Quality Assurance and Safety of Crops & Foods, 2023; 15(2): 59–70

Role of arbuscular mycorrhizal fungi in cadmium tolerance in rice (*Oryza sativa* L): a meta-analysis

Ximei Li[1], Ruiyong Jing[1]*, Liyan Wang[1], Nan Wu[1], Zhenhua Guo[2]*

[1]College of Life Science and Technology, Heilongjiang Bayi Agricultural University, Daqing, P.R. China; [2]Heilongjiang Academy of Agricultural Sciences, Animal Husbandry Research Institute, Harbin, P.R. China

***Corresponding authors**: Ruiyong Jing, College of Life Science and Technology, Heilongjiang Bayi Agricultural University, Daqing 163319, P.R. China. Email: jingruiyong@byau.edu.cn; and Zhenhua Guo, Heilongjiang Academy of Agricultural Sciences, Animal Husbandry Research Institute. No. 368, Xuefu Road, Harbin 150086, P.R. China. Email: gzhh00@163.com

Received: 23 August 2022; Accepted: 17 February 2023; Published: 1 April 2023

RESEARCH ARTICLE

Abstract

Rice is an important agricultural product consumed globally. Rice polluted by cadmium (Cd) poses serious health risks. Numerous studies have shown that arbuscular mycorrhizal fungi (AMF) decrease Cd concentrations in the grain, shoots, and roots of rice. However, one study showed that AMF increased the root Cd concentration in rice. Therefore, a meta-analysis of the contribution of AMF to rice Cd tolerance became necessary. This meta-analysis was conducted to analyze the role of AMF in Cd tolerance in rice by searching the following databases: ProQuest, PubMed, Scopus, and ScienceDirect. A total of 571 studies were found, of which nine studies and 25 datasets were used in the meta-analysis. The period of inclusion of research reports was from January 1992 to April 2022. The results showed that with the addition of *Rhizophagus irregularis*, Cd concentration in the roots was higher than in the control group, although the overall Cd concentration in the plant was reduced. Four species of AMF reduced Cd concentration in rice shoots and grain tissues. These AMF species increased the biomass of rice root and shoot tissues; however, they did not affect grain biomass. AMF decreased the transfer factor (TF), and the TF of *Glomus versiforme* (12.99%) was significantly lower than the other three AMF types. We proposed that Cd could be enriched in rice roots, and the transfer of Cd to the grain could be inhibited. At the time of grain harvesting, rice roots are removed from the soil, thus removing Cd from the soil. This operation can efficiently improve both land-bearing capacity and soil without affecting rice yield. Thus, Cd was enriched in rice roots, and the potential for Cd transfer to the grain was inhibited due to the decreased TF. The future research must focus on how *R. irregularis* could improve the *HMA3* gene expression in rice root, and prevents the transportation of Cd from the roots to shoots.

Keywords: absorption; bacteria; rice; pollution; soil

Highlight

We propose that Cadmium (Cd) can be enriched in rice roots, and the transfer of Cd to grain can be inhibited. When treated with *Rhizophagus irregularis*, the Cd concentration increased in rice roots; however, the transfer of Cd to the grain could be inhibited.

Introduction

Rice is an important agricultural product consumed by people globally (Eslami *et al.*, 2015; He *et al.*, 2021; Ma *et al.*, 2021; Sarmast *et al.*, 2021). Cadmium (Cd) pollution is a serious problem that threatens human health and the ecosystem. In the early 20th century, a notorious incident occurred in Toyama, Japan, where Cd accumulation in rice resulted in mass *itai-itai* disease (Xiao *et al.*, 2020). Our previous report showed that animal manure alters the ecosystem of rice plants (Guo *et al.*, 2022). In fact, at all cultivation stages, from fertilization with animal manure to the cultivation of rice plants, Cd and other heavy metal deposits have been found in all types of crops in farmlands (Majeed *et al.*, 2021). One way to reduce heavy metal-contamination from the soil is to extract heavy metals from the soil using hyperaccumulator plants. In hyperaccumulator plants, shoot Cd reaches 100 mg/kg, and the transfer factor (TF) value is greater than 1 (Jaffre *et al.*, 2013). In China, if the Cd concentration of a farmland is more than 1.0 mg/kg (Luo *et al.*, 2017), the cultivation of crops is forbidden, and the farmland can only be used after improving soil conditions. Scientists have been searching for means to grow food crops that meet certain benchmarks on lands where Cd concentration exceeds these standards.

Arbuscular mycorrhizal fungi (AMF) are a research hotspot in the field of phytoremediation. AMF can regulate *Nramp5* and *HMA3* gene expressions in rice roots, which are responsible for Cd transport from external soil into root cells (Chen *et al.*, 2019). Research has shown that AMF can increase the growth of hyperaccumulator plants, while a small number of reports have shown that AMF inhibit the growth of hyperaccumulator plants or have no significant effect (Cantamessa *et al.*, 2020; Orlowska *et al.*, 2011). Rice plants are not hyperaccumulators. Most studies have shown that AMF reduce Cd concentration in rice tissues and improve biomass. However, some studies have shown that AMF increase Cd concentration in rice roots (Huang *et al.*, 2018), whereas other studies have shown that there is no significant difference in biomass after AMF are used (Chen *et al.*, 2019; Li *et al.*, 2020). AMF have been found to reduce shoot biomass (Yang *et al.*, 2021). Therefore, a meta-analysis of the role of AMF in Cd tolerance in rice became necessary. The aim of the meta-analysis in this work was to explore the contribution of AMF to Cd tolerance of rice. This study would provide theoretical guidance for utilizing different types of AMF in rice subjected to Cd stress.

Methods

Data base search strategy

A systematic search was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Electronic searches of ProQuest, PubMed, Scopus, Web of Sciences, and ScienceDirect databases were conducted. The key terms searched were as follows: (arbuscular mycorrhizal fungi) OR (arbuscular mycorrhizal symbiosis) OR (AM fungi) OR AMF AND (rice OR *Oryza sativa* L) AND (Cadmium OR Cd). The period of inclusion of research reports was from January 1992 to April 2022. Table 1 lists the inclusion and exclusion criteria used for search in the literature. Randomized controlled trials were not strictly required to be mentioned in the research report, because it was assumed that the planting trials described in the report were randomized trials. Two authors independently completed the literature retrieval, inspection, and inclusion. If the results of the literature inclusion were uncertain, the third author made the final decision.

Data extraction

Most of the experimental data were expressed in the form of figures, and specific values could not be found in the paper. Therefore, in the meta-analysis, GetData graph digitizer v2.5 was used to extract specific research data (Zhou *et al.*, 2021). The experimental groups included in the literature were often set up according to different Cd concentrations or for comparisons of the effects of different AMF. Therefore, each dataset group was extracted from the included literature (Maillard and Angers, 2014). All studies included in this meta-analysis contained data expressed as the standard error (SE) or standard deviation (SD). SE was converted to SD before the meta-analysis was conducted.

