



Assessing the impacts of urbanization on stream ecosystem functioning through investigating litter decomposition and nutrient uptake in a forest and a hyper-eutrophic urban stream

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ABSTRACT

Rapid urbanization significantly affects freshwater systems by interfering with important ecological functions. The responses of different ecosystem functions in urban streams and their potential ecological effects remain largely unknown, impeding their management and restoration in many cases. In this study, we simultaneously assessed two important ecosystem functions, litter decomposition and nutrient uptake, and investigated the associated microbial and benthic macroinvertebrate communities in two subtropical streams (i.e., a forest headwater stream as a reference and an urban stream that was hyper-eutrophic). Litter decomposition was estimated using litter bags with two mesh sizes (i.e., 50 μm and 2 mm) and two leaf species with different qualities (i.e., *Alangium chinense* and *Machilus leptophylla*), with a total of 96 litter bags. Nitrogen (N) and phosphorus (P) uptake rates were measured *in situ* based on the spiraling model. We found that the decomposition rate of *A. chinense* was approximately seven times that of *M. leptophylla* in both streams. Moreover, in the urban stream, the litter decomposition rate (0.004 day^{-1}) was one-third that of the forest stream (0.013 day^{-1}), regardless of the litter species. Macroinvertebrates strongly contributed to litter decomposition in the forest stream, where decomposition rates were 1.8-fold higher in the coarse mesh compared to the fine mesh bags, while they had a negligible role in the urban stream (no significant difference between the two mesh bags). P uptake was higher (85-fold) and N uptake was lower (0.13-fold) in the urban compared to forest stream. Litter decomposition and nutrient uptake exhibit decoupled response. These findings show that litter decomposition by $k_{\text{coarse}}/k_{\text{fine}}$ metrics and the uptake of N and P are complementary and should be considered in the management and restoration of urban stream ecosystems.

1. Introduction

Urbanization is becoming a dominant land use form worldwide and 90% of urban population growth over the next 30 years is projected to occur in Asia and Africa (Classen-Rodríguez et al., 2019; United Nations, 2018). Rapid urbanization has brought about a series of changes in the physical (e.g., temperature and geomorphology), chemical (e.g., excessive nutrients and contaminants), and biological parameters, referred as “Urban Stream Syndrome” (Walsh et al., 2005; Walters et al., 2009; Wiederkehr et al., 2020). Under the stress of urbanization, vulnerable

freshwater ecosystems experienced severe declines in aquatic biodiversity and ultimate degradation in ecosystem functions (Gál et al., 2019; Palmer and Ruhi, 2019; Pereda et al., 2021). For example, leaf litter decomposition and nutrient uptake, two key ecosystem functions that support organic matter and nutrient cycling in streams (Chauvet et al., 2016; Johnson et al., 2016; Woodward et al., 2012), are adversely impacted, thereby weakening the critical ecosystem services such as supporting and regulation services to human beings (Chadwick et al., 2006a; Heathwaite, 2010; Levi and McIntyre, 2020).

Leaf litter decomposition rates vary in response to multiple

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environmental and biological factors modulated by urbanization stress (Lin et al., 2020; Wiederkehr et al., 2020). More specifically, higher nutrient concentrations and warmer water in urban streams, facilitate colonization and activity of decomposer communities (i.e., bacteria, fungi and macroinvertebrates) on leaves (Chadwick et al., 2006a; Lin et al., 2020; Silva-Junior et al., 2014). This promoting effect is more pronounced for litter species with lower initial nutrient concentrations (e.g., oak tree leaves) (Ferreira et al., 2006; Gulis et al., 2006). Since microbes growing on such litter species are nutrient-limited, they tend to readily utilize the inorganic nutrients from eutrophic streams (Gulis et al., 2006). However, reduced litter decomposition rates are also reported in urban streams due to toxic compounds (e.g., ammonia), greater sedimentation and limited oxygen (Chadwick et al., 2006; Peng et al., 2020), which could result in a decrease in biodiversity and a shift of decomposer communities (Peng et al., 2020; Wang et al., 2018; Zhang et al., 2021). Among decomposers, shredders are more sensitive to the adverse impact in urban streams (with reduced abundance as a consequence), while bacteria may be boosted in this condition, where an enhanced contribution from the tolerant bacteria to the litter decomposition could be expected (Lin et al., 2020; Pascoal et al., 2005; Pereira et al., 2016).

At the same time, higher nutrient concentrations in urban streams can accelerate nutrient uptake by increasing productivity of microbes and algae, for which the nitrogen (N) and phosphorus (P) are primary biogenic elements (Rode et al., 2016; Wang et al., 2018). Specifically, microbial N removal, including denitrification and anaerobic ammonium oxidation (anammox) processes, can be promoted by hypoxic conditions and abundant substrates in sediments of urban streams (Stoliker et al., 2016; Zhu et al., 2013). However, with continuous increases of nutrient concentrations, the nutrient uptake rate will eventually stop increasing and plateau, as additional nutrient inputs will lead to algal blooms with associated hypoxic conditions, thereby reducing habitat quality (Arnon et al., 2015; Dunck et al., 2015; Liu et al., 2019).

Ecosystem functions of litter decomposition and nutrient uptake are usually investigated separately in streams (Mulholland et al., 2008; Woodward et al., 2012), and rarely examined in tandem. In addition, few studies have examined these ecosystem functions under urbanization (Ferreira et al., 2015; Woodward et al., 2012). In fact, a dramatic increase in nutrient concentrations of streams was observed in subtropical Asia in recent years (Cao et al., 2020; Sarmiento-Sánchez et al., 2017; Shreadah et al., 2020). Typically, the concentration of N and P in many urban streams in developing countries such as China and India has risen to a very high stage (i.e., TN > 1.2 mg L⁻¹ and TP > 0.1 mg L⁻¹), highlighting the occurrence of hyper-eutrophication (Istvánovics, 2009; NOAA, 1996; Yang et al., 2008). However, according to a comprehensive over-review on effects of nutrient enrichment on litter decomposition in freshwater, the related research in tropical areas is scarce (<8% of the total 99 studies) and only 1% were conducted in Asia (Ferreira et al., 2015).

