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目次

2006~2015年北京市不同地区 O ₃ 浓度变化	王占山, 李云婷, 安欣欣, 李倩, 孙乃迪, 王步英, 潘锦秀 (1)
南京北郊黑碳气溶胶的来源解析	肖思略, 于兴娜, 朱彬, 何稼祺, 吕睿, 沙丹丹 (9)
电镀厂周边大气 PM ₁₀ 中重金属季节性分布特征及生态风险评价	赵珍丽, 赵委托, 黄庭, 程胜高, 余葱葱, 尹伊梦 (18)
广西玉林市大气 PM ₁₀ 和 PM _{2.5} 中有机碳和元素碳污染特征分析	黄炯丽, 陈志明, 莫招育, 李宏姣, 杨俊超, 刘慧琳, 毛敬英, 梁桂云, 张达标, 吴熊平, 郝爽 (27)
青岛近海及黄渤海大气气溶胶中不同形态氮磷质量浓度及组成特征	张瑞峰, 祁建华, 丁雪, 谢丹丹 (38)
大气环境分区管理: 以广东省为例	杨柳林, 李敏辉, 廖程浩, 曾武涛, 张晖, 张永波 (49)
基于高分影像的城市黑臭水体遥感识别: 以南京为例	温爽, 王桥, 李云梅, 朱利, 吕恒, 雷少华, 丁萧蕾, 苗松 (57)
基于 SWAT 模型的流域河道硝酸盐 δ ¹⁵ N 和 δ ¹⁸ O 模拟	王康, 冉宁, 林忠兵, 周祖昊 (68)
程海流域非点源污染负荷估算及其控制对策	陈学凯, 刘晓波, 彭文启, 董飞, 黄智华, 冯顺新, 王若男 (77)
陆浑水库饮用水源地水体中金属元素分布特征及健康风险评价	余葱葱, 赵委托, 高小峰, 程胜高, 谢地, 马鹏途 (89)
天津供水系统中抗生素分布变化特征与健康风险评价	张新波, 宋姿, 张丹, 刘楠楠, 李楠, 温海涛 (99)
西南丘陵区村镇典型供水水源有机物分布特征及对饮水水质的影响	王琼, 李乃稳, 李磊, 李龙国, 苟思, 杨凌肖 (109)
北京市地下水有机氯和有机磷农药健康风险评价	陈卫平, 彭程伟, 杨阳, 吴玉梅 (117)
黄河岸边土壤中类二噁英类多氯联苯污染现状及风险	姚宏, 卢双, 张旭, 裴晋, 鲁垠涛 (123)
淹水落干下三峡水库消落带土壤无机磷形态转化特征	周健, 李春辉, 张志永, 胡红青, 万成炎, 胡莲, 潘晓洁 (130)
磷酸盐对铁锰复合氧化膜去除地表水中氨氮的影响	卓瑞双, 黄廷林, 张瑞峰, 文刚 (137)
K ₂ S ₂ O ₈ 强化 g-C ₃ N ₄ 薄膜电极光电催化降解 Cu(CN) ₂ 并同步回收 Cu	党聪哲, 李一兵, 王彦斌, 赵旭 (145)
以 HKUST-1 为模板制备铜氧化物活化过一硫酸氢钾降解罗丹明 B	蒲嘉懿, 万金泉, 王艳, 马邕文, 武书彬 (152)
氨化松香基交联聚合树脂对水中诺氟沙星的吸附性能	马亚红, 黄婉婷, 刁开盛, 李鹏飞, 谭学才, 董慧岭, 覃方夸, 雷福厚, 刘绍刚 (161)
铁锰泥除砷颗粒吸附剂对 As(V) 的吸附去除	曾辉平, 吕赛赛, 杨航, 尹灿, 曹瑞华, 王艳菊, 李冬, 张杰 (170)
水铁矿及其胶体对砷的吸附与吸附形态	马玉玲, 马杰, 陈雅丽, 雷梅, 郭华明, 翁莉萍, 李永涛 (179)
阳(阴)离子复配修饰两性磁性膨润土的表面特征差异及对苯酚吸附的影响	任爽, 孟昭福, 王腾, 张洋, 田凯, 刘伟, 闫东旭 (187)
典型城市污水中对羟基苯甲酸酯的污染特征	赵雪, 张子峰, 祝富杰, 李一凡, 马万里 (195)
水力停留时间对活性炭生物转盘处理污水的影响	许雯佳, 成小英 (202)
环丙沙星对膜生物反应器运行效能的影响及其去除特性	戴琦, 刘锐, 舒小铭, 张永明, 陈吕军 (212)
AAO 工艺低氧条件下的运行及其模拟	曹特特, 王林, 李咏梅 (219)
磷酸盐对亚硝化系统的抑制及恢复	顾澄伟, 陈方敏, 李祥, 张程锦 (227)
碳源对污水处理厂 SAD 工艺小试的影响	李冬, 赵世勋, 王俊安, 朱金凤, 关宏伟, 张杰 (232)
容积负荷对 ABR-MBR 工艺反硝化除磷性能的影响	吕亮, 尤雯, 韦佳敏, 吴鹏, 沈耀良 (239)
DNBF-O ₃ -GAC 组合工艺深度脱除氮磷及代谢产物	钟丽燕, 郝瑞霞, 王卫东, 万京京, 朱晓霞 (247)
交替好氧/缺氧运行模式对生物脱氮效能及活性污泥胞外聚合物的影响	孙洪伟, 陈翠忠, 吴长峰, 赵华南, 于雪, 方晓航 (256)
纳米零价铁对升流式颗粒污泥床反硝化性能的影响	周丰, 王翻翻, 钱飞跃, 黄慧敏, 沈耀良, 周建民 (263)
高含固污泥厌氧消化中 Fe/S 及 pH 对原位抑硫效率影响及其交互作用	韩芸, 曹玉芹, 卓杨, 王晓飞, 韩雅婷, 彭党聪 (269)
浑河流域贮存污泥成分特征及演变规律分析	刘甜甜, 崔崇威, 赫俊国, 唐建 (276)
去除城市生活污泥中有机络合态金属强化其厌氧生物制气	卢怡清, 许颖, 董滨, 戴晓虎 (284)
污泥飞灰中重金属不同浸出方法比较及综合毒性评价	王丰, 李润东, 李彦龙, 赵云斌, 杨天华 (292)
初冬时期闽江河口区养殖塘排水后的 CH ₄ 和 N ₂ O 通量日变化特征	杨平, 谭立山, 黄佳芳, 何清华, 全川 (300)
3 种土壤改良剂对河套灌区玉米田温室气体排放的影响	武岩, 红梅, 林立龙, 刘梅, 刘宇杰 (310)
有机氮替代比例对冬小麦/夏玉米轮作体系作物产量及 N ₂ O 排放的影响	侯苗苗, 吕凤莲, 张弘毅, 周应田, 路国艳, Ayaz Muhammad, 黎青慧, 杨学云, 张树兰 (321)
拔节期水稻光合碳输入的动态变化及其对施氮的响应: ¹³ C-CO ₂ 脉冲标记	陈珊, 祝贞科, 袁红朝, 王久荣, 彭佩钦, 葛体达, 吴金水 (331)
陕北黄土丘陵区不同土地利用方式下土壤碳剖面分布特征	兰志龙, 赵英, 张建国, 李会杰, 司炳成, 焦瑞, Muhammad Numan Khan, Tanveer Ali Sial (339)
不同地表条件下生物炭对土壤氮挥发的影响	邹娟, 胡学玉, 张阳阳, 陈窈君, 王向前, 刘扬 (348)
秸秆与生物炭还田对土壤团聚体及固碳特征的影响	徐国鑫, 王子芳, 高明, 田冬, 黄容, 刘江, 黎嘉成 (355)
县域尺度土壤铜的有效性及相关影响因素评估	李锦芬, 瞿明凯, 刘刚, 黄标 (363)
基于改进 LUR 模型的区域土壤重金属空间分布预测	曾菁菁, 沈春竹, 周生路, 陆春峰, 金志丰, 朱雁 (371)
沈北新区土壤中多环芳烃污染特征及源解析	李嘉康, 宋雪英, 魏建兵, 王颖怡, 李玉双, 郑学昊 (379)
磷酸盐、腐殖酸与粉煤灰联合钝化处理模拟铅镉污染土壤	赵庆圆, 李小明, 杨麒, 陈灿, 钟振宇, 钟宇, 陈飞, 陈寻峰, 王祥 (389)
控源及改良措施对稻田土壤和水稻镉累积的影响	封文利, 郭朝晖, 史磊, 肖细元, 韩晓晴, 冉洪珍, 薛清华 (399)
典型土壤双季稻对 Cd 吸收累积差异	李欣阳, 龙坚, 王树兵, 陈齐, 董霞, 蒋凯, 侯红波, 彭佩钦, 廖柏寒 (406)
松花江上游夹皮沟金矿开采区芦苇叶片汞分布特征	张曼胤, 李梦洁, 崔丽娟, 王贺年, 郭子良, 徐卫刚, 魏圆云, 杨思, 肖红叶 (415)
硝酸盐对土壤反硝化活性及厌氧降解的影响	代军帅, 左小虎, 王明霞, 姚炎红, 周志峰 (422)
长期施肥对稻田土壤微生物量、群落结构和活性的影响	王伟华, 刘毅, 唐海明, 孙志龙, 李宝珍, 葛体达, 吴金水 (430)
海洋沉积物中硫酸盐还原菌和硫酸化菌群落分析方法的比较	张玉, 米铁柱, 甄毓, 陈烨, 付璐璐, 王勋功 (438)
鄱阳湖微囊藻毒素时空分布格局及其与理化及生物因子的关系	袁丽娟, 廖旦根, 张莉, 张大文, 罗林广, 刘聚涛 (450)
畜禽粪便中多重耐药细菌及耐药基因分布特征	张昊, 王盼亮, 杨清香, 俞宁 (460)
烟气净化工艺和焚烧炉类型对生活垃圾焚烧灰灰性质的影响	章骅, 于思源, 邵立明, 何品晶 (467)

