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生物炭对退化蔬菜地土壤及其修复过程中 N₂O 产排的影响*

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摘 要 高氮投入的设施蔬菜地土壤易发生次生盐渍化、酸化、板结化等土壤退化现象, 也会引起地下水污染、N₂O 大量排放等环境后果, 严重影响了蔬菜农业的可持续发展。强还原土壤灭菌 (reductive soil disinfection, RSD) 作为新兴的退化设施蔬菜地土壤修复方法, 能够有效缓解土壤退化, 但也导致 N₂O 大量排放。本研究选用生物炭作为调节剂, 评估不同生物炭施加量对退化设施蔬菜地土壤及其强还原修复过程中 N₂O 排放的影响, 并测定反硝化功能基因 (一氧化氮还原酶基因 *norB* 和氧化亚氮还原酶基因 *nosZ*) 丰度来反映反硝化微生物活性。结果显示: RSD 法显著降低了土壤硝酸盐含量、提高了土壤 pH, 缓解了土壤退化, 但其 N₂O 累计排放量是非修复土壤的 950 倍以上; 施加生物炭具有减排效果, 其中施加 5% 的生物炭显著降低了退化设施蔬菜地土壤及其强还原修复过程中的 N₂O 排放, 其减排量分别为 68.7% 和 16.0%; Pearson 相关分析显示, 非强还原修复过程和强还原修复过程中土壤 N₂O 排放速率均与 pH 显著负相关, 而在强还原修复过程中土壤 N₂O 排放速率还与 NO₃⁻-N 含量显著正相关; 施加生物炭显著改变了土壤 *norB* 和 *nosZ* 基因, 线性回归分析表明, 非强还原修复过程和强还原修复过程中土壤 N₂O 排放的微生物机理不同, 前者显著受 *nosZ* 基因丰度影响, 而后者显著受 *norB* 基因丰度影响。在退化设施蔬菜地土壤中施加 5% 生物炭可有效减低退化设施蔬菜地土壤及其强还原修复过程中的 N₂O 排放。

关键词 N₂O 排放; 生物炭; 退化设施蔬菜地; 土壤修复; 环境效应; 反硝化微生物

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设施蔬菜地生产因其集约化程度高、复种指数高、单位产量高、经济效益高、受季节影响小等优点, 在我国的种植面积持续扩大, 2011 年种植面积达到 2.43×10^7 hm², 占农作物总种植面积的 12.9%^[1]。但是不合理的轮作、大量氮肥施用使得土壤出现严重的次生盐渍化、酸化、板结化等土壤退化现象, 乃至土传病害频繁发生引起蔬菜减产或绝产^[2-3]。硝化作用可以将施入土壤中的铵盐、尿素等氮肥转

化成硝酸盐, 设施蔬菜地大量氮肥的施加导致硝酸盐大量积累, 可导致地下水严重污染、氧化亚氮 (N₂O) 大量排放等, 既造成氮素流失, 也给环境带来巨大的影响^[2, 4]。

退化设施蔬菜地土壤修复研究由来已久, 方式多样^[5-8], 如 (1) 施加石灰等碱性物质来缓解土壤酸化; (2) 施加有机物料来提高土壤有机质含量、缓解土壤板结化; (3) 化学灭菌来减轻土

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传病害；(4)田闲时短期淹水、轮作、间作等缓解土壤退化；(5)培育新品种。近年来，一种称为强还原土壤灭菌(reductive soil disinfestation, RSD)的退化蔬菜地土壤修复技术快速发展，该技术采用短期淹水并添加有机物料的方法，制造强还原环境来快速修复退化设施蔬菜地土壤，达到消除连作障碍、杀灭土传病害、提高作物产量的目的^[9-11]。然而，强还原环境下，退化设施蔬菜土壤高积累的硝酸盐经反硝化作用，可快速生成 N_2O ，给大气环境带来了负面影响。 N_2O 作为仅次于 CO_2 和 CH_4 的第三大温室气体，其增温效应是 CO_2 的296倍~310倍^[12]。据估计，大气中每年有80%~90%的 N_2O 来源于土壤^[13]，而农田土壤是重要排放源，其中高氮投入的设施蔬菜地土壤占农田土壤 N_2O 总排放的20%，是 N_2O 的重要排放源^[14]。因此，研究退化设施蔬菜地土壤 N_2O 减排，特别是强还原修复过程中的 N_2O 减排措施尤为重要。高硝态氮背景下，反硝化过程是 N_2O 产生的主要途径，其排放速率往往受一氧化氮还原酶(NorB，又称氧化亚氮产生酶)和氧化亚氮还原酶(NosZ)活性的影响，因此，土壤 $norB$ 和 $nosZ$ 基因丰度是研究 N_2O 产生功能微生物机理的重要指标^[15-16]。目前大部分研究认为生物炭可能是通过影响 $nosZ$ 基因丰度来影响旱地农业土壤 N_2O 产生^[16-17]，但是在强还原环境中的研究鲜见报道，因此，研究退化设施蔬菜地及其强还原修复过程中的 N_2O 产生功能基因很有必要。

