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《环境科学》征订启事(4061) 《环境科学》征稿简则(4132) 信息(4233, 4293, 4304)

土壤微生物群落结构对生活源和工业源再生水灌溉的差异化响应

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摘要: 为研究不同来源再生水灌溉对土壤微生物群落结构的影响,以地下水灌溉土壤为对照,采用 Illumina MiSeq 高通量测序技术对长期利用生活源和工业源再生水灌溉的土壤微生物群落结构进行分析,进一步探究土壤环境因子及其相互作用对微生物群落结构的影响。结果表明,与地下水灌溉相比,长期生活源再生水灌溉可显著提高土壤中 TOC、DOC、Eh、 NH_4^+ -N 和 TP 的含量,长期工业源再生水灌溉导致 Cd、Cr、Cu、Pb 和 Zn 在表层土壤大量累积。再生水灌溉显著增加了土壤中 Acidobacteria 和 Planctomycetes 的相对丰度,降低了 Firmicutes 和 Tectomicrobia 的相对丰度,且不同来源再生水对土壤中功能微生物的影响不同,生活源再生水灌溉可显著增加 Chloroflexi 和 Nitrospirae 的相对丰度,而工业源再生水灌溉对 Actinobacteria 具有显著的抑制作用。db-RDA 分析结果表明,生活源再生水灌溉土壤菌群主要受 TN、TP、DOC 和 Eh 影响 ($P < 0.05$),工业源再生水灌溉土壤菌群主要受重金属影响 ($P < 0.05$)。长期再生水灌溉可改变土壤环境因子间的相互作用,进而影响微生物群落结构,生活源再生水灌溉土壤中微生物主要受 DOC、TN 和 TP 等营养物质含量的增加和氧化还原条件的改变控制,工业源再生水灌溉土壤中微生物与重金属的积累显著相关。

关键词: 再生水灌溉; 生活源; 工业源; 微生物群落; 环境因子

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Different Responses of Soil Microbial Community Structure to Irrigation with Treated Wastewater from Domestic and Industrial Sources

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Abstract: To investigate the long-term effects of irrigation with treated domestic and industrial wastewater on the microbial community structure of the soil, Illumina MiSeq high-throughput sequencing technology was applied. Groundwater irrigated soil was used as a control. The effects of soil environmental factors and their interactions on the microbial community structure were investigated. Compared with the groundwater irrigation, irrigation with treated domestic wastewater can significantly increase the contents of TOC, DOC, Eh, NH_4^+ -N, and TP, whereas irrigation with treated industrial wastewater can increase the contents of Cd, Cr, Cu, Pb, and Zn in the soil. Irrigation with treated wastewater also increases the relative abundance of Acidobacteria and Planctomycetes, and reduces the relative abundance of Firmicutes and Tectomicrobia. The effects of treated wastewater from different sources on functional microorganisms in soil are also different; irrigation with treated domestic wastewater can increase the relative abundance of Chloroflexi and Nitrospirae, whereas irrigation with treated industrial wastewater has negative effects on the abundance of Actinobacteria. The results of db-RDA analysis show that TN, TP, DOC, and Eh are the main factors that impact the microbial communities in soils irrigated with treated domestic wastewater ($P < 0.05$), and heavy metals are the main factors that impact the microbial communities in soils irrigated with treated industrial wastewater ($P < 0.05$). Compared with groundwater irrigation, treated wastewater irrigation can change the correlations between soil environmental factors, which in turn affect the microbial community structure. The growth of microorganisms in soils irrigated with treated domestic wastewater is mainly controlled by the increase in the nutrients such as DOC, TN, and TP and changes in soil redox conditions. The abundance of microorganisms in soil irrigated with treated industrial wastewater is significantly correlated with the accumulation of heavy metals.

Key words: treated wastewater irrigation; domestic; industrial; microbial community; environmental factors

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随着气候变化、人口的增长和工农业的发展,水资源短缺及水环境污染成为我国水资源可持续利用的两大挑战,也成为农业发展的两大限制性因素^[1,2].再生水是缓解水资源短缺的一种可持续性替代水源,也是我国干旱半干旱地区农业灌溉的重要补充水源^[3].随着再生水在农业灌溉中的广泛应用,长期再生水灌溉对土壤生态环境尤其是微生物群落结构的影响也受到广泛关注.

土壤微生物是陆地生态系统的重要组成部分,微生物群落的动态变化对土壤生态功能具有重要的影响^[4,5],具体表现在调节土壤肥力、养分循环、植物生长及土壤中污染物降解转化等方面^[6,7].有研究表明,土壤微生物群落结构受多重因素的影响,包括土壤形态、植物类型、温度、水分、灌溉水质和农业管理措施等^[8-11].灌溉水质是土壤中微生物群落结构发生变化的主导因素之一,再生水中含有大量溶解性有机质、重金属、抗生素、病原体及其它外源微生物,再生水灌溉可显著提高土壤 pH、EC、K、Na 和 DOC 含量,使得变形杆菌丰度增加,放线菌丰度降低^[12].目前,再生水灌溉对土壤生态环境的影响已得到广泛研究,但多关注于单个环境因子变化对微生物群落结构的影响^[13,14].然而,土壤中微生物群落结构的变化是多种因素共同作用的结果,因此,再生水灌溉引起的环境因子及环境因子间相互作用的变化对微生物群落结构的影响同样不容忽视.