硝酸盐对土壤反硝化活性及蒽厌氧降解的影响

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摘要: 多环芳烃(polycyclic aromatic hydrocarbons, PAHs)在土壤中的反硝化降解是其厌氧去除的重要途径之一,但严格厌氧条件下反硝化电子受体(硝酸盐)对土壤反硝化活性及PAHs降解影响的报道还不多见。通过添加硝酸盐和蒽的厌氧微宇宙培养实验,探讨厌氧条件下硝酸盐对土壤蒽的厌氧降解及反硝化活性的影响。设置了不添加(N_0)和添加硝酸盐(N_{30} : $30\text{ mg}\cdot\text{kg}^{-1}$)的两组处理,每组处理分别含3个蒽浓度(A_0 : $0\text{ mg}\cdot\text{kg}^{-1}$ 、 A_{15} : $15\text{ mg}\cdot\text{kg}^{-1}$ 、 A_{30} : $30\text{ mg}\cdot\text{kg}^{-1}$),共6个处理(N_0A_0 、 N_0A_{15} 、 N_0A_{30} 、 $N_{30}A_0$ 、 $N_{30}A_{15}$ 、 $N_{30}A_{30}$)。厌氧条件下 25°C 黑暗培养45 d,并于第3、7、15及45 d测定土壤 N_2O 和 CO_2 的产生速率、反硝化相关功能基因(*narG*、*nirK*、*nirS*)丰度及蒽含量。结果表明,在培养第3 d检测到较强的反硝化活性,且硝酸盐及蒽均能显著促进土壤的反硝化酶活性。随着培养时间的延续,各处理中土壤反硝化活性急剧下降,蒽对土壤反硝化活性却表现出明显的抑制作用。方差分析的结果也表明,硝酸盐、蒽及其交互作用均能显著影响土壤的反硝化活性。3种反硝化功能基因中,只有*narG*和*nirS*基因的丰度在培养期间呈现逐渐升高的趋势,且它们能够受到硝酸盐、蒽及其交互作用的显著影响。厌氧条件下土壤蒽的最终去除率在33.83%~55.01%之间,添加硝酸盐对土壤蒽的去除率和降解速率均无显著影响,但高蒽含量(N_0A_{30} 、 $N_{30}A_{30}$)处理的降解速率显著高于低蒽含量(N_0A_{15} 、 $N_{30}A_{15}$)处理($P<0.05$)。综上,硝酸盐的添加能显著影响土壤的反硝化活性及与反硝化相关的*narG*和*nirS*基因的丰度,但对土壤蒽的厌氧降解无显著影响。

关键词: 土壤; 厌氧培养; 硝酸盐; 反硝化酶活性; 蒽; 降解

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Effect of Nitrate Amendment on Soil Denitrification Activity and Anthracene Anaerobic Degradation

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Abstract: The degradation of soil polycyclic aromatic hydrocarbons (PAHs) under denitrification is one of the most important pathways for anaerobic PAH elimination, but little is known about the effect of nitrate (the terminal electron acceptor for denitrification) on soil denitrification activity and PAH degradation under anaerobic conditions. In this study, the effect of nitrate on soil anthracene anaerobic degradation and denitrification activity was investigated through an anaerobic microcosm experiment. Two groups of treatments without (N_0) and with (N_{30}) nitrate ($30\text{ mg}\cdot\text{kg}^{-1}$) amendment were conducted. Each group contained three treatments with different anthracene concentrations (0, 15, and $30\text{ mg}\cdot\text{kg}^{-1}$, denoted as A_0 , A_{15} , and A_{30} , respectively). Therefore, a total of six treatments (N_0A_0 , N_0A_{15} , N_0A_{30} , $N_{30}A_0$, $N_{30}A_{15}$, and $N_{30}A_{30}$) were incubated in darkness at 25°C for 45 days, and the production rates of N_2O and CO_2 , abundances of denitrification related genes (*narG*: periplasmic nitrate reductase gene; *nirK*: copper-containing nitrite reductase gene; and *nirS*: *cd*₁-nitrite reductase gene), and soil anthracene content were measured at 3, 7, 14, and 45 days. The results indicated that the intensive denitrification enzyme activity in each treatment was only detected at day 3, which could be significantly enhanced by both nitrate and anthracene amendments. Subsequently, a sharp decline of denitrification enzyme activity was observed in each treatment, while anthracene showed an obvious inhibition of soil denitrification enzyme activity. The result of a two-way ANOVA also indicated that nitrate, anthracene, and their interactions had significant effects on soil denitrification enzyme activity. The result of a quantitative-PCR indicated that, during the incubation, the abundances of *narG* and *nirS* exhibited an increasing tendency, but the abundance of *nirK* was relatively constant compared with its former counterparts. The final removal rate of anthracene under anaerobic soil environment was in the range of 33.83%–55.01%, and neither the final removal rate nor the degradation rate of anthracene could be significantly affected by nitrate amendment during incubation. The anthracene degradation rates in the higher anthracene containing treatments (N_0A_{30} and $N_{30}A_{30}$) were significantly higher than those in the lower anthracene containing treatments (N_0A_{15} and $N_{30}A_{15}$). In summary, nitrate amendments had no effect on soil anthracene anaerobic degradation but could significantly affect soil denitrification enzyme activity and the abundance of denitrification related *narG* and *nirS* genes.