Data analysis

Each dataset was analyzed as an independent study. Four AMF species were identified: *Rhizophagus irregularis, Glomus versiforme*, *Funneliformis mosseae*, and *Rhizophagus intraradices*. These four AMF species were identified from a subgroup analysis, and the effect of AMF on Cd concentration in rice roots and shoots was analyzed. As there were limited grain data, subgroup analysis was not performed. Then, the effects of AMF on the biomass of rice roots, shoots, and grain under

Table 1. Inclusion and exclusion criteria for meta-analysis.

Inclusion	Exclusion
Plant included, but not limited to rice	Rice was not used
English literature	Non-English
AMF treatment alone or with other treatments of rice	No AMF treatment of rice
Cd included	No Cd data

AMF: arbuscular mycorrhizal fungi; Cd: cadmium.
The retrieved papers were selected one by one according to the entries in the table.

Cd stress were analyzed. According to the continuous model and standard mean difference (SMD), meta-analyses were performed using Stata 12.0 (Stata Corp, College Station, TX, USA). Assessing the heterogeneity I^2, if it was greater than 50%, a randomized model was adopted. If the heterogeneity was less than 50%, a fixed model was used. The potential publication bias was also evaluated.

The following equation was used to calculate the average TF:

$$\overline{\text{TF}} = \sum_{i=1}^{n} \frac{C_{\text{shoot}}}{C_{\text{root}}} \times \frac{1}{n} \times 100\%$$

In the above equation, C_{root} and C_{shoot} denote Cd content in rice roots and rice shoots, respectively (Lei *et al.*, 2021). The TF was analyzed with statistical analysis system (SAS) using one-way analysis of variance (ANOVA). Fisher's protected least-significant difference (LSD) test was used to determine significant differences. $P < 0.05$ was considered as statistically significant (Guo *et al.*, 2018).

Results

A total of 571 studies were found, of which nine were used for meta-analysis (Chen *et al.*, 2019; Gao *et al.*, 2021; Huang *et al.*, 2018; Lei *et al.*, 2021; Li *et al.*, 2016, 2020; Luo *et al.*, 2017; Yang *et al.*, 2021; Zhu *et al.*, 2022). The nine studies and 25 datasets are shown in Table 2 and Figure 1.

Most studies showed that treatment with AMF reduced Cd concentration, compared to the control group (Li *et al.*, 2020; Yang *et al.*, 2021). However, when plants were treated with *R. irregularis*, the plant Cd concentration in roots was higher than that in the control group (SMD = 3.17, 95% confidence interval [CI] = 0.04–6.31, $P = 0.05$; Figure 2A). AMF *Funneliformis mosseae* did not affect Cd concentration in roots (SMD = −0.95, 95% CI = −2.24–0.34, $P = 0.15$; Figure 2A). AMF *Glomus versiforme* and *Rhizophagus intraradices* decreased Cd concentration in roots. Overall, 23 datasets showed that AMF reduced Cd concentration in rice roots (SMD = −1.25, 95% CI = −2.24 to −0.26, $P = 0.01$; Figure 2A). Four species of AMF reduced Cd concentration in rice shoots and grain tissues (Figures 2A and 2B). Figure 2C shows that all articles are distributed on both sides of the midline and are concentrated together, indicating a lack of publication bias. AMF increased the biomass of rice root and shoot tissues (Figure 3A). However, it did not affect the grain biomass (Figure 3B).

AMF decreased TF, indicating that Cd transfer from the roots to shoots was inhibited. The results of the meta-analysis showed that the average TFs of *R. irregularis*, *F. mosseae*, *R. intraradices*, and *G. versiforme* were 23.63%, 24.77%, 15.01%, and 12.99%, respectively (Figure 4). TF of *G. versiforme* was significantly lower than that in other three types of AMF.

Table 2. Characteristics of the studies included in the analysis.

	Study ID	Dataset No.	AMF	Stage	Seed	Soil Cd	Soil pH	Duration
1.	Chen *et al.*, 2019	2	*Funneliformis mosseae* *Rhizophagus intraradices*	Not mentioned	Upland rice (Hanyou 3)	33 mg/kg	5.8	50 days
2.	Gao *et al.*, 2021	1	*R. intraradices*	Maturity	Upland rice (Hanyou 3)	0.05 mM in water	Not mentioned	105 days
3.	Huang *et al.*, 2018	8	*F. mosseae* *Rhizophagus irregularis*	Not mentioned	Rice (Beidao 4)	0.5, 1, 2, and 5 mg/kg	6.3	90 days
4.	Lei *et al.*, 2021	1	*Glomus versiforme*	Maturity	Upland rice (Hanyou 3)	5 mg/kg	6.23	115 days
5.	Li *et al.*, 2016	4	*F. mosseae* *R. intraradices*	Not mentioned	Rice (Zhenghan 9)	0.05 mM and 0.1 mM in water	5.9	60 days
6.	Li *et al.*, 2020	4	*F. mosseae* *R. intraradices*	Flowering	Upland rice (Hanyou 3)	2 and 10 mg/kg	5.9	105 days
7.	Luo *et al.*, 2017	2	*R. intraradices*	Ripening	Upland rice (Hanyou 3)	2 and 10 mg/kg	5.8	145 days
8.	Yang *et al.*, 2021	2	*F. mosseae*	Heading Maturity	Upland rice (Hanyou 73)	1.12 mg/kg	7.02	56 days 112 days
9.	Zhu *et al.*, 2022	1	*G. versiforme*	Jointing	Upland rice (Hanyou 3)	10 mg/kg	6.23	70 days

The experimental factors mentioned in each paper that may affect cadmium (Cd) concentrations extracted, and these extracted data were used as the grouping basis for subgroup analysis.

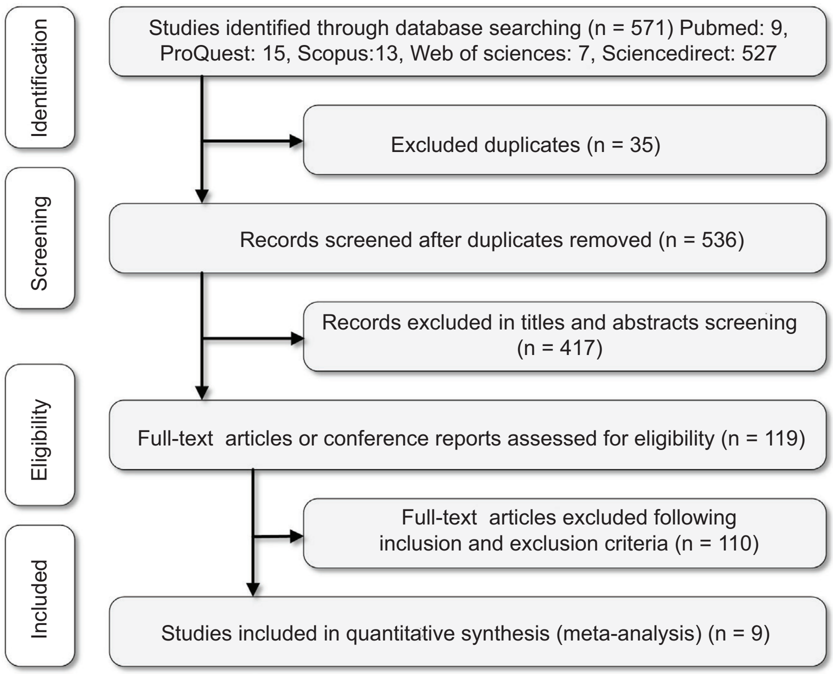

Figure 1. Summary of the study selection procedure.

