factor (BCF) was used to evaluate the efficiency of Cd phytoextraction and was calculated using the following Equation:

$$BCF = C_{Cd\text{-}aboveground}/C_{Cd\text{-}soil}$$

where, $C_{Cd\text{-}aboveground}$ (mg/kg) is the Cd concentration in aboveground parts of the plants including stems and leaves. $C_{Cd\text{-}soil}$ (mg/kg) is the Cd concentration in soil (Ghosh and Singh, 2005).

1.5 Data analysis

A two-way ANOVA was performed for each dependent variable by using the general linear model in the SPSS V.10.0 (SPSS Inc., Chicago, USA). The independent variables were Cd concentrations and types of plant culture (monocultures or mixture). The least significant difference (LSD) at the 5% confidence level was used for comparing treatments. Data for AMF colonization rate and BCF were arcsine transformed before ANOVAs were performed. The difference of types of culture within each Cd treatment was determined by independent-sample *t*-tests.

2 Results

2.1 Biomass of tobacco

Cadmium treatments significantly influenced ($F = 15.13$, $p < 0.01$) tobacco biomass, which increased with the addition of Cd to soil (Fig. 1). Type of culture and the two-way interaction did not influence ($F = 0.01$, $p > 0.05$; $F = 3.63$, $p > 0.05$) tobacco biomass.

2.2 Cd concentration in tobacco tissues

Cadmium concentrations of tobacco tissues were in the order of lower leaf > cutter leaf > root > upper leaf >stem (Fig. 2). Cadmium treatments significantly affected Cd concentrations in the root ($F = 483.85$, $p < 0.01$, Fig. 2E), stem ($F = 882.77$, $p < 0.01$, Fig. 2D), lower leaf ($F = 1104$, $p < 0.01$, Fig. 2C), cutter leaf ($F =$

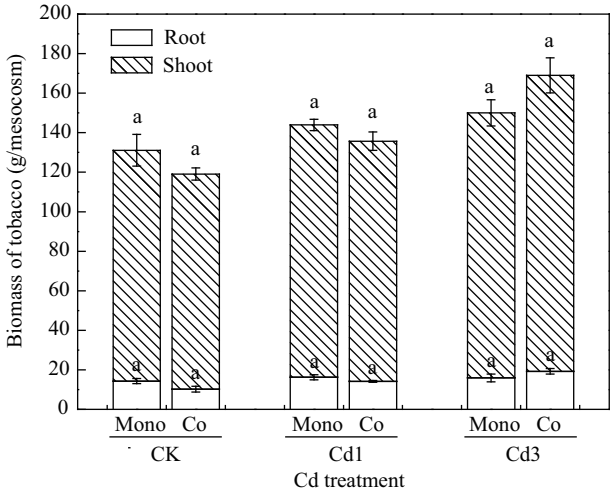

Fig. 1 Biomass of tobacco grown under monoculture (Mono) and co-planting (Co) in the three Cd treatments. Values are means (± SE) of four replicate mesocosms. For each Cd treatment, different letters indicate significant differences between monoculture and co-planting according to LSD at $p < 0.05$.

971.45, $p < 0.01$, Fig. 2B), and upper leaf ($F = 1192$, $p < 0.01$, Fig. 2A). Cadmium concentrations in all these tobacco tissues increased with addition of Cd to soil. Types of culture also significantly affected Cd concentration in tobacco roots ($F = 20.78$, $p < 0.01$, Fig. 2E), stems ($F = 69.89$, $p < 0.01$, Fig. 2D), lower leaves ($F = 106.16$, $p < 0.01$, Fig. 2C), cutter leaves ($F = 148.69$, $p < 0.01$, Fig. 2B), and upper leaves ($F = 101.82$, $p < 0.01$, Fig. 2A). Compared to monoculture, inter-planting significantly increased Cd concentrations in all tobacco tissues in the two treatments in which Cd was added. The two-way interaction was significant for the stems ($F = 23.08$, $p < 0.01$), lower leaves ($F = 24.02$, $p < 0.01$), cutter leaves ($F = 36.13$, $p < 0.01$), and upper leaves ($F = 41.84$, $p < 0.01$).

