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# 水华对沉积物中氮的转化和去除的影响

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**摘要:** 湖泊富营养化和水华暴发已经成为突出的环境问题之一, 了解水华对湖泊氮素的转化和去除的影响, 对于削减湖泊氮负荷至关重要. 本文研究了水华对沉积物中氮转化和氮转化相关功能基因的影响, 并采用结构方程模型分析了水华影响湖泊中氮转化和去除的途径. 结果表明, 与厌氧氨氧化相比, 反硝化作用是太湖沉积物氮削减的主要途径, 对沉积物中总溶解性氮去除率的解释度为 42.3%. 水华可以直接造成沉积物中 TDN 和 TOC 量的增加, 提高厌氧氨氧化菌、*nirS* 和 *nirK* 的基因丰度, 并且间接提高沉积物中氨氮和硝酸盐的浓度, 通过增强厌氧氨氧化和反硝化过程加速沉积物中氮的去除.

**关键词:** 水华; 氮去除; 反硝化作用; 厌氧氨氧化; 结构方程模型

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## Water Bloom Modified Sediment Nitrogen Transformation and Removal

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**Abstract:** Water bloom is a notorious and annual reoccurring problem in eutrophic lakes. Understanding the influence of water bloom on lacustrine nitrogen transformation and removal is crucial for predicting ecosystem functions and taking strategies to reduce in-lake nitrogen budgets. In this study, we investigated the impact of water bloom on the levels and transformation of nitrogen in sediments as well as the pathway to influence the nitrogen removal process. The results of structural equation model analysis showed that water bloom can directly elevate the sediment budget of total dissolved nitrogen (TDN) and total organic carbon (TOC), and the gene abundance of anaerobic ammonium oxidation (ANAMMOX), *nirS*, and *nirK* and can indirectly enhance sediment concentration of ammonia and nitrate as well as nitrogen removal. Moreover, compared with coupled nitrification-ANAMMOX in Lake Taihu sediment, denitrification was the main path of nitrogen removal, with 42.3% explanation of the total nitrogen removal in the sediments. Water bloom can accelerate nitrogen removal in sediment through enhancement of ANAMMOX and denitrification process.

**Key words:** water bloom; nitrogen removal; denitrification; ANAMMOX; structural equation models

湖泊富营养化问题在全球范围内日益严重, 已经引发了水华暴发和水环境质量恶化等一系列问题. 氮是控制初级生产力的限制性营养物质, 也是造成富营养化的主要原因之一. 已有研究表明, 氮在沉积物中的转化包括氨化, 硝化, 厌氧氨氧化, 反硝化和异化 $\text{NO}_3^-$ -N还原(DNRA)等过程, 这些过程影响了水生生态系统中氮的转化和去除<sup>[1,2]</sup>. 其中沉积物中的反硝化作用可去除 82% 左右的硝态氮<sup>[3]</sup>. 水华的暴发使沉积物中氮的转化过程更为复杂, 并影响着水体中氮的去除<sup>[4]</sup>. 随着水华的衰亡, 水华残体沉降到沉积物中可以改变水体与沉积物中营养物质的比例, 同时藻类在降解过程中会消耗大量的溶解氧, 为反硝化过程提供缺氧条件, 从而影响氮转化过程<sup>[5,6]</sup>. 但目前关于水华对湖泊中氮转化和去除影响的认识仍然不足, 这限制了人们对湖泊中氮转化过程的理解以及对湖泊氮含量长期变化及水华暴发的预测能力.

太湖中存在着以水华和水生植物为主的不同的生态类型, 具有高度的异质性, 是研究水华对氮转化和去除影响的良好天然实验场. 本研究通过对水华频发和大型水生植物为主的两个湖区的沉积物样

品的分析, 分析水华对沉积物中氮含量的影响, 探讨水华对氮转化和去除的影响. 为分析水华对沉积物中氮转化的直接和间接影响途径, 采用结构方程模型对水华影响沉积物氮转化和去除的关键因素进行了分析, 以期了解水华对沉积物中氮转化的影响, 并为合理选择氮削减策略提供依据.

### 1 材料与方法

#### 1.1 采样点和样本采集

本研究采样点分别位于西太湖的 N1 点 (31°18'14.0"N, 119°58'17.0"E) 和贡湖湾的 N3 点 (31°27'52.5"N, 120°10'36.9"E). 自 2011 年 5 ~ 12 月, 每月采集沉积物样品, 所有样品在 4℃ 条件下立即运送到实验室进行分析.

