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Can water temperature impact litter decomposition under pollution of copper and zinc mixture

Impact of temperature and metals to litter decomposition

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Abstract: To better understand the impact of warming on heavy metals (HM) associated with plant litter decomposition in streams, we investigated the impact of high and low HM (Cu and Zn) levels and different water temperatures (10, 15 and 20ºC) on microbial decomposition of *Typha angustifolia* L. litter and the associated extracellular enzyme activities. During a 100-day incubation, changes in litter mass losses, chemical composition (lignin and total carbohydrate), and extracellular enzyme activity were determined. The decomposition rates were accelerated by the low HM levels at 20ºC (0.0051 day^{-1} at CK *vs* 0.0061 day^{-1} at low HM levels). The negative effects of Cu and Zn on *Typha* litter decomposition were more pronounced at lower temperatures (10 and 15°C). The enhanced enzyme activities of cellulase and β-glucosidase and the higher lignin/litter weight loss and lignin/carbohydrate ratios were found at 20ºC and low HM treatment. The enzyme activities of β-glucosidase and cellulase were positively correlated with litter mass losses at 20ºC and low HM levels. These results suggest that a 5ºC increase in water temperature may attenuate the inhibition of low HM level on litter decomposition.

Keywords: global warming; heavy metal pollution; litter decomposition; extracellular enzyme activity.

1 Introduction

The biological decomposition of plant litter plays an essential role in organic matter turnover and energy transfer to higher trophic levels [1]. Compared with terrestrial ecosystems, this ecological process in aquatic ecosystems is sensitive to changes in water quality such as the increase of temperature, heavy metal (HM) pollution and eutrophication [2-3], which caused by human activities in the process of human development promoting changes in biotic communities with consequences to the functioning of aquatic ecosystems [4].

The predicted increase in temperature, which may reach 2.4–6.4°C, by the end of this century [5] is expected to affect organisms and ecological processes in both terrestrial and aquatic ecosystems [2-3,6]. As one of the most important ecosystem processes, litter decomposition is likely to be affected by the increased temperature [3, 7]. Previous studies have shown that a higher temperature may increase the decomposition of leaf litter in aquatic ecosystems by promoting the leaching of soluble compounds and enhancing invertebrate and microbial activities [2-3, 6]. Furthermore, the leaf litter decomposition is primarily mediated by the extracellular enzyme activity (EEA) of microbes, such as lignin and cellulase-degrading enzymes [9]. EEA is highly sensitive to environmental conditions, such as pH, salinity, trace metals and UV-B radiation and particularly with temperature as its important indirect effect to the affinity of enzyme systems [6]. Correlative studies have shown that without the interference of other environmental factors, any increase in temperature results in increased enzymatic activity to a certain extent [3,7,10-11]. Apart from the effects of temperature, EEA also reflects and feeds back on community composition [1,12]. The result is a successional loop that is highly responsive to temperature changes: alterations in activity affect substrate composition and population dynamics, and

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changes in community composition affect enzyme activity [12].

Additionally, several factors co-occurring with the increased temperature may modulate the effects of the litter on stream biota and processes. HM pollution is one of the primary concerns. Although some metals, such as copper and zinc, are essential for enzyme activity and electron transport chains, toxic metal concentrations may affect the bioactivity associated with litter decomposition in aquatic ecosystems [2,13-16]. Recent studies have indicated that the interaction of HMs (such as Cu, Zn, and Cd) is a combination of synergistic, additive, and antagonistic effects when these metals are mixed [10,17-18]. Likewise, the combination of single HM pollution (such as Cu or Cd) and temperature change may result in increased metal toxicity for aquatic decomposers [2] or copper tolerance to bacterial communities after short-term exposure [19]. However, the effects of aquatic decomposers simultaneously exposed to several stresses are scared [13].

Thus, the present study aimed to explore whether a 5ºC increase (from 10 to 15ºC in winter or from 15 to 20ºC in spring) in water temperature can impact litter decomposition under pollution of copper and zinc mixture and the impact of this ecological process.

