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富营养化湖泊藻华腐解产生的溶解性有机质动态变化 及其环境效应

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摘要:富营养化和有害藻华是湖泊面临的主要环境问题,富营养化湖泊藻华在后期会发生衰亡和腐解并产生大量藻源溶解性有 机质(DOM),影响水体 DOM 的质量和活性,并对关键元素的生物地球化学循环产生重要调控作用.为探究不同富营养化程度湖 泊水体藻华腐解过程,对藻华腐解过程中水体 DOM 总量、生物有效性、相对分子质量和组分的动态变化进行分析,并探讨了藻 华腐解引发的环境效应.结果表明,藻华腐解显著提高 DOM 浓度、生物有效性和各荧光组分强度.随着腐解的进行,DOM 浓度 逐渐降低,而相对分子质量逐渐增大.在分子水平上,超高分辨率质谱结果显示腐解过程中不饱和烃和脂肪族化合物优先被微 生物利用,并生成木质素、缩合烃和高 O/C 值的单宁酸等惰性分子.藻华腐解过程中细菌群落主要优势种从变形菌门(46%)逐 渐变为拟杆菌门(42%).此外,藻华腐解还导致水体 CO₂和 CH₄排放显著升高 1.2~5倍,且排放量可以由 DOM 光学指标 *a*₂₅预测.该结果为全面揭示藻华腐解过程中 DOM 特征的动态变化,以及湖泊富营养化治理和环境效应预测提供理论依据和科学支撑.

关键词:溶解性有机质;生物有效性;相对分子质量;藻华腐解;温室气体

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Dynamic Changes of Dissolved Organic Matter Derived from Algal Decomposition and the Environmental Effects in Eutrophic Lakes

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Abstract: The global occurrences of lake eutrophication have led to algal bloom and the subsequent algal decomposition, releasing high amounts of algae-derived dissolved organic matter (DOM) into the lake water. Algae-derived DOM could regulate the quantity and composition of DOM in lake water and further impact the biogeochemical cycles of multiple elements. In this study, the dynamic changes in the quantity and quality of DOM during algal decomposition under different eutrophic scenarios (e. g., from oligotrophication to severe eutrophication) were monitored, and the corresponding environmental effects (e. g., microbial responses and greenhouse gas emissions) caused by algal decomposition were further explored. The results showed that algal decomposition significantly increased the DOM levels, bioavailability, and intensities of fluorescent components in the water. The total DOM levels gradually decreased, whereas the average molecular weight increased along the decomposition process. Furthermore, unsaturated hydrocarbon and aliphatic compounds were preferentially utilized by microorganisms during algal decomposition, and some refractory molecules (e. g., lignin, condensed hydrocarbons, and tannin with high O/C values) were synchronously generated, as evidenced by the results from ultra-high-resolution mass spectrometry. The dominant bacterial species during algal decomposition shifted from Proteobacteria (46%) to Bacteroidetes (42%). In addition, algae addition resulted in 1. 2-5 times the emissions of CO_2 and CH_4 from water, and the emission rates could be well predicted by the optical index of a_{234} in water. This study provides comprehensive perspectives for understanding the environmental behaviors of aquatic DOM and further paves the ways for the mitigation of lake eutrophication.

Key words: dissolved organic matter; bioavailability; relative molecular weight; algal decomposition; greenhouse gas

城市化迅速发展导致大量富含氮磷的废污水直接或间接排入湖泊水体,使得湖泊富营养化成为最严重的水环境问题之一^[1].全球面积超过25 km²的湖泊中,有60%都处于富营养化状态,而我国达到富营养化状态的大型湖泊(面积大于10 km²)甚至超过总数的85%^[1].湖泊富营养化不仅对水生生态系统带来严重的危害,同时也对碳等元素生物地球化学循环产生重要调控作用^[2,3].湖泊藻华一般会经历"复苏-暴发-衰亡-休眠"的循环^[4].在藻华暴发阶段,浮游植

物生产力显著增加,导致碳沉降率增加^[5];而在藻华 衰亡和腐解阶段,藻细胞的凋亡会向水体释放大量 糖类、类蛋白和木质素等物质,成为湖水中溶解性有 机质(DOM)的重要来源^[6]. DOM 是连接生命形态碳

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和无机碳的关键纽带,并对污染物(如重金属和有机 污染物等)的生物有效性和环境行为产生重要影 响^[7]. DOM 直接参与水体有机碳动态循环过程,部分 活性和半活性组分可以被微生物利用并以 CO₂和 CH₄ 的形式释放回大气,而另一部分难降解组分则以相 对惰性有机质的形式保存在湖泊水体和沉积物中^[8]. 因此全面了解藻华腐解过程中 DOM 性质的变化,对 理解富营养湖泊碳库的动态变化以及富营养化治理 具有重要意义.

已有研究模拟了蓝藻和刚毛藻等浮游植物腐解 过程中DOM的变化^[9,10],然而,以上研究多以单一手 段表征 DOM 特性,不利于全面揭示 DOM 组成变化. 例如,三维荧光光谱法常用于解析 DOM 的荧光组分 信息,但它缺乏相应的分子结构解释^[4,11]. DOM 相对 分子质量对污染物的生物有效性具有重要影响[12,13], 有研究表明63%~94%的重金属与较高芳香度且相对 分子质量高的 DOM 组分络合^[13]. 而目前对藻华腐解 过程中水体DOM相对分子质量分布及生物有效性的 变化关注较少.富营养化湖泊水体温室气体的产生 和排放也是当前关注的热点[14,15]. 已有研究发现湖 泊富营养化会促进 DOM 的矿化并提升温室气体的排 放[15,16]. 然而,富营养化湖泊水体温室气体排放过程 复杂,受到光合作用、呼吸作用和分解作用等共同影 响^{111]},藻华腐解对湖泊温室气体排放的影响目前尚 未得到充分认知,不利于全面理解和准确预测藻华 引起的湖泊温室气体排放效应.

