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丛枝菌根真菌减少污染土壤Cd淋溶流失的效应研究

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摘 要: 为研究丛枝菌根真菌(AMF)对玉米生长与镉(Cd)吸收、土壤团粒组成、壤中流Cd浓度和Cd淋溶流失的影响,本研究以云南兰坪铅锌矿周边Cd污染农田土壤为基质,玉米为宿主植物,设置接种和不接种AMF处理,开展室内盆栽试验。结果表明:与不接种AMF(CK)相比,接种AMF显著增加玉米生物量,降低植株Cd含量与吸收量。接种AMF显著增加0~40 cm土层总球囊霉素相关蛋白(T-GRSP)和易提取球囊霉素相关蛋白(EE-GRSP)的含量,导致粒径(R)>0.25 mm土壤团聚体含量增加55%,R<0.25 mm团聚体含量降低,土壤有效态Cd含量降低26%~43%。接种AMF导致0~30 cm土层壤中流Cd浓度下降14%~22%,Cd淋溶流失量降低29%。相关分析发现:R>0.85 mm的团聚体含量与T-GRSP含量呈显著正相关,R>0.25 mm的团聚体含量与壤中流Cd浓度、Cd淋溶流失量呈极显著负相关;有效态Cd含量与壤中流Cd浓度、Cd淋溶流失量呈极显著和显著正相关。研究表明,与CK相比,接种AMF不仅能增加土壤球囊霉素相关蛋白和大团聚体的含量,而且有助于降低土壤有效态Cd含量与壤中流Cd浓度,减少Cd的淋溶流失,AMF对土壤Cd淋溶流失效应具有重要影响。

关键词: 丛枝菌根真菌(AMF);球囊霉素相关蛋白;壤中流;Cd淋溶;土壤团粒组成

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Effects of arbuscular mycorrhizal fungi on Cd leaching loss in contaminated soil and its preliminary mechanism

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Abstract: The effects of arbuscular mycorrhizal fungi(AMF)on growth and cadmium(Cd) uptake in maize, soil aggregate composition, Cd concentration in interflows, and leaching loss were investigated. Cd-polluted soils were sampled from farmland around a lead-zinc mine in Lanping County, Yunnan Province. Maize(*Zea mays* L.) with or without AMF inoculation was planted indoors in pots. The results showed that the AMF inoculation significantly increased the maize biomass and decreased maize's Cd content and uptake. AMF significantly increased contents of total glomalin-related soil protein(T-GRSP) and easily extracted glomalin-related soil protein(EE-GRSP) in the 0~40 cm soil layers. Furthermore, AMF increased the content of soil aggregates with a size(R)>0.25 mm by 55% and decreased the content of soil aggregates(R<0.25 mm) as well as the available Cd content by 26%~43%. Moreover, AMF significantly reduced the Cd concentration in the interflow in the 0~30 cm soil layer by 14%~22% and caused a decrease of 29% in Cd leaching from the polluted soil. Correlation

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analysis indicated that the content of soil aggregates(*R*>0.85 mm) was positively correlated with the T-GRSP content, and the aggregate content(*R*>0.25 mm) presented a very significant negative correlation with the Cd concentration in the interflow and Cd leaching from the soil. There was a very significant and significant positive correlation between the available Cd content and Cd concentration in the interflow and Cd leaching loss from the soil, respectively. AMF increased the content of macroaggregates(*R*>0.25 mm) and glomalin related soil protein in soil, which contributed to reducing the available Cd content in soil and the Cd concentration in the interflow thereby decreasing Cd leaching from polluted soil. Therefore, AMF has an important impact on the effects of soil Cd leaching and loss.

Keywords:arbuscular mycorrhizal fungi(AMF); glomalin related soil protein; interflow; Cd leaching; soil aggregate composition

由于金属矿产开采冶炼、污水灌溉和污泥施用等工农业生产活动,农田土壤重金属污染日益严重,成为全球重大环境问题之一[1-2]。镉(Cd)是农田土壤常见的污染元素,具有较强的活性与迁移能力,易随水体淋溶流失[3]。在灌溉、降雨等情形下,污染农田土壤Cd离子发生淋溶流失,迁移到周边水体中,进而导致流域水体Cd污染[4]。因此,污染土壤的Cd淋溶流失问题引起了普遍关注。

丛枝菌根真菌(Arbuscular mycorrhizal fungi,AMF)是土壤中重要的微生物,即使在重金属严重污染的土壤中,AMF也普遍存在[5]。AMF侵染寄主植物根系后,根外菌丝在土壤中分枝延伸形成庞大的菌丝网络,其生态学作用引起人们的广泛关注[6]。AMF菌丝在土壤中能迅速分解转化[7],显著改变土壤结构和理化性质[8],对土壤Cd等元素的环境行为与生态效应产生重大影响[9]。然而,这方面的研究主要集中在AMF增强植物对Cd的耐受性[10]、影响Cd在土壤-植物系统中迁移上[11-12],但对AMF影响污染土壤Cd的淋溶流失及其作用机制的了解很有限。

土壤团聚体组成会显著影响污染土壤Cd的淋溶流失。其中,大团聚体是由微团聚体黏合而成的一类比表面积大、多孔的土壤团粒结构,其黏附力远大于环绕在周围的微小团粒[13-14]。大团聚体对土壤养分和水分具有较好的固持作用,且对Cd离子具有较强的吸附作用,有助于减少污染土壤Cd的淋溶流失[15]。值得注意的是,AMF能够通过菌丝缠绕、分泌球囊霉素相关蛋白(Glomalin related soil protein,GRSP)等作用促进土壤团聚体的形成[16-17]。因此,AMF可能会通过影响污染土壤的团粒组成,改变土壤Cd的淋溶流失,这为研究AMF影响污染土壤Cd淋溶流失的可能机制提供了思路。

