

Freshwater organic micropollutants alter submerged macrophyte litter decomposition dynamics and microbial community in the surface sediments

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Abstract

Purpose Aquatic plant decomposition in aquatic ecosystems is a natural process that releases essential nutrients (C, N and P), which in turn influences the composition and function of sediment microbial communities. Antibiotics, as emerging contaminants widely present in aquatic environment, have the potential to disrupt litter decomposition and microbial activity. However, the ecosystem-level consequences of such alterations remain poorly understood. This study investigated the effects of two model antibiotics, i.e. norfloxacin and doxycycline, present individually and in combination, on nutrient release during the decomposition of *Ceratophyllum demersum* litter, as well as on sediment physicochemical properties and microbial diversity and community structure.

Methods A controlled microcosm experiment was conducted using *C. demersum* litter and antibiotic treatments (norfloxacin, doxycycline, and their combination). We monitored nutrient release dynamics (C, N and P) during decomposition, measured sediment physicochemical parameters and characterized sediment microbial diversity and community composition using high-throughput sequencing.

Results The litter input from *C. demersum* reduced bacterial diversity in the sediment. Antibiotic exposure, whether single or combined, further slowed nutrient release from decomposing litter and decreased microbial diversity. Moreover, both litter addition and antibiotic exposure significantly altered the composition of the sediment microbial community.

Conclusions The combined inputs of plant litter and antibiotics can jointly reshape microbial community structure and reduce diversity, inhibit nutrient mineralisation and modify the physicochemical characteristics of sediments. This study provides an integrative framework for assessing the potential risks posed by antibiotic contamination and organic matter decomposition to aquatic ecosystem functioning and offers guidance for future ecosystem management.

Keywords: Antibiotics; Aquatic ecosystem; *Ceratophyllum demersum*; Lake restoration; Nature-based solution.

1. Introduction

Plant litter decomposition is a key ecosystem process that regulates global carbon cycling and nutrient dynamics (Han et al. 2024). In aquatic ecosystems, the decomposition of macrophyte litter releases dissolved organic matter (DOM) and nutrients, such as nitrogen (N) and phosphorus (P), into the water column and sediment (Zhao et al. 2021; Zhang et al. 2022). These released compounds stimulate microbial metabolism, alter sediment biogeochemistry, and potentially exacerbate eutrophication in nutrient-sensitive systems. The resulting increase in nutrient loading can enhance primary production and promote algal bloom under favourable conditions (Paumelle et al. 2021; Wei et al. 2024; Wang et al. 2024). Microbial respiration and bloom degradation often deplete dissolved oxygen, leading to hypoxic conditions that negatively affect higher trophic levels, including fish and invertebrates (Zhang et al. 2022). Due to these cascading effects, the secondary environmental impacts of aquatic macrophyte decomposition have attracted considerable scientific attention worldwide.

Microbes play a central role in litter decomposition by breaking down plant-derived organic matter (Wu et al. 2024). This process involves a succession of microbial communities, primarily bacteria and fungi (Salis et al., 2023; Zhao et al., 2021), which utilise organic compounds as carbon and energy sources (Wu et al., 2024). The composition and activity of these microbial communities are influenced by the chemical properties of the litter, such as the carbon-to-nitrogen ratio, lignin content, and the presence of secondary metabolites (Zhao et al. 2021;

Zhang et al. 2022). As decomposition proceeds, community structure and diversity shift dynamically (Santschi et al. 2018; He et al. 2024). Certain taxa are dominant during the early stages when labile compounds are abundant, while others prevail in later phases, specializing in the breakdown of recalcitrant polymers, such as lignin and cellulose (Zhou et al. 2018; Paumelle et al. 2021; Zhao et al. 2021). These successional patterns contribute to nutrient cycling and influence overall ecosystem function (Oliveira et al. 2016; Chen et al. 2023). Throughout decomposition, both labile and recalcitrant compounds are sequentially released into the water column and sediments, altering resource availability and stoichiometry, thereby directly influencing microbial growth, community composition and metabolic activity. Concurrently, oxygen consumption during decomposition lowers dissolved oxygen levels and modifies redox conditions, favouring certain metabolic groups and further reshaping the microbial community structure. Thus, litter decomposition and microbial community dynamics are linked by complex bidirectional feedback.

In addition to the well-documented role of microbial processes in macrophyte decomposition, a growing body of evidence indicates that environmental contaminants, such as heavy metals, microplastics and antibiotics, significantly alter the decomposition dynamics of aquatic macrophytes. Among these, antibiotics represent a particularly important class of emerging contaminants, with their environmental prevalence having increased markedly in recent decades owing to their extensive use in human medicine, agriculture, livestock production, and aquaculture (Li et al. 2022; Halawa et al. 2024). This widespread use has led to the accumulation of antibiotic resistance genes and virulence factors in environmental matrices, thereby exacerbating ecological risks (Halawa et al. 2024). Although antibiotics have been shown to influence aquatic plant decomposition (Paumelle et al. 2021; Zhao et al. 2021; Li et al. 2023), the mechanisms by which antibiotic-induced changes in decomposition affect sediment bacterial communities are poorly understood. Therefore, it is important to evaluate how antibiotic exposure affects nutrient release during litter decomposition and to elucidate the resulting shifts in the structure and function of sediment bacterial community.

In this study, norfloxacin and doxycycline were selected as representative antibiotics, due to

their global use in human and veterinary medicine for treating bacterial infections (Sikorski and Bęś 2024). Their frequent detection in aquatic systems, resulting from widespread use and improper disposal, raises concerns regarding their ecotoxicological effects (Pan et al., 2017; Zhao et al., 2021). Previous studies have demonstrated that antibiotics can alter litter decomposition (Li et al. 2023; Tian et al. 2023). For instance, norfloxacin and other antimicrobial agents have been shown to inhibit leaf litter decomposition and nutrient release (Zhao et al. 2021). Based on these findings, we hypothesized that antibiotic exposure would impact nutrient release during litter decomposition and alter sediment physicochemical properties, thereby directly and indirectly shifting the bacterial community structure and function. This study aimed to (i) compare the diversity, composition, structure, assembly mechanisms, and potential ecological functions of sediment bacterial communities under antibiotic exposure and (ii) explore the interrelationships among nutrient release, sediment physicochemical parameters, and bacterial communities during litter decomposition under antibiotic stress. The findings of this study will enhance our understanding of how antibiotic micropollutants influence the decomposition of aquatic macrophyte and support sustainable ecosystem management strategies.

2 Materials and methods

2.1 Experimental organisms and reagents

Norfloxacin (NOR) and doxycycline (DOX) used in this study were obtained from Shanghai Macklin Biochemical Technology Co., Ltd. To attain the target concentrations, NOR and DOX stock solutions were prepared at $180\text{ }\mu\text{g}\cdot\text{mL}^{-1}$ and $160\text{ }\mu\text{g}\cdot\text{mL}^{-1}$ in chromatographic-grade methanol, respectively. To simulate an authentic aquatic ecosystem, the surface water used in this experiment was collected from a pond of Jiangsu University, Zhenjiang City, China ($119^{\circ}30'23''\text{E}$, $32^{\circ}11'58''\text{N}$), and passed through a 30 mm plankton net to remove large and suspended particles. Sediment from the same site was sieved through 20 mesh stainless screens to eliminate coarse debris, thoroughly homogenized and stored at room temperature (Tao et al., 2024). The initial physicochemical properties of sediments are presented in [Table S1](#). C.

demersum, one of the predominant submerged macrophyte in the shallow freshwater lakes of the lower reaches of the Yangtze River reaches, was collected from a lake in Zhenjiang, China (119°28'19"E, 32°14'49"N). Upon delivery to the laboratory, the plant material was thoroughly rinsed with tap water and then air-dried for 15 days until a stable weight. The dried litter was segmented into 4-5 cm pieces and divided into 87 samples (10 g each). Three randomly selected samples were determined the initial nutrient content (C: 3.44 g·kg⁻¹; N: 0.31 g·kg⁻¹; P: 0.69 g·kg⁻¹), while the remainder (n=84) were designated for the subsequent decomposition study.