2.3 Allocation of Cd by tobacco and total metal uptake

Cadmium treatments significantly affected Cd accumulation in roots ($F = 104.99$, $p < 0.01$) and shoots ($F = 279.26$, $p < 0.01$) of tobacco (Table 1). Type of culture also significantly affected Cd accumulation in roots ($F = 6.42$, $p < 0.05$) and shoots ($F = 29.46$, $p < 0.01$) of tobacco (Table 1). Cadmium accumulation in both tobacco shoots and roots was remarkably higher under co-planting than under monoculture, especially in shoots, with an increment of 6.2 μg/ plant in CK, 77.7 μg/plant in Cd1, and 66.8 μg/plant in Cd3. The two-way interaction was not significant for Cd accumulation in tobacco roots or shoots (for root $F = 3.50$, $p > 0.05$; for shoot $F = 5.71$, $p > 0.05$).

Cadmium treatments significantly affected ($F = 16.04$, $p < 0.01$) the ratio of Cd accumulation in shoots vs.

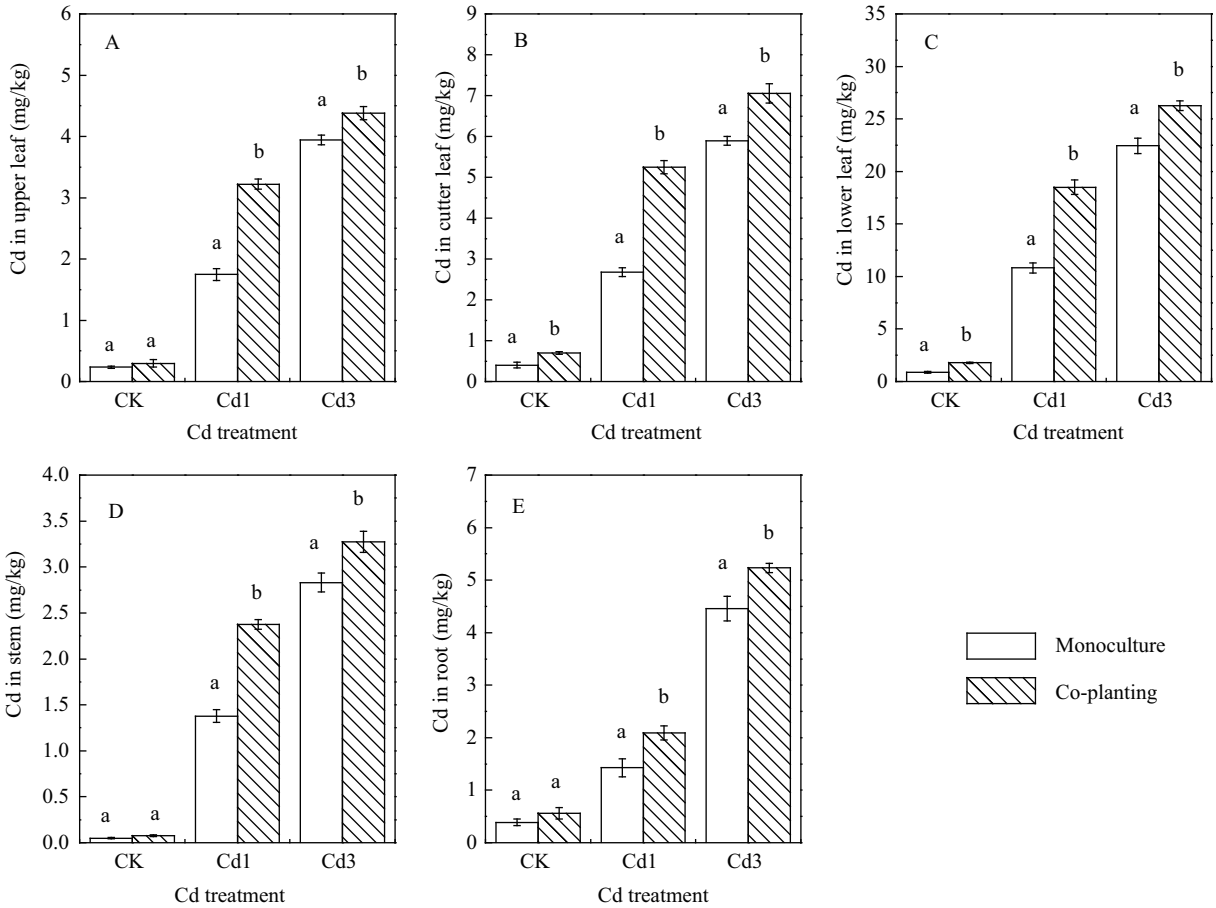

Fig. 2 Cd concentration in upper leaf (A), cutter leaf (B), lower leaf (C), stem (D) and root (E) of tobacco grown under monoculture and co-planting in the three Cd treatments. Values are means (±SE) of four replicate mesocosms. For each Cd treatment, different letters indicate significant differences between monoculture and co-planting according to LSD at $p < 0.05$.

Table 1 Cd allocation in tobacco, and Cd uptake in tobacco, Japanese clover, and tobacco plus clover*

Cd treatment	Culture type	Cd allocation in tobacco (μg/plant)			Cd uptake (μg/mesocosm)		
		Shoot	Root	Shoot/Root	Tobacco	Japanese clover	Total
CK	Monoculture	10.1 ± 0.5 a	1.4 ± 0.3 a	8.1 ± 2.4 a	45.8 ± 1.8 a		45.8 ± 1.8 a
	Co-planting	16.3 ± 1.0 b	1.4 ± 0.3 a	12.7 ± 2.5 a	70.6 ± 4.0 b	6.1 ± 0.4	76.7 ± 3.7 b
Cd1	Monoculture	107.0 ± 5.8 a	5.7 ± 0.5 a	18.9 ± 1.7 a	450.9 ± 24.5 a		450.9 ± 24. 5 a
	C-planting	184.7 ± 13.8 b	7.4 ± 0.6 a	25.4 ± 3.4 a	768.6 ± 53.1 b	12.8 ± 1.7	781.4 ± 54.7 b
Cd3	Monoculture	247.8 ± 11.9 a	17.9 ± 2.0 a	14.1 ± 0.8 a	1062.6 ± 59.5 a		1062.6 ± 59.5 a