本文以云南典型的铅锌矿区周边Cd污染农田土壤为供试基质,制备高度为40 cm的土柱,以玉米为寄主植物,设置接种和不接种AMF处理,开展土壤淋溶试验,研究AMF对玉米生长、Cd吸收、土壤团粒组成、壤中流Cd浓度与Cd淋溶流失的影响,分析土壤团粒组成与壤中流Cd浓度、Cd淋溶流失间的关系,以阐释AMF影响污染土壤Cd淋溶流失的效应及其初步机制,丰富对污染土壤中AMF的生态功能及其作用机制等方面的认识。

1 材料与方法

1.1 供试材料

供试玉米(*Zea mays* L.)为会单4号,购于云南省昆明市小板桥种子市场,所选种子籽粒饱满且大小一致。AMF供试菌种为摩西斗管囊霉(*Funneliformis mosseae*),其菌种保藏号为BGC YN05,1511C0001BG-CAM0013,由北京市农林科学院植物营养与资源研究所提供,主要由土壤颗粒、孢子和细根段组成。

供试土壤采自云南省兰坪铅锌矿(26°22′30.0″N,99°22′27.6″E)周边污染农田,其基本理化性质如下:pH为6.11,有机质为32.12 g·kg^{-1},Cd和Pb全量分别为4.27 mg·kg^{-1}和68.29 mg·kg^{-1},全氮和全磷含量分别为2.87 g·kg^{-1}和1.58 g·kg^{-1},碱解氮和速效磷含量分别为72.54 mg·kg^{-1}和58.03 mg·kg^{-1}。土壤采回后,经自然风干,剔除杂物,捣碎研磨后过2 mm尼龙筛混匀,将土壤放入蒸汽高压灭菌锅,121 ℃高温灭菌120 min后,常温条件放置3 d待用。

1.2 试验处理

2018年6月,在云南农业大学试验大棚内开始土柱试验。试验土柱为直径110 mm、高45 cm的聚氯乙烯(PVC)管;采用灭菌土壤装填土柱,每个土柱土壤装填量为4.0 kg(图1)。土柱底端封闭,设置1个出水阀,用于收集淋溶液;在土柱的10、20 cm和30 cm深度处设置壤中流取样点,用于采集壤中流样品。

玉米种子首先在1%次氯酸钠(NaClO)溶液中浸泡1 min,然后移入75%的乙醇溶液中浸泡3 min,取出后用无菌水冲洗3次,完成玉米种子的表面消毒。将玉米种子置于垫有浸湿滤纸的无菌培养皿中,25 ℃下恒温培养4 d,待种子萌发冒白2 cm左右,挑

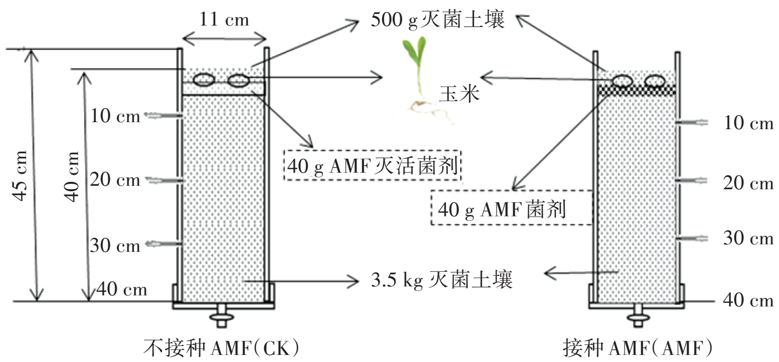


图1 试验装置与取样位置示意图

Figure 1 Schematic diagram of test device

选无污染且生长一致的萌发种子进行种植。

试验设不接种AMF(CK)和接种AMF(AMF)2组处理,每组处理6个平行。先在土柱中装入3.5 kg灭菌土,AMF处理将40 g AMF菌剂平铺在土柱中,CK处理则加入40 g AMF灭活菌剂;种植2粒玉米种子,再均匀加入500 g灭菌土,以覆盖玉米种子(图1)[18]。土柱试验过程中不施用化肥和农药,自然光照,温室温度为14~29 ℃;期间根据土柱土壤水分状况浇灌去离子水20~30 mL,保持土壤湿润。

1.3 样品采集

在玉米种植第45天和第50天,进行人工灌溉,开展淋溶试验。每次灌溉量为2.0 L,每15 min浇灌去离子水500 mL,共浇灌4次,相当于33.3 mL·min^{-1}的降雨强度。

在浇灌开始后,打开土柱底部的出水阀,收集土柱产生的淋溶液,直至出水阀不产生水滴,使用容量筒测量淋溶液体积。浇灌30 min后,采用0.25 μm陶瓷滤管(RHIZON MOM 19.21.21F)负压抽取深度为10、20 cm和30 cm的土柱土壤溶液50 mL。

第2次土柱淋溶试验后,采集玉米植株与根际土壤样品。将玉米整株取出,抖去根部附着的疏松土壤,采集与根系紧密结合的根际土壤(厚度约为1 mm),室内自然风干、研磨后备用。将玉米植株分为地上部(茎叶)和地下部(根系),分别用无菌水漂洗干净、晾干;除选取少量细根用于AMF侵染率指标测定外,玉米植株于105 ℃杀青30 min,再经75 ℃烘干72 h至恒质量,获得植株干质量,碾磨粉碎备用。

