



研究报告

## 电子废弃物拆解区土壤重金属污染对丛枝菌根真菌多样性的影响

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**摘要:**【背景】电子废弃物拆解造成的土壤重金属污染引发的环境问题日益突出,丛枝菌根(arbuscular mycorrhizal, AM)真菌能侵染植物根系并增强植物抵御环境胁迫的能力,具有重要的生态功能和应用潜力。【目的】探究电子废弃物拆解区土壤重金属污染对 AM 真菌群落结构与多样性的影响,甄别可耐受重金属污染的 AM 真菌类群。【方法】从浙江台州某典型电子废弃物拆解场地及其周边区域共采集土壤样品 12 份,针对土壤中 AM 真菌的 18S rRNA 基因进行高通量测序以及可操作分类单元(operational taxonomic unit, OTU)相对丰度和多样性指数计算。【结果】该区土壤中 AM 真菌由原囊霉目(*Archaeosporales*)、球囊霉目(*Glomerales*)和多孢囊霉目(*Diversisporales*)组成,其中球囊霉目占据优势地位。土壤 AM 真菌多样性指数与重金属的浓度、综合污染指数和潜在生态风险指数间均无显著相关性,但疑似泡囊根生囊霉(*Rhizophagus vesiculiferus*)的 OTU 相对丰度与上述重金属污染指标之间均呈显著正相关关系。【结论】*R. vesiculiferus* 可能对重金属污染有极强耐受性,可为今后电子废弃物拆解污染土壤治理提供技术基础。

**关键词:** 电子废弃物拆解, 重金属污染, 泡囊根生囊霉, 丛枝菌根真菌, 群落结构, 多样性

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## Effect of soil heavy metal contamination on arbuscular mycorrhizal fungal diversity in an e-waste dismantling area

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**Abstract: [Background]** The environmental problem caused by soil heavy metal (HM) contamination upon e-waste dismantling is becoming increasingly serious. Arbuscular mycorrhizal (AM) fungi can colonize plant root and enhance the ability of plant to resist environmental stresses, with important ecological function and application potency. **[Objective]** To study soil HM contamination effects on the community structure and diversity of AM fungi in e-waste dismantling areas, and to identify AM fungal species which have strong resistance to HM contamination. **[Methods]** A total of 12 soil samples were collected from a typical e-waste dismantling site and the surrounding areas in Taizhou city, Zhejiang province. The 18S rRNA gene of soil AM fungi was sequenced by pyrosequencing, and the relative abundances of operational taxonomic units (OTUs) and the diversity indices were calculated. **[Results]** Soil AM fungi in the investigated area are mainly composed by *Archaeosporales*, *Glomerales* and *Diversisporales*, with the dominant order of *Glomerales*. Although soil AM fungal diversity indices had no significant correlations with the concentrations, and the comprehensive pollution and potential ecological risk indices of HMs, the relative abundance of *Rhizophagus vesiculiferus*-like OTU positively correlated to all above pollution parameters of HMs. **[Conclusion]** *R. vesiculiferus* may have extremely strong resistance to HM contamination, providing technical basis for the treatment of contaminated soil caused by e-waste dismantling in the future.

**Keywords:** E-waste dismantling, Heavy metal contamination, *Rhizophagus vesiculiferus*, Arbuscular mycorrhizal fungi, Community structure, Diversity

20 世纪末期, 中国东南部地区包括台州温岭、汕头贵屿和清远龙塘等地出现了专门从事电子废弃物拆解的行业<sup>[1]</sup>。然而, 电子废弃物拆解是一把“双刃剑”, 一方面能够回收大量的金属资源, 减轻资源短缺造成的压力, 促进地区经济增长, 具有积极作用; 另一方面, 拆解业长期以来大多采用的是小规模和不规范的拆解方式, 特别是露天拆解, 致使重金属、持久性有机污染物等各种有毒有害物质大量泄露到环境中, 给大气、水体和土壤造成严重污染<sup>[2]</sup>, 造成不容忽视的环境危害。由于大气和水体中的重金属也会以各种形式进入到土壤, 而且它们在土壤中迁移能力

