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棉秆炭调控对碱性镉污染水稻根际土壤真菌群落结构和功能的影响

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摘要: 为研究棉秆炭对碱性镉污染水稻根际土壤真菌多样性、结构及功能的影响, 采用室外水稻盆栽试验, 在土壤中添加为 0、1 和 8 mg·kg⁻¹ 的镉和炭土质量比分别为 0%、1% 和 5% 的棉花秸秆生物炭, 以水稻成熟期根际土壤为研究对象, 借助 Illumina HiSeq 高通量测序手段分析棉秆炭与镉污染条件对碱性水稻根际土壤真菌群落多样性、结构及潜在功能的影响, 探寻不同土壤环境因子与真菌群落结构间的关联性。结果表明: ① 施用棉秆炭显著提高了土壤 pH、速效养分和有机质等指标, 并降低土壤中可还原态镉的含量 ($P < 0.05$)。② 水稻根际土壤真菌菌门分布主要为子囊菌门、Aphelidiomycota 门和壶菌门, 占有菌门的 57%。菌属分布主要为被孢霉属、链格孢属和镰刀菌属。各处理间真菌群落 α -多样性差异显著 ($P < 0.05$)。未添加棉秆炭处理下 (C0), 镉浓度的增加降低了土壤中壶菌门、被孢霉属和链格孢属的相对丰度和真菌多样性指数 (Shannon 指数)。在不同浓度镉处理下 (Cd0、Cd1 和 Cd8), 增加棉秆炭会降低真菌群落丰富度指数 (Chao1 指数) 和 Shannon 指数。镉污染导致土壤中壶菌门相对丰度增多, 却使子囊菌门丰度下降, 施用棉秆炭可显著提升壶菌门的相对丰度 ($P < 0.05$)。镉污染使被孢霉属和链格孢属丰度下降, 但是施用棉秆炭可使链格孢属相对丰度增多。增加棉秆炭施用量, 土壤中会存在更多的内生菌、植物病原体和腐生菌; 而镉污染程度增加会减少土壤中内生菌、植物病原体和腐生菌。③ 影响真菌群落多样性及结构的主要环境因子有土壤速效钾、有机质和 pH。水稻土壤镉含量中占比最大的可还原态镉与轮形动物门、Aphelidiomycota 门和子囊菌门呈显著正相关 ($P < 0.05$), 但与其它菌门呈负相关 ($P < 0.05$)。研究结果说明棉秆炭在碱性镉污染土壤微生态调控方面具有一定的作用。

关键词: 棉秆炭; 镉; 根际土壤; 真菌; 多样性

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Effects of Cotton Stalk Biochar on the Structure and Function of Fungi Community in Alkaline Rhizosphere Soil of Rice Under Cadmium Pollution

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Abstract: To study the effects of cotton stalk biochar on the regulation of fungal diversity, the structure and function of alkaline rice rhizosphere soil under cadmium pollution was investigated. An outdoor pot experiment was conducted by adding cotton stalk biochar (0%, 1%, and 5%) to an alkaline paddy soil with a cadmium concentration of 0.1 and 8 mg·kg⁻¹. Taking rice rhizosphere soil as the research object, Illumina HiSeq sequencing was used to analyze the effects of cotton stalk biochar and cadmium pollution on the diversity, structure, abundance, and function of fungi in an alkaline rhizosphere soil, and to explore the correlation between soil environmental factors and the fungal community under the control of cotton stalk biochar. The results showed that: ① the application of cotton stalk biochar significantly increased the soil pH, available nutrients, and organic matter, and reduced the content of reducible cadmium in the soil ($P < 0.05$). ② The distribution of rice rhizosphere soil fungi was mainly Ascomycota, Aphelidiomycota, and Chytridiomycota, which accounted for 57% of all mycophytes. The genus was mainly *Mortierella*, *Alternaria*, and *Fusarium*. There was a significant difference in the α -diversity of the fungal community among the treatments ($P < 0.05$). In the absence of cotton stalk biochar (C0), the increase in the cadmium concentration reduced the relative abundance and fungal diversity index (Shannon index) of Chytridiomycota, *Mortierella*, and *Alternaria* in the soil. Under different concentrations of cadmium (Cd0, Cd1, and Cd8), increasing cotton stalk biochar reduced the fungal community richness index (Chao1 index) and Shannon index. Cadmium pollution resulted in an increase in the relative abundance of Chytridiomycota in the soil, but decreased the abundance of *Alternaria*. The application of cotton stalk biochar could significantly increase the relative abundance of Chytridiomycota ($P < 0.05$). Cadmium pollution reduced the abundance of *Mortierella* and *Alternaria*, but the application of cotton stalk biochar could increase the relative

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abundance of *Alternaria*. Increasing cotton stalk biochar means that soil will have more endophytes, plant pathogens, and saprophytes; while increasing cadmium pollution will reduce endophytes, plant pathogens, and saprophytes in the soil. ③ The main environmental factors affecting the diversity and structure of fungal communities are the available potassium, organic matter, and pH of the soil. The reducible cadmium content, which comprises the largest proportion of cadmium in rice soil, was significantly positively correlated to Rotifera, Aphelidiomycota, and Ascomycota ($P < 0.05$), but negatively correlated to other mycophytes ($P < 0.05$). The results indicate that cotton stalk biochar plays a certain role in the microecological regulation of alkaline cadmium-contaminated soil.

