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滴灌方式和生物质炭对温室土壤矿质态氮及其微生物 调控的影响

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摘要:滴灌和生物质炭添加均可影响土壤氮的形态及转化. 深入了解滴灌方式和生物质炭对土壤矿质态氮和参与矿质态氮转 化关键功能基因及微生物种群的综合影响,对改善设施农业生产管理方式,提高水分和氮源利用效率,降低硝酸盐积累及其 淋失引起的地下水污染具有重要指导意义. 在日光温室设置地表滴灌(D)、插人式滴灌(ID,插入深度 15 cm)、地表滴灌 + 10 t·hm⁻²生物质炭(DB)和插入式滴灌 + 10 t·hm⁻²生物质炭(IDB,插深 15 cm)这 4 个处理,以收获期辣椒根际和非根际土壤为 研究对象. 结果发现,非根际和根际土壤铵态氮含量不受滴灌方式和生物质炭的影响. 与地表滴灌相比,插入式滴灌显著降低 了非根际土壤硝态氮的含量(P<0.05),但生物质炭削弱了这种差异.同种滴灌方式下,生物质炭添加降低了根际土壤硝态氮 的含量. 生物质炭添加降低了地表滴灌辣椒非根际土壤 AOA、AOB 和 nirK 基因拷贝数以及根际土壤 AOA 基因拷贝数(P< 0.05),提高了两种滴灌方式根际土壤 AOB 和 nirK 基因拷贝数(P<0.05).结构方程模型分析结果显示,在非根际和根际土壤 中,pH 和电导率分别是对铵态氮和硝态氮含量影响最大的环境因子,AOB 基因拷贝数是对硝态氮影响最大的生物因子.基于 PICRUSt 功能预测,γ-变形菌纲菌属对氨单加氧酶基因(K10945)表达的贡献高于其它菌属; α-变形菌纲,尤其是根瘤菌成员 对参与含铜离子的亚硝酸还原酶基因(K00368)表达的贡献高于其他菌属. 生物质炭对非根际土壤 K10945 和根际土壤 K00368 同源基因细菌群落结构的影响显著(P<0.05). 综上所述,相对于滴灌方式,生物质炭的添加对设施农业土壤硝态氮 氮含量及其转化的关键微生物影响更大.

关键词:滴灌方式:生物质炭;设施农业;矿质态氮;细菌

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Effects of Drip Irrigation Patterns and Biochar Addition on Soil Mineral Nitrogen and Microbial Regulation of Greenhouse

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Abstract: Drip irrigation and biochar amendment could affect the nitrogen form and transformation. Creating a deep understanding of the interacting effects of drip irrigation patterns and biochar on soil mineral nitrogen, as well as the key functional genes and microbial community involved in nitrogen transformation is helpful for improving facility agricultural management, increasing water and nitrogen use efficiency, and reducing the nitrate accumulation and groundwater pollution caused by nitrogen leaching. Four treatments [surface drip irrigation (D), insert drip irrigation (ID, insert depth 15 cm), surface drip irrigation + 10 t·hm⁻² of biochar (DB), and insert drip irrigation +10 t·hm⁻² of biochar (IDB)] were conducted in a solar greenhouse, and non-rhizospheric and rhizospheric soils of pepper plants were studied. There was no effect of drip irrigation patterns and biochar on ammonium-nitrogen in the non-rhizospheric and rhizospheric soils. Compared with surface drip irrigation, insert drip irrigation decreased the nitrate-nitrogen concentration in the non-rhizosphere soil (P < 0.05), but biochar addition weakened the difference. Biochar addition decreased the nitrate-nitrogen concentration in the rhizosphere soil under the same drip irrigation patterns. In the D treatment, biochar significantly decreased the number of copies of AOA, AOB, and nirK genes in the non-rhizospheric soil, and AOA gene copies in the rhizospheric soil (P < 0.05); however, there was an increase in the number of copies of AOB and nirK genes in the rhizospheric soil of the D and ID treatments (P < 0.05). Based on the structural equation model (SEM), in the non-rhizospheric and rhizospheric soils, pH and electrical conductivity were the environmental factors with the greatest influence on the ammonium-nitrogen and nitrate concentrations, respectively, and the gene copy number of AOB was the biotic factor with the greatest influence on the nitrate-nitrogen concentration. Based on PICRUSt, the γ -Proteobacteria contributed mostly to ammonia monooxygenase gene (K10945) expression, whereas the α -Proteobacteria, especially the rhizobia members, contributed mostly to nitrite reductase gene (K00368) expression. Biochar addition

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regulated the bacterial community structure that participated in K10945 gene expression in the non-rhizospheric soil and K00368 gene expression in the rhizospheric soil (P < 0.05). Overall, biochar addition contributed more to nitrate-nitrogen and microbial mineral nitrogen-transformation processes in the agricultural soil than did the drip irrigation patterns.

