

Review

# Impacts of Microplastics on Anammox Systems: A Comprehensive Review of Mechanisms and Influences

Baohong Han <sup>1</sup>, Fangxing Zha <sup>1</sup>, Jianying Gong <sup>1</sup>, Huixian Jiang <sup>2,\*</sup>

<sup>1</sup> Department of Municipal and Environmental Engineering, Hebei University of Architecture, Zhangjiakou 075000, China

<sup>2</sup> Shijiazhuang Customs Technical Center, Zhangjiakou 075000, China

\* Corresponding author: [jianghuixian2004@163.com](mailto:jianghuixian2004@163.com)

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**Abstract:** Anaerobic ammonia oxidation (Anammox) technology has emerged as a highly promising biological nitrogen removal approach, exhibiting remarkable technical and economic advantages in treating wastewater with high ammonia nitrogen content and a low carbon-to-nitrogen ratio. However, with the escalating global plastic pollution, microplastics (MPs) have become ubiquitous in wastewater treatment systems. This review systematically collates the research status regarding the sources and classification of microplastics in wastewater treatment plants, and the impacts of MPs on anammox systems. Based on their degradation characteristics, MPs are categorized into two major types: degradable microplastics and non-degradable microplastics. The critical factors influencing anammox processes in the presence of microplastics are comprehensively summarized and analyzed. The impacts exerted by MPs on anammox systems are closely correlated with key factors such as polymer type, particle size distribution, concentration-dependent effects, and exposure duration-dependent responses. Low concentrations of microplastics can act as biofilm carriers or provide carbon sources, thereby facilitating microbial adhesion and growth, and enhancing the nitrogen removal efficiency of the system. In contrast, high concentrations of microplastics usually inhibit anammox activity through pathways including physical clogging, toxic effects, and oxidative stress, which in turn leads to the decline of nitrogen removal performance, damage to sludge structure and alterations of microbial community structure in the system. The underlying mechanisms involve three aspects: shifts in microbial community structure, regulation of functional gene expression, and disturbance of metabolic processes. Finally, this review proposes potential future research directions. This study aims to provide comprehensive theoretical support for the stable operation of anammox systems and environmental risk management against the backdrop of microplastic pollution.

**Keywords:** anammox; microplastics; influencing factors; mechanisms

## 1. Introduction

With the continuous growth and overuse of global plastic production, plastic pollution has evolved into a severe environmental crisis. Microplastics (MPs) generally refer to plastic particles with a particle size of less than 5 mm [1]. They have diverse sources and stable properties, enabling long-term migration and accumulation in various media such as aquatic environments, soil, and atmosphere [2–4]. As centralized treatment facilities for urban sewage, wastewater treatment plants (WWTPs) are not only important sinks for MPs but also key sources of their diffusion into the natural environment. Studies have shown that the removal rate of MPs during wastewater treatment is approximately 90%–98%, yet a considerable amount of MPs still enter water bodies or soil environments along with effluent and sludge [5].

As an innovative biological nitrogen removal technology, anaerobic ammonia oxidation (Anammox) directly converts ammonium nitrogen and nitrite nitrogen into nitrogen gas through the metabolic activity of Anammox bacteria (AnAOB) [6]. Compared with traditional nitrification-denitrification technology, Anammox technology eliminates the need for additional carbon source supplementation, reduces energy consumption by more than 60%, and decreases sludge production by 90%. It exhibits significant technical and economic advantages in treating wastewater with high ammonia nitrogen and low C/N ratios [7].

The impacts of MPs on biological wastewater treatment systems have become a research hotspot in the field of environmental engineering. Mounting evidence indicates that MPs can exert substantial effects on microbial activity and treatment performance of activated sludge systems and biological nitrogen removal processes, primarily through physical adsorption, chemical interactions, and biological interactions [8,9]. However, research on the impacts of MPs on anammox systems is relatively limited, and the conclusions of existing studies are controversial. Some studies suggest that low concentrations of MPs have no significant effect on the nitrogen removal efficiency of anammox systems, and may even serve as biofilm carriers to promote the attachment and growth of AnAOB, while high concentrations of MPs may inhibit AnAOB activity through toxic effects, thereby leading to reduced nitrogen removal performance [10,11]. Moreover, MPs are recognized as potential components in anammox biofilm carrier systems, which are still largely unexplored.

This review provides a comprehensive analysis of the sources, classification, and recent research progress related to MPs in anammox systems. It meticulously elaborates on the key factors influencing the impacts of MPs, including material type, particle size, concentration, and exposure duration, and their underlying mechanisms. Additionally, it identifies and proposes priority areas for future research. The overarching aim is to provide valuable insights for ensuring the stable operation of anammox systems and formulating targeted strategies to mitigate environmental risks associated with microplastic contamination.

## **2. Sources, Classification, and Research Progress of Microplastics**

### **2.1. Sources**

#### **2.1.1. Influent-Derived Sources**

The influent of WWTPs serves as the primary source of MPs in Anammox systems. MPs in urban domestic sewage predominantly originate from the laundering of synthetic fiber garments, the use of personal care products, the degradation of plastic packaging materials, and the abrasion of plastic products [12]. Asia is the dominant source of microplastic fiber (MPF) pollution, emitting 3.71 million tons—far exceeding North America (1.18 million tons) and Europe (0.45 million tons). This high MPF discharge into natural waters is attributed to global disparities in wastewater management and the low MP removal capacity of WWTPs in Asia [13]. Industrial wastewater also constitutes a significant source of microplastics. Industries such as food processing, textiles, and plastic manufacturing release substantial amounts of microplastics during production processes, which are directly discharged into wastewater treatment systems [14]. Furthermore, agricultural wastewater and surface

runoff can transport microplastics from soil and aquatic environments into WWTPs, ultimately facilitating their entry into anammox systems [15].

