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——庆祝厦门大学环境学科创立40周年



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目次

厦门大学环境学科创立 40 周年专栏

新污染物共排放对生态环境监测和管理的挑战	王佩, 黄欣怡, 曹致纬, 吴朝阳, 吕永龙 (4801)
河口-近海环境新污染物的环境过程、效应与风险	王新红, 于晓璇, 王思权, 殷笑晗, 钱韦旭, 林晓萍, 吴越, 刘畅 (4810)
海水痕量营养盐和金属的分子光谱分析方法研究进展	袁东星, 黄勇明, 王婷 (4822)
环境水体中硫化物的分析方法: 从实验室分析到原位监测	李鹏, 林坤德, 袁东星 (4835)
环境水体中无机砷现场分析方法研究进展	薄光永, 陈钊英, 弓振斌, 马剑 (4845)
海洋痕量元素采样技术和分析方法的发展及展望: 厦门大学痕量元素平台建设进展	黄勇明, 周宽波, 陈耀瑾, 张楠, 杨俊波, 戴民汉, 曹知勉, 蔡毅华 (4858)

聚乙烯微塑料的微生物降解研究进展	骆苑蓉, 钱义谦, 齐雅楠 (4869)
水稻土中氮素对微生物固氮的扰动及效应机制	王锋, 张静, 周少余, 王鸿辉, 李建, 赵聪媛, 黄鹏, 陈铮 (4876)
中国海洋生态毒理学研究中的毒性测试生物	史天一, 洪海征, 王明华, 谭巧国, 史大林 (4888)
中国油气系统甲烷逸散排放估算	陈春赐, 吕永龙, 贺桂珍 (4905)
2015 ~ 2020 年厦漳泉地区大气氨排放清单及分布特征	李香, 吴水平, 姜炳祺, 刘怡靖 (4914)
九龙江口微塑料与抗生素抗性基因污染分布特征	程宏, 陈荣 (4924)
厦门湾沙滩沉积物微塑料污染特征	姚蕊, 刘花台, 李永玉, 刘潇雅, 吴海波, 王新红 (4931)
九龙江口-厦门湾海域中溶解态痕量金属的时空分布特征与影响机制	戚柳倩, 岳新利, 钟灏文, 王棋, 王德利, 陈能汪 (4939)
福建省流域-近海溶解氧时空格局与低氧调控机制	杨艾琳, 杨芳, 李少斌, 余其彪, 陈能汪 (4950)
厦门西溪河口沉积物活性磷的分布特征及迁移转化机制	潘峰, 蔡宇, 郭占荣, 王新红 (4961)
改性生物炭固定床对模拟湖水体中 Mn^{2+} 的吸附	赵洁, 叶志隆, 王佳妮, 蔡冠竟 (4971)
基于表面增强拉曼光谱技术的饮用水中痕量恩诺沙星和环丙沙星快速检测	徐婧, 郑红, 卢江龙, 刘国坤 (4982)
紫外驱动高级氧化法降解水体中的磷酸三苯酯	徐子文, 印红玲, 熊远明, 宋娇娇, 譙杨 (4992)

研究报告

2019 年秋季海南省 4 次臭氧污染过程特征及潜在源区分析	符传博, 陈红, 丹利, 徐文帅 (5000)
伊犁河谷夏季 $PM_{2.5}$ 和 PM_{10} 中水溶性无机离子浓度特征和形成机制	陈巧, 谷超, 徐涛, 周春华, 张国涛, 赵雪艳, 吴丽萍, 李新琪, 杨文 (5009)
城区与郊区 $PM_{2.5}$ 污染及传输特征差异性	齐鹏, 周颖, 程水源, 白伟超 (5018)
南京北郊 BTESX 特征及健康风险评估	冯悦政, 安俊琳, 张玉欣, 王俊秀 (5030)
我国陆域水体系统表层水中微塑料生态风险评估	孙晓楠, 陈浩, 贾其隆, 朱奔, 马长文, 叶建锋 (5040)
东江流域不同空间尺度景观格局对水质影响分析	陈优良, 邹文敏, 刘星根, 曾金凤, 李丹, 郑汉奕 (5053)
长江与黄河源丰水期地表水中汞的分布特征、赋存形态及来源解析	刘楠涛, 吴飞, 袁巍, 王训, 王定勇 (5064)
青藏高原湖泊水环境特征及水质评价	刘智琦, 潘保柱, 韩谔, 李刚, 王韬轶 (5073)
基于水化学与硫同位素的卡林型金矿区岩溶水文地球化学特征及控制因素	查学芳, 吴攀, 李学先, 陈世万, 黄家琰, 李清光, 陈思睿 (5084)
丹江口水库真核浮游植物群落分布特征及其与环境因子的关系	贺玉晓, 买思婕, 任玉芬, 李卫国, 赵同谦, 马寅男 (5096)
铜沸石对磷和重金属的吸附与底泥钝化性能	王哲, 朱俊, 李雯, 闫德馨, 董雯, 刘玉玲, 李家科 (5106)
基于宏基因组与宏转录组分析石化废水生物处理系统脱氮功能菌群	章旭, 周佳佳, 周珉, 罗西子, 严新杰, 刘勇弟, 厉巍 (5115)
寒冷地区 IFAS + 磁混凝污水厂菌群结构和抗生素抗性基因分析	杜文琰, 姚俊芹, 马辉英, 胡渊鑫, 张春雷, 陈银广 (5123)
中国旱作农田一氧化氮排放及减排: Meta 分析	田政云, 吴雄伟, 吴媛媛, 魏佳楠, 白鹤, 顾立新 (5131)
硝化抑制剂对我国蔬菜生产产量、氮肥利用率和氧化亚氮减排效应的影响: Meta 分析	刘发波, 马笑, 张芬, 梁涛, 黎亮武, 王军杰, 陈新平, 王孝忠 (5140)
不同施肥措施对热带地区稻菜轮作体系土壤 CH_4 和 N_2O 排放的影响	邵晓辉, 汤水荣, 孟磊, 伍延正, 李金秋, 张广林 (5149)
不同水分条件下土地利用方式对我国热带地区土壤硝化过程及 NO 和 N_2O 排放的影响	唐瑞杰, 胡煜杰, 赵彩悦, 赵炎, 袁新生, 汤水荣, 伍延正, 孟磊 (5159)
基于文献计量分析的长江经济带农田土壤重金属污染特征	刘孝严, 樊亚男, 刘鹏, 吴秋梅, 胡文友, 田康, 黄标 (5169)
基于 EBK 插值预测和 GDM 模型的襄州区耕地土壤重金属时空分布及来源变化分析	高浩然, 周勇, 刘甲康, 程晓明, 郭嵩, 江衍, 谭恒鑫 (5180)
基于 GIS 对宁夏某铜银矿区周边土壤重金属来源解析	张扣扣, 贺婧, 钟艳霞, 魏琪琪, 陈锋 (5192)
老化作用对生物炭钝化白云鄂博矿区碱性土壤中 Cd^{2+} 的影响	王哲, 程俊丽, 卞园, 郑春丽, 王维大, 姜庆宏 (5205)
磁性氧化铁/桑树杆生物炭的制备及其对砷污染土壤溶解性有机碳和砷形态的影响	芦琳, 颜利玲, 梁美娜, 成官文, 朱宗强, 朱义年, 王敦球 (5214)
牡蛎壳粉和石灰改良酸性水稻土对磷有效性、形态和酶活性的影响	赵丽芳, 黄鹏武, 杨彩迪, 卢升高 (5224)
磷、锌和镉交互作用对小白菜生长和锌镉累积的影响	帅祖华, 刘汉燧, 崔浩, 魏世强 (5234)
重庆开州区农地土壤抗生素污染特征及潜在生态环境风险评估	方林发, 叶萃萃, 方标, 范晓霞, 高坤鹏, 李士洋, 陈新平, 肖然 (5244)
基于 InVEST 和 GeoSoS-FLUS 模型的黄河源区碳储量时空变化特征及其对未来不同情景模式的响应	侯建坤, 陈建军, 张凯琪, 周国清, 尤号田, 韩小文 (5253)
黄土丘陵区不同恢复植被类型的固碳特征	许小明, 张晓萍, 何亮, 郭晋伟, 薛帆, 邹亚东, 易海杰, 贺洁, 王浩嘉 (5263)
土壤多功能性对微生物多样性降低的响应	陈桂鲜, 吴传发, 葛体达, 陈剑平, 邓扬悟 (5274)
氮添加对不同坡度退化高寒草甸土壤真菌多样性的影响	苏晓雪, 李希来, 李成一, 孙华方 (5286)
碳减排背景下我国与世界主要能源消费国能源消费结构与模式对比	李辉, 庞博, 朱法华, 孙雪丽, 徐静馨, 王圣 (5294)
中国能源消费碳排放的空间化与时空动态	郝瑞军, 魏伟, 刘春芳, 颜斌斌, 杜海波 (5305)
京津冀及周边地区“2+26”城市结构性调整政策的 CO_2 协同减排效益评估	杨添祺, 王洪昌, 张辰, 朱金伟, 崔宇韬, 谭玉玲, 束轲 (5315)
我国塑料污染防治政策分析与建议	李欢, 朱龙, 沈茜, 贺亚楠, 邓义祥, 安立会 (5326)

