

1 **Recent advances in impacts of microplastics on nitrogen cycling in the environment: A Review**

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13 **Abstract**

14 Nitrogen cycling plays a decisive role in biogeochemistry, and largely depends on microbial driven
15 nitrogen transformation. The environmental problems caused by microplastics are becoming more
16 serious, and the analysis and control of its pollution in the environment have become a research hotspot
17 in the field. The nitrogen transformation and nitrogen cycling in the environment are mainly driven by
18 microorganisms in the environment, and the existence of microplastics can affect the microbial
19 population, abundance and type, thus affecting the transformation of nitrogen. The effect of microplastics
20 on microorganisms involved in nitrogen transformation is briefly described. This paper mainly reviews
21 the research progress on the impacts of microplastics on nitrogen transformation and nitrogen cycling in
22 water, soil, sediment and sewage sludge. Microplastic type, size and concentration can cause obvious
23 difference in the impacts of microplastics on nitrogen transformation. Then, response and mechanism of
24 microplastics to microorganism mediated nitrogen transformation and nitrogen cycling are introduced.
25 Processes of nitrogen transformation are affected by interfering with microorganism diversity and
26 structure, enzyme activities and related coding genes and oxygen flux. Additionally, additives released
27 from microplastics also affect the microbial activity. However, mechanisms of microplastics on
28 environmental nitrogen transformation and nitrogen cycling are not fully understood due to the lack of
29 relevant research. There are effective strategies to evaluate complex environmental systems, prolong
30 action time, strengthen multi factor and multi-level research, and assist molecular biology and stable
31 isotope technology. This review article can provide valuable insights into the impact of microplastics on
32 microorganisms mediated nitrogen transformation processes and evaluate the impact on ecological and
33 environmental health.

34

35 **Keywords:** Microplastics; Nitrogen transformation; Nitrogen cycling; Microorganisms; Coding gene;

36 Environmental pollution

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37 **1. Introduction**

38 The pollution of microplastics and its accumulation and harm in the environment have attracted
39 more attention (Shen et al., 2019d). Due to the small particle size and large specific surface, microplastics
40 can adsorb and enrich various environmental pollutants such as heavy metal, organic compounds and
41 microorganisms from external environment (Hartmann et al., 2017). Microplastics can not only carry the
42 adsorbed pollutants in different environmental media, but also can promote the migration or
43 transportation of pollutants in organisms, tissues and organs through desorption, so as to alter the
44 environmental behavior and ecological risks of microplastics themselves and their adsorbed pollutants
45 (Shen et al., 2019c). Microplastics in the environment may threaten the growth, development and
46 reproduction of microorganisms through their own physical effects, additives release and adsorption of
47 coexisting pollutants (Shen et al., 2019b; Wang et al., 2020). As such, microplastic pollution has become
48 one of the research hotspots and frontier issues in the field of environmental science (Hu et al., 2019).

49 The wide distribution of microplastics is closely related to its migration characteristics in the
50 environment. The sources of microplastics in the water environment mainly include land-based input,
51 shipping, tourism, and aquaculture and fishing (Kumar et al., 2021). The pandemic of COVID-19
52 unexpectedly increased the production of microplastics (Shen et al., 2021b). Microplastics in land
53 environment mainly come from agricultural plastic film residue, compost, sludge application, wastewater
54 irrigation, automobile tire debris (Blasing and Amelung, 2017). In the atmosphere, synthetic fibers (such
55 as clothes, curtains and soft carpets), coating materials, erosion of synthetic rubber tires and urban dust
56 are the important sources of microplastics (Huang et al., 2020b; Petersen and Hubbart, 2020).
57 Microplastics have a cyclic exchange process in the four environments of water, soil, sludge and air
58 (Huang et al., 2020a). Microplastics in the ground environment are washed into the water environment

59 by surface runoff, and the application of irrigation and sludge introduces microplastics in water into the
60 soil environment.

61 Microplastics can not only adsorb nutrients and organic matters from the surrounding environment,
62 provide an ideal niche for environmental microorganisms and promote microbial colonization and
63 biofilm growth, but also help microorganisms resist environmental pressure and accelerate diffusion
64 (Shen et al., 2019d). Microplastics can also change the community structure and function of
65 microorganisms and finally affect the geochemical cycle process of biogenic substances such as carbon,
66 nitrogen and phosphorus (Bank and Hansson, 2019). Nitrogen cycling plays a decisive role in
67 biogeochemistry, and largely depends on microbial driven nitrogen transformation in the environment.
68 Microbial action is the main factor controlling nitrogen transformation and can be recycled orderly
69 through the following processes. Ammonia is assimilated into organic nitrogen, which is degraded and
70 ammoniated to form ammonium salt, then oxidized to nitrate through nitrification, and converted to N_2O
71 or N_2 through denitrification, or nitrate is reduced to ammonia or anaerobic ammonia oxidation to N_2 .
72 The key processes related to nitrogen cycling are driven by microorganisms, and the processes in which
73 microorganisms play a decisive role are enzymatic reactions by various key enzymes, encoded by
74 corresponding functional genes. However, evidence showed that biofilm attached on the surface of
75 microplastics can promote the oxidation and denitrification of ammonia nitrogen and nitrite, but may
76 also inhibit the nitrogen removal by reducing the content of denitrifying bacteria (Chen et al., 2020a). In
77 addition, the presence of microplastics can not only disturb the activity of nitrogen cycling related
78 enzymes (Qin et al., 2020), but restrict the expression of functional genes encoding nitrogen cycling
79 related enzymes (Seeley et al., 2020). This means that microplastics pollution can also affect the earth
80 nitrogen cycling and then threaten the security of global ecosystem.