### 2.1.2. Externally Added Sources

To enhance the operational stability and nitrogen removal efficiency of anammox systems, organic-based carriers are artificially added as biofilm carriers in certain research and engineering applications. Zhang et al. investigated the application of polyvinyl alcohol-sodium alginate composite carriers in anammox membrane bioreactor systems, achieving the activation of free-living planktonic AnAOB with a purity exceeding 95% [16]. This innovative cultivation strategy provides a robust technical basis for in-depth investigations into the microbial, physiological, and biochemical characteristics of anammox bacteria. Han et al. evaluated the performance of polypropylene (PP) carriers in anammox biofilm reactors, demonstrating that PP carriers effectively accelerated anammox biofilm formation and improved the total nitrogen removal efficiency by 10.5% [17]. Their findings highlighted that PP carriers not only facilitate the rapid colonization of anammox consortia but also enhance the metabolic activity of functional microorganisms. Peng et al. reported that the incorporation of polyurethane sponge into an upflow anaerobic sludge blanket reactor shortened the start-up period of the anammox process by 28% and reduced the reactivation time by 50% [18]. This enhancement was attributed to the superior biomass retention capability of polyurethane sponge, which enabled the enrichment of anammox bacteria within the reactor system. Wang et al. realized the successful coupling of anammox immobilization with partial denitrification after 94 days of continuous operation; under this integrated system, a nitrite-nitrogen accumulation rate of 63.5% and a nitrate-nitrogen removal rate of 98.4% were achieved [19].

During long-term operation, these organic-based carrier materials are prone to physical fragmentation and chemical degradation under the synergistic effects of hydraulic shear, biological erosion, chemical oxidation, and other environmental stressors, thereby generating microplastic particles [20]. Traditional plastic-based carriers widely used in the anammox process have become one of the key endogenous sources of microplastics in water treatment systems. However, due to their large specific surface area and hydrophobic properties, the generated MPs can be colonized by AnAOB and other functional microbial communities. This colonization gradually leads to the formation of new biofilm carriers that persist in water treatment facilities for extended periods [21]. Therefore, the environmental fate and ecological risks of these MPs warrant further investigation.

## 2.2. Classification

Based on their degradation characteristics, MPs in the biological nitrogen removal system of WWTPs can be categorized into two major types: degradable microplastics (DMPs) and non-degradable microplastics (NDMPs). The classification diagram is shown in **Figure 1**. Significant differences exist in the degradation characteristics, material properties, and application effects of the two types, which directly affect their environmental fates and roles in anammox systems (**Table 1**).

### 2.2.1. Degradable Microplastics

DMPs refer to microplastic particles that can be decomposed into small-molecule

substances (such as organic acids, alcohols, and CO<sub>2</sub>) under specific environmental conditions (e.g., microbial action, temperature changes, and dissolved oxygen fluctuations). According to their degradation mechanisms, DMPs can be classified into biodegradable microplastics and photo/thermally degradable microplastics, among which biodegradable microplastics have attracted relatively high research attention [22].

Common biodegradable microplastics include polylactic acid (PLA), polycaprolactone (PCL), polybutylene succinate (PBS), polyhydroxyalkanoates (PHA), and polybutylene adipate-co-terephthalate (PBAT) [8,23,24]. PLA is polymerized from lactic acid monomers, with raw materials derived from renewable resources such as corn and potatoes. It exhibits excellent biocompatibility and degradability, and can be gradually converted into CO<sub>2</sub> and CH<sub>4</sub> through anaerobic digestion in typical anaerobic biological processes. PCL is polymerized from caprolactone monomers; it has a relatively slow degradation rate but excellent flexibility and biocompatibility, and can be decomposed into adipic acid and ethylene glycol under microbial action [25].

As mainstream biodegradable carriers, PBS and PBAT have demonstrated remarkable application efficacy in anammox and biological nitrogen removal processes: PBS can construct a solid-phase denitrification system, achieving a nitrogen removal efficiency of up to 99.5% under specific operating conditions while enriching denitrifying bacteria to provide suitable substrates for anammox [26]. PBAT particles integrate the functions of a solid carbon source and microbial carrier, featuring stable carbon release and a denitrification rate of 6.23 mg/(L·d), making them suitable for treating low-load nitrogen-containing wastewater. Consequently, such biodegradable materials are widely applied in denitrification processes [27].

### **2.2.2. Non-Degradable Microplastics**

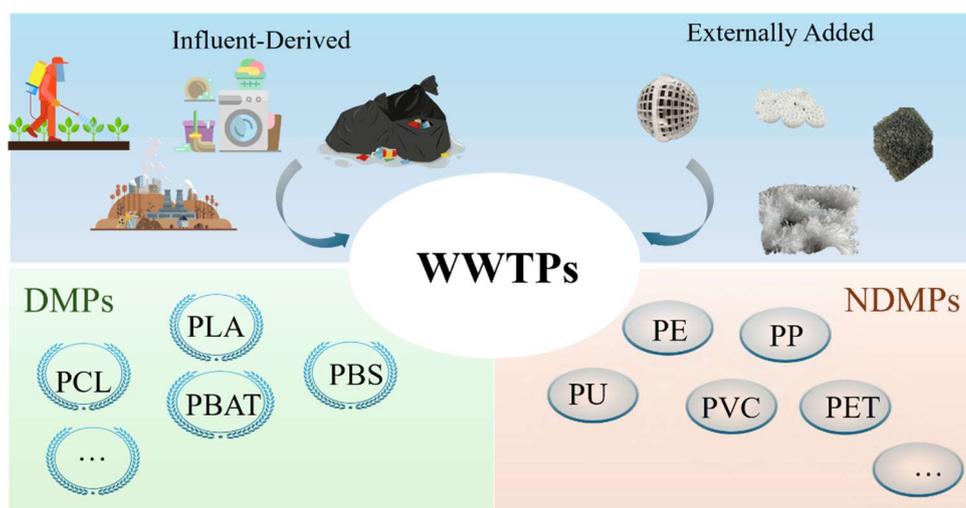
NDMPs refer to microplastic particles that are barely decomposable under natural environmental conditions. Endowed with stable chemical structures, they tend to accumulate in the environment over the long term. Typical examples of NDMPs include polyethylene (PE), PP, polyurethane (PU), polyvinyl chloride (PVC), polystyrene (PS), high-density polyethylene (HDPE), and polyethylene terephthalate (PET) [28–31].