基于宏基因组与宏转录组分析石化废水生物处理系统脱氮功能菌群

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摘要: 石化行业是我国的支柱产业, 长期以来, 石化废水处理一直是水污染治理领域的重难点, 阻碍了石化行业高质量可持续发展。由于石化企业和园区多临河或临海而建, 其废水排放易造成流域性或区域性水生态风险, 尤其石化废水中的氮素污染, 严重威胁了水生态安全及人民群众身体健康。因此, 从上海某化工区的某条石化废水 A/O 脱氮工艺线中采集了污泥样品, 通过宏基因组和宏转录组对比分析了出水水质稳定时期与出水水质波动时期的不同污泥中微生物的群落结构, 脱氮菌群的功能特征和关键的氮代谢途径。结果表明, 工艺的氨氧化功能易波动, 而亚氮和硝氮的去除相对稳定。菌群研究发现该 A/O 工艺主体脱氮途径为硝化-反硝化脱氮途径, 未发现工艺内有厌氧氨氧化相关基因片段。体系脱氮关键功能基因中约有 90% 与反硝化作用相关, 只有约 0.17% 的基因参与了硝化过程中氨氮的转化。进一步研究发现, 氨氧化功能菌在工艺中丰度极低且结构单一, 主要的氨氧化功能菌属是 *Nitrosomonas*。这可能是工艺线出水氨氮浓度易波动的主要原因。

关键词: 石化废水; A/O 工艺; 宏基因组; 宏转录组; 脱氮微生物

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Metagenomic and Metatranscriptomic Analysis of Nitrogen Removal Functional Microbial Community of Petrochemical Wastewater Biological Treatment Systems

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Abstract: Petrochemicals are one of the pillar industries of China. Despite this, the treatment of petrochemical wastewater has long been seen as a massive challenge in the field of water pollution control, hindering the high-quality and sustainable development of the petrochemical industry. The majority of petrochemical enterprises and zones are located near rivers or seas, so their wastewater discharges can easily cause watershed or regional water ecological risks. Specifically, nitrogen pollution in petrochemical wastewater poses a significant threat to water ecological safety and human health. Sludge samples were collected from a petrochemical wastewater A/O nitrogen removal process line in a chemical industry zone in Shanghai. Metagenomic and metatranscriptomic methods were used to analyze the community structure of microorganisms, the functional characteristics of nitrogen removal bacteria, and the key nitrogen metabolism pathways in different sludges during the period when effluent water quality was stable and fluctuating. During the study, it was found that the nitrite and nitrate removal was relatively stable in this process, but ammonia oxidation fluctuated easily. In the study of microbial communities, it was found to be a nitrification-denitrification pathway that primarily removed nitrogen from the A/O process, and no genes related to ANAMMOX were detected. Approximately 90% of the functional genes responsible for removing nitrogen were responsible for denitrification, whereas only 0.17% of them were involved in the conversion of ammonia nitrogen in the nitrification process. Moreover, the abundance of ammonia-oxidizing bacteria in the process was extremely low, and the main genus was *Nitrosomonas*. It is likely that this is the main cause of fluctuations in ammonia nitrogen concentration in effluent due to water quality shocks in the process line.

Key words: petrochemical wastewater; A/O process; metagenomic; metatranscriptomic; nitrogen-removing microorganisms

石油化工产业是国家的支柱产业, 在国民经济中扮演着极其重要的角色^[1]。石油化工废水来源多样, 且排放量巨大^[2,3]。据 2019 年中国石油化工行业报告显示, 我国石化废水年排放量已达到 40 亿 t^[3], 主要来自炼油和石油产品加工过程^[4]。石油化工废水的处理已成为石油化工行业绿色发展主要挑战之一^[5~7]。以石油产品加工过程中产生的废水为例, 该种废水通常含有不同浓度的氨氮与氮氧化物^[2], 直接排放会加剧水体的富营养化污染。在石

化行业逐步削减工业取新水总量, 并极力推行污水资源化利用的大背景下, 石化废水的脱氮处理更是关键环节^[8]。

活性污泥法已被广泛应用于石化废水脱氮处

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理^[9~11],然而活性污泥法中的功能微生物群落高度复杂^[12],相关的脱氮过程交错多样^[13].据报道在活性污泥体系中,最核心的脱氮途径是硝化-反硝化结合的脱氮途径^[12,14,15],但同时也可能伴生异化硝酸盐还原(DNRA)^[16],厌氧氨氧化(ANAMMOX)等过程^[17,18].脱氮微生物的群落组成及状态直接影响废水中氮素生物转化的途径和效率^[19].全面地了解脱氮微生物群落结构和功能特征可为石化废水脱氮处理的过程诊断与定向调控提供微生物学依据.然而,以往对石化废水处理工艺中微生物的研究都基于16S rRNA基因高通量测序分析^[20,21],但由于扩增过程带来的偏向性以及很难直接从功能基因角度获取结果^[22],将导致无法精确地反映复杂系统中实际的菌群结构与功能特征,使人们对石化废水处理中脱氮微生物结构与功能的理解受到限制.