为此,本研究模拟不同富营养化程度的湖泊藻 华腐解过程,以全面揭示水体 DOM 总量、生物有效 性、相对分子质量和组分的动态变化.其中,采用不 同尺寸的透析袋对 DOM 进行相对分子质量分级实验 以探究藻华腐解过程中 DOM 相对分子质量变化.结 合三维荧光光谱和超高分辨率质谱(傅里叶变换离 子回旋共振质谱,FT-ICR MS),从分子水平揭示藻华 腐解过程 DOM 组成变化.此外,还同步关注了藻华 腐解过程中细菌群落结构多样性的变化以及 CO₂和 CH₄排放量的变化.本研究通过全面揭示藻华腐解过 程中 DOM 的动态变化和引发的环境效应,以期为未 来湖泊富营养化治理和修复提供数据基础和科学 支撑.

1 材料与方法

1.1 区域概况

巢湖位于安徽省中部,为长江下游左岸水系湖 泊,是我国第五大淡水湖.巢湖湖面东西长约55 km, 南北宽约21 km,湖岸线周长约180 km,湖面面积约 为780 km²,平均水深2.9 m.巢湖的湖水补给主要来 自于地表径流,沿湖共有35条河流并呈放射状汇入 巢湖,其中南淝河、杭埠河和白石天河3条河流入湖 径流量占总径流量75%以上^[18].近几十年来,随着社 会经济迅速发展,周边城镇产生的大量废污水排放 入湖,加剧了巢湖的富营养化进程^[19]. 巢湖的藻华暴 发主要集中在每年5~8月,并呈现西高东低以及湖周 高湖心低的特点^[19].2019~2020年巢湖水体富营养化 指数在50~70之间,其中,西半湖区水体富营养化 数为61,长期处于中度甚至重度富营养化状态^[20].

1.2 样品采集与处理

巢湖西半湖的北部湖湾主要承接合肥市排放的 生活污水,是富营养化相对严重的区域;同时,受东 南季风的影响,每年夏季有大量藻类聚集在西半湖 的北部湖湾[21]. 因此,选择该区域采集藻华样品[图1 (a)],以重点考察富营养化湖泊藻华腐解对水体 DOM产生的影响和环境效应.采样点设置在南淝河 入湖河口处[图1(b)],使用125目浮游生物采集网在 藻类聚集的水面来回拖动,采集藻类样品置于车载 冰箱并尽快运回实验室.用超纯水清洗样品表面杂 质后冷冻干燥获取藻粉[图1(c)]. 藻粉用于后续藻 华腐解模拟实验,以保证每次藻添加量的均一性和 实验结果的稳定性. 巢湖藻华期间藻类以微囊藻 (Microcystis spp.)为主[22],这也是我国其它淡水湖泊 藻华暴发时的优势种[23~25].因此,从巢湖采集的藻类 样品可以较好地代表我国典型富营养化湖泊状况. 同步选取湖面相对清洁的区域采集湖泊表层水样 品,低温运至实验室作为室内培养实验用水.

1.3 实验设置

为考察湖泊不同富营养化程度下藻华腐解产生 的藻源 DOM 动态变化特征,本研究向 40 mL 未过滤 湖水中分别加入0、1、5和10mg藻粉,以分别模拟湖 泊水体的贫营养(记为"对照组")、轻度富营养(记为 "低加藻组")、中度富营养(记为"中加藻组")和重度 富营养(记为"高加藻组")状态. 各实验组水体中初 始叶绿素 a 浓度分别为(15.3±1.76)、(241±19.4)、 (969±20.0)和(1919±59)µg·L⁻¹,其中各加藻组叶绿 素a浓度与富营养化湖泊水体叶绿素a浓度范围 (150~3 000 μg·L⁻¹)基本一致^[26]. 藻水培养体系用封 口膜密封,充分混匀后置于恒温箱中避光培养,培养 温度为23℃(藻华腐解期9~11月巢湖流域平均气 温),避光以防止培养过程中DOM发生光降解.取样 时间点设定为0、1、3、7、12、18和24d. 各实验组均 设置3个平行.在每个时间点进行破坏性取样,测定 水体 DOC 浓度,同时对 DOM 进行紫外-可见光谱和三 维荧光光谱表征,以观察藻华腐解过程中DOM的动 态变化特征.同步测定藻华腐解过程产生的温室气 Z 31°45

31°40'

31°35'

31°30'



(a)采样点分布示意图,(b)采样点照片,(c)冻干藻粉
 图1 巢湖采样点位
 Fig. 1 Sampling sites of Chaohu Lake

体(包括 CO₂和 CH₄)浓度,以探究藻华腐解带来的环 境效应.此外,分别在腐解前期(1 d 或 3 d)、中期(12 d)和后期(24 d)额外选取对照组和高加藻处理组样 品,用于 DOM 分级测定和 DOM 超高分辨率质谱表 征,以及观察藻华腐解过程中 DOM 相对分子质量和 分子组成变化;同时,对水体细菌丰度和群落结构进 行调查,以考察藻华腐解不同阶段细菌丰度和群落 变化及其对藻源 DOM 的响应.