本研究以地下水灌溉土壤为对照,对比分析了长期生活源再生水灌溉和工业源再生水灌溉对土壤理化特性、重金属累积及微生物群落结构的影响,识别了不同来源再生水灌溉土壤中影响微生物群落结构的主要因素.通过进一步探究不同来源再生水灌溉条件下,环境因子间相互作用的变化及其对微生物群落结构的影响,以期科学指导再生水在农业灌溉中的

应用及改善土壤生态环境提供理论依据.

1 材料与方法

1.1 样品采集

本研究中土壤样品为 2018 年 9 月采集于河北省石家庄市附近,其中生活源再生水灌溉区位于洮河附近(E 114.543 66°~114.546 32°; N 37.891 92°~37.894 22°),该河流主要接收来自城南生活污水处理厂排放的尾水;工业源再生水灌溉区位于辛集总干渠附近(E 115.347 28°~115.347 52°; N 37.945 79°~37.946 24°),该干渠主要接收来自辛集工业园污水处理厂排放的尾水;地下水灌溉区位于藁城市东庄村附近(E 114.532 71°, N 37.883 51°),距离洮河及辛集总干渠约 10 km,可认为不受再生水影响.研究区的土地利用类型为耕地,作物以玉米和小麦为主,为缓解水资源短缺并促进农作物生长,洮河及辛集总干渠附近的耕地常年从河流引水灌溉.

再生水的基本理化性质见表 1,两种再生水中 DOC、BOD、COD、TN、Cd、Cr、Pb 和 Zn 含量显著高于地下水,生活源再生水中 DOC、BOD、COD、TN 和 TP 含量显著高于工业源再生水,而工业源再生水中 Cd、Cr、Cu、Pb 和 Zn 含量显著高于生活源再生水.

3 个灌溉区均采用相同或相近的农业管理措施的,保证灌溉水是土壤质量的主要影响因素.分别采集生活源和工业源再生水灌溉区表层(0~20 cm)土壤样品,每个灌溉区设置 3 个采样区,在每个采样区随机采集 10 个表层土壤样本并混合,然后用 2 mm 筛去除土壤植物根系及碎石.每个样品取两份平行样,一份冻干后置于 -20℃^[15,16]环境中用于土壤理化指标测定,另一份置于 -80℃环境中用于土壤微生物指标测定.

表 1 灌溉水理化指标¹⁾(平均值±标准偏差)/mg·L⁻¹

Table 1 Physicochemical properties of irrigation water (Mean±SD)/mg·L⁻¹

样品 ²⁾	pH	DOC	BOD	COD	TN	TP	Cd	Cr	Cu	Pb	Zn
GW	7.2±0.3ab	1.6±0.5a	2.8±0.7a	13.4±2.1a	0.5±0.1a	0.1±0.0a	0.0±0.0a	2.1±0.5a	2.3±0.6a	0.1±0.0a	1.8±0.3a
DTWW	7.9±0.2a	32.4±4.2b	61.8±12.4b	159.0±31.8b	6.3±1.3b	1.0±0.1b	0.1±0.0b	4.1±0.8b	2.7±0.5a	5.6±1.3b	20.4±3.1b
ITWW	7.2±0.2b	14.7±0.8c	25.2±6.7c	63.2±4.2c	2.2±0.35c	0.1±0.0a	21.4±2.29c	26.6±3.8c	57.8±7.0b	45.6±8.5c	63.7±8.3c

1)采用 Duncan's multiple range test 方法分析,不同字母表示显著性差异($P < 0.05, n = 3$); 2)GW、DTWW 和 ITWW 分别表示地下水、生活源再生水和工业源再生水样品,下同

1.2 理化指标测定

土壤样品 pH、Eh、CEC、TN 和 NH_4^+ -N 指标的测定参照文献[17].使用重铬酸钾氧化-分光光度计测定总有机碳(TOC);使用岛津公司的 5000 TOC 分析仪测定溶解性有机碳(DOC);土壤总磷(TP)浓度采用硫酸-高氯酸消煮法^[18]进

行测定.本研究选取的重金属指标(Cd、Pb、Cu、Cr 和 Zn)在再生水中含量较高且明显超出《地表水环境质量标准》(GB 3838-2002) V 类水质标准,重金属浓度测定参考 Vieira 等^[19]的方法,土壤样品经反王水消解,利用电感耦合等离子体质谱法进行测定.