	C-planting	314.6 ± 20.1 b	25.2 ± 1.6 b	12.6 ± 1.1 a	1359.2 ± 81.6 b	21.3 ± 2.4	1380.5 ± 82.2 b

* Values are means ±SE of four replicate mesocosms. For each Cd treatment, means followed by different letters indicate significant differences between monoculture and inter-planting according to the LSD test at the 0.05 level.

roots (Table 1). The ratio was not significantly affected by type of culture (F = 3.25, p > 0.05) or by the two-way interaction (F = 1.83, p >0.05).

Cadmium treatments (F = 290.73, p < 0.01), type of culture (F = 33.18, p < 0.01), and the two-way interaction (F = 7.2, p < 0.01) significantly affected total Cd uptake (Table 1). Total Cd uptake was obviously higher under co-planting than under monoculture, with an increment of 30.9 μg/mesocosm in CK, 330.5 μg/mesocosm in Cd1, and 317.9 μg/mesocosm in Cd3.

2.4 Bioconcentration factor

The Cd BCF of tobacco ranged from 2.23 to 4.96 (Fig. 3). Cadmium treatments, type of culture, and the two-way interaction significantly affected BCF of tobacco (for Cd treatments F = 38.76, p < 0.01; for type of culture F = 106.22, p < 0.01; for two-way interaction F = 17.61, p < 0.01). BCF was 1.8 times higher (in Cd1) and 1.1 times higher (in Cd3) under inter-planting than under monoculture.

2.5 Soil exchangeable Cd concentration, pH, and AMF colonization rate

Cadmium treatments, type of culture, and the two-way interaction significantly affected soil exchangeable Cd concentration (for Cd treatment F = 297.91, p < 0.01; for type of culture F = 87.70, p < 0.01; for interaction of Cd treatment and type of cultures F = 18.22, p < 0.01). Soil exchangeable Cd concentration was higher under monoculture of Japanese clover than under monoculture of tobacco or co-planting of tobacco and Japanese clover (Fig. 4A). For tobacco, the soil exchangeable Cd concentration was significantly higher under co-planting than under monoculture (F = 5.36, p < 0.05).