81 Recently, experimental studies on the impacts of microplastics on nitrogen transformation and
82 nitrogen cycling in the environment have been reported. However, there is still a lack of timely review
83 of this subject to provide basic understanding and guide further research. Although some studies have
84 been done on the effect of microplastics on environmental nitrogen cycling, most of the research is
85 carried out under laboratory conditions. The existing results show that microplastics in the environment
86 have a significant impact on nitrogen cycling, unfortunately, the current research still focuses on the
87 “phenomenon” and the mechanism is still not fully understood. The research focuses on the impact of a
88 single microplastic on nitrogen transformation and nitrogen cycling in a single environment (sediment
89 or soil), and do not take into account the impact of complex environment (different microplastics and
90 particle size distribution and ecosystem). In the water environment, microplastics can affect the
91 photosynthesis and growth of phytoplankton, inhibit the activities of zooplankton, and affect the
92 biological pump and carbon storage (Shen et al., 2019a). Phytoplankton plays an important role in the
93 process of carbon and nitrogen fixation. Its growth is inhibited by microplastics, which then could
94 interfere with the process of nitrogen transformation. Microplastics can alter the physical and chemical
95 properties of soil environment, and these changes would directly affect the functional and structure
96 diversity of soil microbial community, resulting in more serious soil environmental problems. Compared
97 with the surrounding soil particles, the uneven surface of microplastic particles and various substances
98 attached to the surface will form a completely different microbial community form that in the soil (Chai
99 et al., 2020). The interference of microplastics on nitrogen transformation in environmental media such
100 as submerged zone or farmland using sludge as fertilizer is more complex. The interference effect of
101 microplastics in complex system on nitrogen cycling and its related mechanisms still require to be
102 clarified. Consequently, recent studies about the effect of microplastics on nitrogen cycling are

103 thoroughly reviewed. The effects of microplastics on nitrogen transformation in water soil, sediment and
104 sludge are focused, and the potential influence mode of microplastics on nitrogen cycling process is
105 further revealed. This paper is expected to provide reference for the follow-up exploration of the
106 influence of microplastics on global nitrogen cycling.

107

108 **2. Impacts of microplastics on microorganisms**

109 Microplastics can adsorb organic matter and inorganic nutrients in the environment, so as to attract
110 microorganisms such as bacteria and viruses to adhere to them. On the other hand, microplastic particles
111 can help microorganisms resist environmental pressure, provide relatively stable habitat, and enhance
112 microbial diffusion ability (Shen et al., 2019d). The material circulation and energy flow in the
113 environment are mainly driven by microorganisms, which are closely related to community composition
114 and metabolic function. The accumulation of microplastics can significantly affect the enzyme activity
115 and microbial community structure (Cao et al., 2020a). The effect of microplastics on bacterial
116 community structure can be changed by changing the physical and chemical characteristics or nutritional
117 conditions. A study carried out by Fei et al. (2020) has found that the presence of PE and PVC
118 microplastics in acid cropped soil significantly inhibited the activity of fluorescein diacetate
119 hydrolase, stimulated the activities of acid phosphorus and urease, and reduced the diversity and
120 richness of microbial community, and the effects of PE microplastic on soil was more serious than
121 that of PVC microplastic. Huang et al. (2019) found that LDPE microplastic addition can affect
122 microbial community composition and activity of urease in soil, thus disturbing the hydrolysis of
123 organic nitrogen, and pointed out that microplastics in soil can be used as a unique habitat,
124 potentially altering the ecological function of soil ecosystem. Moreover, a recent study done by

125 Rong et al. (2021) also reported that existence of LDPE microplastics in soil affected niche and
126 nutrient competition and the correlation of soil bacteria, thus altering the nitrogen cycling driven by
127 soil microorganisms. Qian et al. (2018) showed that plastic film residual in agricultural soil could
128 reduce the content of soil inorganic nitrogen and the activity of functional enzymes, and inhibit the
129 expression of functional genes related to nitrogen cycling. Awet et al. (2018) evaluated the effects
130 of PS nanoplastics on soil microbial activity and biomass, as well as the functional enzyme diversity,
131 and the results showed that the presence of nanoplastics significantly decreased the activity of N-
132 (leucine aminopeptidase) and limited the potential utilization ability of nitrogen in soil. Since the
133 main component of microplastics is carbon, it may affect the storage and conversion of carbon in the
134 environment (Ho et al., 2017).

135 Microplastics can improve the functions of cell membrane transporters and movement, and reduce
136 some essential metabolic pathways. As a protein system, membrane transporters play an extremely
137 important role in regulating adverse changes in the bacterial environment (Langille et al., 2013).
138 Microplastics can stimulate the function of bacterial membrane transporters, so as to regulate the
139 mechanism of maintaining the stability of intracellular environment (Fei et al., 2020). The existence of
140 micro plastics makes bacteria have higher cell mobility, which may help to speed up the movement of
141 bacteria to nutrients and avoid toxic substances to adapt to the changeable environment (Kisand et al.,
142 2012). The metabolic pathways of amino acids, cofactors and vitamins in the biofilm of micro plastic
143 matrix are significantly enhanced, which also shows that micro plastic, as a unique microbial habitat, can
144 not only change the community structure, but also affect the microbial function, and potentially affect
145 the ecological function of the microbial community in the ecosystem (Miao et al., 2019b). Additionally,
146 biofilms attached to microplastics are very complex and contain many genes, which may lead to gene

147 exchange between biofilm communities or between biofilm communities and surrounding communities.
148 A variety of bacteria are attached to organic particles. Due to the strong variability of bacterial genes,
149 gene exchange occurs among communities through horizontal gene transfer, such as absorption and
150 utilization of DNA in the environment, gene transformation between cells, transduction through phage.
151 Therefore, microplastics may become a hot area of bacterial gene exchange, which has affected the
152 energy flow of ecosystem and the circulation of nitrogen, phosphorus and other substances (Rong et al.,
153 2021).

