



Microbiota for Nitrogen Removal in Wastewater Treatments and Marine Environments: Advocating Communication and Interactive Research

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INTRODUCTION

Nitrogen (N) is essential for life as all organisms need N for growth. Due to the intensive application of the Haber-Bosch process and cultivation of N-fixation crops on a global scale, the reactive N (mainly as nitrate, nitrite and ammonium) is increasingly accumulated in terrestrial systems worldwide (Zhang et al., 2020). To eliminate the environmental stress and negative ecological feedbacks caused by redundant N, N removal reactions are deemed to be a key factor in its cycling and receive great attention from the scientific community, managers and stakeholders. To avoid further human-derived N enrichment of ecosystems, N removal in wastewater treatment is also pivotal. Currently, different technologies based on a wide range of N processing microbiota, mainly derived from nitrification, anaerobic/aerobic denitrification, anaerobic ammonium oxidation (Anammox) have been used for reducing N content in sewage (Cao et al., 2021), such as single reactor for high activity ammonia removal over nitrite (SHARON), completely autotrophic nitrogen removal over nitrite (CANON) and oxygen-limited autotrophic nitrification and denitrification (OLAND). Nevertheless, N accumulation is still frequently observed in urban and rural areas and a significant quantity of reactive N produced from anthropogenic activities enters into marine systems *via* surface loadings, submarine groundwater discharge and atmospheric deposition (Jiang et al., 2021a). In marine environments, N excess is rapidly removed *via* biogeochemical reactions, such as biological assimilation in the euphotic zone, aerobic/anaerobic denitrification and dissolved oxygen (DO) dependent Anammox in both water parcels and sediments (Jiang et al., 2021b). In fact, the microbial strains for N removal used in wastewater treatments could be frequently observed in marine environments, e.g., *Thiosphaera pantotropha* in denitrification or *Candidatus Brocadia sinica* in Anammox. As research subjects with great similarity, valuable N reaction information from researchers in marine environments and wastewater treatments is barely shared and microbiota involved in N removal process is also limitedly exchanged between subjects. Active communication between academics likely enhances the understanding on a series of key issues in both subjects, such as the efficiency of N removal in brackish wastewater, low temperature conditions and carbon-limited scenarios, as well as *in-situ* N removal along coastal belts. Here, we demonstrate the similarity of N removal in wastewater treatments and marine environments and highlight causal linkages between each other. Furthermore, we advocate the necessity and benefits derived from communication and interactions among scientists in both subjects in the future.

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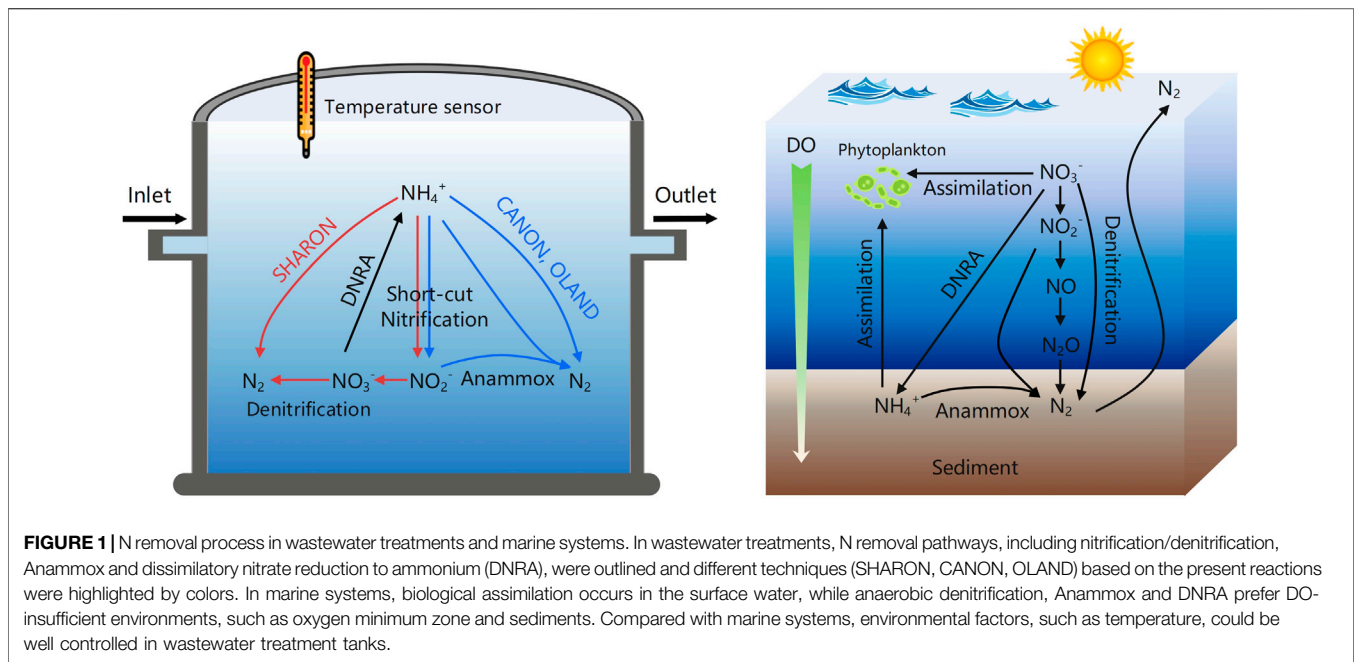
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N REMOVAL IN WASTEWATER TREATMENTS AND MARINE ENVIRONMENTS