农业土壤 N_2O 减排措施主要包括水肥管理、耕作方式、土壤调节剂等^[5]。考虑到设施蔬菜地耕作方式、肥料管理相对固定以及强还原修复过程中的高水分条件，施加土壤调节剂可能是减少退化设施蔬菜地土壤及其强还原修复过程中 N_2O 排放的有效途径。生物炭是富含碳的多孔材料，具有高化学稳定性、热稳定性和生物稳定性等特点，可有效提高土壤pH、增加土壤透气性、提高微域微生物活性^[16, 18]。但是，还有一些研究发现施加生物炭并不能显著减少 N_2O 排放^[19-21]。因此，本研究旨在测定施加不同量生物炭对高氮投入的退化设施蔬菜地土壤及其强还原修复过程中 N_2O 的减排效果并探讨其机理。

1 材料与方法

1.1 供试材料

本研究选取南京麒麟门设施蔬菜地(N 31°14', E 118°22')为研究位点，该地区隶属亚热带季风气候，年降水量1 106 mm，年平均气温15.4 °C。该设施蔬菜地种植年限均在5年以上，每年种植两茬或三茬作物(西红柿、茄子、黄瓜、草莓)，其有机氮肥和无机氮肥年均施用量分别达到 $N_{200} \sim 300$ 和 $600 \sim 800 \text{ kg hm}^{-2}$ 。目前，该蔬菜地土壤硝态氮含量高达 208.5 mg kg^{-1} ，并出现盐渍化、板结等土壤退化现象。供试土壤为该蔬菜地0~20 cm土壤，一部分新鲜土壤过2 mm筛后于4 °C保存备用(试验于两周内进行)，一部分土壤风干后用于理化性质测定。生物炭由中国科学院南京土壤研究所谢祖彬研究员课题组自主研发的秸秆制炭设备(专利号: ZL200920232191.9)在400 °C无氧环境下进行烧制，过1 mm筛备用。水稻秸秆取自南京周边水稻田，80 °C下烘干后剪碎过2 mm筛备用。本研究所用土壤、生物炭和水稻秸秆的基本理化性质见表1。

1.2 研究方法

在250 ml锥形瓶中称取相当于30.0 g干土重的新鲜土壤，分别加蒸馏水调节土壤含水率为16%(设施蔬菜地原位平均含水量)及淹水加稻秆修复状态(淹水2 cm，稻秆加入量为土壤干重的2%)，并按照土壤干重0%、1%和5%的比例施加生物炭，共计6个处理：①CK(含水量16%，对照)、②1% BC(含水量16%，1%生物炭)、③5% BC(含水量16%，5%生物炭)、④RSD(淹水加稻秆修复状态，对照)、⑤RSD + 1% BC(淹水加稻秆修复状态，1%生物炭)和⑥RSD + 5% BC(淹水加稻秆修复状态，5%生物炭)。每个处理设置32个重复。锥形瓶使用保鲜膜封口(膜上有小孔透气)，置于30 °C培养箱中避光培养。

处理CK、1% BC和5% BC，于培养的0、1、3、7、10、14 d随机选择4个重复锥形瓶测定土壤 N_2O 排放速率；处理RSD、RSD + 1% BC和RSD + 5% BC，于培养的0、4、7、14 d随机选择4个重复锥形瓶用于测定土壤 N_2O 排放速率。土壤 N_2O 排放

表1 供试土壤、生物炭和水稻秸秆的理化性质

Table 1 Physicochemical properties of the soil, biochar and rice straw used in this study									
样品 Samples	来源 Sources	总有机碳 TOC (%)	总碳 TC (%)	总氮 TN (%)	C : N	pH	硝态氮 NO ₃ -N (mg kg ⁻¹)	铵态氮 NH ₄ -N (mg kg ⁻¹)	电导率 EC (mS m ⁻¹)
土壤 Soil	南京麒麟门设施 蔬菜地	2.03	3.51	0.26	13.5	6.43	208.5	16.53	54.4
生物炭 Biochar	实验室自制	48.2	50.3	1.07	47	10.04	ND	ND	28.4
水稻秸秆 Rice straw	南京周边收集	51.9	53.4	0.89	60	ND	ND	ND	ND

注：ND，未检测 Note: ND, not determined

速率采用累计法测定，测定前使用合成空气（80% N₂和20% O₂）冲刷锥形瓶换气后，使用硅胶塞将瓶口密封，置于30 ℃培养箱中培养。于培养的0和4 h抽取20 ml气体样品，N₂O浓度测定采用含有ECD检测器的气相色谱法（Agilent 7890A），检测器温度为300 ℃，载气（N₂）流速为2 ml min⁻¹，柱温为55 ℃。根据锥形瓶4 h内N₂O的积累速率来计算土壤N₂O排放速率。于培养的0、1、2、3、7、10、14 d，采集完气体后（采样日时间点保持一致），每个处理随机选择4个重复进行破坏性采样，一部分样品用于测定土壤无机氮含量和土壤pH，一部分土壤保存在-70 ℃用于分子生物学分析。

1.3 指标测定

土壤和水稻秸秆的理化性质测定方法参照文献[22]。采用H₂SO₄-K₂CrO₇消解法测定总有机碳含量，采用元素分析仪（VARIO EL III）法测定总碳和总氮含量，采用玻璃电极法（METTER TOLEDO InLab Expert Pro pH detector）测定土壤pH（水土比为2.5:1），采用电导仪法测定土壤电导率（雷磁DDSJ-308F）。使用2 mol L⁻¹ KCL提取土壤无机氮，采用流动分析仪（SKALAR SAN++）来测定滤液中的NH₄⁺-N和NO₃⁻-N含量。生物炭的总有机碳、总碳、总氮、pH等指标测定参照European Biochar Certificate^[23]。