2.2 Litter decomposition experiment design and setup

The incubation experiment comprised five distinct treatments, each with three replicates: (1) control group (CK), containing only sediment and water; (2) litter decomposition group (LD) amended with plant litter but no antibiotics; and (3) three antibiotic-litter decomposition groups (ALD), which contained plant litter and were separately spiked with one of the following: (i) 180 ng·g⁻¹ norfloxacin (NOR) as the N-ALD group, (ii) 160 ng·g⁻¹ doxycycline (DOX) as the D-ALD group, or (iii) a combination of both antibiotics (180 ng·g⁻¹ NOR + 160 ng·g⁻¹ DOX) as the ND-ALD group. The selected antibiotic concentrations are environmentally relevant, based on levels reported for freshwater ecosystems such as lakes and river (Siedlewicz et al. 2016; Li et al. 2022). All microcosms were destructively sampled three times (days 1, 35, and 110) during incubation.

A total of 105 microcosms (2 L volume; 120 mm diameter × 185 mm height) were established, each loaded with 1 kg of sediment (approximately forming 5 cm layer) and 1.6 L of pond water. For each treatment, 1 mL of the NOR stock solution and 1 mL of the DOX stock solution were simultaneously added to 50 g of sediment and thoroughly stirred for initial mixing. The mixture was then transferred to a fume hood and turned frequently until the methanol had completely evaporated (approximately 24 h). After evaporation, the spiked sediment portion was gradually incorporated into the remaining 950 g of sediment, followed by repeated stirring to ensure homogeneity. Subsequently, 84 portions of the prepared plant litter were individually placed on the sediment surface of the microcosms. Pond water was then slowly added along the walls of

the container and maintain an overlying water depth at approximately 12 cm to simulate a shallow-lake environment. The experiment was conducted in an outdoor laboratory with a roof from November 2022 to March 2023 for a total of 110 days. Based on our previous field studies, this duration (over 110 days) allows approximately 80.68% decomposition of the *C. demersum* litter.

Surface sediments and plant litter were randomly collected from each container on days 1, 35 and 110. Each sample was divided into two subsamples: one stored at -25°C for bacterial community analysis, and the other was oven-dried to a constant weight for the determination of nutrient contents (litter) and the physicochemical properties of the sediments.

2.3 Determination of litter nutrient contents and sedimental physicochemical properties

The dried litter was homogenized using Mixer Mill MM400 (Retsch, Germany) before analysis. The total carbon (TC) and nitrogen (TN) contents were determined by elemental analyzer (Vario EL cube, Germany). Total phosphorus (TP) of litter was determined after H₂SO₄-H₂O₂ digestion by colorimetric analysis. The ratios (C: N, C: P and N: P) were calculated based on these measured values following established methodologies (Zhao et al. 2021). Sediment pH was determined in supernatant with water-to-solid ratio of 2.5:1 using pH meter (PHS-25, INESA Analytical Instrument Co., Ltd., Shanghai, China). Sediment TC and TN were analyzed by the Vario EL cube elemental analyzer. The Ammonia nitrogen (NH₄⁺-N) and nitrate nitrogen (NO₃⁻-N) contents were determined the indophenol blue and phenol sulfonic acid methods, respectively. The organic matter (O.M.) content was determined using the dichromate oxidation method. Sediment TP was measured using the ascorbic acid-molybdate blue method after appropriate digestion (Tao et al. 2024).

2.4 Microbial analysis

DNA was extracted from the sediment samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, USA), according to the manufacturer's protocol. The extracted DNA was identified using 1% agarose gel electrophoresis and ultraviolet (UV) spectrophotometry. The

hypervariable V3-V4 region of the bacterial 16S rRNA gene was amplified using the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') for bacterial communities (Song et al. 2024; Tao et al. 2024). The PCR products were detected by 2% agarose gel electrophoresis, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using a Quantus™ Fluorometer (Promega, USA). The purified amplicons were paired end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, USA) by Shanghai Majorbio Biopharm Technology Co., Ltd. (Shanghai, China).

Raw sequencing reads were processed through quality control, filtering, and double-ended splicing steps. To mitigate biases from uneven sequencing depth, all samples were subsampled to 37585 sequences each, to adjust to the minimum number of sequences observed before subsequent analysis. The high Good's coverage values (average 98.66%; range 97.91%-99.86%) confirmed that the sequencing depth adequately captured the bacterial diversity. Furthermore, the plateauing Shannon curve indicated that bacterial community richness was sufficiently represented in our samples (Fig. S1). The normalized sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using the Ribosomal Database Project (RDP) classifier. A total of 26,337 operational taxonomic units (OTUs) were identified. Subsequent analyses of alpha diversity, beta diversity, and bacterial community composition were based on this OTU table.

2.5 Statistical analysis

The physicochemical properties of sediment and the nutrients of the plant litter were calculated and statistically analyzed using IBM SPSS Statistics 25 software (IBM Corporation, Armonk, NY, USA) and Origin 2024b (Origin Lab Corporation, Northampton, MA, USA). The sediment bacteria composition was analyzed using Venn, Bar/Pie and Heatmap diagrams. The significance of dissimilarity in community structure was analyzed by beta diversity analysis (PCoA) and Anoism/Adonis/PERMANOVA (Bray-Curtis). Redundancy analysis (RDA), Spearman correlation heatmap and Mantel test were performed to evaluate the relationships

between the bacterial communities and functional genes with the sediment physicochemical properties. PICRUST2 predicted the functions and metabolic pathways of the bacteria. FAPROTAX predicted the bacterial ecological functional groups related to the biochemical cycle processes. The community assembly process was evaluated using the beta-nearest taxon index (β NTI) based on a null model with 999 randomizations. Differential analysis of the alpha diversity indices, dominant bacterial groups and predicted metabolic pathways and functional genes of the bacterial community were determined by Wilcoxon rank-sum test. Statistical differences were identified at $P < 0.05$ or 0.01 for all analyses. Microbial correlation analyses and graphs were generated using the online Majorbio Cloud Platform (<http://cloud.majorbio.com>).

3 Results

3.1 Nutrient contents and their ratios of plant litters

Antibiotic exposure in aquatic environments consistently impeded nutrient release from decomposing litter, as evidenced by the-reduced net losses of TC, TN and TP in the ALD group compared to the LD group (Table 1). This retardation of decomposition was further reflected in stoichiometric shifts, specifically lower C:N ratio and higher N:P ratio. Since one-way ANOVA showed no significant differences ($P > 0.05$) in nutrient content among the N-ALD, D-ALD, and ND-ALD treatments (Table 1), the specific type of antibiotic (NOR, DOX, or their mixture) selected in this study had minimal differential effects. Thus, the overarching impact on nutrient dynamics can be attributed to antibiotic exposure per se rather than the distinct modes of action of NOR, DOX, or their combination.

3.2 Changes in physicochemical properties of sediments

Analysis of sediment physicochemical properties revealed significant treatment-induced variations following the 110-day incubation period (Fig. S2). The ALD group exhibited significantly higher TC, TN, and TP levels than the LD and CK groups (Fig. S2a-c, $P < 0.05$). This trend was consistent across additional parameters: with O.M. content decreasing in the

order ALD > LD > CK (Fig. S2d), whereas sediment NO₃⁻-N, NH₄⁺-N and pH content displayed the same trend (Fig. S2e-g).

Distinct response patterns were observed among the different antibiotic treatments (N-ALD, D-ALD, and ND-ALD). The D-ALD treatment demonstrated particularly dynamic temporal changes, with the sediment TC content reaching its lowest value (8.12 g·kg⁻¹) on day 35 and the highest value (10.18 g·kg⁻¹) on day 110 (Fig. S2a). Similarly, TN content in the D-ALD group remained consistently elevated throughout the experiment (Fig. S2b). In contrast, the TP content in D-ALD exhibited an inverse trend, declining from the highest value (0.24 g·kg⁻¹) on day 35 to the lowest value (0.21 g·kg⁻¹) on day 110 (Fig. S2c). Sediment pH remained within the neutral range (7.18–7.68) across all microcosms, with the D-ALD group consistently recording the lowest values (Fig. S2g). The O.M. content at both points was ranked as ND-ALD > D-ALD > N-ALD (Fig. S2d). On day 110, the NH₄⁺-N content increased by 27–65% relative to day 35 across the antibiotic treatments (Fig. S2e), whereas the NO₃⁻-N content decreased by 6% to 29% (Fig. S2f). These results indicate that the specific type of antibiotic, in addition to litter decomposition, exerts a significant influence on the temporal evolution of the physicochemical properties of sediments.