Key words: drip irrigation; biochar; facility agriculture; mineral nitrogen; bacteria

土壤中的矿质态氮,尤其是铵态氮和硝态氮是影响作物生长的重要氮素形态,其在土壤中的累积与转化受农业生产管理活动的影响.不合理的管理模式,如:氮肥的大量施入会导致土壤硝酸盐的积累;不合理的灌溉方式不仅会造成水资源浪费,同时会引起氮淋失和地下水污染[1,2].因此,如何实现资源节约和减少环境污染一直是农业生产管理探寻的问题.

最大程度地提高灌溉水利用效率,节约水资源 是设施农业生产中重要目的. 这不仅有利于缓解土 壤板结,减少深层渗漏,降低养分流失和病虫害,还 对增产增效有一定的作用[3,4]. 尤其是在缺水地区, 滴灌因其水分利用效率高,用水量少和节省劳力等 优点而备受农业生产者的青睐. 但有研究发现,滴灌 方式的差异会导致土壤湿润体体积和土体垂直及水 平方向上的水分分布均匀度的不同[5,6],最终影响 作物根系的分布范围和作物产量. 与地表滴灌相比, 地下滴灌能促进茄科类根系向土层更深处生长及根 系干物质和产量的增加[7,8]. 土壤水分含量的改变 势必影响土壤微生物群落、功能菌群及其作用、土壤 养分形态及迁移规律的差异. 譬如, 充足的水分条件 可以提高微生物的丰富度和均匀度[9]; 氨氧化古菌 较氨氧化细菌更能适应缺水环境[10]. 在一定条件 下,土壤水分含量的降低会促进微生物硝化过程,在 降低土壤铵态氮含量同时会增加硝态氮的迁移与淋 失风险[11,12]. 生物质炭作为一种在完全或部分缺氧 条件下的芳香化热解产物,因其发达的孔隙结构、比 表面积,使其成为农业生产中的一种改善土壤环境 的资源再利用物质[13]. 生物质炭的添加可以提高土 壤持水能力[14,15],提高水分利用效率和低肥力产田 作物产量[16],并改善果实内部品质[17].生物质炭较 强的吸附能力可以吸附土壤中的无机氮,从而降低 了土壤中的氨和硝酸盐的损失,同时潜在地控制被 吸附养分缓慢释放到植物根部,提高氮利用 率[14,18,19]. 而生物质炭的多孔性能够改善土壤通气 状况,其本身含有的营养物质可以为参与固氮和硝 化过程的好氧微生物提供良好的生长环境,同时抑 制土壤反硝化作用,进而影响土壤不同形态无机氮 含量. 也有研究认为无论是短期还是长时间内,生物 质炭对土壤氮含量都没有影响[20]. 因此,人们需要 结合环境因素综合分析生物质炭在无机氮形态及其 转化的作用.

氨氧化过程作为硝化过程的限速步骤,影响铵 态氮向硝态氮的转化速率,主要受氨氧化古菌 (AOA)和氨氧化细菌(AOB)的调控.含有 nirK 基因 的微生物控制反硝化过程的速度,影响亚硝酸盐的 含量. 亚硝酸盐作为硝酸盐的前物质不仅受氨氧化 作用的调控,同时也受反硝化过程中亚硝酸盐还原 作用的影响[21~23]. 根际土壤作为植物与土壤物质能 量交换活跃区域,其土壤微环境有别于非根际土 壤[24].滴灌方式的不同对作物根际与非根际土壤无 机氮形态以及参与矿质态氮转化的关键功能微生物 调控机制的影响有何差异? 生物质炭的添加是否会 通过改变土壤湿润体体积及水分分布均匀度影响矿 质态氮的转化值得深入研究.基于此,本研究以滴灌 方式(地表滴灌和插入式滴灌)和生物质炭添加与 否作为处理因子,结合 MiSeq 测序技术以及 COG (clusters of orthologous groups)数据库比对,重点解 析不同滴灌方式和生物质炭添加条件下,辣椒根际 与非根际土壤矿质态氮与土壤理化性质及参与矿质 态氮转化的关键功能基因的关系,以期为深入理解 设施农作物根际与非根际土壤微生物群落参与氮转 化生理生态过程提供一定的理论依据,也为提高设 施农业土壤水分和氮素利用效率,缓解设施土壤硝 酸盐累积和因氮素流失导致的地下水污染提供可靠 的技术参考.