Key words: cotton stalk biochar; cadmium; rhizosphere soil; fungus; diversity

根际土壤微生物指生活在植物根系土壤中的细菌、放线菌、真菌、藻类和原生动物等^[1],因受植物根系和分泌物影响^[2],它们的数量是非根际微生物数量的几倍或几十倍^[3].真菌在土壤-植物生态系统中发挥着重要作用^[4],一方面真菌可参与植物残体的分解,推动土壤养分的循环^[5],另一方面真菌也受到植物和土壤理化性质的影响^[6],如植物种类、土壤 pH^[7]、水分^[8]、温度^[9]和营养状况^[10]等.真菌对环境污染有很好的指示作用^[11],重金属镉污染不仅影响土壤理化性质^[12],抑制植物生长,而且对土壤真菌群落多样性和组成结构也有重要影响^[13].有研究表明,土壤中真菌会随着镉污染浓度的增加与污染时间的延长,真菌数量和多样性均降低^[14];但也有研究发现镉污染增加了植物根际土壤中真菌群落的多样性^[15];还有研究认为镉对根际土壤真菌群落影响不明显^[16].真菌对重金属污染较细菌更敏感^[17],土壤真菌群落结构特征可以从侧面反映土壤重金属的污染程度,预测土壤环境质量的改变^[18],因此开展植物根际土壤真菌群落多样性相关研究,对深入揭示土壤真菌对重金属等外来干扰的响应机制有着重要的意义.

生物质炭(biochar, BC)是生物质在缺氧条件下高温裂解生成的一类新型环境功能材料^[19],有研究表明生物质炭在改善土壤理化性质,降低土壤真菌群落的丰富度和均匀度^[20],促进植物的生长发育^[21]以及降低重金属生物有效性方面具有良好的效果^[22].目前生物质炭的研究多集中在中国东南部的酸性重金属污染农业土壤区域^[23],而新疆土壤的 pH 值普遍大于 8.0^[24],因而在碱性镉污染土壤中施用棉秆炭对作物根际土壤真菌群落会有怎样的影响?近年来,农田土壤镉超标问题导致水稻(*Oryza sativa* L.)生产安全受到威胁^[25],新疆水稻主产区之一的米东区曾有稻田土壤镉超标的报道^[26].稻田环境与旱地相比可能更复杂^[27],真菌数量虽不如细菌和放线菌多,但是真菌对稻田土壤的温室气体排放、有机碳固定及污染物的降解有着极其重要的作用^[28].目前有关碱性水稻根际土壤中真菌对镉污染和生物质炭的双重作用下的响应机制尚不明确.本研究利用 Illumina HiSeq 高通量测序技术分析了在

不同浓度的碱性镉污染土壤中施用棉花秸秆生物质炭对水稻根际土壤真菌 α -多样性、结构和潜在功能的影响,探讨各处理下土壤环境因子与真菌间的关系,以期对棉秆炭对碱性镉污染水稻根际土壤微生物生态调控提供科学参考.

1 材料与方法

1.1 试验材料与盆栽设计

供试水稻根际土壤取自室外盆栽试验,盆栽试验土壤为乌鲁木齐市米东区稻田土壤.试验设计参考《土壤环境质量农用地土壤污染风险管控标准(试行)》(GB 15618-2018),各处理土壤中添加镉浓度分别为 0、1 和 8 $\text{mg}\cdot\text{kg}^{-1}$,分别记为 Cd0、Cd1 和 Cd8,稳定 2 个月后,按照占土壤的质量分数分别施入 0%、1% 和 5% 的棉秆炭,分别记为 C0、C1 和 C5,共 9 处理,每处理 3 重复.棉秆生物质炭由新疆农业科学院提供,炭化温度为 360℃,炭化时间为 16 h,过 0.5 mm 筛备用.棉秆生物质炭 pH 值为 9.37,全氮 21.76 $\text{g}\cdot\text{kg}^{-1}$,全磷 10.58 $\text{g}\cdot\text{kg}^{-1}$,全钾 21.45 $\text{g}\cdot\text{kg}^{-1}$,碱解氮 5.38 $\text{mg}\cdot\text{kg}^{-1}$,速效磷 200.94 $\text{mg}\cdot\text{kg}^{-1}$,镉含量 0.083 $\text{mg}\cdot\text{kg}^{-1}$,种植特丰优 2 号水稻,在水稻成熟期采集根际土壤待测.

1.2 水稻根际土壤的采集

根际土壤采集方法参考文献[29]中的抖落法,根际土壤过 20 目尼龙筛后,用四分法保留 3 g 的土壤鲜样装入已灭菌的 EP 管中,立即放入 -80℃ 液氮中保存,共 27 份样品用于土壤真菌分析.

1.3 试验方法

1.3.1 土壤理化性质及镉形态测定方法

土壤 pH、电导率、速效钾、速效磷、碱解氮和有机质测定方法参考文献[30].采用 BCR 逐步提取法^[31]测定土壤镉形态.