154

155 **3. Interference ways of microplastics on environmental nitrogen transformation and nitrogen** 156 **cycling**

157 Microorganisms are the engine of nitrogen and other nutrient elements circulation. Ammoniation,
158 nitrogen fixation, nitrification and denitrification constitute the main links of nitrogen transformation and
159 nitrogen cycling, and each process requires the participation of corresponding microorganisms.
160 Microbial community structure controls nitrogen cycles in different ecosystem, and then regulates the
161 function and stability of ecosystems. The number, activity, and flora structure of microorganisms
162 involved in nitrogen cycles control the direction and process of nutrient mineralization and fixation, and
163 then affect the process and products of nitrogen cycles (**Fig. 1**). Dai et al. (2020) found that the presence
164 of PVC microplastics aerobic sewage sludge obviously reduced the content of denitrifying bacteria,
165 thereby affecting the relative removal of total nitrogen in the reaction system. Huang et al. (2021)
166 reported that PE microplastics in sediments could promote the growth of denitrifying bacteria and
167 anaerobic ammonia oxidizing bacteria, and at the same time, the health and activity of benthos in
168 sediments is also affected by microplastics.

169 Environmental media, polymer type, particle size and concentration of microplastics can also affect
170 the functional bacteria related to nitrogen cycling, which may be related to the fact that microplastics can
171 act as organic substrates and release of additives in microplastics (Seeley et al., 2020). As an organic
172 substance, microplastics can be utilized by microorganisms with the participation of oxygen. It can not
173 only promote the growth of related functional bacteria, but also form an oxygen concentration gradient
174 conducive to nitrogen transformation on its inner surface (Huang et al., 2021). Li et al. (2020) have
175 revealed that PP microplastics produce additional anaerobic atmosphere on its inner surface, which
176 contributes to the growth and denitrification activity of denitrifying bacteria. Additionally, plastics used
177 in daily life often add a variety of plasticizers to improve the performance of plastics. Evidence showed
178 that the additives released by microplastics can affect microbial activities by directly destroying
179 microbial cells (Mohammad Mirsoleimani Azizi et al., 2021; Shen et al., 2019c). For example, bisphenol
180 A released from PVC is the key inhibition mechanism to promote the cell wall rupture of anaerobic
181 digestion microorganisms (Wei et al., 2019). Antibacterial PE microbeads used in cosmetic scrub also
182 inhibit the process of sediment bacteria transforming nutrient into bioavailable forms, resulting in
183 changes in nutrient cycling (Cluzard et al., 2015). PVC plastic products used in the medical field have
184 antibacterial properties because they contain plasticizers, which are selective to specific types of
185 microorganisms such as Gram-negative bacteria and sulfate reducing bacteria, and are resistant to
186 nitrifying bacteria. Furthermore, microplastics in the environment can be further degraded into
187 nanoplastics (Shen et al., 2019c). nanoplastics can catalyze the production of reactive oxygen species
188 such as hydroxyl radical ($\cdot\text{OH}$), which can directly destroy microbial cells and inhibit metabolic function
189 (Azizi et al., 2021). PS-NH₂ nanoplastics could bind to lipid bilayers on the cell membrane with high
190 affinity and show the highest toxicity to its surface biofilm, resulting in excessive reactive oxygen species,

191 destroying the basic ecological function of microorganisms and affecting the process of nitrogen cycles
192 (Miao et al., 2019a).

193 Moreover, microplastics can inhibit the activity of enzymes related to nitrogen transformation, and
194 then affect the nitrogen cycling (**Fig. 1**). The enzymes involved in nitrogen cycling mainly include nitrate
195 reductase, nitrite reductase, nitrite oxidoreductase, nitric oxide reductase, and nitrous oxide reductase.
196 Evidence showed that the presence of PS microplastics significantly decreased the activity of N-(leucine
197 aminopeptidase), a key enzyme of nitrogen cycling, which disturbed the nitrogen cycling in freshwater
198 (Miao et al., 2019a) and soils (Awet et al., 2018). Gao et al. (2021) found that LDPE microplastics could
199 enhance the activity of urease in soil environment and improve the utilization potential of nitrogen, and
200 Huang et al. (2019) reported that microplastic addition had little impact on soil nitrite reductase and
201 nitrous oxide reductase. The participation of enzymes in a series of redox reaction is the key to the
202 nitrogen cycling. Influencing the activity and quantity of related enzymes would inevitably interfere with
203 the nitrogen transformation processes.