1.4 指标分析

1.4.1 DOC浓度测定及 DOM 生物可利用性组分的 确定

取部分水样经 0.45 μ m 滤膜过滤,所得滤液即为 待测 DOM 样品.利用总有机碳分析仪(TOC-V_{cen}, Shimadzu, Japan)测量 DOC 浓度(mg·L⁻¹,以C计),以 表示藻华腐解产生的藻源 DOM 总量的变化.DOM 生 物降解过程中 DOC 浓度随时间(t)的动态变化符合 G 模型,即:DOC= $c_1e^{-k_1}+c_2e^{-k_2t}+c_3e^0$.该模型确定了 DOM 由 3 种不同生物可利用性组分的指数衰减项之和组 成,包括易降解组分(c_1)、半易降解组分(c_2)和难降解 组分(c_3), k_1 和 k_2 分别表示易降解和半易降解组分的 一级衰减常数^[27].该模型已被广泛用于水体 DOM 生 物 降 解 过程中不同生物可利用性组分的定量 评估^[28, 29].

1.4.2 DOM紫外-可见吸收光谱测定

利用紫外-可见分光光度计(UV-1800, Shimadzu) 测量水体 DOM 紫外-可见吸收光谱,测量波长范围为 200~800 nm,间隔为1 nm,以 Milli-Q 超纯水作为空 白.分别选取 *a*254、SUVA254和光谱斜率 *S*275-295 以表示 DOM 浓度^[30]、DOM 芳香性^[31]和 DOM 相对分子质 量^[32]的变化.

1.4.3 DOM 相对分子质量分级测定

对DOM进行相对分子质量分级测定以探究藻华

腐解过程中DOM 相对分子质量变化.分别使用相对 分子质量为3500和10000的透析袋(SnakeSkin[®] Dialysis Tubing, Thermo Scientific, USA), 将藻源 DOM 分为相对分子质量<3 500组分(小分子)、相对分子 质量为3 500~10 000 组分(中分子)和相对分子质量> 10 000 组分(大分子)^[33]. 剪取一定长度相对分子质 量为3500的透析袋,向其中加入20mL浓缩过滤藻 源 DOM 溶液, 然后将其放入含 400 mL去离子水烧杯 中,在恒温(4℃)和黑暗条件下持续搅拌48h.透析结 束后,烧杯中溶液即为相对分子质量<3 500组分.而 后将透析袋中的样品(相对分子质量>3 500)转移到 相对分子质量为10000的透析袋中进行下一步分 级. 重复上述过程,最终透析袋中的样品为相对分子 质量>10 000组分,而透析袋外的样品为相对分子质 量3500~10000组分.取样结束后,每个组分样品测 定 DOC 浓度以评估不同分子组分的比例.分级 DOC 的整体回收率在93.6%~100%之间.

1.4.4 DOM 三维荧光光谱测定

利用荧光分光光度计(F-7000, Hitachi)测定水体 DOM 三维荧光光谱.采用1 cm 石英比色皿,光源为 150 W 氙灯,电压设定为700 V,波长误差为±1 nm. 其 中,波长的扫描范围为:激发波长(E_x)200~400 nm 和 发射波长(E_m)250~580 nm,波长间隔均为5 nm,扫描 速度为12 000 nm·min⁻¹,以 Milli-Q 超纯水作为空白. 采用荧光区域积分进行 DOM 组分分析,根据荧光峰 的位置不同,可将三维荧光光谱划分为5个区域^[34], 分别对应5类物质,包括酪氨酸类蛋白质(E_x :200~ 250 nm; E_m :280~330 nm)、色氨酸类蛋白质(E_x :200~ 250 nm; E_m :330~380 nm)、类富里酸(E_x :200~250 nm; E_m :380~550 nm)、溶解性微生物代谢产物(E_x :250~400 nm; E_m :380~550 nm).

1.4.5 DOM 超高分辨质谱表征

利用傅立叶变换离子回旋共振质谱仪(FT-ICR MS, Bruker SolariX, 15.0 T)表征水体 DOM 分子组 成^[29,35].将待测水样酸化至 pH 值<2,然后通过固相 萃取柱(Agilent Bond Elute PPL, 0.5g, 6mL)进行有机 组分的富集和浓缩.样品负载后,用甲醇洗脱,收集 洗脱液进行上机分析.根据分子式中是否存在杂原 子 N 和 S 将分子分为4类:CH0、CH0N、CHOS 和 CH0NS,根据分子式的 H/C和 0/C将分子分为7类,包括脂类(0/C:0~0.3; H/C:1.5~2.0)、蛋白质类(0/C:0.3~0.67; H/C:1.5~2.2)、木质素类(0/C:0.3~0.67; H/C:1.5~2.2)、本质素类(0/C:0.3~1.5)、缩合烃类(0/C:0~0.67; H/C:0.2~0.7)和单宁 酸类(0/C:0.67~1.0; H/C:0.5~1.5)^[35].