土壤总DNA采用试剂盒法进行提取（FastDNA SPIN Kit for Soil, MP Biomedicals），DNA浓度和纯度采用NanoDrop 2000测定。采用基于SYBR green染料法的定量PCR（qPCR）技术检测土壤

反硝化功能基因*norB*和*nosZ*的丰度，引物分别为*cnorB_BF/cnorB_BR*^[24]和*nosZ2F/nosZ2R*^[25]，SYBR green试剂选用SYBR® Premix Ex Taq Kit（Takara, China），测定机器为CFX96 Real-time PCR system（Bio-rad, USA）。qPCR反应体系包括10.0 μl的2 × SYBR® Premix Ex Taq buffer、上下游引物（20 μmol μl⁻¹）各0.5 μl、1.0 μl样品DNA模版（6.7~13.5 ng μl⁻¹），最后加入灭菌超纯水补足至20 μl。qPCR反应程序为：95 ℃预变性3 min，95 ℃变性20 s，退火30 s（*norB*和*nosZ*基因扩增的退火温度分别为57和56 ℃），72 ℃延伸30 s，共40个循环，每循环结束后采集荧光数据；40轮扩增后，从65 ℃到95 ℃，每上升0.5 ℃便采集荧光数据，制作溶解曲线以判断扩增特异性^[26]。分别以含有*norB*和*nosZ*基因的重组质粒作为标准DNA模版，连续10倍稀释后制作标注曲线，浓度范围分别为1.35 × 10²~1.35 × 10⁸ μl⁻¹和2.17 × 10²~2.17 × 10⁸ μl⁻¹。每个样品做3个技术重复，*norB*和*nosZ*基因定量PCR的扩增效率分别为100.9%和104.7%，R²分别为0.994和0.992。

1.4 数据分析

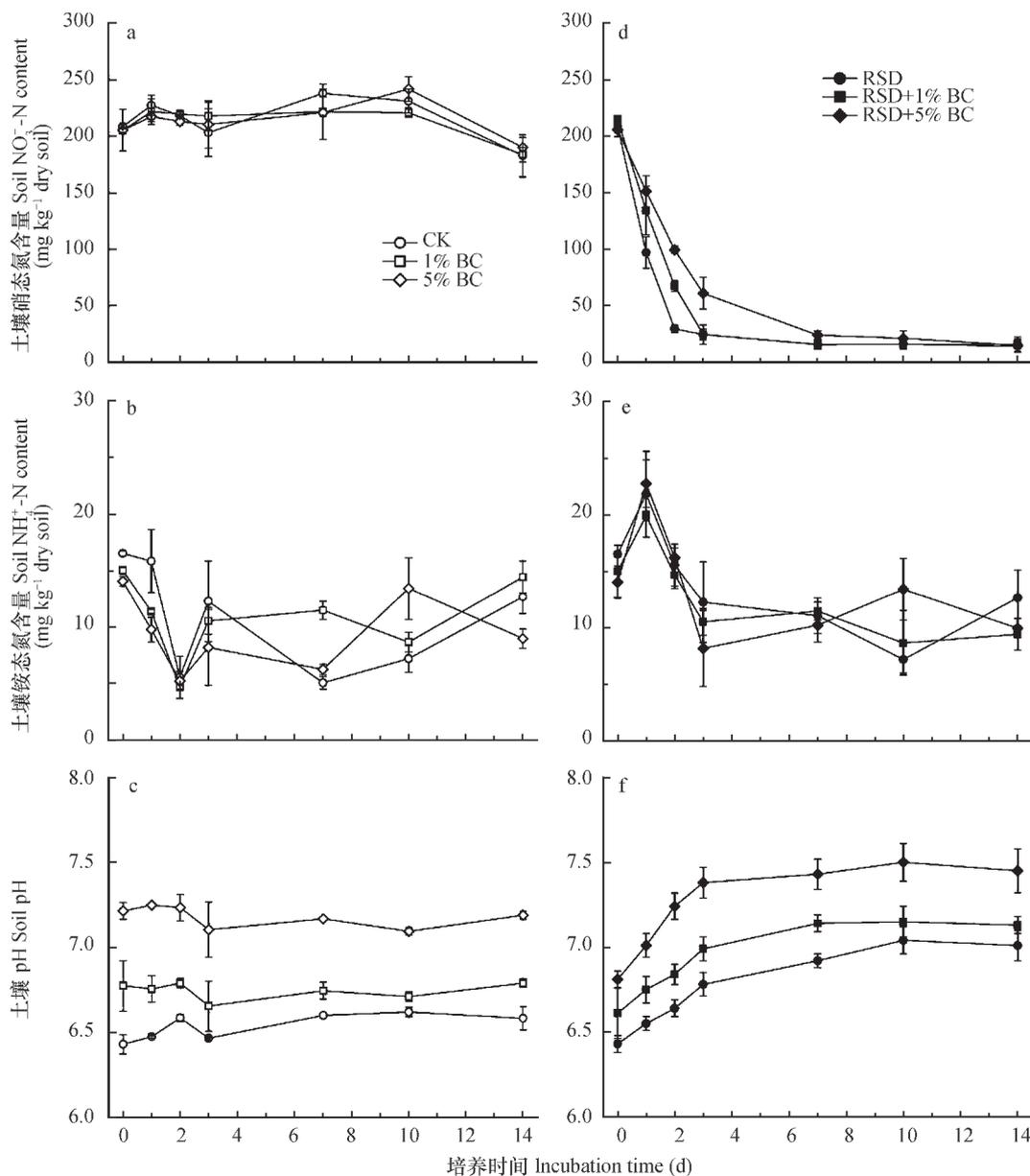
本研究采用SPSS 16.0进行统计分析，采用单因素方差分析比较处理间的差异显著性（*p* < 0.05，LSD 检验），采用Pearson相关分析研究N₂O排放速率、反硝化基因丰度和环境因子间的关系，采用线性拟合来研究N₂O排放速率和功能基因丰度间的关系。使用Origin 8.0、Adobe Illustrator CS6.0进行图像绘制。