204 Furthermore, the functional genes encoding enzymes related to nitrogen cycling are also affected
205 by microplastics (**Fig. 1**). Nitrification is a series of oxidation processes from NH_3 or NH_4^+ to NO_3^- under
206 aerobic conditions, mainly involving three nitrogen transformation stages ($\text{NH}_3/\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow$
207 $\text{NO}_2^- \rightarrow \text{NO}_3^-$). The transformation of $\text{NH}_3/\text{NH}_4^+$ to hydroxylamine (NH_2OH) is mainly catalyzed by
208 ammonia monooxygenase, encoded by *amoA* gene, produced by ammonia oxidizing bacteria and
209 Archaea (Pjevac et al., 2017), and the transformation of NH_2OH to NO_2^- is controlled by hydroxylamine
210 reductase, encoded by *hao* gene, metabolized by ammonia oxidizing bacteria (Simon and Klotz, 2013).
211 Most ammonifying bacteria belong to β - and γ - *Proteus*, which are chemoautotrophs that can oxidize
212 NH_4^+ to NO_2^- . The transformation from NO_2^- to NO_3^- is completed by nitrite oxidizing bacteria, light

213 energy utilization bacteria and nitrite oxidoreductase metabolized by anaerobic ammonia oxidizing
214 bacteria, and its coding gene is *nxrAB* (Schweiger, 2016). Nitrite oxidoreductase can be produced by
215 aerobic nitrous oxidizing bacteria (such as *Curvularia*, *Spirochetes*, α -*Proteus*, β -*Proteus*, and γ -*Proteus*)
216 (Daims et al., 2016), anaerobic light energy utilization bacteria (*Rhodospseudomonas*) (Griffin et al.,
217 2007), and anaerobic ammonia oxidizing bacteria (Strous et al., 2006). Denitrification is a series of
218 reduction reaction in which NO_3^- is reduced to $\text{N}_2\text{O}/\text{N}_2$ under anoxic conditions, mainly including four
219 nitrogen transformation stages ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$). The transformation from NO_3^- to
220 NO_2^- is mainly catalyzed by cytoplasmic membrane bound nitrate reductase (encoded by *narG* gene) or
221 extracellular peripheral nitrate reductase (encoded by *napA* gene) (Butala and Folkinham, 2018), which
222 generally occurs in an anoxic environment rich in NO_3^- and most microorganisms contain both enzymes
223 (Philippot et al., 2007). The transformation of NO_2^- to NO is catalyzed by two unrelated nitrite reductase
224 (NIRS) in peripheral stromal cells, including cd1-NIR encoded by *nirS* gene and Cu-NIR encoded by
225 *nirK* gene, which are common in bacteria and Archaea (Graf et al., 2014). The transformation of NO to
226 N_2 is catalyzed by nitric oxide reductase, encoded by *narB* gene, which widely exists in various
227 environments (Yang et al., 2014) and the transformation of N_2O to N_2 is catalyzed by nitrous oxide
228 reductase, encoded by *nosZ* gene, which is found in a variety of bacteria (*Proteus*, *Bacteroides* and
229 *Chloromycetes*) and Archaea (*Halophilia*) (Zumft and Kroneck, 2006). Ammoniation is a process of
230 transformation of organic nitrogen into NH_4^+ ($\text{organic N} \rightarrow \text{NH}_4^+$), which is catalyzed by keratinase
231 (encoded by *cynS* gene) and urease (encoded by *ureABC* gene) (Kuypers et al., 2018). A series of
232 nitrogen redox reactions catalyzed by enzymes secreted by microorganism are the main drivers of
233 nitrogen transformation and nitrogen cycling, and the genes encoding enzymes are the key to control
234 microbial processes. Qian et al. (2018) have revealed that agricultural residual plastic films increased the

235 abundance of nitrogen fixation related functional gene (*nifH*), N₂O reduction related gene (*nosZ*) and
236 denitrification related functional gene (*nirS*), but decreased denitrification gene (*nirK*). Seeley et al.
237 (2020) reported that PLA microplastics also increased the abundance of *amoA* gene and *nirS* gene, and
238 decreased the abundance of *nirK* gene in sediments, while PVC microplastics decreased the abundance
239 of all of them (*amoA*, *nirS* and *nirK* gene). Rong et al. (2021) showed that microorganisms containing
240 AOA*amoA* and *nirS* genes were more vulnerable to the presence of LDPE microplastics, and LDPE
241 addition increased the abundance of *nifH*, AOB*amoA* and *nirK* genes involved in nitrogen cycle at
242 different culture stages. According to the changes of functional gene abundance combined with the
243 changes of enzymes, functional microbes and nitrogen concentration in certain ecosystem, the influence
244 mode of microplastics on nitrogen transformation and nitrogen cycling can be evaluated.

245 Finally, oxygen content is also a key in the process of nitrogen transformation and nitrogen cycling
246 (**Fig. 1**). Nitrification mainly occurs under aerobic conditions, while denitrification, anaerobic ammonia
247 oxidation and dissimilatory reduction of nitrate to ammonium are more common under anaerobic
248 conditions. Kojima et al. (2012) found that the functional genes of anaerobic bacteria were detected in
249 deep lake sediments, but they were difficult to detect in shallow lake sediments. The microplastics in the
250 environment have the characteristics of small particle size, easy to migrate vertically to the deep layer of
251 the sediment, and may remain in the sediment due to the change on the surface (Dong et al., 2018). When
252 microplastics settle into sediments, it would affect the porosity of sediments (Seeley et al., 2020), and
253 the increase of porosity not only promotes the rate of sediment nutrient flux, but also increases the
254 diffusion of oxygen (Cluzard et al., 2015). However, limited information can be available on the effect
255 of oxygen content on nitrogen transformation, and whether it would affect the process and its potential
256 mechanism needs to be further explored.