1.4.6 水体细菌丰度以及群落结构测定

使用0.22µm滤膜对藻华腐解溶液进行抽滤,将 滤膜于-80℃中保存待测.采用DNA提取试剂盒 (MO BIO Laboratories, Carlsbad, CA, USA)对样本 DNA进行提取,以稀释后的基因组DNA为模板,使用 515F(5'-GTGCCAGCMGCCGCGGTAA-3')和909R(5' -CCCCGYCAATTCMTTTRAGT-3')作为PCR 扩增引 物,随后进行细菌16SrRNA荧光定量以及Illumina高 通量测序^[36]测序结果利用Qiime(V1.9.0)进行质量 控制、OUT聚类、物种注释以及均一化处理^[37]. 1.4.7 水体温室气体浓度测定

将50 mL离心管的盖子预先打孔并用硅橡胶(南 大704 硅橡胶,室温固化硅橡胶).待加入湖水和适量 藻粉后,拧紧离心管盖并用硅橡胶将离心管盖周围 一圈密封随后进行培养.在每个取样点,用注射器和 三通阀定量抽取培养系统中顶空气体,然后将气体 迅速注入气相色谱(HP-5890 Series II, Agilent Technologies Inc., USA),得到样品的色谱图.以色谱 标准物质 CH₄和 CO₂保留时间为定性依据,以峰面积 作为响应值,计算藻华腐解过程中产生 CH₄和 CO₂ 的量^[38].

1.5 统计分析

采样点位图由 ArcGIS 10.8 制作,数据图由 Origin 8.5 制作, Mental 检验相关关系图由 RStudio 4.2.0 制作.利用 IBM SPSS Statistics 26 进行统计分 析,包括单因素方差分析(ANOVA)和平均值 *t* 检验, 显著性水平为 *P*<0.05.

2 结果与讨论

2.1 DOM总量及生物有效性变化 随着藻华腐解的进行,水体 DOC 浓度均呈现出

指数衰减的趋势[图2(a)]. 所有实验组 DOC 的降解 均遵循先快后慢的特点,其中,前3d的DOC降解率 可达总降解率的54%~87%.随着藻密度的增加,水体 初始ρ(DOC)(以C计,下同)从对照组7.99 mg·L⁻¹增 加到高加藻组 32.2 mg·L⁻¹,这说明藻华腐解可以显 著提高水体 DOM 总量. 经过 24 d 的腐解, 对照组、 低、中和高加藻处理组 $\rho(DOC)$ 分别降至5.02、 5.12、6.68和11.4 mg·L⁻¹, 对应 DOC 降解率分别为 37%、36%、63%和65%,即DOC初始浓度越高,DOC 降解速率越快,最终DOC降解率越高.这表明水体富 营养化程度越高,藻华腐解向水体释放 DOM 越多,进 而促进微生物的增长,以加剧 DOM 的生物降解^[39]. 进一步通过G模型分析发现,对照组水体DOM易降 解(c₁)和半易降解(c₂)组分占比分别为10%和28%, 而高加藻处理组这两者占比分别达到43%和22% [图2(b)],这说明随着富营养化程度的加剧,藻华腐 解会向水体输入更高比例的活性有机质组分^[28].以 上结果表明,随着水体富营养化程度的加剧,藻华腐 解会向水体释放更多数量和更高比例的活性有机 质,从而驱动微生物的增长并对DOM的动态变化产 生重要调控作用[40,41]



2.2 DOM光谱指标变化

DOM 吸收系数 *a*₂₅₄会随其含有化合物的芳香环 缩合程度、含碳比例以及总含碳量增加而增加,主要 表征 DOM 相对浓度^[30].本研究中,低、中和高加藻处 理组水体 *a*₂₅₄随着腐解的进行下降趋势非常明显,24 d 腐解过程中分别降低了 41%、71% 和 83%,且下降 主要集中在腐解前 3 d[图 3(a)]. 对照组水体 *a*₂₅₄在腐 解过程中变化不大.

SUVA₂₅₄主要用于指示 DOM 芳香性^[31]. 加藻组水 体SUVA₂₅₄表现出不同的变化趋势.其中,低和中加藻 组水体 SUVA₂₅₄在腐解前 3 d 分别从 1.69 L · (mg·m)⁻¹ 和 2.34 L·(mg·m)⁻¹ 降至 1.13 L·(mg·m)⁻¹ 和 1.29 L·(mg·m)⁻¹,随后又逐渐上升到1.60 L·(mg·m)⁻¹和 1.70 L·(mg·m)⁻¹左右并保持稳定[图 3(b)]. 而高加 藻组水体 SUVA234则一直处于降低趋势,从初始 2.40 L·(mg·m)⁻¹降低至1.28 L·(mg·m)⁻¹[图3 (b)]. SUVA₂₅₄的降低可能与部分含氧官能团的芳香 性物质被微生物生长利用有关[31,42],这部分物质的 降解速率可能高于非腐殖组分的分解以及芳香类腐 殖组分的生成速率. 当低和中加藻组中引入的这部 分物质消耗殆尽后,水体腐殖化逐渐增加.而高加 藻组中引入的这部分芳香性物质过多,从而使 SUVA₃₅₄一直处于下降趋势. 对照组水体 SUVA₃₅₄在 3 d 后逐渐增大并于 12 d 趋于稳定,到腐解末期 SUVA₃₄增大了约1.5倍[图3(b)],这表明随着腐解 的进行,容易分解的物质被逐步消耗从而使富含芳 香环结构的腐殖质占比逐步升高,导致对照组水体 DOM腐殖化程度增强^[43].