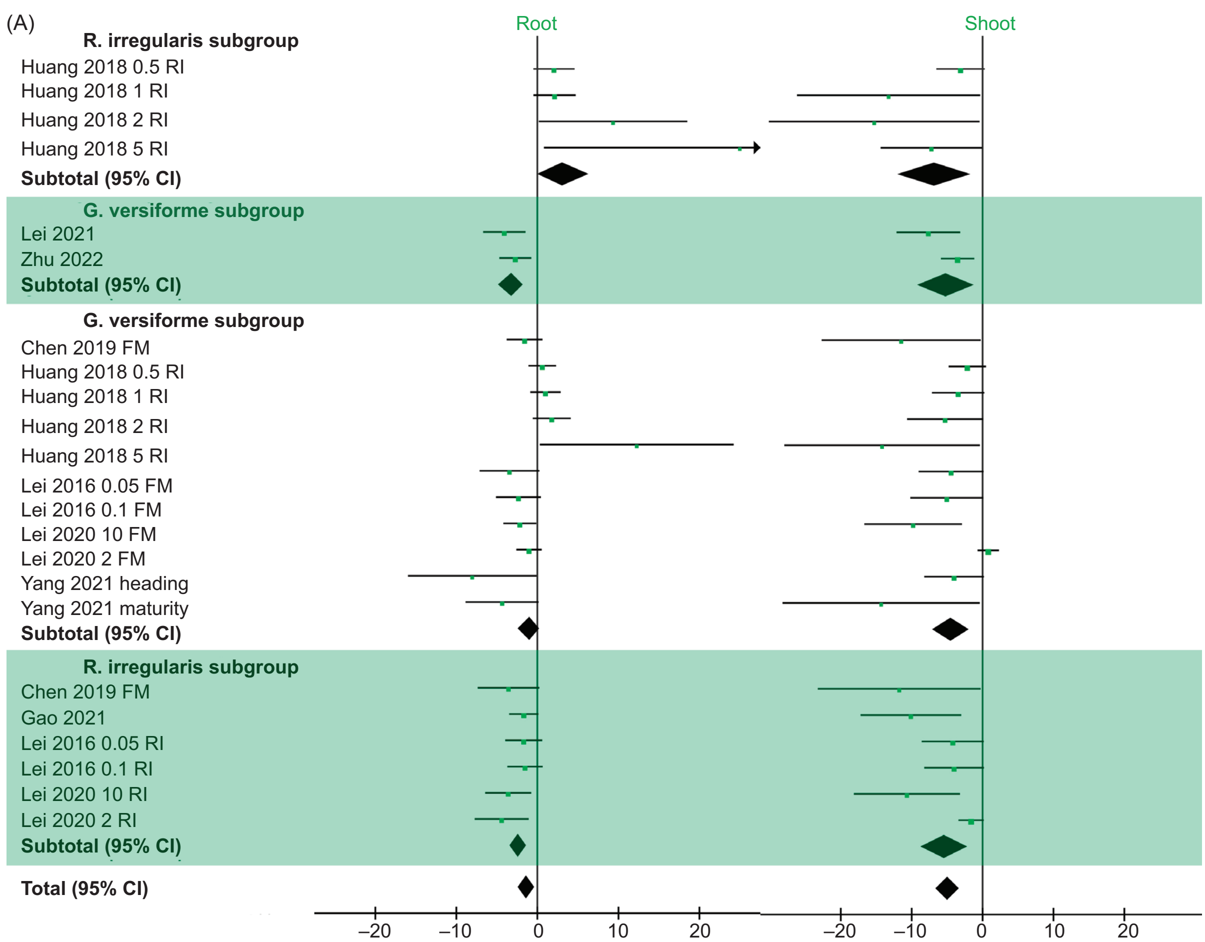

Figure 2. Effects of arbuscular mycorrhizal fungi (AMF) treatment on rice cadmium (Cd) concentrations. (A) Forest plot of the roots and shoots.

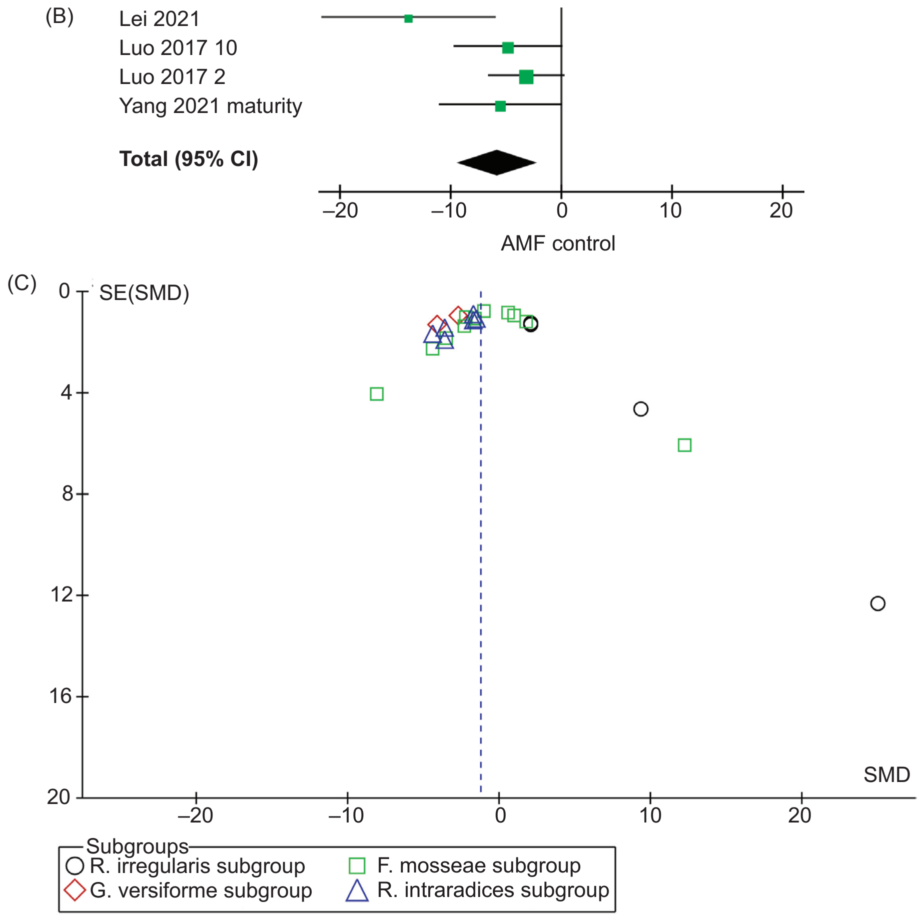

Figure 2. Effects of arbuscular mycorrhizal fungi (AMF) treatment on rice cadmium (Cd) concentrations. (B) grain, CI = 95%. (C) Funnel plot of root Cd concentration.