1.3.2 真菌 18ITS 测序

采用微生物 Marker 基因高通量测序评估真菌群落结构和多样性,使用 MN NucleoSpin 96 Soil 试剂盒/强力土壤 DNA 提取试剂盒(PowerMag™ Soil DNA isolation Kit, MO BIO)从样品中提取真菌总 DNA.利用特异引物 ITS1F(5'-CTTGCTCATTTAGAG GAAGTAA-3')和 ITS2(5'-GCTGCGTTCTTCATCGAT

GC-3'),结合适配器序列和条形码序列进行扩增.本研究样品的测序和生物信息服务在北京百迈客生物科技有限公司 Illumina HiSeq 2500 平台完成.

1.4 数据处理与分析

基于 R(v3.3.2)的 agricolae 软件包进行正态分布和方差齐性检验(normal distribution and homogeneity of variance test)及单因素方差分析(One-way ANOVA),并在 5% 显著性水平下做 LSD (least significant difference)多重比较.

1.5 生物信息学分析

使用 FLASH(v1.2.11)对原始数据进行拼接,通过 Trimmomatic(v 0.33)将拼接得到的序列进行质量过滤,借助 UCHIME(v8.1)去除嵌合体,获得高质量 Tags 序列.使用 USEARCH(v10.0)对 OTU 进行相似度为 97% 的聚类,以测序所有序列数的 0.005% 作为阈值过滤 OTU.使用 RDP Classifier(v2.2)对分类群进行分类,最小可信度估计为 80%.

借助 Mothur(v1.30)分析真菌群落 α -多样性,通过 ggpubr 软件包中 Kruskal-Wallis 检验比较组间差异,并使用 ggplot2 软件包完成绘图.冗余分析(Reundancy analysis, RDA)环境因子对真菌群落组成的影响,蒙特卡罗置换检验(Monte Carlo permutation, $n = 999$)测试分析结果的显著性.基于 KEGG 数据库,使用 STAMP(v2.1.3)进行真菌功能差异显著性分析及绘图.

2 结果与分析

2.1 棉秆炭调控对碱性镉污染水稻根际土壤养分的影响

由表 1 可知,在相同炭量处理下,水稻土壤 pH、电导率、有机质和速效养分(碱解氮、速效钾和速效磷)含量随镉浓度递增而逐渐减少($P < 0.05$).相同镉浓度处理下,与对照 C0 相比,添加棉秆炭可以显著提高水稻土壤 pH 电导率和土壤养分($P < 0.05$).

表 1 棉秆炭对碱性镉污染水稻土壤养分的影响¹⁾

Table 1 Effects of cotton stalk biochar on nutrients in an alkaline cadmium-contaminated paddy soil

处理	pH	电导率 / $\mu\text{S}\cdot\text{cm}^{-1}$	碱解氮 / $\text{mg}\cdot\text{kg}^{-1}$	速效磷 / $\text{mg}\cdot\text{kg}^{-1}$	速效钾 / $\text{mg}\cdot\text{kg}^{-1}$	有机质 / $\text{g}\cdot\text{kg}^{-1}$
Cd0C0	8.06 $\pm$ 0.03d	931.00 $\pm$ 1.00c	17.37 $\pm$ 1.58e	15.30 $\pm$ 0.26d	1 075.72 $\pm$ 0.97f	39.47 $\pm$ 0.31e
Cd0C1	8.17 $\pm$ 0.03c	1019.33 $\pm$ 1.53b	58.06 $\pm$ 1.58b	17.92 $\pm$ 0.25c	1 255.63 $\pm$ 1.92d	41.67 $\pm$ 0.28d
Cd0C5	8.37 $\pm$ 0.02a	1202.67 $\pm$ 6.11a	73.15 $\pm$ 2.10a	26.97 $\pm$ 0.64a	4 474.93 $\pm$ 11.92a	58.23 $\pm$ 0.26a
Cd1C0	7.89 $\pm$ 0.00f	731.67 $\pm$ 2.52g	14.17 $\pm$ 2.10f	11.67 $\pm$ 0.12e	923.32 $\pm$ 0.32h	38.26 $\pm$ 0.80f
Cd1C1	7.93 $\pm$ 0.03e	829.67 $\pm$ 2.05e	31.09 $\pm$ 1.58d	17.47 $\pm$ 0.05c	1 205.67 $\pm$ 1.40e	39.85 $\pm$ 0.52e
Cd1C5	8.29 $\pm$ 0.02b	891.67 $\pm$ 2.08d	71.78 $\pm$ 2.86a	26.67 $\pm$ 0.25a	4 225.11 $\pm$ 23.75b	53.38 $\pm$ 0.34b
Cd8C0	7.74 $\pm$ 0.01g	563.33 $\pm$ 3.51i	6.86 $\pm$ 0.00g	6.33 $\pm$ 0.58f	685.26 $\pm$ 1.85i	36.78 $\pm$ 0.02g
Cd8C1	7.89 $\pm$ 0.01f	633.00 $\pm$ 1.00h	8.23 $\pm$ 0.01g	6.50 $\pm$ 0.00f	1 048.21 $\pm$ 0.28g	38.98 $\pm$ 0.45ef
Cd8C5	8.16 $\pm$ 0.02c	805.00 $\pm$ 2.65f	34.28 $\pm$ 2.38c	23.00 $\pm$ 1.00b	3 596.17 $\pm$ 5.43c	46.25 $\pm$ 1.11c