The classical technology for N removal in wastewater treatments is based on anaerobic denitrification (**Figure 1**). The microbiota involved in anaerobic denitrification include several strains, such as *Pseudomonas* and *Bacillus* strains and function enzymes are usually detected as NAS, NAR, NIR, etc. (Kuyppers et al., 2018). Aerobic denitrification and Anammox are also key pathways in wastewater treatments and direct ammonia oxidation (Dirammox) is a newly recovered process for ammonia removal (Mai et al., 2021; Wu, M. R. et al., 2021). The microbiota of aerobic denitrification used in wastewater treatments are frequently identified as *Alcaligenes faecalis*, *Zoogloea* sp. and *Shinella zoogloeoides* (Ji et al., 2015), while Anammox bacteria, *Ca. Brocadia sinica* strains, are usually observed in wastewater treatments (Speth et al., 2016). In terms of other processes implemented in wastewater treatment, e.g., OLAND, SHARON and CANON, the participated microorganisms are frequently identified as strains found in nitrification, denitrification and Anammox, such as *Sphingomonas* sp., *Ca. Kuenenia* and *Nitrosomonas* sp. (Schmidt et al., 2003).

N removal, in terms of reaction pathways and microbiota, between wastewater treatments and marine environments shows high similarity (**Figure 1**). Denitrification, especially anaerobic denitrification, is also identified as the dominant pathway for the permanent N removal in oceans. Compared with the microbiota found in wastewater treatment (mainly to be several genera, e.g., *Pseudomonas*, *Terrimonas*, and *Paracoccus*), denitrifiers in the marine environment are highly diverse. More than 50 strains for anaerobic denitrification, belonging to 10 classes, have been

identified in different oceanic environments (Mills et al., 2008). Several strains with active N metabolism activities, isolated from deep oceans and trenches, are barely observed in artificial treatments. In addition, the electron providers (pelagic carbon and sulfide) are significantly different from those found in wastewater (Pajares and Ramos, 2019).

The significance of Anammox in marine environments was overlooked in the 20th century, while the importance on N removal (accounting for 70% N removal in several sites) is gradually emphasized (Devol, 2015). Currently, the microbiota that participates in Anammox mainly include six genera in the biosphere. *Ca. Scalindua* and *Ca. Brocadia sinica* are frequently obtained in marine environments (Amano et al., 2007), while the remaining genera are mainly found in wastewater treatments (**Table 1**). For aerobic nitrification (key steps in OLAND), a wide range of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), e.g., *Nitrosopumilus*, *Proteobacteria* and *Crenarchaeota* have been identified in aerobic sediments along coastal belts and the ocean surface (Francis et al., 2005; Jiang et al., 2020). Several of these nitrifiers, mainly as AOB, appear in wastewater treatments for the oxidation of ammonium, while the appearance of AOA is limited in sewage environments.