1.3 DNA 提取和 16S rRNA 高通量测序

DNA 提取采用 E. Z. N. A 土壤 DNA 试剂盒 (Omega Bio-Tek, Norcross, GA, US) 进行, 方法和步骤参照试剂盒说明书. 以 338F (5'-ACTCCTACGG GAGGCAGCAG-3') 和 806R (5'-GGACTACHVGGG TWTCTAAT-3') 为引物^[20,21], 对细菌 16S rRNA 基因 V3-V4 可变区进行 PCR 扩增^[22]. 纯化后的 PCR 产物交由上海美吉生物科技有限公司在 Illumina MiSeq 测序平台进行高通量测序.

1.4 数据分析

原始 DNA 序列采用 QIIME 软件进行序列的拼接和去杂, 使用 USEARCH (version 7.0 <http://drive5.com/uparse/>) 对序列进行嵌合体检测, 采用 UPARSE-OTU 算法以 97% 的序列相似性划分分类单元 (OTUs, operational taxonomic units)^[23,24]. 通过与 Silva 9 (Release128 <http://www.arb-silva.de>) 分类数据库对比对 OTUs 进行注释.

1.5 统计学分析

本研究中统计学分析采用 R 语言 (3.6.1)、STAMP 及 SPASS 22.0 完成. 利用 SPASS 22.0 完成土壤环境因子单因素方差分析 (ANOVA); 利用“vcd”和“ggtern”包进行了微生物 (科水平) 群落分布情况分析; 利用 STAMP 完成了土壤中微生物 (门水平相对丰度排序 Top 10) 组间差异显著性检验; 基于相似性分析 (analysis of similarities: ANOSIM) 及微生物群落的组间差异性分析, 利用 R 语言中“vegan”包完成环境因子与微生物群落的 db-RDA 分析; 利用“ggcor”包完成了基础理化指标、重金属和微生物群落 Pearson 相关分析.

2 结果与讨论

2.1 土壤理化特性

不同灌溉土壤的基础理化指标如表 2 所示. 3 种土壤均呈现弱碱性, pH 和 TN 差异不显著, 这可能与土壤及农作物类型有关. 长期再生水灌溉对土壤 pH

的影响因土壤类型或种植作物的不同而呈现不同的结果. 如: 放牧土壤 pH 显著升高^[25], 而种植生菜、柑橘等作物的土壤 pH 下降^[26]. 与地下水相比, 生活源再生水灌溉显著提高土壤中 TOC、DOC、Eh、NH₄⁺-N 和 TP 的含量 ($P < 0.05$), 工业源再生水灌溉显著提高土壤中 DOC、Eh、CEC 及 NH₄⁺-N 的含量 ($P < 0.05$). 这与已有的研究结果相符, 再生水灌溉可提高土壤 pH、EC、K、Na、DOC、TOC、NH₄⁺-N、TP 及其它养分元素的含量^[27~29]. 然而, 对比两种再生水灌溉土壤发现, 除 CEC 外, 生活源再生水灌溉土壤中基础理化指标值均高于工业源再生水灌溉土壤, 这主要与灌溉水理化性质和土壤特性的差异有关.

不同灌溉土壤重金属含量如表 3 所示, 按照《土壤环境质量农用地土壤污染风险管控标准 (试行)》(GB 15618-2018) 的要求, 3 种灌溉土壤中的 Cd 含量和工业源再生水灌溉土壤中 Pd、Zn 含量均明显超出农用地土壤污染风险筛选值, 且工业源再生水灌溉土壤中 Cd、Pb 和 Zn 的含量显著高于其它两种灌溉土壤, 表明长期工业源再生水灌溉导致 Cd、Pb 和 Zn 在表层土壤中大量积累. 再生水灌溉土壤中 Cu 和 Cr 的含量显著高于对照组, 且在工业源再生水灌溉土壤中含量最高. 上述结果表明, 长期再生水灌溉可明显提高土壤中重金属的含量^[30]. 本研究中, 工业源再生水主要来源于制革企业排放的废水, 重金属含量高于生活源再生水, 是造成该区域土壤中 Pb、Zn 含量超出国家标准及 Cu、Cr 大量积累的重要原因.

综合比较, 再生水灌溉可改变土壤理化特性, 不同来源再生水灌溉对土壤理化特性的影响不同. 除 CEC 外, 生活源再生水灌溉土壤中的基础理化指标值均高于工业源再生水灌溉土壤, 而工业源再生水灌溉土壤中重金属累积程度明显高于生活源再生水灌溉土壤. 因此, 长期生活源再生水灌溉可显著影响土壤的基础理化指标, 长期工业源再生水灌溉导致表层土壤重金属大量累积.