1 材料与方法

1.1 试验设计

本试验在中国农业科学院新乡综合试验基地日光温室内开展,土壤质地为壤土.本试验设置 4 个处理,分别为:地表滴灌(D)、插入式滴灌(ID,插入深度 15 cm)、地表滴灌 + 10 t·hm -² 生物质炭(DB)和插入式滴灌 + 10 t·hm -² 生物质炭(IDB,插深 15 cm),每个处理设置 3 个重复. 在种植辣椒前,将生物质炭均匀施入耕层土壤. 生物质炭产自河南商丘三利新能源有限责任公司,为花生壳在 500℃左右条件下制成. 供试土壤与生物质炭基本理化性质如表 1 所示. 供试青椒品种为新查理皇,于 2019 年 3 月 29 日移栽,行距 60 cm,株距 40 cm,每小区 6 行,每行 20 株. 移栽前施入基肥 750 kg·hm -² 复合肥料(氮、磷、钾比例为 19、19、19),有机肥 15 t·hm -² (有机质含量 46%),追肥(复合肥料,氮、磷、钾比例为 19、19、19)在开花期(移栽后 30 d)和结果期

(移栽后59 d和79 d)分3次施入,每次施入量为 375 kg·hm⁻². 地表滴灌和插入式滴灌总灌水量和滴 头流量保持一致,每行辣椒一条滴灌带,每株作物配 一个滴箭. 移栽后第一次灌足水, 在移栽后每 10 d 灌一次水,灌水量35 mm,总灌水量455 mm. 其他田 间管理手段保持一致.

表 1 供试土壤(0~20 cm)及生物质炭基本理化性质1)

Table 1	D :		£ :1		1 1. : 1	f	1	
rabie i	Dasic	properties o	I SOII	samples an	a biochar	ior t	ne ext	periment

项目	有机碳 /g·kg ⁻¹	全氮 /g·kg ⁻¹	全磷 /g·kg ⁻¹	全钾 /g·kg ⁻¹	容重 /g·cm ⁻³	рН	砂粒 /%	粉粒 /%	黏粒 /%
土壤	9. 16	1. 02	0. 86	21. 20	1. 32	7. 58	23.5	69. 7	6. 8
生物质炭	427. 05	11.70	7. 50	27. 60	0. 18	10.08	_	_	

1)"一"表示没有数据

1.2 样品采集与测定

1.2.1 样品采集

在辣椒植株收获前,选择长势均一的代表性植 株,轻轻拔出整棵植株,然后将与根系紧密结合的土 样刷下作为根际土;取两行辣椒中间区域土样作为 非根际土. 每个小区均按照多点取样法,分别将非根 际和根际土混合均匀,去除土壤中可见动植物残体, 然后按照四分法取一部分经过液氮速冻处理后,保 存在-80℃冰箱,用于功能基因定量 PCR 和细菌群 落多样性分析. 一部分新鲜土样用于测定土壤水分、 铵态氮和硝态氮含量,剩余土样风干测定相关理化 指标.

1.2.2 样品测定

采用 Fast DNA® SPIN 试剂盒(Qbiogene Inc., USA) 提取土壤总 DNA, DNA 浓度和纯度利用 NanoDrop2000 进行检测,利用 1% 琼脂糖凝胶电泳 检测 DNA 提取质量. 在设计测序引物时,在序列中 添加 bar-code 序列以区分各个样品测序数据. 用 338F(5'-ACTCCTACGGGAGGCAGCAG-3') 和 806R (5'-GGACTACHVGGGTWTCTAAT-3')引物对 V3-V4 可变区进行 PCR 扩增,扩增程序为:95℃ 预变性 3 min, 27 个循环(95℃变性 30 s, 55℃ 退火 30 s, 72℃延伸 30 s),最后 72℃延伸 10 min(PCR 仪:ABI GeneAmp® 9700型). 扩增体系为 20 μL, 4 μL 5 × FastPfu 缓冲液, 2 μL 2.5 mmol·L⁻¹ dNTPs, 0.8 μL 引物(5 μmol·L⁻¹), 0.4 μL FastPfu 聚合酶; 10 ng DNA 模板. 使用 2% 琼脂糖凝胶回收 PCR 产物,利 用 AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) 进行纯化, Tris-HCl 洗脱, 2% 琼脂糖电泳检测. 利用 QuantiFluor™-ST(Promega, USA)进行检测定量. 根据定量结果和 测序量要求,取 PCR 产物构建测序文库. 构建好的 文库在 Illumina MiSeq PE300 测序平台测序. AOA 基因实时定量 PCR 扩增引物为 Arch-amoAF (5'-STAATGGTCTGGCTTAGACG-3')和 Arch-amoAR(5'-GCGGCCATCCATCTGTATGT-3')[25]; AOB 基因实

时定量 PCR 扩增引物为 amoA-1F(5'- GGGGTTTC TACTGGTGGT-3') 和 amoA-2R (5'- CCCCTCKGSA AAGCCTTCTTC-3')^[25]; nirK 基因实时定量 PCR 扩 增引物为 FlaCu (5'-ATCATGGTSCTGCCGCG-3') 和 R3Cu(5'-GCCTCGATCAGRTTGTGGTT-3')^[25]. 扩增 条件为:95℃ 预变性 3 min, 95℃ 5 s, 55℃ 30 s, 72℃ 1 min, 40 个循环.

