


## Article

# Effects of Plant Growth Form and Water Substrates on the Decomposition of Submerged Litter: Evidence of Constructed Wetland Plants in a Greenhouse Experiment

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**Abstract:** Wetland plants are important components in constructed wetlands (CWs), and one of their most important functions in CWs is to purify the water. However, wetland plant litter can also increase eutrophication of water via decomposition and nutrient release, and few studies have focused on the interspecific variation in the decomposition rate and nutrient release of multiple plant species in CWs. Here a greenhouse litter-bag experiment was conducted to quantify the decomposition rates and nutrient release of 7 dominant macrophytes (2 floating plants and 5 emergent plants) in three types of water substrate. The results showed that plant litter species and growth forms significantly affected the litter mass losses. The nutrient release was significantly different among plant litter species, but not between floating and emergent plants. Litter traits, such as litter lignin, total nitrogen (TN) and total phosphorus (TP) can well predict the decomposition rates of submerged litter. These results indicated that submerging litter in water did not change the relationships between litter traits and litter decomposition rates, and leaching might play a more important role in the decomposition of submerged litter in CWs than that in other terrestrial ecosystems. These findings can provide suggestions for managers about the maintenance of constructed wetlands.

**Keywords:** constructed wetlands; decomposition; ecosystem functioning and services; plant litter

## 1. Introduction

Plant litter decomposition is a key ecosystem process in constructed wetlands (CWs) because it can release nutrients for plant growth [1] and control the dynamics of the carbon (C) stock [2,3]. The process of plant litter decomposition includes all the physical and chemical changes that occur after tissue senescence and death, starting with complex organic molecules and ending in simple inorganic elements [4]. Plant litter decomposition is the result of three interlinked processes: leaching, fragmentation and catabolism [5]. In constructed wetlands where moisture is not constraining, the most important determinants of decomposition are chemical traits of the decomposing material (litter quality), nutrient availability, and decomposer activity at the site where the decomposition occurs (external environment) [6–9]. Nutrient release can promote the growth and development of wetland plants; however, a large amount of plant litter decomposition might lead to eutrophication in CWs [10] via the continuous release of nutrients. Therefore, quantifying decomposition rates (mass loss and

nutrient release) is essential for understanding the role of wetland plants in affecting the ecosystem functioning of aquatic habitats [11]. Several studies have dealt with the change of nutrients affected by plant litter decomposition and its relation to litter traits [11–15], but few studies have focused on the interspecific variation in the decomposition rates and nutrient releases of wetland plants in CWs.

Based on a literature review of near 600 CWs, there are about 200 plant species used in CWs worldwide; and among those species, the most commonly used species are *Typha*, *Scirpus*, *Phragmites*, *Juncus* and *Eleocharis* [16]. Different plant species have been proven to decompose at different rates due to the large interspecific variation in litter qualities, such as lignin/P, lignin/N, and N/P ratios [17–19]. Moreover, previous research has also shown that plant growth form might affect litter decomposition rates when the decomposition process does not happen in the water. For example, trees, shrubs and grasses have significantly different decomposition rates from one another [20], as do coniferous and broad-leaved tree species [21]. In wetland ecosystems, the submerged species have faster decomposition rates than emergent species at wetlands [11,19,22]. However, few studies have examined whether plant growth forms might affect the nutrient release of plant species in CWs, and whether the relationship between plant growth forms and litter decomposition rates will change when the decomposition processes happen in water.

Previous studies have indicated that plant litter decomposition rates and nutrient release can be affected by the concentration of nutrients (nitrogen and phosphorus) in the water [23–25]. Those effects can be either positive or negative. For example, it is recognized that higher nutrient availability in the water generally supports faster decomposition rates [26–29] because nutrient enrichments increase microbial and enzymatic activities. However, some other studies have also suggested that high nutrient levels in the water may not stimulate detritus decomposition or slow down the decomposition rates of plant litter [30–32] due to the effects of high detritus quality and variations in microbial compositions and activities. Therefore, the effect of water quality on litter decomposition is still not clear, especially in CWs, where litter decomposition processes mostly happen in water.