Key words: soil; anaerobic incubation; nitrate; denitrification enzyme activity; anthracene; degradation

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多环芳烃 (polycyclic aromatic hydrocarbons, PAHs) 是一类广泛分布于环境中由两个或两个以上的苯环组成的有机化合物,具有强烈的三致作用,且可在环境中长期滞留,因此美国环境保护署和欧盟均已将其列为重点监控的典型有机污染物^[1~3]. 土壤是 PAHs 最大的汇,源于焚化燃烧,汽车尾气,工业等人为活动的 PAHs 均可通过直接或间接的途径进入土壤,进而威胁土壤生态系统并危害人类健康^[4,5]. 因此,土壤 PAHs 的污染及修复一直是环境科学研究的热点. 土壤中的 PAHs 在好氧及厌氧条件下均能被微生物降解. 其中,好氧 PAHs 的降解具有高效、迅速的特点,且多数 PAHs 的好氧降解机制也已被阐明^[6~8]. 然而,土壤环境的复杂性决定了其中必然存在大量的微氧及厌氧微环境,因而厌氧代谢在去除土壤中 PAHs 的作用也不可忽视^[9]. 尽管,多数 PAHs 厌氧降解的机制还不清楚,但其在反硝化、硫酸盐还原、铁还原及产甲烷体系下的厌氧降解已经被证实^[10]. 其中,以硝酸盐为电子受体的反硝化过程在土壤中广泛存在^[11],该过程是反硝化微生物在微氧或厌氧条件下,由一系列酶促反应将硝酸盐逐级还原,最终形成一氧化氮(NO)、氧化亚氮(N_2O)或分子氮(N_2)的生物化学过程,编码这些酶促反应关键酶的功能基因也常被用作研究环境中反硝化微生物群落组成的分子标识物^[12,13]. 当前,从清洁及 PAHs 污染的土壤或沉积物中均获取到能够有效降解典型 PAHs (萘、菲、芘)的反硝化菌株或富集菌群^[14~18],且有研究表明厌氧条件下土壤中菲、蒽、芘及苊降解的同时均伴随着反硝化过程^[19]. 可见,清洁和污染土壤中均存在具备反硝化降解 PAHs 能力的微生物,其在反硝化条件下具有降解 PAHs 的潜力. 然而,土壤反硝化过程对 PAHs 的响应机制及其在 PAHs 去除中的作用还不清楚.

蒽 (Anthracene) 是一种典型的含 3 个苯环的稠环 PAHs,水溶性低,性质稳定,具有急性毒性及致癌性,是美国环保总署所列的 16 种优先控制 PAHs 之一,也常被用作研究 PAHs 污染毒性及降解的模式化合物^[20]. 鉴于此,本研究通过添加硝酸盐 (反硝化过程的电子受体) 和蒽的厌氧土壤微宇宙培养实验,测定培养过程中反硝化酶活性、反硝化相关功能基因 (*narG*: periplasmic nitrate reductase gene; *nirK*: copper-containing nitrite reductase gene; *nirS*: *cd*₁-nitrite reductase gene) 丰度及土壤蒽含量的变化,探讨厌氧条件下硝酸盐对反硝化微生物活性、丰度及土壤蒽降解的影响,以期为进一步深入研究土壤反硝化

过程与 PAHs 去除的偶联机制打下基础.

1 材料与方法

1.1 土壤的采集及微宇宙培养

用于培养的土壤样品采集于地处江汉平原腹地的湖北省汉川市的稻麦轮作农田 ($113^{\circ}30'3''\text{E}$, $30^{\circ}42'37''\text{N}$) 表层土 (0 ~ 20 cm). 去除鲜土中的碎石和植物残体后,过 2 mm 筛,备用. 主要理化性质如下: pH 7.58、有机质 $21.86 \text{ mg}\cdot\text{kg}^{-1}$ 、有效磷 $15.94 \text{ mg}\cdot\text{kg}^{-1}$ 、硝酸根 $51.33 \text{ mg}\cdot\text{kg}^{-1}$ 、PAHs 未检出.

厌氧微宇宙培养实验中设置了不含硝酸盐 (N_0) 和含 $30 \text{ mg}\cdot\text{kg}^{-1}$ 硝酸盐 (N_{30}) 的两组处理,每组处理分别设置 3 个含不同蒽浓度的处理 (0、15、 $30 \text{ mg}\cdot\text{kg}^{-1}$, 分别表示为 A_0 、 A_{15} 、 A_{30}), 共 6 个处理 (N_0A_0 、 N_0A_{15} 、 N_0A_{30} 、 N_{30}A_0 、 $\text{N}_{30}\text{A}_{15}$ 、 $\text{N}_{30}\text{A}_{30}$), 每个处理 3 次重复. 实验过程简述如下: 取上述备用的鲜土于 25°C 避光活化一周,然后将其等分成添加和不添加硝酸盐两组,前者均匀喷洒硝酸钠溶液 ($1000 \text{ mg}\cdot\text{L}^{-1}$),后者喷洒相同体积的去离子灭菌水,过 2 mm 筛; 随后,将上述两组处理土壤分别进行三等分,根据设置的蒽浓度,喷洒等体积不同蒽浓度的丙酮溶液 (以 $500 \text{ mg}\cdot\text{L}^{-1}$ 的蒽丙酮溶液为母液) 并充分混匀,最终在两组处理中分别得到不同蒽含量 (0、15、 $30 \text{ mg}\cdot\text{kg}^{-1}$) 的 3 个处理; 将 6 个处理的土壤置于通风橱中 1 h,待丙酮充分挥发后,分别分装至 100 mL 血清瓶 (每瓶 30 g, 以土计),并用丁基胶塞和铝盖封口; 向血清瓶中通入高纯氮气吹洗 5 min 后,用装水注射器调节平衡,最后置于 25°C 避光恒温培养. 培养过程中,每 7 d 或取样前的 24 h 用高纯氮气冲洗换气一次,分别于培养的第 3、7、15 及 45 d 测定血清瓶上方 N_2O 和 CO_2 的浓度,同时取样 (非破坏性取样) 测定各处理土壤中蒽的含量,并提取 DNA 进行后续的定量 PCR 测定.

1.2 N_2O 和 CO_2 的测定

在培养的第 3、7、15 及 45 d (24 h 前进行换气处理),用注射器抽取血清瓶上方的气体,用气相色谱 (Agilent Technologies 7890A, USA) 测定各处理 24 h 内产生的 N_2O 和 CO_2 的浓度,并计算 N_2O 和 CO_2 的产生速率.

1.3 土壤 DNA 提取及定量 PCR

使用 Fast DNA[®] SPIN Kit for Soil (MP Biomedicals, USA) 试剂盒并参照其说明书来提取各土壤样品中的 DNA. 获取的 DNA,经 1% 的琼脂糖凝胶电泳检测后, -20°C 保存备用.