基于土壤农化分析方法[26],称取新鲜土样,按 照水土比5:1,采用2 mol·L-1 KCl 浸提,连续流动 分析仪(Tecator FIA Star 5000 Analyzer, Foss Tecator, Sweden)测定铵态氮和硝态氮含量. 土壤水分含量 采用105℃烘干称重法测定. 土壤 pH 按照水土比 2.5:1混匀静置 30 min 后 Metro-pH320 计测定上清 液. 土壤电导率按照水土比 5:1振荡 3 min, 静置澄 清后取上清液,用雷磁 DDSJ-308A 型电导仪测定.

1.3 数据分析

数据统计在 Excel 2007 中完成、香浓多样性的 计算在 QIME1.80 中完成;细菌群落功能基因预测 基于 PICRUSt 分析实现[27],通过 PICRUSt; PICRUSt 软件存储了 greengene ID 对应的 KEGG Ortholog (KO)信息,对 OTU 丰度表进行标准化,并去除 16S marker gene 在物种基因组中的 copy 数目的影响; 然后通过每个 OTU 对应的 greengene ID, 获得 OTU 对应的(KO)信息; 并计算各功能基因对应的 KO 丰度,该分析在 http://huttenhower. sph. harvard. edu/galaxy/中在线实现. 基于 https://www.kegg.jp/ 分析同源基因物种丰度. 基于 SPSS16.0 配对 T 检验 研究处理间或同一处理根际与非根际间土壤理化性 质、功能基因拷贝数、丰度及功能微生物丰度的差 异:基于双因素方差分析研究滴灌方式和生物质炭 对土壤理化性质、功能基因拷贝数和功能菌群的影 响. 结构方程模型的构建基于 SEM Amos 21.0 软件 完成 (Small Waters Corp., Chicago, IL, USA),用 于评估模型拟合度的主要指标包括 P值、卡方值 χ^2 、拟合优度指数(GFI)和渐进残差均方和平方根 (RMSEA)^[28]. 基于 R3.4.3 中的 pheatmap 包作热 图,基于 Origin 8.5 作柱形图.

2 结果与分析

2.1 根际与非根际土壤矿质态氮和其它理化指标的差异

如表 2 所示,同一处理根际土壤硝态氮含量显著低于非根际土壤(P < 0.05). DB 处理非根际土壤水分含量显著高于根际土壤(P < 0.05), ID 和 DB 处理非根际土壤水分含量显著高于 D 处理(P <

0.05). ID 和 DB 处理根际土壤 pH 显著高于非根际土壤(P < 0.05). 除 IDB 处理,其余处理根际土壤电导率均显著高于非根际土壤;生物质炭显著提高 ID 处理中非根际土壤电导率,但显著降低根际土壤电导率(P < 0.05). 双因素方差分析的结果(表 3)表明,滴灌方式、生物质炭及滴灌方式与生物质炭互作均显著影响非根际土壤硝态氮(P < 0.01);在根际土壤中仅有生物质炭对硝态氮的影响显著(P < 0.05).

表 2 土壤矿质态氮和其它理化指标的差异1)

Table 2 Difference of soil mineral nitrogen and other physiochemical properties

TK T-	Г)	ID)	DB	1	ID	В
指标	非根际	根际	非根际	根际	非根际	根际	非根际	根际
铵态氮/mg·kg ⁻¹	10. 78 ± 2. 13	8. 00 ± 1. 03	9. 10 ± 0. 77	8. 77 ± 0. 96	9. 64 ± 0. 80	9. 45 ± 1. 31	9. 42 ± 2. 20	9. 30 ± 0. 72
硝态氮/mg·kg-1	270. 76 ± 6. 20aA	5.38 ± 1.26 b	211. 51 ± 5. 02aB	5. $44 \pm 0.73 \text{bA}$	$276.01 \pm 2.05a$	$3.95 \pm 0.84 \mathrm{b}$	276. 46 ± 3.06 aA	$3.88\pm0.12\mathrm{bB}$
水分含量/%	12.02 ± 0.55 B	11. 82 ± 0.10	14.98 ± 0.79 A	12. 76 ± 1. 19	15. 26 ± 0.81 Aa	13. 37 \pm 1. 32b	15. 00 ± 0.34	13.79 ± 0.70
pН	7.58 ± 0.25	7.98 ± 0.02	7.49 ± 0.03 b	7.98 ± 0.05 a	$7.47 \pm 0.02b$	8. 01 ± 0. 01 a	7. 56 ± 0.30	8.02 ± 0.04
电导率/μS·cm ⁻¹	82. 90 \pm 2. 31b	119. 23 ±9. 74a	72. 30 ± 7.41 bB	116. 67 ± 6. 99aA	85. 32 ± 4. 76b	109. 43 ± 2. 14a	99. 21 ± 8. 17A	106. 03 ± 5. 17B