2 Materials and Methods

2.1 Leaf conditioning

Typha (Typha angustifolia L.) leaves were collected in October 2011 along Qinhuai River (Nanjing, China) and oven dried at 40°C for 72 hours. They were subsequently rinsed using deionized water and cut into sections (10 cm diameter). Sets of 5 g of Typha leaves were placed in 111 net bags (15 cm × 23 cm; 0.5 mm mesh) to prevent the access of macro-invertebrate colonization. The litter bags were then put into the stream for 15 days to allow microbial colonization. A set of three bags was randomly retrieved from the stream after 30 minutes to determine initial leaf mass weight. The stream water had an average pH of 7.6, water temperature of 8ºC, conductivity of 19 µS cm^{-1} and the following contents: < 0.05 µg L^{-1} $N\text{-}NH_4^+$, 32 µg L^{-1} $N\text{-}NO_3^-$, and 40 µg L^{-1} $P\text{-}PO_4^{3-}$. Additionally, 60 L of stream water was collected and stored in a fridge (4°C) for water renewal throughout the whole experiment.

2.2 Microcosm experiment

After 15 days, the litter bags were retrieved from the stream and leaf sections from each of the remaining 108 bags were rinsed with deionized water and placed in sterile 250 ml Erlenmeyer flask with 150 ml of filtered and sterilized stream water (121ºC, 20 minutes). The microcosms were supplemented with low (15 µmol $ZnCl_2$ and 8 µmol $CuCl_2$) or high (150 µmol $ZnCl_2$ and 80 µmol $CuCl_2$) HM concentration solutions and the control treatment without any the above concentration solutions. All microcosms were kept on a shaker (150 rpm) at three temperatures (10, 15, and 20ºC, which based on a common temperature 15 ± 2.3ºC measured in Qinhuai River in spring, another set at 10°C and 20°C to simulate possible scenarios in a climate change) for 100days and solutions were renewed every 5 days. After 10, 30, 60, and 100 days of exposure, a set of 27 microcosms (three replicates) was sacrificed to determine leaf mass loss and EEA**. The experimental process is shown in Figure 1.**

2.3 Leaf mass loss and chemical characteristics

Leaf sections from each replicate of each treatment were dried at 40ºC to a constant mass (±72h) and weighed to the nearest 0.001 g. Lignin concentration was determined using the King and Heath method [20].

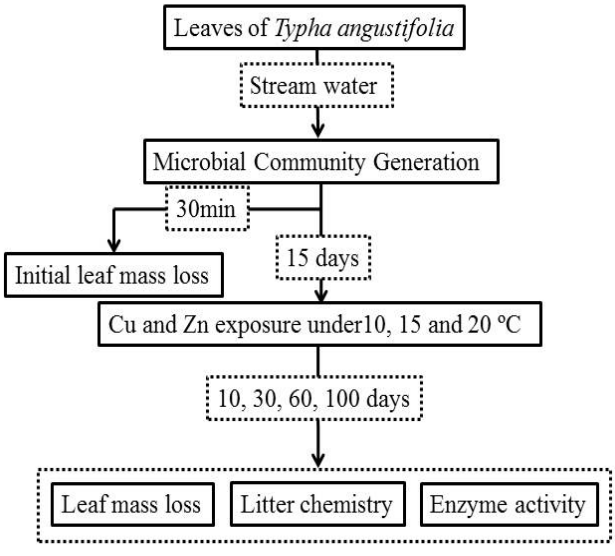

Figure 1: Graphical representation of the experimental process.

2.4 Enzyme assays

The enzymatic activities associated with leaf litter decomposition involved in carbon (β-glucosidase and cellulose), nitrogen (nitrate reductase), phosphorus (alkaline phosphatase) cycling and polyphenol metabolism (phenol oxidase and peroxidase) were treated as following: two small (2 cm-long) sections were randomly cut from the surface of the same leaf and blended in a digital ultrasonic cleaner with 10 mL of sodium acetate buffer (50 mM, pH 7.6) and ice (40 kHz, 2 minutes, interval of 10 seconds). The enzymatic activities were determined either by standard ELISA or spectrophotometry with slight modifications: β-glucosidase and oxidative enzymes [9], cellulose [21], nitrate reductase [22], alkaline phosphatase [23].