近年来,基于Illumina测序的宏基因组测序分析技术发展迅速,测序分析成本逐渐降低,在微生物群落深度分析中显示出巨大的优势,已被广泛应用于饮用水、土壤、实验室模拟反应器和城市污水处理厂等自然或工程环境中微生物群落分析^[13,14,23,24].宏基因组测序分析已经被证明是一个强有力的工具,可以助力解开具有较高多样性的工程生态系统中的“暗物质”^[25,26].然而,宏基因组测序只是潜能而非实际功能或活性的测量,联合采用宏基因组和宏转录组的多组学方法能更好地揭示活性污泥微生物内部的物种相互作用和基因表达活性对环境的响应^[27,28].基于多组学分析方法的石化废水处理厂中脱氮微生物的分析研究未见报道^[29,30].本研究通过宏基因组和宏转录组技术对一实际石化废水生物处理系统中脱氮微生物进行探究,将有助于明晰石化废水生物脱氮处理的生物过程.

1 材料与方法

1.1 污水处理厂运行数据比较及污泥样品采集

在2021年1~8月期间,对位于上海某化工区污水处理厂的某条生物脱氮A/O工艺线,监测了运行数据并收集了污泥样本.该条工艺线主要处理化工区内多家石化企业排出的数十种废水源的混合废水,工艺日处理水量平均值为 $(6\,788 \pm 99) \text{ m}^3 \cdot \text{d}^{-1}$.该A/O工艺缺氧池和好氧池水力停留时间分别为9 h和27 h,污泥停留时间约为20 d.

工艺线的运行数据由在线水质监测探头定时监测,并收集了这期间工艺运行的实际监测数据,通过对进出水氨氮($\text{NH}_4^+ \text{-N}$)、硝态氮($\text{NO}_3^- \text{-N}$)和亚硝态氮($\text{NO}_2^- \text{-N}$)浓度的统计比较,厘清工艺运行效能.污泥样本的采集,选择了工艺运行的两个典型时

期作为采样的时间点,即工艺出水水质稳定时期与工艺出水水质波动时期,以反映工艺由“稳定”状态到“失稳”状态的过程,聚焦此过程中的微生物特征.第一次样本采集在3月底,此期间出水水质稳定;第二次样本采集在6月初,某家石化企业在5月31日至6月5日间进行的检修工作造成了工艺线进水中总油浓度的增加,此期间进水 ρ (总油)为 $35.4 \text{ mg} \cdot \text{L}^{-1}$,增加了近3倍,是工艺“失稳”期间主要的进水水质变化,并在6月1~10日间出水 $\text{NH}_4^+ \text{-N}$ 浓度出现波动.每次污泥采样在好氧池首尾两端各设置一个采样位点,共计2个采样点,记为 O_f 和 O_b ;缺氧池设置1个采样位点,记为A.为降低系统误差,每个采样点均进行两次重复采样.污泥样品采集后用液氮快速冷冻,立即放入 -80°C 冰箱保存.

1.2 DNA及RNA的提取及测序

利用FastDNA™ Spin及RNA PowerSoil® (MoBio Laboratories Inc, USA)试剂盒对所采集的污泥样品进行总DNA和RNA提取.总DNA的提取步骤为:适量污泥混合液经低温离心后取约0.5 g加入到裂解介质管,并向其中依次加入磷酸钠缓冲液和MT Buffer,振荡离心后保留上清液至洁净离心管.加入250 μL PPS试剂混匀,离心后保留上清液至洁净离心管并加入1.0 mL Binding Matrix,振荡静置后小心去除500 μL 上清液,剩余混合物转移到SPIN™ Filter.多次洗涤离心后,加入100 μL ddH₂O洗脱离心,所得液体即为提取的总DNA,测序前置于 -20°C 冰箱保存.总RNA的提取步骤为:适量污泥混合液经低温离心后去除上清液,加入100 μL TE缓冲液和350 μL RL裂解液,振荡混匀后加入250 μL 的无水乙醇,混合液转移至CR3吸附柱.低温离心后去除收集管中废液,多次洗涤离心后,加入30 μL ddH₂O洗脱离心,所得液体即为提取的总RNA,测序前置于 -80°C 冰箱保存.污泥样品的DNA及RNA序列分别由HiSeq-PE150和HiSeq xten sequencer (Illumina)测序平台进行测序(派森诺,上海).具体测序流程为:将DNA样品加入Fragmentation Buffer,采用超声破碎仪进行随机打断,将打断后得到的短片段DNA用于文库构建,对于质检合格的文库将采用Illumina HiSeq 2500高通量测序平台进行PE150测序.RNA的测序流程类似,合格的RNA样品用以文库的构建,然后采用Illumina HiSeq Xten测序平台进行测序.