1)小写字母代表同一处理根际与非根际土壤指标差异,大写字母代表根际或非根际土壤不同滴灌方式或同种滴灌方式生物质炭添加处理之间指标差异

表 3 滴灌方式和生物质炭对辣椒根际与非根际土壤矿质态氮及其转化关键基因拷贝数的影响

Table 3 Effect of drip irrigation and biochar addition on soil mineral nitrogen and the copies of functional genes participating in the transformation of soil mineral nitrogen cycling

处理	因素	H D D T	根际		艮际
Ce VIV	四系	F F	Sig.	F	Sig.
720	铵态氮	1.03	0.341	0.27	0.615
(0)	硝态氮	134.28	0.000	0.00	0.988
滴灌方式	AOA 基因拷贝数	16.54	0.004	4.29	0.072
	AOB 基因拷贝数	81.46	0.000	0.51	0.495
	nirK 基因拷贝数	0.01	0.915	5.14	0.053
	铵态氮	0.19	0.675	2.77	0.135
	硝态氮	191.38	0.000	9.41	0.015
生物质炭	AOA 基因拷贝数	7.37	0.026	0.06	0.815
	AOB 基因拷贝数	125.76	0.000	14.48	0.004
	nirK 基因拷贝数	11.42	0.010	3.64	0.093
	铵态氮	0.60	0.460	0.60	0.461
	硝态氮	137.41	0.000	0.02	0.902
滴管方式×生物质炭	AOA 基因拷贝数	0.20	0.670	12.57	0.008
	AOB 基因拷贝数	14.50	0.005	11.99	0.009
	nirK 基因拷贝数	0.23	0.646	3.81	0.086

2.2 根际与非根际土壤矿质态氮转化关键功能基因变化特征

本研究表明(图1),生物质炭显著降低滴灌处理根际与非根际土壤 AOA 基因拷贝数,却显著提高插入式滴灌处理根际土壤 AOA 基因拷贝数(P < 0.05),同时削弱了两种滴灌方式下根际与非根际土壤 AOA 基因拷贝数差异.在 DB 和 IDB 两个处理

中,辣椒根际土壤 AOB 基因拷贝数显著高于非根际土壤(P < 0.05). 生物质炭显著提高了 D 和 ID 两个处理中根际土壤 AOB 基因拷贝数(P < 0.01), 显著降低了非根际土壤 AOB 基因拷贝数(P < 0.05). 与非根际土壤相比,生物质炭显著提高根际土壤 nirK 基因拷贝数(P < 0.05). 在非根际土壤中, IDB 处理显著低于DB处理中nirK基因拷贝数(P < 0.05).

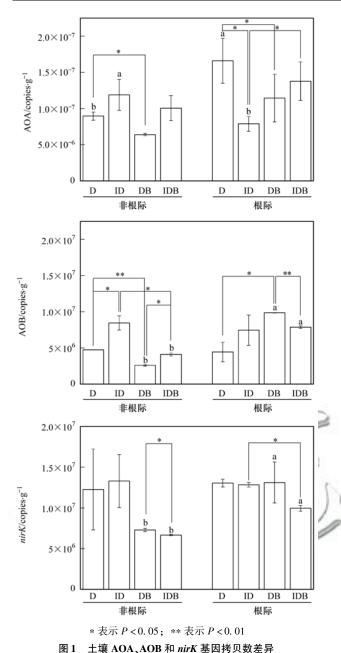


Fig. 1 Difference of gene copies of AOA, AOB, and nirK in soil

在根际土壤中,生物质炭显著提高 ID 处理 nirK 基因拷贝数(P<0.05). 双因素方差分析结果(表3)表明,非根际土壤 AOA 基因拷贝数受滴灌方式和生物质炭的影响,在根际土壤中受二者交互影响(P<0.01). 滴灌方式、生物质炭及滴灌方式与生物质炭交互作用均对非根际土壤 AOB 基因拷贝数的影响显著(P<0.01); 仅有生物质炭对 nirK 基因拷贝数影响显著(P<0.01). 在根际土壤中,nirK 基因拷贝数受生物质炭以及滴灌方式与生物质炭交互影响(P<0.01).

2.3 细菌对参与矿质态氮转化关键功能基因的潜在贡献

基于 PICRUSt 功能预测和同源基因物种丰度的结果(图 2),变形菌门、放线菌门和硝化螺旋菌门为参与氨单加氧酶基因(K10945)表达的三大门类,其中变形菌门中的亚硝化螺菌属对该基因表达的贡献高于其它菌属.参与含铜离子的亚硝酸还原酶基因(K00368)表达的细菌门类有 9 种,其中变形菌门、放线菌门和拟杆菌门丰度相对较高,如变形菌门中的中慢生根瘤菌属、慢生根瘤菌属、微枝形杆菌属、粘着箭菌属;放线菌门中的微杆菌属、芽球菌属以及拟杆菌门的 Pontibacter 属等. 双因素方差分析的结果发现,生物质炭对非根际土壤 K10945 和根际土壤 K00368 同源基因物种群落结构的影响显著(表 4, P < 0.05).