S275-205可反映 DOM 相对分子质量大小,且值越大 表示 DOM 相对分子质量越小^[32]. 各加藻组水体 S275-295 随腐解时间均呈下降趋势[图3(c)],表明腐解过程 中藻源 DOM 的相对分子质量逐渐增大. 与本研究结 果类似,Balch等[44]也发现藻源DOM的相对分子质量 随培养时间逐渐增大,45 d DOM的相对分子质量为 6 d 的两倍. 这表明藻华腐解产生的小分子物质会优 先被微生物利用而消耗,最终剩下一些难降解的相 对分子质量较高的物质[45].此外,富营养化水体 S275-295显著升高并与藻密度呈正相关,低、中和高加 藻组水体初始S275~295分别达到37.1、52.3和58.1 μm⁻¹[图3(c)],这可能是由于内源性有机质含有较多 的小分子氨基酸和碳水化合物,从而导致相对分子 质量随藻密度增加而逐渐降低^[46]. 对照组水体 S275-295 稳定在 21.5~32.4 µm⁻¹之间,平均值为 25 µm⁻¹,保持 相对稳定[图3(c)],表明对照组水体DOM相对分子 质量变化不大.

2.3 DOM 相对分子质量变化

随着藻华腐解的进行,DOM小分子组分(相对分 子质量<3 500)、中分子组分(相对分子质量3 500~ 10 000)和大分子组分(相对分子质量>10 000)浓度 均呈下降趋势.小、中和大分子组分 $\rho(DOC)$ 分别从 12.0、6.61 和 9.77 mg·L⁻¹降至 2.92、1.01 和 1.67 mg·L⁻¹[图4(a)]. 其中,小分子组分和中分子组分 ρ(DOC)共减少了14.7 mg·L⁻¹,减少量要高于大分子



parameters during algal decomposition

组分的减少量(8.10 mg·L⁻¹),这表明随着藻华腐解的 进行,水体 DOM 相对分子质量逐渐增大,与上述 S775-295变化趋势保持一致[图3(c)]. 在藻华腐解不同 阶段,不同分子组分比例变化不大,小、中和大分子 组分的平均占比分别为45%、34%和21%[图4(b)], 这也表明了藻华腐解会向水体输入大量氨基酸等小 分子物质,这些小分子物质一般具有较高的生物活 性,从而对水体微生物增长和污染物的转化产生重 要影响[45].

2.4 DOM 组分变化

2.4.1 DOM 荧光组分变化

藻华腐解过程中,加藻组湖水 DOM 各组分的荧 光强度均有不同程度的下降,而对照组中相关组分





各组分荧光强度下降了 30%~60% [图 5(b)], 中和高

weight during algal decomposition 的荧光强度没有明显变化[图 5(a)]. 低加藻组 DOM 保持一致[图3(b)]. 🔜 类腐殖酸类物质 🛛 🔜 溶解性微生物代谢产物 🛽 $1.8 imes 10^5$ 4.8×10^{4} (a) 对照组 3.6×10^{5} 荧光区域积分/a.u. 荧光区域积分/a.u. 1.2×10^{5} 2.4×10^{4} 6×10^{4} 1.2×10^{5} 0 0 1 3 7 12 18 24 0 1 3 7 12 2.0×10^{6} 3.5×10^{6} (c) 中加藻组 1.6×10^{6} 2.8×10^{6} 荧光区域积分/a.u. 荧光区域积分/a.u. 1.2×10^{9} 2.1×10^{6} Ι 4.0×10^{5} 7.0×10^{5} 0 0 0 1 3 7 12 18 24 0 1 3 7 12 时长/d 时长/d

加藻组各组分荧光强度减少量都超过70%[图5(c) 和 5(d)],这与 DOC 和 a254下降规律类似,表明藻源 DOM 的输入加剧了细菌对不同 DOM 组分的降解利 用.随着富营养化程度的增加,DOM各组分荧光强 度均显著升高.例如,与对照组相比,高加藻处理组 中络氨酸类蛋白质、色氨酸类蛋白质、类富里酸类 物质、溶解性微生物代谢产物和类腐殖酸类物质荧 光强度分别提高了 200%~1 689%、183%~1 309%、 203%~1653%、129%~1153%和210%~3160% [图5 (d)],说明藻华腐解会向水体中输入大量活性迥异 的有机质.其中,类腐殖酸类物质荧光强度增量最 大,表明藻华腐解可以产生大量含氧官能团类腐殖 酸芳香性物质[42].随着藻华腐解的进行,此类物质的 减少量是其余4种组分总减少量的2倍,这与上述高 加藻组水体SUVA24随腐解时间逐渐降低的变化趋势



图 5 藻华腐解过程中水体 DOM 荧光组分强度的动态变化

Fig. 5 Dynamic changes in relative intensities of DOM fluorescent components during algal decomposition

2.4.2 DOM分子组成变化

对藻华腐解过程中 DOM 进行 FT-ICR MS分析, 进一步从分子水平解析 DOM 组成的变化.从元素组 成上来看,藻华腐解显著提高了水体 DOM 中生物可 利用性高的 CHON 类化合物占比(表1). 从分子组成 上看,藻华腐解显著提高了水体 DOM 中相对分子质

量低的脂肪类和碳水化合物的比例,碳水化合物、脂 质、类蛋白和不饱和烃的比例分别从1.8%、3.1%、 11.8% 和 8.7% 提高到 2.7%、5.3%、17.5% 和 14.1%(表1).从时间趋势上看,高加藻组水体DOM 随着腐解的进行,脂质、类蛋白和不饱和烃等组分呈 现降低趋势,而缩合烃、木质素和单宁酸的比例随着

表1 基于 FT-ICR MS 分析的藻华腐解过程中 DOM 各组分比例变化

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腐解程度有所提高(表1),这表明生物活性高的类蛋白、类糖和脂质等组分可以在短期内参与微生物代谢和物质循环,而高0/C值的木质素和单宁酸难以被

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生物降解^[28,47,48].对照组水体 DOM 分子组成整体变 化不大,脂质、碳水化合物和类蛋白的比例略有降 低,同时,木质素的比例略有增加(表1).