2 结果

2.1 施加生物炭对土壤理化性质的影响

退化设施蔬菜地土壤培养14 d内土壤 NO_3^- -N、 NH_4^+ -N含量和土壤pH变化见图1。14 d培养期内，土壤 NO_3^- -N、 NH_4^+ -N含量和pH无显著变化；施加不同量的生物炭对退化设施蔬菜地土壤 NO_3^- -N和 NH_4^+ -N含量无显著影响， NO_3^- -N含量始终处于较高水平；施加生物炭提高了土壤pH，施加量越大，

pH提升的幅度越高（图1c）。退化设施蔬菜地土壤强还原修复过程中的土壤 NO_3^- -N、 NH_4^+ -N含量和土壤pH变化见图1。14 d的强还原修复过程中，土壤 NO_3^- -N含量持续下降，培养结束3个处理土壤 NO_3^- -N含量水平一致；施加生物炭显著减缓了 NO_3^- -N含量的下降速率，RSD处理土壤 NO_3^- -N含量于2 d内下降至最低水平，RSD + 1% BC处理于3 d内下降至最低水平，而RSD + 5% BC处理于7 d内下降至最低水平（图1d）。14 d的修复期内，3



注：图中值为平均值，误差线为标准差 ($n=4$)。下同 Note: Values and error bars prefer means and standard deviations ($n=4$).

The same below

图1 施加生物炭对退化蔬菜地土壤 (a, b, c) 及其强还原修复过程 (d, e, f) 中的土壤无机氮含量和pH的影响
Fig. 1 Effects of biochar amendment on soil inorganic nitrogen contents and pH in degraded vegetable soil (a, b, c) and the soil under reductive soil disinfestation (RSD) (d, e, f)

个处理土壤NH₄⁺-N含量无显著差异。施加生物炭提高了土壤pH，施加量越大，pH提升的幅度越高；强还原修复后，各处理土壤pH显著上升（图1f）。

2.2 施加生物炭对土壤N₂O排放的影响

退化设施蔬菜地土壤培养14 d内土壤N₂O排放速率和累计排放量结果见图2和图3。14 d培养期内，添加生物炭土壤N₂O排放速率低于CK处理，5% BC处理土壤N₂O排放速率显著最低（图2a），5% BC处理的N₂O累计排放量也显著低于CK处理

（N₂O减排量为68.7%）（图3a）。强还原修复过程中，施加5%生物炭也降低了土壤N₂O排放速率（图2b），其N₂O累计排放量也显著低于CK处理（N₂O减排量为16.0%）（图3b）。强还原修复环境下的N₂O累计排放量显著较高，是非强还原修复环境下的951倍~2 554倍（图3）。Pearson相关分析（表2）显示，非强还原修复过程中土壤N₂O排放速率仅和pH显著相关（ $p = 0.001$ ），而强还原修复过程中土壤N₂O排放速率和pH、NH₄⁺-N、NO₃⁻-N含量均显著相关（ $p < 0.05$ ）。