To address these knowledge gaps, we investigated the response of nutrient uptake, leaf litter decomposition, and associated microbial and macroinvertebrate community structures to urbanization. Our study was conducted in two subtropical streams in China using a forest stream and a hyper-eutrophic urban stream. In this work, we hypothesized that urbanization has stronger negative effects on macroinvertebrate shredders than microbes (e.g., fungi and bacteria). Then, considering the fact that litter decomposition is closely linked with activities of shredder and fungi, with nutrient uptake closely linked with bacteria, respectively, we hypothesized that these two functions would respond differently. Overall, our study aims to provide complementary information about stream ecosystem functioning under urban stream syndrome conditions that may be helpful for management and/or restoration measures.

2. Materials and methods

2.1. Study sites

The study was conducted in a forest headwater stream (Jinfoshan stream) and an urban stream (Qingshuixi stream) in Chongqing (southwest China, Fig. S1) between October 2017 and January 2018. The region has a subtropical humid climate. The forest stream flows through a mountain area with little human disturbance, located in the Jinfoshan Mountain National Nature Reserve (28°46'–29°38'N, 106°54'–107°27'E). It served as the reference stream for this study. The Qingshuixi stream is located downstream from a main urban area of the city (29°27'–29°46'N, 106°14'–106°31'E), where it is surrounded by an agricultural area with a high population density (2,083 km²). Qingshuixi stream represented the typical hyper-eutrophic urban streams in Chongqing city (27 streams) and even the whole of China (approximately 2000 streams) (Cao et al., 2020). This study could serve as a reference to the hyper-eutrophication of urban streams in China and other developing countries. Two sites with 200–300 m distance at the upstream were selected as representative reaches for each stream. The characteristics (i.e., stream order, size, and morphology) other than nutrients were standardized as far as possible: the two streams were low order, 7–9 m wide, and < 50 cm deep at the winter baseflow (Table 1).

2.2. Water parameters

Three water samples were collected from each stream on a monthly basis from October 2017 to January 2018 during mid-afternoon. For each stream, the dissolved oxygen (DO), conductivity, temperature, and pH were determined *in situ* (YSI plus, Yellow Spring, USA). The concentrations of chlorophyll-a (Chl-a) and total nitrogen (TN), ammonium (NH₄⁺), nitrate (NO₃⁻), total phosphorus (TP), and phosphate (PO₄³⁻) were measured by a spectrophotometer (UV2600, Shimadzu, Japan) (APHA, 2005). Hydro-morphological parameters (i.e., stream depth, width, and current velocity) were measured following Gorden et al. (1992).

2.3. Macroinvertebrates

Benthic macroinvertebrates were collected when we deposited and retrieved the litter bags. Three samples characterizing different microhabitats present in pools were collected using a Surber net (30 × 30 cm, mesh size 250 μm). Macroinvertebrate samples were preserved in 75% ethanol and then determined to the lowest possible taxonomic level (mainly to genus or species), except for the *Diptera* (Family or subfamily) and *Oligochaeta* (Class). Functional feeding groups were classified according to Tachet et al. (2002).

Table 1
Characteristics of streams (shown as mean ± SE, n = 4). R = reference or study control; U = urban.

Stream Characteristics		Jinfoshan (R)	Qingshuixi (U)
Mean annual precipitation	mm	1,395.5	1,113.4
Air temperature	°C	12.5	18.2
Distance to source	km	8.6	7.0
Riparian shading	%	95 ± 4.6	56.2 ± 3.9
Bedrock		Limestone and conglomerate	Conglomerate and mudstone
Streambed		Stones and pebbles	Silt and sand
Width	m	8.4 ± 0.4	8.3 ± 0.3
Average slope	‰	29 ± 1.6	13 ± 0.5
Depth	m	0.1 ± 0.05	0.25 ± 0.02
Velocity	m s ⁻¹	0.08 ± 0.01	0.18 ± 0.04

2.4. Leaf bag preparation and litter decomposition

Two riparian trees (i.e., *Alangium chinense* and *Machilus leptophylla*) were selected according to their contrasting leaf chemical characteristics (Table 2) and representation in the region. Since *A. chinense* had lower concentrations of N, P, lignin, and the lower toughness, C/N and lignin/N ratios ($p < 0.05$ for all) compared to the values for *M. leptophylla* (Table 2), we regarded *A. chinense* as the herein higher-quality species compared to *M. leptophylla*. We first collected freshly fallen leaf litter in September 2017, dried the leaves at 60°C for 48 h, and then enclosed the dried leaves (3 g) in nylon bags (15 × 10 cm) with two mesh sizes (50 µm and 2 mm). The coarse mesh (2 mm) allowed access for macro-invertebrates, while the fine mesh (50 µm) excluded macro-invertebrates. Finally, 96 litter bags (2 streams × 2 species × 2 mesh sizes × 4 replicates × 3 times) were tied to nylon lines that were anchored randomly to the stream bed using house bricks in pools of the stream channel, and on days 21, 47, and 78, four replicates for each treatment were retrieved into a cool box. The retrieved leaves were taken out of the bag, gently rinsed with distilled water, and then transferred into a vacuum freeze-dryer. Afterwards, a subsample of 0.6 g was stored at −80°C for microbial measurements (described in next the section). In addition, the remaining litter was dried at 60°C for 48 h and then weighed, to give the final dry mass (DM), taking into account the subsample removed for microbial measurements.

2.5. Microbes

2.5.1. Microbial abundance

Microbial DNA was extracted from the leaf subsamples via a FastDNA® Spin Kit for Soil (MP, USA). The abundances of fungi and bacteria were estimated using real-time quantitative PCR (qPCR). Primers targeting the bacterial 16S V4 hypervariable region were: 515F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGACTACHVGGGTWCTAAT-3') (Caporaso et al., 2011). Meanwhile, primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify the fungal ITS ITS1 hypervariable region (Tolkinen et al., 2015). Amplification was performed with an ABI Prism® 7500 Real Time PCR system (Applied Biosystems, USA) as detailed in Wang et al. (2020).