2.4 环境因子与生物因子对根际与非根际土壤矿质态氮的影响

基于结构方程模型的结果(图 3),在非根际土壤中环境因子和生物因子对铵态氮的解释率为70%,对硝态氮的解释率为99%.铵态氮与水分含量及pH 呈显著负相关; AOB 基因拷贝数与电导率呈显著负相关.硝态氮与电导率及nirK基因拷贝数呈

表 4 滴灌方式和生物质炭对辣椒根际与非根际土壤矿质态氮转化功能微生物群落结构的影响

Table 4 Effect of drip irrigation and biochar addition on functional microbial communities participating

in the transformation of soil mineral nitrogen

处理	基因	非相	根际	根际	
处理	本 囚	\overline{F}	Sig.	\overline{F}	Sig.
滴灌方式	K10945	0.901	0.494	1.128	0.352
间准刀八	K00368	0.478	0.943	1.145	0.283
生物质炭	K10945	2.819	0.032	0.211	0.957
生物灰灰	K00368	0.772	0.625	2.133	0.027
滴管方式×生物质炭	K10945	2.850	0.042	0.637	0.657
闹官刀式×生物灰灰	K00368	1.163	0.310	0.882	0.558

显著正相关,与水分含量及 AOB 基因拷贝数呈显著 负相关.基于表 5 中的 λ 值,pH 对铵态氮的直接负作 用最大,其次是水分含量; AOB 基因拷贝数在所有因

子中对硝态氮直接负作用最大,电导率对硝态氮的直接与间接正作用均最大.在根际土壤中环境因子和生物因子对铵态氮和硝态氮的解释率分别为70%和

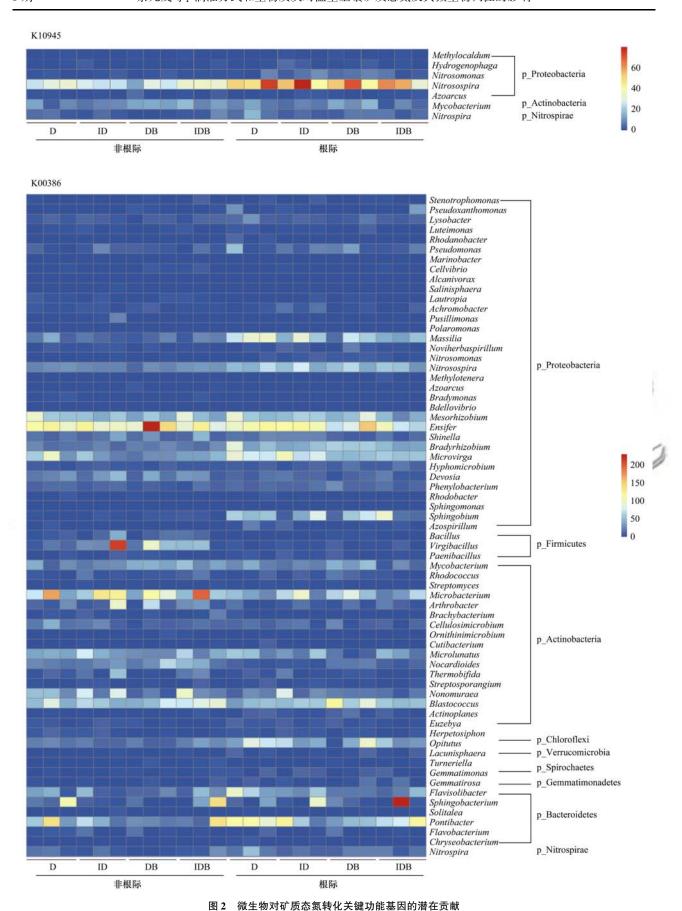
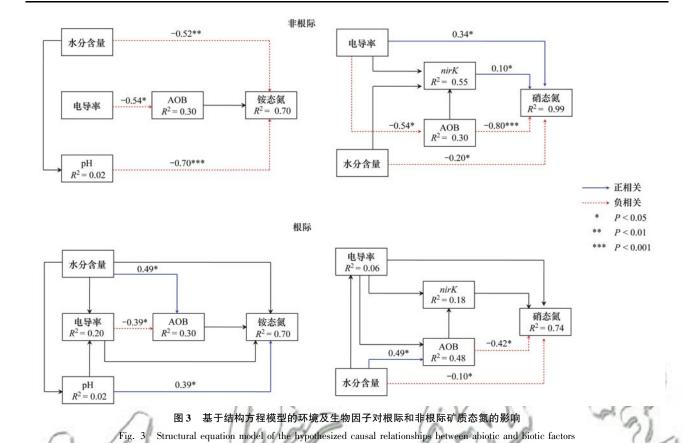


Fig. 2 Potential contribution of microbe to functional genes expression participating in the transformation of soil mineral nitrogen

74%. 硝态氮与 AOB 基因拷贝数呈显著负相关, pH 对铵态氮的直接正作用最大, 电导率的直接负作用最

大. AOB 基因拷贝数在所有因子中对硝态氮直接负作用最大; 电导率对硝态氮的直接正作用最大.