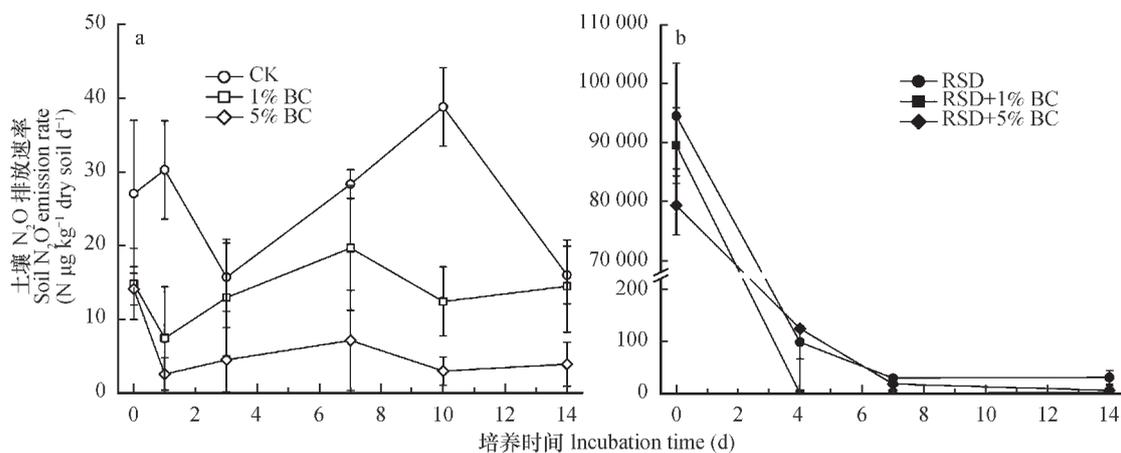
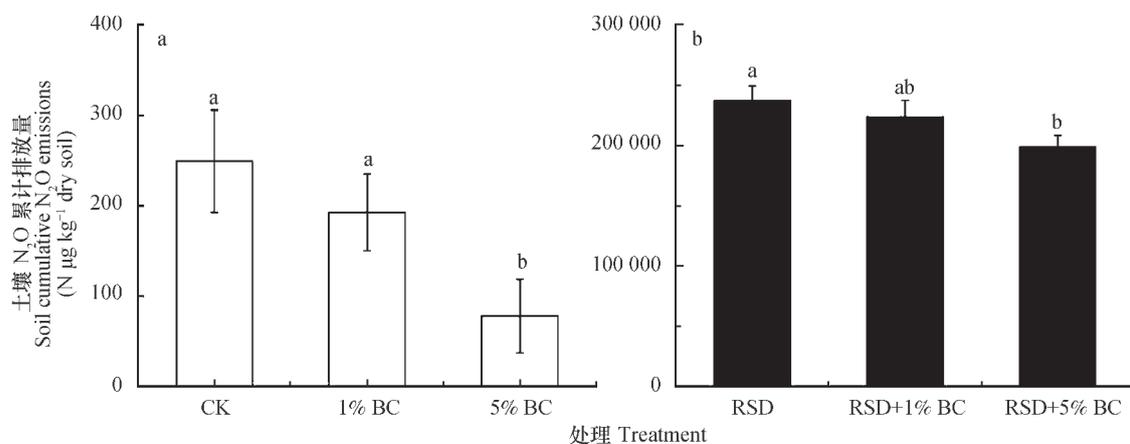


图2 施加生物炭对退化蔬菜地土壤（a）及其强还原修复过程（b）中的土壤N₂O排放速率的影响

Fig. 2 Effects of biochar amendment on soil N₂O emission rates in degraded vegetable soil (a) and in the soil under reductive soil disinfestation (RSD) (b)



注：字母不同代表处理间的显著差异（ $p < 0.05$ ） Note: Different letters above the columns indicate a significant difference between treatments at 0.05 level

图3 施加生物炭对退化蔬菜地土壤（a）及其强还原修复过程（b）中的土壤N₂O累计排放量的影响

Fig. 3 Effects of biochar amendment on soil cumulative N₂O emissions in degraded vegetable soil (a) and in the soil under reductive soil disinfestation (RSD) (b)

表2 土壤N₂O排放速率、反硝化功能基因丰度与环境因子间的皮尔森相关分析Table 2 Pearson's correlation analysis between soil N₂O emission rates, the abundances of denitrifying functional genes and environmental factors

	参数 Parameter	pH	铵态氮 NH ₄ ⁺ -N	硝态氮 NO ₃ ⁻ -N
非强还原修复过程 non-RSD process	N ₂ O排放速率 N ₂ O emission rate	-0.734 (0.001)	0.084 (0.741)	0.228 (0.362)
	<i>norB</i> 基因丰度 Abundance of <i>norB</i> gene	0.107 (0.784)	0.288 (0.453)	-0.172 (0.659)
	<i>nosZ</i> 基因丰度 Abundance of <i>nosZ</i> gene	0.647 (0.060)	-0.409 (0.274)	-0.286 (0.456)
强还原修复过程 RSD process	N ₂ O排放速率 N ₂ O emission rate	-0.606 (0.037)	-0.660 (0.020)	0.580 (0.048)
	<i>norB</i> 基因丰度 Abundance of <i>norB</i> gene	-0.925 (0.008)	0.887 (0.001)	0.927 (<0.001)
	<i>nosZ</i> 基因丰度 Abundance of <i>nosZ</i> gene	0.354 (0.350)	-0.256 (0.506)	-0.241 (0.532)

注：表中括号外数值代表皮尔森相关系数，括号内数值代表相关分析的显著性（双侧）Note: Value outside the parentheses is Pearson correlation coefficient, and value inside the parentheses indicates the sig. (2-tailed)

2.3 施加生物炭对土壤功能微生物群落丰度的影响
退化设施蔬菜地土壤及其强还原修复过程中的土壤一氧化氮还原酶基因 (*norB*) 和氧化亚氮还原酶基因 (*nosZ*) 丰度变化见图4。14 d培养期内，CK处理土壤*norB*基因丰度先下降后上升，但施加生物炭处理土壤*norB*基因丰度先上升后下降（图4a）；而强还原修复的3个处理中土壤*norB*基因丰度呈下降趋势（图4b）。14 d培养期内，CK、1% BC和5% BC处理土壤*nosZ*基因丰度呈持续上升趋势，施加生物炭处理上升速度高于CK处理（图4c）；而强还原修复的3个处理中土壤*norB*基因丰度无显著变化（图4d）。Pearson相关分析（表2）显示，非强还原修复过程中土壤*norB*基因和*nosZ*基因丰度与pH、NH₄⁺、NO₃⁻含量均不显著相关；在强还原修复过程中土壤*norB*基因与pH、NH₄⁺、NO₃⁻含量显著相关 ($p < 0.01$)，而*nosZ*基因丰度与环境因子不显著相关。