257

258 **4. Impacts of microplastics on nitrogen transformation and nitrogen cycling in different** 259 **environments**

260 4.1 Water

261 Freshwater is not only an important source and sink of microplastics, but also an important channel
262 and bridge of microplastics from the land to the ocean. In general, the process of water nitrogen
263 transformation is mediated by microorganisms, and the formation of biofilms on the surface of
264 microplastics may affect the process of water nitrogen cycling. A research performed by Chen et al.
265 (2020b) has revealed that the formation of biofilm on polypropylene (PP) microplastic surface can
266 promote the oxidation and denitrification of ammonia ($\text{NH}_4^+\text{-N}$) and nitrite ($\text{NO}_2^-\text{-N}$), thus affecting the
267 nitrogen cycling in the freshwater system (Table 1). The study also pointed out that when the biofilm
268 was mature and disintegrated, the nitrogen and phosphorus elements constituting microorganisms would
269 also be released back to the freshwater environment. Additionally, the release of chemical additives, such
270 as bisphenol A and phthalate, in the microplastics may further harm the microbial community, thereby
271 disturbing the nitrogen cycle in the environment. Miao et al. (2019a) have studied that acute effect of
272 microplastic and nanoplastic polystyrene (PS) with particle size range of 100 nm - 9 mm on five
273 biological endpoints in biofilms. The finding showed that PS with large particle size (500 nm, 1 mm and
274 9 mm) had little effect on the biological endpoints determined in the biofilms, while high concentration
275 nanoplastic PS significantly reduced the content of chlorophyll a and the functional enzyme activities of
276 β -glucosidase and N-(leucine aminopeptidase), demonstrating a negative impact on the carbon and
277 nitrogen cycles of freshwater biofilm.

278 The marine nitrogen cycling is mainly driven by a variety of nitrogen transformation processes

279 mediated by microorganisms, including nitrogen sequestration and retention processes, and
280 denitrification processes (Pajares and Ramos, 2019). Microplastics can affect the photosynthesis and
281 growth of phytoplankton, inhibit zooplankton activities, disturb the marine biological pump and global
282 carbon storage in the ocean (Shen et al., 2019a). Phytoplankton plays a key role in the process of global
283 carbon and nitrogen sequestration, and the inhibition of phytoplankton growth by microplastics may
284 interfere with the marine nitrogen cycles. Furthermore, microplastics can also alter the feeding selectivity
285 of copepods, and interfere with the important links of marine food chain/web and nutrient cycles (Shen
286 et al., 2019d). Cluzard et al. (2015) applied laboratory micro-research to control of microplastic
287 concentration, organic matter content and bivalves to determine the impact of sediment microplastics on
288 the ammonium cycles in intertidal zone, and the research showed that microplastic amended made the
289 ammonium concentration in overlying water significantly higher compared with the treatment without
290 microplastics. This preliminary study indicated that high concentration of microplastics may alter key
291 deposition processes of ammonium flux, having significant consequences such as eutrophication events
292 and red tides because of the increase of ammonium content in the water column. The mechanism of
293 marine nitrogen fixation is relatively complex, but limited information is available on the direct impact
294 of microplastics on marine nitrogen cycles. Most of these conclusions are derived from indirect evidence
295 and analysis, that is, they are expounded by evaluating the impact of microplastics on global marine
296 organisms, and its direct impact on global marine nitrogen cycles is still not fully understood.
297 Consequently, it is urgent to further strengthen the exploration and research on the influence of
298 microplastics on nitrogen cycles in water environment.

299

300 4.2 Soil

301 Microplastic in soil environment mainly comes from agricultural film mulching, sludge utilization,
302 wastewater/river irrigation and composting (Bläsing and Amelung, 2017). Microplastics could
303 significantly reduce the physical and key ecological functions of soil, such as nutrient cycling and soil
304 microbial activity, after entering the soil (Fei et al., 2020; Iqbal et al., 2020). Although some studies on
305 the impact of microplastics and nanoplastics on nitrogen transformation and nitrogen cycling have been
306 done, there are still many limitations, such as the type and single particle size of microplastics and
307 nanoplastics (**Table 1**). Limited by the differences of microplastic concentrations and polymer types, the
308 effects of microplastics on soil nitrogen cycling are not consistent. Awet et al. (2018) investigated the
309 short-term effects (28 d) of PS nanoplastics on soil microbial activity and biomass, as well as the
310 functional diversity of soil enzymes at low levels related to the environment, and the findings
311 demonstrated that nanoplastic addition obviously decreased the activity of N-(leucine aminopeptidase),
312 a key nitrogen cycling enzyme, and affected the potential utilization ability of nitrogen in soil
313 environment. Although nanoplastics have a significant effect on the activity of leucine aminopeptidase,
314 microplastics account for the main type in the environment, and they affect the soil nitrogen
315 transformation by affecting the microbial activity in the soil in a short time. Gao et al. (2021) evaluated
316 the effects of LDPE microplastic pollution on soil microbial community structure and soil nutrient
317 cycling, and the results suggested that microplastic addition had little impact on soil nitrous oxide (N₂O)
318 emission, but significantly promoted carbon dioxide (CO₂) emission. The presence of LDPE
319 microplastics had little effect on soil functional genes of ammonia oxidizing archaea, nitrous oxide
320 reductase and nitrite reductase, while the abundance of ammonia oxidizing bacteria and nitrous oxide
321 reductase was reduced. The addition of microplastics affects the growth of tolerant microorganisms in
322 soil, and indirectly affects nitrogen transformation by affecting the abundance of some important bacteria.