差、滞留时间长、不能被微生物降解<sup>[3]</sup>, 所以电子废弃物拆解造成的土壤重金属污染问题已经引起了人们的高度重视。电子废弃物拆解、堆卸导致周边土壤普遍受到重金属污染, 其中拆解、倾倒点位呈重度污染状态<sup>[4-5]</sup>, 这会对植物、土壤动物以及微生物等产生威胁。例如, 台州某电子废弃物拆解尾渣倾倒点土壤中高浓度的重金属显著抑制向日葵生长并破坏其叶片类囊体层状结构<sup>[6]</sup>, 贵屿某电子废弃物拆解区域附近水稻土中线虫的数量随着重金属浓度升高而呈现波动下降<sup>[7]</sup>, 龙塘某电子废弃物集中处理区土壤中真菌和细菌的数量分别下降了 85.6% 和 99.7%<sup>[8]</sup>。另外, 与周边

稻田相比,电子废弃物拆解现场表层土壤中含有更高浓度的镉和铜,土壤过氧化氢酶、蔗糖酶、酸性磷酸酶和脲酶等的活性下降<sup>[9]</sup>。因此,由电子废弃物拆解造成的土壤重金属污染引发的其他环境问题尤其需要关注。

丛枝菌根(arbuscular mycorrhizal, AM)真菌广泛存在于农田和自然生态系统中,能与绝大多数陆生植物的根系建立互惠共生关系<sup>[10]</sup>,在重金属污染土壤中也能够侵染植物根系,改善植物的生长状况<sup>[11]</sup>。但是,重金属污染会对AM真菌群落结构和多样性产生影响,而且随着土壤重金属浓度的升高,定殖在植物根部的AM真菌多样性也会降低<sup>[12]</sup>。例如,铜污染不仅降低了海洲香薷根际土壤中AM真菌的香农指数,同时改变了其群落组成<sup>[13]</sup>。在锰污染土壤中,AM真菌的香农指数与总锰、可提取态锰的浓度均呈显著负相关关系<sup>[14]</sup>。在铅锌矿区土壤中,重金属复合污染降低了AM真菌的物种丰富度、香农指数和辛普森指数,导致刺槐根际土壤中AM真菌的群落结构发生变化<sup>[15]</sup>。研究发现,球囊霉属(*Glomus*)和无梗囊霉属(*Acaulospora*)是重金属污染土壤中最常见的AM真菌属<sup>[16-18]</sup>。尽管国内外已有大量学者关注土壤重金属与AM真菌之间的相互关系并开展了很多研究,但电子废弃物拆解造成的土壤重金属污染对AM真菌群落结构和多样性的影响尚不明确。因此,本研究以浙江省台州市路桥区某典型电子废弃物拆解场地及其周边区域为对象,采用Illumina高通量测序技术来探究电子废弃物拆解造成的土壤重金属污染对AM真菌多样性的影响,并试图甄别具有高重金属耐受性的AM真菌,以便为电子废弃物拆解区重金属污染土壤的治理提供理论依据。

## 1 材料与方法

### 1.1 样点信息

2016年1月,在浙江省台州市路桥区某典型电子废弃物拆解场地及其周边区域,针对拆解/倾

倒和扩散共设置12个样点(范围为28°30'39"N-28°31'31"N, 121°22'07"E-121°24'34"E),采集表层土壤(0-10 cm)。其中,拆解/倾倒区包括拆解园区(C1)、下脚料拆解点(C2)、尾渣倾倒点(Q1)和尾渣排放点(Q2)共计4个样点,扩散区包括1个池塘底泥样点(D1)和7个农田样点(N1-N7)。每个土样混匀和去除碎石等杂物后,一式两份,其中一份自然风干过筛后测定Cd、Cu、Pb、Cr、Zn、Ni等浓度<sup>[4]</sup>,另一份在-40℃下存储以提取土壤总DNA。具体的采样点信息、土壤pH值、重金属浓度、综合污染指数及潜在生态风险指数见表1。