1) 同列数据后不同小写字母表示差异显著($P < 0.05$)

2.2 棉秆炭调控对碱性镉污染水稻根际土壤镉赋存形态的影响

由图 1 可知,在 Cd1 和 Cd8 处理下,土壤中镉的形态分布情况为:可还原态(41.43%~74.63%) > 残渣态(19.79%~54.98%) > 可氧化态(2.26%~5.06%) > 酸提取态(0.63%~2.17%),水稻土壤中镉主要以可还原态存在,酸提取态较少.在相同镉浓度处理下,随棉秆炭施用量的增加可显著降低还原态镉的含量($P < 0.05$);酸提取态和可氧化态镉含量随炭量变化的趋势与可还原态镉一致,残渣态镉含量变化趋势相反($P < 0.05$),说明棉秆炭的添加能有效降低土壤中易被植物吸收利用的有效态镉(酸提取态和可还原态镉)含量,增加残渣态镉含量.

2.3 棉秆炭调控对碱性镉污染水稻根际土壤真菌 α -多样性的影响

测得所有样品覆盖度超过 99.95%,说明测序深度足够,能满足后续分析.各处理间真菌群落 α -多

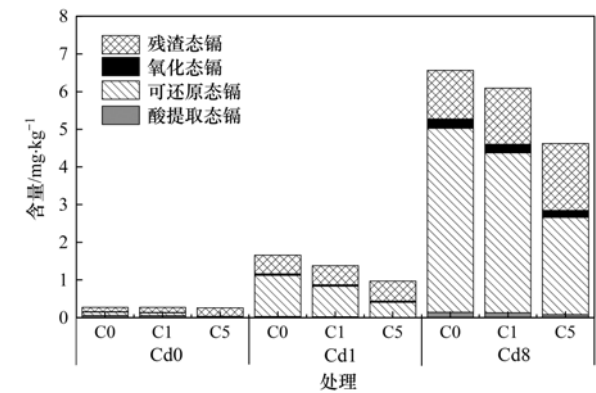


图 1 棉秆炭对碱性水稻根际土壤中镉赋存形态的影响

Fig. 1 Effect of cotton stalk biochar on the fractions of cadmium in an alkaline paddy soil

样性均达到差异显著水平($P < 0.05$).由 α -多样性箱型图得出(见图 2),在 C0 处理下,随着镉浓度的递增,Chao1 指数(丰富度指数)呈 Cd1 > Cd0 > Cd8 的趋势,Shannon 指数(多样性指数)呈 Cd0 > Cd1 >

Cd8 的逐渐递减趋势. C1 处理下, 真菌 OTUs、Chao1 指数和 Shannon 指数均随镉浓度递增而逐渐提高 ($P < 0.05$). 而在不同镉浓度处理下 (Cd0、Cd1 和 Cd8), 与 C0 相比, 施用棉秆炭处理均使真菌多样性和丰富度下降 ($P < 0.05$).