Discussion

Rice is an irreplaceable component of the global food supply (Eslami *et al.*, 2015; He *et al.*, 2021; Ma *et al.*, 2021; Sarmast *et al.*, 2021). Cd getting into the food chain through rice poses serious health risks. In particular, Cd in the soil is easily absorbed by rice plants (Kumar *et al.*, 2019). The content of heavy metals in paddy soil is higher than that in dry land soil (Huang *et al.*, 2019). According to the Codex Alimentarius Commission (CAC) of Food and Agriculture Organization (FAO)/World Health Organization (WHO) (CXS 193-1995), the rice grain Cd concentration must be lower than 0.4 mg/kg (Yang *et al.*, 2021). According to farmland standards for Cd concentration in soils (GB 15618-1995) in China, Cd concentration must be lower than 1.0 mg/kg (Luo *et al.*, 2017). Reducing the concentration of Cd in rice has always been a research hotspot (Yan *et al.*, 2019). Two approaches are currently used to address this problem. First, new rice varieties are being created through gene editing. Second, auxiliary agents are added to the cultivated land to reduce the absorption of Cd by rice plants. Both methods can reduce Cd content in rice. However, these methods have disadvantages that can result in reduced Cd absorption but increased arsenic (As) absorption (Li *et al.*, 2022).

Arbuscular mycorrhizal fungi may reduce Cd uptake in rice, kenaf (*Hibiscus cannabinus*), and maize to produce safer grain varieties (Pan *et al.*, 2022; Yu *et al.*, 2022). One study showed that in the soil with 1.12 mg/kg Cd, adding AMF reduced the rice grain Cd concentration to 0.38 mg/kg (Yang *et al.*, 2021). Another study showed that in the soil with 2 mg/kg Cd, AMF helped to reduce the rice grain Cd concentration by more than 0.4 mg/kg (Luo *et al.*, 2017). This suggested that rice growth could be promoted by the application of AMF only in land with slight Cd pollution. Heavily Cd-polluted land must be improved before it is used safely.

Comparative screening of the AMF currently in use showed that *R. irregularis* could enrich Cd in rice roots compared to the control group. Moreover, the application of *R. irregularis* prevented the transfer of Cd from the roots to shoots, reduced Cd concentration in the grain, and did not affect its yield. This suggested that rice could be cultivated on the land that was slightly polluted

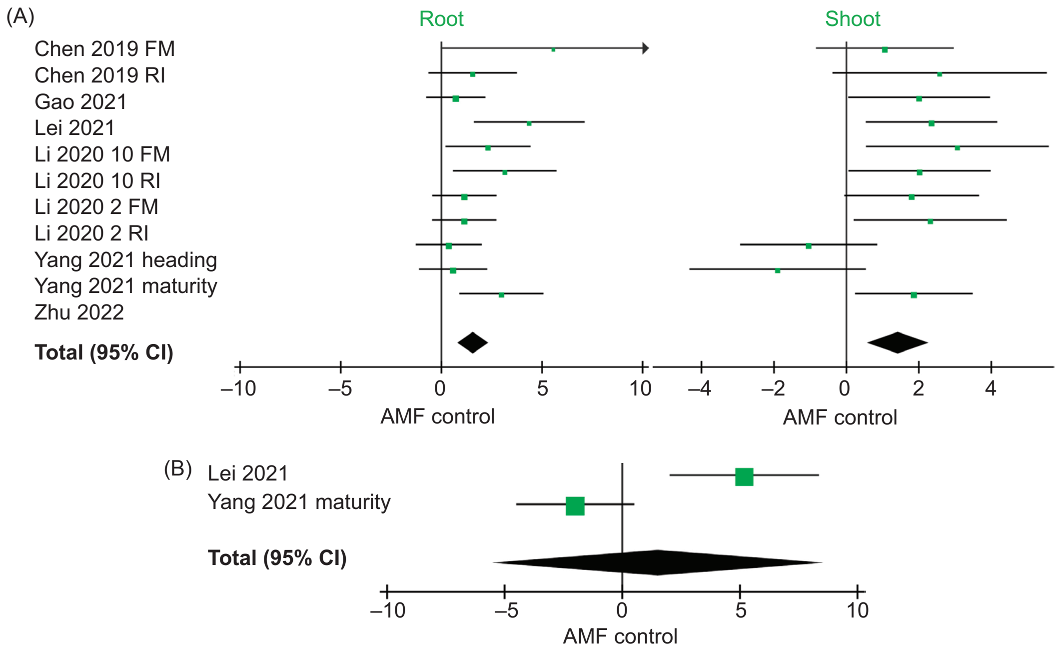

Figure 3. Forest plot of arbuscular mycorrhizal fungi (AMF) treatment effects on rice biomass. (A) Roots and shoots; (B) grain, CI = 95%.

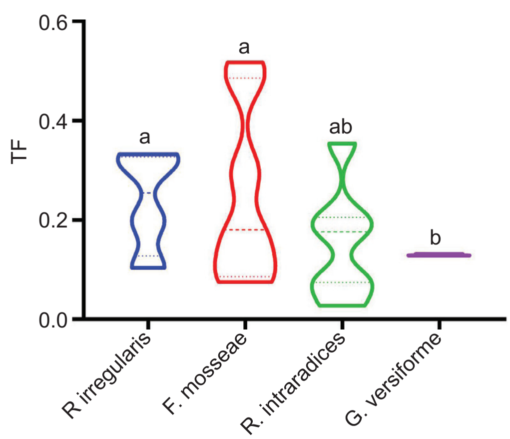

Figure 4. Effects of four arbuscular mycorrhizal fungi (AMF) treatments on the rice transfer factor (TF).

with Cd. After harvest, the roots and shoots could be removed to reduce Cd content in the soil, thereby improving the soil without affecting the rice yield.