#### 1.2 沉积物和水样的化学分析

100 g 沉积物 4 000 r·min<sup>-1</sup> 离心 30 min, 所得上清为孔隙水, 过 0.22 μm 滤膜后, 分析孔隙水中总

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溶解氮 (TDN)、铵态氮 ( $\text{NH}_4^+\text{-N}$ )、硝态氮 ( $\text{NO}_3^-\text{-N}$ ) 和亚硝态氮 ( $\text{NO}_2^-\text{-N}$ )<sup>[7]</sup>. 使用 TOC 分析仪 (Elementar, 德国) 测定总有机碳 (TOC) 含量<sup>[8]</sup>.

### 1.3 DNA 提取和氮转化相关功能基因丰度分析

每个样品取 0.5 g 沉积物用 MoBio PowerSoil™ DNA Isolation Kit (San Diego, CA) 提取沉淀物中的总 DNA. 总 DNA 经电泳和 NanoDrop ND 2000 (NanoDrop Technologies, 美国) 检测后, 通过定量 PCR 进行细菌 16S rRNA (338F/518R)、厌氧氨氧化 16S rRNA (AMX809F/1066R<sup>[9]</sup>)、氨氧化细菌 (*amoA1F/amoA2R*-TC)、氨氧化古菌 (Arch-*amoA*F/Arch-*amoA*R)、反硝化菌 (*nirS*, *cd3af/r3cd*)、反硝化菌 (*nirK*, *nirK-1F/nirK-5R*) 基因丰度的测定<sup>[4]</sup>.

### 1.4 统计分析

不同采样位点和时间点之间的差异采用单因素方差分析 (ANOVA) 和 HSD 事后检验分析. 氮循环基因的丰度与环境变量之间的关系通过 SPSS 进行 Spearman 相关分析. 水华对沉积物中氮转化和去除之间的直接和间接影响通过结构方程模型 (SEM)

分析<sup>[10]</sup>.

## 2 结果与讨论

### 2.1 沉积物孔隙水中不同形态氮含量的变化

两个采样点沉积物中 TDN 和  $\text{NH}_4^+\text{-N}$  的浓度均在夏季达到最高, 这与夏季水华的暴发相一致, 表明水华的暴发可能引发了大量的氮输入至沉积物中. 与 N3 点相比, N1 点的沉积物中总溶解性氮 (TDN)、 $\text{NH}_4^+\text{-N}$  和  $\text{NO}_3^-\text{-N}$  浓度更高, 而 C/N 相对较低, 表明与 N3 相比, N1 点的污染更为严重<sup>[11]</sup>. N1 点沉积物中氮浓度较高, 可能是由藻华碎屑沉积造成的. 藻华碎屑蛋白质含量高、C/N 低, 很容易被沉积物中的微生物分解<sup>[12]</sup>, 可能是沉积物中 TDN 和  $\text{NH}_4^+\text{-N}$  在夏季浓度较高的原因之一. 同时, 沉积物中的低氧环境可能减缓藻华碎屑的分解速率, 使得 N1 点沉积物中有机物和氮的含量更高<sup>[13]</sup>. 与 TDN 和  $\text{NH}_4^+\text{-N}$  相比,  $\text{NO}_3^-\text{-N}$  的浓度呈波动性变化. 两个地点的  $\text{NO}_2^-\text{-N}$  浓度在 5~7 月较高, 而在上覆水中的  $\text{NO}_2^-\text{-N}$  浓度较低 (图 1), 这表明沉积物中的  $\text{NO}_2^-\text{-N}$  虽有可能来自上覆水的扩散, 但也