In the anammox process, carriers made of the aforementioned NDMPs are widely applied in nitrogen removal systems such as moving bed biofilm reactors and sequencing batch biofilm reactors. PE carriers can enhance hydrophilicity through surface modification to facilitate the attachment of AnAOB. A micro-anaerobic environment can be formed on their surface, meeting the anaerobic metabolic requirements of AnAOB. In denitrification systems, PE carriers can enrich denitrifying bacteria, promote the contact between substrates and microbial communities through adsorption, and improve total nitrogen removal efficiency [7].

PP is commonly used in the anammox-denitrification combined process and is suitable for fluidized bed reactors. AnAOB and denitrifying bacteria on the carriers form a synergistic effect to simultaneously remove ammonia nitrogen and nitrate nitrogen. In the treatment of low C/N ratio wastewater, PP carriers can enhance biomass retention capacity and reduce the loss of functional microbial communities

[32]. PU is a commonly used immobilization carrier in the anammox process, particularly suitable for packed bed reactors. The porous structure of PU carriers not only meets the anaerobic requirements of AnAOB but also provides living space for certain nitrifying bacteria, enabling the synergistic nitrogen removal via partial shortcut nitrification-anammox. In biological nitrogen removal systems, PU carriers can enrich numerous denitrifying bacteria, retain organic matter through internal pores, and provide a continuous carbon source for denitrification reactions [33].

PET is often used in composite carrier systems for anammox and biological nitrogen removal processes. For example, PET nonwoven fabric, as a biofilm carrier, possesses both filtration and biodegradation functions. In anammox reactors, the high specific surface area of PET fibers is conducive to the attachment of AnAOB, and the network structure formed by fiber interweaving can retain biofilm fragments and increase biomass concentration [34]. As described in Section 2.1.2, these non-degradable carriers can decompose into MPs and persist in wastewater treatment systems, posing potential environmental risks.



**Figure 1.** Sources and classification of MPs in wastewater treatment plants.

## 2.3. Current Research Progress

### 2.3.1. Research Progress on Degradable Microplastics

The effects of DMPs on the anammox system are dualistic. On the one hand, DMPs can serve as biofilm carriers and carbon sources, promoting the growth and metabolism of AnAOB and denitrifying bacteria, and thereby improving the nitrogen removal efficiency of the system [27]. On the other hand, the degradation process of DMPs may generate intermediate products, which exert an inhibitory effect on the activity of AnAOB.

Qin et al. demonstrated positive short-term effects of PLA microplastics on the anammox system: PLA microplastics increased nitrogen removal efficiency by 8.21% (100 mesh) and 6.54% (1000 mesh), enhanced the enzyme activity of the key hydrazine dehydrogenase genes (HDH) enzyme activity, and promoted extracellular polymeric substances (EPS) secretion to protect AnAOB and strengthen sludge aggregation [35]. Metabolically, PLA-enriched benzoic acid-related metabolites

activated carbon and amino acid biosynthesis pathways, while these metabolites were catabolized by AnAOB themselves. Nie et al. noted distinct impacts of PLA and PBAT in freshwater sediment: both slightly elevated total organic carbon content and reduced denitrifying/anammox bacterial diversity [27]. PLA degraded to lactic acid, enriching *nirS* denitrifying and anammox bacteria on its surface. PBAT induced variable responses, with a higher *nirK* denitrifying bacteria abundance but lower *nirS* and anammox bacteria levels, due to the differing properties of their monomers. Tang et al. found concentration-dependent effects of PBS [26]: low concentrations (0.1 g/L) minimally affected ammonia nitrogen removal and slightly promoted nitrite removal, while high concentrations (0.5 g/L) reduced nitrite removal efficiency, increased the polysaccharide content in sludge. PBS stimulated the growth of *Candidatus Brocadia*, *Proteobacteria*, and *Planctomycetes*, upregulated nitrogen metabolism modules and genes, and decreased methanogen *Methanosaeta* abundance, which is linked to its biodegradation intermediates and changes in sludge structure.

### 2.3.2. Research Progress on Non-Degradable Microplastics

Currently, research on the impacts of NDMPs on biological nitrogen removal and anammox systems has achieved considerable progress. Relevant findings indicate that these impacts are generally concentration-dependent, with some also exhibiting time-dependent characteristics. The effects of PET microplastics remain controversial and inconsistent: Fan et al. found that short-term exposure (7 days) induced cellular oxidative damage but had no significant impact on nitrogen removal performance; in fact, a slight improvement in total nitrogen removal efficiency was observed at low concentrations [36]. However, long-term (282 days) exposure to high concentrations significantly inhibited nitrogen removal efficiency by 28.7%, disrupted the granular sludge structure, and reduced EPS secretion. This inhibition was reversible: after a 3-month recovery period, the system regained 87% of its initial nitrogen removal efficiency. The core mechanism involves inhibiting the growth of AnAOB and disrupting metabolic pathways, while the evolution of microbial communities and metabolic adaptation are key to system recovery. In contrast, Hong et al. reported that low concentrations ( $\leq 0.2$  g/L) of PET microplastics had no significant effects on nitrogen removal efficiency or specific anammox activity (SAA); instead, they promoted EPS secretion and enhanced sludge aggregation. However, inhibition occurred at high concentrations ( $\geq 0.5$  g/L): at 1.0 g/L, SAA decreased by 16.2% and total nitrogen (TN) removal efficiency declined by 4.2% [10]. For PU microplastics, short-term impacts were generally negligible. Xie et al. showed that total inorganic nitrogen removal efficiency remained above 80% at concentrations of 1 mg/L and 50 mg/L, with no significant changes in the abundance of AnAOB functional genes [37]. Nevertheless, 50 mg/L PU microplastics induced granular sludge fragmentation, weakened sludge aggregation, promoted the proliferation of mobile genetic elements, and increased the risk of horizontal transfer of resistance genes. PE microplastics exhibited concentration-dependent inhibitory effects. Xue et al. demonstrated that low concentrations (0.05–0.1 g/L) had minimal effects on core system functions, while high concentrations (0.2–1.0 g/L) reduced SAA by up to 20% [38]. This inhibition was attributed to PE microplastics adsorbing onto the surface of sludge, blocking mass transfer channels, and decreasing the abundance of AnAOB. PVC microplastics