本研究中,参照 He 等^[21]的方法制备 *narG*、*nirK* 和 *nirS* 基因的质粒及标准曲线,在荧光定量 PCR 仪(ABI 7500, Applied Biosystems, USA)上用 SYBR GREEN 法进行. 25 μL 的反应体系组成为:2 $\times$ SYBR Premix Ex *Taq* II (TaKaRa, Japan)12.5 μL , 上下游引物各 1 μL ,ROX-Reference Dye II (TaKaRa,

Japan)0.5 μL ,DNA 模板 1 μL ,无菌水补足 25 μL . 各基因扩增所用的引物序列及反应条件见表 1. 扩增反应完成后,设置了溶解曲线程序(60 ~ 95 $^{\circ}\text{C}$),用来检测定量 PCR 产物的特异性,最后通过 ABI 7500 Software (version 2.0.6)来分析所得的数据并计算各样品中基因的拷贝数.

表 1 定量 PCR 中所使用的引物及扩增程序

Table 1 Primer sets and amplification programs used in the quantitative PCR				
基因	引物	序列 (5'-3')	扩增程序	文献
<i>narG</i>	1960F	TYACTSGGSCARGARAA	94 $^{\circ}\text{C}$ 5 min; 94 $^{\circ}\text{C}$ 30 s, 58 1 min, 72 $^{\circ}\text{C}$ 45 s, 40 cycles; 72 $^{\circ}\text{C}$ 10 min	[22]
	2650R	TYTCRTACCABGTBGC		
<i>nirK</i>	nirk1F	GCMATGGTKCCSTGGCA	94 $^{\circ}\text{C}$ 4 min; 94 $^{\circ}\text{C}$ 1 min, 53 $^{\circ}\text{C}$ 1 min, 72 $^{\circ}\text{C}$ 1.5 min, 35 cycles; 72 $^{\circ}\text{C}$ 10 min	[23]
	nirk5R	GCCTCGATCAGRITRTGG		
<i>nirS</i>	Cd3aF	G TSAACG TSAAGGARACSGG	94 $^{\circ}\text{C}$ 4 min; 94 $^{\circ}\text{C}$ 30 s, 52 $^{\circ}\text{C}$ 40 s, 72 $^{\circ}\text{C}$ 1 min, 40 cycles; 72 $^{\circ}\text{C}$ 10 min	[24]
	R3cd	GASTTCGGRTGSGTCTTGA		

1.4 土壤萘含量的测定及相关计算

土壤萘的提取参照姚等^[25]的方法,采用经典的索氏提取法进行提取和浓缩^[26]. 随后以萘的标准品(Dr. Ehrenstorfer, Germany)配制标准曲线,用高压液相色谱仪(HITACHI L-7100, Japan)测定各样品中萘含量. 检测条件为:Allsphere C_{18} 柱(250 mm $\times$ 4.6 mm),进样量 10 μL ,流速 0.6 $\text{mL} \cdot \text{min}^{-1}$,温度 30 $^{\circ}\text{C}$,以甲醇(A相)和去离子水(B相)为流动相进行梯度洗脱,检测波长 274 nm. 萘的最终去除率及降解速率分别通过公式(1)和(2)来计算:

萘最终去除率 = $\left(\frac{A_0 - A_{45}}{A_0}\right) \times 100\%$ (1)

萘降解速率 = $\frac{A_0 - A_i}{i}$ (2)

式中, A_0 为第 0 d 土壤萘含量($\text{mg} \cdot \text{kg}^{-1}$), A_{45} 为培养 45 d 后土壤萘含量($\text{mg} \cdot \text{kg}^{-1}$), A_i 为第 i d 土壤萘含

量($\text{mg} \cdot \text{kg}^{-1}$), i 为培养时间(d).

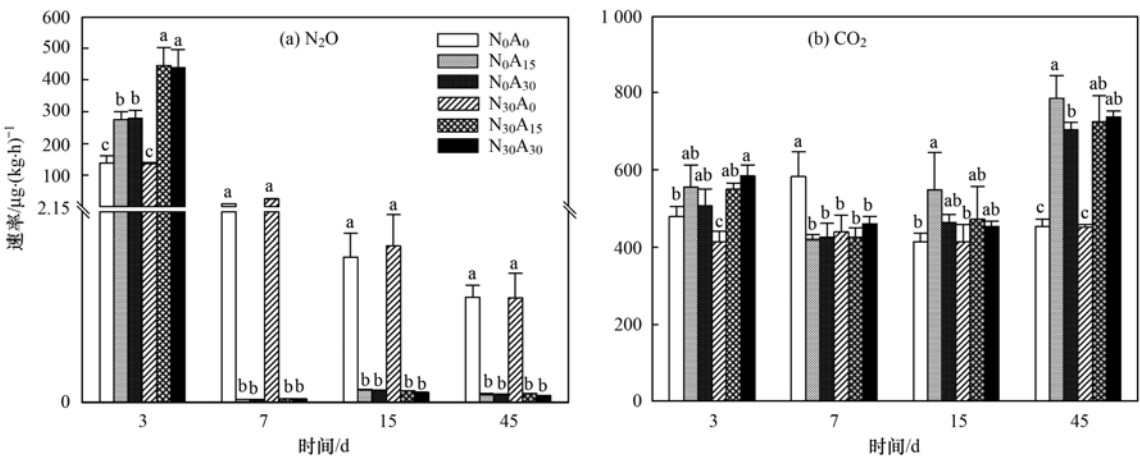
1.5 数据处理

实验数据的统计分析在 SPSS 19.0 软件中进行,各处理差异显著性检测采用单因素方差分析(S-N-K 检验, $P < 0.05$ 表示差异显著). 用 Two-way repeated ANOVA 检验硝酸盐,萘及其交互作用对土壤气体(N_2O 、 CO_2)产生速率、反硝化功能基因(*narG*、*nirK*、*nirS*)丰度及萘降解速率影响的显著性. 上述指标间的相关性用直线相关分析(Bivariate 过程),采用 Spearman 相关系数计算,双尾显著性检验.

2 结果与分析

2.1 培养期间土壤 N_2O 和 CO_2 的产生速率

图 1 为厌氧培养期间各处理土壤 N_2O 及 CO_2 的产生速率. 由图 1(a)可知,各处理土壤在培养初期



图中的误差棒代表标准偏差($n = 3$),不同小写字母表示同一取样天的不同处理间的显著性差异($P < 0.05$)

图 1 不同处理培养期间 N_2O 和 CO_2 的产生速率

Fig. 1 Production rates of N_2O and CO_2 for different treatments during the incubation period

(3 d) 具有较高的 N_2O 产生速率 [$135.6 \sim 445.3 \mu g \cdot (kg \cdot h)^{-1}$, 以土计], 同时添加硝酸盐和蒽 ($N_{30}A_{15}$ 、 $N_{30}A_{30}$) 的处理显著高于只添加蒽 (N_0A_{15} 、 N_0A_{30}) 的处理 ($P < 0.05$), 且它们均显著高于不含蒽 (N_0A_0 、 $N_{30}A_0$) 处理 ($P < 0.05$). 在其它各取样时间各处理土壤中均只检测到较低的 N_2O 产生速率 [$0.074 \sim 24.96 \mu g \cdot (kg \cdot h)^{-1}$, 以土计], 但不添加蒽 (N_0A_0 、 $N_{30}A_0$) 的处理均显著高于含蒽 (N_0A_{15} 、 N_0A_{30} 、 $N_{30}A_{15}$ 、 $N_{30}A_{30}$) 的各处理 ($P < 0.05$). 相关分析表明蒽含量与土壤 N_2O 产生速率呈显著负相关 ($r = -0.343$, $P < 0.05$), 且硝酸盐 ($F = 2.38$, $P < 0.01$), 蒽 ($F = 5.29$, $P < 0.01$) 及其交互作用 ($F =$