323 Rong et al. (2021) found that presence of LDPE microplastics in soil environment affected niche and
324 nutrient competition and the response of microbial community driven by resistance and resilience to
325 interference was also limited, thereby influencing the correlation of soil bacteria and changing nitrogen
326 cycling driven by soil microorganisms. Microplastic exposure had little effect on soil bacterial diversity
327 even at high concentrations (7%), but it changed the process of soil nitrogen cycling by affecting the
328 structure of soil bacterial network. Additionally, LDPE microplastics can interfere with the hydrolysis of
329 organic nitrogen by affecting microbial community composition and activity of urease in soil
330 environment (Huang et al., 2019). The long-term residual plastic film in agricultural production activities
331 can also reduce the content of soil inorganic nitrogen, down regulate microbial genes related to nitrogen
332 transformation and nitrogen cycling and reduce the activity of related enzymes (Qian et al., 2018).

333 Moreover, the existence of biodegradable plastic could also have different effects on nitrogen
334 transformation and nitrogen cycling in soil. Despite considered as a promising alternative to conventional
335 plastics, biodegradable plastics may produce new microplastics, thereby bringing greater microplastic
336 pollution (Shen et al., 2020a). A study carried out by Chen et al. (2020a) has revealed that PLA
337 microplastics had no obvious effect on the overall diversity and composition of bacterial community and
338 related ecosystem functions and processes, but could promote the transformation rate of ammonia in soil,
339 resulting in faster reduction of NH_4^+ concentration. Therefore, the gradual application of biodegradable
340 plastics in agriculture requires the improvement of test methods to ensure its environmental safety.
341 Furthermore, the additives released by microplastics may also accelerate soil pollution and destroy
342 nutrient circulation by limiting the activity of key enzymes such as nitrite reductase and nitrous oxide
343 reductase. In addition to nitrification and denitrification, whether microplastics will affect the nitrogen
344 cycling processes such as anaerobic ammonia oxidation and nitrate dissimilatory reduction to ammonium

345 and its potential mechanism in soil environment still remains to be explored.

346

347 4.3 Sediment

348 Sediment plays a dual role of source and sink in aquatic ecosystem. In addition to being absorbed
349 and utilized by ascending water organisms, the nitrogen input from external sources will also settle at the
350 bottom of the water body through physical, chemical and biological effects. The nitrogen enriched in
351 sediment will release to the overlying water under certain conditions, which will affect the water
352 environment quality. Meanwhile, sediment is also the natural carrier of microorganisms. The single cycle
353 driven by microorganisms in sediment plays a vital role in maintaining the ecological balance of water
354 body. Single cycle microorganisms are involved in ecological processes such as ammoniation,
355 nitrification and denitrification. The microplastics entering the water body can settle in the sediment
356 through colonizing biofilms in the natural environment, which will affect the structure and function of
357 sediment microbial community, thus interfering with the process of nitrogen cycling. Seeley et al. (2020)
358 have revealed that microplastic could change the microbial community composition and nitrogen
359 cycling processes in sediments, and they could be used as organic carbon matrix of microbial community
360 (Table 1). The findings indicated that the addition of PLA and PUF microplastics in sediments promoted
361 nitrification and denitrification, while PVC microplastics significantly inhibited both processes. The
362 author furtherly demonstrated that considering increasing global microplastic pollution and this powerful
363 evidence, it is worth critically studying the impact of microplastics on ecosystems and nitrogen cycling.
364 Huang et al. (2021) studied the effect of microplastics on nitrogen removal in freshwater sediments with
365 the coexist of microorganisms and benthic invertebrate, and the results showed that microplastic addition
366 can accelerate the growth of denitrifying and anaerobic ammonia oxidizing bacteria, so as to improve the

367 total nitrogen removal rate, implying that the increase of microplastics concentration in freshwater
368 ecosystem is related to the nitrogen cycling mediated by benthic invertebrates. In addition to microbial
369 mediation, benthos in sediments also plays a vital role in the process of nitrogen transformation and
370 nitrogen cycling. Microplastics can also interfere with the nitrogen cycling of sediments by affecting
371 benthos in sedimentary habitats, the findings showed that 1% PE microplastics could have an adverse
372 effect on the biological denitrification mediated by macroinvertebrates (Huang et al., 2021). Green et al.
373 (2016) have assessed the effects of PLA, HDPE and PVC microplastic pollution on the health and
374 biological activity of *Arenicola marina* as well as the nitrogen cycling and primary productivity of the
375 sediments, and the results indicated that the metabolic rate of *Arenicola marina* increased and its
376 burrowing behavior was also disturbed, having an adverse effect on the health and biological activity of
377 benthos and the nitrogen cycling processes dominated by them. Additionally, Hope et al. (2020) have
378 also reported that the increase of microplastic fiber concentration would affect the multiple ecological
379 functions mediated by benthos in offshore sediments, and then further disturb the nitrogen transformation
380 and nitrogen cycles.

381 Microplastics of different types mostly promote the nitrification and denitrification processes in
382 sediments. In the aspect of mechanism research, microplastics indirectly interfere with the nitrogen
383 cycling in the sediment environment through adverse effects on the metabolic behavior and biological
384 activity of benthos. Unfortunately, however, relevant studies mainly focus on the short-term impact of
385 microplastics, and it is still unknown whether the nitrogen transformation process in sediments would
386 continue to deteriorate or recover under the long-term effect. Moreover, most microplastics in the actual
387 environment have different degrees of aging and biofilm adhesion, whether these phenomena would
388 change their impact on nitrogen transformation requires to be further considered. In addition, follow-up

389 studies should pay more attention to the direct impact and path of microplastics in sediments on nitrogen
390 transformation and nitrogen cycling.