Table 1 Changes in percentages of DOM components during argai decomposition based on FT-ICK M5 analysis								
组分	高加藻组/%			对照组/%				
	1 d	12 d	24 d	1 d	12 d	24 d		
CHO类化合物	35.6	29.7	38.1	38.5	39.4	38.7		
CHON类化合物	47.7	51	42.9	43.5	41.5	42.7		
CHOS类化合物	16.7	19.3	19	18	19.1	18.6		
碳水化合物	2.7	4.1	3.2	1.8	2.1	1.5		
缩合烃	1.6	2.4	2.7	3.7	3.7	3.5		
木质素	51.4	53.8	57	62.1	62.7	66.1		
脂质	5.3	3.8	3.4	3.1	3	2.7		
类蛋白	17.5	16	13.9	11.8	11.6	9.4		
单宁酸	6.3	8.2	6.8	7.9	8.3	7.1		
不饱和烃	14.1	10.6	11.6	8.7	7.7	8.9		

DOM 分子的 van Krevelen 图进一步解析藻华腐 解期间水体 DOM 组成的变化.高加藻组水体 DOM 中易降解组分主要为具有低 O/C 值的木质素(16%)、 不饱和烃(35%)和脂肪族化合物(脂质、蛋白质和碳 水化合物,共占比约44%[图6(a)],表明这些物质优 先被水体微生物消耗,其中占比25%的含氮和含硫 的不饱和分子很可能来源于藻类功能细胞成分的亚 基或片段,例如,核苷酸(DNA和RNA)、叶绿素II和 藻蓝蛋白都含有杂环,H/C值为1~1.3⁴⁹.而在腐解 后期消失的分子(代表半易降解组分)不仅数量比初 期少了一半以上,而且主要属于蛋白质和木质素(总 占比约70%)[图6(b)],表明当低O/C值的木质素和 不饱和烃被优先消耗完后,部分高O/C值的结构多糖 也可供细菌降解利用. 对照组易降解和半易降解的 组分主要属于蛋白质类和部分木质素,且这两种组 分仅占腐解期间所有 DOM 分子的 12% [图 6(e) 和 6 (f)],说明对照组中可生物利用DOM比例很低,导致 腐解期间对照组水体 DOM 特性变化不大.

高加藻组和对照组水体中腐解全程都存在的 DOM分子是微生物难利用的组分,这些分子绝大多 数都属于木质素和单宁酸[图6(c)和6(g)].其中,多 数物质落入富羧酸脂环分子(carboxyl-rich alicyclic molecules, CRAM)区域, CRAM 是一类广泛分布于 DOM中的惰性分子,其结构具有多样性且含有大量 的带支链的脂环族化合物^[50]. CRAM可以在原核和真 核生物中的膜成分和次级代谢产物中检测到,因此 湖水中CRAM的来源应该包括藻类和一些水生植物 细胞壁和膜成分的分解^[50]. 对照组水体 DOM 中难降 解分子的比例(59%)高于加藻组(41%),说明藻源 DOM 为水体微生物提供了更多可利用的活性和半活 性组分,该比例也与基于 DOC 浓度变化的 G 模型拟 合得到的结果保持一致[图2(b)].此外,无论是高加 藻组还是对照组,新生成的分子主要为木质素、缩合 烃和具有高 O/C 值的单宁酸[图6(d)和6(h)],代表藻 华腐解过程中微生物代谢生成的难降解分子,这可 能也是导致对照组水体腐解过程中 DOM 腐殖化程度 加重的原因.

2.5 藻华腐解引发的环境效应

2.5.1 水体细菌丰度和群落结构

藻华腐解产生的藻源 DOM 输入显著增加了水体 细菌的丰度.对照组水体细菌丰度在藻华腐解期间 没有发生明显变化;而在高加藻组,腐解前期(1 d)水 体细菌丰度相对于对照组就提高了一个数量级,并 且随着藻华腐解的进行,细菌 DNA 丰度呈上升趋势 [图 7(a)],从 1.14×10⁸ copies • mL⁻¹增加到 1.82×10⁸ copies • mL⁻¹,这是因为藻华腐解产生大量活性有机 质,促进了微生物的增长繁殖.

藻华腐解产生的藻源 DOM 显著改变了水体细菌的群落结构.对照组水体中细菌主要为变形菌门(Proteobacteria, 29%)和放线菌门(Actinobacteria, 28%),且随着藻华腐解的进行,两者分别增加到 38%和 36%;而拟杆菌门(Bacteroidetes)从初始 23%降低到 8%[图 7(b)].高加藻组腐解前期变形菌门、拟杆菌门和厚壁菌门(Firmicutes)为优势种类,其相对丰度分别为 46%、25%和 24%[图 7(b)],表明藻源 DOM的输入显著提升了它们的初始丰度^[51].随着藻华腐解的进行,变形菌门和厚壁菌门的相对丰度降低到 30%和 11%,而拟杆菌门相对丰度增加到 42%.此外,酸杆菌门(Acidobacteria)和螺旋体门(Spirochaetes)在腐解中后期的相对丰度也较高(1.65%~7.04%)[图 7



在藻华腐解过程中,仅在1d存在的分子表示为易降解组分,在1d和12d存在的分子表示为半易降解组分,全程都存在的分子表示为难降解 组分,腐解24d新出现的分子表示为新生成组分;DOM组分按H/C和0/C值分成7类,1~7分别指脂类、蛋白质类、木质素类、碳水化合物、不 饱和烃类、缩合烃类和单宁酸类