For microbes in the sediments, 5 g of sediment was collected and preserved at −80°C for further analysis. The DNA extraction of microbes was performed as detailed in our previous publication (Huang et al., 2021). The 16S rRNA gene, denitrifying functional gene *nirS*, and anammox functional gene (hydrazine synthase gene, *hzsB*) were amplified using primers 338F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGACTACHVGGGTWCTAAT-3') (Wu et al., 2019), cd3aF (5'-GTSACGTSAAGGARACSGG-3') and R3cdR (5'-GASTTCGGRTGSGTCTTG A-3') (Palmer et al., 2012), and *hzsB* 396F (ARGGHTGGG-GHAGYTGAAG) and *hzsB* 742R (GTYCCHACRTCATGVGTCTG) (Zhu et al., 2015). Details of the amplification can be found in Huang et al. (2021).

2.5.2. Microbial community structure

Microbial community structures were analyzed using a high-

Table 2

Concentration of carbon, nitrogen, phosphorus, and lignin, ratios of lignin:N and the toughness of *A. chinense* and *M. leptophylla* leaf litter (mean ± SE; $n = 5$). Comparison of litter characteristics between leaf species was based on the *t*-test.

Species		<i>A. chinense</i>	<i>M. leptophylla</i>	<i>p</i> value
C	(% DM)	36.49 ± 0.25	40.08 ± 0.22	<0.0001
N	(% DM)	1.55 ± 0.06	1.22 ± 0.03	0.001
P	(% DM)	0.19 ± 0.002	0.10 ± 0.004	<0.0001
lignin	(% DM)	3.81 ± 0.05	25.52 ± 0.99	<0.0001
Lignin:N		2.46 ± 0.10	28.89 ± 1.00	<0.0001
toughness	(N mm ^{−1})	0.24 ± 0.02	0.35 ± 0.03	0.02

throughput sequencing technique. For each sample, equimolar amounts of amplicons were sequenced and paired-end formatted with the Illumina MiSeq platform (Illumina, San Diego, USA). Microbial community structures in the leaves were detected using 515F and 806R for bacteria, and ITS1F and ITS2R for fungi (see details of pyrosequencing and analysis in Qin et al. (2018)), and bacterial structures in the sediments were detected using the primers 338F and 806R (Wu et al., 2019) (see details in Huang et al. (2021)).

2.6. Nutrient uptake

Nutrient uptake experiments were conducted for the two streams according to the nutrient spiraling model (Ensign and Doyle, 2006; Stream Solute Workshop, 1990) and using the slug addition technique (Ruggiero et al., 2006; Sánchez-Pérez et al., 2009; Yao et al., 2017). At the upstream (release point), a known volume of solution (i.e., nutrients NO₃[−] and PO₄^{3−} plus a conservative tracer NaCl) were added simultaneously and at once in the mid-channel. Subsequently, water samples were collected with increasing frequency (i.e., from every minute to every 15 s) at the downstream (monitoring point, approximately 120–150 m from the release point), to record the conductivity data profile of the streams. Sample collection was stopped when conductivity values decreased to those of the background value (i.e., the conductivity measured before the slug addition experiment). In total, 50–60 samples were collected in each stream. Concentrations of nutrients and Cl[−] were plotted on time curves. Based on the obtained concentrations, the mass (mg) of retained nutrient was estimated for the investigated reach. Uptake length (*Sw*, describing the mean distance that a nutrient ion has traveled before the final uptake) was estimated by the following equation:

$$Sw(m) = ([Nut]b \times Q) \div (u \times w) \quad (1)$$

where *u* (mg m^{−2} min^{−1}) denotes the mass of removed nutrients per unit area of the stream bottom, *A* (m²), in the experiment duration, *T* (min), *[Nut]b* is the background concentration of nutrient (mg L^{−1}) in the stream water, *Q* is the discharge (m³ s^{−1}), and *w* (m) is the mean width of the stream reach.

The mass transfer velocity (*Vf*) was determined according to:

$$Vf = u \times h \div Sw \quad (2)$$

where *h* denotes the depth.

The gross nutrient uptake rate at ambient level (*U*) was used to quantify the benthic nutrient removal since it is relatively independent of the hydrologic characteristics of the stream and nutrient concentrations (Yao et al., 2017). Uptake rate (*U*, mg m^{−2} min^{−1}) was determined according to:

$$U = Q \times [Nut]b \div Sw \quad (3)$$

2.7. Statistical analysis

Stream characteristics, total abundance and Shannon-Wiener index (*H*) of macroinvertebrates, and bacterial abundance (i.e., abundance of the bacterial 16S gene, denitrifying *nirS* gene, and anammox *hzsB* gene) in sediments between two streams were examined using Student's *t*-tests, as well as litter characteristics between two leaf species.

Litter decomposition rates (*k*), were calculated according to the negative exponential model:

$$M_t = M_0 e^{-kt} \quad (4)$$

where *M*₀ indicates the initial mass and *M*_t indicates the remaining mass at time *t* (in days) (Graça et al., 2005).

Calculations were performed for decomposition rates for fine (*k_{fine}*) and coarse mesh (*k_{coarse}*) bags, and for the ratio of *k_{coarse}/k_{fine}* (Gessner and Chauvet, 2002). A three-way ANOVA test was performed to analyze

the effects of sites, leaf species, mesh size, and their interactions on litter decomposition rates (k), and the bacterial and fungal abundance on leaves. Tukey's post hoc test was used to compare the differences between mesh sizes at each stream for each species. Data analysis was performed using a log ($x + 1$) (fungal abundance) or a square root transformation (decomposition rate) when necessary to achieve normality and homogeneity. All statistical analyses were performed with R software (version 3.4.2, 2016).