表 2 土壤基础理化指标 (平均值 ± 标准偏差)

Table 2 Basic physiochemical properties of soils (Mean ± SD)

样品	pH	TOC /mg·kg ⁻¹	DOC /mg·kg ⁻¹	Eh	CEC /mol·kg ⁻¹	TN /mg·kg ⁻¹	NH ₄ ⁺ -N /mg·kg ⁻¹	TP /mg·kg ⁻¹
GWs	7.4 ± 1.0a	12.4 ± 0.2a	1.2 ± 0.0a	64.4 ± 5.9a	17.1 ± 1.4a	2.0 ± 0.1a	22.3 ± 0.6a	0.7 ± 0.1a
DTWWs	7.7 ± 1.2a	15.1 ± 1.4b	1.9 ± 0.2b	96.6 ± 4.9b	17.9 ± 1.1a	3.0 ± 1.1a	35.7 ± 3.5b	0.9 ± 0.1b
ITWWs	7.4 ± 1.0a	14.1 ± 3.5ab	1.3 ± 0.1c	80.3 ± 5.6c	24.0 ± 1.8b	1.8 ± 0.3a	32.8 ± 6.1b	0.7 ± 0.0a

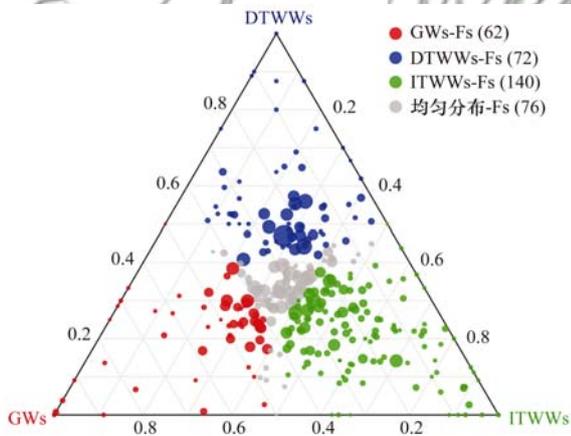
表 3 土壤重金属指标 (平均值 ± 标准差) /mg·kg⁻¹

Table 3 Heavy metal properties of soils (Mean ± SD) /mg·kg⁻¹

样品	Cd	Cr	Cu	Pb	Zn
GWs	1.1 ± 0.2a	51.2 ± 0.7a	23.7 ± 0.7a	21.0 ± 0.9a	69.2 ± 0.5a
DTWWs	0.8 ± 0.5a	56.5 ± 1.0b	30.9 ± 0.3b	11.3 ± 0.8b	86.0 ± 2.1b
ITWWs	2.6 ± 0.2b	70.1 ± 1.8c	60.1 ± 0.4c	182.5 ± 3.0c	585.7 ± 5.1c

2.2 土壤微生物的群落结构

通过对 16S rRNA 高通量测序结果进行分析,共得到 311 086 条有效序列,它们分别属于 41 个门、99 个纲、192 个目及 366 个科. 为了解再生水灌溉对土壤中微生物群落结构的影响,本研究利用三元相图对 3 种灌溉土壤中微生物(科水平)群落结构进行对比分析(图 1),地下水、生活源再生水和工业源再生水灌溉土壤中分别富集了 62、72 和 140 个科的细菌,它们分别属于 12、17 和 29 个门类;此外,有 76 个科的细菌在 3 种灌溉土壤中分布较为均衡. 结果表明,相较于地下水,再生水灌溉可明显增加土壤中微生物的种类和丰度. 研究表明,再生水中含有大量溶解性有机质和多种营养物质,可为微生物提供速效碳源和氮源,激发某些微生物的生长和繁殖^[23];同时,再生水中外源微生物的输入也是土壤中微生物种类和丰度增加的原因之一^[31]. 对比两种再生水灌溉土壤中微生物群落结构发现,不同来源再生水对土壤中微生物群落的影响不同,工业源再生水灌溉土壤中富集了种类更多和相对丰度更高的微生物,对微生物群落分布的影响更为显著.



3 个顶点分别表示地下水(GW)、生活源再生水(DTWW)和工业源再生水(ITWW)灌溉土壤中的微环境区域,每个点代表一类科水平微生物,点的大小代表微生物在 3 种土壤环境中相对丰度的高低,点的位置由土壤微环境对微生物相对丰度的贡献来确定,Fs 表示科水平微生物的种类

图 1 微生物在 3 种土壤中的分布图(科水平)