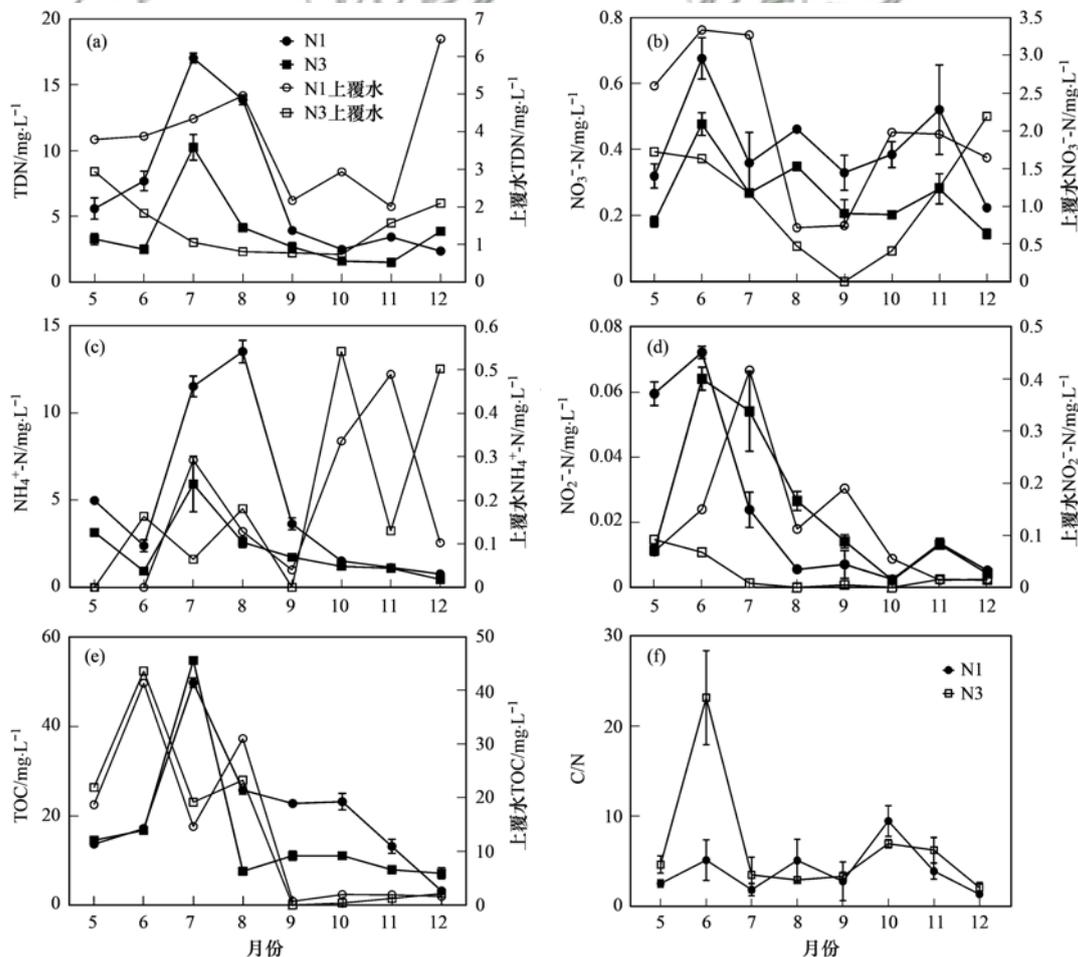


图 1 沉积物孔隙水与上覆水中 TDN、 $\text{NO}_3^-\text{-N}$ 、 $\text{NH}_4^+\text{-N}$ 、 $\text{NO}_2^-\text{-N}$ 、TOC 的浓度和 C/N 的变化

Fig. 1 Monthly variation in TDN,  $\text{NO}_3^-\text{-N}$ ,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , and TOC concentration and C/N in the pore water of sediments and overlying water at the two sampling sites

可能是由于沉积物中的硝化作用大于反硝化作用<sup>[14]</sup>所造成的。

## 2.2 沉积物中不同形态氮的转化率

图2结果表明,不同形态氮转化率在N1与N3点的变化趋势大致相同.7月和8月的N1点 $\text{NH}_4^+$ -N分别增加了396.20%和189.90%,表明有机氮的矿化在氮转化过程中占主导地位.有机氮矿化作用中释放的 $\text{NH}_4^+$ -N易于结合到大生物和微生物的生物质中<sup>[15]</sup>,在氮的再循环中起着核心作用,

释放的 $\text{NH}_4^+$ -N可能会影响包括沉积物硝化和反硝化的整个氮转化过程.TDN在9月去除率最高为71%,此时C/N为1.24[图2(a)和图1(f)],表明沉积物中在这种情况下,C/N比对反硝化没有显著影响.7~9月, $\text{NH}_4^+$ -N的转化率呈下降趋势, $\text{NO}_3^-$ -N呈上升趋势,与N1和N3点的潜在硝化速率(PNR)在夏季较高相符(图3).在整个研究过程中 $\text{NO}_2^-$ -N积累量较低,这表明沉积物中的反硝化活性能够去除两个地点的 $\text{NO}_3^-$ -N和 $\text{NO}_2^-$ -N.