Cadmium treatments and types of culture significantly affected soil pH (F = 15.60, p < 0.01; F = 127.06, p < 0.01), but the two-way interaction did not affect soil pH (F = 2.91, p > 0.05). Soil pH was lower under monoculture of Japanese clover (5.47–5.74) than under both monoculture (6.05–6.11) and co-planting (5.79–5.94) of tobacco (Fig. 4B). For tobacco, soil pH was lower under co-planting than under monoculture (F = 69.48, p< 0.01).

Type of culture significantly affected the AMF colonization rate of tobacco (F = 52.26, p < 0.01, Fig. 4C). AMF colonization rate was higher in Japanese clover under monoculture (54.1%–61.2%) than in tobacco under monoculture (34.2%–36.6%) or in tobacco under co-planting (38.7%–44.2%). Cadmium treatments and the two-way interaction did not affect the AMF colonization rate of tobacco (for Cd treatment F = 2.68, p > 0.05; for the two-way interaction F = 0.29, p > 0.05). For tobacco, the AMF colonization rate was higher under co-planting than under monoculture (F = 11.86, p < 0.01).

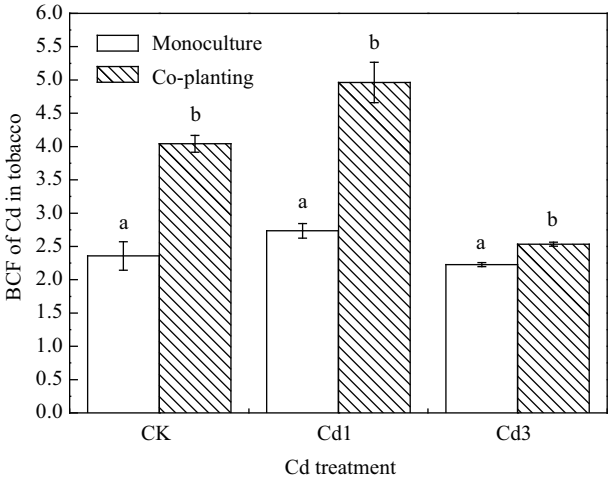

Fig. 3 Bio-concentration factor (BCF) of tobacco grown under monoculture and co-planting in three Cd treatments. Values are means (± SE) of four replicate mesocosms. For each Cd treatment, different letters indicate significant differences between monoculture and co-planting according to LSD at p < 0.05.

3 Discussion

The efficiency of phytoextraction is determined by two key factors: biomass production and metal concentration of plants (Giordani et al., 2005). In our experiment, the two Cd polluted treatments did not reduce tobacco biomass. Also no significant difference in tobacco biomass was found between monoculture and co-planting, indicating that tobacco did not suffer from competition for nutrients and other resources with Japanese clover. Moreover, Cd concentrations in all tobacco tissues and Cd accumulation in shoots and roots were significantly (p < 0.01) higher under co-planting than under monoculture (Fig. 2 and Table 1), indicating that the efficiency of phytoremediation could be optimized by co-planting. Other experiments have also demonstrated that total uptake of Pb by plants increased when plots were co-planted with multiple rather than single plant species (Wu et al., 2005). Similarly, Li et al. (2009) reported that maize (*Zea mays*) accumulated more Cd when inter-planted with legumes (cowpea (*V. unguiculata* (L.) Walp.), purple haricot (*L. purpureus* (L.) Sweet.) and chickpea (*C. arietinum* L.)) than that under monoculture.