线性回归分析显示，非强还原修复过程中土壤N₂O排放速率的log值和*nosZ*基因丰度的log值呈显著线性相关 ($p = 0.010$)（图5a），与*norB*基因丰度的log值无显著线性关系 ($p = 0.748$)；但是在强还原修复过程中，土壤N₂O排放速率的log值和*norB*基因丰度的log值呈显著线性相关 ($p <$

0.001)（图5b），与*nosZ*基因丰度的log值无显著线性关系 ($p = 0.801$)。

3 讨论

3.1 施加生物炭减少了土壤N₂O排放

国内外研究显示施加生物炭能够有效减少旱地或水田土壤N₂O的排放，其减排量从20.2%^[27]至89%^[28]不等。本研究中施加生物炭在非强还原修复环境下减少了68.7%的N₂O排放，在强还原修复环境下减少了16.0%。考虑到强还原修复环境下的N₂O累计排放量是非强还原修复环境下的950倍以上，因此在强还原修复环境中施加生物炭的N₂O绝对减排能力处于领先水平。本研究中施加1%生物炭也有减排效果，但数据统计不显著，而施加了5%生物炭的处理具有显著的减排效果。可见，生物炭的减排效果还与施加量相关，同样的结果也有文献报道^[29]。

生物炭为碱性，添加生物炭可有效提高土壤pH^[16; 29]。本研究中发现施加生物炭提高了退化设施蔬菜地土壤及其强还原修复过程起始状态下的土壤pH，生物炭施加量越大，pH提升的幅度越高，且相关分析显示非强还原修复过程和强还原修

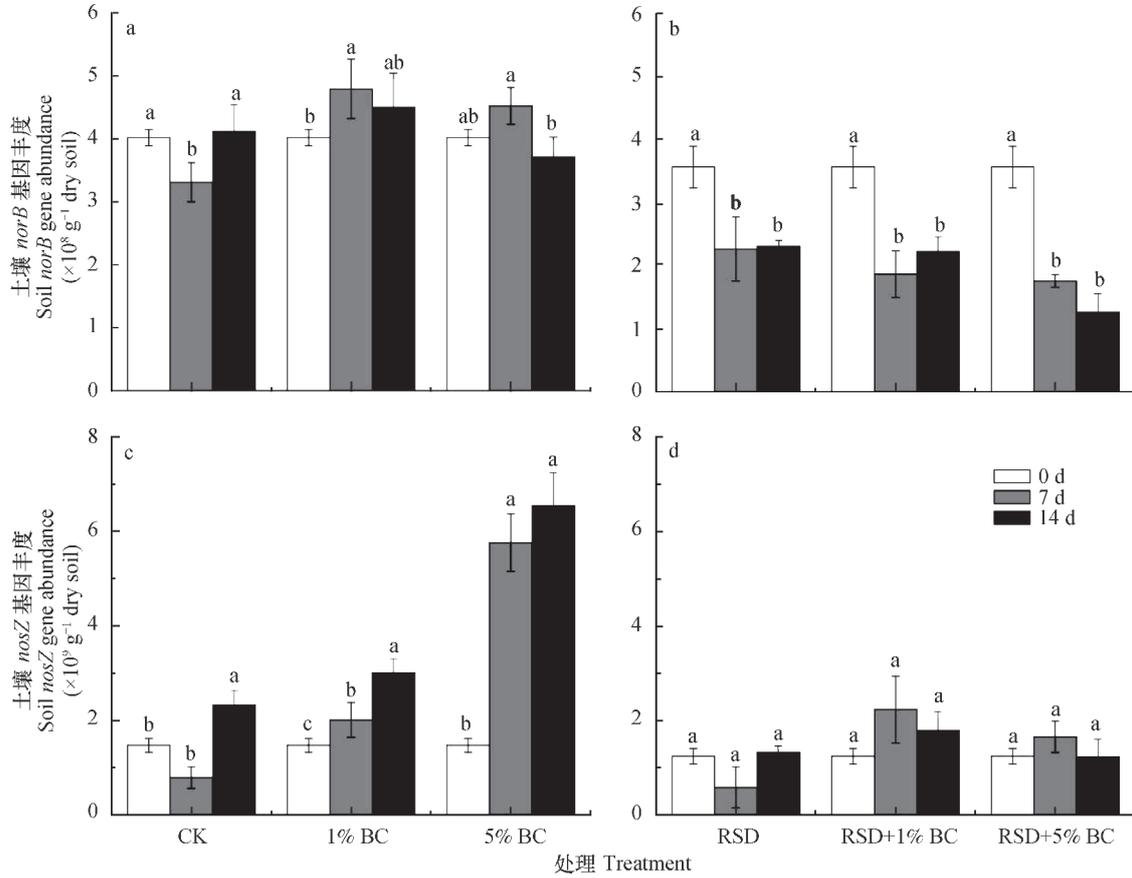


图4 施加生物炭对退化蔬菜地土壤 (a, c) 及其强还原修复过程 (b, d) 中的土壤*norB*基因丰度 (a, b) 和*nosZ*基因丰度 (c, d) 的影响