1.4 测定方法

将根系鲜样剪成1 cm左右的根段,碱解离、蓝墨水染色后,采用曲利苯蓝染色改良法测定AMF的侵染率[19]。采用蔗糖离心法测定AMF孢子数[20]:称取10.0 g土样,反复冲洗过0.25 mm筛,将残留物转移至100 mL的离心管中,3 000 r·min^{-1}离心10 min,去掉上清液后加入50%蔗糖溶液,充分摇匀,3 000 r·min^{-1}离心10 min得到上清液,用滤纸过滤上清液,在立体显微镜下观察滤纸上AMF孢子的数量。

称取1.0 g风干土样,在0.11 MPa、121 ℃条件下,采用20 mmol·L^{-1}柠檬酸缓冲液(pH 7.0)提取30 min,得到易提取球囊霉素相关蛋白(Easy extractive glomalin related soil protein,EE-GRSP)的待测液;50 mmol·L^{-1}柠檬酸钠缓冲液(pH 8.0)提取60 min,连续提取3次,得到总球囊霉素相关蛋白(Total glomalin related soil protein,T-GRSP)的待测液。采用Braford法测定土壤EE-GRSP和T-GRSP的含量[21]。

称取过2 mm筛的风干土样30 g,放置于土壤团粒分析仪中,套筛孔径依次为0.85、0.25 mm和0.075 mm,采用湿筛法分离不同粒径的土壤团聚体,于105 ℃下烘至恒质量,分别称量,获得粒径(R)>0.85、0.25~0.85、0.075~0.25 mm和<0.075 mm的土壤团聚体含量[22]。

称取1.0 g土样,采用0.1 mol·L^{-1} HCl浸提-火焰原子分光光度法测定土壤有效态Cd的含量。称取0.5 g植株干样,采用H_2SO_4-H_2O_2消解-凯氏定氮法-钒钼黄比色法测定植株全氮和全磷的含量。称取0.1 g植株干样,采用HNO_3-$HClO_4$消解-火焰原子吸收分光光度法测定植株的Cd含量[23]。

吸取10 mL壤中流或20 mL淋溶液,采用HNO_3-H_2O_2消解,石墨炉原子吸收法测定溶液的Cd浓度[24]。将淋溶液Cd浓度乘以其体积,获得土柱Cd的淋溶流失量。

1.5 统计分析

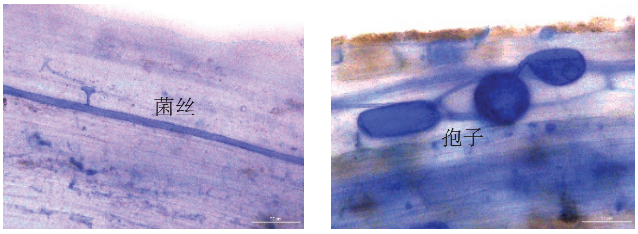
采用Microsoft Excel 2018对数据进行处理,用SPSS 23.0软件进行数据统计分析;用LSD法检验处理间差异显著性,P<0.05为显著,P<0.01为极显著;

采用Person法分析相关性，用OriginPro 9.0绘图。

2 结果与分析

2.1 玉米根系AMF侵染特征

AMF在玉米根系中的结构见图2。经检测，接种AMF土壤的根系侵染率为25%，菌丝密度为28.36 cm·g^{-1}，孢子数为18.82个·g^{-1}。



图片为显微镜下放大20倍

The image is magnified 20 times under the microscope

图2 AMF在玉米根系中的结构

Figure 2 Typical structures of AMF in maize roots

由图3可知，与CK相比，AMF处理0~10 cm和10~20 cm土层土壤EE-GRSP含量显著上升，分别增加24%和33%，0~10、10~20、20~30 cm和30~40 cm土层土壤T-GRSP含量分别显著增加44%、36%、16%和11%。综上可知，重金属胁迫下接种AMF能不同程度增加0~40 cm不同土层土壤中EE-GRSP和T-GRSP的含量。

2.2 接种AMF对土壤团聚体粒径分布的影响

由图4可知，与CK相比，AMF处理0~10、10~20 cm和30~40 cm土层土壤中R>0.85 mm团聚体含量分别显著增加28%、46%和13%，0~10、20~30 cm和30~40 cm土层土壤中0.85 mm>R>0.25 mm团聚体含量分别显著增加10%、22%和55%。而与CK相比，AMF处理0~40 cm土层土壤中0.25 mm>R>0.075 mm团聚体含量均显著降低，降幅为10%~12%，0~10 cm和10~20 cm土层土壤中R<0.075 mm团聚体含量分别显著降低13%和16%。综上可知，重金属胁迫下土壤接种AMF能增加土壤R>0.25 mm团聚体含量，降低R<0.25 mm团聚体含量。

2.3 接种AMF对土壤有效态Cd含量和植物Cd吸收量的影响

由图5可知，与CK相比，AMF处理0~10、10~20 cm和30~40 cm土层土壤中有效态Cd含量分别显著降低26%、16%和27%，表明接种AMF能不同程度降低土壤不同土层的有效态Cd含量。