NECESSITY OF COMMUNICATION AND INTERACTIONS

Apart from cross-inoculation among different treatment containers/pools, N removal microbiota accumulated in wastewater treatments are frequently isolated from terrestrial systems or coastal belts (e.g., aquaculture ponds) due to the convenience in sample collection and storage. Despite the

TABLE 1 | A comparison of reaction pathways, techniques, involved microbiota, environmental settings and research challenges between wastewater treatments and marine environments.

	Marine environments	Wastewater treatments
Pathways	Denitrification, nitrification, DNRA, Anammox	Denitrification, nitrification, Anammox, Dirammox
Techniques	None	SHARON, CANON, OLAND, etc.
Microbiota	Abundant denitrifiers and nitrifiers, including AOA, AOB; Anammox, including <i>Ca. Scalindua</i> and <i>Ca. Brocadia Sinica</i> . Facultative DNRA strains are available	Several denitrifiers and nitrifiers, mainly AOB; Anammox, including <i>Ca. Brocadia Sinica</i> , <i>Ca. Kuenenia</i> , <i>Ca. Anammoxoglobus</i> , <i>Ca. Jettenia</i> , <i>Ca. Anammoximicrobium moscowii</i> . DNRA strains are overlooked. <i>Alcaligenes ammonioxydans</i> for Dirammox is available
Environment	Variable: substrate limitation, extreme temperatures and ionic strength, both aerobic and anaerobic conditions, sunshine exposure	Relatively stable: available diverse substrates, controlled temperature and DO, fresh to brackish
Challenges	Dynamic reactions, <i>in-situ</i> remediation	Functional nitrogen pollution removal strains, extreme environment impacts

success for N elimination in many cases, N removal processes in wastewater treatment face several challenges (Table 1), especially low efficiencies when dealing with brackish wastewater, unsteady carbon supply, redox potential variability and temperature decreases triggered by cold weather (Kiani et al., 2020; Gao and Xiang, 2021). Pollutants in sewage, such as heavy metals and antibiotics, also commonly depress the efficiency of N removal in those treatments (Buelow et al., 2020). All these add uncertainties to the N processing capability in artificial treatments and highlight the necessity of further strain isolation, especially for those species adapted to “unfavorable” environmental conditions.

Interestingly, the above-mentioned environmental pressures that decrease N removal efficiency in sewage treatments are typical conditions in marine environments. Accordingly, the N removal microbiota in marine environments is commonly suitable for these unfavorable conditions. Apart from saline water adaptability, microbial genera in nitrification and denitrification, such as *Nitrosopumilus*, *Nitrospira* and *Nitrosomonas* show high N reaction capability in carbon-variable sediments (Jiang et al., 2021c). Moreover, the activity of denitrification and Anammox conductors (e.g., *Acidithiobacillia*, *Sedimenticola thiotaurini* and *Ca. Brocadia Sinica*) showed to be constant during both low and high salinity in a tidal subterranean estuary (terrestrial groundwater extrusion vs recycled seawater injection; Wu, J. et al. 2021b). In addition, the microbiota that participate in denitrification, nitrification and Anammox are active at high-water pressure (e.g., 200–800 m depth below ocean surface) and the heterotrophic reaction there (e.g., denitrification) could rely on low-carbon supply (from the settlement of phytoplankton; Pajares and Ramos, 2019). The Anammox microbes in the marine environment displayed active functioning in a wide range of temperatures (<20 or >40°C), with an extreme temperature of approximately 0°C at Greenland coasts (Dalsgaard et al., 2005), while this reaction might be muted at similar temperature conditions in wastewater treatments (Gonzalez-Martinez et al., 2018). Given the importance of the isolation of effective strains and their subsequent inoculation for the optimization of N removal in wastewater treatments (Shen et al., 2017), microbiota from marine environments, especially from deep oceans and sea trenches, could be potential solutions. Even in shallow marine environments, such as high-latitude