### 1.2 主要试剂和仪器

FastDNA<sup>®</sup> SPIN Kit for Soil, MP Biomedicals公司; Premix Taq酶, TaKaRa公司; QIAquick PCR纯化试剂盒, Qiagen公司。PCR仪, TaKaRa公司; NanoDrop ND-1000, Thermo Scientific公司。

### 1.3 测定方法

#### 1.3.1 DNA提取

土壤总DNA提取采用FastDNA<sup>®</sup> SPIN Kit for Soil,称取0.5 g冷冻土样,参照试剂盒内的使用说明提取总DNA。提取的总DNA用无菌水稀释10倍,储存在-30℃冰箱待用。

#### 1.3.2 Illumina高通量测序

以土壤总DNA的10倍稀释液为模板,以AMV4.5NF (5'-AAGCTCGTAGTTGAATTTGG-3')和AMDGR (5'-CCCAACTATCCCTATTAATCAT-3')为引物,对土壤样品中AM真菌的18S rRNA基因进行扩增<sup>[22]</sup>。此外,为了区分扩增产物,在每个样品的引物中添加一个5 bp的Barcode序列。PCR反应体系(50 μL): DNA模板(10 ng/μL) 5 μL, Premix Taq酶 25 μL,正、反向引物(10 μmol/L)各1 μL,无菌水 18 μL。PCR反应条件: 95℃ 5 min; 95℃ 45 s, 58℃ 45 s, 72℃ 1 min,共35个循环; 72℃ 7 min<sup>[23]</sup>。使用QIAquick PCR纯化试剂盒纯化PCR产物,并用NanoDrop ND-1000测定其含量。混合等量后在Illumina MiSeq<sup>™</sup> System平台上测序。

表 1 土壤pH值与重金属浓度、综合污染指数与潜在生态风险指数<sup>[4]</sup>Table 1 Soil pH and the concentrations, and comprehensive factor pollution and potential ecological risk indices of heavy metals<sup>[4]</sup>

样点 Sampling site	pH	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Cr (mg/kg)	Zn (mg/kg)	Ni (mg/kg)	综合污染生态风险 指数CPI	指数ERI
拆解园区Waste recycling plant	8.35	8.24	3 611.0	9 386.0	818.0	4 470	278.0	161.00	2 685.0
下脚料拆解点Scraps dismantling point	6.33	0.44	107.0	80.2	103.0	231	49.4	2.16	88.0
尾渣倾倒点Tailings dumping point	8.06	46.6	3 243.0	3 087.0	423.0	12 985	279.0	153.00	6 914.0
尾渣排放点Tailings discharge point	6.66	1.07	167.0	452.0	99.2	1 055	48.7	8.34	227.0
池塘底泥Sediment in pond	8.77	0.13	35.1	31.0	104.0	121	48.7	0.95	32.5
农田Farmland	6.23	0.28	52.6	43.8	95.6	160	48.1	1.20	56.3
农田Farmland	6.16	0.51	76.8	50.8	262.0	326	42.7	2.30	92.9
农田Farmland	5.52	0.24	50.3	51.0	177.0	144	44.4	1.53	52.9
农田Farmland	7.23	0.30	65.6	52.3	122.0	161	46.7	1.42	61.7
农田Farmland	5.10	0.51	280.0	79.2	90.7	295	47.1	5.00	117.0
农田Farmland	6.52	0.82	188.0	78.6	131.0	229	75.2	3.59	150.0
农田Farmland	5.47	1.47	179.0	87.2	120.0	262	65.3	4.94	233.0