2.5 Data analyses

Assuming exponential decay, the decomposition rates of Typha leaf sections were calculated as following:

$$x_t = x_0 e^{-kt}$$

where x_0 is the initial mass, x_t is the remaining mass at time t, and k is the decomposition rate [7]. Lignin/weight loss ratio (L/W) and lignin/carbohydrate loss ratio (L/C) were calculated as follows,:

$$L/W = \frac{m_{lignin\ weight\ loss}}{m_{litter\ weight\ loss}}$$

$$L/C = \frac{m_{lignin\ weight\ loss}}{m_{carbon\ weight\ loss}}$$

Two-way analyses of variance were used to compare the effects of temperature and HM level on decomposition rates and EEA. The EEAs were calculated with trapezoid integration. Statistically significant differences were set at $P < 0.05$.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results

3.1 Litter decomposition

The mass loss of *Typha* litter after incubation in microcosms for 100 days varied between 25% (at 10ºC and high HM) and 40% (at 20ºC and low HM) (Figure 2a-c), which corresponded to decomposition rates between 0.0016 day^{-1} and 0.0061 day^{-1} ($P < 0.05$; Table 1). Decomposition rates were stimulated by low HM level only at 20ºC (Figure 2a-b), but restrained by low HM levels at low temperature (10 and 15ºC, Figure 1a-b) and high HM levels at all temperature treatments (Figure 2a-b; $P < 0.05$, Table 1); for both control and low HM treatments, decomposition rates were faster at 20ºC than at lower temperatures (20 > 15 >10ºC at control; 20 > 15ºC and 20>10 ºC at low HM; $P < 0.05$; Table 1). The calculated ANOVAs showed that temperature affected litter mass losses significantly (F=17.325, $P < 0.001$).

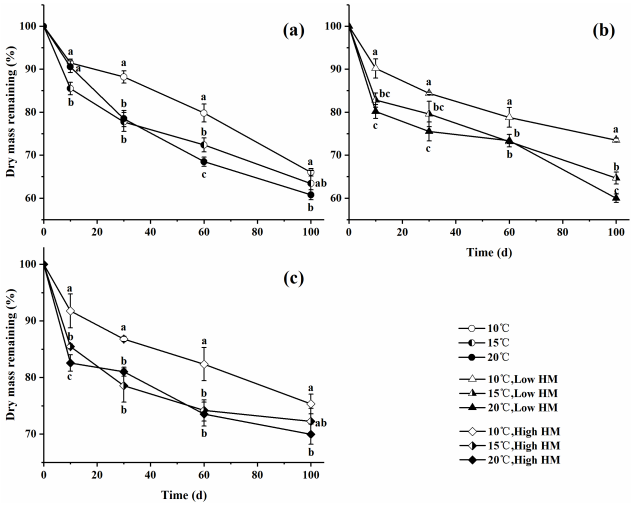

Figure 2: Mass remaining of *Typha* incubated at three temperatures and two heavy metal (HM) levels for 100 days. Legend: (a) control treatment; (b) three temperatures and low heavy metal levels; (c) three temperatures and high heavy metal levels. Different lowercase letters on the top of the bars denote significant differences among different treatments at the same sampling time (n=3, $P < 0.05$).

The L/W ratio was highest at 20ºC and low HM treatment (Figure 3a-c). The L/C ratio was highest at 20 ºC and high HM treatment (Figure 4a-c) while the lowest values over time at 15ºC and high HM levels (Figure 4c). ANOVA analysis showed that both temperature and HM levels affected L/W and L/C ratio significantly ($P < 0.001$).

3.2 Changes in enzyme activities during decomposition

The enzyme activities of alkaline phosphatase and β-glucosidase, which were integrated throughout the course of the experiment, were increased with the increase in temperature under CK treatment (Figure 5b, d). With the increase in temperature, the accelerated effects of low HM levels on the enzyme activities of cellulase

Table 1: Decomposition rates (*k*) of Typha leaf incubated in microcosms at different temperature (T) and low or high heavy metal (HM) level for 100 days, and coefficient of determination (R^2) of the regression.

T (ºC)	HM Level	*k*	R^2
10	Control	0.0041 ^{d}	0.93
	Low HM	0.0030 ^{de}	0.85
	High HM	0.0016 ^{e}	0.94
15	Control	0.0046 ^{c}	0.57
	Low HM	0.0042 ^{bcd}	0.95
	High HM	0.0034 ^{de}	0.92
20	Control	0.0051 ^{b}	0.85
	Low HM	0.0061 ^{a}	0.93
	High HM	0.0033^{cd}	0.68

Table 2: Summary table for two-way ANOVAs performed on enzymatic activities, associated with Typha leaf sections incubated in microcosms at three temperatures (T) and two heavy metal (HM) levels for 100 days.

