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Effects of Different-Sized Polyethylene Microplastics on Nitrogen Transformation in River Sediment-Water Systems

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Abstract: As an emerging pollutant, the impact of microplastics' physical properties on key biogeochemical cycles remains unclear. This study investigated the effects and mechanisms of four particle sizes (25, 150, 200, 500 μm) of polyethylene microplastics (PE-MPs) on nitrogen transformation in river systems through a 120-day indoor micro-cultivation experiment. Results indicate that microplastic addition significantly enhanced nitrogen partitioning from the water phase to sediments, leading to reduced total nitrogen and nitrate concentrations in overlying water. Small-sized (25 μm) microplastics strongly promoted ammonium nitrogen accumulation in sediments during long-term exposure, while large-sized (500 μm) microplastics induced dramatic fluctuations in sediment total nitrogen content, revealing complex adsorption-desorption dynamics. Correlation analysis indicates that sediment ammonium accumulation is significantly positively correlated with iron content. This study reveals that microplastics primarily disrupt nitrogen transformation processes by altering sediment microenvironments and providing microbial attachment sites, ultimately inhibiting nitrification and leading to the accumulation of nitrogen in the sediment as reduced ammonium salts. The study emphasizes that microplastic size is a key physical parameter governing its interference with the nitrogen cycle, providing crucial evidence for scientifically assessing its ecological risks.

Key words: Microplastics, size effect, nitrogen form, sediment-water system.

1. Introduction

Microplastics (MPs) refer to plastic particles smaller than 5 mm in size. They originate from diverse sources, exhibit complex properties (such as varying shapes, sizes, densities, and types), degrade slowly, and continuously accumulate in the environment, posing significant challenges to biological health and environmental safety. Research on microplastics began in marine environments, yet terrestrial ecosystems serve as important sinks for microplastics, potentially harboring quantities 4 to 23 times greater than those found in the ocean [1-6]. Therefore, it has gradually attracted the attention of scholars both domestically and internationally [5, 6]. MPs pollution in freshwater environments is also becoming increasingly severe, with MPs contamination prevalent in freshwater bodies such as rivers and lakes worldwide. The abundance of MPs detected in freshwater bodies can reach 10^7 pieces per square kilometer, surpassing the abundance of MPs detected in marine environments.

River ecosystems serve as vital links connecting land and sea, and their health and stability are crucial to global biogeochemical cycles and human well-being [7]. Nitrogen is a vital biogenic element in aquatic ecosystems. The distribution and transformation processes of its various forms, including ammonium nitrogen, nitrate nitrogen, nitrite nitrogen, and organic nitrogen, directly influence primary productivity, water quality, and even the risk of eutrophication in downstream estuaries and coastal environments [8]. In recent years, with the acceleration

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of industrialization and urbanization, river systems are facing the combined pressures of traditional pollutants and emerging contaminants [9].

Existing research indicates that microplastics can disrupt material cycles and energy flows in aquatic ecosystems through direct or indirect pathways. Regarding the nitrogen cycle, microplastics due to their large specific surface area and hydrophobic properties can adsorb nitrogen compounds and associated conversion enzymes from water bodies, serving as microbial attachment hotspots. This may significantly alter the pathways and rates of nitrogen transformation [10]. However, current research has primarily focused on the occurrence characteristics of microplastics, their ecotoxicology, or their impact on the migration of pollutants such as antibiotics. The key processes by which the physical properties of microplastics themselves—particularly size variations—regulate nitrogen transformation and migration in rivers remain poorly understood. Microplastics of different sizes may exhibit significant variations in environmental suspension, migration capacity, surface properties, and interactions with microbial communities. These differences are likely to result in distinct impacts on nitrogen transformation processes such as nitrification, denitrification, ammonification, and nitrogen fixation, thereby reshaping the distribution and ultimate fate of nitrogen forms within rivers.

To date, systematic research on the effects of PE-MPs of different particle sizes on sediment nitrogen cycling processes and their driving microorganisms remains scarce. To address this gap, this study employs laboratory simulation experiments to investigate the influence of four distinct PE-MPs particle sizes on nitrogen speciation in overlying water and sediments.