Cadmium in the soil is first absorbed by the rice root system and transferred to the shoots, ultimately accumulating in the grain. *IRT1/IRT2*, *ZIP4*, *LCT1*, *YSL*, *Cd1*, *Nramp1*, and *Nramp5* in root apoptosis are transporters that play a crucial role in Cd accumulation in rice (Tang *et al.*, 2017, 2022; Yan *et al.*, 2019; Yang *et al.*, 2021. They can transfer Cd from the soil to rice root tissues. P-type *ATPase HMA2* in root tissues transport Cd to the xylem (Yamaji *et al.*, 2013). *CAL1* transports Cd in the xylem to the shoots (Luo *et al.*, 2018). In shoot tissues, *LCT1* distributes Cd to various organs (Uraguchi *et al.*, 2014). *HMA3* in the roots chelates Cd and deposits it in root tissues, preventing its transport to the shoots (Wang *et al.*, 2021). This is similar to the function of AMF in *R. irregularis*. Currently, no study has directly shown that *R. irregularis* enhances *HMA3* expression in rice roots. However, studies by other groups have shown that *F. mosseae* and *R. intraradices* can regulate *Nramp5*, *HMA3*, and *HMA2* gene expressions (Chen *et al.*, 2019; Yang *et al.*, 2021). Interestingly, under Cd stress, *F. mosseae* increased the expression of *HMA3* whereas *R. intraradices* inhibited it (Chen *et al.*, 2019) (Figure 5).

Arbuscular mycorrhizal fungi have existed for more than 400 million years, coinciding with the settlement of plants on land (Martin *et al.*, 2017). The symbiosis between AMF and plants establishes a pathway for material exchange. The area of AMF mycelia is much larger than that of rice roots, which can help plants absorb mineral nutrients that cannot be obtained from the roots. AMF symbiosis improves plant resistance to pathogens, poor conditions, drought, and pollution (Li *et al.*, 2022). There are many types of AMF, and those with a completed genome sequence include *Rhizophagus irregularis* (Yildirir *et al.*, 2022), *Rhizophagus clarus* (Kobayashi *et al.*, 2018), *Diversispora epigaea* (Sun *et al.*, 2019), *Rhizophagus cerebriforme*, *Rhizophagus diaphanus*, and *Gigaspora rosea* (Morin *et al.*, 2019), *Geosiphon pyriformis* (Malar *et al.*, 2021), and *Gigaspora margarita* (Venice *et al.*, 2020). Currently, only *R. irregularis*, *F. mosseae*, *R. intraradices*, and *G. versiforme* have been

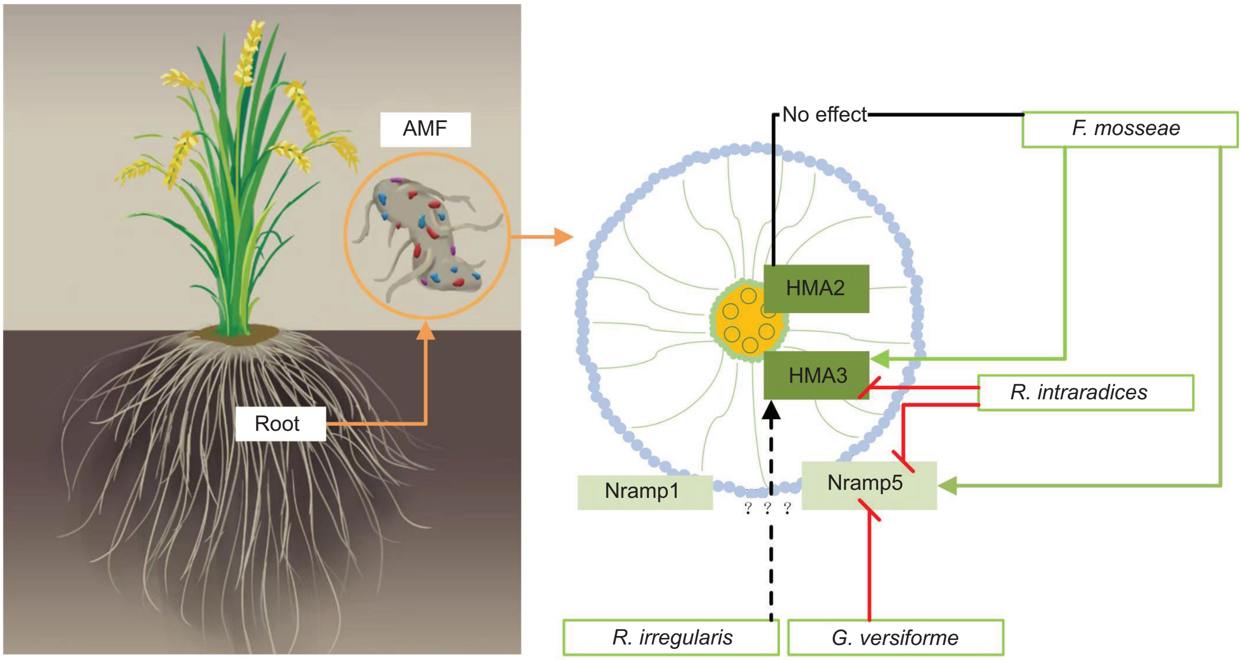

Figure 5. Role of arbuscular mycorrhizal fungi (AMF) in absorbing nutrients from the soil.

applied in research on Cd tolerance in rice. In addition, similar reports have described the use of *Glomus mosseae* in improving the tolerance of antimony (Sb) pollution in rice (Zhou *et al.*, 2022). *Glomus etunicatum*, *Glomus geosporum*, and *Glomus Mosseae* have been used to increase salt stress tolerance in rice (Tisarum *et al.*, 2020). However, *F. mosseae* did not decrease Cd concentration in the roots (Chen *et al.*, 2019; Yang *et al.*, 2021).

Whether AMF can promote the uptake of elements that are beneficial to the human body is still unknown. However, some studies have discovered that *F. mosseae* and *G. versiforme* can enrich selenium (Se) in rice (Chen *et al.*, 2020), suggesting that AMF not only inhibit the uptake of Cd and manganese (Mn) in rice but also increase the amount of other metals transported by uninhibited transporters. Different types of AMF have different effects (Chen *et al.*, 2019). The meta-analysis results show that the TF of *G. versiforme* is also low, thus reduces Cd concentration in rice grains.

Significant differences in the Cd accumulation capacity are discovered among different rice varieties (Yan *et al.*, 2019). The collected rice variety data are shown in Table 2. Some studies have determined that the activity of fungi is not affected under flooding conditions (Vallino *et al.*, 2014). Different conditions affect the absorption of Cd by rice. The Cd concentration in rice can also be reduced by switching between drained and undrained conditions (Wang *et al.*, 2020). The lower the soil pH value, the higher the Cd mobility (Zhu *et al.*, 2016). AMF increase the pH value of the soil. By adjusting the pH, AMF reduce the content of inorganic Cd in the soil and increase the amount of residual Cd (Li *et al.*, 2022). In addition, AMF mycelia adsorb large amounts of heavy metals and reduce their entry into plants (Zhang *et al.*, 2009).