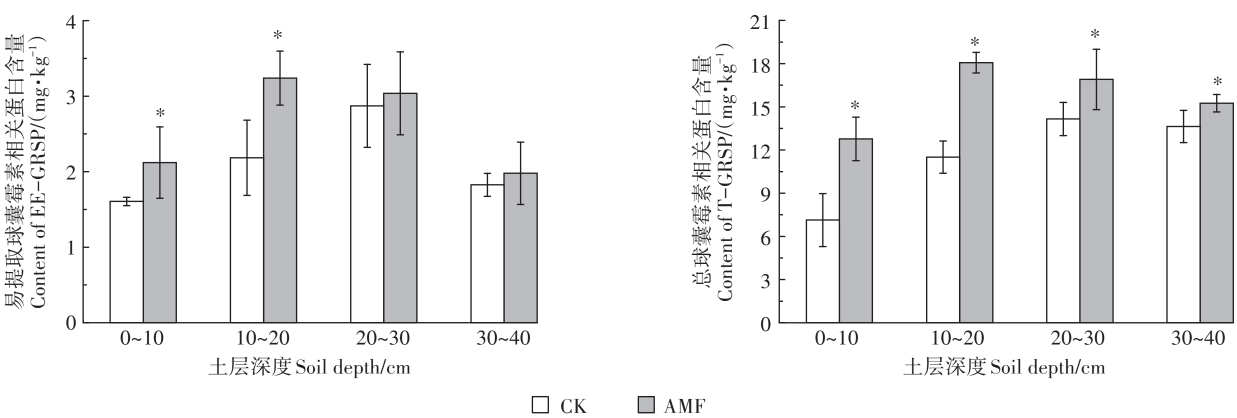
由表1可知，与CK相比，AMF处理玉米地上部生物量显著增加36%，地上部和地下部Cd含量分别显著降低45%和40%，地上部和地下部Cd吸收量分别显著降低25%和31%。

2.4 接种AMF对壤中流Cd含量的影响

由图6可知，与CK相比，第一次淋溶试验后，AMF处理0~10 cm和20~30 cm土层的壤中流Cd含量显著降低20%和23%，第二次淋溶试验后，AMF处理10~20 cm和20~30 cm土层的壤中流Cd含量显著降低22%和14%。表明重金属污染胁迫下土壤接种AMF能减少不同土层壤中流的Cd含量。

2.5 接种AMF对Cd淋溶流失的影响

由表2可知，与CK相比，第一次淋溶试验后，AMF处理对淋溶液Cd含量和Cd流失量均没有显著影响；第二次淋溶试验后，AMF处理下淋溶液Cd含量和Cd流失量分别显著降低30%和34%；第二次淋溶试验



*代表处理间差异达到显著水平（P<0.05）。下同

* indicate significant differences between AMF and CK treatments（P<0.05）. The same below