2. Materials and Methods

2.1 Sediment Sample Collection and Microscopic Experiment Design

Sediment samples for the experiment were collected from the Chishui River basin in July 2024. Surface sediments (0-10 cm) were obtained using a Peterson grab sampler. Collected sediments were immediately placed into pre-chilled sterile glass containers, stored in ice packs, and rapidly transported to the laboratory. In the laboratory, sediment samples were thoroughly mixed after removing visible impurities such as gravel, shells, and plant/animal debris to ensure homogeneity. The homogenized sediment was then aliquoted and stored at 4 °C for later use. All samples were utilized within one week of collection for establishing microculture experiments. PE-MPs used in this study were sourced from Dongguan Shuangfu Plastic Raw Material Co., Ltd. The micro-scale experimental design comprised five microplastic size gradient treatment groups: (1) blank control (N0); (2) 25 μm (N25); (3) 150 μm (N150); (4) 200 μm (N200); (5) 500 μm (N500), with three replicates per group. Experiments were conducted in 15 glass columns (diameter 0.14 m, height 0.2 m, volume 2 L).

Microplastics were added at a dose of 7.5 g MPs/1500 g sediment and thoroughly mixed prior to water addition. To minimize sediment disturbance, water samples were slowly injected along the column walls using syringes at a water-to-sediment ratio of 2,000 mL/1,500 g. All control and treatment groups were established in August 2024 and commenced cultivation (Fig. 1). Surface water samples were collected at 0 days, 5 days, 15 days, 20 days, 30 days, 45 days, 60 days, 90 days, and 120 days. Sediment samples were collected at 0 days, 30 days, 60 days, and 120 days.

2.2 Determination of Physical and Chemical Properties

Overlying water temperature (T), pH, electrical conductivity (EC), dissolved oxygen (DO), and total dissolved solids (TDS) were measured using portable water quality analyzers (WTW, Germany; HACH HQ30d, USA; Leizhi, China). Total nitrogen (TN) and ammonium nitrogen (NH_4^+-N) were determined using the potassium persulfate method and the Nessler's reagent method, respectively [11].

Sediment samples were freeze-dried, ground, and then extracted using 1 mol/L potassium chloride solution at a liquid-to-solid ratio of 5 mL:1 g. At 25 °C, the mixture was shaken at 200 rpm for 1 hour. Potassium chloride solution extraction-spectrophotometry was employed to determine sediment concentrations of NH_4^+, nitrate nitrogen (NO_3^-), and nitrite nitrogen (NO_2^-) [12-14]. pH and ORP (oxidation-reduction potential) were measured on-site using a portable soil analyzer (Leizhi, China). Total nitrogen (TN) was determined by the Kjeldahl digestion method [15]. Total iron (Fe) and ferrous iron (Fe^{2+}) content were determined by the o-phenanthroline method [16]. The trivalent iron (Fe^{3+}) content was calculated using the difference method (total Fe content minus Fe^{2+} content). Organic matter (OM) was determined by the low-temperature external heating potassium dichromate oxidation-colorimetric method [17]. Total phosphorus (TP) was determined by the sodium hydroxide fusion-molybdenum antimony colorimetric method [18]. The moisture content (ω) is determined by the gravimetric method [19].

2.3 Statistical Analysis

Organize and calculate experimental data using Excel software. Plot data using Origin Pro 2025, with all figures presenting data as mean ± standard deviation. Employ the pheatmap and plspm packages to perform Spearman correlation analyses, investigating the impact of microplastic-induced changes in the cosmic microenvironment on nitrogen transformation processes.