3. Results

3.1. Water characteristics

Temperature, conductivity, and concentrations of TN, TP, NH_4^+ , NO_3^- , and PO_4^{3-} were substantially higher in Qingshuixi than in the Jinfoshan stream (t -test, $p < 0.05$) (Table 3). Considering the high values for TN (15.1–16.8 mg L^{-1}) and TP (0.59–0.66 mg L^{-1}), the Qingshuixi stream was regarded as a hyper-eutrophic urban stream, according to the reference data of National Oceanic and Atmospheric Administration for rivers and estuaries (NOAA, 1996). As listed in Table 3, DO concentration in Qingshuixi was approximately half that of the Jinfoshan stream (9.6 mg L^{-1}). In addition, pH varied little between the streams.

3.2. Macroinvertebrate communities

Our study demonstrates that macroinvertebrates had significantly lower diversity (1/4th, t -test, $p < 0.0001$) but greater total abundance (2-times, t -test, $p = 0.001$) in the urban stream (Qingshuixi) than in the reference stream (Jinfoshan) (Fig. 1a and b). In addition, the macroinvertebrate assemblage structures and dominance shifted under urbanization. Insect larvae were the most dominant organisms in the reference stream (97.4% of abundance), while *Oligocheta* and *Hiruta* dominated the macrofaunal density in the urban stream (Qingshuixi stream, 70.1% and 25.4% of abundance) (Fig. 1b). For the functional feeding group, the percentage of deposit feeders in the urban stream (70.8%) was approximately 4-times that of the reference stream (17.5%). Shredders accounted for 18.2% of the total abundance in the reference stream, while they were absent in the urban stream (Fig. 1c).

3.3. Litter decomposition and associated microbial communities

The average abundance of fungi ($F_{1,16} = 39.22$, $p < 0.0001$) and bacteria ($F_{1,16} = 24.55$, $p = 0.0001$) associated with leaf litter varied between the streams (Table S1, Fig. 2). Specifically, the average fungal abundance decreased significantly from the reference stream ($6.6 \text{ E} + 10$) to the urban stream ($7.5 \text{ E} + 08$) (Tukey's test, $p = 0.0001$) (Fig. 2a), while the average bacterial abundance was similar for two streams ($2.8 \text{ E} + 11$ and $2.5 \text{ E} + 11$) (Fig. 2b). Thus, a much higher average bacterial abundance (up to three orders of magnitude) than the fungal abundance in the urban stream was found. Regarding the influence of mesh size,

fungal abundance in the coarse mesh bags was considerably lower than that in the fine mesh bags for *A. chinense* at both sites (Tukey's test, $p = 0.006$) (Fig. 2a). Further, the bacterial abundance in the coarse mesh bags was significantly higher than that in the fine mesh bags on *A. chinense* in the urban stream (Tukey's test, $p = 0.0001$), while the opposite trend was found for *M. leptophylla* (Tukey's test, $p = 0.004$) (Fig. 2b).

In both streams, *Ascomycota* was the dominant fungus phyla (87.2% and 53.7% for the reference and urban stream, respectively) (Fig. 3a). At the genus level, fungal assemblages depended on the leaf type and mesh sizes, yet exhibited completely different community structures in the two streams (Table S5 and S6). Specifically, in the reference stream, *Tetracladium marchalianum* and *Setophaeosphaeria* accounted for most genus on *A. chinense* and *Mycocleptodiscus* sp. dominated on *M. leptophylla*. In the urban stream, *Geotrichum* and *Hydnellum* were the dominant genus on *A. chinense* for both mesh size, and on *M. leptophylla* for its fine mesh size, on which *Neopestalotiopsis* dominated for its coarse mesh size (>60%).

Proteobacteria and γ -*Proteobacteria* were the most abundant bacteria phylum and class, respectively in the litter bags in both streams (Fig. 3b). At the class level, in the reference stream, *Alphaproteobacteria* and *Bacteroidia* accounted for more of the bacterial community on *A. chinense* (30.8% and 18.8%, respectively) than on *M. leptophylla* (19.9% and 6.0%, respectively) (Table S7 and S8). In the urban stream, *Bacteroidia* (17.6%) and *Clostridia* (17.0%) were the dominant groups on *A. chinense* and *Alphaproteobacteria* (24.7%) dominated on *M. leptophylla*.

Litter decomposition rates (k) were significantly influenced by litter species ($F_{1,24} = 141.36$, $p < 0.0001$) and urbanization ($F_{1,24} = 59.33$, $p < 0.0001$), and the interactions (species $\times$ stream ($F_{1,24} = 0.99$, $p = 0.33$) and mesh $\times$ stream ($F_{1,24} = 10.25$, $p = 0.0043$)) (Three-way ANOVA, Table S1). In both streams, the rate of decomposition of *A. chinense* was approximately seven times that of *M. leptophylla*. Moreover, for both litter species, average decomposition rates for the urban stream were one-third that of the reference stream (Fig. 4). Specifically, decomposition rates in the coarse mesh bags were significantly greater compared to those in the fine mesh bags in the reference stream (Tukey's test, $p < 0.001$ for *A. chinense* and *M. leptophylla*, respectively), while no significant differences were found between mesh types in the urban stream (Tukey's test, $p = 1.00$ and 0.23 for *A. chinense* and *M. leptophylla*, respectively). The mean ratios of k in coarse mesh and k in fine mesh bags ($k_{\text{coarse}}/k_{\text{fine}}$) in the reference stream were similar for *A. chinense* and *M. leptophylla* (i.e., 1.9 and 1.8, respectively). In the urban stream, however, this ratio was 0.9 and 0.4 for *A. chinense* and *M. leptophylla*, respectively (Fig. 4).