Limitations

Certain limitations were confronted in the research on rice and AMF. For example, agricultural cultivation encompasses a large and complex system that includes tillage, fertilization, crops, the plant rhizosphere microbial environment, and the addition of AMF. Chemical or organic fertilizers are used concurrently during plantation of crops. Our previous research demonstrated that the application of animal manure to the soil changes the original microbial population, resulting in the formation of a new microbial ecology (Guo *et al.*, 2022). Similarly, research by other teams established that under the application of different fertilizers, the biomass of AMF increased along with the biomass of fungal protozoa and nematodes, thereby significantly altering the composition of AMF community (Jiang *et al.*, 2020).

The studies included in this work focused on the extent to which AMF contributed to rice yield and reduced Cd content. These factors did not account for how much AMF was benefited by the rice. In the symbiotic relationship between AMF and plants, AMF obtain fixed carbon resulting from plant photosynthesis, which is approximately 4–20% of the total carbon fixation in a whole plant (Zhang *et al.*, 2022). The roots of plants recruit AMF, and the mycelia of AMF recruit soil microorganisms. The results of a ^{13}C tracer study showed that plant roots provided a carbon source for AMF and that AMF provided a carbon source for soil microorganisms (Zhou *et al.*, 2020). AMF secrete proteins to supply soil microorganisms. *R. irregularis* secrete SP7, which affects the gene expression of host plants (Kloppholz *et al.*, 2011). In addition to its effects on host plants, AMF-secreted proteins can also manipulate the activities of other microorganisms (Snelders *et al.*, 2020).

Arbuscular mycorrhizal fungi treatment concentration, duration, and soil Cd concentration are shown in Table 2. This study focused on the summary effect of the included data and overlooked the fact that treatment concentrations may have varied from one study to another study. Only publicly published data were collected, while unpublished studies with negative results were not considered. Figure 2C displays a function plot demonstrating no publication bias in the root concentration data. Other sample data could have the probability of including publication bias and sketched data.

Although only discussing the relationship between AMF and rice has limitations, the present authors believed that the experimental design of the included literature followed the following three principles: (1) single-factor analysis; (2) repeated trials; and (3) randomized trials. Therefore, the authors believe that the results of this research are credible.

Conclusions

Achieving a balance between the reasonable cultivation of land and soil improvement has always been a challenge. Although *F. mosseae* promotes the expression of *Nramp5* and *HMA3* gene expressions, the results of the meta-analysis showed that *F. mosseae* did not improve the root Cd concentration. Based on the findings of the analysis, the future studies should focus on *R. irregularis*, as this AMF can improve *HMA3* expression in rice roots. Thus, Cd can be enriched in rice roots, and the transfer of Cd to the grain can be inhibited. Thus, this method can efficiently improve both land-bearing capacity and soil conditions without affecting the yield of rice.

Acknowledgements

The authors thank Accdon (www.accdon.com) for its linguistic assistance for preparing this manuscript.

Funding

This work was supported by the “Three Longitudinal” scientific research support program of Heilongjiang Bayi Agricultural University (ZRCPY202114) and the Daqing Guided Science and Technology Project (zd-2021-78).

Competing interests

The authors declare that they had no conflict of interest to report.

Availability of data

Please contact corresponding authors for data requests.

References

Cantamessa, S., Massa, N., Gamalero, E. and Berta, G., 2020. Phytoremediation of a highly arsenic polluted site, using Pteris vittata L. and arbuscular mycorrhizal fungi. Plants (Basel) 9(9): 1211. https://doi.org/10.3390/plants9091211

Chen, X.W., Wu, L., Luo, N., Mo, C.H., Wong, M.H. and Li, H., 2019. Arbuscular mycorrhizal fungi and the associated bacterial community influence the uptake of cadmium in rice. Geoderma 337: 749–757. https://doi.org/10.1016/j.geoderma.2018.10.029

Chen, X., Zhang, Z., Gu, M., Li, H., Shohag, M.J.I., Shen, F., et al. 2020. Combined use of arbuscular mycorrhizal fungus and selenium fertilizer shapes microbial community structure and enhances organic selenium accumulation in rice grain. Science of the Total Environment 748: 141166. https://doi.org/10.1016/j.scitotenv.2020.141166

Eslami, M., Mashak, Z., Heshmati, A., Shokrzadeh, M. and Mozaffari Nejad, A.S., 2015. Determination of aflatoxin B1 levels in Iranian rice by ELISA method. Toxin Reviews 34(3): 125–128. https://doi.org/10.3109/15569543.2015.1074925

Gao, M.Y., Chen, X.W., Huang, W.X., Wu, L., Yu, Z.S., Xiang, L., et al. 2021. Cell wall modification induced by an arbuscular mycorrhizal fungus enhanced cadmium fixation in rice root. Journal of Hazardous Materials 416: 125894. https://doi.org/10.1016/j.jhazmat.2021.125894

Guo, Z., Islam, M.S., Liu, D., Liu, G., Lv, L., Yang, Y., et al. 2018. Differential effects of follistatin on porcine oocyte competence and cumulus cell gene expression in vitro. Reproduction in Domestic Animals 53(1): 3–10. https://doi.org/10.1111/rda.13035

Guo, Z., Lv, L., Liu, D., He, X., Wang, W., Feng, Y., et al. 2022. A global meta-analysis of animal manure application and soil microbial ecology based on random control treatments. PLoS One 17(1): e0262139. https://doi.org/10.1371/journal.pone.0262139

He, G., Liu, X. and Cui, Z., 2021. Achieving global food security by focusing on nitrogen efficiency potentials and local production.

Global Food Security 29: 100536. https://doi.org/10.1016/j.gfs.2021.100536

Huang, X., An, G., Zhu, S., Wang, L. and Ma, F., 2018. Can Cd translocation in *Oryza sativa* L. be attenuated by arbuscular mycorrhizal fungi in the presence of EDTA? Environmental Science and Pollution Research (ESPR) 25(10): 9380–9390. https://doi.org/10.1007/s11356-017-1157-x

Huang, Y., Wang, L., Wang, W., Li, T., He, Z. and Yang, X., 2019. Current status of agricultural soil pollution by heavy metals in China: a meta-analysis. Science of the Total Environment 651(Pt 2): 3034–3042. https://doi.org/10.1016/j.scitotenv.2018.10.185

Jaffre, T., Pillon, Y., Thomine, S. and Merlot, S., 2013. The metal hyperaccumulators from New Caledonia can broaden our understanding of nickel accumulation in plants. Frontiers in Plant Science 4: 279. https://doi.org/10.3389/fpls.2013.00279

Jiang, Y., Luan, L., Hu, K., Liu, M., Chen, Z., Geisen, S., et al. 2020. Trophic interactions as determinants of the arbuscular mycorrhizal fungal community with cascading plant-promoting consequences. Microbiome 8(1): 142. https://doi.org/10.1186/s40168-020-00918-6