1.3 宏基因组与宏转录组分析

基于DIAMOND(版本0.8.34)^[31]分析手段,将污泥样本的DNA和RNA序列与氮循环关键酶的基

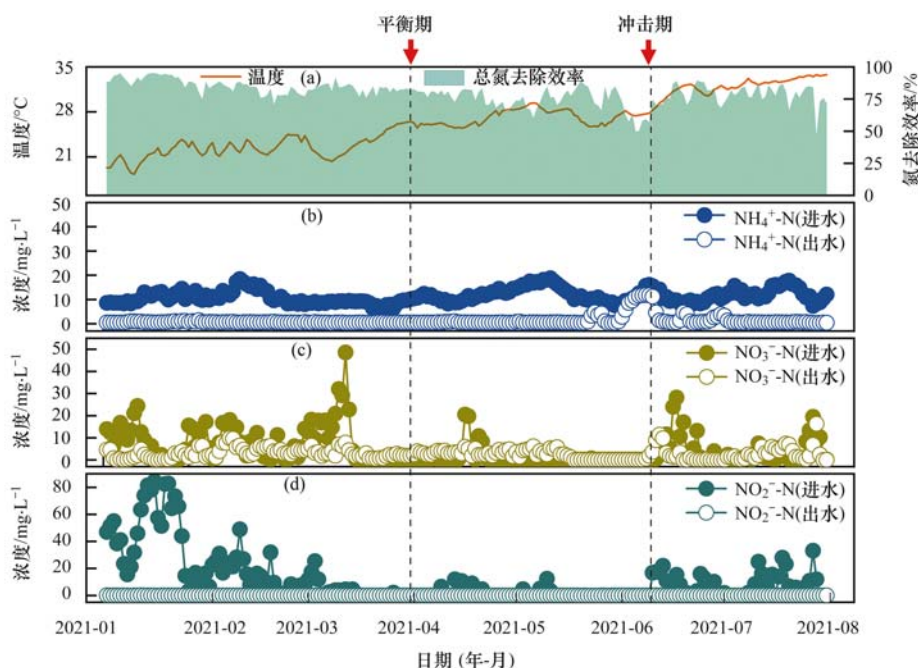
因数据库进行比较, 该数据库包含 13 个氮循环关键酶基因^[32]. 基因组的组装使用 Megahit, 超过 80% 的测序序列组装成重叠区, 大于 500 bp 的重叠区将用于进一步的分析. CheckM (版本 1.0.11)^[33] 用于评估拼接出的基因组草图的完整性和污染情况. 基于 Kaiju 分析手段, 对微生物物种的组成与比例特征进行分析, 评价不同样品中微生物的多样性差异^[34].

2 结果与讨论

2.1 工艺脱氮效能

系统分析了两次污泥采样前后 A/O 工艺共 7 个月的脱氮效能相关数据, 对进出水 NH_4^+ -N、

NO_3^- -N 和 NO_2^- -N 浓度以及脱氮效率进行整理, 如图 1 所示. 工艺进水 ρ (总氮) 平均值为 $(52.5 \pm 1.7) \text{ mg} \cdot \text{L}^{-1}$ (图 1). 监测期内, 总氮去除率平均值为 $(79.9 \pm 0.6) \%$, 去除率随温度变化无明显波动. 出水 $\rho(\text{NH}_4^+$ -N) 平均值为 $(1.3 \pm 0.1) \text{ mg} \cdot \text{L}^{-1}$, 在 6 月初的出水 NH_4^+ -N 浓度波动期间, 出水 $\rho(\text{NH}_4^+$ -N) 平均值达到 $(9.8 \pm 0.8) \text{ mg} \cdot \text{L}^{-1}$. 出水 $\rho(\text{NO}_2^-$ -N) 和 $\rho(\text{NO}_3^-$ -N) 平均值分别为 $< 0.2 \text{ mg} \cdot \text{L}^{-1}$ 和 $(2.8 \pm 0.2) \text{ mg} \cdot \text{L}^{-1}$. 结果表明在监测期内, 体系中 NO_2^- -N 和 NO_3^- -N 的转化效果良好, 但 NH_4^+ -N 转化效果的波动幅度大, 初步判断 NH_4^+ -N 的转化可能是 A/O 体系脱氮过程中的薄弱环节.



(a) 温度变化和总氮去除效率, (b) 进、出水 NH_4^+ -N 浓度, (c) 进、出水 NO_3^- -N 浓度, (d) 进、出水 NO_2^- -N 浓度; 虚线标记为污泥采样时间点

图 1 A/O 工艺脱氮效能

Fig. 1 Nitrogen removal efficiency of the A/O process

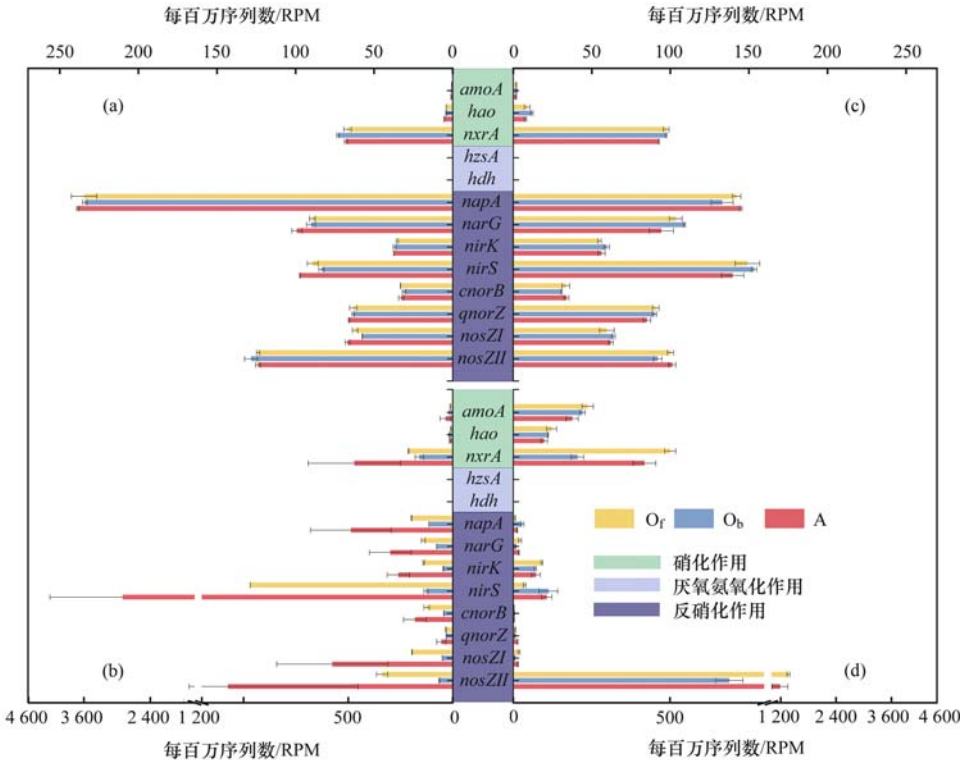
2.2 氮代谢的关键基因分析

为了深入分析体系中氮代谢过程, 以测序获得的 DNA 及 RNA 序列, 比对硝化过程关键基因(氨氧化: *amoA* 和 *hao*; 亚硝酸盐氧化: *nxrA*)、厌氧氨氧化过程关键基因(*hzsA* 和 *hdh*)、反硝化过程关键基因(*napA*、*narG*、*nirK*、*nirS*、*cnorB*、*qnorZ*、*nosZ I* 和 *nosZ II*), 用标准化序列丰度及表达活性代表样品中关键功能基因的丰度与表达活性, 用每百万序列数 (reads per million, RPM), 即每百万条测得的序列中比对到的目标物种基因组的序列条数作为评估标准, 衡量氮代谢中关键功能基因丰度的大小与表达活性的强弱^[24,27].