图3 接种AMF对土壤球囊霉素相关蛋白含量的影响

Figure 3 Effects of AMF on contents of GRSP in soil

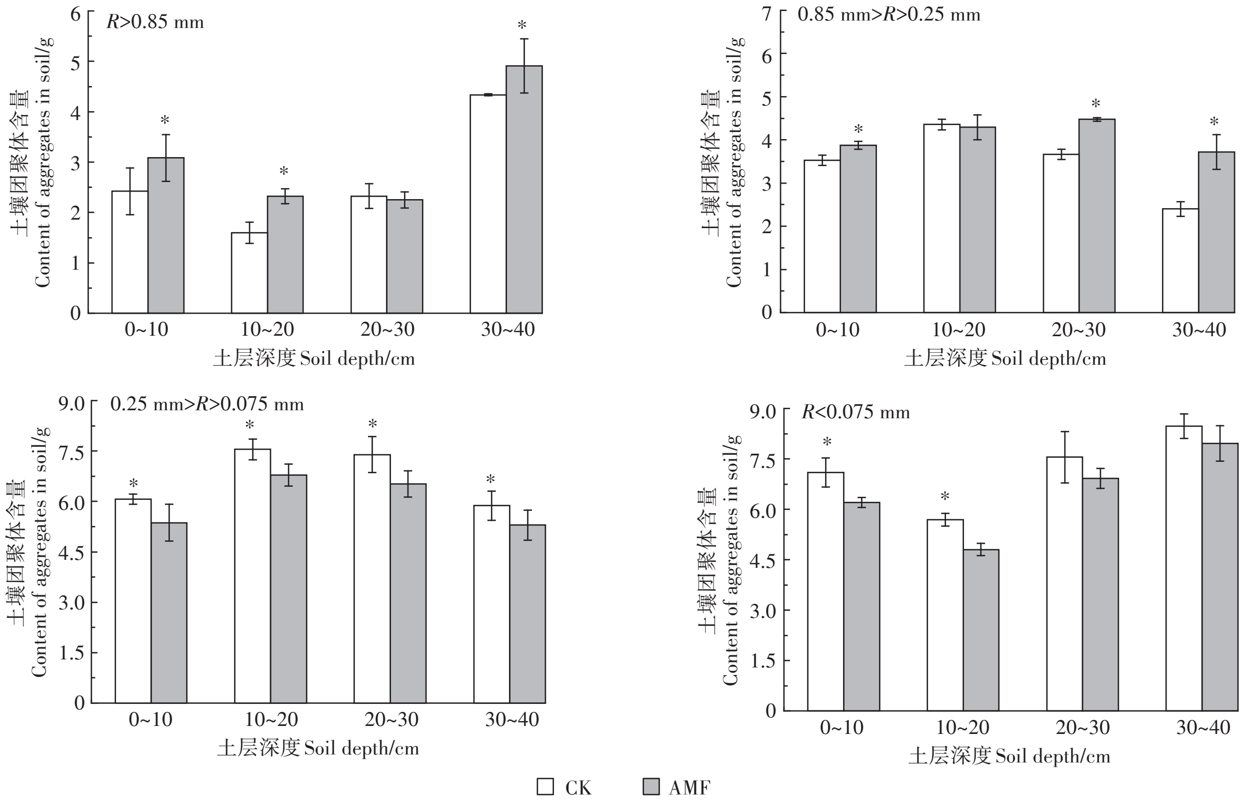


图4 接种AMF对不同土层土壤不同粒径团聚体含量的影响

Figure 4 Effects of AMF on aggregate contents in soil

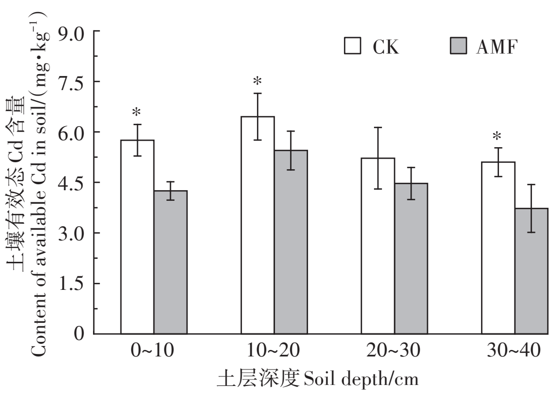


图5 接种AMF对不同土层土壤有效态Cd含量的影响

Figure 5 Effects of AMF on available Cd contents in soil

后，AMF处理下淋溶流失总量降低29%。表明接种AMF能减少淋溶液中有效态Cd含量与流失量。

2.6 相关性分析

由表3可知，*R*>0.85 mm的团聚体含量与壤中流Cd含量呈显著负相关；0.85 mm>*R*>0.25 mm的团聚体含量与T-GRSP呈显著正相关，与壤中流Cd含量和Cd淋溶流失量呈极显著负相关；0.25 mm>*R*>0.075 mm的团聚体含量与Cd淋溶流失量呈显著正相关；*R*<0.075 mm的团聚体含量与T-GRSP和EE-GRSP含量呈显著负相关，与Cd淋溶流失量呈显著正相关。土壤有效态Cd含量与壤中流Cd含量和Cd淋溶流失量分别呈极显著与显著正相关。

表1 接种AMF对玉米Cd含量和累积量的影响

Table 1 Effects of AMF inoculation on biomass, Cd content and accumulation in maize

处理 Treatment	生物量/(g·柱$^{-1}$) Biomass/(g·pot^{-1})		Cd含量 Cd content/(mg·kg^{-1})		Cd吸收量/(mg·柱$^{-1}$) Cd accumulation/(mg·pot^{-1})		Cd转运系数 Cd transport coefficient
	地上部 Shoots	地下部 Roots	地上部 Shoots	地下部 Roots	地上部 Shoots	地下部 Roots	
CK	33.98±1.10	5.70±0.80	0.31±0.05*	1.35±0.61*	10.50±1.32*	7.69±1.04*	0.23±0.17
AMF	46.31±2.10*	6.59±0.68	0.17±0.05	0.81±0.17b	7.87±1.53	5.34±1.20	0.21±0.04

注：表中数值为平均值±标准差（*n*=6）；*代表处理间差异显著（*P*<0.05）。表2同。

Note: Data are presented as means±SD (*n*=6); * indicate significant differences between AMF and CK treatments (*P*<0.05). The same as table 2.

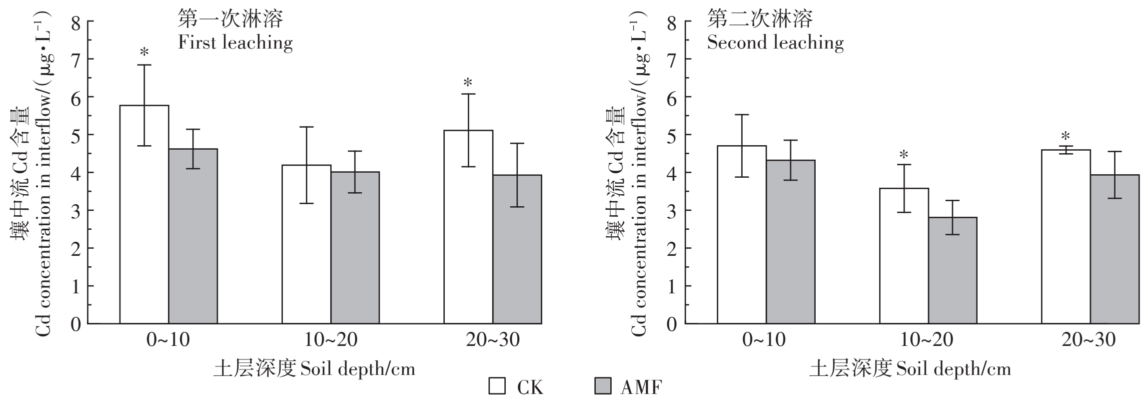


图6 接种AMF对壤中流Cd含量的影响

Figure 6 Effects of AMF on Cd concentrations in interflow

3 讨论

在重金属胁迫下，与CK相比，接种AMF土壤的T-GRSP和EE-GRSP含量显著增加，土壤有效态Cd含量显著降低。GRSP是AMF代谢分泌的一类糖蛋白[25]，能与土壤中重金属结合，改变重金属的生物有效性，GRSP含有的芳香烃、羟基等不稳定活性官能团[26-27]，能够通过离子交换吸附-配合-共沉淀作用，促使土壤中的重金属由离子态向残渣态转变[28-29]。有研究表明，GRSP能固定土壤中Cd、Pb等重金属离子，且GRSP与土壤有效态Cd、Pb含量呈负相关[30]，其结果与本试验研究结果一致。