3.3 Bacterial community responses under antibiotic exposure during litter decomposition

3.3.1 Alpha diversity of the bacterial communities

The alpha diversity of the sediment bacterial communities differed significantly among the main treatment groups (CK, LD and ALD) (Fig. 1). The CK group exhibited the highest mean values for the Sobs, Chao, Ace, and Shannon indices, followed by the LD group, with the ALD group displaying the lowest values; In contrast, the Simpson index showed an inverse trend ($P < 0.01$). Significant reductions in these indices from CK to LD ($P < 0.01$), indicate that litter input alone substantially altered the sediment bacterial community. A further significant decrease in the ALD group confirmed that antibiotic exposure reduced bacterial richness and diversity in the presence of decomposing litter. Among the specific antibiotic treatments (N-

ALD, D-ALD and ND-ALD), no significant differences were observed in any of the alpha diversity indices ($P > 0.05$) (Fig. S3), indicating that the type of antibiotic (NOR, DOX or their mixture) had no statistically distinct impact on microbial community diversity.

Temporal dynamics varied across the treatment groups (Fig. S4). In the LD group, the Sobs, Ace, Chao and Shannon indices significantly decreased from day 1 to day 110, indicating continuous impact of litter decomposition on microbial diversity. In contrast, the CK group remained stable over time for all indices. The ALD displayed more complex and compound-specific temporal patterns. For instance, in the D-ALD group, the Shannon index peaked, whereas the Simpson index reached its lowest value by day 35. The N-ALD and ND-ALD groups also showed distinct results over time. Collectively, these results highlight that both the duration of antibiotic exposure and litter decomposition are significant factors driving fluctuations in alpha diversity of sediment microbial communities.

3.3.2 Beta diversity of the bacterial communities

Principal coordinate analysis (PCoA) revealed distinct clustering patterns in the sediment bacterial community structure across treatments and over time (Fig. 2 and S5). As shown in Fig. 2a, the CK group formed a tight and separate cluster, indicating a stable community. In contrast, both the litter decomposition (LD) and antibiotic-litter decomposition (ALD) groups were clearly separated from CK and exhibited more dispersed distributions (Fig. 2a), reflecting the increased community variation induced by litter input and antibiotic exposure, respectively. Furthermore, the bacterial communities among the different antibiotic types (N-ALD, D-ALD, ND-ALD) showed substantial overlap in their confidence ellipses (Fig. 2b), suggesting that the three antibiotic types had a minor effect. The results were statistically supported by significant ANOSIM and PERMANOVA results (Table S2).

Exposure time was another major factor shaping the community structure. In the PCoA plot, samples from different incubation days (1, 35, and 110) were distinctly separated (Fig. 2c-d and S5). ANOSIM and PERMANOVA further confirmed that community structures at these three time points were significantly different ($P < 0.01$) in the LD and all ALD groups (Table S2).

This demonstrates that incubation time is a critical factor driving the succession of sediment bacterial communities during litter decomposition.

3.3.3 Bacterial community composition

Venn diagrams illustrate the distribution of shared and unique operational taxonomic units (OTUs) across the treatment groups and overtime (Fig. 3a-c). A core microbiome comprising 8,947 OTUs (34.01% of the total) was shared among the CK, LD, and ALD groups (Fig. 3a), indicating that these microorganisms possessed broad environmental adaptability. The ALD group harboured the highest number of unique OTUs (28.06%), substantially exceeding those of the CK (12.12%) and LD (8.89%) groups (Fig. 3a). This suggests that antibiotic exposure promote the development of a more distinct bacterial assembly during litter decomposition. Analysis of antibiotic-specific treatments revealed a similar trend (Fig. 3b). The N-ALD, D-ALD, and ND-ALD groups exhibited 3,263 (16.10%), 3,423 (16.89%), and 2,817 (13.90%) unique OTUs, respectively, while sharing a common core of 7,349 OTUs (36.27%). Furthermore, the number of unique OTUs declined as decomposition progressed (Fig. 3c and S6). This temporal decline indicates that antibiotic exposure act as key selective forces during litter decomposition that progressively shape the composition of the unique bacterial community in sediments over time.

Bacterial 16S rRNA gene sequencing of all sediment samples identified 69 phyla, 214 classes, 504 orders, 849 families, 1,691 genera, and 4,292 species. Among these 69 phyla, the greatest number of differentially abundant phyla was observed between the CK and ALD groups (49 phyla), followed by the CK and LD groups (40 phyla), whereas the fewest differences were detected between the LD and ALD groups (9 phyla). Although the dominant phyla (relative abundance >1%) were largely consistent across the CK, LD and ALD groups (Fig. 3d), their relative abundances exhibited significant variation. Specifically, statistical analyses revealed notable shifts in the abundance of several major phyla, including Firmicutes, Chloroflexi and Acidobacteriota (Kruskal-Wallis H test, $P < 0.05$) (Fig. S8a). Rare phyla with low abundance (< 0.01% relative abundance) also increased in treatment-dependent manner, accounting for

21%, 27% and 29% of the total phyla in the CK, LD, and ALD groups, respectively. Furthermore, two phyla (WS4 and CK-2C2-2) showed distinct variations among the N-ALD, D-ALD and ND-ALD groups (Fig. S8b). Similarly, this pattern was consistently observed at the class and genus levels across the CK, LD and ALD groups as well as among the different antibiotic types (N-ALD, D-ALD and ND-ALD groups) (Fig. S7). Collectively, these findings demonstrate that antibiotic exposure is significant drivers of the sediment bacterial community composition during litter decomposition.

In addition, 16 bacterial phyla (Fig. S8c), including Firmicutes, Proteobacteria, Bacteroidota, Actinobacteriota, Myxococcota and Nitrospirota, showed significant differences among days 1, 35 and 110 within all experimental groups ($P < 0.05$). The temporal of litter decomposition significantly influenced microbial community composition ($P < 0.05$). Notably, the relative abundance of Firmicutes increased from 26.54% on day 1 to 33.07% on day 110. Bacteroidota showed consistent increase from 4.51% on day 1 to 12.89% on day 110, whereas Proteobacteria decreased from 28.48% on day 1 to 15.07% on day 110 (Fig. 3e and S8e). These results indicate the pivotal role of time in shaping the community composition.

3.3.4 Bacterial metabolic-pathway analysis

The metabolic potential of the sediment bacterial communities was predicted from 16S rRNA gene sequences using PICRUSt2 and functionally annotated against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Fig. 4 and S9). At Level 1 functional classification, metabolism consistently dominated across all samples, accounting for 76.40%–77.33% of the predicted gene families, followed by Genetic Information Processing (7.38%–7.71%), Environmental Information Processing (5.21%–6.14%), Cellular Processes (4.67%–4.75%), Human Diseases (3.31%–3.57%), and Organismal Systems (1.76%–1.81%). Statistical analyses revealed that the main treatment groups (CK, LD, ALD) induced significant differences in four Level 1 categories, including Metabolism, Environmental Information Processing, Human Diseases and Organismal System pathways ($P < 0.05$, Fig. 4a). This trend became more pronounced at a higher taxonomic resolution, with 31 out of 46 Level 2 metabolic

pathways showing significant alterations among the treatment groups (Fig. 4b). In contrast, the type of antibiotic (N-ALD, D-ALD and ND-ALD) exerted minimal impact, resulting in no significant differences at Level 1 and only one significantly pathway at Level 2 (Fig. S9a). Furthermore, significant temporal shift in functional potential was observed, with 31 metabolic pathways and several core cellular processes exhibiting dynamic changes throughout the decomposition period (Fig. 4c and d).