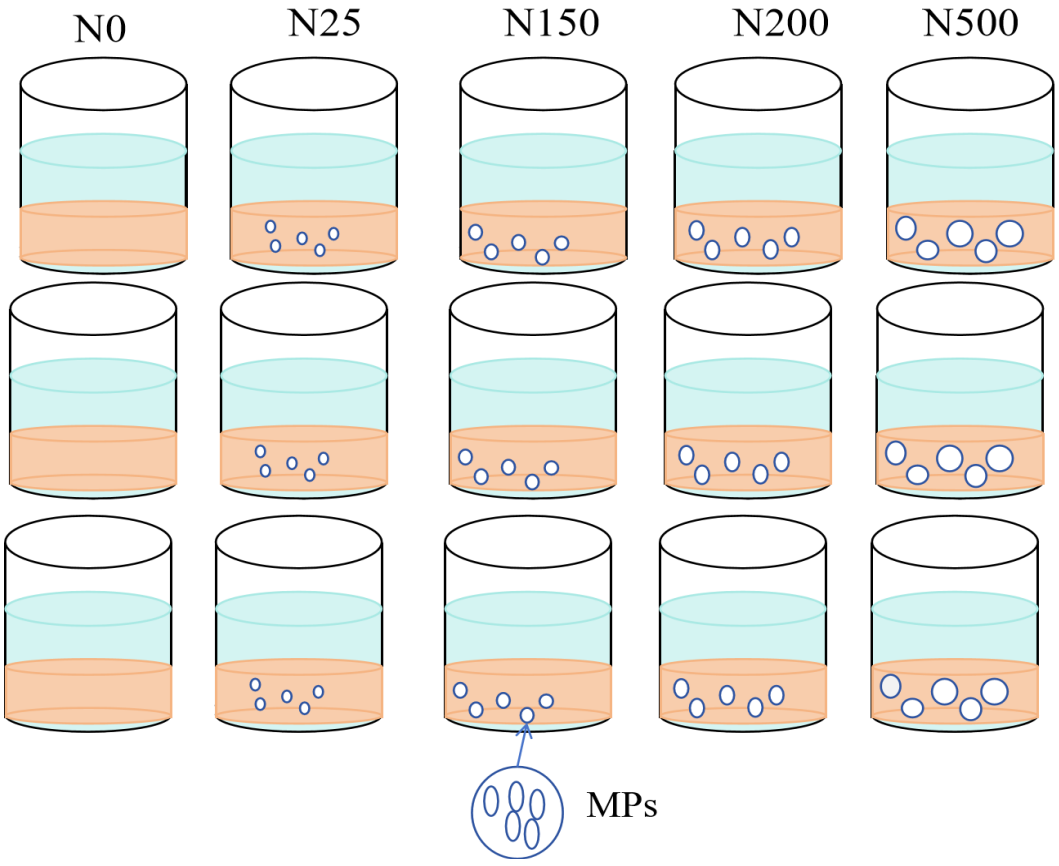

Fig. 1 Schematic diagram of the simulation experiment.

3. Results and Discussion

3.1 Changes in Nitrogen Forms in Overlying Water

Experimental results indicate (Fig. 2) that the total nitrogen concentration in the blank control group (N0) remained at a relatively high level (3.236-3.672 mg/L), significantly higher than that in all microplastic-added treatment groups (N25, N150, N200, N500). The presence of microplastics may accelerate nitrogen removal or inhibit its accumulation in aquatic environments through mechanisms such as adsorption, promoting transformation, or influencing microbial processes. The significant decrease in total nitrogen concentration during the initial phase (Day 15) may be attributed to the rapid adsorption of dissolved nitrogen by microplastics and the swift utilization of available nitrogen by early microorganisms. The subsequent slight rebound observed on Day 20 could result from gradual saturation of adsorption on microplastic surfaces, desorption of partially adsorbed nitrogen, or phased adjustments in microbial community structure. After 90 days, concentrations stabilized, indicating that the system gradually reached a new dynamic equilibrium in the presence of microplastics, with microbial metabolism and nitrogen transformation processes entering a relatively stable adaptation phase. Notably, the N200 group reached its lowest total nitrogen concentration (1.172 mg/L) on day 30, while the N150 group exhibited a significant increase at day 150.

NH_4^+-N concentrations in the overlying water remained at relatively high levels in the N0 group (0.318-0.387 mg/L), while they were generally lower in the microplastic-treated groups (especially N200 and N150). Among these, N200 reached its lowest value (0.028 mg/L) on day 120. Overall, the trends in the control group were similar to those in the experimental groups, indicating that under the experimental conditions, the addition of microplastics did not significantly affect the transformation and accumulation of ammonia nitrogen.

Overall, the blank control group (N0) exhibited the highest NO_3^--N concentrations at all time points, ranging from 2.422 to 3.163 mg/L, significantly higher than all microplastic-amended treatment groups (N25, N150, N200, N500). where concentrations generally ranged from 0.59 to 1.985 mg/L. Over time, most treatment groups exhibited fluctuating decreases in NO_3^--N concentrations, with further reductions observed at 120 days, suggesting potential gradual transformation or migration of nitrates. The N200 group recorded only 0.59 mg/L at 30 days but subsequently rose steadily, reaching 1.139 mg/L by 120 days. The N150 group peaked at 1.985 mg/L at 45 days, representing the highest mid-term value among all treatment groups.