本试验中，在重金属胁迫下，接种AMF后，0~40 cm土层的R>0.25 mm团聚体含量显著高于CK。AMF侵染寄主植物后形成的发达的根外菌丝与其分泌物GRSP的机械缠绕，联合腐植酸类物质的超强黏合作用将土壤颗粒缠绕黏合形成土壤团聚体[31]。菌丝、GRSP以及腐植酸在团聚体稳定性中扮演着重要的角色，其能将小土壤颗粒黏成R<0.25 mm的微团聚体，进而形成水稳态团聚体（R>0.25 mm）[32-33]。有学者研究不同真菌对土壤结构的影响发现[16]，土壤接种AMF后稳定性团聚体含量远高于微团聚体含量；有学者研究接种不同真菌对水稳性大团聚体形成的作用得出，接种真菌的土壤形成水稳性大团聚体的含量显著高于未接种土壤[14,29]。但目前对于团聚体的形成因素中，AMF根外菌丝、GRSP以及腐植酸类物质哪种因素占主导地位或者为主要因素还未有准确结果，还需进一步验证。

表2 接种AMF对土壤Cd含量和流失量的影响

Table 2 Effects of AMF inoculation on Cd content and loss in soil

项目 Item	处理 Treatment	淋溶液Cd含量 Cd concentration/(μg·L⁻¹)	体积 Leachate volume/mL	Cd总流失量 Total Cd loss/μg
第一次淋溶 First leaching	CK	6.62±1.12	225±35	1.48±0.22
	AMF	5.29±0.93	205±28	1.08±0.11
第二次淋溶 Second leaching	CK	5.60±1.06*	216±39	1.21±0.14*
	AMF	3.94±0.83	202±29	0.80±0.28

表3 GRSP、淋溶液Cd含量、Cd流失量与土壤水稳态团聚体、有效态Cd含量的相关性

Table 3 Correlations between contents of available Cd and GRSP, Cd concentration in interflow and leachate with contents of aggregate and available Cd in soil

项目 Item	土壤团聚体 Soil aggregates with a diameter				土壤有效态Cd含量 Available Cd content in soil
	R>0.85 mm	0.85 mm>R>0.25 mm	0.25 mm>R>0.075 mm	R<0.075 mm	
T-GRSP	0.554	0.708*	−0.631	−0.777*	−0.724*
EE-GRSP	0.178	0.585	−0.332	−0.716*	−0.478
壤中流Cd含量 Cd concentration in interflow	−0.800*	−0.890**	−0.808*	0.693	0.938**
Cd流失量 Total Cd leaching loss	−0.663	−0.870**	0.734*	0.777*	0.792*

注：*代表显著相关（P<0.05），**代表极显著相关（P<0.01）。

Note: * indicates significant correlation (P<0.05), and ** indicates extremely significant correlation (P<0.01).

本研究结果表明，与CK相比，接种AMF后，0~40 cm土层的R>0.25 mm团聚体含量显著增加，0~40 cm土层土壤有效态Cd含量显著下降。这可能是由于不同粒径团聚体对土壤中重金属吸附固定作用有明显的差异，此外，不同粒径土壤团聚体中AMF菌丝、GRSP以及腐植酸类物质含量不同以及团聚体结构也存在差异[34]。接种AMF能改善土壤团聚体的水稳定性，吸附固定土壤有效态重金属。团聚体是农田土壤中重金属吸持的关键因素，0.2~2 mm粒径的团聚体能够集聚大量的植物根系以及腐植酸类物质等，对重金属有很强的吸附固持能力[33,35]。

土壤中游离态Cd离子极易在土壤中迁移流动，在灌溉、降雨、降雪等淋溶作用下，Cd离子下渗到地下水，或扩散到流域出水口，影响地下水水质，扩大农田土壤Cd污染面积[3,36]。降低Cd的移动性，将Cd固定在土壤中以减少Cd的流失量是解决Cd流失的关键性因素[29,34]。GRSP是菌根共生体分泌物，含有大量土壤有机质、多环芳烃以及阴离子基团，其特殊的组成结构能改变土壤中有效态重金属的生物有效性，将游离态金属离子转变为残渣态金属沉淀物[37]。大量相关研究表明，重金属胁迫下，接种AMF后能降低土壤环境中重金属的生物有效性，其分泌的GRSP能够固定土壤中的Cd；当土壤中Cd含量较高时，GRSP对Cd的固定量也较高[2,5]。本研究结果表明，重金属污染胁迫下，接种AMF土壤的GRSP浓度显著上升，不同土层土壤有效态Cd含量显著降低。