3.4. Nutrient uptake and microbial communities in sediments

For the nutrient uptake function, the reference stream retained more NO_3^- -N than for PO_4^{3-} -P, while the urban stream showed the opposite trend (Fig. 5). In the reference stream, the uptake length (S_w) was shorter and the uptake velocity (V_f) and uptake rate (U) were higher for NO_3^- -N than for PO_4^{3-} -P (Table S2 and Fig. 5). The urban stream showed a much higher PO_4^{3-} -P concentration, exhibiting an 85-fold increase in uptake capacity for PO_4^{3-} -P compared to the reference stream ($U_P = 0.17$ and 0.002 , respectively). Nevertheless, the NO_3^- -N uptake rates in the urban stream were approximately one-tenth those of the value of the reference stream ($U_N = 0.02$ and 0.15 , respectively).

In general, the total abundance of bacteria was approximately 1.5-times higher in the urban stream than in the reference stream (t -test, $p = 0.0001$) (Fig. 6a). Further, the abundances of the *nirS* and *hnsB* gene in the urban stream were approximately two orders of magnitude greater than those in the reference stream (t -test, $p < 0.0001$) (Fig. 6a). For the bacterial community structures (Fig. 6b and c), the *Proteobacteria* was the major bacteria phyla in the sediments of the reference stream (60.4%) and the urban stream (50.6%), with α -*Proteobacteria* being the most abundant class for both streams (31.5% and 21.3%, respectively). At the phylum level, more *Actinobacteria*, and less *Acidobacteria*,

Table 3

Physicochemical characteristics of stream water during the experimental period (shown as mean $\pm$ SE, $n = 4$). R = reference or study control; U = urban.

Stream Characteristics		Jinfoshan (R)	Qingshuixi (U)	p value
Conductivity	$\mu\text{S cm}^{-1}$	181 $\pm$ 8.4	968 $\pm$ 46.7	<0.0001
DO	mg L^{-1}	9.7 $\pm$ 0.32	4.5 $\pm$ 0.32	<0.0001
pH		7.9 $\pm$ 0.18	7.7 $\pm$ 0.04	0.32
NH_4^+	mg L^{-1}	0.2 $\pm$ 0.07	7.3 $\pm$ 1.67	0.0054
NO_3^-	mg L^{-1}	0.8 $\pm$ 0.48	15.4 $\pm$ 1.32	<0.0001
PO_4^{3-}	mg L^{-1}	0.04 $\pm$ 0.01	0.7 $\pm$ 0.08	0.0002
TN	mg L^{-1}	1.9 $\pm$ 0.13	19.2 $\pm$ 0.80	<0.0001
TP	mg L^{-1}	0.14 $\pm$ 0.01	0.53 $\pm$ 0.03	<0.0001
Mg^{2+}	mg L^{-1}	5.7 $\pm$ 0.26	19.4 $\pm$ 0.80	<0.0001
Ca^{2+}	mg L^{-1}	4.1 $\pm$ 0.22	18.0 $\pm$ 0.74	<0.0001
Chl-a	mg L^{-1}	0.03 $\pm$ 0.01	0.16 $\pm$ 0.01	<0.0001

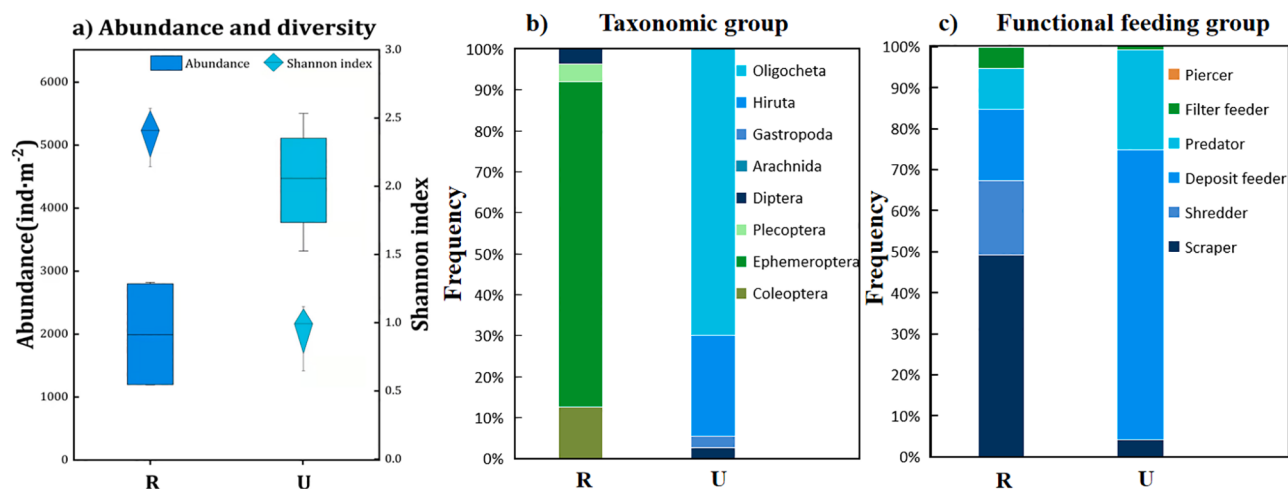


Fig. 1. (a) Values of abundance and the Shannon index (H'), (b) relative abundance of taxonomic group, and (c) functional feeding groups of macroinvertebrates in the two study streams (R = reference stream, U = urban stream).