注: 综合污染指数采用内梅罗指数法, 以温黄平原土壤环境背景上限值为污染评价标准<sup>[19]</sup>, Cd、Cu、Pb、Cr、Zn和Ni的权重分别为3、2、3、3、2、2<sup>[20]</sup>; 生态风险指数参照徐争启等<sup>[21]</sup>进行计算, 为多种重金属的潜在生态风险指数之和。

Note: The Nemerow index method was used to calculate the comprehensive factor pollution index (CPI). The upper limit of soil environmental background in the Wenhuan Plain was taken as the pollution evaluation standard<sup>[19]</sup>. The weights of Cd, Cu, Pb, Cr, Zn and Ni were 3, 2, 3, 3, 2 and 2, respectively<sup>[20]</sup>. The ecological risk index (ERI) was calculated according to Xu et al<sup>[21]</sup>, referring to the sum of the potential ecological risk indices of various heavy metals.

## 1.4 数据处理

测序结果使用微生物生态定量指标 (the quantitative insights into microbial ecology, QIIME) 软件包进行分析 ([http://qiime.org/tutorials/processing\\_18S\\_data.html](http://qiime.org/tutorials/processing_18S_data.html))。首先, 对18S rRNA基因序列进行质控(质量分>25、序列长度>200 bp), 根据Barcode序列将所有序列分配到相应样品后, 去除Barcode和引物序列。然后, 以97%为阈值筛分可操作分类单元(operational taxonomic unit, OTU)<sup>[24]</sup>, 并从中选择丰度最高的序列作为代表序列, 通过与Silva 128 数据库(<https://www.arb-silva.de/download/archive/qiime/>)比对获得每个OTU的分类学信息后, 分析AM真菌群落组成; 采用香农(Shannon)、辛普森(Simpson)和Chao1 指数来比较AM真菌多样性。采用Pearson相关分析对AM真菌多样性、各OTU的相对丰度与重金属污染指标进行相关性分析, 解析具有重金属高耐受性的OTU,

并使用*t*检验分析拆解/倾倒区和扩散区之间的差异显著性。所有统计分析均用SPSS 19进行, 数据处理和绘图使用Excel 2013 和Origin 2017 进行。

## 2 结果与分析

### 2.1 AM 真菌群落结构

所测土壤样品AM真菌由原囊霉目(Archaeosporales)、球囊霉目(Glomerales)和多孢囊霉目(Diversisporales)组成, 其中处于优势的球囊霉目约占94% (图1A); 在科水平上包括巨孢囊霉科(Gigasporaceae)、球囊霉科(Glomeraceae)和3个分类地位未定的科, 其中球囊霉科占据优势地位。各样点土壤AM真菌不同科的相对丰度存在一定差异(图1B), 但按样点类型来看, 拆解/倾倒区与扩散区未见明显的规律性差异。其中, 除拆解点C1 和C2 以外, 其余各土壤样品中均出现了多孢囊霉目或原囊霉目。