and soil mineral nitrogen in the rhizosphere and non-rhizosphere soils of pepper plants 表 5 环境及生物因子对辣椒根际与非根际土壤矿质态氮的直接和间接影响¹⁾

Table 5 Direct, indirect, and total effects of abiotic and biotic factors on soil mineral nitrogen

土壤	矿质态氮	影响	A	/		λ 值	
一块	7 贝心氨	彩啊	水分含量	电导率	pН	AOB 基因拷贝数	nirK 基因拷贝数
		直接影响	-0.523	0.000	-0.701	-0.225	_
11	铵态氮	间接影响	0.109	0.122	0.000	0.000	_
非根际		总影响	-0.414	0. 122	-0.701	-0.225	_
		直接影响	-0.199	0.342	_	-0.799	0.097
	硝态氮	间接影响	-0.032	0.376	_	0.031	0.000
		总影响	-0.231	0.718	_	-0.768	0.097
		直接影响	0.118	-0.304	0.390	0.204	_
	氨态氮	间接影响	0.251	-0.079	0.145	0.000	_
根际		总影响	0.369	-0.383	0.535	0.204	_
		直接影响	-0.096	0.372	_	-0.425	0.283
	硝态氮	间接影响	-0.344	0.285	_	-0.045	0.000
		总影响	-0.441	0.656	_	-0.380	0.283

1)"一"表示没有数据

3 讨论

3.1 根际与非根际土壤矿质态氮对滴灌方式与生物质炭的响应

生物质炭由于其多孔性及较大的表面积增加了生物质炭颗粒与水分之间的吸附力;同时,生物质炭的添加可以增加土壤毛细孔隙,从而提高土壤水分含量^[15].因此,生物质炭添加削弱了滴灌方式对

非根际土壤水分含量的影响. 土壤中辣椒根系对水分的吸收及其在土壤中的分布和对土壤物理性状的改变可能会削弱生物质炭对水分含量的影响,从而导致不同滴灌方式下,生物质炭添加与否对土壤水分含量的影响并不显著. 滴灌方式和生物质炭添加与否对辣椒根际或非根际土壤铵态氮含量均无显著影响. 但不同处理方式可以通过改变土壤其它理化性质直接或间接影响矿质态氮的含量. 研究发现在

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非根际土壤中,铵态氮与水分含量及 pH 呈显著负 相关. 水分含量的升高可能会削弱土壤氮的矿化作 用,减少铵态氮的积累[25].有研究认为,并不是水分 含量升高就会削弱土壤的硝化过程,当土壤水分含 量低于某一个临界点时,与反硝化作用相比,硝化作 用依然占主导地位. 本研究中土壤水分含量可能对 硝化作用有一定的激发作用,促进铵态氮向硝态氮 的转化,从而导致土壤铵态氮含量的降低[11]. 土壤 pH 值的升高促进土壤水溶液 NH, 的形成和挥发, 导致土壤铵态氮的减少;另一个可能原因是土壤 pH 的升高会削弱矿化作用,同时增强硝化作用,从 而使土壤中矿化产生的铵态氮减少,更多的铵态氮 转化成硝态氮[23,29]. 但是,在根际土壤中铵态氮与 水分含量和 pH 的关系并不明显,这可能与根际和 非根际土壤微环境(如:根际土壤中辣椒根系分布、 根系对养分的吸收及根系分泌物等)的差异有 关[30].

生物质炭添加对非根际和根际土壤硝态氮含量 均有显著影响,但生物质炭的添加显著提高同一滴 灌方式下非根际土壤硝态氮含量,却降低了根际土 壤硝态氮含量. 非根际土壤硝态氮含量的增加可能 与生物质炭本身吸附作用以及滴灌方式带来的水分 差异有关[6,15],而根际硝态氮含量的降低可能与根 系对氮素的吸收有关[24,31]. 有研究发现,非根际与 根际土壤硝态氮含量均与水分含量呈显著负相关关 系. 在本研究中水分含量的增加导致硝态氮淋失的 风险增大,同时生物质炭对硝态氮的吸附固定可能 会降低其在土壤溶液中的含量[12,14,18]. 生物质炭的 添加促进同一种滴灌方式下非根际土壤电导率的增 加和硝态氮含量的积累,这可能是由于生物质炭本 身带有大量的阳离子,如 K+和 Ca2+等,阳离子的释 放促进土壤溶液电导率的提升以及土壤溶液中阳离 子吸附位点,从而有利于对 NO; 的吸附,促进土壤 硝态氮含量的增加[11,30]. 总体来讲,滴灌方式和生 物质炭可以通过自身的吸附作用或者调控土壤水分 含量、电导率等指标影响辣椒根际与非根际土壤硝 态氮含量.