不同粒径的土壤团聚体对土壤中Cd吸收率和淋溶流失的影响存在差异。R>0.25 mm的水稳性团聚体具有多孔性、比表面积大等特性，且富含大量的腐植酸类物质，能够增加对Cd离子的吸附-络合-螯合等作用[17,38]，从而改变重金属Cd在土壤中的存在形态，将游离态Cd固定在土壤中，减少Cd的淋溶流失。有研究表明，R>0.25 mm的水稳态团聚体吸附有效态重金属的效率显著高于R<0.25 mm的微团聚体，能使淋溶液中的有效态重金属含量降低，重金属流失量减少[3,33]。本研究结果显示，0~40 cm土层水稳态团聚体含量显著增加，两次淋溶0~30 cm土层壤中流有效态Cd含量显著降低，第二次淋溶的淋溶液中有效态Cd含量和流失量均显著降低，且Cd淋溶流失量与R>0.25的团聚体含量呈显著负相关，与R<0.25 mm的团聚体含量和土壤有效态Cd含量呈显著正相关。综上可知，重金属污染胁迫下，接种AMF能增加土壤水稳态团聚体含量，降低淋溶液中有效态Cd浓度和流失量。

4 结论

（1）重金属污染胁迫下，接种丛枝菌根真菌能够降低土壤中有效态Cd含量，从而减少土壤有效态Cd在玉米体内的富集，缓解重金属对植物的毒害，促进植物生长。

（2）接种丛枝菌根真菌能够强化土壤对壤中流Cd的固持效应，降低壤中流有效态Cd浓度，从而减少灌溉等淋溶作用下土壤Cd的流失量和扩散效应。

参考文献：

[1] SUN Z H, XIE X D, WANG P. Heavy metal pollution caused by small-scale metal ore mining activities：A case study from a polymetallic mine in south China[J]. *Science of the Total Environment*, 2018, 639：217-227.

[2] ZHAN F D, ZENG W Z, YUAN X C, et al. Field experiment on the effects of sepiolite and biochar on the remediation of Cd and Pb-polluted farmlands around a Pb-Zn mine in Yunnan Province, China[J]. *Environmental Science and Pollution Research*, 2019, 26（8）：7743-7751.

[3] LU Y Y, LUO D G, LAI A. Leaching characteristics of EDTA-enhanced phytoextraction of Cd and Pb by *Zea mays* L. in different particle-size fractions of soil aggregates exposed to artificial rain[J]. *Environmental Science and Pollution Research*, 2017, 24（2）：1845-1853.

[4] FANG W, WEI Y, LIU J. Comparative characterization of sewage sludge compost and soil：Heavy metal leaching characteristics[J]. *Journal of Hazardous Materials*, 2016, 310：1-10.

[5] 游义红, 李博, 李明锐, 等. 重金属污染胁迫下接种AMF对玉米生长和镉铅含量的影响[J]. 山地农业生物学报, 2019, 38（3）：38-45. YOU Y H, LI B, LI M R, et al. Effects of AMF Inoculation on maize growth and cadmium and plumbum content under heavy metal pollution stress[J]. *Journal of Mountain Agriculture and Biology*, 2019, 38（3）：38-45.

[6] ZHAN F D, LI B, JIANG M, et al. Arbuscular mycorrhizal fungi enhance antioxidant defense in the leaves and the retention of heavy metals in the roots of maize[J]. *Environmental Science and Pollution Research*, 2018, 25（24）：24338-24347.

[7] VODNIK D, GRCMAN H, MACEK I, et al. The contribution of glomalin- related soil protein to Pb and Zn sequestration in polluted soil[J]. *Science of the Total Environment*, 2008, 392（1）：130 -136.

[8] WANG Q, BAO Y, LIU X, et al. Spatio-temporal dynamics of arbuscular mycorrhizal fungi associated with glomalin-related soil protein and soil enzymes in different managed semiarid steppes[J]. *Mycorrhiza*, 2014, 24（7）：525-538.

[9] HU J L, WU S C, WU F Y. Arbuscular mycorrhizal fungi enhance both absorption and stabilization of Cd by Alfred stonecrop（*Sedum alfredii Hance*）and perennial ryegrass（*Lolium perenne* L.）in a Cd-contaminated acidic soil[J]. *Chemosphere*, 2013, 93（7）：1359-1365.

[10] 伍松林, 张莘, 陈保冬. 丛枝菌根对土壤-植物系统中重金属迁移转化的影响[J]. 生态毒理学报, 2013, 8（6）：847-856. WU S L, ZHANG X, CHEN B D. Effects of arbuscular mycorrhizal fungi on heavy metal translocation and transformation in the soil-plant contin-

uum[J]. *Asian Journal of Ecotoxicology*, 2013, 8(6): 847–856.

[11] MEDINA A, VASSILEV N, AZCON R. The interactive effect of an AM fungus and an organic amendment with regard to improving inoculum potential and the growth and nutrition of *Trifolium* repens in Cd-contaminated soils[J]. *Applied Soil Ecology*, 2010, 44(2): 181–189.

[12] HUANG X C, HO S H, ZHU S S, et al. Adaptive response of arbuscular mycorrhizal symbiosis to accumulation of elements and translocation in *Phragmites australis* affected by cadmium stress[J]. *Journal of Environmental Management*, 2017, 197(4): 448–455.

[13] RILLIG M C. Arbuscular mycorrhizae, glomalin, and soil aggregation [J]. *Canadian Journal of Soil Science*, 2004, 84: 355–363.

[14] RAO Z X, HUANG D Y, WU J S, et al. Distribution and availability of cadmium in profile and aggregates of a paddy soil with 30-year fertilization and its impact on Cd accumulation in rice plant[J]. *Environmental Pollution*, 2018, 239(8): 198–204.

[15] GLASSMAN S I, CASPER B B. Biotic contexts alter metal sequestration and AMF effects on plant growth in soils polluted with heavy metals[J]. *Ecology*, 2012, 93(7): 1550–1559.