391

392 4.4 Sewage sludge

393 Sewage treatment plants are an important collecting and distributing center of microplastics in the
394 environment. The primary treatment and secondary treatment in municipal sewage treatment plant can
395 remove more than 90% of microplastics in the sewage, but if excessive sewage is discharged into the
396 environment, a large number of microplastics will still enter the water environment (Shen et al., 2020b;
397 Sun et al., 2019). Although the microplastics in sewage can be treated these times to achieve a certain
398 effect, these processes are not specially designed to remove microplastics. At present, the removal rate
399 of microplastics in sewage plants is generally calculated by the concentration of microplastics in inlet
400 and outlet water (Shen et al., 2021a). Without the establishment of a special technology to remove
401 microplastics, more microplastics removed in sewage treatment plants are retained in sewage sludge.
402 Denitrification is an important part of sewage treatment process, and due to the influence of microplastics,
403 the denitrification capacity of activated sludge and aerobic granular sludge is disturbed (**Table 1**). A
404 research performed by Li et al. (2020) has revealed that five microplastics (PE, PES, PP, PS, and PVC)
405 with different concentrations (1000, 5000, and 10000 particles/L) have similar effects on activated sludge
406 nitrification and denitrification. These microplastics have an adverse effect on ammonia oxidation rate
407 in activated sludge nitrification process, while have little effect on nitrite oxidation rate, and promote the
408 denitrification process. In addition, high concentration of PVC microplastics significantly increased the
409 emission of N₂O in the denitrification process, and the findings showed that the N₂O emission with
410 addition of PVC microplastics 10000 particles/L was about 4.6 times than that of the control group. Dai

411 et al. (2020) also found that PVC microplastics could promote the release and absorption of phosphorous
412 by phosphorous accumulating bacteria, and inhibit the removal of total inorganic nitrogen by reducing
413 the content of denitrifying bacteria. Qin et al. (2020) reported that PES microplastics had little inhibitory
414 effect on ammonia nitrogen removal in aerobic granular sludge, while the total nitrogen removal
415 increased by 5.6% with addition of PES. Moreover, PES microplastics inhibited the activity of nitrite
416 oxidase in the nitrification process of aerobic granular sludge and promoted the growth of nitrate
417 reductase, thus resulting in the accumulation of nitrite and disturbing the process of nitrogen metabolism.
418 Cui et al. (2021) reported that PA microplastics had little short-term and long-term effect on activated
419 sludge partial nitrification system, but the ammonia oxidation rate decreased slowly with the increase
420 concentration of PA microplastics. The microplastic amended could also reduce microbial activity and
421 change the microbial community structure and reproduction of the system. Song et al. (2020) investigated
422 the effect of PVC microplastics on partial nitrification process with different concentration range of 0 –
423 10000 particles/L, and the findings showed that with the increase of PVC concentration, the mobility rate
424 of nitrite in the system was significantly reduced, and the ammonia oxidation rates and average emission
425 of dissolved N₂O were also decreased in carrying degree compared with the control group because of the
426 limitation on the activities of ammonia oxidation bacteria and nitrite oxidizing bacteria.

427 At present, the research on the effect of microplastics on nitrogen transformation of sewage sludge
428 is still in its infancy, and there is no consistent conclusion. The impacts of microplastics on sewage sludge
429 nitrogen transformation and nitrogen cycling mainly affect the process of nitrification and denitrification.
430 The nitrification process of sewage sludge may be inhibited because the ammonia oxidation process or
431 nitrite oxidation process is limited by the presence of microplastics, and the denitrification rate is usually
432 improved accordingly. In addition, microplastics not only can act as an important microbial carrier, but

433 also hinder the absorption of dissolved oxygen by sewage sludge, from anaerobic atmosphere, and
434 promote the denitrification process to a certain extent. The existence of microplastics will interfere with
435 the denitrification performance of sewage sludge in sewage treatment system, mainly resulting in the
436 accumulation of nitrite and the increase of greenhouse gas N₂O emission. In addition, the monomers and
437 additives released from microplastics could affect the nitrogen transformation process of sewage sludge,
438 but the relevant mechanism needs to be further explored.

439

440 **5. Perspectives and challenges**

441 Microplastics can directly affect the nitrogen cycling by disturbing the microbial community
442 structure and productivity, changing the quality and quantity of organic matter input and the
443 decomposition rate of organic matter, and indirectly affect the nitrogen cycling processes by altering
444 biological activity. Although some studies have proved the impact of microplastics on the key processes
445 of environmental nitrogen cycling, and the current research still focuses on the “phenomenon” and the
446 mechanism is still not fully understood. Therefore, it is necessary to observe and study the response
447 characteristics and mechanisms of microplastics to the change of global nitrogen cycling at different
448 scales in the future.

449

450 **5.1 Evaluating complex environmental systems**

451 The research on the influence of nitrogen transformation in microplastics is mainly focuses on soil
452 and sewage sludge, while it is still in its infancy in other environmental media. This limits the accurate
453 evaluation of the change trend of nitrogen cycling caused by microplastic pollution under global change
454 to a certain extent. The overall environment is relatively single, and the interference effect of

455 microplastics in complex system, such as intertidal zone, mangrove area and other key zones, on nitrogen
456 cycling and its related mechanisms still require to be clarified, especially the influence process and
457 mechanism of horizontal and vertical distribution of microplastics on nitrogen cycling. It should be to
458 explore the effects of physical, chemical, environmental and biological factors involved in the whole
459 cycle process, to develop and construct nitrogen cycling models, to master the dynamic changes of
460 nitrogen cycling in each reaction system, to realize the effective monitoring and provide scientific basis
461 regulation and management.