3.2 滴灌方式与生物质炭对根际与非根际土壤矿质态氮转化微生物调控的影响

氨氧化细菌和古菌参与亚硝化过程,为硝化过程提供必要的亚硝酸盐,是硝化过程的主要驱动者,同时也受环境因子的调控^[25].在本研究中,与不添加生物质炭处理相比,生物质炭显著降低了地表滴灌处理根际或非根际土壤 AOA 基因拷贝数,却显著提高了插入式滴灌处理根际土壤 AOA 基因拷贝数(P<0.05),这可能与滴灌方式带来的土壤微环境

的差异(如:氧环境)以及作物根系分泌物(如:酸类 物质的量等)的差异有关,有待深入研究[32]. 生物质 炭的添加显著降低同种滴灌方式下非根际土壤 AOB 基因拷贝数,显著提高了根际土壤 AOB 基因 拷贝数,因此,生物质炭提高了非根际与根际土壤 AOB 基因拷贝数的差异. 综合以上结果, 生物质炭 的添加可能会削弱非根际土壤氨氧化作用,有利于 铵态氮在土壤中的存留.辣椒作为喜硝态氮的植物, 根际土壤 AOA 和 AOB 基因拷贝数的增加有利于土 壤铵态氮向硝态氮转化,有利于提高辣椒根际对氮 源的利用率. 普遍认为铵态氮是氨氧化细菌在进行 硝化作用过程中所必需的营养物质,它直接影响硝 化细菌的生长[33],但是在本研究中铵态氮在各处理 中的变化不显著,同时也不是模型构建的关键因子, 可能在一定程度上说明本研究中供试土壤铵态氮的 含量充裕,并未成为影响 AOA 和 AOB 基因拷贝数 的限制性因子. 有研究发现在 pH 为中性和碱性环 境中,AOB 是硝化作用的主要驱动者,而 AOA 主要 在如低氮、强酸性和高温等较苛刻的环境中发挥功 能活性[21,22],说明在土壤矿质态氮转化过程中,氨 氧化微生物功能的发挥对环境具有一定的选择性. 基于结构方程模型的结果,生物质炭的添加可以通 过改变根际与非根际土壤中电导率,影响 AOB 基因 拷贝数. 有研究发现土壤盐分的升高会削弱硝化作 用强度,这可能是由于盐分升高会在一定程度上破 坏微生物的细胞膜和菌体内的酶,抑制微生物的生 长,从而导致氨氧化细菌基因拷贝数的降低[34].同 时,本研究发现生物质炭添加提高了两种滴灌方式 下根际土壤水分含量,进而增加 AOB 基因拷贝数. 可能是由于生物质炭添加在一定水平上提高了土壤 铵态氮的含量,为硝化作用提供了更多可利用氮源, 从而有效提高 AOB 基因拷贝数^[23,25].

nirK 基因作为反硝化过程中参与亚硝酸盐还原的关键基因,在调控硝酸盐含量中也起着重要作用^[21].总体来讲,生物质炭添加显著降低了同种滴灌方式下辣椒非根际土壤 nirK 基因拷贝数.这可能与生物质炭具有大量的孔隙结构,可以提高土壤通气性有关.因为土壤通气性的增加会削弱参与反硝化作用功能微生物活性及数量^[23].土壤电导率的升高可能会削弱氨氧化细菌参与的硝化过程,减少亚硝酸盐的形成,作为 nirK 基因的反应底物,亚硝酸盐的减少可能会影响到参与该过程功能微生物的数量.另外,生物质炭特殊的物理化学特性刺激了辣椒根系分泌物的分泌、根际微环境的改变,进而影响到根际与非根际土壤 nirK 基因拷贝数^[35].

基于 PICRUSt 功能预测和同源基因物种丰度,

参与硝化作用中氨氧化过程的菌群主要属于 γ-变形菌纲,其中亚硝化螺菌属对该基因(K10945)表达的贡献高于其它菌属;而丰度较高,且参与含铜离子的亚硝酸还原酶基因(K00368)表达的菌属主要属于 α-变形菌纲,其中根瘤菌成员的丰度相对较高. 双因素方差分析表明,滴灌方式不影响辣椒根际与非根际土壤参与矿质态氮转化的关键功能菌群的稳定性,但生物质炭添加却因为其对土壤理化形状的改变影响着非根际土壤氨氧化菌群和根际土壤参与亚硝化还原菌群的稳定性.

4 结论

- (1)生物质炭削弱了地表滴灌与插入式滴灌非根际土壤硝态氮的差异,促进了同种滴灌方式下非根际土壤硝态氮的积累,降低了根际土壤硝态氮的含量.
- (2)生物质炭可以通过改变根际与非根际土壤水分含量和电导率影响 AOB 基因拷贝数和矿质态氮含量,有利于降低同种滴灌方式非根际土壤的亚硝酸盐还原作用。
- (3)辣椒根际与非根际土壤参与矿质态氮转化 关键功能菌群的稳定性不受滴灌方式的影响,却受 生物质炭的调控.

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