[16] XIE H T, LI J W, ZHANG B, et al. Long-term manure amendments reduced soil aggregate stability via redistribution of the glomalin-related soil protein in macroaggregates[J]. *Scientific Reports*, 2015, 5 (10): 1–9.

[17] BOTTINELLI N, ANGERS D A, HALLAIRE V, et al. Tillage and fertilization practices affect soil aggregate stability in a humic cambisol of northwest France[J]. *Soil and Tillage Research*, 2017, 170: 14–17.

[18] 张金秀, 湛方栋, 王灿, 等. AMF对铅锌矿区农田土壤部分理化性质、玉米生长和镉铅含量的影响[J]. 农业资源与环境学报, 2020, 37(5): 727–735. ZHANG J X, ZHAN F D, WANG C, et al. Effects of AMF on some soil physical and chemical properties of farmland from a lead-zinc mine area, growth, cadmium and lead contents in maize[J]. *Journal of Agricultural Resources and Environment*, 2020, 37 (5): 727–735.

[19] PHILLIPS J M, HAYMAN D S. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection[J]. *Transactions of the British Mycological Society*, 1970, 55: 158–161.

[20] GERDEMANN J W, NICOLSON T H. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting[J]. *Transactions of the British Mycological Society*, 1963, 46: 235–244.

[21] WRIGHT S F, FRANKE S M, MORTON J B, et al. Time course study and partial characterization of a protein on hyphae of arbuscular mcyrrohzal fungi during active colonization of roots[J]. *Plant and Soil*, 1996, 181: 193–203.

[22] MANNA M C, SWARUP A, WANJARI R H, et al. Long-term fertilization, manure and liming effects on soil organic matter and crop yields[J]. *Soil & Tillage Research*, 2007, 94(2): 397–409.

[23] 鲍士旦. 土壤农化分析[M]. 3版. 北京: 中国农业出版社, 2000. BAO S D. Agricultural soil analysis[M]. 3rd Edition. Beijing: China Agriculture Press, 2000.

[24] 王心芳. 水和废水监测方法[M]. 北京: 中国环境科学出版社, 2002. WANG X F. Methods for monitoring and analysis of water and waste water[M]. Beijing: China Environmental Science Press, 2002.

[25] CORNEJO P, SEBASTIÁN M, BORIE G, et al. Glomalin-related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn sequestration[J]. *Science of the Total Environment*, 2008, 406(1/2): 154–160.

[26] VIJAY G, MATTHIAS C. RILLIG M C. The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60 [J]. *FEMS Microbiology Letters*, 2006, 263(1): 93–101.

[27] HE Y M, FAN X M, ZHANG G Q, et al. Effects of arbuscular mycorrhizal fungi and dark septate endophytes on maize performance and root traits under a high cadmium stress[J]. *South African Journal of Botany*, 2019, 24(2): 18494–18504.

[28] STEFANO B, CHIARAFRANCESCA R, MANUELA G. Molecular characterization and glomalin production of arbuscular mycorrhizal fungi colonizing a heavy metal polluted ash disposal island, downtown Venice[J]. *Soil Biology & Biochemistry*, 2010, 42(5): 758–765.

[29] BAN Y, XU Z, ZHANG H. Soil chemistry properties, translocation of heavy metals, and mycorrhizal fungi associated with six plant species growing on lead-zinc mine tailings[J]. *Annals of Microbiology*, 2015, 65(1): 503–515.

[30] WU Z P, MCGROUTHER K, HUANG J D, et al. Decomposition and the contribution of glomalin-related soil protein (GRSP) in heavy metal sequestration: Field experiment[J]. *Soil Biology & Biochemistry*, 2014, 68: 283–290.

[31] ŠIMANSKÝ V, BALASHOV E, HORÁK J. Water stability of soil aggregates and their ability to sequester carbon in soils of vineyards in Slovakia[J]. *Archives of Agronomy and Soil Science*, 2016, 62(2): 177–197.

[32] LI X, ZHANG J, GAI J, et al. Contribution of arbuscular mycorrhizal fungi of sedges to soil aggregation along an altitudinal alpine grassland gradient on the Tibetan Plateau[J]. *Environmental Microbiology*, 2015, 17(8): 2841–2857.

[33] YANG Y, HE C, HUANG L, et al. The effects of arbuscular mycorrhizal fungi on glomalin-related soil protein distribution, aggregate stability and their relationships with soil properties at different soil depths in lead-zinc contaminated area[J]. *PLoS One*, 2017, 12(8): 1–19.

[34] WANG M, CHEN S B, HAN Y, et al. Responses of soil aggregates and bacterial communities to soil-Pb immobilization induced by biofertilizer[J]. *Chemosphere*, 2019, 220(4): 828–836.

[35] MATTHIAS C, RILLIG M C, SARA F, et al. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: Comparing effects of five plant species[J]. *Plant and Soil*, 2002, 238(2): 325–333.

[36] ZHANG J, LI H, ZHOU Y, et al. Bioavailability and soil-to-crop transfer of heavy metals in farmland soils: A case study in the Pearl River Delta, south China[J]. *Environmental Pollution*, 2018, 235(4): 710–719.

[37] CHEN S, WANG J, MICHAEL G W, et al. Glomalin-related soil protein influences the accumulation of polycyclic aromatic hydrocarbons by plant roots[J]. *Science of the Total Environment*, 2018, 644(12): 465–473.

[38] QUENEA K, LAMY I, WINTERTON P. Interactions between metals and soil organic matter in various particle size fractions of soil contaminated with waste water[J]. *Geoderma*, 2009, 149(4): 217–223.

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