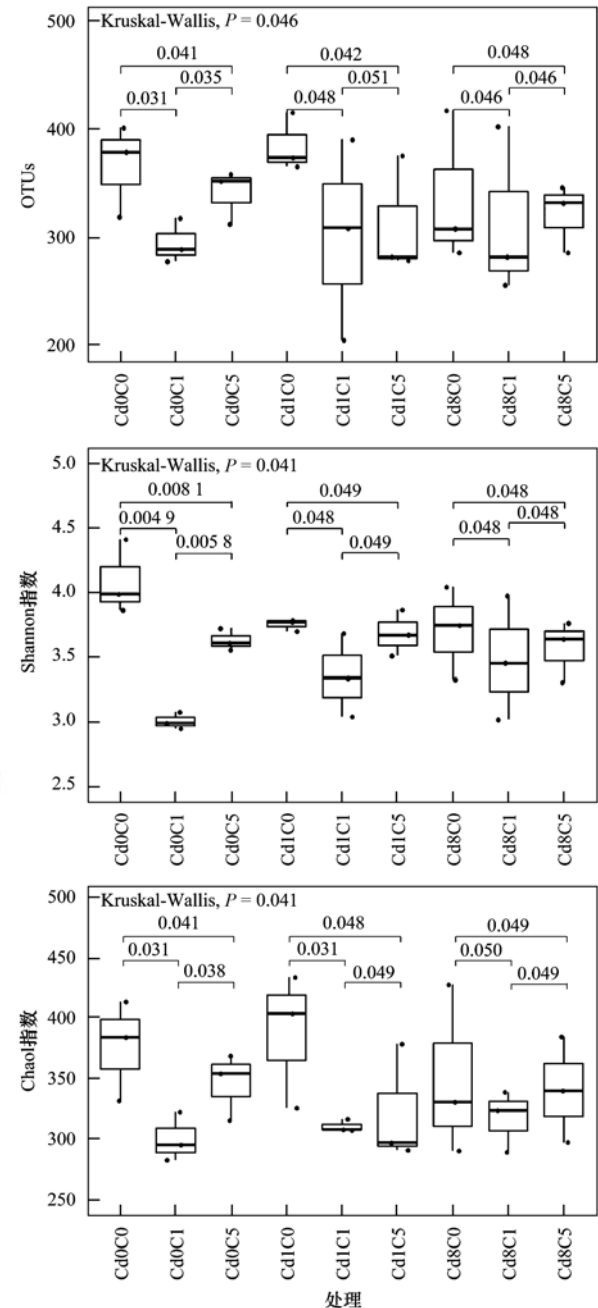


图2 棉秆炭对碱性水稻根际土壤真菌群落 α -多样性的影响
Fig. 2 Effects of cadmium and cotton stalk biochar on the α -diversity of fungal communities in an alkaline rhizosphere soil of rice

2.4 棉秆炭调控对碱性镉污染水稻根际土壤真菌群落物种丰度的影响

物种组成分析反映样品在不同分类学水平上的群落结构, 真菌群落组成对不同浓度镉污染和棉秆炭的响应不同. 9 个处理水稻根际土壤的真菌共检测出 11 个门和 72 个属. 图 3 为门水平上水稻根际

土壤真菌物种丰度分布图, 剔除了 495 条 unclassified 序列, 显示了前 10 位的菌门. 分别为子囊菌门 (Ascomycota)、Aphelidiomycota 门、壶菌门 (Chytridiomycota)、被孢霉门 (Mortierellomycota)、担子菌门 (Basidiomycota)、隐真菌门 (Rozellomycota)、油壶菌门 (Olpidiomycota)、单毛壶菌门 (Monoblepharomycota)、轮形动物门 (Rotifera) 和球囊菌门 (Glomeromycota). 在未被镉污染土壤中 (Cd0), 壶菌门和轮形动物门的丰度随生物质炭施用量的增加 (0~5%) 而增多, 与空白 C0 相比, 各菌门丰度在 C5 处理下分别增加了 1.63 倍和 6.29 倍. Aphelidiomycota 门和油壶菌门丰度随炭量增多而逐渐降低, C5 处理下, 各菌门丰度分别比空白组 C0 降低了 1.42 倍和 7.14 倍. Cd1 浓度中, 油壶菌门丰度随炭量增加而减少, 被孢菌门和隐真菌门丰度反之增多. Cd8 浓度中, 隐真菌门丰度随炭量递增而递减, 壶菌门丰度反之增多. 而在未添加生物质炭条件下 (C0), 壶菌门丰度随镉浓度递增而逐渐增加, Cd8 处理下比 Cd0 增加了 24.9%, 子囊菌门和单毛壶菌门丰度随镉浓度递增而逐渐降低, 在 Cd8 处理下比 Cd0 降低了 0.41 倍和 1.61 倍.

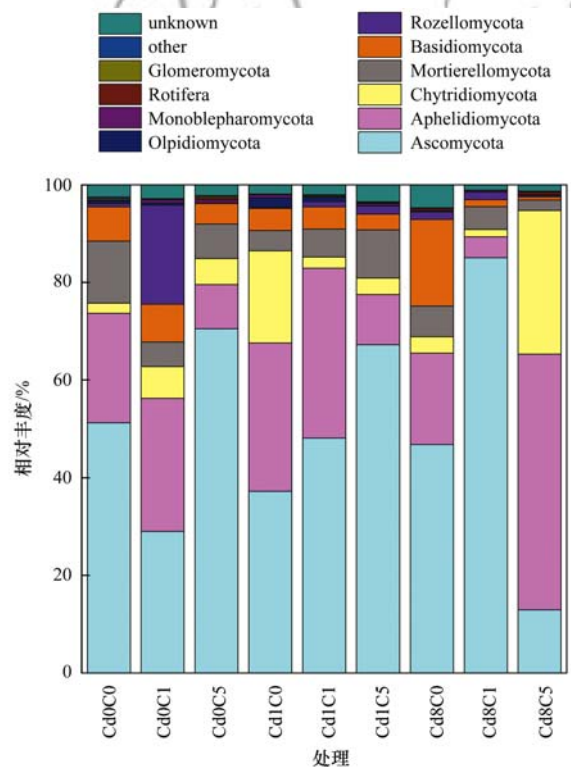


图3 门水平上水稻根际土壤真菌结构
Fig. 3 Relative abundance distribution of Fungus species in a rhizosphere soil of rice at the phylum level

表 2 显示了前 9 位的菌属, 分别为被孢霉属 (*Mortierella*)、链格孢属 (*Alternaria*)、镰刀菌属 (*Fusarium*)、维希尼克氏酵母属 (*Vishniacozyma*)、枝

孢属 (*Cladosporium*)、线黑粉酵母属 (*Filobasidium*)、曲霉属 (*Aspergillus*)、拟棘壳孢属 (*Pyrenochaetopsis*) 和 *Naganishia* 属. 在未被镉污染土壤中 (Cd0), 链格孢属丰度随生物质炭量的增多而逐渐降低, C5 处理下其丰度较空白 C0 下降了 2.70 倍. 在 Cd1 浓度下, 被孢霉属、链格孢属、镰刀菌属和枝孢属的丰度

随炭量递增而增多, 线黑粉酵母属丰度反之减少. 在 Cd8 浓度下, 枝孢属丰度随炭量增多而减少. 而在未添加生物质炭条件下, 被孢霉属、链格孢属、曲霉属和 *Naganishia* 属丰度随镉浓度递增而逐渐降低, 各菌属丰度在 C5 处理下较空白 C0 分别下降了 1.61、1.28、0.96 和 3.85 倍.