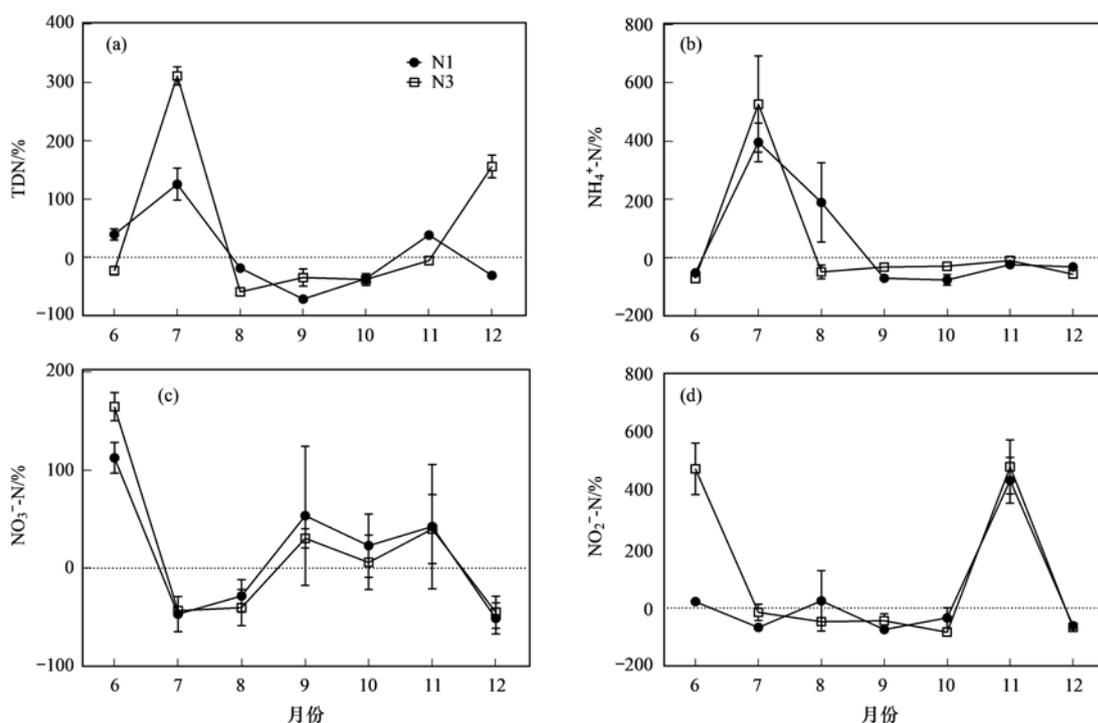


图2 沉积物中TDN,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N和 $\text{NO}_2^-$ -N的氮转化率

Fig. 2 Nitrogen transformation ratio of TDN,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, and  $\text{NO}_2^-$ -N in sediments at the two sampling sites

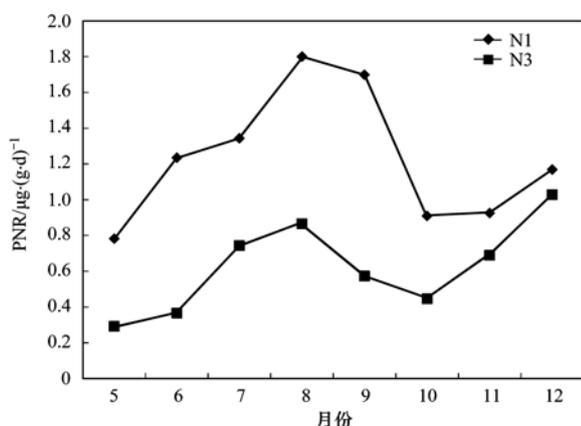


图3 两个采样点沉积物PNR的变化

Fig. 3 Monthly variation of PNR in sediments at the two sampling sites

## 2.3 沉积物中硝化和反硝化功能基因的变化

图4(a)结果显示,在采样期间氨氧化细菌 $\text{amoA}$ (AOB)丰度相对稳定,氨氧化古菌 $\text{amoA}$

(AOA)丰度存在显著的季节性变化( $P < 0.05$ ),并在冬季最高.这可能是因为冬季太湖沉积物中氨氮含量相对较低,AOA适合在氨氮含量较低时生长<sup>[16]</sup>.AOA和AOB丰度在N1点整体上均高于N3( $P < 0.05$ ).5~8月,N1和N3的 $\text{amoA}$ 基因分别增加7.88倍和5.89倍,氨氧化古菌占据优势[图4(a)].同时,在N1和N3点中均检测到了厌氧氨氧化菌,但两个位点之间的丰度不存在显著性差异,且其丰度均低于氨氧化古菌.