exhibited the most significant and irreversible inhibitory effects. Zheng et al. found that long-term exposure to 0.5 mg/L PVC microplastics reduced nitrogen removal efficiency by 20.64%, resulting in a final efficiency of only 62.56% [39]. Furthermore, acute toxicity was more severe under short-term high-concentration exposure, with a half-maximal inhibitory concentration lower than that of biodegradable microplastics.

**Table 1.** Comparison table of core parameters: impacts of biodegradable and non-biodegradable microplastics on anammox systems.

| Category                        | Microplastic Type | Key Concentration                      | Nitrogen Removal Impact  | Core Microbial/Enzyme                                     | Sludge/EPS Change                                     | Reference |
|---------------------------------|-------------------|--|--|---|---|-----------|
| Biodegradable Microplastics     | PLA               | 100/1000 mesh                          | Increased (8.21%–6.54%)  | enhanced HDH; enriched AnAOB/ <i>nirS</i>                 | Promoted EPS; strengthened aggregation                | [35]      |
|                                 | PBAT              | 100 µm–2 mm                            | -  | Enriched <i>nirK</i> ; reduced AnAOB/ <i>nirS</i>         | -   | [27]      |
|                                 | PBS               | Low (0.1 g/L); High (0.5 g/L)          | Low: Slight nitrite promotion; High: Nitrite inhibition        | Stimulated nitrogen-related microbes; upregulated N genes | High: Increased polysaccharides; impaired aggregation | [26]      |
| Non-Biodegradable Microplastics | PET               | Low/Short-term; High/Long-term         | Low: No impact/slight promotion; High: Inhibition (reversible) | High: Inhibited AnAOB; reduced SAA                        | High: Reduced EPS; damaged sludge structure           | [10,36]   |
|                                 | PU                | 1/50 mg/L                              | No significant impact  | -   | 50 mg/L: Sludge fragmentation                         | [40]      |
|                                 | PE                | Low (0.05–0.1 g/L); High (0.2–1.0 g/L) | Low: No impact; High: Inhibited SAA (up to 20%)                | High: Decreased AnAOB abundance                           | High: Potential mass transfer blockage                | [38]      |

PLA: polylactic acid; PBS: Polybutylene succinate; PBAT: polybutylene adipate-co-terephthalate; PET: polyethylene terephthalate; PU: polyurethane; PE: polyethylene.

### 3. Influencing Factors of Microplastics' Impacts on Anammox Systems

The effect of MPs on the anammox system is not dominated by a single factor, but rather the result of the synergistic interaction of multiple factors, including material type, particle size, concentration, and exposure time, as shown in **Figure 2**. Differences in these factors directly lead to significant variations in the impact effects and mechanisms [41].

#### 3.1. Material Differences

The chemical composition and structure of microplastics form the basis of their environmental behaviors and effects. MPs of different materials exert varying impacts on the anammox system due to differences in hydrophobicity, biocompatibility, degradability, and other properties [42].

DMPs (e.g., PLA and PHA) contain ester bonds in their chemical structures and can be decomposed into small-molecule substances such as lactic acid and hydroxy fatty acids by microorganisms, thus possessing both carrier functions and carbon source supply potential. The degradation rate of PLA microplastics is greatly influenced by pH, temperature, and microbial communities. In the anaerobic environment of the anammox system, the degradation cycle is approximately 30–60

days. Its degradation product, lactic acid, can serve as a carbon source for denitrifying bacteria, thereby promoting the synergistic nitrogen removal of denitrification and anammox [35]. PHA microplastics exhibit superior biocompatibility, and their non-toxic degradation products can be directly utilized by microorganisms to synthesize cellular substances and EPS, exerting a more significant growth-promoting effect on AnAOB [43]. Zhang et al. found that PHA microplastics enhanced the total nitrogen removal rate of the anammox system. This is attributed to the fact that the degradation rate of PHA is more compatible with the microbial metabolic cycle of the anammox system [44].

NDMPs are typically represented by PE and PS. They exhibit high chemical stability, are difficult to degrade in the environment, and tend to accumulate over the long term. PE microplastics have strong hydrophobicity and lack active functional groups on their surface, mainly affecting the anammox system through physical effects of adsorption and blocking [38]. At low concentrations, PE microplastics can adsorb trace inhibitory substances in water, indirectly improving the survival environment of AnAOB. In contrast, PS microplastics have a benzene ring structure on their surface, which may release trace toxic substances such as styrene monomers. Meanwhile, their surface charge characteristics are prone to electrostatic interactions with sludge particles, leading to a decrease in sludge aggregation capacity [45].

### 3.2. Particle Size Effects

The particle size of microplastics directly determines their migration pathways and modes of action in the anammox system, and MPs with different particle size ranges exert significantly distinct impacts on the system.

Nano-scale microplastics (NPs,  $<1 \mu\text{m}$ ) possess a high specific surface area and strong surface activity. They can penetrate into the interior of sludge granules through the pores, and even cross the cell membrane of AnAOB to enter the cells [46]. A study confirmed that 50 mg/L PS-NPs inhibited the total EPS content of anammox sludge by 42.3%, which was significantly higher than that of micro-scale PS microplastics [47].