8.52 , $P < 0.01$) 均能显著影响土壤 N_2O 产生速率 (表 2). 从图 1(b) 可知, 与 N_2O 产生速率不同, 培养期间各处理中均能持续检测到较高的 CO_2 产生速率 [$415.26 \sim 785.03 \mu g \cdot (kg \cdot h)^{-1}$, 以土计], 其中前 3 次取样天各处理中 CO_2 产生速率的变化无明显规律 (多数处理间差异不显著), 但在第 45 d 各含蒽 (N_0A_{15} 、 N_0A_{30} 、 $N_{30}A_{15}$ 、 $N_{30}A_{30}$) 处理中均显著高于不添加蒽 (N_0A_0 、 $N_{30}A_0$) 各处理 ($P < 0.05$). 方差分析表明蒽含量与土壤 CO_2 产生速率呈显著正相关 ($r = 0.658$, $P < 0.01$), 且蒽 ($F = 34.61$, $P < 0.01$) 及其与硝酸盐的交互作用 ($F = 5.73$, $P < 0.05$) 能够显著影响土壤 CO_2 的产生速率 (表 2).

表 2 双因素重复测量方差分析检验硝酸盐(N)、蒽(A)及其交互作用(N×A)对气体(N_2O 、 CO_2)产生速率及蒽降解速率的影响¹⁾

Table 2 Two-way repeated measures ANOVA examining the effects of nitrate (N), anthracene (A), and their interaction (N×A) on the degradation rate of anthracene and gases (N_2O and CO_2) production rates

变量	df	N_2O		CO_2		降解速率	
		F	P	F	P	F	P
N	1	2.38	<0.01 *	2.61	0.13	2.77	0.12
A	2	5.29	<0.01 *	34.61	<0.01 *	187.87	<0.01 *
N×A	2	8.52	<0.01 *	5.73	0.02 *	1.43	0.28

1) * 表示 $P < 0.05$ 水平显著相关, 下同

2.2 培养期间土壤 *narG*、*nirK* 和 *nirS* 基因丰度的动态变化

各处理土壤 *narG*、*nirK* 及 *nirS* 基因在厌氧培养期间的丰度变化如图 2 所示. 由图 2(a) 可知, 培养期间 *narG* 基因的丰度呈逐渐升高的趋势 ($2.1 \times 10^8 \sim 4.6 \times 10^9 \text{ copies} \cdot g^{-1}$, 以干土计), 第 3 d 时只添加硝酸盐 ($N_{30}A_0$) 处理中的丰度显著高于其它处理 ($P < 0.05$), 而到培养后期 (45 d) 各含蒽处理中的丰度均显著高于不含蒽处理 ($P < 0.05$). 培养期间 *nirK* 基因丰度变化的趋势不明显 [图 2(b)], 第 3 d 时 N_0A_0 及 N_0A_{15} 中的丰度显著低于其它各处理 ($P < 0.05$), 第 7 d 时 N_0A_{15} 中的丰度显著低于其它各处理 ($P < 0.05$), 但在第 15 及 45 d 各处理中

该基因的丰度均无显著差异. 培养期间 *nirS* 基因的丰度变化趋势与 *narG* 相似, 且在培养的第 15 及 45 d 各含蒽 (N_0A_{15} 、 N_0A_{30} 、 $N_{30}A_{15}$ 、 $N_{30}A_{30}$) 处理中的丰度均显著高于不添加蒽 (N_0A_0 、 $N_{30}A_0$) 的处理 ($P < 0.05$) [图 2(c)]. 此外, 双因素重复检测方差分析的结果表明, 硝酸盐、蒽及它们交互作用均能显著影响土壤中 *narG* 和 *nirS* 基因的丰度, 而对 *nirK* 基因丰度无显著影响 (表 3). 由 2.1 节可知, 各处理第 3 d 均检测到最高的 N_2O 产生速率, 然而相关分析的结果表明, 此时 N_2O 产生速率与 *narG* ($r = -0.404$, $P > 0.05$)、*nirK* ($r = -0.053$, $P > 0.05$) 及 *nirS* ($r = -0.220$, $P > 0.05$) 基因丰度之间均无显著相关.

表 3 双因素重复测量方差分析检验硝酸盐(N)、蒽(A)及其交互作用(N×A)对土壤反硝化功能基因(*narG*、*nirK*、*nirS*)丰度的影响

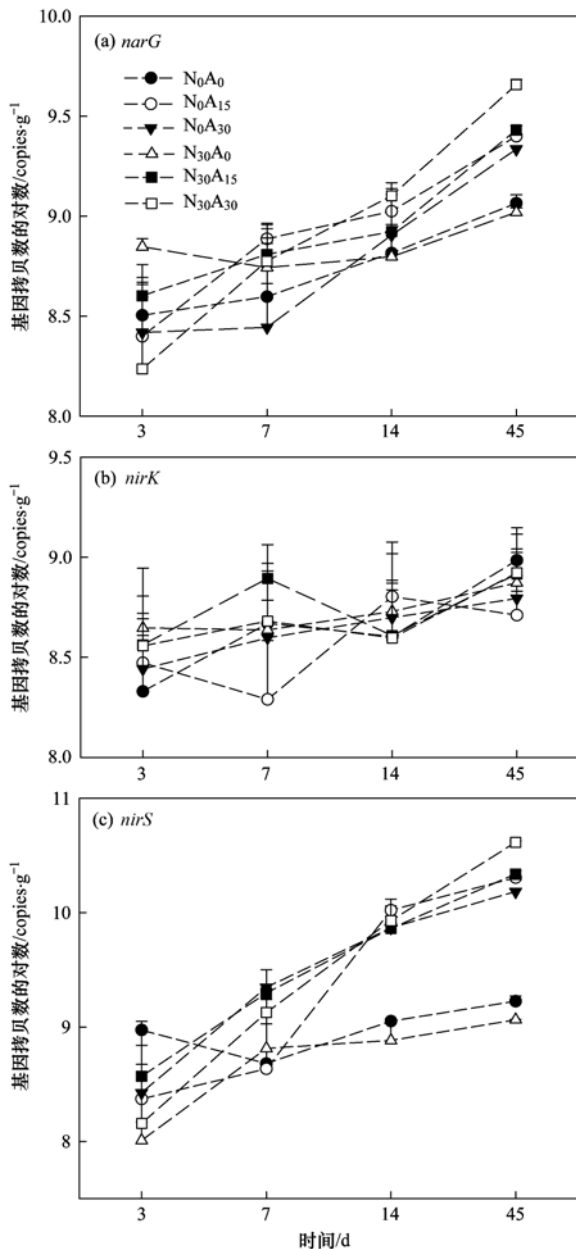
Table 3 Two-way repeated measure ANOVA examining the effects of nitrate (N), anthracene (A), and their interaction (N×A) on the abundance of denitrification related genes (*narG*, *nirK*, and *nirS*) during the incubation period

变量	df	<i>narG</i>		<i>nirK</i>		<i>nirS</i>	
		F	P	F	P	F	P
N	1	64.42	<0.01 *	3.37	0.08	653.86	<0.01 *
A	2	100.3	<0.01 *	0.24	0.79	359.25	<0.01 *
N×A	2	48.12	<0.01 *	0.83	0.45	5.69	0.01 *