图 6 藻华腐解过程中 DOM 组分 van Krevelen 图

Fig. 6 van Krevelen diagram of the identified components in DOM during algal decomposition



(b)].在纲水平上,藻源DOM的输入显著提高了 γ -变 形菌(Gammaproteobacteria)、芽孢杆菌(Bacilli)和拟 杆菌(Bacteroidia)的相对丰度,随着藻华腐解的进行, γ -变形菌和芽孢杆菌的相对丰度逐渐降低,拟杆菌和 梭菌(Clostridia)的相对丰度逐渐上升[图7(c)].而对 照组水体微生物的优势菌为 γ -变形菌、 α -变形菌 (Alphaproteobacteria)和放线菌等,其中,拟杆菌和产 氧光合细菌(Oxyphotobacteria)的相对丰度随腐解进 行而明显降低,而嗜热油菌(Thermoleophilia)的相对 丰度则从 1. 1%提高到 10. 4% [图7(c)].

本研究表明藻华腐解过程中拟杆菌门、厚壁菌 门和变形菌门为优势菌,而这几类菌也是刚毛藻和 蓝藻等腐解过程中的优势菌^[10,51].在水环境中,拟杆 菌门通常与纤维素等多糖的分解有关,产生低聚糖 或短链脂肪酸,例如乙酸和丙酸等有机酸,并能快速 在植物表面定殖和生长^[52].在藻类腐解前期,黄杆 菌(*Flavobacterium*)是拟杆菌门的优势属(占细菌总 丰度的18%),前期研究认为黄杆菌在富营养化湖泊 中具有重要的生态功能,可有效降解蛋白质和纤维 素等物质^[53].厚壁菌门是一个快速生长的富营养型 菌(r策略),可以在富含活性碳底物的环境中快速分 类增殖^[54],这也是富营养化水体中厚壁菌门的相对 丰度要远高于对照组的原因[图7(b)].随着藻源 DOM浓度、活性组分的降低和难降解分子的富集, 厚壁菌门的相对丰度逐渐降低并被其它门属所取 代.其中,芽孢杆菌在初期是优势菌群,该类典型的 反硝化细菌可以产生胞外纤维素酶,与碳和氮的代 谢密切相关^[55,56].随着腐解过程中溶解氧的消耗, 芽孢杆菌逐渐被厌氧的梭菌所取代,Xing等^[57]也发 现在微囊藻水华厌氧降解过程中梭菌占据主导地位 (高达72%),而梭菌在DOM发酵过程中产生的小分 子中间体(如乙酸盐和乙醇等)也可以被产甲烷古菌 利用生成 CH₄,影响富营养化湖泊水体温室气体的 排放.

变形菌门可以分解植物凋落物中的顽固碳化合物,且与氮循环过程密切相关^[s8,s9].本研究中藻华腐解初期变形菌门中的优势属是气单胞菌属(Aeromonas),相对丰度为22.3%.气单胞菌在地表水

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中很常见,具有广泛的外切酶(淀粉酶、蛋白酶、脂肪酶和核酸酶等)^[60],甚至可以高效分解有机污染化合物,如杀虫剂溴氰菊酯^[61].以上结果表明,由于可利用的养分类型和环境因子的变化,水体细菌群落随藻华腐解的进行发生明显的变化^[52].在此过程中细菌及其之间的共生作用又驱动了碳和氮等营养元素循环,如增强固碳、发酵、产甲烷和反硝化等过程,反过来又改变了它们的环境,导致湖泊营养水平的变化^[62].因此,需要进一步研究藻华腐解输入的DOM与微生物群落之间的相互作用机制,包括对微生物相关功能基因的预测分析,为湖泊藻华暴发的治理提供理论依据.

2.5.2 温室气体排放

随着藻华腐解的进行,各加藻组水体 CO₂累积产量逐渐增加,在12d达到峰值趋势稳定;而对照组水体的 CO₂累积产量保持相对不变.从整个腐解过程来看,对照组、低、中和高加藻处理组 CO₂累积产量的平均值分别为 25.4、50.9、105.4 和 149.7 mol·L⁻¹

[图 8(a)],表明藻源 DOM 输入可以显著加剧水体 CO,释放,且藻密度越高,水体CO,产量也越高.齐天 赐等^[63]研究也发现巢湖表层水体CO,浓度与溶解性 有机碳浓度呈显著正相关. CH₄生成对藻源 DOM 输 入的响应要小于CO,,其中,低加藻组和中加藻组水 体的 CH₄累积产量与对照组没有明显差异,并且随 着腐解时间逐渐降低. 仅高加藻处理组水体 CH 累 积产量有所上升,在腐解7d达到峰值并趋于稳定; 高加藻组水体CH。累积产量平均值为37.1 mmol·L⁻¹, 比对照组高1.3倍[图8(b)],说明湖泊重度藻华堆 积区是CH,重要的排放源^[14,64].同时,进一步研究发 现,不同富营养化水体CO,产量与湖水DOM光学指 标 a34有较好的正相关关系 [图 8(c)],水体 CH4产量 与a254也存在类似的正相关关系[图8(d)].考虑到水 体 a254 指标测定的便捷性以及与湖泊富营养化状态 的良好关联性[65],这为预测全球尺度的不同营养状 态湖泊温室气体排放及贡献提供了理论支撑和数据 基础.