Micro-scale microplastics (1–10  $\mu\text{m}$ ) represent the most common particle size range in the aquatic environment. Unable to penetrate cell membranes, they mainly adhere to the surface of sludge granules or fill the sludge pores. At low concentrations, large-sized micro-scale microplastics (5–10  $\mu\text{m}$ ) can act as the core skeleton of sludge granules, promoting the adhesion and aggregation of microorganisms and enhancing the granulation degree [48]. At high concentrations, the accumulation of a large number of microplastic particles blocks the sludge pores, leading to a decrease in substrate mass transfer efficiency. Consequently, the metabolism of AnAOB is hindered due to substrate limitation [49].

Millimeter-scale microplastics ( $>10 \mu\text{m}$ ) have a low content in sewage. Due to their relatively large volume, they are difficult to penetrate into the interior of sludge granules and mainly exist as biofilm carriers. Studies have shown that 5 mm microplastics can promote bacteria to form a dense biofilm on their surface [50]. Compared with the control group, the biofilm thickness increased by 40%, bacterial abundance increased by 25%, and total nitrogen removal rate improved by 3.8% [51].

However, when the concentration is excessively high (>500 mg/L), millimeter-scale microplastics occupy the effective volume of the reactor, leading to a decrease in sludge concentration and thereby inhibiting the nitrogen removal efficiency [52].

### **3.3. Concentration-Dependent and Time-Cumulative Effects**

The performance of the anammox process under MPs stress is jointly regulated by MPs concentration and exposure duration, with these two factors exerting synergistic rather than independent effects.

#### **3.3.1. Low-Concentration Microplastics**

Short-term exposure to low-concentration MPs generally exerts no obvious inhibitory effect on the anammox process, and even elicits a promotional effect in some cases. Under such stress conditions, the activity of AnAOB and nitrogen removal efficiency (NRE) of the anammox system remain stable, as validated by multiple studies. Han et al. revealed that 0.1 mg/L polystyrene nanoplastics (PS-NPs) did not inhibit anammox activity [17]; similarly, Hong et al. confirmed that polyethylene terephthalate microplastics (PET-MPs) at concentrations of 0.1–0.2 g/L did not interfere with the NRE of anammox systems [53]. Liu et al. reported that 1 mg/L polyvinyl chloride microplastics (PVC-MPs) significantly increased the network size and complexity of the anammox bacterial community [54]. Huang et al. further observed that the addition of 0.1 wt% polyethylene microplastics to freshwater sediments promoted the growth and proliferation of AnAOB, as well as the abundance of functional genes associated with denitrification and anammox [55].

Two specific pathways mediate the promotional effects of low-concentration MPs: first, MPs can act as carriers for AnAOB aggregation, thereby strengthening interspecific interactions [56]; second, biodegradable MPs can be degraded into dissolved organic carbon, which acts as a nutrient source for denitrifying bacteria and consequently synergistically improves the nitrogen removal efficiency of anammox systems [55,57].

In contrast, long-term exposure to low-concentration MPs significantly impairs the stability of the anammox process, with inhibitory effects arising from cumulative cytotoxicity. Xu et al. demonstrated that 200 days of exposure to 1–2 mg/L PS-NPs caused a significant decrease in the NRE of the anammox reactor [47]; by day 225, the SAA of anaerobic granular sludge (AnGS) was markedly reduced, and the extent of cell membrane damage in AnAOB was significantly aggravated. These results confirm that even low-concentration MPs may induce irreversible damage to anammox systems through cumulative effects during long-term exposure.

#### **3.3.2. High-Concentration Microplastics**

High-concentration MPs exert significant inhibitory effects on the anammox process regardless of exposure duration, with such effects primarily manifested in three aspects: deterioration of AnGS characteristics, reduction in AnAOB activity, and disruption of microbial community structure.

In terms of AnGS characteristics, high-concentration MPs induce granular sludge fragmentation, structural loosening, and reduced stability. Liu et al. found that exposure to 15 and 50 mg/L PVC-MPs significantly increased the proportion of AnGS with particle sizes of 0–500  $\mu\text{m}$ , indicating exacerbated sludge fragmentation [54].

Huang et al. pointed out that high-concentration MPs significantly elevate the integrity coefficient of AnGS, a parameter positively correlated with the reduced structural stability of granular sludge [58]. The core cause of AnGS structural damage lies in the inhibited secretion of extracellular polymeric substances (EPS)—key components that maintain AnGS stability and protect microorganisms from external stress [59]. Consistently, Hong et al. confirmed that high-concentration PET-MPs suppress EPS secretion by AnAOB, resulting in the loss of the AnGS protective barrier and further aggravating sludge loosening and fragmentation [10].

Regarding AnAOB activity and NRE, high-concentration MPs directly inhibit SAA and NRE in a concentration-dependent manner. Hong et al. showed that short-term exposure to PET-MPs at concentrations >0.2 g/L led to a significant decrease in the SAA of AnGS [10]; Han et al. further documented that increasing the concentration of PS-NPs from 0.5 mg/L to 2.0 mg/L caused marked deterioration in the NRE of the anammox process [17]. This inhibitory effect is further amplified by the suppression of key enzyme activity in AnAOB, which impairs the core metabolic capacity of these bacteria and thus exacerbates the decline in system performance [54].