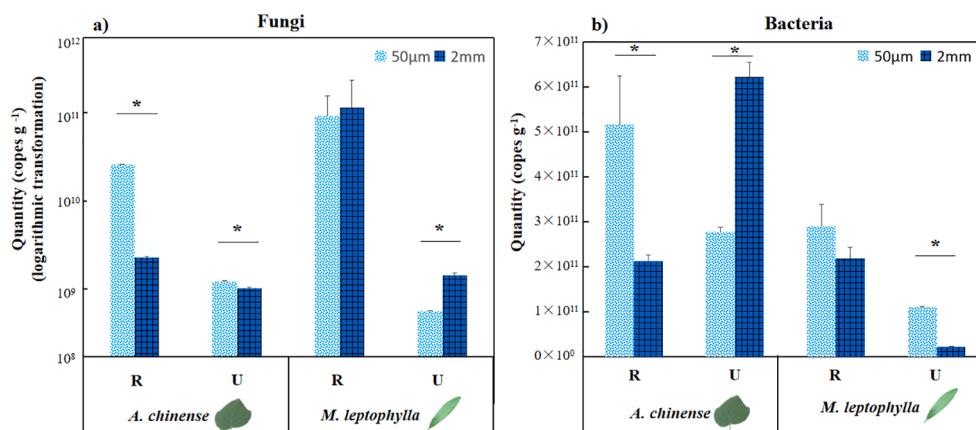


Fig. 2. Abundance of (a) fungal ITS gene and (b) bacteria 16S gene in *A.chinense* and *M.leptophylla* leaf litter enclosed in fine (50 μm) and coarse (2 mm) mesh bags incubated in the two study streams (mean ± SE, n = 3; R = reference stream, U = urban stream). For a given species and stream, star and horizontal line symbol denote significant differences between the mesh sizes, $p < 0.05$.

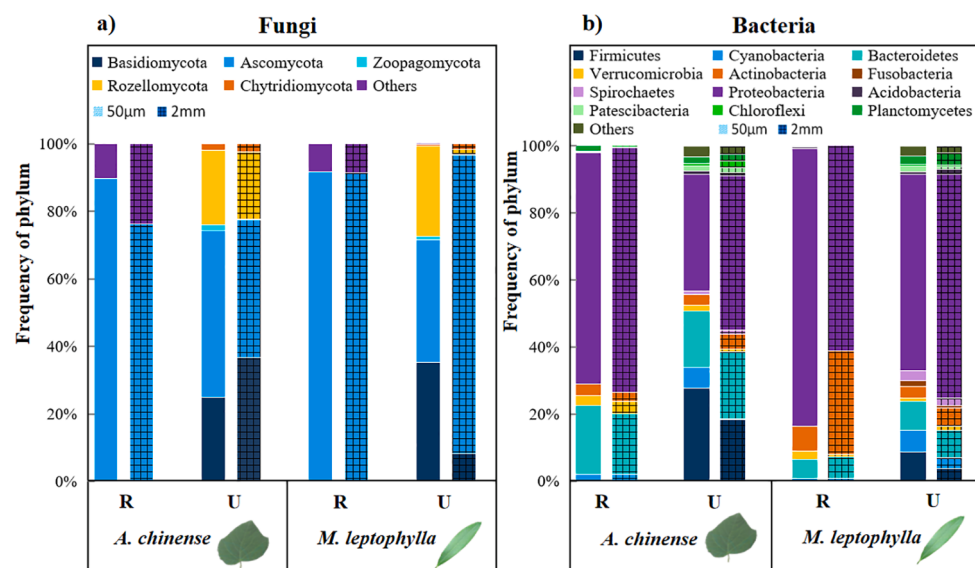


Fig. 3. (a) Relative abundances of fungal and (b) bacterial dominant phylum in *A.chinense* and *M.leptophylla* leaf litter enclosed in fine (50 μm) and coarse (2 mm) mesh bags incubated in the two study streams (R = reference stream, U = urban stream).

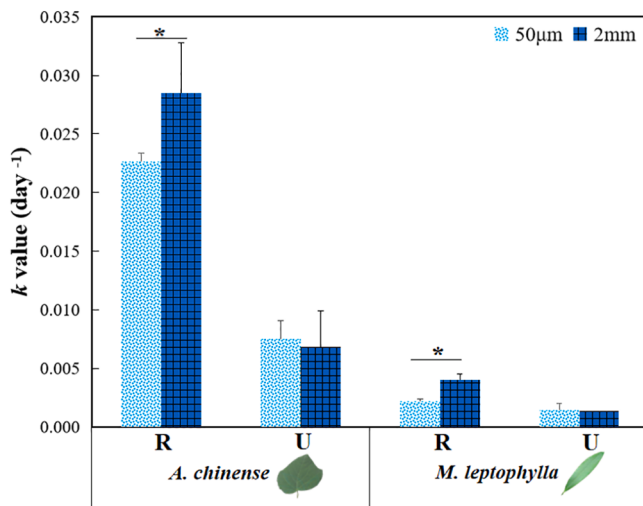


Fig. 4. Litter decomposition rates (k , day^{-1} , mean $\pm$ SE, $n = 4$) for *A. chinense* and *M. leptophylla* leaf litter enclosed in fine (50 μm) and coarse (2 mm) mesh bags incubated in the two study streams (R = reference stream, U = urban stream). For a given species and stream, star and horizontal line symbol denote the significant differences for the mesh size, $p < 0.05$.

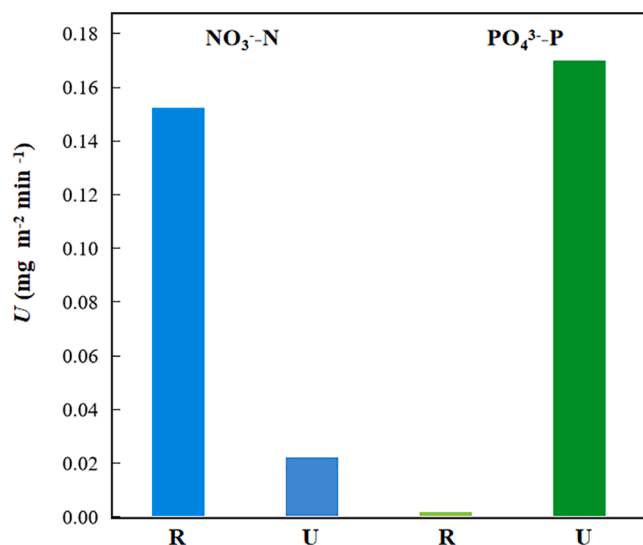


Fig. 5. Nitrate and phosphorus uptake rates in the two streams (R = reference stream, U = urban stream).