The microbial functional potential, predicted using the FAPROTAX database, encompassed 73 ecologically relevant metabolic groups. Significant treatment effect was observed, with 41 of these groups differing substantially ($P < 0.05$) among the CK, LD, and ALD groups (Fig. S9b). The LD and ALD groups were enriched in functions associated with carbon metabolism (e.g., chemoheterotrophy and fermentation) as well as reductive nitrogen processes (e.g., denitrification and nitrate reduction). In contrast, the CK group was characterized by higher abundance of oxidative nitrogen pathways, including nitrification and aerobic nitrite oxidation (Fig. S9b). The type of antibiotic had comparatively minor influence, with only four functional groups showing significant differences across the N-ALD, D-ALD, and ND-ALD treatments (Fig. S9c). Incubation time was another major driver of functional succession, significantly altering 35 functional groups during the experimental period (Fig. S9d). Over time, processes such as chemoheterotrophy and fermentation increased, accompanied by dynamic shifts in nitrogen transformation potentials. Overall, these findings indicate that plant litter addition and antibiotic exposure are the primary factors reshaping sediment microbial function, with incubation time acting as secondary modulating factor, while the type of antibiotic plays a negligible role.

3.3.5 Assembly mechanism of the microbial community in surface sediments

The assembly mechanisms of sediment bacterial communities were distinctly influenced by litter decomposition, antibiotic type and incubation time (Fig. 5). Heterogeneous selection was the dominant process across all major treatment groups, with its relative contribution increasing from 61.73% in the CK group to 83.95% and 81.48% in the LD and ALD groups, respectively

(Fig. 5a). Drift represented the secondary mechanism, whereas dispersal limitation, homogeneous selection and homogenizing dispersal played negligible roles. Under different antibiotic treatments, heterogeneous selection remained dominant but exhibited greater variability, accounting for 46.91%, 66.67% and 44.44% in the N-ALD, D-ALD and ND-ALD groups, respectively (Fig. 5b). Stochastic processes (dispersal limitation, homogenizing dispersal) demonstrated relatively higher contributions compared to the CK groups. A pronounced temporal shift in the community assembly processes was observed over time (Fig. 5c). The influence of heterogeneous selection decreased from 71.60% on day 1 to 0% on day 110, whereas homogeneous dispersal increased from 2.47% to 19.75%. Homogeneous selection emerged as a significant deterministic force (reaching 29.63 %) only at the end of the incubation period. These findings highlight that deterministic selection primarily governs community assembly under the combined pressures of litter decomposition and antibiotic exposure.

3.3.6 Multiple factors affecting the bacterial community in surface sediments during the submerged macrophyte debris decomposition processing

The physicochemical properties of sediments and plant litter were identified as direct drivers of bacterial community diversity, structure and composition in the surface sediments (Fig. 6 and S10). In the LD group, bacterial richness (Sobs, Ace, Chao) and diversity (Shannon) were significantly positively correlated with C, N, P and N:P ratios of litter, but negatively correlated with C: N ratio of litter (Fig. 6c), and bacterial diversity (Simpson) were significantly negatively correlated with TP and NO₃⁻-N of sediment and the C, N and N: P ratios of the litter. Accordingly, RDA confirmed that sediment NO₃⁻-N and litter nutrients (C, N and P) were the primary factors shaping community structure in the LD groups (Fig. 6d). Specific bacterial phyla, such as *Firmicutes*, *Proteobacteria*, *Chloroflexi* and *Acidobacteriota*, exhibited significant relationships ($P < 0.05$) with these key environmental variables (Fig. S10b). Thus, the input of macrophyte litter was an indirect factor driving changes in the bacterial community.

Antibiotic exposure was another significant indirect factor (Table S3, Fig.6 and S10). In the ALD groups, RDA revealed that community structure was co-driven by NH₄⁺-N and O.M. of

sediment, litter nutrients and antibiotics (Fig. 6f). The association of specific phyla with distinct environmental niches, such as *Proteobacteria* and *Bacteroidota* with antibiotics, C and N of litter nutrients and sediment (Fig. S10c), demonstrating functional partitioning within the community. In addition, the sediment microbial community is primarily influenced by sedimentary physicochemical factors, including C, N and O.M., as well as litter nutrients and antibiotics. These factors exhibited significant correlations with microbial alpha diversity and several dominant groups (Fig. S11).

4 Discussion

4.1 Antibiotic-Induced delay in litter nutrient release and alteration of sediment physicochemical properties

Plant litter decomposition is a critical process in the cycling of carbon, nitrogen, and phosphorus within ecosystems, supplying sediments with essential nutrients while simultaneously altering the chemical composition of the litter (Oliveira et al. 2016; Zhao et al. 2021). C: N: P ratio serves as an indicator of energy flow and nutrient dynamics. The decomposition of aquatic macrophyte litter typically occurs in three distinct phases: rapid initial leaching of water-soluble compounds, followed by microbial degradation and fragmentation of recalcitrant organic materials (lignin, cellulose, etc.). During the initial stage, labile components, such as sugars, proteins, organic acids, and minerals, are rapidly lost (Oliveira et al. 2016; Wu et al. 2024), which aligns with our observation that the reduction in litter C, N and P was less on day 110 than on day 35 (Table 1). This pattern reflects the progression of decomposition. On day 35, the process was predominantly in the initial leaching phase, during which nutrient loss was primarily attributed to the release of cytoplasmic components and water-soluble compounds from macrophytes (Oliveira et al. 2016). By day 110, decomposition had advanced into the latter two stages, characterized by the breakdown of high-molecular-weight organic matter into smaller molecules, resulting in a reduced litter C: P and N: P ratio.

Antibiotics, among the most frequently detected emerging contaminants in aquatic environments, have been shown to impair plant litter decomposition. For instance, Zhao et al.

(2021) demonstrated that norfloxacin affects leaf biomass retention, mass loss, and bacterial abundance. In our study, exposure to norfloxacin and doxycycline in sediment hindered nutrient release from aquatic litter by reducing microbial abundance, thereby suppressing the decomposition. Strong correlations were observed between bacterial alpha diversity and litter nutrients and sediment physicochemical properties (Fig. 6 and S11) support the hypothesis that antibiotics indirectly influence sediment geochemistry by suppressing microbial communities responsible for both litter decomposition and nutrient transformation. Specifically, this effect propagated into the sediment environment, where the antibiotic-treated group (ALD) exhibited significant alterations in physicochemical properties, including elevated TC, TN, and TP levels (Fig.S2). Consequently, antibiotic contamination may alter aquatic ecosystem functioning by decelerating nutrient cycling from litter and modifying the biogeochemical conditions of the sediment.

4.2 Antibiotic exposure changed alpha diversity composition and function of sediment bacteria communities during litter decomposition

Several studies have reported that microbial diversity and richness typically increase during decomposition under natural conditions (Yin et al. 2022; Wu et al. 2025). However, in contrast to this commonly observed pattern, the results of this experiment demonstrated that litter input led to a reduction in diversity (Fig. 1). The underlying causes of this phenomenon are likely to be multifaceted. First, excessive litter accumulation may intensify competition for microbial resources, leading to the inability of certain microorganisms to effectively utilize these resources and ultimately resulting in community homogenization (Santschi et al. 2018). Combined with findings from other studies (Yin et al. 2022; Kaźmierczak et al. 2024) suggest that environmental fluctuations and complex ecological factors are essential for the microbial community succession and diversity. Additionally, Cason et al. (2019) demonstrated that hypoxic or anaerobic environments can reduce microbial diversity. As shown in Fig. S12, the dissolved oxygen (DO) levels ranged from approximately 0.27 to 1.73 mg·L⁻¹ and less than 2 mg·L⁻¹, remaining below 2 mg·L⁻¹ after 20 days of litter decomposition. This suggests that decomposition predominantly occurs under hypoxic or anaerobic conditions. Consequently, the

rapid degradation of litter creates an environment that limits the growth of aerobic microorganisms. Therefore, variations in environmental controls and resource availability, coupled with litter availability, may lead to a decline in microbial diversity.