表 2 属水平上水稻根际土壤真菌结构/%

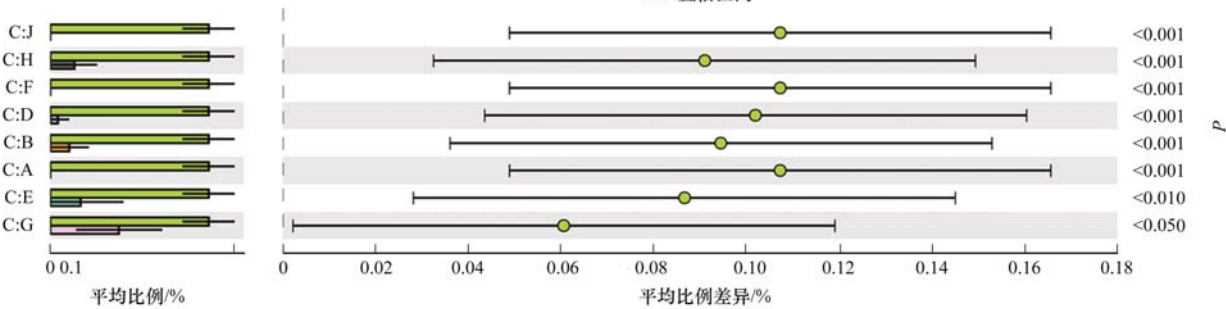
Table 2 Relative abundance of fungal species in a rhizosphere soil of rice under different treatments/%		处理								
门	属	Cd0C0	Cd0C1	Cd0C5	Cd1C0	Cd1C1	Cd1C5	Cd8C0	Cd8C1	Cd8C5
被孢霉门 (Mortierellomycota)	被孢霉属 (<i>Mortierella</i>)	2.27	0.47	1.30	0.92	1.58	3.61	0.87	3.58	0.86
	链格孢属 (<i>Alternaria</i>)	0.71	0.23	0.19	0.48	0.77	1.17	0.31	0.36	0.17
半知菌亚门 (Deuteromycotina)	镰刀菌属 (<i>Fusarium</i>)	0.17	0.11	0.44	0.17	0.40	0.93	0.53	0.85	0.22
	枝孢属 (<i>Cladosporium</i>)	0.42	0.10	0.14	0.19	0.23	0.27	0.28	0.26	0.13
担子菌门 (Basidiomycota)	线黑粉酵母属 (<i>Filobasidium</i>)	0.30	0.04	0.09	0.49	0.18	0.10	0.02	0.14	0.10
	维希尼克氏酵母属 (<i>Vishniacozyma</i>)	0.12	0.04	0.20	0.20	0.01	0.28	1.83	0.02	0.03
	长西氏酵母属 (<i>Naganishia</i>)	0.42	0.10	0.14	0.19	0.23	0.27	0.28	0.26	0.13
子囊菌门 (Ascomycota)	曲霉属 (<i>Aspergillus</i>)	0.23	0.06	0.18	0.13	0.03	0.19	0.12	0.27	0.10
	拟棘壳孢属 (<i>Pyrenochaetopsis</i>)	0.10	0.07	0.19	0.13	0.09	0.25	0.01	0.38	0.07

2.5 棉秆炭调控碱性水稻根际土壤的真菌群落功能预测分析

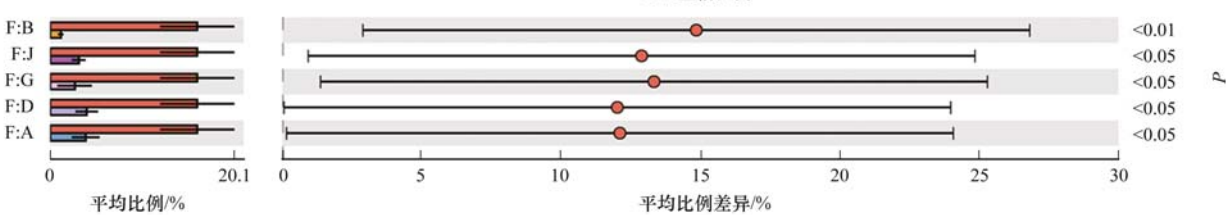
通过 PICRUST 功能注释软件将测序所得真菌群落组成“映射”到 Greengenes 数据库中, 基于基因功能谱预测真菌基因组的潜在功能. 使用 FUNguild (v1.0, <https://github.com/UMNFun/FUNGuild>) 获得结果 (见图 4), 由内生菌根-植物病原体-未定义腐生真菌 (endomycorrhizal-plant pathogen-undefined saprotroph) 分析可见: C (Cd0C5) > A (Cd0C0),