Chloroflexi and *Planctomycetes* were found in the sediments of the reference stream compared to the urban stream. At the class level of the bacterial community, more *Actinobacteria* and *Deltaproteobacteria*, and less *Anaerolineae*, *Planctomycetacia*, and *Subgroup_6*, and comparable *Gammaproteobacteria* were found in the sediments of the reference stream compared to the urban stream.

4. Discussion

4.1. Response of the litter decomposition function to urbanization

First, this study documented variations of the fungi and macroinvertebrates communities in the urban stream in respect with that in the forest stream. Specifically, we observed a decrease in abundance of aquatic hyphomycetes (such as *Tetrachaetum*) (Fig. 3a). Moreover, the main functional feeding groups shifted from scrappers, shredders, and deposit feeders to deposit feeders and predators, with an increase in

pollution-tolerant species (mainly *Oligocheta* and *Hiruta*) (Fig. 1). Previous findings have shown that urbanization often causes increased eutrophic level, fine sediments, accumulation of toxic substances (e.g., nitrite and ammonia), and decreased concentration of dissolved oxygen (Camargo and Alonso, 2006; Chadwick et al., 2006; Peng et al., 2020). Under such conditions, the abundance of some key species (e.g., aquatic hyphomycetes and shredders) has been reported to be reduced (Dunck et al., 2015; Lecerf et al., 2006; Woodward et al., 2012).

In this context, decomposition rates of both *A. chinense* and *M. leptophylla* decreased by two-thirds in the urban stream (Fig. 4). We speculate that the adverse effects of urbanization on litter decomposition in our study far outweighed the differences between the leaf characteristics (Pereira et al., 2016). Considering that the herein total impervious area (TIA) for the urban stream catchment was over 80% (He et al., 2017) where litter loss decreased as impervious catchment area exceeded 40% TIA (Chadwick et al., 2006), our finding coincided with this previous report. Such a decrease can be mainly attributed to the reduced fungal abundance (especially of aquatic hyphomycetes) (Figs. 2 and 3) and the lack of shredders (Fig. 1), since fungi and macroinvertebrate shredders are known to be essential in decomposition processes (Pereira et al., 2016). Fungi can improve the litter palatability for macroinvertebrates by metabolizing the less-degradable molecules (e.g., lignin) into more assimilable compounds (Graça et al., 2016; Seena et al., 2019), and shredders can break apart coarse particulate organic matter into small fragments (Graça, 2001; Martins et al., 2015; Villanueva et al., 2012).

For the decomposer communities reflected by different mesh sizes, microbes were major contributors to litter decomposition while the macroinvertebrates impacted differently in the two streams (Fig. 4). The $k_{\text{coarse}}/k_{\text{fine}}$ metric detected clear shifts in the contribution of invertebrates and microbial decomposers in the urban stream. In particular, in the reference stream, the macrofauna significantly promoted litter decomposition for both leaf species in the reference stream, suggesting a direct shredding effect and/or positive interactions between the macroinvertebrates and microbial communities (Wang et al., 2020). Nevertheless, the macroinvertebrates had a negligible role in litter decomposition in the urban stream, where no significant difference in decomposition rates was found between bag types. This decreased contribution of macroinvertebrates to litter decomposition in the urban stream was mainly due to the fact that high ammonia/nitrite concentrations inhibited macroinvertebrate shredders, while they are not significantly detrimental for microbial decomposers (Pereira et al., 2016; Piscart et al., 2009).

Indeed, previous studies have shown a contribution of macroinvertebrates that decreases with the nutrient levels (Pascoal et al., 2005; Woodward et al., 2012). Further studies are needed to better understand the contributions of macroinvertebrates under conditions of urbanization, especially in the condition of hyper-eutrophication, and to further explore the $k_{\text{coarse}}/k_{\text{fine}}$ values (Gessner and Chauvet, 2002).

In this study, we observed a significant decrease in bacterial abundance on *M. leptophylla* in coarse mesh bags compared to the fine ones in the urban streams, (Fig. 2b). *Branchiura sowerbyi* accounted for 70% of the macroinvertebrate abundance in the urban stream. This suggests a top-down control of bacteria from the macrofauna, which agrees with a previous study that documented *B. sowerbyi* consumed saprotrophic bacteria that proliferated on foliar tissue (Aston, 1984). We should also note that there may be other explanation as well. For example, in coarse mesh bags, bacteria may be more easily rushed away, leading to a decrease of bacterial abundance. The actual mechanisms should be further elucidated with indoor controlled experiments.

4.2. Response of nutrient uptake function to urbanization

N and P uptake rates in this study were within the range for forest and urban streams, as reported by Mulholland et al. (2008) and Ensign & Doyle (2006). For the nutrient uptake function, decoupling results were found for N (reference stream > urban stream) and P (reference stream