2.3 微宇宙培养中土壤蒽的最终去除率及降解速率

培养 45 d 后, 各含蒽土壤蒽的最终去除率如图

3(a) 所示. 从中可见, 厌氧条件下土壤蒽的最终去除率在 33.83% ~ 55.01% 之间, 且各处理中蒽的去



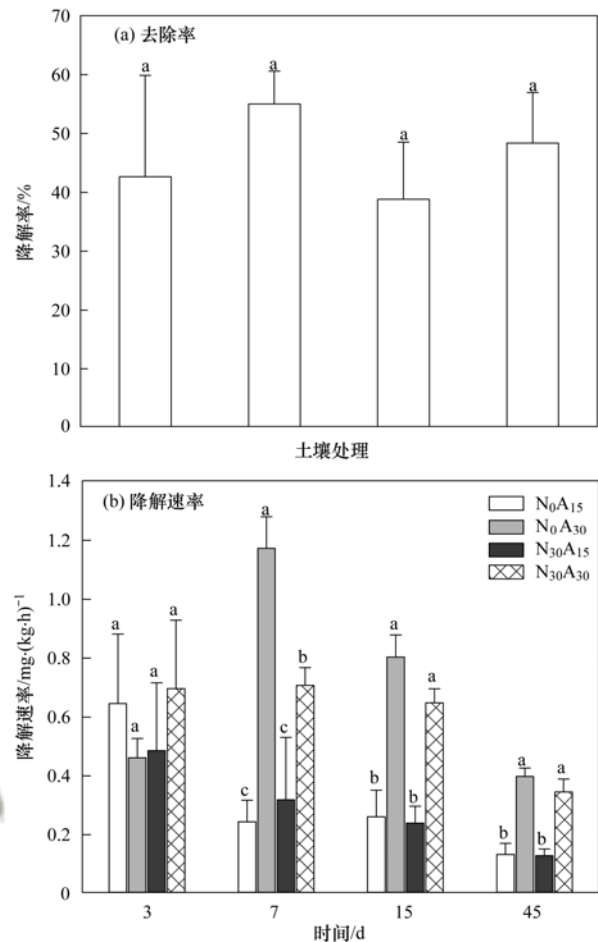
图中的误差棒代表标准偏差 ($n=3$)

图2 不同处理培养期间 *narG*、*nirK* 及 *nirS* 丰度的动态变化

Fig. 2 Dynamic changes in the abundance of *narG*, *nirK*, and *nirS* for different treatments during the incubation period

除率无显著差异。图3(b)为含蒽处理在各取样天蒽的降解速率。培养初期(3 d)各含蒽处理中蒽的降解速率无显著差异,但在随后的第7、15及45 d,高蒽含量(N_0A_{30} 、 $N_{30}A_{30}$)处理中的降解速率均显著高于低蒽含量(N_0A_{15} 、 $N_{30}A_{15}$)处理($P < 0.05$)。相关分析的结果也表明蒽的降解速率与土壤蒽含量呈极显著正相关($r = 0.741$, $P < 0.01$)。此外,低蒽含量(N_0A_{15} 、 $N_{30}A_{15}$)处理之间的降解速率在各取样时间均无显著差异,而高蒽含量处理之间也仅在 第 7 d 有显著差异,即 N_0A_{30} 处理中蒽的降解速率显

著高于 $N_{30}A_{30}$ 处理($P < 0.05$)。可见硝酸盐对土壤蒽的降解速率无明显促进作用,双因素重复检测方差分析的结果也表明只有蒽浓度能显著影响蒽的降解速率($F = 187.87$, $P < 0.01$),而硝酸盐及其与蒽的交互作用均对土壤蒽的降解速率无显著影响(表2)。



图中的误差棒代表标准偏差 ($n=3$) 不同小写字母表示处理间的显著性差异 ($P < 0.05$)

图3 培养结束时蒽的最终去除率和培养期间的降解速率

Fig. 3 Final removal rate of anthracene at the end of the incubation period and the degradation rate of anthracene during the incubation period

3 讨论

土壤中 N_2O 及 CO_2 产生速率可分别表征土壤反硝化酶活性及土壤呼吸速率。本研究结果表明,培养初期(3 d)硝酸盐和蒽的添加均能提高土壤反硝化酶活性,而随后蒽的添加却显著抑制了土壤反硝化酶活性。当前,严格厌氧条件下 PAHs 对土壤反硝化活性影响的报道还不多见,而好氧条件下相关报道的结果也不尽一致^[27-29]。如,Contreras-Ramos 等^[28]发现菲、蒽及苯并芘的添加在培养初期(2 ~

48 h)能够提高农田土壤的反硝化酶活性,之后反硝化活性逐渐降低。而 Guo 等^[29]的研究结果却发现芑的添加能显著抑制土壤反硝化酶活性,且土壤芑浓度与反硝化酶活性之间存在清晰的剂量效应关系。这些研究均能检测到连续的反硝化酶活性,但均是从培养土壤中取样后进行单独的厌氧培养(24 h)来测定反硝化酶活性,这与本研究中原位的检测不同。本研究中,仅在培养的第 3 d 检测较高的反硝化酶活性,且硝酸盐和蒽的添加均能提高土壤反硝化酶活性。其中,硝酸根作为反硝化过程的电子受体理应能够促进土壤的反硝化活性,而蒽的添加则可能通过快速杀死部分微生物细胞,为反硝化微生物提供了更多的速效碳源,进而促进土壤的反硝化酶活性^[30]。培养 3 d 后,各处理土壤反硝化酶活性迅速下降,原因可能是严格厌氧条件下,土壤微生物代谢过程中积累了某些抑制反硝化酶活性的产物。本研究的结果表明,培养的 3、7 及 15 d 蒽均没有明显影响土壤的呼吸速率,而好氧条件下,PAHs 的添加通常能够抑制土壤微生物的呼吸速率^[31~33]。如 Yang 等的报道^[33],典型 PAHs(萘、菲、芑)的添加能改变水稻土中的微生物群落结构并抑制微生物的代谢活性。可能是由于严格厌氧条件下微生物对污染物不敏感^[34],使得本研究中蒽的添加没有表现出对土壤 CO₂ 产生速率的影响。此外,本研究中培养后期(45 d)蒽能促进土壤呼吸速率,可能的原因是随着培养时间的延长,部分蒽的中间代谢产物可能成为多数微生物能够利用的速效碳源,从而体现出对 CO₂ 产生速率的促进效应。

本研究中,*narG* 和 *nirS* 基因的丰度在厌氧培养期间均呈现出逐渐升高的趋势,且硝酸盐、蒽及其交互作用在培养后期均体现出对它们的促进效应,硝酸根既是反硝化微生物的电子受体同时也可作为微生物的氮源,因此其对上述基因的丰度体现出明显的促进作用,这也与前人的研究结论一致^[35,36]。而培养后期,蒽对上述基因的促进作用,可能的原因与此时土壤呼吸速率升高的原因相同,即土壤中含 *narG* 及 *nirS* 的微生物可能以蒽代谢过程的某些中间产物或微生物细胞的内含物为碳源快速生长。与上述基因相比,*nirK* 基因的丰度在培养过程中的波动较小,且硝酸盐和蒽均对其没有显著的影响。该基因与 *nirS* 基因具有相同的功能(催化亚硝酸根的还原),但它们对污染物却常具有不同的响应^[30,37,38]。如, Hallin 等^[37]发现在活性淤泥中添加甲醇能够明显提升 *nirS* 基因丰度并改变其群落结