如图 2 所示, 从基因丰度来看, 3 月底和 6 月初

不同采样时期的基因丰度变化差异不明显, 说明系统中脱氮菌群结构具有一定的稳定性. 其中, 厌氧氨氧化过程功能基因丰度平均值低于 $(0.05 \pm 0.01) \text{ RPM}$ [图 2(a) 和 2(c)]. 反硝化过程功能基因丰度平均值为 $(91.65 \pm 7.43) \text{ RPM}$, 约占工艺内氮代谢关键基因丰度的 90%. 相比之下, 氨氧化过程功能基因丰度相对较低, *amoA* 基因约占工艺内氮代谢关键基因丰度的 0.17%, 比以往的研究报道都要小^[35~37].

此外, 本研究还测定了硝化、厌氧氨氧化和反硝化关键基因的表达活性 [图 2(b) 和 2(d)]. 但几乎监测不到厌氧氨氧化过程基因的表达. 对于硝化作用来说, 6 月初采样点氨氧化功能基因 (*amoA*



(a) 3 月底样品硝化、厌氧氨氧化和反硝化的关键基因丰度, (b) 3 月底样品硝化、厌氧氨氧化和反硝化的关键基因表达活性, (c) 6 月初样品硝化、厌氧氨氧化和反硝化的关键基因丰度, (d) 6 月初样品硝化、厌氧氨氧化和反硝化的关键基因表达活性; O_f 、 O_b 和 A 分别表示好氧池前端、好氧池后端和缺氧池采样点

图 2 A/O 系统中氮代谢功能基因分析

Fig. 2 Analysis of nitrogen metabolism genes in the A/O system

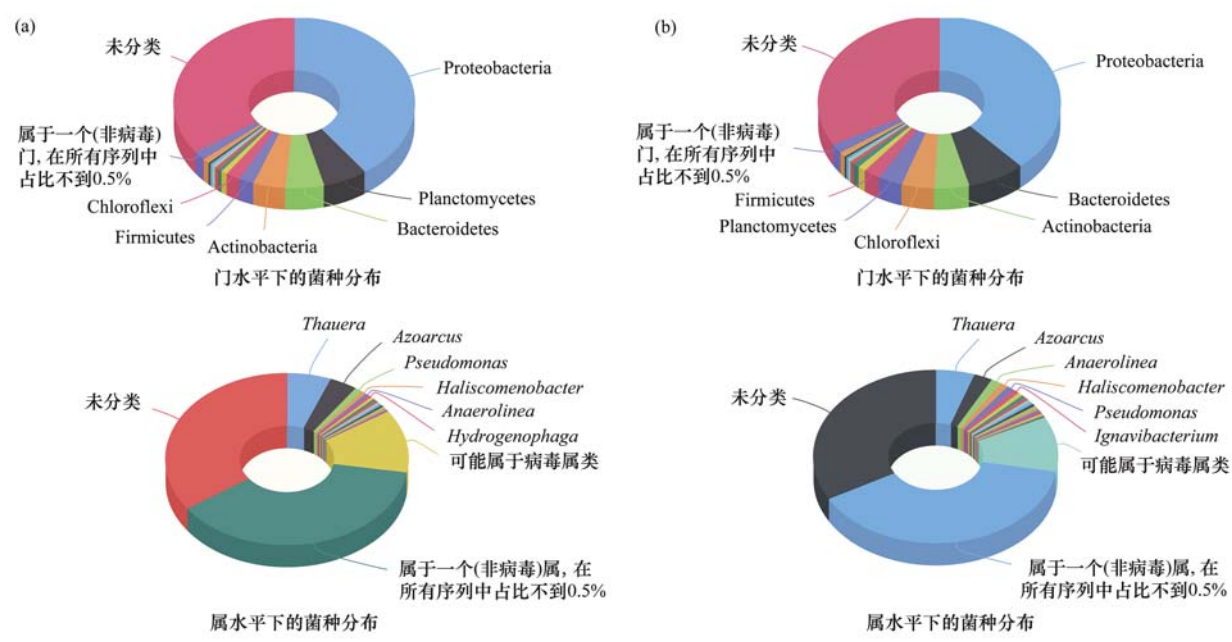
和 *hao*) 的表达活性是 3 月底采样点的近 10 倍, 6 月初采样点亚硝酸盐氧化功能基因 (*nxrA*) 的表达活性是 3 月底采样点的近 1.5 倍, 硝化作用的功能基因表达活性都有增长, 可能是由于反应池内 NH_4^+ -N 浓度的增加, 促进了相应氨氧化功能基因的表达. 虽然 6 月初 *amoA* 基因的表达活性更高, 但体系中 *amoA* 基因的低丰度情况并未改变, 因此出水 NH_4^+ -N 浓度依旧升高.

2.3 功能微生物组成分析

对原始样品的宏基因组序列数据通过 Kaiju 分析手段, 计算体系中微生物物种组成与比例特征, 如图 3 所示. 原始样品中有近 50% 的序列不能被注释, 活性污泥内仍有大部分生物不能被识别. 在门水平中, 变形菌门 (Proteobacteria) 占可注释细菌总数的近 60%, 这与以往报道相似^[38,39], 在污水处理厂中变形菌门是活性污泥中最主要的门类. 在属水平中, 陶厄氏菌属 (*Thauera*) 和固氮弧菌属 (*Azoarcus*) 是主要的优势菌属, 占注释菌属比例为 62%, 是常见的反硝化菌属^[40-42], 而且都属于变形菌门 (Proteobacteria) 中的 β -变形菌 (β -Proteobacteria). β -变形菌通常被认为是活性污泥法群落中的主要成员, 在其他的活性污泥微生物研究

中也报告了这一发现^[42]. 同时, Kaiju 分析结果发现氨氧化功能菌的丰度小于 0.5%.

通过原始 DNA 序列数据, 利用 Megahit 组装共获得约 1 255 238 条 500 bp 以上的重叠区 (contigs), 通过质量筛选 (完整度 > 70%, 污染度 < 10%), 获得 126 个高质量的基因组草图 (draft metagenome-assembled genomes). 对这些基因组草图进行分类注释, 发现共涉及近 20 个门类, 其中, 主要门类为变形菌门 (Proteobacteria)、浮霉菌门 (Planctomycetes) 和拟杆菌门 (Bacteroidetes). 同时, 利用脱氮功能基因与筛选出的基因组草图做比对, 进行功能注释进而筛选出脱氮功能微生物. 发现 126 个高质量基因组草图中携带反硝化过程基因的草图个数为 81 个, 且未发现有携带 *amoA* 基因的基因组草图. 为了更深入地了解系统中的优势功能菌, 本研究将基因组草图与原始序列数据进行比对, 计算得到相对丰度与表达活性, 以此判断体系中的优势菌, 筛选相对丰度与表达活性最高的前 5 株菌, 如表 1 所示. 结果发现, 这 5 株优势菌都携带有反硝化过程的功能基因, 尤其是 PRO1 这株菌携带有硝酸盐还原酶 (*napA/narG*)、亚硝酸盐还原酶 (*nirK/nirS*) 及一氧化氮还原酶 (*cnorB/qnorZ*) 的编码基因.