Fig. 4 Effects of biochar amendment on the gene abundances of *norB* (a, b) and *nosZ* (c, d) genes in degraded vegetable soil (a, c) and in the soil under reductive soil disinfestation (RSD) (b, d)

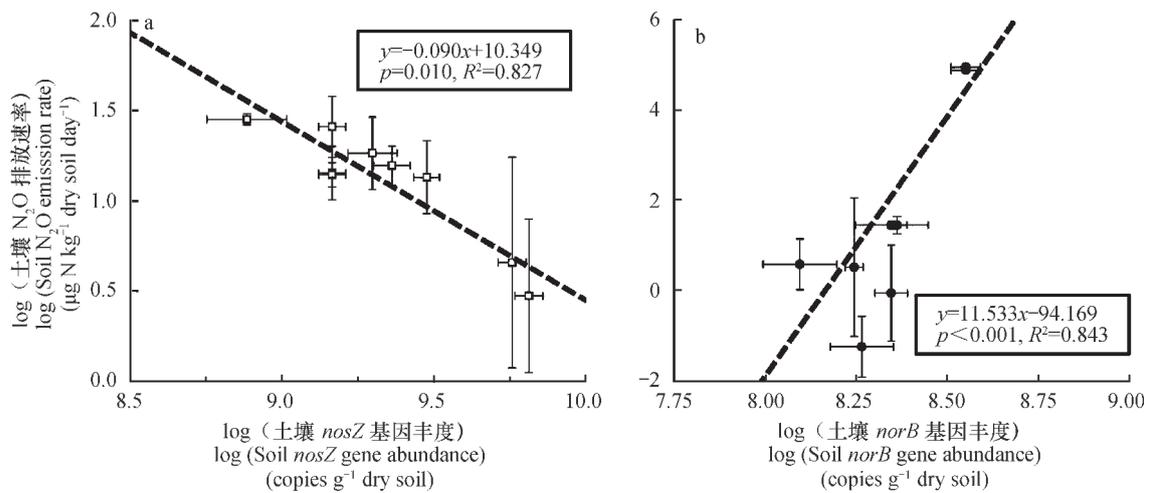


图5 退化设施蔬菜地土壤 (a) 及其强还原修复过程 (b) 中土壤反硝化功能基因 (*norB*和*nosZ*) 丰度和N₂O排放速率 log值间的线性回归

Fig. 5 The linear regression analysis between the log values of denitrifying functional gene (*norB* and *nosZ*) abundances and soil N₂O production rates in degraded vegetable soil (a) and in the soil under Reductive Soil Disinfestation (RSD) (b)

复过程中土壤 N_2O 排放速率均与pH呈显著负相关,即提升pH可能是施加生物炭减少 N_2O 排放速率的重要因素之一。而在强还原修复过程中土壤 N_2O 排放速率还与 NO_3^- 含量呈显著正相关,这是因为 NO_3^- 是 N_2O 产生的底物,其含量直接决定 N_2O 的产生量;但是强还原修复过程中土壤 N_2O 排放速率还与 NH_4^+ 含量呈显著负相关,然而该过程中 NH_4^+ 含量变化幅度较小,其原理尚不可知。此外,强还原修复过程中添加秸秆等有机物料可以加速强还原环境的形成^[4, 11],导致土壤 NO_3^- 含量急剧下降,含量从 $N 208.5 \text{ mg kg}^{-1}$ 干土下降至约 15 mg kg^{-1} 干土。但是施加生物炭显著减缓了 NO_3^- 含量的下降速率,可能是因为生物炭的多孔结构在含氧量低的强还原淹水环境中形成了含氧量较高的微域环境^[16, 30],降低了 NO_3^- 被还原的速率。除秸秆外,黑麦草、鸡粪等有机物料也能快速降低土壤 NO_3^- 含量^[9-10],其研究结果与本研究类似。

3.2 施加生物炭减少土壤 N_2O 排放的机理

众多研究表明生物炭对土壤 N_2O 排放的影响机制主要包括物理机制和生物机制。物理机制主要表现为有生物炭对 N_2O 的吸附, Cornelissen等^[31]的研究表明生物炭对 N_2O 有吸附效应,其吸附能力与 N_2O 排放之间存在Langmuir吸附曲线。生物机制主要包括施加生物炭引起的土壤pH升高、土壤微域环境含氧量高、生物炭对可溶性碳的吸附等因素对反硝化微生物的影响^[30]。其中土壤pH是影响反硝化微生物群落生长及活性的重要因素;土壤微域环境含氧量是决定微域反硝化微生物是否能发挥反硝化功能;反硝化微生物是异养微生物,其生长和活性也受可溶性有机碳含量的影响^[31, 33]。本研究中,施加生物炭可提高土壤pH,而水稻秸秆的加入也可提高土壤可溶性有机碳含量,且结果也显示施加生物炭显著改变了反硝化功能基因丰度,且强还原修复过程中 $norB$ 基因丰度的变化与pH显著相关。许多研究也表明,土壤中添加生物炭后会影响到反硝化活性和相关反硝化功能基因丰度^[17, 33]。Ducey等^[33]发现生物炭增加了土壤中 $nosZ$ 基因的丰度。目前在稻田土壤和果园土壤中有很多关于生物炭通过对微生物活性的影响而减少土壤 N_2O 排放的研究^[16, 34],但在退化设施蔬菜地土壤修复过程中关于生物炭对相关功能微生物影响的研究还鲜有报道。目前大部分研究认为生物炭是通过影响 $nosZ$ 基因丰度来是影响 N_2O 产生^[16-17],与