Kloppholz, S., Kuhn, H. and Requena, N., 2011. A secreted fungal effector of Glomus intraradices promotes symbiotic biotrophy. Current Biology 21(14): 1204–1209. https://doi.org/10.1016/j.cub.2011.06.044

Kobayashi, Y., Maeda, T., Yamaguchi, K., Kameoka, H., Tanaka, S., Ezawa, T., et al. 2018. The genome of Rhizophagus clarus HR1 reveals a common genetic basis for auxotrophy among arbuscular mycorrhizal fungi. BMC Genomics 19(1): 465. https://doi.org/10.1186/s12864-018-4853-0

Kumar, S., Prasad, S., Yadav, K.K., Shrivastava, M., Gupta, N., Nagar, S., et al. 2019. Hazardous heavy metals contamination of vegetables and food chain: role of sustainable remediation approaches—a review. Environmental Research 179(Pt A): 108792. https://doi.org/10.1016/j.envres.2019.108792

Lei, L.L., Zhu, Q.Y., Xu, P.X. and Jing, Y.X., 2021. The intercropping and arbuscular mycorrhizal fungus decrease Cd accumulation in upland rice and improve phytoremediation of Cd-contaminated soil by Sphagneticola calendulacea (L.) Pruski. Journal of Environmental Management 298: 113516. https://doi.org/10.1016/j.jenvman.2021.113516

Li, H., Chen, X.W., Wu, L., Luo, N., Huang, W.X., Mo, C.H., et al. 2020. Effects of arbuscular mycorrhizal fungi on redox homeostasis of rice under Cd stress. Plant and Soil 455(1): 121–138. https://doi.org/10.1007/s11104-020-04678-y

Li, H., Gao, M.Y., Mo, C.H., Wong, M.H., Chen, X.W. and Wang, J.J., 2022. Potential use of arbuscular mycorrhizal fungi for simultaneous mitigation of arsenic and cadmium accumulation in rice. Journal *of* Experimental Botany 73(1): 50–67. https://doi.org/10.1093/jxb/erab444

Li, H., Luo, N., Zhang, L.J., Zhao, H.M., Li, Y.W., Cai, Q.Y., et al. 2016. Do arbuscular mycorrhizal fungi affect cadmium uptake kinetics, subcellular distribution and chemical forms in rice? Science of the Total Environment 571: 1183–1190. https://doi.org/10.1016/j.scitotenv.2016.07.124

Luo, J.S., Huang, J., Zeng, D.L., Peng, J.S., Zhang, G.B., Ma, H.L., et al. 2018. A defensin-like protein drives cadmium efflux and allocation in rice. Nature Communications 9(1): 645. https://doi.org/10.1038/s41467-018-03088-0

Luo, N., Li, X., Chen, A.Y., Zhang, L.J., Zhao, H.M., Xiang, L., et al. 2017. Does arbuscular mycorrhizal fungus affect cadmium uptake and chemical forms in rice at different growth stages? Science of the Total Environment 599–600: 1564–1572. https://doi.org/10.1016/j.scitotenv.2017.05.047

Ma, S., Wang, Z., Guo, X., Wang, F., Huang, J., Sun, B., et al. 2021. Sourdough improves the quality of whole-wheat flour products: mechanisms and challenges—a review. Food Chemistry 360: 130038. https://doi.org/10.1016/j.foodchem.2021.130038

Maillard, E. and Angers, D.A., 2014. Animal manure application and soil organic carbon stocks: a meta-analysis. Global Change Biology 20(2): 666–679. https://doi.org/10.1111/gcb.12438

Majeed, A., Niaz, A., Rizwan, M., Imran, M., Alsahli, A.A., Alyemeni, M.N., et al. 2021. Effects of biochar, farm manure, and pressmud on mineral nutrients and cadmium availability to wheat (*Triticum aestivum* L.) in Cd-contaminated soil. Physiologia Plantarum 173(1): 191–200. https://doi.org/10.1111/ppl.13348

Malar, C.M., Kruger, M., Kruger, C., Wang, Y., Stajich, J.E., Keller, J., et al. 2021. The genome of *Geosiphon pyriformis* reveals ancestral traits linked to the emergence of the arbuscular mycorrhizal symbiosis. Current Biology 31(7): 1570–1577 e1574. https://doi.org/10.1016/j.cub.2021.01.058

Martin, F.M., Uroz, S. and Barker, D.G., 2017. Ancestral alliances: plant mutualistic symbioses with fungi and bacteria. Science 356(6340): eaad4501. https://doi.org/10.1126/science.aad4501

Morin, E., Miyauchi, S., San Clemente, H., Chen, E.C.H., Pelin, A., de la Providencia, I., et al. 2019. Comparative genomics of Rhizophagus irregularis, R. cerebriforme, R. diaphanus and Gigaspora rosea highlights specific genetic features in glomeromycotina. New Phytologist 222(3): 1584–1598. https://doi.org/10.1111/nph.15687

Orlowska, E., Przybylowicz, W., Orlowski, D., Turnau, K. and Mesjasz-Przybylowicz, J., 2011. The effect of mycorrhiza on the growth and elemental composition of Ni-hyperaccumulating plant Berkheya coddii Roessler. Environmental Pollution 159(12): 3730–3738. https://doi.org/10.1016/j.envpol.2011.07.008

Pan, J., Cao, S., Xu, G., Rehman, M., Li, X., Luo, D., et al. 2022. Comprehensive analysis reveals the underlying mechanism of arbuscular mycorrhizal fungi in kenaf cadmium stress alleviation. Chemosphere 314: 137566. https://doi.org/10.2139/ssrn.4267813

Sarmast, E., Fallah, A.A., Jafari, T. and Mousavi Khaneghah, A., 2021. Occurrence and fate of mycotoxins in cereals and cereal-based products: a narrative review of systematic reviews and meta-analyses studies. Current Opinion in Food Science 39: 68–75. https://doi.org/10.1016/j.cofs.2020.12.013

Snelders, N.C., Rovenich, H., Petti, G.C., Rocafort, M., van den Berg, G.C.M., Vorholt, J.A., et al. 2020. Microbiome manipulation by a soil-borne fungal plant pathogen using effector proteins. Nature Plants 6(11): 1365–1374. https://doi.org/10.1038/s41477-020-00799-5

Sun, X., Chen, W., Ivanov, S., MacLean, A.M., Wight, H., Ramaraj, T., et al. 2019. Genome and evolution of the arbuscular mycorrhizal fungus *Diversispora epigaea* (formerly *Glomus versiforme*) and its bacterial endosymbionts. New Phytologist 221(3): 1556–1573. https://doi.org/10.1111/nph.15472

Tang, L., Dong, J., Qu, M., Lv, Q., Zhang, L., Peng, C., et al. 2022. Knockout of *OsNRAMP5* enhances rice tolerance to cadmium toxicity in response to varying external cadmium concentrations via distinct mechanisms. Science of the Total Environment 832: 155006. https://doi.org/10.1016/j.scitotenv.2022.155006