(a) 3 月底采样门和属水平下的物种组成; (b) 6 月初采样门和属水平下的物种组成

图 3 宏基因组数据分类评价不同层次微生物群落组成

Fig. 3 Metagenomic data were used to assess the composition of microbial communities at different levels

为了进一步探究体系中的氨氧化功能微生物,利用宏转录组的序列数据进行深度挖掘,筛选出携带有 *amoA* 基因的序列,通过 Kaiju 工具分析体系中氨氧化功能菌的组成情况,如图 4 所示.由分析结果可知,两个采样时期下,体系中主要的氨氧化功能菌属是亚硝化单胞菌(*Nitrosomonas*),约占注释序列的近 80%.而在第一次采样点中,主要的氨氧化菌种为 *Nitrosomonas communis*,而在第二次采样点中,主

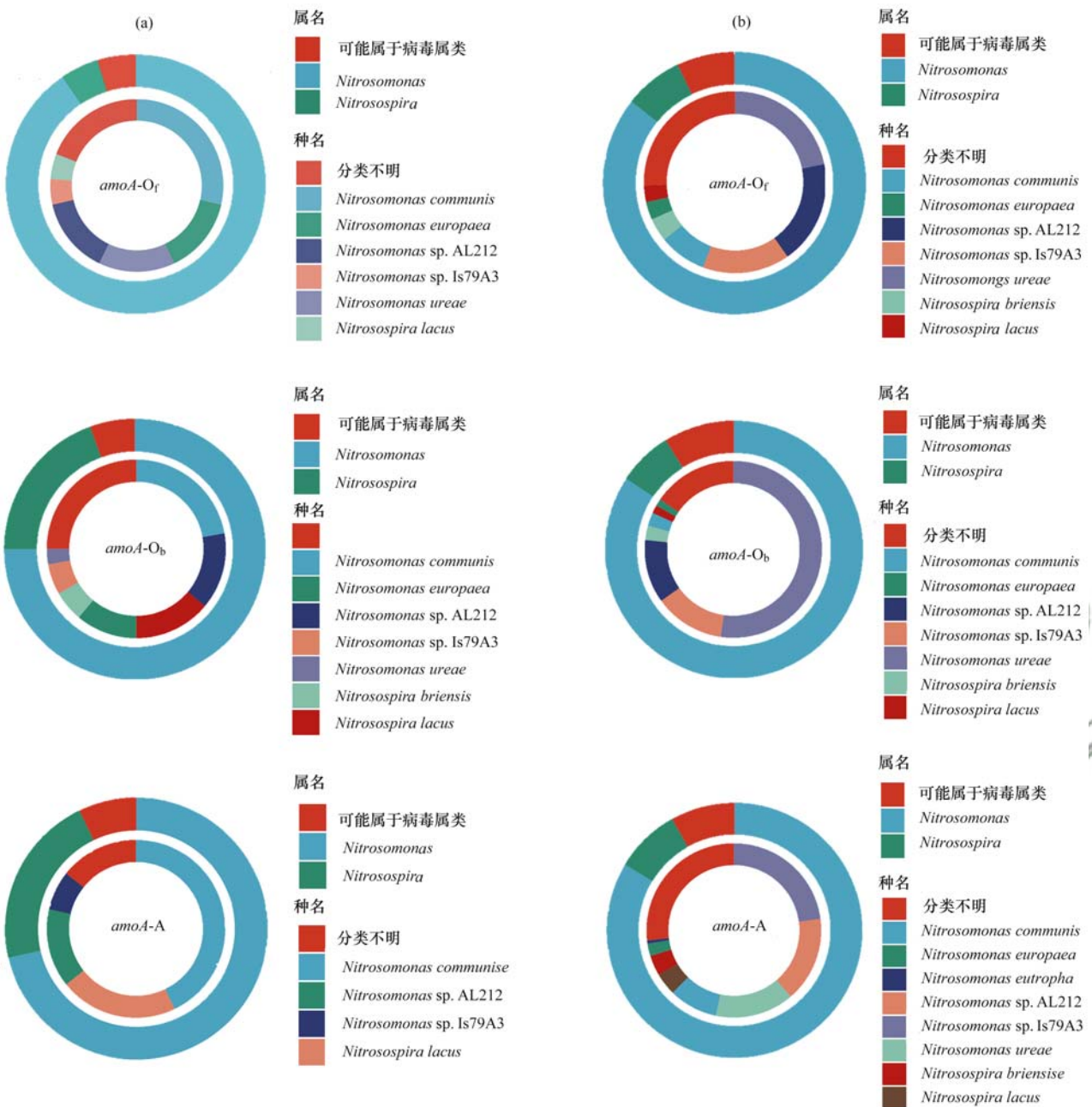
要的氨氧化菌种为 *Nitrosomonas ureae*,据以往的研究报告,*N. communis* 和 *N. ureae* 都是 *Nitrosomonas* 属中常见的物种,在代谢过程中未发现特殊差异,在实际污水厂中常被检测到^[43].体系中氨氧化菌种的变化可能是由于季节变化发生的演替,据报导,季节温度的变化是硝化菌群落演替的关键因素^[26,4].工艺线中氨氧化菌群丰度低和结构单一,这可能是体系中氨氧化作用处于劣势的主要原因^[44,45].

表 1 A/O 工艺体系中的 5 株优势菌

Table 1 Five dominant bacteria in the system

名称	分类	完整度 /%	污染度 /%	GC 含量 /%	基因组大小 /Mbp	S1 基因丰度/S1 表达活性 ¹⁾			S2 基因丰度/S2 表达活性		
						O _f	O _b	A ²⁾	O _f	O _b	A
PRO1	Proteobacteria; γ-Proteobacteria; Burkholderiales; Rhodocyclaceae; <i>Azoarcus_C</i>	93.45	1.58	66.80	4.26	2.85/2.27	2.61/2.38	2.66/1.43	1.39/0.02	1.41/0.01	1.49/0.01
CYB1	Cyanobacteria; Vampirovibrionia; Obscuribacterales	95.73	4.27	50.80	5.89	1.78/0.66	2.34/0.81	1.45/0.73	1.04/0.04	1.27/0.05	0.78/0.03
BCD1	Bacteroidota; Bacteroidia; Chitinophagales	95.32	0.99	44.10	3.89	0.33/0.57	0.43/0.61	0.23/0.40	1.29/0.53	1.20/0.58	1.41/0.62
MYX1	Myxococcota; Polyangia; Polyangiales; Polyangiaceae	92.04	1.61	69.20	7.53	1.76/1.56	1.60/1.41	2.19/1.61	2.18/0.02	2.03/0.01	2.04/0.01
BCD2	Bacteroidota; Bacteroidia; Chitinophagales; Saprospiraceae; UBA6168	98.99	2.78	52.60	6.92	2.07/1.61	2.25/1.30	1.94/1.62	1.19/0.22	1.11/0.16	1.25/0.14