C (Cd0C5) > B (Cd0C1); C (Cd0C5) > F (Cd1C5), C (Cd0C5) > J (Cd8C5), 即在 Cd0 浓度下, C5 处理与 C0 和 C1 相比较, 存在更多的内生菌和植物病原体; 而在 C5 处理下, 随镉污染浓度增高, 内生菌、植物病原体和腐生菌会减少. 由未定义腐生真菌分析可见: F (Cd1C5) > D (Cd1C0), F (Cd1C5) > J (Cd8C5), 即在 Cd1 浓度下, C5 处理比 C0 有更多的腐生菌; 而在 C5 处理下, Cd1 比 Cd8 处理存在更多的腐生菌.

内生菌根-植物病原体-未定义腐生真菌



未定义腐生真菌



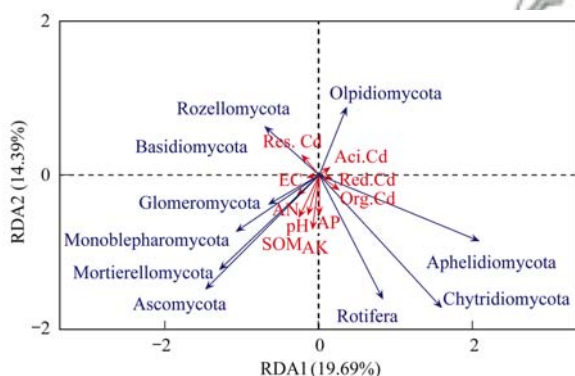
方便测序分析: A 表示 Cd0C0, B 表示 Cd0C1, C 表示 Cd0C5, D 表示 Cd1C0, E 表示 Cd1C1, F 表示 Cd1C5, G 表示 Cd8C0, H 表示 Cd8C1 和 J 表示 Cd8C5; C:J 表示两个处理间的比较, 以此类推

图 4 棉秆炭对碱性水稻根际土壤真菌群落的功能影响预测

Fig. 4 Effects of cotton stalk biochar on fungal community function in an alkaline rice rhizosphere soil of rice

2.6 环境因子与真菌群落间相关性分析

由 RDA 分析得出(见图 5),在镉污染和棉秆炭处理下,真菌群落结构与水稻土壤镉的赋存形态和土壤 pH、养分等 10 个环境因子之间的关联性. 速效钾、有机质和 pH 是影响真菌群落结构最主要的环境因子,其次是速效磷、碱解氮和残渣态镉含量. 图 5 中土壤 pH、电导率和各养分指标分别与子囊菌门(Ascomycota)、被孢菌门(Mortierellomycota)、单毛壶菌门(Monoblepharomycota)、球囊菌门(Glomeromycota)和轮形动物门(Rotifera)呈显著正相关($P < 0.05$). 酸提取态、可还原态和可氧化态镉含量分别与轮形动物门、Aphelidiomycota 门和子囊菌门呈显著正相关,与其它菌门呈负相关($P < 0.05$). 残渣态镉含量与隐真菌门(Rozellomycota)、油壶菌门(Olpidiomycota)和担子菌门(Basidiomycota)呈显著正相关,与其它菌门呈显著负相关.



Aci.Cd 表示酸提取态镉, Red.Cd 表示可还原态镉, Oxi.Cd 表示可氧化态镉, Res.Cd 表示残渣态镉, SOM 表示有机质, pH 表示酸碱度, EC 表示电导率, AK 表示速效钾, AP 表示速效磷, AN 表示碱解氮

图 5 环境因子与水稻根际土壤真菌群落间 RDA 分析

Fig. 5 RDA analysis between environmental factors and fungal communities in a rhizosphere soil of rice

3 讨论

3.1 棉秆炭调控对碱性镉污染水稻根际土壤养分和镉形态的影响

棉秆炭的施入对土壤、养分以及理化性质具有一定的调控作用. 5% 量的棉秆炭有效提升了土壤养分($P < 0.05$). 生物质炭自身呈碱性, 含有大量营养成分, 当施用到土壤后, 其附带的碱性物质和 N、P 和 K 等元素释放出来, 显著提升了土壤的 pH、电导率、有机质和速效养分含量^[32]. 另一方面, 生物质炭具有多孔隙和高度芳香化结构, 使生物质炭具备了良好的吸附性^[33], 可使重金属向更稳定的形态转化, 降低其生物有效性^[34]. 有研究表明 BCR 逐步提取法中, 由于酸提取态和可还原态镉含量较不稳定,

易于被植物吸收利用, 故将酸提取态和可还原态镉作为有效态镉^[35], 本试验中棉秆炭的添加降低了土壤中有有效态镉, 这与前人研究一致^[36], 这可能由于棉秆炭的施入提高了土壤 pH 值, 土壤吸附反应下形成的氢氧化物为重金属提供了大量吸附位点, 同时土壤及生物质炭释放的有机质成分, 与 Cd^{2+} 形成稳定不易被吸收的配位络合物, 从而降低了镉的有效性^[37].