coasts, the marine microbiota may also be a “treasury vault” for wastewater research. For instance, the wide range of AOA with high activity in seawater and sediments have the potential to improve nitrification steps in wastewater treatments. In addition, apart from these two relatively well-gauged reactions, dissimilatory nitrate reduction to ammonium (DNRA), an internal cycling path for dissolved N (Figure 1), is frequently observed in marine sediments, including permeable sediments at oxic conditions (Ibáñez and Rocha, 2017). Though this reaction could not directly reduce the levels of dissolved N, it rapidly transfers NO_3^- to NH_4^+ and likely stimulates the activity of anaerobic denitrification and Anammox (Ibáñez and Rocha, 2017). Accordingly, it might be potentially interesting in combination with other pathways for treating sewage tanks receiving NO_3^- enriched wastewater. Currently, the DNRA microbiota is frequently abundant (e.g., *Nitrospira*) in many sewage tanks, while its contribution to N removal is supposed to be minor due to the unsuitable factors (Wang et al., 2020). The identification of controlling factors for DNRA in marine research would benefit the decoding of low-efficiency N removal in wastewater treatment microbiota, which might subsequently improve N removal strategies and techniques.

The research on the microbial community structure and performance in wastewater treatments also benefits the N cycling research in marine environments. High frequency monitoring of activated sludge during N transformation processes in wastewater treatment plants or bioreactors could be excellent in determining macroecological theories (Wu et al., 2019). In particular, the reaction dynamics of the dominant strains would provide crucial information to understand their functioning in marine environments. In addition, microbiota in sewage treatment pools and marine environments include a wide range of facultative species. The microbial community composition including N removal participants would be dynamic and dependent on environment settings. The microbial community variability found in sewage environments, triggered by changes in substrates, temperature, DO or pH, could help to understand microbial community composition in the marine environment, particularly in face of the projected climate and environmental changes.

Apart from these potential feedbacks, information on the functioning of these microbiota would be highly beneficial to marine researchers. For instance, *in-situ* remediation is one of the

main challenges for marine researchers to deal with N pollution. Specially, researchers have developed permeable reactive barrier systems with suitable microbiota in coastal zones to remove nitrate in fertilizer-contaminated groundwater (Tian et al., 2016). They introduced artificial balls or nanospheres with microbial biofilms on the surface into coastal environments to reduce excessive N, likely from aquaculture ponds along coastal belts. The core of these *in-situ* mediation technologies is dependent on high-efficiency N removal microbiota. Accordingly, the information obtained on the performance of these microbes from wastewater treatments would be a key reference for the selection of suitable strains to deal with significant N removal in natural environments.

As aforementioned, despite the importance and significant benefits from knowledge sharing, interactions between academic activities in wastewater and marine environments are still limited. Analysis of publications from the Web of Science database showed that the co-existence of “wastewater,” “marine” and “coastal” keywords is always limited though the quantity of publications in the microbial subject markedly increased during the past 20 years. To this end, we advocate the necessity of communication and interactions of researchers from these two distant areas. In addition, the encouragement of microbiologists covering these two research topics would be also highly beneficial.

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CONCLUSION

N removal processes, including metabolic pathways, microbial community and function genes, in both wastewater treatments and marine environments are summarized and compared. Between these two different research fields, a great similarity in metabolic pathways and associated microbiota has been witnessed. In contrast, the academic communication and interactive research between these two research subjects is limited, likely due to the difference in research questions and focuses. However, the advantage and research achievements from each subject are likely to address the challenges from the other subject, indicating a significant benefit and necessity of academic communication. Accordingly, more active knowledge sharing between these two subjects needs to be significantly enhanced. Moreover, some novel strategies, such as synthetic biology and synthetic microbiota, will further enhance the understanding of N removal processes in marine and wastewater treatment microbiota, which might benefit for the N cycling in natural and artificial environments.

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All authors wrote the paper together.

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