AMF are ubiquitous in soil, forming symbiotic associations with the roots of most plant species (Smith and Read, 2008). The AMF community composition in soil and the AMF colonization rate of specific plants are affected by the composition of the plant community (Chen et al., 2005; Hartnett and Wilson, 1999; Jastrow and Miller, 1993; Mummey et al., 2005). Mycorrhizae play a crucial role in plant tolerance and uptake of metals, including Cd, Zn, Pb, Cu, and Al (Khan et al., 2000; Leyval et al., 1997). Previous experiments have demonstrated that the extra-radical hyphae of mycorrhizae can enhance metal uptake of host plants in slightly metal-contaminated soil by increasing root absorption surface (Chen et al., 2005; Redon et al., 2008). Other experiments have shown that mycorrhizae can also enhance plant tolerance of metals and can reduce metal concentration in the aboveground plant parts in highly contaminated soil by hindering metal transport from roots to shoots (Soares and Siqueira, 2008). Our experiment showed that AMF colonization of tobacco was enhanced by co-planting of Japanese clover. The enhanced mycorrhizal colonization could have two impor-

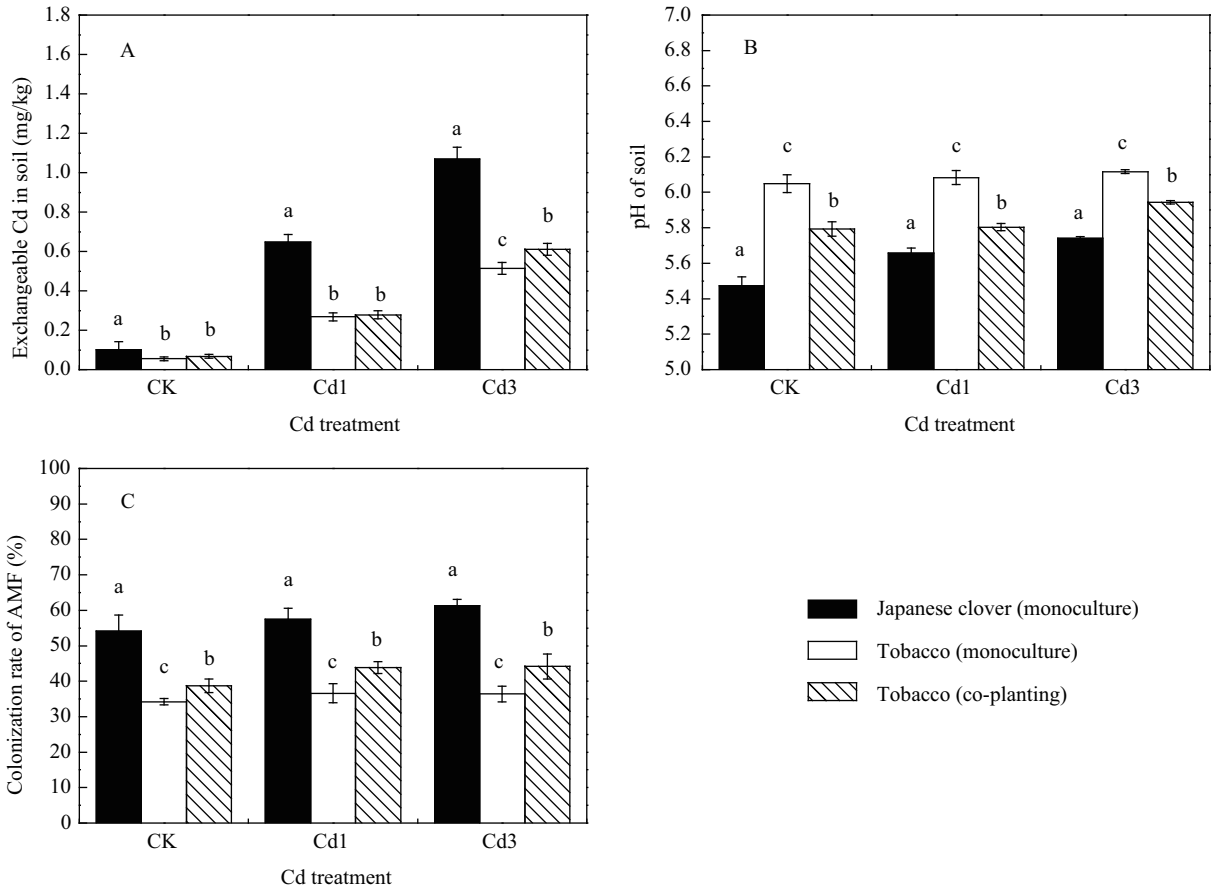

Fig. 4 Soil exchangeable Cd content (A) and pH value (B) in mesocosms planted, and arbuscular mycorrhizal fungal (AFM) colonization rate (C) with tobacco and Japanese clover growth under monocultures or co-plantings in the three Cd treatments. Values are means (± SE) of four replicate mesocosms. For each Cd treatment, different letters indicate significant differences between types of culture (monoculture of tobacco, monoculture of Japanese clover, and co-planting) according to LSD at $p < 0.05$.

tant effects on tobacco growing in Cd-contaminated soil: the enhanced colonization could increase the tolerance of tobacco to Cd and could also facilitate the uptake of Cd by tobacco.