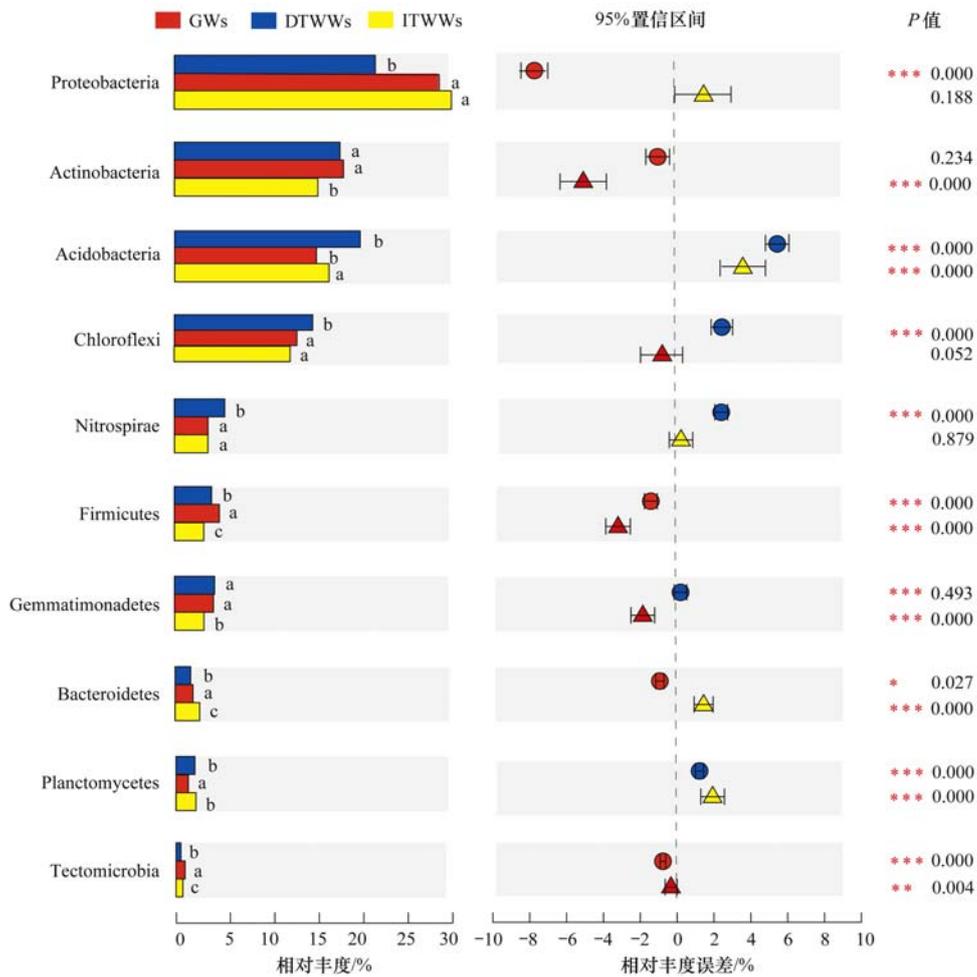
Fig. 1 Distribution of microorganisms in the three types of soil (family level)

优势微生物在土壤中相对丰度较高且在生态功能调控中发挥着重要作用. 为更直观地反映再生水灌溉对土壤优势微生物的影响,选取丰度排名前 10 的门类,包括:变形菌门(Proteobacteria)、放线菌门(Actinobacteria)、酸杆菌门(Acidobacteria)、绿弯菌门(Chloroflexi)、硝化螺杆菌门(Nitrospirae)、厚壁菌门(Firmicutes)、芽单胞菌门(Gemmatimonadetes)、拟杆菌门(Bacteroidetes)、浮霉菌门

(Planctomycetes) 和护微菌门(Tectomicrobia). 对 3 种灌溉土壤中优势微生物相对丰度的差异进行显著性检验(图 2),3 种灌溉土壤中优势微生物相对丰度差异明显. 与地下水灌溉相比,再生水灌溉显著增加了土壤中 Acidobacteria 和 Planctomycetes 的相对丰度,并使得 Firmicutes 和 Tectomicrobia 相对丰度显著降低. Acidobacteria 是土壤微生物的重要类群,占比超过 50%^[32,33],其丰度与土壤有效氮含量呈正相关^[34]. 再生水灌溉土壤中 Acidobacteria 相对丰度的显著增加,可能是因为生活源再生水和工业源再生水灌溉土壤中铵态氮和硝态氮含量显著高于地下水灌溉土壤(铵态氮含量分别为 35.7、32.8 和 22.3 mg·kg⁻¹;硝态氮含量分别为 179.9、168.6 和 130.8 mg·kg⁻¹). Planctomycetes 在厌氧条件下可将铵态氮转化为氮气并以此来获取能量,是污水处理中重要的脱氮功能菌^[35],因此,本研究中 Planctomycetes 相对丰度显著增加可能和灌溉水的外源输入有关. 与地下水相比,生活源再生水灌溉使得土壤中 Acidobacteria、Chloroflexi、Nitrospirae 及 Planctomycetes 相对丰度显著增加,Proteobacteria、Firmicutes、Bacteroidetes 及 Tectomicrobia 相对丰度显著降低;工业源再生水灌溉使得土壤中 Acidobacteria、Bacteroidetes 及 Planctomycetes 相对丰度显著增加,Actinobacteria、Firmicutes、Gemmatimonadetes 及 Tectomicrobia 相对丰度显著降低(图 2),这可能与灌溉水的外源微生物输入、土壤中营养物质及重金属的含量变化有关. Frenk 等^[36]的研究也发现,与地下水相比,再生水灌溉可显著降低土壤中 Actinobacteria 和 Firmicutes 的丰度,且 Actinobacteria 丰度与土壤中硝态氮和铵态氮含量呈负相关. Gemmatimonadete 具有很强的脱氮功能,其相对丰度随着土壤中氮素水平的增加为降低^[37]. 生活源再生水和工业源再生水灌溉土壤中优势微生物相对丰度的差异,主要与再生水水质有关^[12]. 不同来源再生水中营养物质类型不同,根据营养物质的输入类型会促进或抑制特定微生物的生长繁殖^[38]. 此外,再生水灌溉可显著影响土壤理化特性,而优势微生物的相对丰度与土壤理化特性密切相关.