3.2 Changes in Nitrogen Forms in Sediments

Total nitrogen (TN) content in sediments exhibited significant variations across different treatment groups and incubation periods, with overall values ranging from 168 to 476 mg/kg. The TN content in the microplastic-free N0 group remained relatively stable, whereas the TN levels in the microplastic-amended groups (N25, N150, N200, N500) exhibited distinct trends over the cultivation period, indicating that microplastic addition influenced sediment nitrogen accumulation and transformation. Except for the N500 group, TN levels in all addition groups were significantly higher at 120 days compared to initial or 30-day levels. Notably, the N25 group increased from 168 to 448 mg/kg, representing a 167% increase. This suggests that low-to-moderate microplastic concentrations may enhance sediment TN through adsorption, suppression of nitrogen volatilization, or promotion of organic nitrogen deposition. The N500 group reached 476 mg/kg TN at 30 days, significantly higher than other groups, then decreased to 280 mg/kg at 60 days before rebounding to 392 mg/kg at 120 days. This sharp fluctuation likely reflects intense initial adsorption of nitrogen-containing substances by high-concentration microplastics, followed by partial nitrogen release due to environmental disturbance or microbial activation,

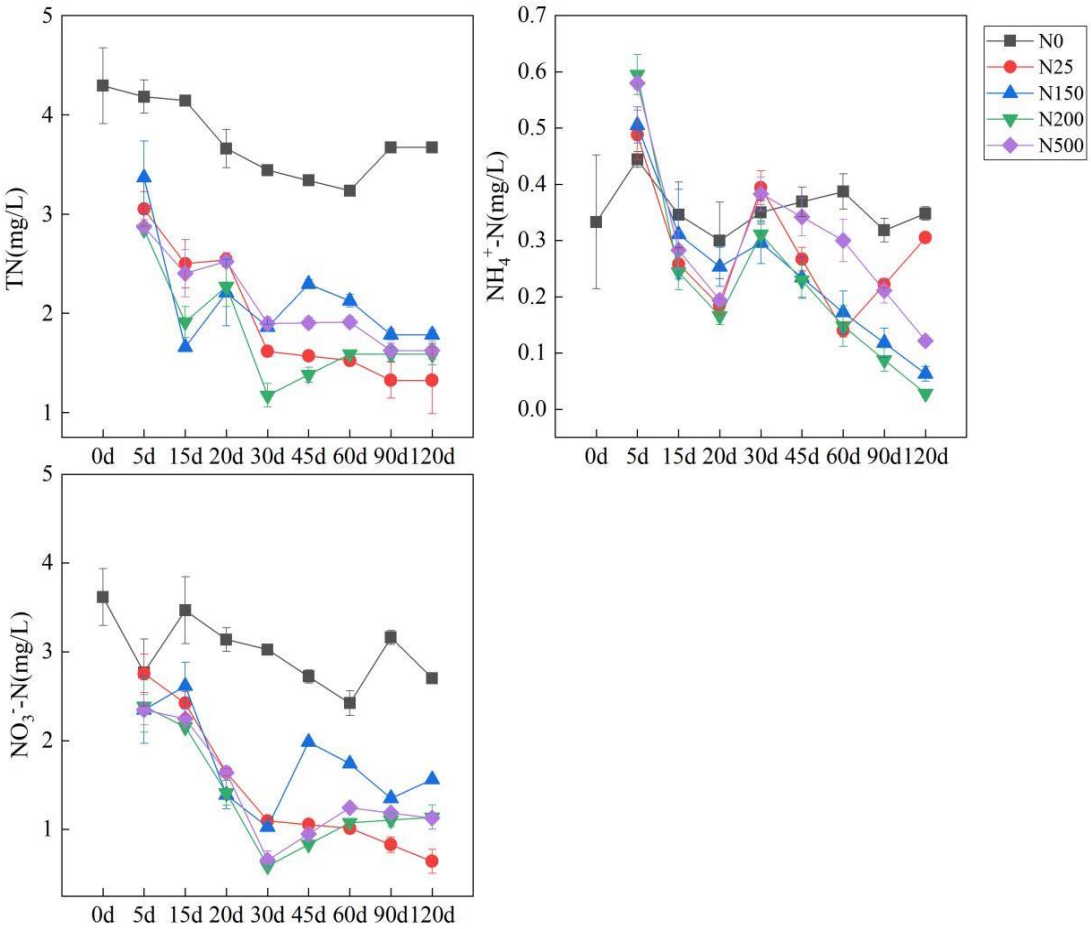

Fig. 2 Changes in nitrogen forms in overlying water.

and subsequent re-accumulation. Overall, as incubation time increased (particularly at 120 days), most microplastic-treated groups showed an upward trend in TN, indicating that prolonged exposure to microplastics enhances the sediment nitrogen sink function.