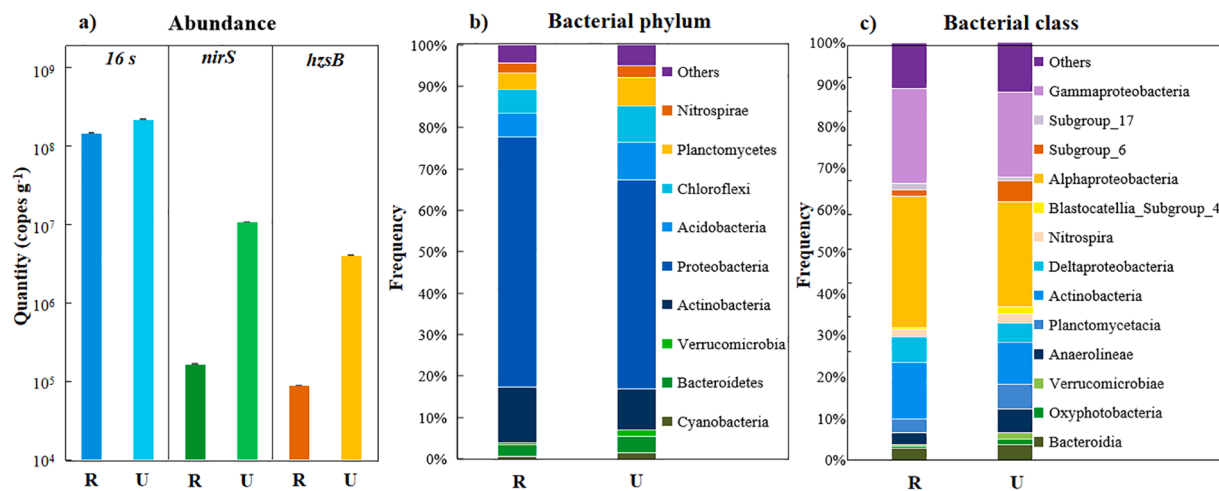


Fig. 6. (a) Abundance of the bacterial 16 s gene, denitrifying *nirS* gene, and anammox *hzsB* gene in sediments of the two streams (note that the Y-axis is in logarithmic scale; mean $\pm$ SE, $n = 3$; R = reference stream, U = urban stream); (b) Relative abundances of bacterial dominant phylum and (c) genera in sediments of the two streams.

< urban stream) (Fig. 5). Such decoupled results can be mainly caused by the extremely high background concentration of N (TN: 19.2 mg/L) and the N:P ratio (26) in the urban stream. These results indicated that N was saturated while P was limiting in the urban stream, especially when considering that a N:P ratio of 20 has been proposed in the previous study associated with P limiting cases (Earl et al., 2006). Ultimately, nutrient saturation occurs when the availability of a nutrient increases to a point at which another factor critical to growth becomes limiting.

In particular, P uptake was considerably stimulated in the urban stream, compared to the reference stream (Fig. 5). The increased P uptake rates in our study could stem from enhanced assimilation as well as through physical interaction with sediments (e.g., absorption and precipitation reactions) (Painter et al., 2020). On one hand, the concentration of Chl-a in the urban stream that is 4-times greater indicates that P uptake due to algae assimilation could be stimulated by excess nutrients (Andersen et al., 2020). On the other hand, since the divalent cation concentration (e.g., Ca^{2+} and Mg^{2+} , Table 1) was 4-times greater, one might assume that more P would be precipitated in the urban stream (Madrid et al., 2020).

In contrast, the N uptake capacity in the urban stream was considerably weaker than in the reference stream (Fig. 5). In fact, a greater abundance of NO_3^- removing bacteria in the urban stream suggests greater denitrification and anammox processes to remove NO_3^- (Fig. 6) and more algae/bacteria could also lead to an increase in N assimilation (Andersen et al., 2020). The decreased N uptake that was observed was due to the following reasons. First, excessive N (approximately >1 mg/L) may act as a stressor to stream communities and can result in N uptake saturation, precluding the biological system from assimilating N in masses corresponding to their concentrations in the stream (Arnon et al., 2015; Gücker and Pusch, 2006; Merseburger et al., 2011). Second, in urban freshwaters, the release of internal N loading cannot be neglected (release rate up to $175 \text{ mg N m}^{-3} \text{ day}^{-1}$ has been reported in a typical hyper-eutrophic stream (Wu et al., 2017)), which possibly offset the role of the intrinsic N uptake, resulting into a decreased uptake rate as found in our study. Furthermore, more *Nitrospira* in the urban stream (Table S9) suggested that oxidation of NH_4^+ to NO_3^- occurred which could lead to increased NO_3^- concentration and a reduction of NO_3^- uptake, which should be explored in further studies.

In highly urbanized streams, litter decomposition rates in coarse and fine mesh bags both decreased. Noticeably a declined $k_{\text{coarse}}/k_{\text{fine}}$ was also observed, which is due to the decreased contribution of macro-invertebrates. Taking this phenomenon as a sign of severe degradation of ecosystem, we propose that determining $k_{\text{coarse}}/k_{\text{fine}}$ could indicate the influence of urbanization stress on freshwater ecosystems. Meanwhile,

we observed the decoupled uptake rates of N and P in the urban stream, which was highly associated to N:P ratio in our invested condition, and interplay of other in-stream variables. A deep understanding of the biogeochemical distortions created by N and P uptake and the circumstances that foster a return to more normal nutrient cycling conditions could provide reference for restoration of water purification capacity in urban streams. Finally, a combined use of these ecological indicators could shed light on assessing stream conditions, determining the interactions between biological and ecological factors and, accordingly, effective avenues for restoring urban stream ecosystems. Future studies are suggested to compare more multi-functional stream systems with different urbanization gradients, so that their statistical analyses would allow the identification of the contributions of environmental factors to the litter decomposing and nutrient uptake from different streams.

5. Conclusions

This is the first study to report the simultaneous responses of two ecosystem functions, litter decomposition and nutrient uptake, in a highly urbanized stream ecosystem under hyper-eutrophic conditions. Results from this study demonstrated that the litter decomposition rates in the urban stream decreased by two thirds, regardless of the litter species. A negligible role of the macroinvertebrates in litter decomposition occurred in the urban stream. With regard to nutrient uptake function, urbanization led to a strong P uptake and a weak N uptake, mainly because of the high ratio of N:P in the investigated stream. Based on these findings, management or restoration strategies that focus on single ecosystem function may not be fully effective, given the decoupled performance of the two measured ecosystem functions that we observed in the urban stream. By investigating both stream ecosystem functions simultaneously and analyzing their interactions with biological factors, we believe these findings help contribute to obtain a better baseline for evaluating the impacts of urbanization on freshwater ecosystems which are important for management and restoration strategies.

CRedit authorship contribution statement

Jie Gao: Investigation, Writing – original draft. **Yuyue Huang:** Investigation, Writing – original draft. **Yue Zhi:** Writing – review & editing. **Jingmei Yao:** Conceptualization, Writing – original draft. **Fang Wang:** Investigation. **Wei Yang:** Investigation. **Le Han:** Writing – review & editing. **Dunmei Lin:** Validation. **Qiang He:** Investigation. **Bing Wei:** . **Khara Grieger:** Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2022.108859>.

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