2.3 土壤环境因子对微生物群落结构的影响

基于 Bary-Curtis 距离估算了 3 种灌溉土壤中微生物群落结构的差异(ANOSIM). 结果如图 3 所示,3 种灌溉土壤之间微生物群落结构差异显著($P < 0.05$). 为进一步探究造成这一差异的环境因素,本研究基于 Bary-Curtis 距离,将主坐标分析(PCOA)计算的样本得分应用到冗余分析(RDA)中,进行更适用于微生物群落与环境因子的基于距离的冗余分



圆形表示生活源再生水灌溉与地下水灌溉土壤中优势微生物相对丰度误差；三角形表示工业源再生水灌溉与地下水灌溉土壤中优势微生物相对丰度误差；* 表示 $P < 0.05$ ，** 表示 $P < 0.01$ ，*** 表示 $P < 0.001$ ，为微生物在两种土壤中相对丰度差异显著；不同字母表示 3 种土壤中微生物相对丰度差异显著性 ($P < 0.05, n = 3$)

图 2 土壤中优势微生物相对丰度对比分析 (门水平)

Fig. 2 Comparative analysis of the relative abundance of dominant microorganisms in soil (phylum level)

析 (db-RDA). 结果如图 4 所示, 3 种灌溉土壤样品点分区不同, 表明地下水、生活源再生水和工业源再生水灌溉对土壤微生物群落结构的影响存在显著差异, 这与 ANOSIM 分析结果一致.

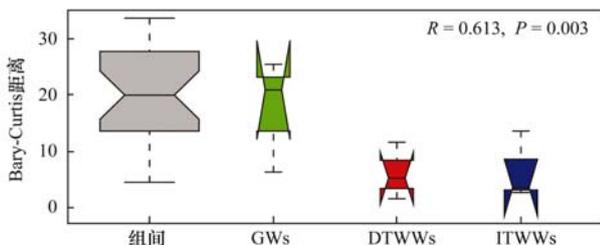


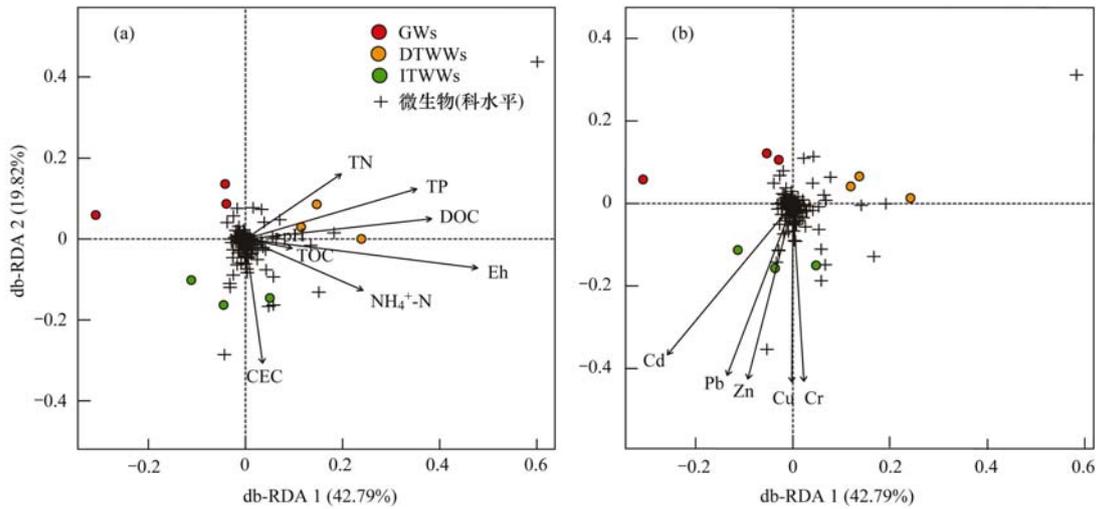
图 3 土壤中微生物群落差异分析

Fig. 3 Analysis of microbial community differences in soil

db-RDA 分析结果表明 (图 4), 轴一和轴二的解释率分别为 42.79% 和 19.82%, 总解释率超过 60%. 基础理化指标与微生物群落的 db-RDA 分析表明 [图 4(a)], 生活源再生水灌溉土壤中微生物群落与 TN、TP、DOC 及 Eh 呈正相关, 表明 TN、

TP、DOC 及 Eh 是生活源再生水灌溉土壤菌群的关键影响因子 ($P < 0.05$). 重金属与微生物群落的 db-RDA 分析结果表明 [图 4(b)], 各项重金属指标与工业源再生水灌溉土壤中微生物群落呈正相关, 表明工业源再生水灌溉土壤菌群主要受重金属累积的影响 ($P < 0.05$). 以往的指纹技术研究也表明, 重金属可显著影响土壤微生物群落结构^[39]. 本研究中重金属与微生物群落间的显著正相关关系, 可能是由于在长期的工业源再生水灌溉条件下, 重金属不断累积, 诱导了具有重金属耐受性细菌的生长和大量繁殖^[40], 后续研究中有待于对这类重金属耐受细菌的种类和功能进行更详细和深入的研究.