Micropollutant inputs can alter the bacterial diversity of sediments. For instance, studies have reported that fungicides (Salis et al. 2023) and nanoparticles (Herruzo-Ruiz et al. 2024) tend to reduce the diversity and richness of aquatic microbial communities. In this study, antibiotic exposure to sediments was also associated with a reduction in microbial diversity (Fig. 1, 6 and S11); however, the effect was not statistically significant. This may be attributed to the relatively low concentrations of norfloxacin ($180 \text{ ng} \cdot \text{g}^{-1}$) and doxycycline ($160 \text{ ng} \cdot \text{g}^{-1}$) applied individually and in combination, which may have been insufficient to induce substantial shifts in the microbial community structure. At low concentrations, antibiotics may primarily affect only susceptible microbial taxa, while resistant or highly tolerant populations remain largely unaffected, thereby preserving overall community diversity (Santschi et al. 2018; Yin et al. 2022). Consistent with these findings, Tian et al. (2023) demonstrated that exposure to $0.404 \text{ mg} \cdot \text{L}^{-1}$ tetracycline and $0.004 \text{ mg} \cdot \text{L}^{-1}$ ciprofloxacin did not significantly alter the diversity of microbial communities in aquatic samples. As shown in Fig. 2, the bacterial structures under norfloxacin and doxycycline treatments exhibited no clear separation, supporting the conclusion that antibiotic exposure at these concentrations does not significantly restructure sediment microbial assemblages. These results indicate that low-intensity disturbances, such as those induced by subthreshold antibiotic concentrations, exert a limited influence on microbial dynamics during litter decomposition in aquatic environments.

The ability of microbial communities to regulate litter decomposition processes has evolved through long-term adaptation to environmental conditions and biotic interactions (Paumelle et al. 2021). In the present study, the bacterial communities across all experimental groups were predominantly composed of Firmicutes, Proteobacteria, and Bacteroidetes (Fig. 3d). Firmicutes are known to play a critical role in the degradation of complex organic compounds, such as cellulose, and exhibit high resilience to environmental stressors, due to the presence of spore-forming taxa capable of surviving under adverse conditions (Zeng et al. 2019; Wu et al. 2024).

An elevated relative abundance of Firmicutes under low-oxygen conditions or exposure to stressors, as many antibiotic-resistant bacteria belong to endospore-forming lineages within this phylum (Zhao et al., 2021). Proteobacteria contribute significantly to organic matter decomposition and serve as key players in carbon, nitrogen, and sulfur cycling in aquatic ecosystems (Zeng et al. 2019; Raza et al. 2023; Jing et al. 2025). Meanwhile, Bacteroidetes are instrumental in plant litter breakdown through the production of extracellular enzymes, particularly hydrolases, which facilitate the hydrolysis of complex polymers (Zeng et al. 2019; Raza et al. 2023). Thus, the microbial community structure and composition during litter decomposition are shaped by various environmental factors. Notably, Firmicutes and Bacteroidota exhibited significant negative correlations with the C, N and P of *C. demersum* litter ($P<0.05$), and Proteobacteria showed a significant positive correlation with litter nutrient ($P<0.05$) (Fig. S11).

More importantly, plant litter input and antibiotic exposure significantly restructured the sediment bacterial functional portfolio, which potentially modulates carbon and nitrogen cycling processes. Chen et al. (2023) reported that leaf litter alters diversity and abundance of soil microbes involved in nitrification and denitrification. Li et al. (2023) also described that sulfamethoxazole inhibits AOB and nitrification genes. In this study, significant differences in predicted microbial functions at level1 and level 2 potential were observed among the CK, LD, and ALD groups in the FAPROTAX database (Fig. 4 and S9). The input of plant litter and antibiotic exposure diverse metabolic and anaerobic fermentation pathways, this consists with the LD and ALD groups that were enriched in functions associated with carbon metabolism as well as reductive nitrogen processes (e.g., denitrification, nitrate reduction), which suggests a transition towards nitrogen reduction during the litter decomposition process. The LD and ALD groups exhibited enrichment in functions related to carbon metabolism and reductive nitrogen processes, such as denitrification and nitrate reduction, implying a shift towards nitrogen reduction during litter decomposition. In contrast, the CK group showed higher nitrification, indicating nitrogen-oxide transformation in the untreated sediments. These findings suggest that litter input and antibiotic exposure affect aerobic nitrification.

The results of this study highlight the underscore the importance of dynamics in shaping the sediment microbial community structure in aquatic environments. Specifically, during litter decomposition, C, N and P emerge as key drivers influencing microbial assemblages in sediments. In parallel, both litter input and antibiotic exposure altered bacterial diversity and abundance throughout the decomposition process. As revealed by the correlation analysis between environmental variables and alpha diversity, sediment microbial diversity was significantly associated with the C, N, and P content of litter as well as with the physicochemical properties of the sediment (Fig. 6 and S11). These findings suggest a bidirectional relationship: litter decomposition influences microbial community composition, while microbial communities in turn mediate nutrient release from litter and modify sediment physicochemical characteristics (Santschi et al. 2018; Paumelle et al. 2021)

4.3 Antibiotic exposure significantly altered bacterial communities' assembly mechanism during litter decomposition

The mechanisms underlying microbial community assembly have been a major focus in microbial ecology (Luan et al. 2020; Wu et al. 2025). Microbial community composition is primarily governed by niche-based processes, which shape functional traits and ecological dynamics through environmental filtering and species interactions, and play a critical role in maintaining species composition across spatial scales, from local to global (Lv et al. 2023). Community assembly is widely recognized to be driven by the interplay between deterministic and stochastic forces (Luan et al. 2020). Deterministic processes are influenced by environmental factors, biotic interactions and niche selection, leading to predictable and nonrandom community structures (Luan et al. 2020; Lv et al. 2023; Wu et al. 2025). In contrast, stochastic processes originate from random events such as dispersal limitation, birth-death dynamics and ecological drift, which introduce unpredictability and variation into community assembly. The relative importance of these two sets of processes in shaping and sustaining microbial communities varies with environmental context and disturbance regimes, despite their simultaneous operation across ecosystems (Luan et al. 2020; Lv et al. 2023; Wu et al. 2025).

Plant litter decomposition is a fundamental ecological process in aquatic ecosystems that significantly influences the assembly of microbial communities (Wu et al. 2025). In this study, litter inputs primarily drove microbial community assembly through deterministic mechanisms, with heterogeneous selection being the dominant process (Fig. 5). Decomposition of litter releases substantial quantities of soluble organic matter and nutrients, generating pronounced resource gradients and microenvironmental heterogeneity (e.g., localized low-oxygen niches), that selectively favor microbial taxa capable of utilizing these substrates or thriving under high organic matter and low oxygen conditions. The addition of antibiotics in combination with litter input further intensified environmental filtering by eliminating sensitive taxa and enriching resistant or tolerant lineages, thereby explaining the markedly higher contribution of heterogeneous selection observed in the LD and ALD treatments (Fig. 5); Moreover, different antibiotic types (N-ALD, D-ALD, ND-ALD groups) exerted distinct effects on the strength and direction of selection. Temporal dynamics (Fig. 5) revealed that a transition from divergent, niche-driven communities to more convergent, dispersal-mediated assemblages over time. This trend toward homogenization may result in functional concentration and reduced functional redundancy, potentially compromising ecosystem resilience in the face of environmental perturbations.

4.4 Shortcomings and Prospects

Numerous studies have demonstrated that the type and concentration of antibiotics influence microbial diversity and community structure. However, no consistent pattern was observed in our experiment, and several factors may explain this. First, the antibiotic concentrations used in this study were relatively low and may have been insufficient to induce detectable shifts at the community level. Second, laboratory settings inherently lack many dynamic and multifaceted environmental variables present in natural systems. Thirdly, our study focused exclusively on bacterial communities, thereby excluding the potential contributions of fungi, protists and other microfauna to litter decomposition. The use of 20-mesh-sized sediment also precluded interactions with meso and macrofauna. Fourthly, our study was intentionally designed to evaluate ecological effects at environmentally realistic concentrations ($180 \text{ ng} \cdot \text{g}^{-1}$

for norfloxacin and 160 ng·g⁻¹ for doxycycline), rather than to establish a concentration-response relationship. This approach limits our capacity to detect dose-dependent shifts. Furthermore, we did not quantify antibiotic metabolites, which may exhibit different mobility and bioactivity compared to parent compounds, due to analytical constraints, including the ng·g⁻¹ levels and the absence of LC-MS/MS. To comprehensively assess the effects of antibiotics on decomposition processes, future research should expand spatial and temporal scales, test broader concentration ranges and environmentally relevant mixtures, and incorporate fungal and protist communities through ITS and 18S rRNA gene sequencing. Functional responses, including decomposition rates, extracellular enzyme activities, and resistome profiling using (meta) genomic approaches, should also be evaluated. Integrated mesocosm and in situ studies of this nature will enhance our understanding of the relative roles of litter composition, redox/oxygen conditions, antibiotic exposure, and biotic interactions in shaping microbial communities and ecosystem functioning.