图4(b)显示 $\text{nirK}$ 和 $\text{nirS}$ 基因的丰度在5~8月增加,这可能导致沉积物反硝化能力的增加.9月 $\text{nirK}$ 和 $\text{nirS}$ 的基因丰度降低.与前人的研究结果类似, $\text{nirS}$ 基因丰度在N1和N3点均高于 $\text{nirK}$ 基因( $P < 0.01$ )<sup>[17,18]</sup>,这也表明 $\text{nirS}$ 型的反硝化菌是太湖沉积物中的主要生态类型.

## 2.4 水华对氮转化和去除的影响

结构方程模型分析结果表明水华对沉积物氮循

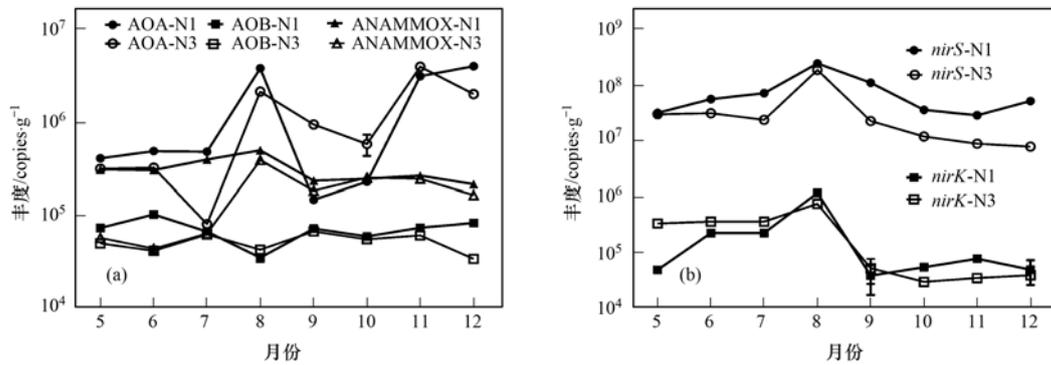


图4 *amoA*、厌氧氨氧化 16S rRNA 和 *nirK/nirS* 基因丰度的变化

Fig. 4 Abundance of the *amoA* gene from ammonia-oxidizing archaea and ammonia-oxidizing bacteria and that of the 16S rRNA gene of ANAMMOX and *nirK* gene, and *nirS* gene at the two sampling sites

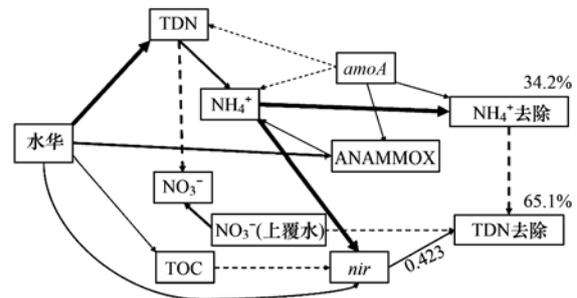
环的直接和间接影响对总溶解性氮去除的解释度为 65.1% (图 5)。水华对沉积物中氮去除没有直接影响, 但水华对沉积物中的 TOC ( $\lambda = 0.34$ ;  $P < 0.001$ )、TDN ( $\lambda = 0.73$ ;  $P < 0.001$ )、厌氧氨氧化菌 16S rDNA 丰度 ( $\lambda = 0.53$ ;  $P < 0.001$ ) 和 *nirS/nirK* 基因丰度 ( $\lambda = 0.49$ ;  $P < 0.001$ ) 呈显著的正相关。