同时,进一步开展 Mantel 检验发现不同营养状态 湖泊水体的 DOC、叶绿素 a、a₂₅₄、络氨酸类物质、色 氨酸类物质和溶解性微生物代谢产物显著影响湖泊 水体温室气体 CO_2 和 CH_4 的产量(Mantel's *P*<0.01, Mantel's *r*>0.4),表明 CO_2 和 CH_4 的产生与生物可利用 度高的 DOM 组分密切相关(图 9).在富营养湖泊中,

藻源 DOM 的输入显著提高了这部分物质的量,从而 提高了产甲烷菌等微生物的数量^[16,66],进而促进了 DOM 的矿化过程,导致温室气体产量增加.藻华腐 解促进 CO₂产生的另一个可能原因是 pH 值的变化 (图 9,与 pH 显著相关, Mantel's P<0.05),藻源 DOM 的输入造成水体 pH 值下降,改变水体中碳酸盐平 衡,导致游离 CO₂相对于碳酸根和碳酸氢根的浓度增 加^[67].因此,富营养湖泊藻华腐解会增加温室气体的 排放^[15,16].



API、APII、FA、SMP和HA是基于三维荧光光谱的荧光区域积分方 法解析的5类物质,分别为络氨酸类、色氨酸类蛋白质、类富里酸 类、溶解性微生物代谢产物和类腐殖酸类组分;BIX、FI和HIX分别 表示生物源指数^[68]、荧光指数^[69]和腐殖化指数^[70];热图的颜色梯 度表示皮尔逊等级相关系数(对应于Pearson's r),正值越大(深蓝 色)表示正相关性越强,负值越大(深红色)表示负相关性越强;两种 温室气体产量使用 Mantel 分析与每个理化性质和光谱指数相关联, 连线的宽窄和颜色对应于 Mantel's r的统计量和显著性 P值 **图9 Mental分析CO₂和CH**₄产量与水体理化性质及DOM光谱指 数的相关关系 Fig. 9 Correlation between CO₂ and CH₄ production and

physicochemical properties of water and DOM spectral indices by Mental tests

此外,Zhou等^[71]发现太湖溶解CH₄浓度与表示陆 地腐殖物质的C2-C4荧光峰和DOM的芳香性呈显著 正相关,认为陆源类腐殖质的输入可能在促进富营 养湖泊CH₄的生产释放方面超过藻华.因此未来需 要重点关注并探究不同来源和组成的有机质对温室 气体排放的贡献及机制.同时,预计未来大气CO₂浓 度升高和变暖场景下湖泊营养负荷的增加,未来湖 泊藻华可能进一步扩大并加剧^[72,73],变暖和富营养化 之间的协同作用可能会成比例地增加CO₂和CH₄产 量与排放导致气候变暖加速,起到正反馈的作用^[74], 因此,需要进一步探索湖泊富营养化和气候变暖之 间的关系,这对准确预测未来气候变化和富营养化 情景下湖泊在全球温室气体收支中的作用至关 重要.

需要注意的是,本研究结果主要是基于室内微 宇宙封闭培养体系获取的;而在实际原位真实条件 下,多种环境因素会影响DOM的环境行为和生物有 效性. 例如,本研究主要关注考察藻华腐解过程中 DOM的生物降解过程,光降解对DOM组分的变化并 未纳入考虑.这是因为在水体富营养化严重的区域, 藻华堆积会导致水体透明度显著下降[75],但真实条 件下光降解与生物降解对 DOM 变化相对贡献仍应予 以关注.此外,发生富营养化的湖口区域一般还会接 收陆源河流输入,因而此区域水体 DOM 主要来自于 多种来源 DOM 的混合. 而最新研究发现,不同来源 DOM 不同比例的混合会显著影响 DOM 的生物活性 (包括生物降解能力、光降解能力、自由基产率和氧 化还原能力等),且呈现明显的非线性变化[76],这说 明真实场景中富营养化湖泊中水体DOM的变化和环 境效应可能更为复杂.如何从室内模拟结果拓展到 野外原位真实条件下 DOM 的动态变化,还需要在后 续研究中进一步关注.

3 结论

(1)随着藻华腐解的进行,水体 DOC 浓度呈现出 指数衰减的趋势且降解速率先快后慢.水体 DOC 和 *a*254</sub>结果指示藻华腐解显著提高水体 DOM 总量,而 G 模型发现藻华腐解显著提高了水体 DOM 的生物有效 性,其中水体 DOM 易降解和半易降解组分占比从 38%提高到 65%.

(2)藻华腐解过程中水体 S₂₇₅₋₂₉₅逐渐降低,水体 DOM小、中和大分子组分浓度均呈下降趋势,但水体 DOM的相对分子质量逐渐增大.

(3)藻源 DOM 输入增大了水体 DOM 各荧光组分的强度,并随藻华腐解的进行而逐渐降低.在分子水平上,藻华腐解过程中不饱和烃和脂肪族化合物(脂质、蛋白质和碳水化合物)优先被微生物利用,并生成木质素、缩合烃和具有高 O/C 值的单宁酸等惰性分子.

(4)藻源 DOM 的输入显著提高了水体细菌的丰度并改变了细菌群落,使细菌优势菌门为变形菌门、 拟杆菌门和厚壁菌门.不同藻华腐解阶段细菌群落 有明显差异,优势种从前期的变形菌门变为拟杆菌 门.此外,富营养湖泊藻华腐解也会显著增加温室气 体 CO₂和 CH₄的排放,且湖水 DOM 光学指标 *a*₂₅₄与 CO₂ 和 CH₄产量均有良好的正相关关系.

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