5 Conclusion

In this study, we investigated the effects of norfloxacin and doxycycline on the decomposition of aquatic macrophyte litter, sediment physicochemical parameters, and diversity and structure of microbial communities in aquatic environments. Litter inputs and antibiotic exposure reduced microbial diversity in the sediment. Venn diagram analyses revealed that the addition of litter and antibiotics significantly altered the bacterial community composition of the sediments. Collectively, the input of aquatic macrophyte litter and exposure to antibiotics reduced microbial diversity and reshaped community composition, thereby significantly disrupting litter decomposition processes and the dynamic cycling of nutrients in the sediments. These findings highlight the potential threat posed by antibiotic contamination to sediment ecosystem functioning and provide a scientific foundation for the risk assessment and management of antibiotic pollution in aquatic environments. Future research should assess the effects of antibiotics on decomposition across spatial/temporal scales, concentration gradients, and mixtures, including fungi/protists, and quantify functional, enzymatic, and resistance gene

responses in mesocosm and field studies.

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CRedit authorship contribution statement

Fan Wu: Writing – Original draft, Data curation, Investigation, Validation, Formal analysis, Conceptualization. Ziyi Yang: Investigation, Data curation. Tao Lyu: Writing – review & editing, Supervision, Conceptualization, Project administration. Xiaohong Zhou: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. Qingjie Xie: Supervision, Conceptualization, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data available within the article and its supplementary materials.

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Tables

Table 1 Variations (mean $\pm$ SD) in the amounts of C, N, and P in the plant litter after 1, 35 and 110 days

Time	Group	C (g·kg ⁻¹)	N (g·kg ⁻¹)	P (g·kg ⁻¹)	C: N	C: P	N: P
Day 1	LD	3.04 $\pm$ 0.11	0.30 $\pm$ 0.02	0.64 $\pm$ 0.09	10.06 $\pm$ 0.70	4.82 $\pm$ 0.60	0.48 $\pm$ 0.04
	LD	2.37 $\pm$ 0.06a	0.18 $\pm$ 0.01a	0.54 $\pm$ 0.01a	12.99 $\pm$ 0.84a	4.36 $\pm$ 0.08b	0.34 $\pm$ 0.02b
Day 35	N-ALD	2.65 $\pm$ 0.03b	0.24 $\pm$ 0.00a	0.61 $\pm$ 0.01b	10.84 $\pm$ 0.02b	4.33 $\pm$ 0.10b	0.40 $\pm$ 0.01ab
	D-ALD	2.67 $\pm$ 0.02b	0.25 $\pm$ 0.01a	0.58 $\pm$ 0.03b	10.59 $\pm$ 0.23b	4.60 $\pm$ 0.09a	0.43 $\pm$ 0.01a
	ND-ALD	2.65 $\pm$ 0.03b	0.25 $\pm$ 0.01a	0.59 $\pm$ 0.08b	10.46 $\pm$ 0.37b	4.47 $\pm$ 0.13ab	0.43 $\pm$ 0.02a
	ALD	2.66 $\pm$ 0.01b	0.25 $\pm$ 0.00a	0.60 $\pm$ 0.02b	10.63 $\pm$ 0.05b	4.46 $\pm$ 0.13ab	0.42 $\pm$ 0.01a
	LD	2.18 $\pm$ 0.01a	0.16 $\pm$ 0.00a	0.49 $\pm$ 0.07a	13.35 $\pm$ 0.18b	4.42 $\pm$ 0.04a	0.33 $\pm$ 0.01a
Day 110	N-ALD	2.31 $\pm$ 0.05b	0.17 $\pm$ 0.01a	0.59 $\pm$ 0.01c	13.39 $\pm$ 0.32b	3.91 $\pm$ 0.14b	0.29 $\pm$ 0.02a
	D-ALD	2.30 $\pm$ 0.07b	0.16 $\pm$ 0.01a	0.52 $\pm$ 0.01b	14.21 $\pm$ 1.21a	4.41 $\pm$ 0.14a	0.31 $\pm$ 0.03a
	ND-ALD	2.30 $\pm$ 0.06b	0.17 $\pm$ 0.01a	0.58 $\pm$ 0.05c	13.22 $\pm$ 0.36b	3.96 $\pm$ 0.15b	0.30 $\pm$ 0.02a
	ALD	2.31 $\pm$ 0.07b	0.17 $\pm$ 0.00a	0.56 $\pm$ 0.03c	13.61 $\pm$ 0.29b	4.09 $\pm$ 0.20ab	0.30 $\pm$ 0.01a

(Litter decomposition group (LD), antibiotic-litter decomposition group (ALD), norfloxacin-litter decomposition group (N-ALD), doxycycline-litter decomposition group (D-ALD), a combination of both antibiotics-litter decomposition group (ND-ALD). Means with a common letter are significantly different ($P < 0.05$)

Figure Captions

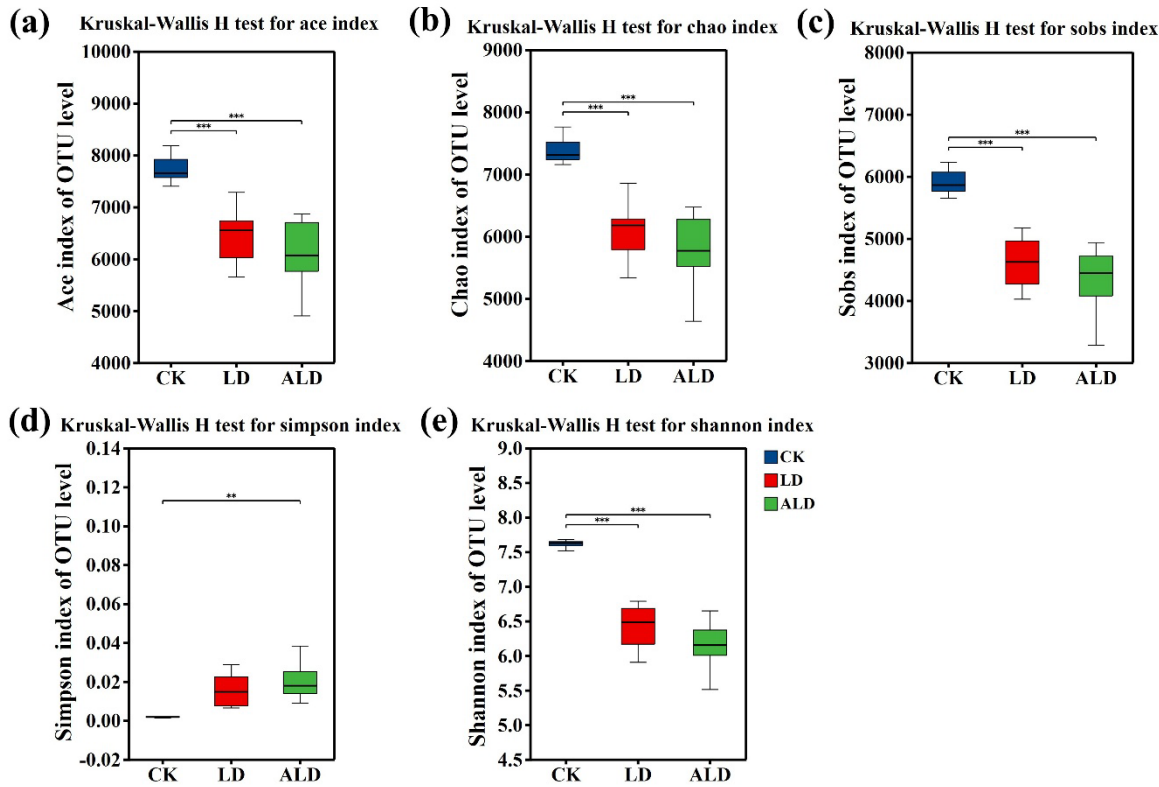


Fig. 1 Kruskal-Wallis H test bar plot of alpha diversity indices of attached bacteria in the litter decomposition group (LD), antibiotic-litter decomposition group (ALD), and control group (CK) in the sediment (* $P<0.05$, ** $P<0.01$)