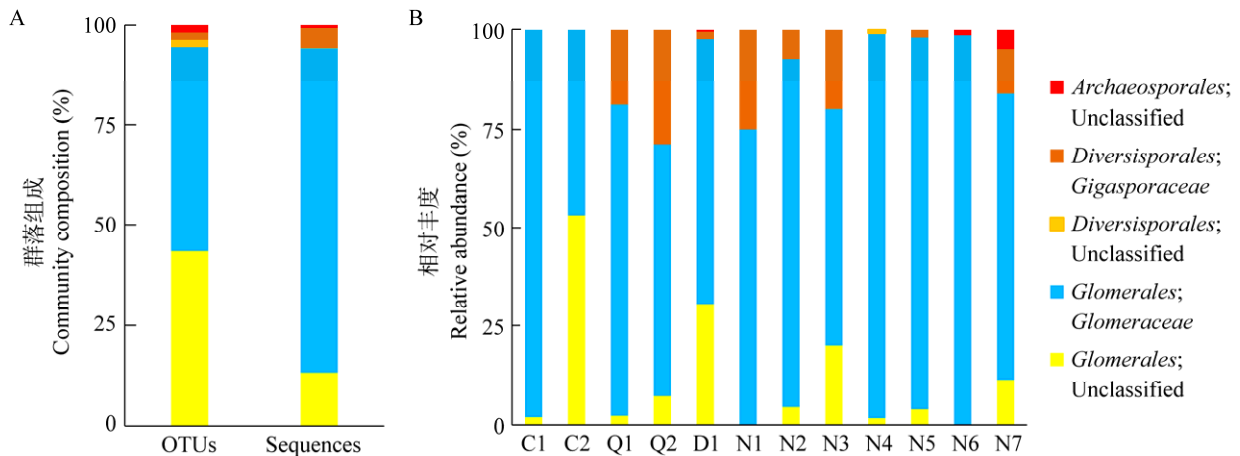


图1 土壤AM真菌的群落组成(A)及其相对丰度(B)

Figure 1 Soil arbuscular mycorrhizal fungal community composition (A) and the relative abundance (B)

## 2.2 AM 真菌多样性及与重金属污染的相关性分析

拆解/倾倒地土壤AM真菌的香农、辛普森和Chao1 指数与扩散区没有显著差异(图 2); 从中位值和均值来看, 拆解/倾倒地高于扩散区(辛普森指数中位值除外), 表明电子废弃物拆解、倾倒并没有降低土壤AM真菌多样性, 甚至刺激了一些高重金属耐受型AM真菌的生长。通过Pearson相关分析发现(表 2), AM真菌多样性指数与重金属浓度、综合污染指数及潜在风险指数间并不存在显著的相关关系。

## 2.3 疑似泡囊根生囊霉的 OTU 相对丰度及与重金属污染的关系

通过相关性分析发现(表 3), 疑似*Rhizophagus vesiculiferus*的OTU (相似度 $\geq 97\%$ )相对丰度与重金属浓度、综合污染指数及潜在生态风险指数间均呈极显著正相关关系。进一步对比中位值和均值发现(图 3), 该疑似*R. vesiculiferus*的OTU在拆解/倾倒地土壤中的相对丰度均远高于扩散区土壤, 这表明该AM真菌对土壤重金属污染具有较强的耐受性, 或可作为电子拆解造成的重金属污染土壤的潜在修复材料。

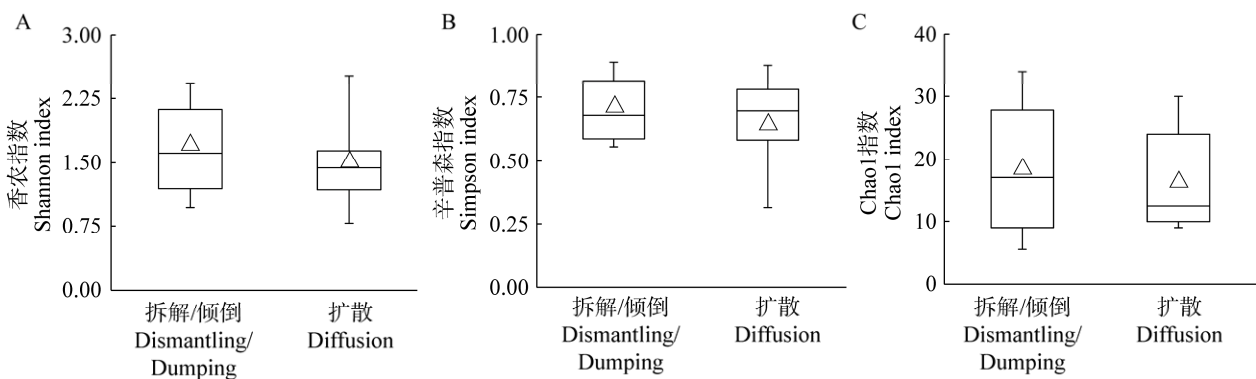


图2 土壤AM真菌香农(A)、辛普森(B)与Chao1 (C)指数

Figure 2 Soil arbuscular mycorrhizal fungal Shannon (A), Simpson (B) and Chao1 (C) indices

注:  $\triangle$ 代表均值。

Note:  $\triangle$  represents the mean value.