462

463 5.2 Exploring impact of long-term effects

464 The inference about the effect of microplastics on nitrogen transformation and nitrogen cycling in
465 environmental media is mainly based on short-term theoretical experiments. In the short term,
466 microplastics addition would affect the nitrogen transformation processes, but it is still unknown whether
467 the nitrogen transformation process would continue to deteriorate or recover in the long term. The short-
468 term experiment is an accelerated experiment, and the difference between the experimental conditions
469 and the real conditions will increase, which is not conducive to the conclusion of real phenomena and
470 results. As such, the research on the impact of microplastics on nitrogen transformation process under
471 long-term action is more helpful to deeply understand the impact of microplastic pollution on global
472 nitrogen transformation and nitrogen cycling.

473

474 5.3 Strengthening exploration of potential mechanism

475 Nitrogen cycling processes mainly include nitrogen fixation, nitrification, denitrification, anaerobic
476 ammonia oxidation and dissimilatory reduction of nitrate to ammonium. Microplastics could affect the

477 nitrogen transformation processes by disturbing microbial colonization, releasing additives, broken into
478 nanoplastics and inducing reactive oxygen species. Nevertheless, at present, the existing studies mainly
479 focused on the impact of microplastics on nitrification and denitrification processes, and have achieved
480 remarked results, while limited information is available on whether microplastics would affect other
481 nitrogen transformation processes, especially on related functional microorganism, enzymes activities
482 and functional genes. The coupling mechanism of nitrogen fixation, nitrification, denitrification,
483 anaerobic ammonium oxidation and dissimilatory reduction of nitrate to ammonium in the same reaction
484 system should be strengthened. Enzymes produced by microbial metabolism are the key to control the
485 transformation process, and the gene encoding enzymes is the driver of the whole process. The interaction
486 between microplastics and microorganisms and its impact on nutrient cycles involves metabonomics,
487 microbiology, transcriptomics and biochemistry, which requires the joint efforts of multiple disciplines.

489 5.4 Carrying out multi-factor and multi-level research

490 The rise of temperature, the increased of atmospheric CO₂ concentration and nitrogen deposition
491 and the change of precipitation pattern are important characteristics of global climate change. Climate
492 change has a significant impact on biogeochemical cycle, ecosystem and aboveground and underground
493 biological interaction. These changes can alter microbial species distribution, population dynamics and
494 habitat, resulting in the loss of biodiversity on a global scale. Warming can affect the decomposition of
495 organic matter, respiration and element mineralization, so as to affect the stability of carbon pool and
496 nitrogen pool. Therefore, in order to reveal the response process and mechanism of microplastics to
497 nitrogen transformation and nitrogen cycling under global change, it is necessary to comprehensively
498 study the interaction of microplastics with other driving factors such as temperature, climate, moisture

499 and nitrogen deposition.

500

501 5.5 Comprehensive applying isotope and molecular biology combined with indoor simulation and field

502 test

503 At present, the main research of microplastics on nitrogen cycling is indoor simulation test. However,

504 unfortunately, in terms of controlling experimental conditions, indoor culture is quite different from the

505 actual situation in the field, and there are many uncontrollable factors. Accordingly, in the future research,

506 the experimental scheme combining field in situ and indoor culture should be added so as to improve the

507 scientificity of the results and deeply and comprehensively reveal the regulation mechanism of

508 microplastics on the key ecological processes of nitrogen cycling. To fully understand how microplastics

509 and microorganisms affect the nitrogen cycling, systematic research needs to be carried out in a variety

510 of ecosystems, such as farmland, grassland, wetland and forest ecosystem, to clarify the feedback

511 mechanism of animals and microorganisms on environmental changes. Additionally, multi-factors and

512 multi-level long-term tracking control test will help to ensure the accuracy and integrity of the test results.

513 Stable isotope analysis provides a powerful research tool for revealing and quantifying the nutritional

514 relationship in the food chain/web, while molecular technology can further understand the interaction

515 and functional significance between microplastics and microorganisms. In the mechanism research, the

516 means and methods of the combination of molecular biological and macrogenomics should be full used

517 to explore the diversity of functional flora and enzymes in the key process of nitrogen cycling. Exploring

518 the effects of functional genes and nitrogen transformation processes has important guiding significance

519 for studying the microbial driving mechanism of various nitrogen cycling processes in the future.

520

521 **6. Conclusions**

522 In summary, as a new environmental pollutant, microplastics can not only threaten the health of
523 organisms, but also affect the normal nitrogen cycling through disturbing colonized microorganisms on
524 microplastics. This paper reviews the potential effects of microplastics in four environmental media
525 (water, soil, sediment and sewage sludge) on nitrogen transformation and nitrogen cycling and the
526 corresponding mechanisms. Microplastics mainly affect nitrogen transformation via influencing
527 microorganism diversity and structure, enzyme activities, related functional genes and oxygen flux of
528 the system. Due to the lack of relevant research, the more mechanisms of microplastics on environmental
529 nitrogen transformation and nitrogen cycling are not very clear. It is an effective strategy to further
530 explore the impact of microplastics on environmental nitrogen cycling by evaluating complex
531 environmental systems, prolonging the action time and assisting multi-factor and multi-level research.
532 In addition, molecular biology and stable isotope technology have been used to explore the role of
533 microorganisms in nitrogen transformation. The isotopic effect analysis of microbial functional genes
534 and transformation processes would further clarify the driving mechanism of microplastics in the
535 nitrogen cycling, which can provide support for the comprehensive evaluation of the impact of
536 microplastics on ecological environmental health, and have important guiding significance in exploring
537 the nitrogen cycling processes of global biogeochemistry.

538

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544 **Declaration of interest**

545 The authors have no conflict of interest to declare regarding this article.

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