构,而对 *nirK* 基因的群落组成无明显影响,这与本研究的结果一致。值得注意的是,本研究中反硝化相关基因的最高丰度均未出现在培养初期(3 d),但此时却具有最高 N₂O 产生速率。当前,也有不少关于土壤反硝化相关基因的丰度与反硝化活性不相关的报道^[30,39]。可见,基于 DNA 水平的定量 PCR 或许不能有效体现土壤中真正具有活性的反硝化微生物丰度。

本研究中,经过 45 d 的厌氧培养,土壤蒽的最终去除率在 33.83%~55.01% 之间,该结果低于已有的关于好氧或微氧环境中蒽的去除率的报道^[40~43]。如, Castro-Sliva 等^[42]研究了蒽在盐碱土中的自然降解过程,发现培养 14 d 时土壤蒽的去除率高达 64%。而据 Wang 等报道^[43],在硝酸盐还原条件下培养 80 d 后,沉积物中的蒽几乎被完全去除。可见,氧气是影响土壤蒽去除的重要因素,且土壤中蒽的自然衰减率作用会随培养时间的延续而升高。此外,在培养第 7、15 及 45 d,高蒽含量处理中蒽的降解速率均显著高于低蒽含量处理,已有的研究也表明 PAHs 的浓度通常与其降解速率呈正相关^[14]。有研究表明,添加硝酸盐能有效促进土壤或沉积物中 PAHs 的去除^[44,45]。如, Macrae 等^[45]发现硝酸盐的添加能够促进沉积物中低分子量多环芳烃的降解。然而,本研究中硝酸盐的添加对蒽的降解速率没有影响,且 Yuan 等^[46]也发现硝酸盐的添加不能有效促进沉积物中芑、芴、蒽、菲和芑的降解。可见,硝酸盐电子受体的添加并不一定能促进土壤 PAHs 的降解。可能原因有以下两点:本研究中未添加硝酸盐的处理同样检测到较强的 N₂O 产生速率,表明硝酸盐并不是该土壤反硝化过程的限制因子;其次,本研究中 N₂O 产生速率与蒽的降解速率呈显著负相关($r = -0.343$, $P < 0.05$),表明反硝化过程并不一定在该土壤蒽的厌氧代谢中起主要作用。

4 结论

(1) 厌氧条件下,硝酸盐与蒽的交互作用及蒽在培养初期能显著促进土壤反硝化酶活性,而在培养 7~45 d 后,蒽对土壤反硝化酶活性则有显著的抑制作用。

(2) 厌氧条件下,硝酸盐、蒽及其交互作用均能显著影响土壤 *narG* 和 *nirS* 反硝化微生物功能基因的丰度,主要体现在培养后期的促进效应,但它们对 *nirK* 基因的丰度无显著作用。