Low bioavailability of heavy metals in soil is a key factor limiting metal accumulation by plants (Marques et al., 2009; Pilon-Smits, 2005). Metals can exist in the soil as discrete particles or can be associated with different soil components (Marques et al., 2009). Some studies have found that plant roots can exude hydrion and root exudates, which can increase metal mobility directly through acidification and chelation or indirectly through effects on microbial activity (Duarte et al., 2007; Ghosh et al., 2007; Khan et al., 2009). In our present experiment, exchangeable Cd concentration in the soil increased but soil pH decreased under co-planting with Japanese clover (Fig. 4A, B). The results indicated that Japanese clover substantially enhanced the bioavailability of Cd in soil, possibly because Japanese clover root exudates caused soil acidification or metal chelation during the process of biological N_2 fixation.

BCF indicating the ability of a plant to take up is therefore important in assessing the feasibility of phytoextraction (McGrath and Zhao, 2003). BCF value depends on various soil physicochemical properties (e.g., soil pH and soil texture), the type and bioavailability of metals, and the species of plants (Arthur et al., 2005; McGrath and Zhao, 2003). Generally, BCF value >1 is considered ideal for phytoextraction (Marques et al., 2009; McGrath and Zhao, 2003). In the present experiment, BCF value in all treatments was greater than 1 (Fig. 3). This result agree with other studies indicating that tobacco, as a high biomass plant, has a high ability to absorb Cd (Barazani et al., 2004; Evangelou et al., 2007; Janouskova et al., 2005). Our experiment also showed that co-planting with the legume, Japanese clover, enhanced BCF suggesting that co-planting can enhance Cd uptake by tobacco from soil by increasing Cd bioavailability because of reduced pH (Figs. 4A, B) or increased colonization by mycorrhizae (Fig. 4C).

As an accompanying species in co-planting, Japanese clover also showed a high ability to accumulate Cd in our study, and total uptake of Cd by the plants was substantially higher in the co-planted than in the monocultured plots (Table 1). Therefore, it is suggested that co-planting could be a new strategy for enhancing Cd phytoremediation.

4 Conclusions

Our experiments demonstrated that Cd concentrations in all tobacco tissues and Cd accumulation in tobacco shoots and roots were substantially increased when tobacco was co-planted with Japanese clover. Co-planting also decreased soil pH and increased Cd bioavailability

of soil. Co-planting increased BCF of Cd by tobacco grown in mildly Cd-contaminated soil. Japanese clover also accumulated substantial Cd in its shoots and roots. Thus, total Cd uptake by the plants was much greater under co-planting than under monoculture. The results suggest that the efficiency of phytoextraction could be optimized by co-planting tobacco and Japanese clover grown in mildly Cd-contaminated soil.

Acknowledgments

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References

Arthur E L, Rice P J, Anderson T A, Baladi S M, Henderson K L D, Coats J R, 2005. Phytoremediation – an overview. *Critical Reviews in Plant Sciences*, 24(2): 109–122.

Barazani O, Sathiyamoorthy P, Manandhar U, Vulkan R, Golan-Goldhirsh A, 2004. Heavy metal accumulation by *Nicotiana glauca* Graham in a solid waste disposal site. *Chemosphere*, 54(7): 867–872.

Chaney R L, Malik M, Li Y M, Brown S L, Brewer E P, Angle J S et al., 1997. Phytoremediation of soil metals. *Current Opinion in Biotechnology*, 8(3): 279–284.

Chen H, Cutright T, 2001. EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. *Chemosphere*, 45(1): 21–28.