		Intercept	T	HM	T * HM
df		1	2	2	4
β-Glucosidase	F	21778.07	5.52	3.58	1.75
	P	< 0.001	0.01	0.03	0.15
Cellulase	F	2175.92	12.17	25.94	8.11
	P	< 0.001	< 0.001	< 0.001	< 0.001
Alkaline phosphatase	F	3227.71	2.93	1.77	0.31
	P	< 0.001	0.06	0.18	0.87
Nitrate reductase	F	4346.79	0.52	7.88	3.11
	P	< 0.001	0.59	0.01	0.02
Phenol oxidase	F	48632.46	0.55	0.04	1.32
	P	< 0.001	0.58	0.96	0.27
Peroxidase	F	25948.33	0.01	8.01	1.43
	P	< 0.001	0.99	0.01	0.23

and β-glucosidase were enhanced (Figure 5c, d). ANOVA analysis revealed that individual variables were significant for cellulase ($P < 0.001$; Table 2), and the interaction of temperature with HM level were significant for cellulase ($P < 0.001$; Table 2) and nitrate reductase ($P < 0.05$; Table 2). Additionally, the enzyme activites of β-glucosidase and cellulase were positively correlated with litter mass losses at 20ºC and low HM levels ($P < 0.01$; Table 3).

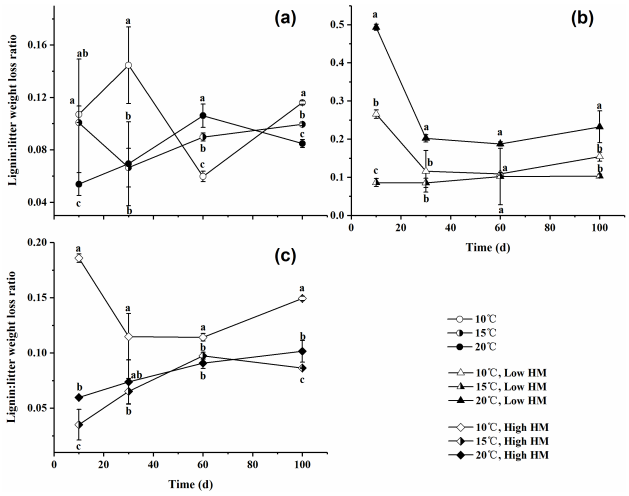

Figure 3: Changes in chemistry characters of lignin: litter weight loss ratio (L/W) in Typha incubated at three temperatures and two heavy metal (HM) levels. Legend: (a) control treatment; (b) three temperatures and low heavy metal levels; (c) three temperatures and high heavy metal levels. Different lowercase letters on the top of the bars denote significant differences among different treatments at the same sampling time (n=3, $P < 0.05$).

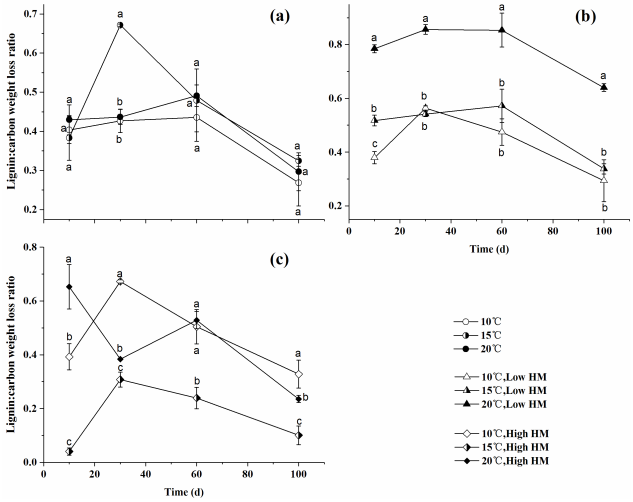

Figure 4: Changes in chemistry characters of lignin: carbon weight loss ratio (L/C) in Typha incubated at three temperatures and two heavy metal (HM) levels. Legend: (a) control treatment; (b) three temperatures and low heavy metal levels; (c) three temperatures and high heavy metal levels. Different lowercase letters on the top of the bars denote significant differences among different treatments at the same sampling time (n=3, $P < 0.05$).