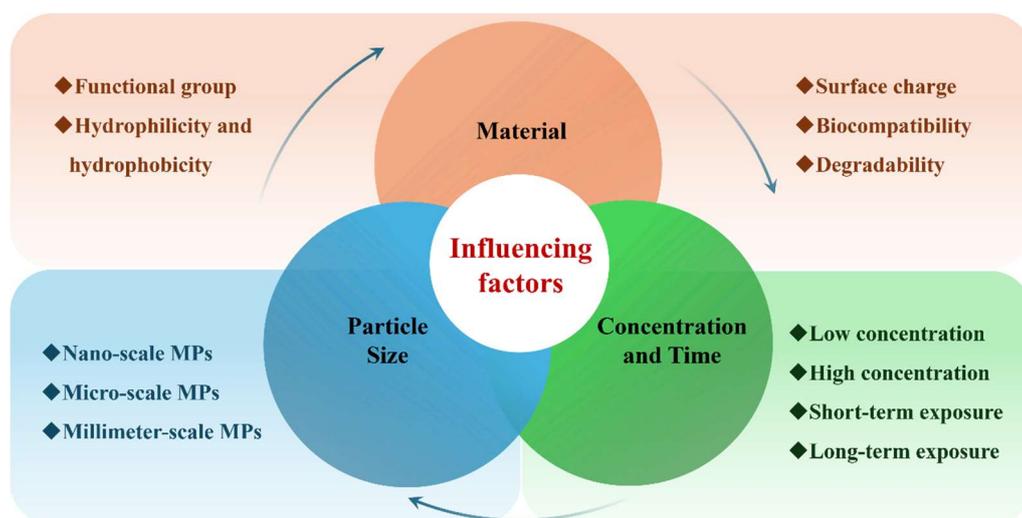
At the microbial community level, high-concentration MPs significantly reduce the diversity of anammox systems and alter the composition of dominant functional genera. Liu et al. reported that the Shannon index of AnGS in the control group was 4.16; this index slightly increased under 1 mg/L PVC-MPs stress but decreased to 3.51 under 50 mg/L PVC-MPs stress [54]. The abundance of operational taxonomic units showed a similar trend, confirming the negative impact of high-concentration MPs on community diversity. This reduction in diversity leads to a decrease in the AnAOB population, which in turn lowers the microbial diversity of the entire system [54,60]. For dominant AnAOB genera, Hong et al. identified three main taxa (*Candidatus Kuenenia*, *Candidatus Brocadia*, and *Candidatus Anammoxoglobus*) in AnGS, and their relative abundances gradually decreased with the increasing PET-MPs concentration [10]. Notably, *Candidatus Kuenenia*—the dominant genus in the control group—exhibited the most significant decline, with its relative abundance dropping from 45.0% at 0 g/L to 11.9% at 1.0 g/L. This result highlights the selective inhibitory effect of high-concentration MPs on key functional bacteria.

### 3.4. Inhibitory Mechanisms of Microplastics on Anammox

High concentrations of MPs generally exert inhibitory effects on the nitrogen removal efficiency of anammox systems, with three primary and interconnected mechanisms underlying this phenomenon [61]. First is the physical clogging effect: high-concentration MPs adhere to the surface of anammox sludge, block its pores, and reduce the mass transfer efficiency of key substrates (i.e., ammonia and nitrite), thereby impairing the metabolic activities of AnAOB. Second is the direct toxic effect, which is particularly pronounced in nanoscale MPs. Owing to their strong penetration capacity, NPs can cross microbial cell membranes, disrupt cellular structures, and cause the leakage of intracellular enzymes and nutrients, directly suppressing AnAOB activity [61,62]. Beyond such direct structural damage, NPs can further trigger cascading adverse effects: reactive oxygen species (ROS) are easily generated on their surfaces, inducing cellular oxidative stress and downregulating the expression of g

EPS synthesis-related genes and key enzyme genes, which in turn exacerbates metabolic disorders in AnAOB [62].

Notably, MP-induced oxidative stress is widely recognized as one of the core mechanisms leading to AnAOB metabolic inhibition [63], with its molecular pathways well characterized and experimental evidence validating its severity. Exposure to typical MPs not only stimulates the excessive production of ROS but also promotes the release of toxic additives such as bisphenol A, which collectively elevate AnAOB mortality [63–65]. The intrinsic mechanism of this oxidative damage involves MPs penetrating AnAOB cell membranes, accumulating intracellularly, disrupting the bacterial electron transport chain, and ultimately inducing excessive ROS generation [66]. Subsequently, accumulated ROS trigger lipid peroxidation, DNA damage, and cell membrane rupture, directly compromising the cellular structure and metabolic functions of AnAOB, and even leading to cell death [64,65,67]. Experimental data further confirm the significant inhibitory effect of oxidative stress: under exposure to high concentrations (500 mg/L) of polyethylene terephthalate nanoplastics (PET-NPs), the cell membrane integrity of AnAOB is markedly reduced, and its apoptosis rate increases by more than 30% [37].



**Figure 2.** Influencing factors of microplastics' impacts on anammox systems.

#### 4. Mechanisms Underlying Microplastics' Impacts on Anammox Systems

As shown in **Figure 3**, the mechanisms by which microplastics affect anammox systems mainly include reshaping microbial community structure, regulating the expression of functional genes involved in nitrogen and carbon metabolism, and interfering with intracellular metabolic processes.

##### 4.1. Shifts in Microbial Community Structure

The microbial community structure of anammox systems is complex. In addition to AnAOB, symbiotic microorganisms such as denitrifying bacteria, nitrifying bacteria, and heterotrophic bacteria coexist synergistically. The interactions among these microorganisms collectively maintain the stable operation of the system [68]. By

altering the living environment of these microbes, microplastics exert significant impacts on the microbial community structure of anammox systems, thereby regulating the nitrogen removal performance of the system.

#### 4.1.1. Abundance and Diversity of AnAOB

The effects of microplastics on the diversity and abundance of AnAOB exhibit a significant concentration-dependent characteristic. Exposure to low concentrations (1 mg/L) of PVC-MPs could expand the scale and complexity of the AnAOB community network, enhance the efficiency of inter-microbial information exchange, and exert no significant inhibitory effect on AnAOB diversity. Similarly, short-term exposure to 0.1 mg/L PS-NPs and 0.1–0.2 g/L PET-MPs maintained the stability of AnAOB diversity and abundance [47].