Tang, L., Mao, B., Li, Y., Lv, Q., Zhang, L., Chen, C., et al. 2017. Knockout of *OsNramp5* using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. Scientific Reports, 7(1): 14438. https://doi.org/10.1038/s41598-017-14832-9

Tisarum, R., Theerawitaya, C., Samphumphuang, T., Polispitak, K., Thongpoem, P., Singh, H.P., et al. 2020. Alleviation of salt stress in upland rice (*Oryza sativa* L. ssp. indica cv. leum pua) using arbuscular mycorrhizal fungi inoculation. Frontiers in Plant Science 11: 348. https://doi.org/10.3389/fpls.2020.00348

Uraguchi, S., Kamiya, T., Clemens, S. and Fujiwara, T., 2014. Characterization of OsLCT1, a cadmium transporter from indica rice (*Oryza sativa*). Physiologia Plantarum 151(3): 339–347. https://doi.org/10.1111/ppl.12189

Vallino, M., Fiorilli, V. and Bonfante, P., 2014. Rice flooding negatively impacts root branching and arbuscular mycorrhizal colonization, but not fungal viability. Plant, Cell & Environment 37(3): 557–572. https://doi.org/10.1111/pce.12177

Venice, F., Ghignone, S., Salvioli di Fossalunga, A., Amselem, J., Novero, M., Xianan, X., et al. 2020. At the nexus of three kingdoms: the genome of the mycorrhizal fungus Gigaspora margarita provides insights into plant, endobacterial and fungal interactions. Environmental Microbiology 22(1): 122–141. https://doi.org/10.1111/1462-2920.14827

Wang, H.Q., Xuan, W., Huang, X.Y., Mao, C. and Zhao, F.J., 2021. Cadmium inhibits lateral root emergence in rice by disrupting OsPIN-mediated auxin distribution and the protective effect of Os*HMA3*. Plant & Cell Physiology 62(1): 166–177. https://doi.org/10.1093/pcp/pcaa150

Wang, P., Yamaji, N., Inoue, K., Mochida, K. and Ma, J.F., 2020. Plastic transport systems of rice for mineral elements in response to diverse soil environmental changes. New Phytologist 226(1): 156–169. https://doi.org/10.1111/nph.16335

Xiao, W., Ye, X., Zhu, Z., Zhang, Q., Zhao, S., Chen, D., et al. 2020. Evaluation of cadmium (Cd) transfer from paddy soil to rice (*Oryza sativa* L.) using DGT in comparison with conventional chemical methods: derivation of models to predict Cd accumulation in rice grains. Environmental Science and Pollution Research (ESPR) 27(13): 14953–14962. https://doi.org/10.1007/s11356-020-07976-1

Yamaji, N., Xia, J., Mitani-Ueno, N., Yokosho, K. and Feng Ma, J., 2013. Preferential delivery of zinc to developing tissues in rice is mediated by P-type heavy metal ATPase OsHMA2. Plant Physiology 162(2): 927–939. https://doi.org/10.1104/pp.113.216564

Yan, H., Xu, W., Xie, J., Gao, Y., Wu, L., Sun, L., et al. 2019. Variation of a major facilitator superfamily gene contributes to differential cadmium accumulation between rice subspecies. Nature Communications 10(1): 2562. https://doi.org/10.1038/s41467-019-10544-y

Yang, X., Qin, J., Li, J., Lai, Z. and Li, H., 2021. Upland rice intercropping with Solanum nigrum inoculated with arbuscular mycorrhizal fungi reduces grain Cd while promoting phytoremediation of Cd-contaminated soil. Journal of Hazardous Materials 406: 124325. https://doi.org/10.1016/j.jhazmat.2020.124325

Yildirir, G., Sperschneider, J., Malar, C.M., Chen, E.C.H., Iwasaki, W., Cornell, C., et al. 2022. Long reads and Hi-C sequencing illuminate the two-compartment genome of the model arbuscular mycorrhizal symbiont Rhizophagus irregularis. New Phytologist 233(3): 1097–1107. https://doi.org/10.1111/nph.17842

Yu, Z., Zhao, X., Liang, X., Li, Z., Wang, L., He, Y., et al. 2022. Arbuscular mycorrhizal fungi reduce cadmium leaching from sand columns by reducing availability and enhancing uptake by maize roots. Journal of Fungi (Basel), 8(8):866. https://doi.org/10.3390/jof8080866

Zhang, X.-H., Lin, A.-J., Gao, Y.-L., Reid, R.J., Wong, M.-H. and Zhu, Y.-G., 2009. Arbuscular mycorrhizal colonisation increases copper binding capacity of root cell walls of *Oryza sativa* L. and reduces copper uptake. Soil Biology and Biochemistry 41(5): 930–935. https://doi.org/10.1016/j.soilbio.2008.08.011

Zhang, L., Zhou, J., George, T.S., Limpens, E. and Feng, G., 2022. Arbuscular mycorrhizal fungi conducting the hyphosphere bacterial orchestra. Trends in Plant Science 27(4): 402–411. https://doi.org/10.1016/j.tplants.2021.10.008

Zhou, X., Cao, Q., Orfila, C., Zhao, J. and Zhang, L., 2021. Systematic review and meta-analysis on the effects of Astaxanthin on human skin ageing. Nutrients 13(9):2917. https://doi.org/10.3390/nu13092917

Zhou, J., Chai, X., Zhang, L., George, T.S., Wang, F. and Feng, G., 2020. Different arbuscular mycorrhizal fungi cocolonizing on a single plant root system recruit distinct microbiomes. mSystems (ASM Journals) 5(6): e00929-20. https://doi.org/10.1128/mSystems.00929-20

Zhou, M., Li, X., Liu, X., Mi, Y., Fu, Z., Zhang, R., et al. 2022. Effects of antimony on rice growth and its existing forms in rice under arbuscular mycorrhizal fungi environment. Frontiers in Microbiology 13: 814323. https://doi.org/10.3389/fmicb.2022.814323

Zhu, H., Chen, C., Xu, C., Zhu, Q. and Huang, D., 2016. Effects of soil acidification and liming on the phytoavailability of cadmium in paddy soils of central subtropical China. Environmental Pollution 219: 99–106. https://doi.org/10.1016/j.envpol.2016.10.043

Zhu, Q., Xu, P., Lei, L. and Jing, Y., 2022. Transcriptome analysis reveals decreased accumulation and toxicity of Cd in upland rice inoculated with arbuscular mycorrhizal fungi. Applied Soil Ecology 177: 104501. https://doi.org/10.1016/j.apsoil.2022.104501

Supplementary

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	No
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis.	No
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	No
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	6
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	6
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	6

(*continues*)

Section/topic	#	Checklist item	Reported on page #
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	7
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	10

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097
For more information, visit: www.prisma-statement.org.