4 Discussion

Within the physiological range, litter decomposition is positively related to temperature [2-3,7,11]. This observation was confirmed in our studies by the acceleration of microbial mediated leaf litter decomposition. One possible

Table 3: Pearson's correlation coefficients between enzymatic activities and litter mass losses in microcosms at three temperatures and two heavy metal (HM) levels for 100 days.

			AKP	NR	PEP	PO	β-G	CMC
10ºC	CK	*r*	-0.43	-0.71	0.73	0.34	0.88	-0.67
		P	0.56	0.29	0.27	0.66	0.12	0.33
	Low HM	*r*	-0.27	0.19	0.49	0.08	0.55	0.61
		P	0.73	0.81	0.50	0.92	0.45	0.39
	High HM	*r*	-0.96*	-0.90	0.78	0.81	0.95*	-0.16
		P	0.04	0.10	0.22	0.19	0.04	0.84
15ºC	CK	*r*	0.82	-0.52	0.42	0.47	0.87	-0.01
		P	0.18	0.48	0.58	0.53	0.13	0.99
	Low HM	*r*	0.99**	-0.98*	0.29	0.49	0.95	0.32
		P	0.00	0.02	0.71	0.51	0.05	0.68
	High HM	*r*	-0.60	-0.42	0.73	0.75	0.89	0.94
		P	0.39	0.58	0.27	0.25	0.11	0.06
20ºC	CK	*r*	0.95	-0.82	0.65	0.41	0.94	0.16
		P	0.05	0.18	0.35	0.59	0.06	0.84
	Low HM	*r*	0.95	-0.71	0.94	0.97*	0.75	0.87*
		P	0.05	0.29	0.06	**0.03**	0.25	**0.02**
	High HM	*r*	0.85	-0.49	0.94	0.62	0.63	-0.88
		P	0.15	0.50	0.06	0.38	0.37	0.12

Footnotes: * and ** indicate significant correlations at the 0.05 and 0.01 probability level, respectively. *P* values equal to or lower than 0.05 are in bold face print. Abbreviations:, β-Glucosidase (β-G);, Cellulase (CMC);, alkaline phosphatase (AKP);, nitrate reductase (NR) ;, phenol oxidase (PO); , Peroxidase (PE).

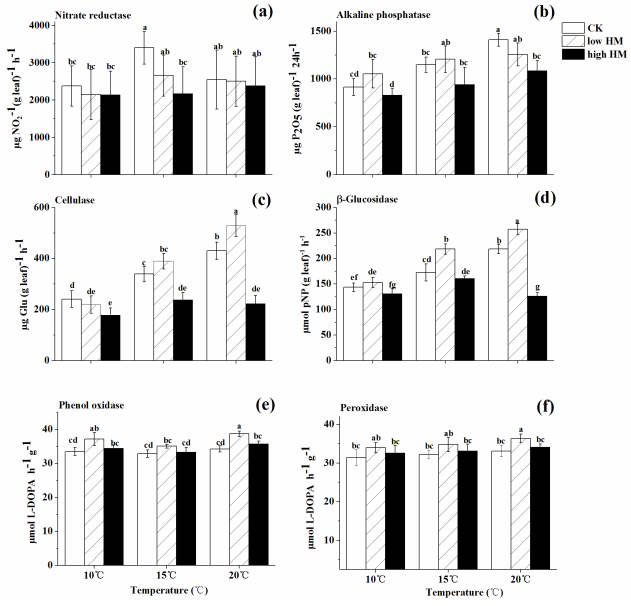

Figure 5: Integrated enzyme activities at three temperatures and two heavy metal (HM) levels for the whole experimental period (100 days). Legend: (a) nitrate reductase; (b) alkaline phosphatase; (c) cellulase; (d) β-glucosidase; (e) phenol oxidase; (f) peroxidase. Different lowercase letters on the top of the bars denote significant differences (n=3, $P < 0.05$).

reason may be that, with the increase of temperature, both the leaching of litter and microbial activity i.e., increasing EEA were enhanced [2,24-26]. Our results showed that the enzyme activities of alkaline phosphatase and β-glucosidase were enhanced by higher temperatures, suggesting that the mineralization of carbon and phosphorus were accelerated since alkaline phosphatase and β-glucosidase play significant roles in phosphorus and carbon cycles, respectively [27]. Another reason may be that the majority organisms may be comfortable at higher temperate than that at lower temperature.