(3) 厌氧条件下,添加硝酸盐对蒽的厌氧降解

没有显著影响, 萘的厌氧降解速率与土壤初始萘含量呈正相关。

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CONTENTS

Variation of O ₃ Concentration in Different Regions of Beijing from 2006-2015	WANG Zhan-shan, LI Yun-ting, AN Xin-xin, <i>et al.</i> (1)
Source Apportionment of Black Carbon Aerosol in the North Suburb of Nanjing	XIAO Si-han, YU Xing-na, ZHU Bin, <i>et al.</i> (9)
Seasonal Characteristics and Ecological Risk Assessment of Heavy Metals in PM ₁₀ Around Electroplating Plants	ZHAO Zhen-li, ZHAO Wei-tuo, HUANG Ting, <i>et al.</i> (18)
Characteristics of Organic and Elemental Carbon in PM ₁₀ and PM _{2.5} in Yulin City, Guangxi	HUANG Jiong-li, CHEN Zhi-ming, MO Zhao-yu, <i>et al.</i> (27)
Concentrations and Compositions of Different Forms of Nitrogen and Phosphorus in Atmospheric Aerosols in the Qingdao Coastal Region and over the Yellow and Bohai Sea	ZHANG Rui-feng, QI Jian-hua, DING Xue, <i>et al.</i> (38)
Air Quality Subarea Management: A case study of Guangdong Province	YANG Liu-lin, LI Min-hui, LIAO Cheng-hao, <i>et al.</i> (49)
Remote Sensing Identification of Urban Black-Odor Water Bodies Based on High-Resolution Images; A Case Study in Nanjing	WEN Shuang, WANG Qiao, LI Yun-mei, <i>et al.</i> (57)
Simulation of Nitrate Isotopic ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) by Coupling the Hydrology and Transport Processes Described by the SWAT Model	WANG Kang, RAN Ning, LIN Zhong-bing, <i>et al.</i> (68)
Estimation of and Control Strategies for Pollution Loads from Non-point Sources in the Chenghai Watershed	CHEN Xue-kai, LIU Xiao-bo, PENG Wen-qi, <i>et al.</i> (77)
Distribution Characteristics and Health Risk Assessment of Metals in Drinking Water Sources from the Luhun Reservoir	YU Cong-cong, ZHAO Wei-tuo, GAO Xiao-feng, <i>et al.</i> (89)
Distribution Characteristics and Health Risk Assessment of Antibiotics in the Water Supply System in Tianjin	ZHANG Xin-bo, SONG Zi, ZHANG Dan, <i>et al.</i> (99)
Organic Distribution Characteristics and Influence on Drinking Water Quality in the Typical Water Sources for Towns in the Southwest Hilly Area of China	WANG Qiong, LI Nai-wen, LI Lei, <i>et al.</i> (109)
Health Risk Evaluation of Organochlorine and Organophosphorous Pesticides in Groundwater in Beijing	CHEN Wei-ping, PENG Cheng-wei, YANG Yang, <i>et al.</i> (117)
Pollution Status and Risks of Dioxin-like Polychlorinated Biphenyls in the Soil of the Yellow River	YAO Hong, LU Shuang, ZHANG Xu, <i>et al.</i> (123)
Effects of Flooding and Drying on the Transformation of Soil Inorganic Phosphorus in the Water-Level-Fluctuating Zone of the Three Gorges Reservoir, China	ZHOU Jian, LI Chun-hui, ZHANG Zhi-yong, <i>et al.</i> (130)
Effect of Phosphate on the Ammonium Removal Performance of Iron-Manganese Co-oxide Film in Surface Water Treatment	ZHUO Rui-shuang, HUANG Ting-lin, ZHANG Rui-feng, <i>et al.</i> (137)
Enhanced Photoelectrocatalytic Oxidation of Cu(CN) ₃ ²⁻ and Synchronous Cathodic Deposition of Cu by Peroxydisulfate	DANG Cong-zhe, LI Yi-bing, WANG Yan-bin, <i>et al.</i> (145)
Using HKUST-1 as a Template for Copper Oxides Preparation to Activate Peroxymonosulfate for RhB Degradation	PU Jia-yi, WAN Jin-quan, WANG Yan, <i>et al.</i> (152)
Evaluation of Performance of an Aminated Rosin-based Resin for Adsorption of Norfloxacin from Aqueous Solutions	MA Ya-hong, HUANG Wan-ting, DIAO Kai-sheng, <i>et al.</i> (161)
Arsenic(V) Removal by Granular Adsorbents Made from Backwashing Residuals from Biofilters for Iron and Manganese Removal	ZENG Hui-ping, LÜ Sai-sai, YANG Hang, <i>et al.</i> (170)
Arsenic Adsorption and Its Species on Ferrihydrite and Ferrihydrite Colloid	MA Yu-ling, MA Jie, CHEN Ya-li, <i>et al.</i> (179)
Comparison of Amphoteric-Cationic and Amphoteric-Anionic Modified Magnetic Bentonites: Characterization and Sorption Capacity of Phenol	REN Shuang, MENG Zhao-fu, WANG Teng, <i>et al.</i> (187)
Pollution Characteristics of Parabens in Typical Sewage Wastewater	ZHAO Xue, ZHANG Zi-feng, ZHU Fu-jie, <i>et al.</i> (195)
Influence of Hydraulic Retention Time on the Treatment of Polluted River Water by an Activated Carbon Rotating Biological Contactor	XU Wen-jia, CHENG Xiao-ying, <i>et al.</i> (202)
Removal and Influence of Ciprofloxacin in a Membrane Bioreactor	DAI Qi, LIU Rui, SHU Xiao-ming, <i>et al.</i> (212)
Operation of the AAO Process Under Low Dissolved Oxygen Conditions and Its Simulation	CAO Te-te, WANG Lin, LI Yong-mei, <i>et al.</i> (219)
Inhibitory Effects of Phosphate and Recovery on a Nitrification System	GU Cheng-wei, CHEN Fang-min, LI Xiang, <i>et al.</i> (227)
Effect of Carbon Source on Lab-scale SAD Process in a Wastewater Treatment Plant	LI Dong, ZHAO Shi-xun, WANG Jun-an, <i>et al.</i> (232)
Effect of Volume Loading Rate (VLR) on Denitrifying Phosphorus Removal by the ABR-MBR Process	LÜ Liang, YOU Wen, WEI Jia-min, <i>et al.</i> (239)
Combined Process of DNB-F-O ₃ -GAC for Nitrogen and Phosphorus and Metabolite Advanced Removal	ZHONG Li-yan, HAO Rui-xia, WANG Wei-dong, <i>et al.</i> (247)
Influence of Operating Modes for the Alternating Anoxic/Oxic Process on Biological Nitrogen Removal and Extracellular Polymeric Substances of Activated Sludge	SUN Hong-wei, CHEN Cui-zhong, WU Chang-feng, <i>et al.</i> (256)
Effects of Nanoscale Zero-valent Iron (nZVI) on Denitrifying Performance of an Upflow Granular Sludge Bed Reactor	ZHOU Feng, WANG Fan-fan, QIAN Fei-yue, <i>et al.</i> (263)
Influence on Desulfurization Efficiency and Interactions of Fe/S and pH During H ₂ S in situ Depression of High Solid Anaerobic Digestion	HAN Yun, CAO Yu-qin, ZHUO Yang, <i>et al.</i> (269)
Analysis of Storage Sludge Composition Characteristics and Evolutionary Regularity in the Hunhe River Basin	LIU Tian-tian, CUI Chong-wei, HE Jun-guo, <i>et al.</i> (276)
Enhancement of Anaerobic Methane Production by Removal of Organic-bonding Metals from Sewage Sludge	LU Yi-qing, XU Ying, DONG Bin, <i>et al.</i> (284)
Comparison of Different Leaching Methods for Heavy Metals in Sludge Fly Ash and Comprehensive Toxicity Evaluation	WANG Feng, LI Run-dong, LI Yan-long, <i>et al.</i> (292)
Diurnal Variations of CH ₄ and N ₂ O Fluxes from the Drained Aquaculture Pond in the Minjiang River Estuary During Early Winter	YANG Ping, TAN Li-shan, HUANG Jia-fang, <i>et al.</i> (300)
Effects of Three Soil Amendments on Greenhouse Gas Emissions From Corn Fields in the Hetao Irrigation District	WU Yan, HONG Mei, LIN Li-long, <i>et al.</i> (310)
Effect of Organic Manure Substitution of Synthetic Nitrogen on Crop Yield and N ₂ O Emission in the Winter Wheat-Summer Maize Rotation System	HOU Miao-miao, LÜ Feng-lian, ZHANG Hong-tao, <i>et al.</i> (321)
Dynamics of Rice Photosynthesized Carbon Input and Its Response to Nitrogen Fertilization at the Jointing Stage: ¹³ C-CO ₂ Pulse-labeling	CHEN Shan, ZHU Zhen-ke, YUAN Hong-zhao, <i>et al.</i> (331)
Profile Distribution of Soil Organic and Inorganic Carbon Under Different Land Use Types in the Loess Plateau of Northern Shaanxi	LAN Zhi-long, ZHAO Ying, ZHANG Jian-guo, <i>et al.</i> (339)
Effect of Biochar on Ammonia Volatilization from Soils of Different Surface Conditions	ZOU Juan, HU Xue-yu, ZHANG Yang-yang, <i>et al.</i> (348)
Effects of Straw and Biochar Return in Soil on Soil Aggregate and Carbon Sequestration	XU Guo-xin, WANG Zi-fang, GAO Ming, <i>et al.</i> (355)
Assessment of the Availability of Soil Copper and Related Influencing Factors at a County Scale	LI Jin-fen, QU Ming-kai, LIU Gang, <i>et al.</i> (363)
Application of the LUR Model in the Prediction of Spatial Distributions of Soil Heavy Metals	ZENG Jing-jing, SHEN Chun-zhu, ZHOU Sheng-lu, <i>et al.</i> (371)
Pollution Characteristics and Source Apportionment of Polycyclic Aromatic Hydrocarbons in Soils of Shenyang North New Area	LI Jia-kang, SONG Xue-ying, WEI Jian-bing, <i>et al.</i> (379)
Passivation of Simulated Pb- and Cd-Contaminated Soil by Applying Combined Treatment of Phosphate, Humic Acid, and Fly Ash	ZHAO Qing-yuan, LI Xiao-ming, YANG Qi, <i>et al.</i> (389)
Distribution and Accumulation of Cadmium in Paddy Soil and Rice Affected by Pollutant Sources Control and Improvement Measures	FENG Wen-li, GUO Zhao-hui, SHI Lei, <i>et al.</i> (399)
Differences in Cd Accumulation in Typical Soils Under the Double Rice System	LI Xin-yang, LONG Jian, WANG Shu-bing, <i>et al.</i> (406)
Distribution Characteristics of Mercury in Reed Leaves from the Jiapiougou Gold Mine in the Songhua River Upstream	ZHANG Man-yin, LI Meng-jie, CUI Li-juan, <i>et al.</i> (415)
Effect of Nitrate Amendment on Soil Denitrification Activity and Anthracene Anaerobic Degradation	DAI Jun-shuai, ZUO Xiao-hu, WANG Ming-xia, <i>et al.</i> (422)
Effects of Long-term Fertilization Regimes on Microbial Biomass, Community Structure and Activity in a Paddy Soil	WANG Wei-hua, LIU Yi, TANG Hai-ming, <i>et al.</i> (430)
Analysis of Sulfate-Reducing and Sulfur-Oxidizing Prokaryote Community Structures in Marine Sediments with Different Sequencing Technologies	ZHANG Yu, MI Tie-zhu, ZHEN Yu, <i>et al.</i> (438)
Seasonal and Spatial Variations of Microcystins and Their Relationships with Physicochemical and Biological Factors in Poyang Lake	YUAN Li-juan, LIAO Qie-gen, ZHANG Li, <i>et al.</i> (450)
Distribution of Multidrug-Resistant Bacteria and Antibiotic-Resistant Genes in Livestock Manures	ZHANG Hao, WANG Pan-liang, YANG Qing-xiang, <i>et al.</i> (460)
Influence of Air Pollution Control (APC) Systems and Furnace Type on the Characteristics of APC Residues from Municipal Solid Waste Incinerators	ZHANG Hua, YU Si-yuan, SHAO Li-ming, <i>et al.</i> (467)