1) S1 为 3 月底采样, S2 为 6 月初采样; 2) O_f、O_b 和 A 分别表示好氧池前端、好氧池后端和缺氧池采样点



(a) 3月底属和种水平下的携带 *amoA* 基因的物种组成, (b) 6月初属和种水平下的携带 *amoA* 基因的物种组成;
O_f、O_b 和 A 分别表示好氧池前端、好氧池后端和缺氧池采样点

图4 宏转录组数据分析携带 *amoA* 基因的微生物群落组成

Fig. 4 Composition of the microbial community carrying the *amoA* gene according to metatranscriptomic analysis

3 结论

本研究通过宏基因组和宏转录组,对上海某化工区的某条石化废水 A/O 脱氮工艺线的活性污泥样品进行了深入分析,聚焦工艺运行由“稳定”状态到“失稳”状态的过程,发现工艺主要的脱氮途径为硝化-反硝化脱氮,且未发现工艺内有厌氧氨氧化发生.反硝化功能基因占关键脱氮功能基因比例约 90%,其次是亚硝酸盐氧化功能基因约 9%,氨氧化功能基因 *amoA* 基因最少,仅占约 0.17%.氨氧化功能菌在工艺中丰度极低且结构单一,主要的氨氧化

功能菌属是 *Nitrosomonas*,这可能是工艺的 NH_4^+ -N 转化“失稳”的主要原因.提高工艺中的氨氧化菌丰度以及丰富氨氧化功能菌的种类可能对石化废水 A/O 工艺出水的稳定达标具有一定的积极作用.