Chen X, Tang J J, Zhi G Y, Hu S J, 2005. Arbuscular mycorrhizal colonization and phosphorus acquisition of plants: effects of coexisting plant species. *Applied Soil Ecology*, 28(3): 259–269.

Chen Y X, Lin Q, Luo Y M, He Y F, Zhen S J, Yu Y L et al., 2003. The role of citric acid on the phytoremediation of heavy metal contaminated soil. *Chemosphere*, 50(6): 807–811.

Cheng S P, 2003. Heavy metal pollution in China: origin, pattern and control. *Environmental Science and Pollution Research*, 10(3): 192–198.

Chiang P N, Wang M K, Chiu C Y, Chou S Y, 2006. Effects of cadmium amendments on low-molecular-weight organic acid exudates in rhizosphere soils of tobacco and sunflower. *Environmental Toxicology*, 21(5): 479–488.

Duarte B, Delgado M, Cacador I, 2007. The role of citric acid in cadmium and nickel uptake and translocation, in *Halimione portulacoides*. *Chemosphere*, 69(5): 836–840.

Ernst W H O, 1996. Bioavailability of heavy metals and decontamination of soils by plants. *Applied Geochemistry*, 11(1-2): 163–167.

Evangelou M W H, Ebel M, Schaeffer A, 2006. Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco *Nicotiana tabacum*. *Chemosphere*, 63(6): 996–1004.

Evangelou M W H, Bauer U, Ebel M, Schaeffer A, 2007. The influence of EDDS and EDTA on the uptake of heavy metals of Cd and Cu from soil with tobacco *Nicotiana tabacum*. *Chemosphere*, 68(2): 345–353.

Everhart J L, McNear D, Peltier E, van der Lelie D, Chaney R L, Sparks D L, 2006. Assessing nickel bioavailability in smelter-contaminated soils. *Science of the Total Environment*, 367(3): 732–744.

Ghosh M, Singh S P, 2005. A comparative study of cadmium phytoextraction by accumulator and weed species. *Environmental Pollution*, 133(2): 365–371.

Ghosh P K, Bandyopadhyay K K, Wanjari R H, Manna M C, Misra A K, Mohanty M et al., 2007. Legume effect for enhancing productivity and nutrient use-efficiency in major cropping systems – an Indian perspective: a review. *Journal of Sustainable Agriculture*, 30(1): 59–86.

Giordani C, Cecchi S, Zanchi C, 2005. Phytoremediation of soil polluted by nickel using agricultural crops. *Environmental Management*, 36(5): 675–681.

Giovannetti M, Mosse B, 1980. Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, 84(3): 489–500.

Hartnett D, Wilson G, 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, 80(4): 1187–1195.

Janouskova M, Pavlikova D, Macek T, Vosatka M, 2005. Influence of arbuscular mycorrhiza on the growth and cadmium uptake of tobacco with inserted metallothionein gene. *Applied Soil Ecology*, 29(3): 209–214.

Jastrow J, Miller R, 1993. Neighbor influences on root morphology and mycorrhizal fungus colonization in tallgrass prairie plants. *Ecology*, 74(2): 561–569.

Jensen E S, Hauggaard-Nielsen H, 2003. How can increased use of biological N_2 fixation in agriculture benefit the environment? *Plant and Soil*, 252(1): 177–186.

Jin T Y, Wu X W, Tang Y Q, Nordberg M, Bernard A, Ye T T et al., 2004. Environmental epidemiological study and estimation of benchmark dose for renal dysfunction in a cadmium-polluted area in China. *Biometals*, 17(5): 525–530.

Karpenstein-Machan M, Stuelpnagel R, 2000. Biomass yield and nitrogen fixation of legumes monocropped and intercropped with rye and rotation effects on a subsequent maize crop. *Plant and Soil*, 218(1-2): 215–232.

Khan A, Kuek C, Chaudhry T, Khoo C, Hayes W, 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, 41(1-2): 197–207.

Khan A G, 2005. Rote of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology*, 18(4): 355–364.

Khan M S, Zaidi A, Wani P A, Oves M, 2009. Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental Chemistry Letters*, 7(1): 1–19.

Kirkham M B, 2006. Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. *Geoderma*, 137(1-2): 19–32.