本研究结果一致,线性回归分析发现非强还原修复过程中土壤 N_2O 排放显著受 $nosZ$ 基因丰度影响;但是在强还原修复过程中土壤 N_2O 排放却显著受 $norB$ 基因丰度的影响,这一结果并未在施加生物炭的研究中发现,仅在酸性土壤中有报道^[35]。非强还原修复过程与强还原修复过程 N_2O 产排的微生物机理不一致,可能是有两个原因:(1)淹水和非淹水所造成的环境不一样,后者会快速形成强烈的还原环境;(2) NO_3^- 含量变化趋势不一致,且在强还原修复过程中土壤 $norB$ 基因丰度与 NO_3^- -N含量显著相关。

4 结论

本研究表明,施加5%生物炭提升了土壤pH,改变了反硝化关键功能微生物丰度,有效降低了退化设施蔬菜地土壤及其强还原修复过程中的 N_2O 排放。该结果为退化设施蔬菜地土壤 N_2O 减排提供了实验数据,也为退化设施蔬菜土壤强还原修复方法提供了环境效应评价及可借鉴的解决措施。

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Effects of Biochar Application on N₂O Emission in Degraded Vegetable Soil and in Remediation Process of the Soil

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Abstract Excessive application of nitrogen fertilizers in facilitated vegetable fields may easily lead to soil degradation (such as soil salinization, acidification, hardening, etc.), and even further to some serious environmental consequences like pollution of groundwater and emission of large volumes of N₂O, thus seriously affecting sustainable development of the vegetable industry. Recently, a novel soil remediation technology, called RSD (reductive soil disinfection) has been developed of adding organic substances (e.g., crop straw, molasses, manure, etc.) in flooding water. The RSD method can effectively alleviate the risk of soil degradation, such as removing the nitrate accumulated in the soil, increasing

pH, lowering soil electrical conductivity and improving soil structure. However, in strong reducing environments the high amount of nitrate accumulated in the degraded facilitated vegetable fields was rapidly reduced by denitrification, and high nitrous oxide (N₂O) emission was observed in RSD process. N₂O is the third major greenhouse gas, and is 296 ~ 310 times as high as CO₂ in global warming potential. It is estimated that N₂O emitted from agricultural soil accounts for 60% of the annual anthropogenic emission into the atmosphere, and the facilitated vegetable soil with high N input is one of the most important agricultural N₂O sources, contributing about 20% to the emission from agricultural soil. Therefore, it is essential to investigate how to mitigate N₂O emission from degraded facilitated vegetable soil, especially in its strong reductive remediation process. In this study, Biochar was selected as soil regulator in evaluating effects of the use of biochar on N₂O emission in degraded vegetable soil and its RSD process relative to rate of its use. Six treatments were designed and conducted, Treatment CK (16% in soil moisture content and no biochar used), Treatment 1% BC (16% in soil moisture content and 1% biochar applied, w/w), Treatment 5% BC (16% in soil moisture content and 5% biochar applied), Treatment RSD (flooded, rice straw incorporated and no biochar used), Treatment RSD + 1% BC (RSD and 1% biochar applied) and Treatment RSD + 5% BC (RSD and 5% biochar applied). By determining the abundance of nitrifying functional genes (*norB* gene and *nosZ* gene encoding nitric oxide reductase and nitrous oxide reductase, respectively), activities of denitrifying microorganisms were characterized. Soil samples were collected from the treatments and incubated at 30 °C in dark for 14 days, and N₂O emission rate was measured during the incubation using the accumulative method. Besides, soil samples were also collected for analysis of inorganic nitrogen contents, soil pH and abundance of denitrifying functional genes. Each sampling had four replicates. Results show that RSD significantly lowered soil nitrate content, raising soil pH and retarded soil degradation, but the cumulative N₂O emission from the RSD-treated soils was over 950 times as high as that from their non-RSD treated counterparts. Application of 5% Biochar reduced the N₂O emission in degraded vegetable soil and its RSD process by 68.7% and 16.0%, respectively. Pearson correlation analysis reveals a negative relationship between N₂O emission rate and soil pH in both degraded vegetable soil and its RSD process, and a positive relationship between N₂O emission rate and soil ammonium content in RSD process. The abundance of *norB* and *nosZ* genes was also significantly changed after biochar application. Linear regression analysis shows that soil N₂O emission was mainly related to *nosZ* gene abundance in degraded vegetable soil, but to *norB* gene abundance in RSD process. To sum up, the application of 5% biochar in degraded vegetable soil and its RSD process can elevate soil pH and alter abundances of the critical denitrifying functional genes, thus efficiently mitigating N₂O emission. All the findings in this study may serve as experimental evidence for N₂O mitigation in degraded vegetable soil, and also can be regarded as important indices for assessment of environmental effects of the remediation of degraded vegetable soil.

Key words N₂O emission; Biochar; Degraded vegetable soil; Soil remediation; Environmental effect; Denitrifiers

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