沉积物中 TDN 的含量对氮的矿化作用表现出强烈的正效应 ( $\lambda = 0.48$ ;  $P < 0.05$ )。厌氧氨氧化菌 ( $\lambda = 0.31$ ;  $P < 0.001$ ) 和 *amoA* ( $\lambda = -0.28$ ;  $P < 0.001$ ) 均对氨氮浓度有很大影响, 氨氮浓度则对 *nirS/nirK* 基因丰度有很大影响 ( $\lambda = 0.75$ ;  $P < 0.001$ )。 *nirS/nirK* 基因丰度,  $\text{NH}_4^+$ -N 去除率和上覆水中  $\text{NO}_3^-$ -N 浓度对脱氮效率有直接影响, 分别为 0.423、-0.674 和 -0.447 (图 5)。水华可以直接提高 TDN 和 TOC 的浓度, 厌氧氨氧化菌和 *nirS/nirK* 的丰度, 并间接提高沉积物氨氮和硝态氮浓度, 从而影响氮的去除。首先, 水华生物量可以通过直接促进细胞的存活和维持, 同时减少细胞生长的滞后时间来加速分解过程<sup>[19, 20]</sup>。其次, 在水华暴发和沉积期间, 与有机物质降解有关的功能基因, 微生物酶活性和微生物都受到刺激<sup>[21, 22]</sup>, 导致有机质矿化能力增强, 氮利用率增高<sup>[23]</sup>。

*amoA* 基因丰度对厌氧氨氧化菌的丰度具有显著正效应 ( $\lambda = 0.37$ ;  $P < 0.001$ ), 表明  $\text{NH}_4^+$ -N 氧化所产生的  $\text{NO}_2^-$ -N 可能是激发沉积物中厌氧氨氧化潜能的重要因素 (图 5), 这表明了沉积物中硝化与厌氧氨氧化作用耦合脱氮的可能性。这两个途径的耦合已经在污水处理厂<sup>[24]</sup>、人工湿地<sup>[25]</sup> 以及海洋<sup>[26]</sup> 和淡水生态系统<sup>[27]</sup> 中的脱氮过程中都发挥着重要作用, 但需要更多地实证研究来证实太湖沉积物中这种耦合作用对沉积物中氮的转化和去除的作用。

此外, 结构方程模型分析显示 *nirS/nirK* 丰度是唯一与 TDN 去除正相关的因素, *nirK/nirS* 丰度

可以解释沉积物中总溶解性氮去除的 42.3%, 表明反硝化是太湖沉积物脱氮的主要过程 (图 5)。反硝化和厌氧氨氧化都消耗了通过 *amoA* 硝化或通过 *narG/napA* 反硝化产生的  $\text{NO}_2^-$ -N<sup>[28]</sup>。因此, 厌氧氨氧化菌和反硝化细菌之间存在资源利用竞争。然而, 在本研究中, *nirS/nirK* 和厌氧氨氧化菌基因之间并没有显著的负相关关系, 表明在太湖沉积物中厌氧氨氧化和反硝化之间的竞争是可以忽略的。这可能是由于 *nirK/nirS* 的丰度 ( $7.91 \times 10^6 \sim 2.43 \times 10^8$  copies·g<sup>-1</sup>) 远高于厌氧氨氧化菌的基因丰度 ( $4.44 \times 10^4 \sim 4.99 \times 10^5$  copies·g<sup>-1</sup>) 造成的 (图 4)。水华与厌氧氨氧化基因丰度 ( $\lambda = 0.53$ ,  $P < 0.001$ ) 和 *nirK/nirS* 基因丰度 ( $\lambda = 0.49$ ,  $P < 0.001$ ) 的显著正相关表明, 水华可通过增强厌氧氨氧化和反硝化过程加速沉积物中氮的去除。



图中仅显示了显著的效果; 实线箭头表示正效应, 虚线箭头表示负效应; 箭头的宽度表示因果关系的强度; 箭头上的数字是标准化路径系数; 内生变量的百分比 ( $R^2$ ) 表示模型解释的方差

图5 水华对沉积物中氮转化和去除影响的结构方程模型分析

Fig. 5 Structural equation model for the effects of water bloom on nitrogen transformation and removal in sediment

### 3 结论

N1 和 N3 点的沉积物中 TDN 和  $\text{NH}_4^+$ -N 的浓度在夏季达到最高, 并且 N1 点沉积物中氮浓度高于 N3 点, 表明水华的暴发与沉积造成沉积物中氮的输入。结构方程分析结果表明水华可直接

对沉积物中的 TDN 和 TOC 浓度产生正向影响, 从而间接影响沉积物中  $\text{NH}_4^+$ -N 浓度. 在太湖沉积物中硝化作用通过与厌氧氨氧化耦合促进了氮去除过程. 然而, 与厌氧氨氧化相比, 通过 *nirK/nirS* 进行的反硝化作用是太湖沉积物中氮去除的主要途径.

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