Leyval C, Turnau K, Haselwandter K, 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza*, 7(3): 139–153.

Li N Y, Li Z A, Zhuang P, Zou B, McBride M, 2009. Cadmium uptake from soil by maize with intercrops. *Water Air and Soil Pollution*, 199(1-4): 45–56.

Liao M, Xie X M, 2004. Cadmium release in contaminated soils due to organic acids. *Pedosphere*, 14(2): 223–228.

Lu R K, 1999. Analytical Methods of Agricultural Chemistry in Soil. China Agricultural Science and Technology Press,

Beijing, China.

Marques A, Rangel A, Castro P M L, 2009. Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. *Critical Reviews in Environmental Science and Technology*, 39(8): 622–654.

McGrath S P, Zhao F J, 2003. Phytoextraction of metals and metalloids from contaminated soils. *Current Opinion in Biotechnology*, 14(3): 277–282.

McGrath S P, Zhao F J, Lombi E, 2001. Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. *Plant and Soil*, 232(1-2): 207–214.

Meers E, Ruttens A, Hopgood M J, Samson D, Tack F M G, 2005. Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. *Chemosphere*, 58(8): 1011–1022.

Mummey D, Rillig M, Holben W, 2005. Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. *Plant and Soil*, 271(1): 83–90.

Murakami M, Ae N, Ishikawa S, 2007. Phytoextraction of cadmium by rice (*Oryza sativa* L.), soybean (*Glycine max* (L.) Merr.), and maize (*Zea mays* L.). *Environmental Pollution*, 145(1): 96–103.

Nehnevajova E, Herzig R, Federer G, Erismann K, Schwitzguébel J, 2005. Screening of sunflower cultivars for metal phytoextraction in a contaminated field prior to mutagenesis. *International Journal of Phytoremediation*, 7(4): 337–349.

Noble A D, Suzuki S, Soda W, Ruaysoongnern S, Berthelsen S, 2008. Soil acidification and carbon storage in fertilized pastures of Northeast Thailand. *Geoderma*, 144(1-2): 248–255.

Olowe V I O, Adeyemo A Y, 2009. Enhanced crop productivity and compatibility through intercropping of sesame and sunflower varieties. *Annals of Applied Biology*, 155(2): 285–291.

Pilon-Smits E, 2005. Phytoremediation. *Annual Review of Plant Biology*, 56: 15–39.

Prasad M N V, Freitas H M D, 2003. Metal hyperaccumulation in plants-biodiversity prospecting for phytoremediation technology. *Electronic Journal of Biotechnology*, 6(3): 285–321.

Raskin I, Smith R D, Salt D E, 1997. Phytoremediation of metals: using plants to remove pollutants from the environment. *Current Opinion in Biotechnology*, 8(2): 221–226.

Redon P O, Beguiristain T, Leyval C, 2008. Influence of *Glomus intraradices* on Cd partitioning in a pot experiment with *Medicago truncatula* in four contaminated soils. *Soil Biology & Biochemistry*, 40(10): 2710–2712.

Smith S E, Read D J, 2008. Mycorrhizal Symbiosis (3rd ed.). Academic Press, San Diego.

Soares C, Siqueira J O, 2008. Mycorrhiza and phosphate protection of tropical grass species against heavy metal toxicity in multi-contaminated soil. *Biology and Fertility of Soils*, 44(6): 833–841.

Temperton V M, Mwangi P N, Scherer-Lorenzen M, Schmid B, Buchmann N, 2007. Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. *Oecologia*, 151(2): 190–205.

Tessier A, Campbell P G C, Bisson M, 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry*, 51(7): 844–851.

van Kessel C, Hartley C, 2000. Agricultural management of grain legumes: has it led to an increase in nitrogen fixation? *Field Crops Research*, 65(2-3): 165–181.

Wu C H, Chen X, Tang J J, 2005. Lead accumulation in weed communities with various species. *Communications in Soil Science and Plant Analysis*, 36(13-14): 1891–1902.

Yang R Y, Tang J J, Chen X, Hu S J, 2007. Effects of coexisting plant species on soil microbes and soil enzymes in metal lead contaminated soils. *Applied Soil Ecology*, 37(3): 240–246.