Additionally, our results showed that Cu and Zn exposure depressed leaf decomposition at lower temperature (10 and 15ºC), which is in agreement with previous reports which found that metal (Cu and Zn) exposure depressed leaf decomposition at 15ºC demonstrated by a reduction in microbial decomposing activities [13]. However, our results also showed that decomposition rates were stimulated by low HM level at 20ºC, suggesting that changes in temperature can affect metal toxicity to litter decomposition without changing the total amount of accumulated metals in stream water [28]. One possible reason may be that the induction of

tolerance of microbial was enhanced at a temperature of 20ºC compared to temperatures of 10 and 15ºC in a certain concentration range of heavy metal (Cu and Zn) [28]. Previous studies indicated that, under high temperatures, the active transport of substrates increased and membranes were more permeable, which may accelerate Cu and Zn uptake in microbial cells and thus increase the microbial community tolerance response [15,18,29]. Therefore, having lower internal exposure to Cu and Zn may explain the observation of increased tolerance development at high temperatures [15,18]. The other reason may be the enhanced enzyme activities. Previous studies indicated that microbial taxa express a wide range of uses for Cu and Zn as enzymatic and electron transport cofactors [30-32], thus, moderate metal concentrations can potentially promote overall metabolic activity [33]. In the present study, the enzyme activities of cellulose, alkaline phosphatase and β-glucosidase were stimulated by low HM levels at 20ºC, suggesting that the mineralization of carbon and phosphorus were accelerated since alkaline phosphatase, cellulase and β-glucosidase play significant roles in phosphorus and carbon cycles, respectively [18,26-27]. In addition, the enzyme activities of β-glucosidase and cellulase were positively correlated with litter mass losses at 20ºC and low HM levels, suggesting that the effect of β-glucosidase and cellulase activities on the litter decomposition of Typha was stronger than that of other enzymes under the same given condition. Previous studies showed that metal toxicity to a body can be affected by changes in temperature without changing the total amount of accumulated metals [6,34]. This suggests that higher temperatures, such as 20ºC compared to 10 and 15ºC, may accelerate the mineralization of carbohydrates by changing the toxicity of Cu and Zn. At the same time, because litter decomposition requires extracellular enzymes which break down the structural components of plant litter and recover organic N and P, the enzyme activities are linked to both community dynamics and ecosystem perspectives [9,12]. Thus, the changes in litter decomposition under low HM at 20ºC possibly contributed to the associated hydrolytic enzymes due to the extracellular enzymes controlling the decomposition rate [25].

Finally, faster litter decomposition at 20ºC and low HM may contribute to the lignin decomposing ability of fungi occurring in litter decomposition processes since the decomposition of lignin is a key factor controlling litter decomposition rates [26,35-36]. Our results showed that the highest L/W and L/C ratio were measured at 20ºC and low HM levels, suggesting that an increase in the attack of lignin by associated fungi may be expected at low HM levels and high temperature. A possible explanation for this result was that at 20ºC, low levels of HM pollution cause a shift in decomposition community composition and destroy the labile litter components protected by lignin [37]. Furthermore, lignin may be more sensitive to temperature increase than other plant-derived compounds [35] and may therefore reduce the negative effects of lignin on microbially driven decomposition in stream water [38] probably due to the chemical complexity of lignin and the high activation energy required for micro-decomposers to decompose lignin [36].

5 Conclusions

This study investigated whether a 5°C increase in water temperature may attenuate the inhibition of low HM on litter decomposition rates in streams. A significant decrease of litter decomposition rate was observed in treatments of low HM level at 20°C. The enhanced enzyme activities of cellulase and β-glucosidase and the higher ratios of lignin/litter weight loss and lignin/carbohydrate weight loss suggested that the mineralization of phosphorus and carbon were increased and the attack of lignin was enhanced, which leads to an increase in Typha litter decomposition. These results suggest that a 5°C increase in water temperature may attenuate the inhibition of low HM on litter decomposition rates in a stream. It is plausible that if water quality of presently heavy metal polluted streams is improved, the potential stimulatory effects of future increases in water temperature on aquatic biota and processes could be mitigated.

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Conflict of interest: Authors state no conflict of interest.

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