3.2 棉秆炭调控对碱性镉污染水稻根际土壤真菌群落的影响

真菌是一类种类多、分布广的真核微生物, 它们具有分解有机质, 为植物提供养分的功能, 是生态系统健康的指示物^[38]. 有研究将真菌作为病原菌, 认为它不利于植物生长, 会降低作物产量^[39], 而镉的增加会降低土壤真菌多样性^[40]. 生物质炭的施用会改善土壤根系环境, 对根际微生物群落有显著影响, 如降低真菌的多样性^[41]. 生物质炭可通过直接或间接作用影响土壤真菌群落多样性及结构, 其直接影响机制主要为: ① 生物质炭可为真菌提供充足栖息地; ② 生物质炭自身富含的营养元素可为真菌生长提供必要条件. 间接影响机制体现在: ① 生物质炭通过改变土壤理化性质而间接影响土壤真菌; ② 生物质炭通过改变其他微生物种群的活性, 间接影响真菌的生长.

真菌群落对土壤酸性和碱性环境的响应不同, 目前大多研究为镉污染对酸性土壤真菌的影响, 研究发现真菌数量与镉污染浓度极显著相关^[42], 且随镉浓度递增, 真菌部分类群出现抗性^[43]. 阎海涛等^[44]的研究认为小麦秸秆生物质炭的添加对酸性土壤真菌 α -多样性影响不大, 但能显著改变真菌群落结构, 表现为子囊菌门和担子菌门相对丰度的降低; 而顾美英等^[45]在碱性土壤中施用棉秆炭后, 发现真菌多样性显著降低, 且镰刀菌属等病原菌数量明显降低. 本试验中, 添加棉秆炭处理与未添加棉秆炭处理比较, 真菌群落的 α -多样性指数均明显下降($P < 0.05$), 与黄修梅等^[20]的研究一致. 本研究得出在水稻根际土壤中真菌群落主要有 11 个菌门, 与大部分农田土壤真菌群落情况一致^[20, 46]. 棉秆炭的添加使壶菌门和轮形动物门的丰度增加, 使 Aphelidiomycota 门和油壶菌门丰度降低, 这与前人的研究结果相似^[47]. 链格孢属^[48]和枝孢属^[49]在自然界分布广, 能引起多种植物病害, 镰刀菌^[50]能产生植物激素(赤霉素), 可使农作物增产. 本研究发现, 未被镉污染土壤中施用棉秆炭可以降低链格孢属的丰度, 但在镉污染条件下, 棉秆炭的增多会导致被孢霉属、链格孢属、镰刀菌属和枝孢属的丰度增

高. 因此生物质炭的添加能使有益真菌丰度增多的同时, 也会促进病原菌真菌类群的生长.

环境因子与植物根际土壤真菌群落间也有一定关系, 阎海涛等^[44]的研究认为土壤 DOC、pH 和含水率是影响褐土真菌群落的主要环境因子. 本研究中速效钾、SOM 和 pH 是影响水稻根际土壤真菌群落的主要环境因子, 可见环境因子与真菌间的响应也因土壤类型而不同. 基于基因功能谱预测, 镉污染使水稻根际土壤中内生菌、植物病原菌和腐生菌减少, 而棉秆炭处理下则能观察到更多的内生菌、植物病原菌及腐生菌. 对于生物质炭能提高真菌功能的结果, Jenkins 等^[51]也有类似结论. 棉秆炭对镉污染下水稻根际土壤真菌群落的调控可能受多方面复杂因子的影响, 作用机制是复杂的, 需要深入开展相关研究.

4 结论

(1) 施用棉秆炭显著提高了土壤 pH、速效养分和有机质等指标, 并降低土壤中可还原态镉的含量 ($P < 0.05$), 改变了水稻根际土壤微生境.

(2) 镉污染增加降低了水稻根际土壤真菌群落多样性和丰富度 ($P < 0.05$). 各镉浓度下施用棉秆炭后, 真菌群落 Chao1 指数和 Shannon 指数均下降. 水稻根际土壤真菌在门水平上主要为子囊菌门、Aphelidiomycota 门和壶菌门, 占有菌门的 57%. 镉污染导致土壤中壶菌门相对丰度增多, 却使子囊菌门丰度下降, 施用棉秆炭可显著提升壶菌门的相对丰度 ($P < 0.05$). 对于真菌群落属水平而言, 水稻根际土壤真菌的主要菌属为被孢霉属、链格孢属和镰刀菌属, 镉污染使被孢霉属和链格孢属丰度下降, 但是施用棉秆炭可使链格孢属相对丰度增多, 增加棉秆炭土壤中会存在更多的内生菌、植物病原体和腐生菌; 而镉污染程度增加会减少土壤中内生菌、植物病原体和腐生菌.

(3) 影响真菌群落多样性及结构的主要环境因子有土壤速效钾、有机质和 pH. 水稻土壤镉含量中占比最大的可还原态镉与轮形动物门、Aphelidiomycota 门和子囊菌门呈显著正相关 ($P < 0.05$).

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