NH_4^+ content in sediments exhibited significant variations across treatment groups and different incubation periods, ranging overall between 58.34-392.24 mg/kg. Overall, NH_4^+ levels in the microplastic-amended groups were consistently higher than the control group (N0) during the later cultivation period (120 days), exhibiting an upward trend with prolonged incubation. This indicates that microplastics promote the accumulation of ammonium nitrogen in sediments. The NH_4^+ concentration in the blank control group gradually increased from 93.19 mg/kg (30 days) to 144.83 mg/kg (120 days), indicating that ammonium nitrogen exhibits some natural accumulation in systems without microplastics, albeit at a limited rate. All microplastic-treated groups exceeded 300 mg/kg NH_4^+ at 120 days, with the N25 group reaching 392.24 mg/kg —approximately 2.7 times that of the N0 group during the same period. This indicates that low-to-moderate microplastic concentrations strongly promote ammonium nitrogen retention or generation. Except for the N0 group, NH_4^+ levels in all treatment groups increased significantly between 60 and 120 days, suggesting that microplastics may exert a sustained influence on nitrogen mineralization or ammonification processes during long-term cultivation. Notably, during the initial cultivation phase (30 days), NH_4^+ levels in all microplastic-treated groups were lower than those in the N0 group.

The NO_3^- content in sediments remained generally low (0.08-19.74 mg/kg), significantly lower than the NH_4^+ and TN concentrations during the same period. During the initial cultivation phase, NO_3^- levels in the N150, N200, and N500 groups all exceeded 14 mg/kg, with N500 reaching 19.74 mg/kg on day 30. This suggests microplastics may promote nitrification or inhibit denitrification in the short term. The N150 group exhibited a NO_3^- concentration of 14.14 mg/kg at day 30, while NH_4^+ was only 69.45 mg/kg during the same period. By day 120, NO_3^- decreased to 0.29 mg/kg while NH_4^+ increased to 353.37 mg/kg, potentially reflecting a dynamic equilibrium in nitrification-denitrification or ammonium nitrogen transformation processes.

NO_2^- concentrations in sediments ranged from 20.66 to 51.72 mg/kg, generally significantly higher than NO_3^- but consistently lower than NH_4^+. Trends in NO_2^- levels were largely consistent across treatment groups, showing an overall decline over time. This decrease was particularly pronounced after long-term incubation (120 days), with most treatment groups exhibiting lower NO_2^- concentrations compared to initial measurements. Compared to the N0 control, NO_2^- levels in all microplastic-amended groups were generally lower at 30 days and exhibited a slower decline over time. Notably, the N200 group even showed a slight increase in NO_2^- at 120 days, suggesting that microplastics may either delay nitrite conversion or promote its formation.