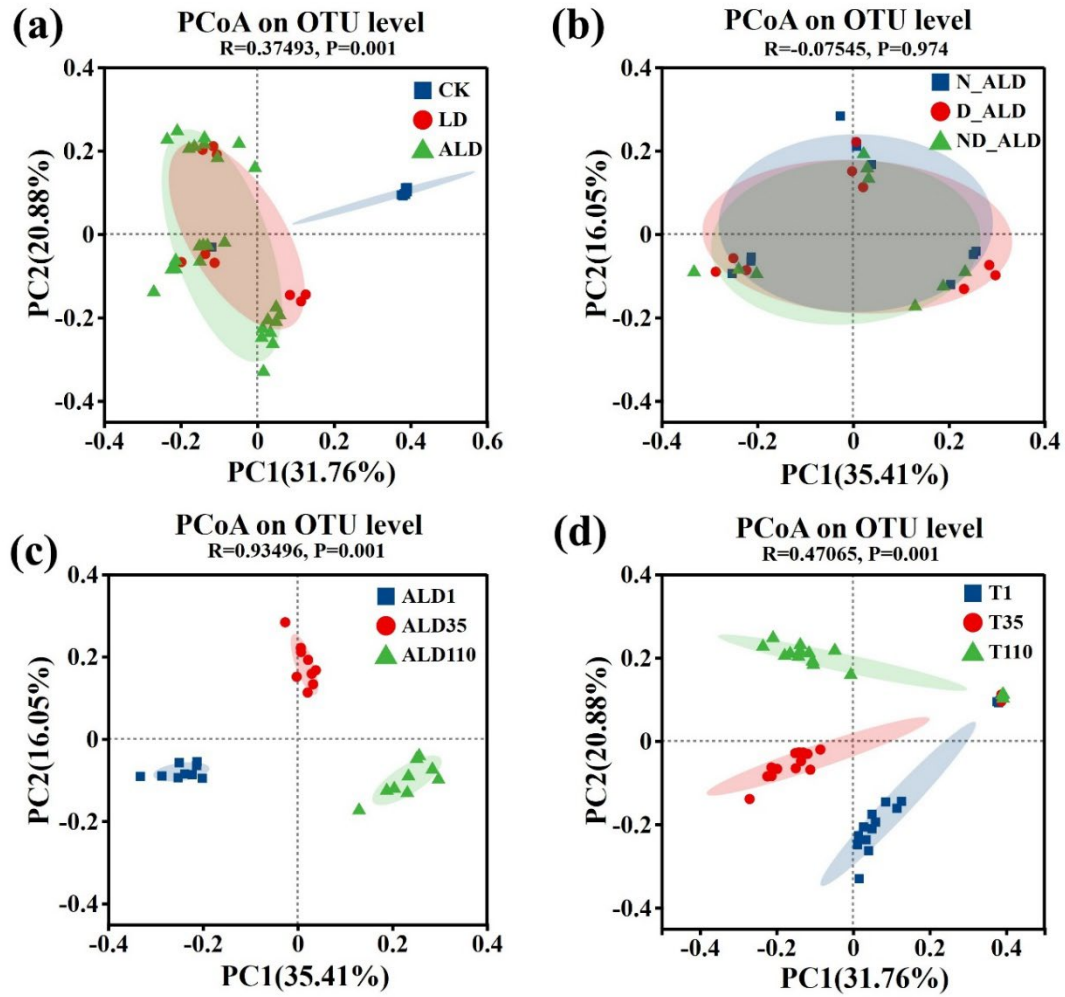


Fig. 2 Principal co-ordinate analysis (PCoA) of the bacterial community structure. (a: Control group (CK), litter decomposition group (LD), and antibiotic-litter decomposition group (ALD); b: norfloxacin-litter decomposition group (N-ALD), doxycycline-litter decomposition group (D-ALD), a combination of both antibiotics-litter decomposition group (ND-ALD); c: three decomposition times of three antibiotic-litter decomposition groups; d: three decomposition times across all experimental groups)

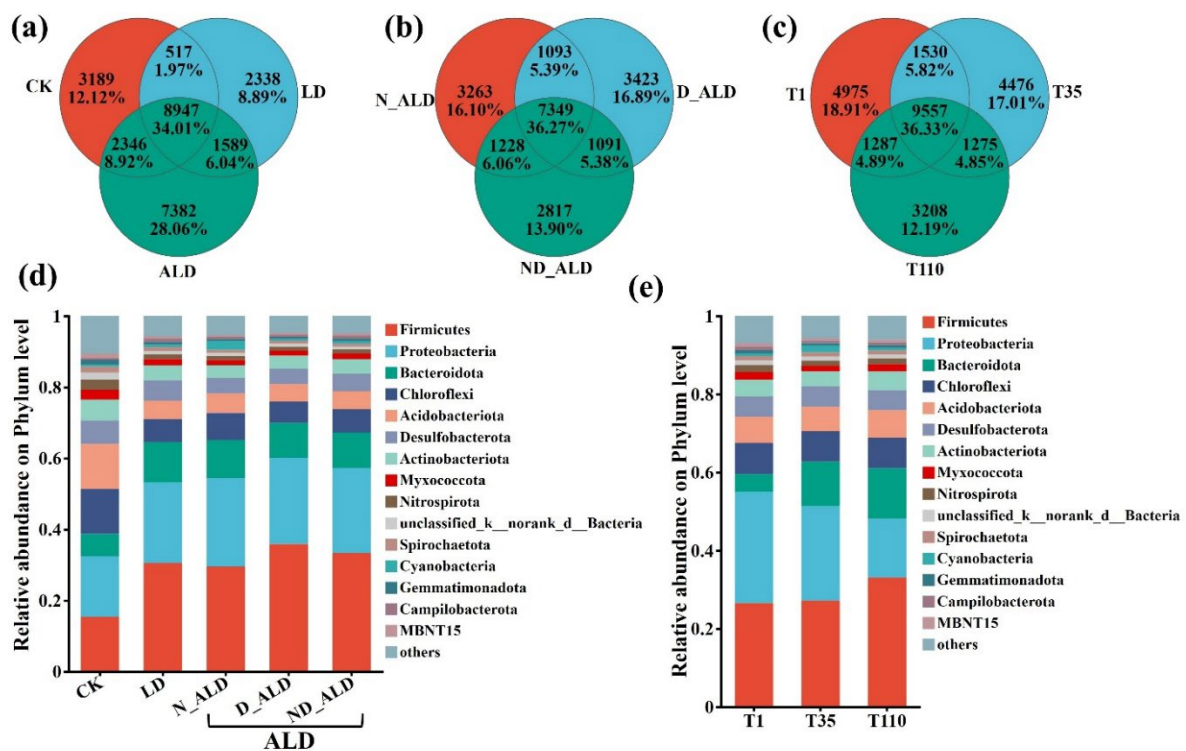


Fig. 3 Venn diagram (a-c) and the relative abundance (d and e) of bacterial communities in freshwater sediments. (Control group (CK), litter decomposition group (LD), antibiotic-litter decomposition group (ALD), norfloxacin-litter decomposition group (N-ALD), doxycycline-litter decomposition group (D-ALD), a combination of both antibiotics-litter decomposition group (ND-ALD))