In contrast, high concentrations of MPs significantly inhibit the diversity and abundance of AnAOB. When the concentration of PVC-MPs increased to 50–500 mg/L, the Shannon diversity index of AnAOB decreased from 4.16 to 3.51, and the total abundance of *Candidatus Kuenenia* and *Candidatus Brocadia* reduced by 6.1%–13.9%. After exposure to 500 mg/L polyethylene terephthalate micro-nanoplastics (PET-MNPs), the relative abundance of AnAOB decreased more significantly, with the inhibitory effect of nanoscale PET being stronger than that of microscale PET. Under long-term exposure (>63 d), even low concentrations (1–2 mg/L) of PS-NPs could induce a continuous reduction in AnAOB population size through the cumulative effect and even trigger cell death [37].

#### 4.1.2. Other Functional Microbial Communities

Other functional microbial communities in the anammox system include symbiotic functional flora, such as denitrifying bacteria, and sludge structure-related flora, such as filamentous bacteria. Changes in their community composition, driven by alterations to the living environment of these microbes, can indirectly affect the operation of the anammox system [69].

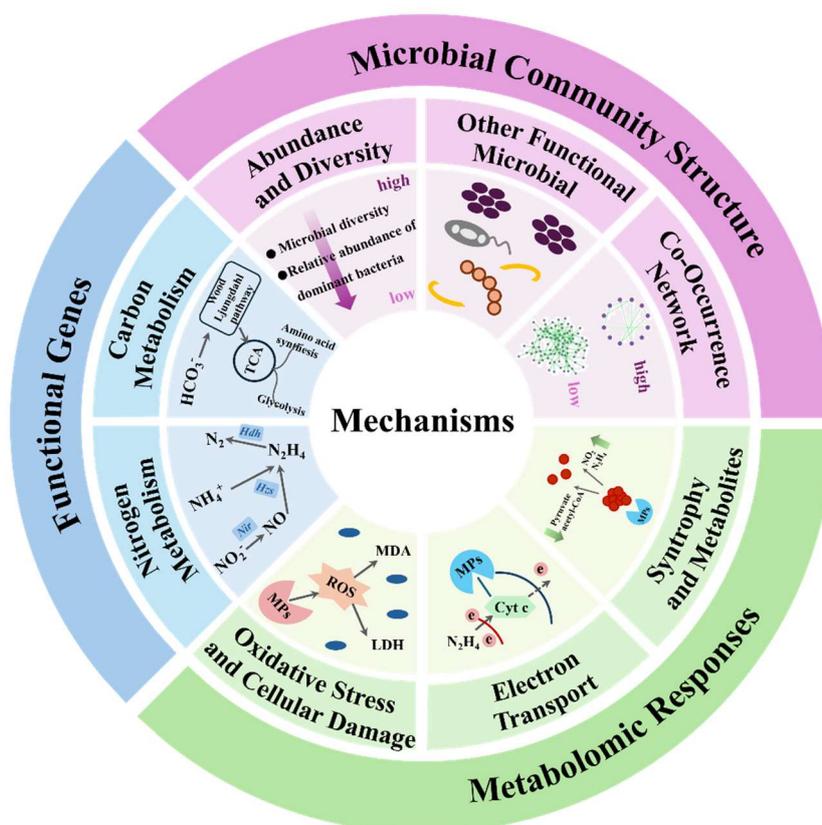
As a key symbiotic flora of AnAOB, the abundance of denitrifying bacteria is crucial for the synergistic nitrogen removal of the anammox system. Exposure to high concentrations (>0.2 g/L) of PET-MPs and 50 mg/L of PVC-MPs leads to a significant decrease in the abundance of denitrifying bacteria such as *Rhodocyclaceae* and *Zoogloeaceae*, disrupting the syntrophic relationship between AnAOB and denitrifying bacteria. However, under the stress of high-concentration PET-MPs, the abundance of genera such as *Ignavibacterium* and *Armatimonadetes\_gp5* increases. These genera may convert nitrate to ammonium through the dissimilatory nitrate reduction to ammonium process, indirectly supplementing the substrate for AnAOB and partially offsetting the inhibitory effect caused by the altered living environment [36].

Filamentous bacteria (e.g., *norank\_c\_OLB14*, *norank\_o\_SBR1031*) are involved in the sludge granulation process, and changes in their abundance directly affect the stability of AnGS. After 63 days of continuous stress under 50–500 mg/L of PVC-MPs, the total abundance of filamentous bacteria decreased by 4.7–18.9 percentage points, resulting in reduced sludge granulation degree, a loose, fragmented structure. The response of EPS secretion-related microbial communities to microplastics shows a concentration-dependent pattern: low concentrations (0.1–0.2 g/L) of PET-MPs

stimulate the activity of EPS-secreting flora, promoting the synthesis of proteins and polysaccharides. High concentrations ( $>0.5$  g/L) significantly inhibit such flora, leading to a decrease in PS content, an increase in the proteins/polysaccharides ratio, and a reduction in sludge adhesion [54].

#### 4.1.3. Microbial Co-Occurrence Network Structure

The complexity and stability of microbial networks directly reflect the disturbance resistance of the community. Under exposure to low-concentration (1 mg/L) PVC-MPs, the node number, link number, and average degree of the AnAOB community network reached 320, 585, and 3.66, respectively, indicating a significant enhancement in network complexity. However, when the concentration of PVC-MPs increased to 50 mg/L, the network node number decreased to 194, the link number dropped to 211, and the average degree reduced to 2.18, with the network structure tending to simplify. High-concentration PVC-MPs shortened the average network distance from 7.85 to 4.51, accelerating the energy transfer and information exchange among microorganisms, which might represent an adaptive strategy of the community to resist external stress [70]. Meanwhile, the composition of keystone species in the network underwent a significant change, with low-abundance functional microbial groups playing a more prominent role under high-concentration microplastic stress [54].



**Figure 3.** Mechanisms underlying microplastics' impacts on anammox system.

#### 4.2. Metagenomic Analysis of Functional Genes

Metagenomics reveals the effects of microplastics on the expression of functional

genes in anammox systems and clarifies changes in key metabolic pathways by analyzing the entire genetic information of microbial communities.