以上结果表明, 土壤微生物群落结构受环境因子的影响. 长期再生水灌溉改变土壤理化特性, 进而改变微生物群落结构. 结合表 2 和表 3 可知, 生活源再生水灌溉主要通过改变基础理化指标从而影响微生物群落结构, 工业源再生水灌溉主要通过增加重



(a) 微生物与基础理化指标的 db-RDA 分析; (b) 微生物与重金属的 db-RDA 分析

图 4 微生物与环境因子之间的 db-RDA 分析

Fig. 4 The db-RDA analysis of microbial and environmental factors

金属含量从而影响微生物群落结构。

2.4 土壤环境因子相互作用对微生物群落结构的影响

目前,土壤中单个环境因子对微生物群落结构的影响已得到广泛研究,因土壤质地、灌溉水质、气候条件等的差异,得出的结论也不同,如土壤 pH 是影响微生物群落的重要因素,尤其是对 Acidobacteria 影响显著^[41];外源易分解有机物质的输入是造成土壤细菌生物量升高的重要原因^[42]。然而,土壤是一个复杂的动态体系,由于多因素相互作用的潜在复杂性,势必给准确评估微生物群落结构的影响因素带来困难^[43]。在不考虑非环境因素条件下,环境因子间相互作用可能对微生物群落结构具有重要影响。因此,本研究基于物种丰度,对微生物(门水平)进行层次聚类(图 5),共得到 3 个不同的组群 (Group1 ~ Group3)。其中, Group1 以

Proteobacteria、 Acidobacteria、 Actinobacteria、 Chloroflexi 和 Bacteroidetes 为主, Group2 以 Verrucomicrobia、 Cyanobacteria、 Parcubacteria 和 Armatimonadetes 为主, Group3 以 Chlamydiae、 SBR1093 和 GAL15 为主。通过 Pearson 相关建立环境因子相互作用与微生物群落的关系(图 6)。

结果表明,与对照组相比,生活源再生水灌溉土壤中 DOC 对 Group2 和 Group3 影响显著,且对 Group2 表现为正效应,对 Group3 表现为负效应; TN 对 Group1 和 Group2 影响显著,且对 Group1 表现为负效应,对 Group2 表现为正效应; TP 对 Group2 表现为显著正效应; pH、 Eh、 CEC、 TOC 及 NH₄⁺-N 对微生物群落的影响也发生明显变化。这可能与该土壤中 DOC、 TN 和 TP 等养分元素含量的增加有关,生活源再生水灌溉为土壤微生物提供足够的碳源、氮源等易于代谢的养分元素,激发了特定微生物的

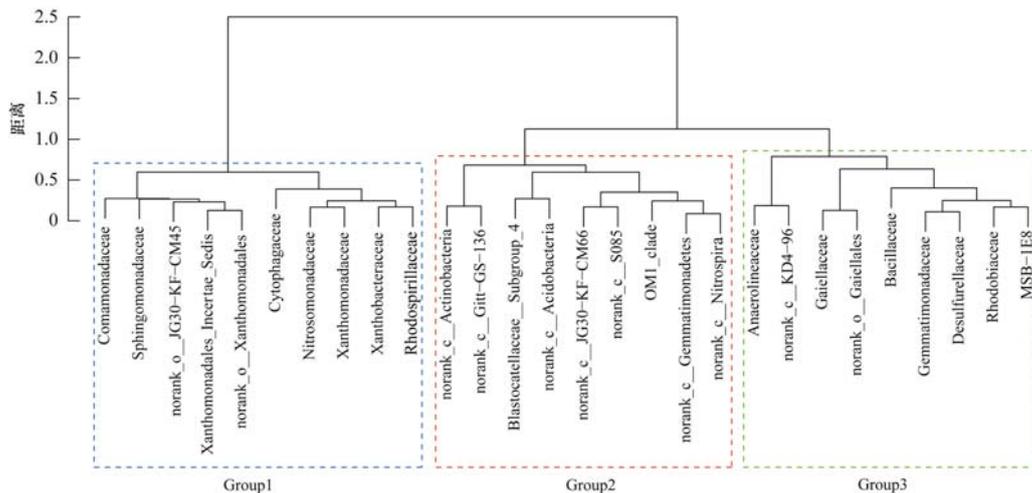
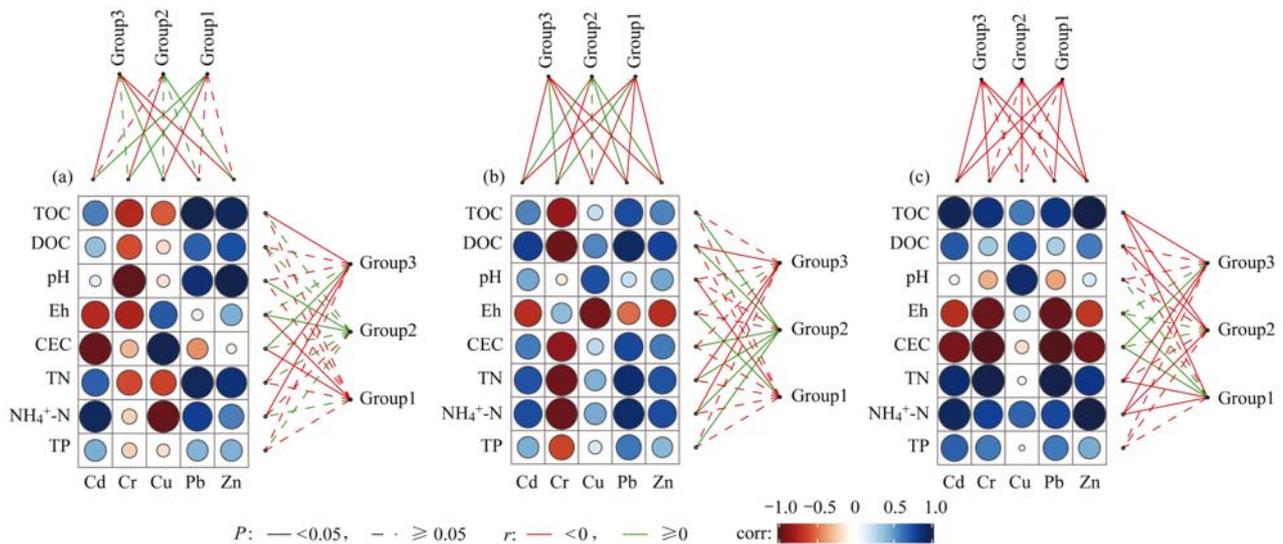