表 2 土壤AM真菌多样性指数与重金属浓度、综合污染指数(CPI)及潜在生态风险指数(ERI)的相关性

Table 2 The correlations between soil AM fungal diversity indices and the concentrations, the comprehensive factor pollution index (CPI), and the potential ecological risk index (ERI) of heavy metals

重金属污染指标 Pollution parameters of heavy metals	香农指数Shannon index		辛普森指数Simpson index		Chao1 指数Chao1 index	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
CPI	-0.313	0.161	-0.261	0.206	-0.411	0.092
ERI	-0.199	0.268	-0.198	0.268	-0.292	0.179
Cd	-0.014	0.337	-0.157	0.313	-0.220	0.246
Cu	-0.320	0.155	-0.262	0.205	-0.426	0.083
Pb	-0.350	0.132	-0.261	0.206	-0.425	0.084
Cr	-0.466	0.063	-0.420	0.087	-0.365	0.122
Zn	-0.018	0.289	-0.187	0.280	-0.267	0.201
Ni	-0.316	0.158	-0.263	0.204	-0.427	0.083

注: *r*: Pearson相关性; *P*: 显著性(单侧).Note: *r*: Pearson correlation; *P*: Significance (one-side).

表 3 疑似泡囊根生囊霉的OTU相对丰度与重金属浓度、综合污染指数(CPI)及潜在生态风险指数(ERI)的相关性

Table 3 Correlations between the relative abundance of *Rhizophagus vesiculiferus*-like OTUs and the concentrations, the comprehensive factor pollution index (CPI), and the potential ecological risk index (ERI) of heavy metals

重金属污染指标 Pollution parameters of heavy metals	疑似泡囊根生囊霉的OTU相对丰度 Relative abundance of <i>R. vesiculiferus</i> -like OTUs	
	<i>r</i>	<i>P</i>
CPI	0.977	0.000
ERI	0.762	0.004
Cd	0.614	0.034
Cu	0.982	0.000
Pb	0.962	0.000
Cr	0.954	0.000
Zn	0.729	0.007
Ni	0.964	0.000

注: *r*: Pearson相关性; *P*: 显著性(单侧).Note: *r*: Pearson correlation; *P*: Significance (one-side).

### 3 讨论

电子废弃物拆解/倾倒地和扩散区土壤AM真菌群落结构、多样性受到重金属复合污染的影响, 但AM真菌多样性指数与重金属污染指标间并无显著相关性(表2)。这与丁苏丽等<sup>[25]</sup>和赵祥伟等<sup>[26]</sup>的报道相一致, 可能是因为土壤中不同种类微生物对重金属复合污染的敏感性不同, 而且微生物群落之间存在着多种相互关系, 偏利或竞争

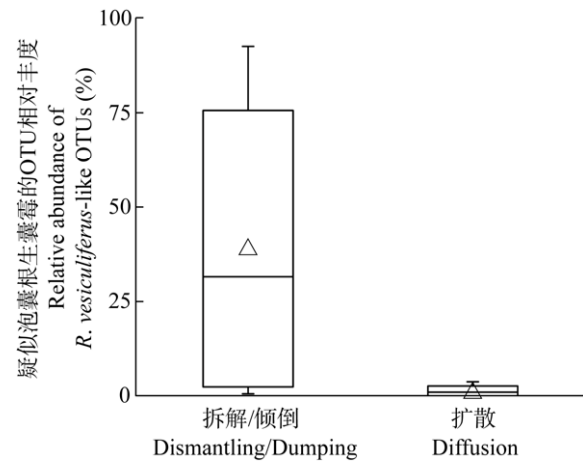


图 3 土壤AM真菌群落中疑似泡囊根生囊霉的OTU相对丰度

Figure 3 Relative abundance of *Rhizophagus vesiculiferus*-like OTUs in soil arbuscular mycorrhizal fungal community

注: △代表均值.