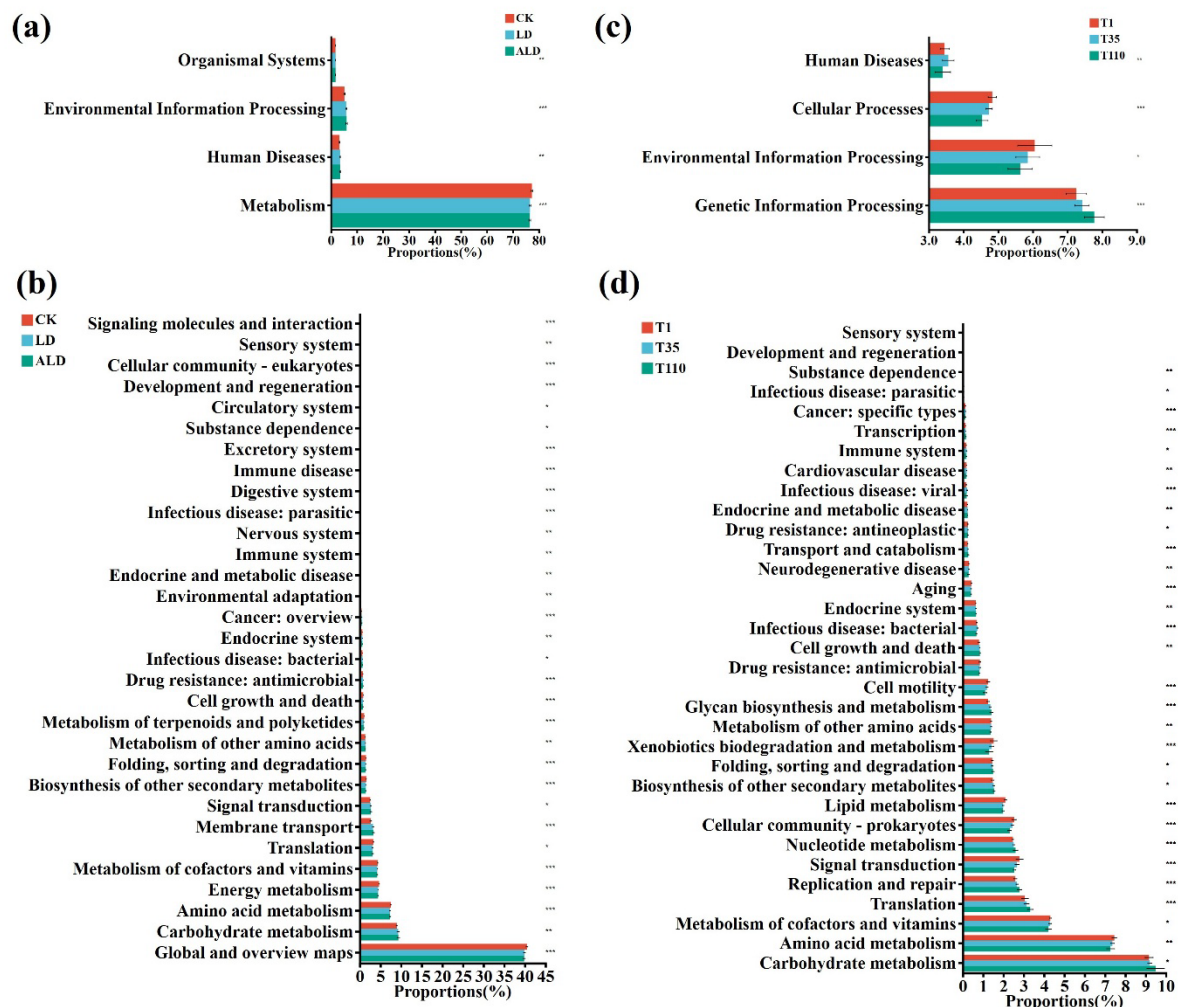


Fig. 4 Bacterial functional pathways showing significant differences at Level 1 and 2 by PICRUST2 analysis based on the KEGG database in the CK, LD and ALD groups (a and b) and three litter decomposition times (c and d) of all groups. (Control group (CK), litter decomposition group (LD), antibiotic-litter decomposition group (ALD); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

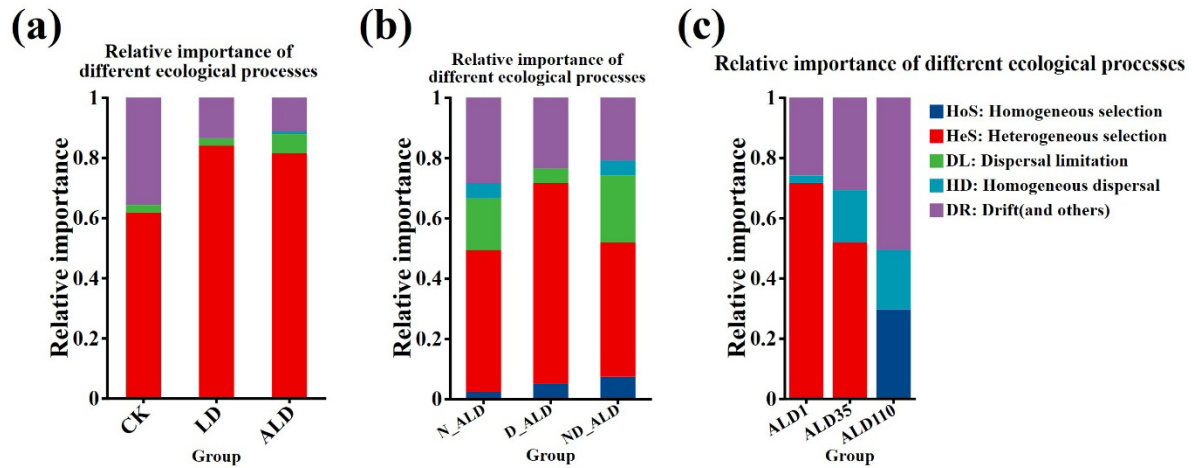


Fig. 5 The assembly processes of sediment microbial communities. (a: Control group (CK), litter decomposition group (LD), and antibiotic-litter decomposition group (ALD); b: norfloxacin-litter decomposition group (N-ALD), doxycycline-litter decomposition group (D-ALD), a combination of both antibiotics-litter decomposition group (ND-ALD); c: three decomposition times of three antibiotic-litter decomposition groups)

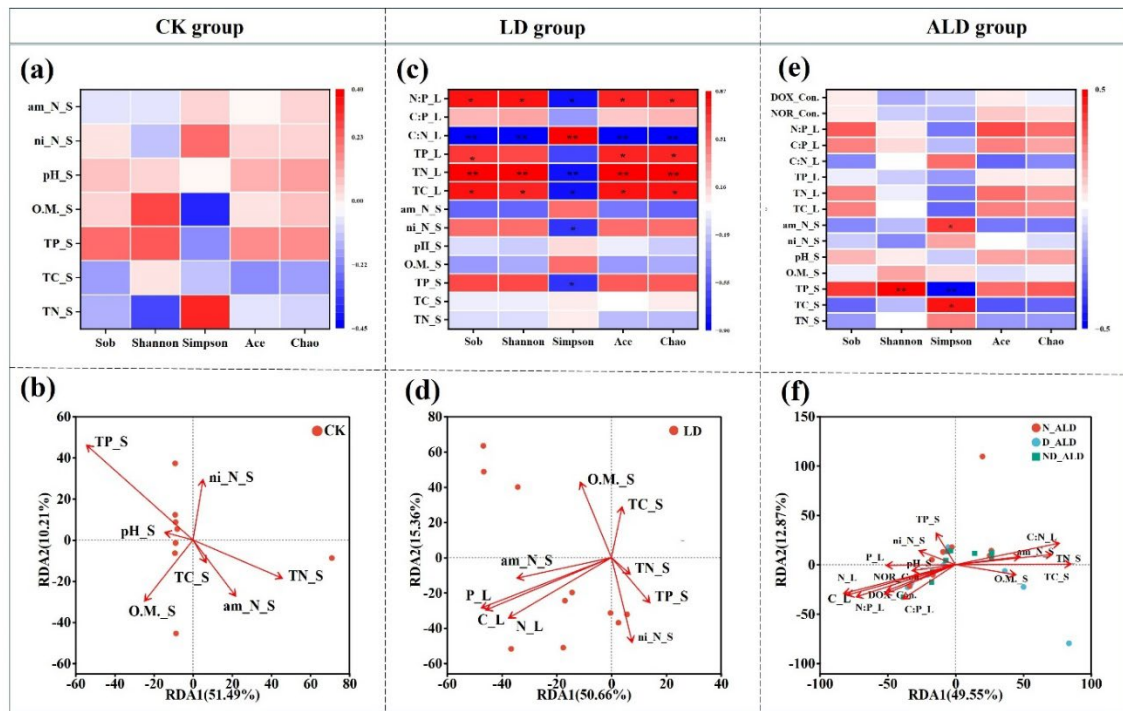


Fig. 6 Heatmap correlation at the phylum level between the bacterial community Alpha diversity and physicochemical properties, and Redundancy analysis (RDA) at the OTU level between the bacterial community structure and the physicochemical properties. (a and b: control group (CK); c and d: litter decomposition group (LD); e and f: antibiotic-litter decomposition group (ALD); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)



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Freshwater organic micropollutants alter submerged macrophyte litter decomposition dynamics and microbial community in the surface sediments

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