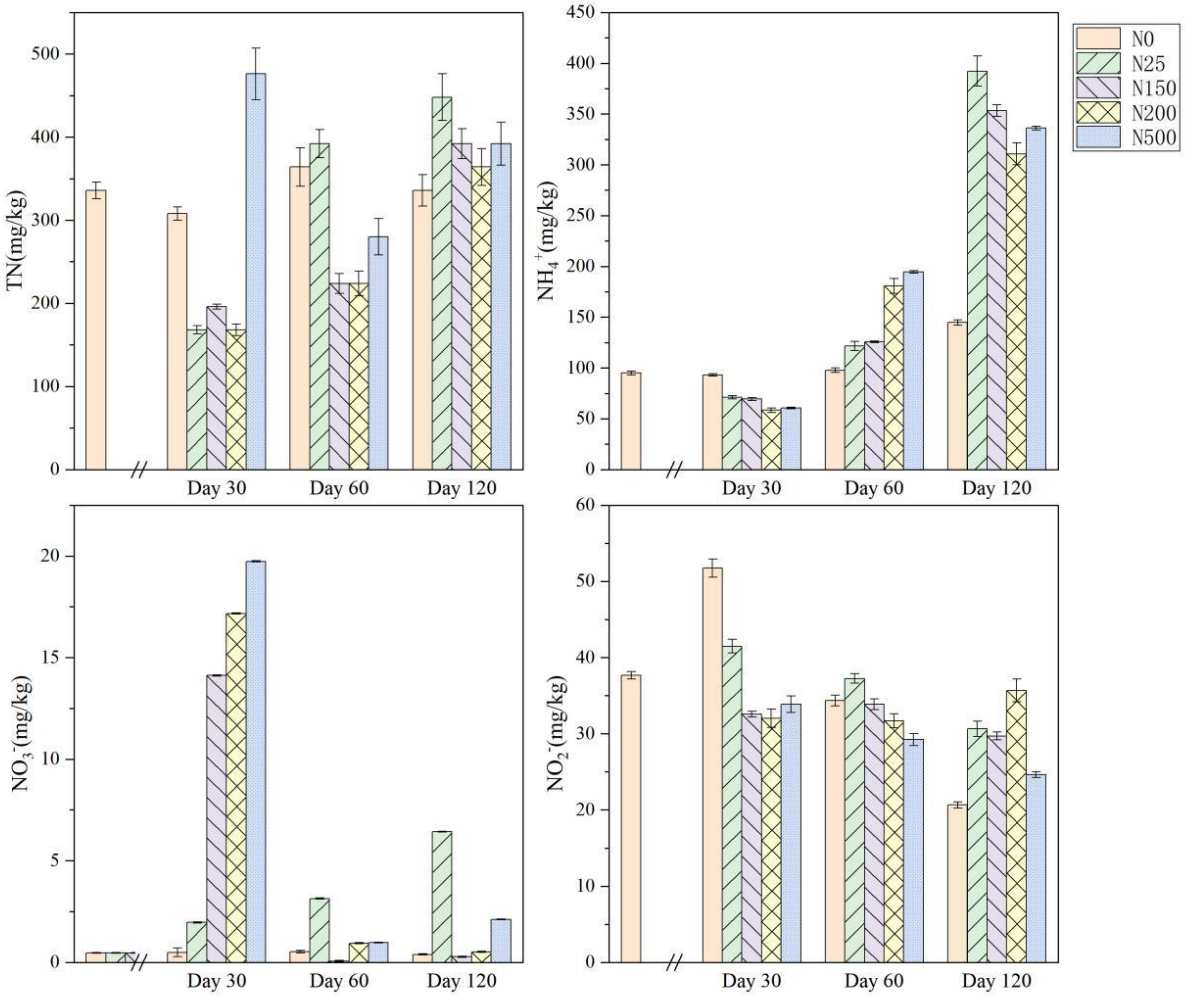

Fig. 3 Changes in nitrogen forms in sediments.

3.3 Effects of Microcosmic Environments on Pair Morphology

As shown in Fig. 4a, the significant positive correlation between TN and NH_4^+-N and NO_3^--N indicates that nitrogen in the overlying water primarily exists as inorganic nitrogen ($p < 0.05$), and the three nitrogen forms share common origins or transformation pathways. The significant negative correlation between NH_4^+-N and DO confirms its greater accumulation in hypoxic environments, potentially linked to enhanced ammonification at the sediment-water interface or inhibited nitrification ($p < 0.01$) [20]. Conversely, positive correlations with ORP and TDS suggest NH_4^+-N may remain stable in microenvironments with higher redox potentials and greater ionic strength ($p < 0.05$) [21]. The highly significant positive correlation between NO_3^--N and TN further indicates that under relatively favorable oxidation conditions in the overlying water, the nitrification process is active, making NO_3^--N one of the primary forms of inorganic nitrogen ($p < 0.001$).

The significant positive correlation between TN and iron in different valence states (Fe, Fe^{2+}, Fe^{3+}) may reflect the adsorption-fixation of nitrogen by iron oxides ($p < 0.05$). In particular, iron-bound nitrogen formed on sediment particle surfaces could constitute a major component of TN. The highly significant positive correlations between NH_4^+ and pH, Fe, Fe^{2+}, and Fe^{3+} indicate that in alkaline sediment environments with active iron cycling, iron reduction processes may couple with organic matter mineralization, jointly promoting NH_4^+ production and accumulation ($p < 0.001$) [22]. The significant negative correlation between NO_3^- and OM/TP suggests that in sedimentary areas enriched with organic matter and phosphorus, denitrification may be more pronounced, leading to the reduction and removal of NO_3^- ($p < 0.05$). The negative correlation between NO_2^- and pH aligns with the chemical behavior characteristic of nitrite, which accumulates more readily at lower pH levels ($p < 0.05$), potentially related to the conversion equilibrium of intermediate products in the nitrification process [23].