Note: △ represents the mean value.

可能会抵消其对重金属的响应<sup>[26]</sup>, 因此, 土壤微生物多样性指数与重金属指标间往往不呈现显著相关关系。当然, 环境因子差异也会掩盖重金属污染对土壤微生物的影响。在本研究中, 各采样点土壤pH存在一定差异(表1), 一方面会直接影响重金属的生物有效性和毒性作用<sup>[27-28]</sup>, 另一方面会因为AM真菌对pH值的适应范围不同而导致群落组成不同<sup>[29]</sup>。此外, 土壤有机质、氮、磷等的差异也可能会影响AM真菌对重金属的响应<sup>[15,30-31]</sup>,

多种因素互作最终导致土壤AM真菌多样性与重金属指标间无显著关系。

电子废弃物拆解/倾倒区和扩散区土壤AM真菌均由原囊霉目、球囊霉目和多孢囊霉目组成,其中球囊霉目占据优势地位(图 1A),这与广东大宝山矿区重金属污染土壤中AM真菌的群落组成情况相似<sup>[32]</sup>。尽管拆解/倾倒区比扩散区具有更加严重的土壤重金属污染(表 1),但土壤AM真菌多样性指数却未呈现显著差异(图 2),表明重金属含量升高对AM真菌群落的组成并没有造成显著的影响,这可能与部分AM真菌类群对重金属具有较强的耐受性有关<sup>[33-34]</sup>。例如,拆解/倾倒区土壤AM真菌群落中疑似*R. vesiculiferus*的OTU相对丰度远高于扩散区土壤,而且与重金属浓度、综合污染指数及潜在生态风险指数间均呈显著正相关关系(表 3)。在Pb (1 585 mg/kg)、Zn (525 mg/kg)和Cd (8.8 mg/kg)复合污染土壤的修复试验中发现,*R. vesiculiferus*的丰度随着重金属浓度的下降而降低<sup>[35]</sup>。重金属浓度升高能促进*R. vesiculiferus*相对丰度的增长,表明*R. vesiculiferus*对重金属污染具有强耐受性,或可作为重金属污染土壤的潜在修复材料。然而,目前对*R. vesiculiferus*的了解仍停留在污染场地调查采样及分子生物学分析阶段,其对重金属的响应机理和耐受机制尚缺乏系统研究。此外,AM真菌生态功能的发挥还会受到植物种类及土壤性质等因素的影响<sup>[29,36]</sup>。因此,将*R. vesiculiferus*用于电子废弃物拆解区重金属污染土壤修复的可行性仍有待进一步探究。

#### 4 结论

(1) 电子废弃物拆解场地及周边区域土壤中均存在一定丰度的AM真菌,其中球囊霉目和球囊霉科分别为目和科水平上的优势类群。

(2) 拆解/倾倒区和扩散区土壤AM真菌群落结构存在一定差异,但土壤重金属污染对AM真菌多样性指数的影响较小,两者之间没有显著差异。

(3) 拆解/倾倒区土壤AM真菌群落中疑似

*R. vesiculiferus*的OTU相对丰度远高于扩散区,而且与重金属浓度、综合污染指数及潜在生态风险指数间均呈显著正相关关系。

(4) 土壤AM真菌中*R. vesiculiferus*对重金属污染可能具有极强的耐受性,或可为重金属污染土壤的修复提供技术支撑。

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