图 5 微生物聚类分析(门水平)

Fig. 5 Microbial cluster analysis (phylum level)



(a)、(b)和(c)分别表示 GW、DTWW 和 ITWW 灌溉土壤基础理化指标与重金属指标相互作用对微生物群落结构的影响

图 6 基础理化指标与重金属相互作用对微生物群落结构的影响

Fig. 6 Effects of interaction between basic physiochemical indexes and heavy metals on microbial community structure

活性^[44]. 土壤 pH 和氧化还原条件的改变也会影响微生物群落结构^[13,14], 生活源再生水灌溉增强了 Group1 对土壤 pH 和氧化还原条件的敏感性. 此外, 生活源再生水灌溉改变了 Cd 与 Group1、Group2, Cu 与 Group3 之间的相关性, 增强了 Cr、Pb 和 Zn 对微生物群落结构的影响. 这可能是 Eh 与重金属相互作用的结果, 生活源再生水灌溉改变了土壤氧化还原条件, 从而影响了重金属的环境行为^[45].

与地下水相比, 工业源再生水灌溉使得土壤中 TOC、TN 和 $\text{NH}_4^+\text{-N}$ 对 Group1 表现为显著负效应; TOC 和 DOC 对 Group2 表现为显著负效应; Eh 和 CEC 对微生物群落结构的影响也发生明显变化. 有研究表明, 重金属的大量累积不利于土壤中 C、N 循环(如: 加剧 C、N 矿化)^[46], 这可能是导致上述指标对微生物群落结构表现为负效应的原因. 此外, 工业源在再生水灌溉土壤中重金属与微生物群落均表现为负相关, 推测是土壤中重金属大量累积造成的. 重金属含量较高, 土壤中, Cu、Zn、Pb 和 Cd 与微生物量均呈现负相关^[47]. 不同生物类群对重金属的耐受程度不同^[48], 耐受性较差的微生物会因重金属含量的增加而受到抑制.

以上结果表明, 再生水灌溉改变了土壤中环境因子间的相互作用, 进而影响微生物群落结构. 生活源再生水灌溉主要通过改变土壤基础理化指标进而影响微生物群落结构. 工业源再生水灌溉土壤中微生物群落结构主要受重金属影响.

3 结论

(1) 不同来源再生水灌溉对土壤理化特性影响

不同, 生活源再生水灌溉显著提高了土壤中 TOC、DOC、Eh、 $\text{NH}_4^+\text{-N}$ 和 TP 的含量, 而工业源再生水灌溉导致 Cr、Cd、Cu、Pb 和 Zn 在表层土壤中的大量积累.

(2) 再生水灌溉显著增加土壤中 Acidobacteria 和 Planctomycetes 的相对丰度, 降低 Firmicutes 和 Tectomicrobia 的相对丰度. 然而不同来源再生水对土壤中功能微生物的影响也不同, 生活源再生水灌溉可显著增加 Chloroflexi 和 Nitrospirae 的相对丰度, 而工业源再生水灌溉对 Actinobacteria 具有显著的抑制作用.

(3) 不同来源再生水灌溉导致微生物群落结构发生变化的驱动因子不同, 生活源再生水灌溉通过改变土壤的基本理化指标, 从而驱动微生物群落结构改变; 而重金属含量则是工业源再生水灌溉导致微生物群落结构改变的主要驱动因子.

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