#### 4.2.1. Nitrogen Metabolism-Related Functional Genes

The nitrogen metabolism process of the Anammox system involves a series of functional genes, and the ammonia oxidation function of AnAOB depends on the expression of hydrazine synthase genes (HZS) and hydrazine dehydrogenase genes HDH. Exposure to high concentrations ( $>0.2$  g/L) of PET-MPs, 50 mg/L of PVC-MPs, and 2.0 mg/L of PS-NPs significantly downregulates the expression levels of HZS and HDH genes, resulting in a 16.2%–33.7% decrease in specific anammox activity (SAA). Studies have demonstrated that 500 mg/L of PET-MNPs can significantly reduce the abundance of genes related to cytochrome C (Cyt c) synthesis, indirectly inhibiting the transcriptional efficiency of HZS and HDH genes and further impairing the nitrogen metabolism capacity of AnAOB [37]. Long-term exposure to low concentrations (1–2 mg/L) of PS-NPs induces irreversible inhibition of HZS and HDH genes through the intracellular accumulation effect [47].

The impacts of microplastics on denitrification genes exhibit gene-specific differences. Exposure to high concentrations of PVC-MPs and PET-MPs significantly suppresses the abundance of key denitrification genes *nirS*, *nirK*, and *nosZ*, with the recovery of *nirS/nirK* genes lagging behind that of the core AnAOB genes. Additionally, research has confirmed that long-term exposure to 5 mg/L of PVC-MPs leads to a decrease in the abundance of ammonia oxidation-related genes and an increase in the abundance of denitrification reductase genes in the nitrosation system, ultimately promoting  $N_2O$  emission [39].

#### 4.2.2. Carbon Metabolism-Related Functional Genes

The expression of carbon metabolism-related functional genes directly affects the energy supply and material synthesis of microorganisms. Exposure to high concentrations of MPs significantly downregulates the expression of genes associated with glycolysis, the tricarboxylic acid cycle and energy production, thereby reducing the efficiency of carbon source utilization [13]. Genes involved in amino acid biosynthesis are also inhibited under high-concentration microplastic stress, leading to a decrease in protein synthesis in EPS and further impairing the structural stability of sludge.

Biodegradable microplastics exhibit distinct effects: the dissolved organic carbon produced during their degradation can upregulate the expression of genes related to acetic acid synthesis and folate biosynthesis, providing additional carbon sources for AnAOB and symbiotic bacteria, and thus partially alleviating the inhibitory effects of non-biodegradable microplastics.

### 4.3. Metabolomic Responses of Anammox Systems

#### 4.3.1. Inhibition of the Electron Transport System

The Cyt c-mediated electron transport system is a key component of AnAOB energy metabolism [65]. Studies have confirmed through molecular docking and molecular dynamics simulations that PET-MNPs can form stable binding with Cyt c, altering its spatial conformation and leading to loss of activity. After exposure to high concentrations (500 mg/L) of PET-MNPs, the Cyt c content in AnGS decreases by

73.4%–78.5%, and the electron transport activity reduces by 34.3%–72.7%, directly inhibiting the energy generation and substrate conversion efficiency of AnAOB [40].

#### 4.3.2. Dysregulation of Microbial Syntrophy and Metabolites

Microplastic exposure disrupts the metabolic interaction network between AnAOB and symbiotic bacteria. Acetic acid secreted by AnAOB cannot be efficiently supplied to denitrifying bacteria, while the production of folate (an essential nutrient for AnAOB) synthesized by symbiotic bacteria decreases, leading to obstruction of cross-trophic syntrophic pathways. Reduced secretion of metabolites related to the quorum sensing pathway further decreases the efficiency of inter-microbial communication, impairing sludge structural stability and the synergistic nitrogen removal effect.

Metabolite analysis shows that high concentrations of microplastics lead to the accumulation of nitrogen metabolism intermediates (hydrazine, nitrite), a reduction in the content of key carbon metabolism products (pyruvate, acetyl coenzyme A), and lipid metabolism disorder-induced damage to cell membrane phospholipid components, ultimately affecting substrate transmembrane transport and electron transport chain function. In addition, long-term exposure to PVC-MPs increases N<sub>2</sub>O emissions by 9.22%–88.36% through the hydroxylamine oxidation pathway and heterotrophic denitrification pathway, exacerbating the risk of greenhouse gas emissions [71].

## 5. Conclusions and Outlook for Further Research

This review systematically summarizes the sources, classification, and impacts of microplastics on Anammox systems, revealing concentration-dependent, time-cumulative, and particle-size-differential effects, as well as the underlying mechanisms involving microbial community disruption, functional gene suppression, oxidative stress, and physical clogging.

Existing research remains inconsistent regarding the concentration thresholds for microplastic-induced promotion/inhibition, and the interactive effects of microplastics with other pollutants in complex wastewater require further investigation. To promote the sustainable implementation of anammox technology in MP-contaminated environments, future investigations should prioritize the following key research directions:

- 1) The combined pollution effects of multiple microplastics, heavy metals, and antibiotics in practical wastewater treatment systems;
- 2) The long-term impacts of microplastics on the anammox system and corresponding N<sub>2</sub>O emission reduction strategies;
- 3) Microbial regulation-based system remediation technologies, including the screening and enrichment of functional flora resistant to microplastic stress, and the rational addition of quorum sensing signal molecules to enhance the metabolic activity of AnAOB and the structural resilience of anammox consortia;
- 4) The migration and transformation rules, as well as biotoxicity differences of microplastics with different particle sizes.

In-depth exploration of these research avenues will not only fill the existing

knowledge gaps regarding the complex interactions between microplastics and anammox systems, but also provide a more comprehensive, science-based theoretical foundation for maintaining the stable operation of anammox processes and implementing precise environmental risk prevention and control measures under microplastic pollution stress.

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