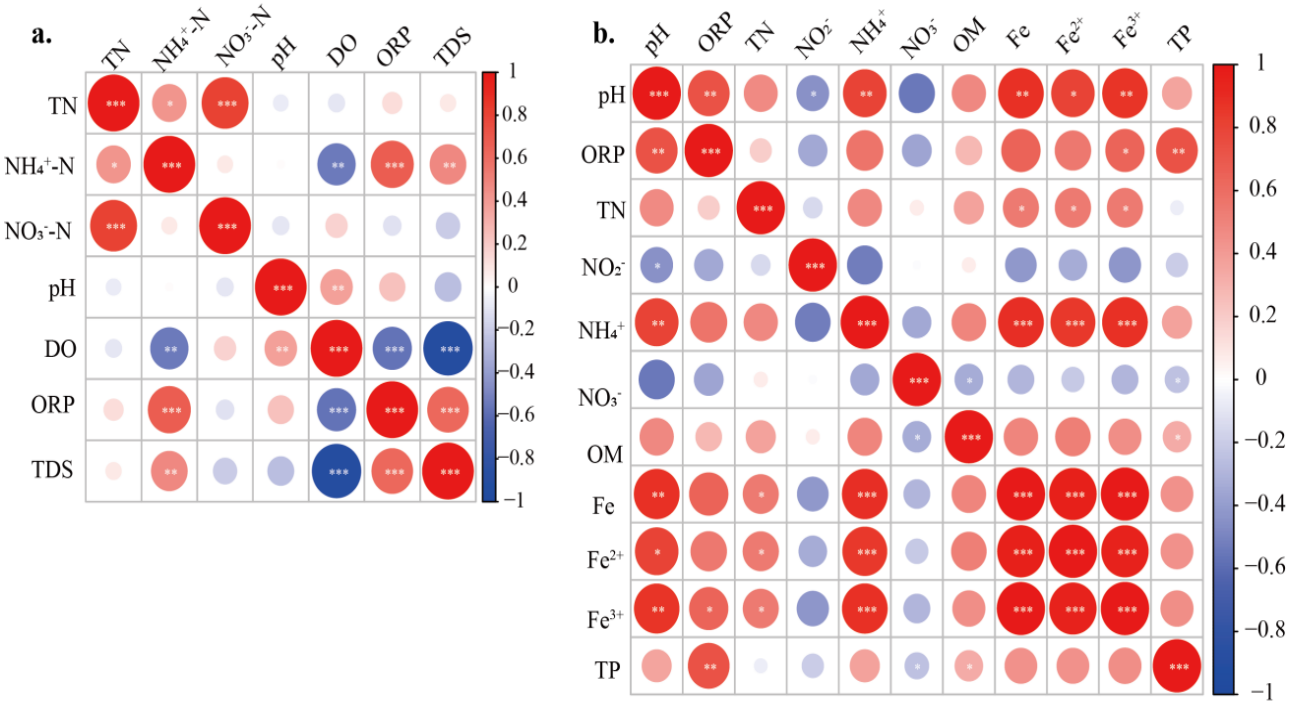

Fig. 4 Correlation analysis of environmental factors. a. Overlying water. b. Sediment. (*R* values are displayed in different colors in the figure, while p-values are marked with asterisks: * indicates $p < 0.05$; ** indicates $p < 0.01$; * indicates $p < 0.001$).**

4. Conclusion

This study investigated the effects of polyethylene microplastics of varying sizes (25, 150, 200, 500 μm) on riverine nitrogen cycling through simulation experiments. Results indicate that microplastics significantly promoted nitrogen transfer from the water phase to sediments, reducing total nitrogen and nitrate nitrogen concentrations in overlying water. Long-term exposure to small-sized microplastics (25 μm) strongly promoted ammonium nitrogen accumulation in sediments, while large-sized microplastics (500 μm) induced dramatic fluctuations in total nitrogen, exhibiting complex adsorption-desorption dynamics. Mechanistically, microplastics primarily disrupted nitrogen transformation processes by altering sediment microenvironments (promoting iron reduction) and providing microbial attachment sites. This ultimately inhibited nitrification, leading to the accumulation of nitrogen in sediments as reduced ammonium salts. These findings reveal that the physical properties of microplastics are critical factors in assessing their ecological risks.

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