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CONTENTS

Challenges Regarding the Co-emission of Emerging Pollutants to Eco-environmental Monitoring and Management	WANG Pei, HUANG Xin-yi, CAO Zhi-wei, <i>et al.</i> (4801)
Environmental Process, Effects and Risks of Emerging Contaminants in the Estuary-Coastal Environment	WANG Xin-hong, YU Xiao-xuan, WANG Si-quan, <i>et al.</i> (4810)
Research Progress of Analytical Methods with Molecular Spectroscopy for Determination of Trace Nutrients and Metals in Seawaters	YUAN Dong-xing, HUANG Yong-ming, WANG Ting (4822)
Research Progress on the Determination of Sulfide in Natural Waters: From Laboratory Analysis to In-Situ Monitoring	LI Peng, LIN Kun-de, YUAN Dong-xing (4835)
Advances in On-site Analytical Methods for Inorganic Arsenic in Environmental Water	BO Guang-yong, CHEN Zhao-ying, GONG Zhen-bin, <i>et al.</i> (4845)
Advances and Prospect of Sampling Techniques and Analytical Methods for Trace Elements in the Ocean; Progress of Trace Element Platform Construction in Xiamen University	 HUANG Yong-ming, ZHOU Kuan-bo, CHEN Yao-jin, <i>et al.</i> (4858)
Biodegradation of Polyethylene Microplastic: A Review	 LUO Yuan-rong, QIAN Yi-qian, QI Ya-nan (4869)
Mechanism and Environmental Effect on Nitrogen Addition to Microbial Process of Arsenic Immobilization in Flooding Paddy Soils	WANG Feng, ZHANG Jing, ZHOU Shao-yu, <i>et al.</i> (4876)
Toxicity Testing Organisms for Marine Ecotoxicological Research in China	SHI Tian-yi, HONG Hai-zheng, WANG Ming-hua, <i>et al.</i> (4888)
Estimating Methane Fugitive Emissions from Oil and Natural Gas Systems in China	CHEN Chun-ci, LÜ Yong-long, HE Gui-zhen (4905)
Atmospheric NH ₃ Emission Inventory and Its Tempo-spatial Changes in Xiamen-Zhangzhou-Quanzhou Region from 2015 to 2020	LI Xiang, WU Shui-ping, JIANG Bing-qi, <i>et al.</i> (4914)
Distribution of Microplastic and Antibiotic Resistance Gene Pollution in Jiulong River Estuary	CHENG Hong, CHEN Rong (4924)
Pollution Characteristics of Microplastics in Sediments of Xiamen Bay Beach	YAO Rui, LIU Hua-tai, LI Yong-yu, <i>et al.</i> (4931)
Spatial and Temporal Distribution and Influencing Factors of Dissolved Trace Metals in Jiulong River Estuary and Xiamen Bay	QI Liu-qian, YUE Xin-li, ZHONG Hao-wen, <i>et al.</i> (4939)
Spatiotemporal Characteristics of Dissolved Oxygen and Control Mechanism of Hypoxia (Low Oxygen) in the Watershed-Coastal System in Fujian Province	 YANG Ai-lin, YANG Fang, LI Shao-bin, <i>et al.</i> (4950)
Distribution, Migration, and Transformation Mechanism of Labile Phosphorus in Sediments of Xixi River Estuary, Xiamen	PAN Feng, CAI Yu, GUO Zhan-rong, <i>et al.</i> (4961)
Adsorption of Mn ²⁺ by Modified Biochar Fixed Bed in Simulated Lakes and Reservoir Waters	ZHAO Jie, YE Zhi-long, WANG Jia-ni, <i>et al.</i> (4971)
Rapid Detection of Trace Enrofloxacin and Ciprofloxacin in Drinking Water by SERS	XU Jing, ZHENG Hong, LU Jiang-long, <i>et al.</i> (4982)
Degradation of Triphenyl Phosphate in Water by UV-driven Advanced Oxidation Processes	XU Zi-wen, YIN Hong-ling, XIONG Yuan-ming, <i>et al.</i> (4992)
Characteristics and Potential Sources of Four Ozone Pollution Processes in Hainan Province in Autumn of 2019	FU Chuan-bo, CHEN Hong, DAN Li, <i>et al.</i> (5000)
Characterization and Formation Mechanism of Water-soluble Inorganic Ions in PM _{2.5} and PM ₁₀ in Summer in the Urban Agglomeration of the Ili River Valley	 CHEN Qiao, GU Chao, XU Tao, <i>et al.</i> (5009)
Difference in PM _{2.5} Pollution and Transport Characteristics Between Urban and Suburban Areas	QI Peng, ZHOU Ying, CHENG Shui-yuan, <i>et al.</i> (5018)
Characteristics and Health Risk Assessment of BTEX in the Northern Suburbs of Nanjing	FENG Yue-zheng, AN Jun-lin, ZHANG Yu-xin, <i>et al.</i> (5030)
Ecological Risk Assessment of Microplastics Occurring in Surface Water of Terrestrial Water Systems across China	SUN Xiao-nan, CHEN Hao, JIA Qi-long, <i>et al.</i> (5040)
Scale Effects of Landscape Pattern on Water Quality in Dongjiang River Source Watershed	CHEN You-liang, ZOU Wen-min, LIU Xing-gen, <i>et al.</i> (5053)
Mercury Speciation, Distribution, and Potential Sources in Surface Waters of the Yangtze and Yellow River Source Basins of Tibetan Plateau During Wet Season	 LIU Nan-tao, WU Fei, YUAN Wei, <i>et al.</i> (5064)
Water Environmental Characteristics and Water Quality Assessment of Lakes in Tibetan Plateau	LIU Zhi-qi, PAN Bao-zhu, HAN Xu, <i>et al.</i> (5073)
Karst Hydrogeochemical Characteristics and Controlling Factors of Carlin-type Gold Mining Area Based on Hydrochemistry and Sulfur Isotope	 ZHA Xue-fang, WU Pan, LI Xue-xian, <i>et al.</i> (5084)
Characteristics of Eukaryotic Phytoplankton Community Structure and Its Relationship with Environmental Factors in Danjiangkou Reservoir	HE Yu-xiao, MAI Si-jie, REN Yu-fen, <i>et al.</i> (5096)
Adsorption of Phosphate and Heavy Metals by Lanthanum Modified Zeolite and Its Performance in Sediment Inactivation	WANG Zhe, ZHU Jun, LI Wen, <i>et al.</i> (5106)
Metagenomic and Metatranscriptomic Analysis of Nitrogen Removal Functional Microbial Community of Petrochemical Wastewater Biological Treatment Systems	 ZHANG Xu, ZHOU Jia-jia, ZHOU Min, <i>et al.</i> (5115)
Bacterial Community Structure and Antibiotic Resistance Gene Changes in IFAS + Magnetic Coagulation Process Wastewater Treatment Plant in Cold Regions	 DU Wen-yan, YAO Jun-qin, MA Hui-ying, <i>et al.</i> (5123)
Nitric Oxide Emissions from Chinese Upland Cropping Systems and Mitigation Strategies: A Meta-analysis	 TIAN Zheng-yun, WU Xiong-wei, WU Yuan-yuan, <i>et al.</i> (5131)
Impact of Nitrification Inhibitors on Vegetable Production Yield, Nitrogen Fertilizer Use Efficiency and Nitrous Oxide Emission Reduction in China: Meta Analysis	 LIU Fa-bo, MA Xiao, ZHANG Fen, <i>et al.</i> (5140)
Effect of Different Fertilization Treatments on Methane and Nitrous Oxide Emissions from Rice-Vegetable Rotation in a Tropical Region, China	 SHAO Xiao-hui, TANG Shui-rong, MENG Lei, <i>et al.</i> (5149)
Effects of Land-use Conversion on Soil Nitrification and NO & N ₂ O Emissions in Tropical China Under Different Moisture Conditions	TANG Rui-jie, HU Yu-jie, ZHAO Cai-yue, <i>et al.</i> (5159)
Characteristics of Heavy Metal Pollution in Farmland Soil of the Yangtze River Economic Belt Based on Bibliometric Analysis	LIU Xiao-yan, FAN Ya-nan, LIU Peng, <i>et al.</i> (5169)
Spatial and Temporal Distribution and Source Variation of Heavy Metals in Cultivated Land Soil of Xiangzhou District Based on EBK Interpolation Prediction and GDM Model	 GAO Hao-ran, ZHOU Yong, LIU Jia-kang, <i>et al.</i> (5180)
Identification of Soil Heavy Metal Sources Around a Copper-silver Mining Area in Ningxia Based on GIS	ZHANG Kou-kou, HE Jing, ZHONG Yan-xia, <i>et al.</i> (5192)
Effect of Aging on Stabilization of Cd ²⁺ Through Biochar Use in Alkaline Soil of Bayan Obo Mining Area	WANG Zhe, CHENG Jun-li, BIAN Yuan, <i>et al.</i> (5205)
Preparation of Magnetic Iron Oxide/Mulberry Stem Biochar and Its Effects on Dissolved Organic Carbon and Arsenic Speciation in Arsenic-Contaminated Soils	 LU Lin, YAN Li-ling, LIANG Mei-na, <i>et al.</i> (5214)
Effects of Oyster Shell Powder and Lime on Availability and Forms of Phosphorus and Enzyme Activity in Acidic Paddy Soil	ZHAO Li-fang, HUANG Peng-wu, YANG Cai-di, <i>et al.</i> (5224)
Effects of Interaction of Zinc and Cadmium on Growth and Cadmium Accumulation of <i>Brassica campestris</i> L.	SHUAI Zu-ping, LIU Han-yi, CUI Hao, <i>et al.</i> (5234)
Pollution Characteristics and Ecological Risk Assessment of Antibiotics in Vegetable Field in Kaizhou, Chongqing	FANG Lin-fa, YE Ping-ping, FANG Biao, <i>et al.</i> (5244)
Temporal and Spatial Variation Characteristics of Carbon Storage in the Source Region of the Yellow River Based on InVEST and GeoSoS-FLUS Models and Its Response to Different Future Scenarios	HOU Jian-kun, CHEN Jian-jun, ZHANG Kai-qi, <i>et al.</i> (5253)
Carbon Sequestration Characteristics of Different Restored Vegetation Types in Loess Hilly Region	XU Xiao-ming, ZHANG Xiao-ping, HE Liang, <i>et al.</i> (5263)
Response of Soil Multifunctionality to Reduced Microbial Diversity	CHEN Gui-xian, WU Chuan-fa, GE Ti-da, <i>et al.</i> (5274)
Effect of Nitrogen Addition on Soil Fungal Diversity in a Degraded Alpine Meadow at Different Slopes	SU Xiao-xue, LI Xi-lai, LI Cheng-yi, <i>et al.</i> (5286)
Comparative Energy Consumption Structure and Mode between China and Major Energy-Consuming Countries Under the Background of Carbon Emission Reduction	 LI Hui, PANG Bo, ZHU Fa-hua, <i>et al.</i> (5294)
Spatialization and Spatio-temporal Dynamics of Energy Consumption Carbon Emissions in China	HAO Rui-jun, WEI Wei, LIU Chun-fang, <i>et al.</i> (5305)
Carbon Dioxide Mitigation Co-effect Analysis of Structural Adjustment Measures in the "2 + 26" Cities in the Jing-Jin-Ji Region and Its Surroundings	 YANG Tian-qi, WANG Hong-chang, ZHANG Chen, <i>et al.</i> (5315)
Policy Analysis in Plastic Pollution Governance and Recommendations in China	LI Huan, ZHU Long, SHEN Qian, <i>et al.</i> (5326)