A greenhouse experiment was carried out in order to examine the litter decomposition rates and nutrient release of 7 dominant macrophytes with two growth forms (floating and emergent plants). Plant litter was incubated in three different water substrates: polluted water, purified water and natural tap water; and the litter mass loss, TN, TP changes (the change in the concentrations) and the TN, TP losses (the change in the amounts) of those 7 plant litter species (*Salvinia natans*, *Lemna minor*, *Iris wilsonii*, *Zizania latifolia*, *Sparganium stoloniferum*, *Typha orientalis* and *Phragmites australis*) were quantified. The following questions should be answered: (1) Do the decomposition rate (i.e., litter mass loss) and nutrient release (i.e., nutrient changes and losses) depend on the plant species identity and/or plant growth form? (2) Which plant traits might predict the decomposition rates and nutrient release of those wetland plant species when they are incubated in water? (3) How do different water substrates affect plant litter decomposition rates and nutrient release? The final aim of this study is to understand the role of wetland plant species in CWs and provide suggestions to wetland managers about the maintenance of constructed wetlands.

## 2. Materials and Methods

### 2.1. Study Site

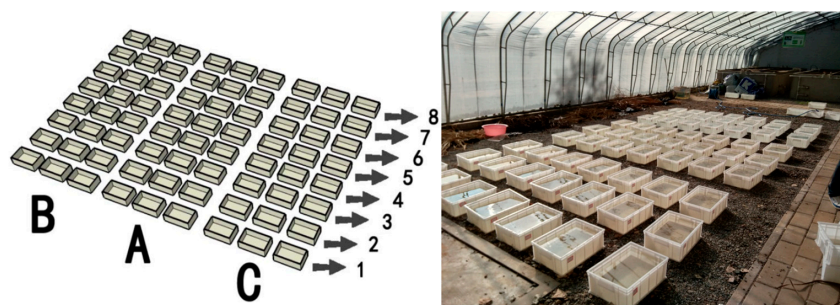
The study site was located in the Beijing Wildlife Rescue and Rehabilitation center in Shunyi district, Beijing, China (Latitude: 40°6′14.40″ N, Longitude: 116°42′35.71″ E). The built-up area of the center is 4359 m<sup>2</sup>, and cages of wildlife are 2563 m<sup>2</sup>. In addition, there is an artificial lake for wildlife perching and sporting, and the area is 1 hm<sup>2</sup>. The water in the artificial lake is polluted by the water bird sewage. In this center, there is a constructed wetland. The wetland consists of three treatment cells and the lengths are 20 m, 22 m, 25 m respectively [33], which is used to improve the water quality of the artificial lake in the site. The CW is composed of surface flow constructed wetland (A–I) and subsurface flow constructed wetland (J–L). The water in the artificial lake flows into the CW from

the section A, and goes back to the artificial lake from the section L. In the surface flow constructed wetland and subsurface flow constructed wetland, the main plant species are *T. orientalis*, *P. australis*, *Z. latifolia*, *I. wilsonii* and *Oenanthe javanica*.

## 2.2. Plant Litter Decomposition Experiment

Newly senesced litter was sampled across the whole CW, and 7 species of litter with two growth forms (floating and emergent plants) were collected, in total. Litter was air-dried for one week in the greenhouse, and three subsamples for each species litter were selected for initial trait measurements (lignin concentration, TN concentration, TP concentration) and initial water content of the air-dried litter. The initial water content was used to estimate the initial oven-dried weight of litter. Each plant litter was placed into 9 nylon litter bags, corresponding to 3 replicates for each water treatment (water A, water B, water C). Water A was collected at section A of the constructed wetland, water B was collected at the end of the surface flow of the same constructed wetland (section I), and water C was natural tap water, which was collected on the same day. The mesh size of the litterbags was 1 mm × 1 mm or 5 mm × 5 mm, depending on the growth form of the different species. For *L. minor*, around 20 g litter for each litter bag was weighed; but for *S. natans* and emergent plant species, around 50 g litter for each litter bag was weighed, depending on the quantities of the collected plant litter.

The prepared litter bags were submerged in 72 plastic boxes, which contained three types of water substrates (water A, water B and water C, Figure 1). The length, width and height of the plastic boxes were 40 cm, 30 cm and 15 cm, respectively. There were 63 plastic boxes that contained only one litter bag, with the other 9 plastic boxes being the control treatment with no litter bag. In order to decrease the gas exchange between the water and the air, and also decrease the evaporation of water, all the boxes were covered with fresh-keeping film. During the experiment, the fresh-keeping film was changed every two weeks. The whole experiment lasted from 28 October 2015 to 24 December 2015, with a duration of 57 days. The litter mass loss, the TN and TP changes, and the TN and TP losses of those 7 plant litter species were monitored at the end of experiment.



**Figure 1.** Experimental set-up and photo of experimental design. A, B, C represented three types of water substrates: polluted water, purified water and natural tap water. Numbers 1–7 represent the 7 species placements, and number 8 represents the control treatment (no litter submergence).

## 2.3. Measurements of the Initial and Harvested Litter

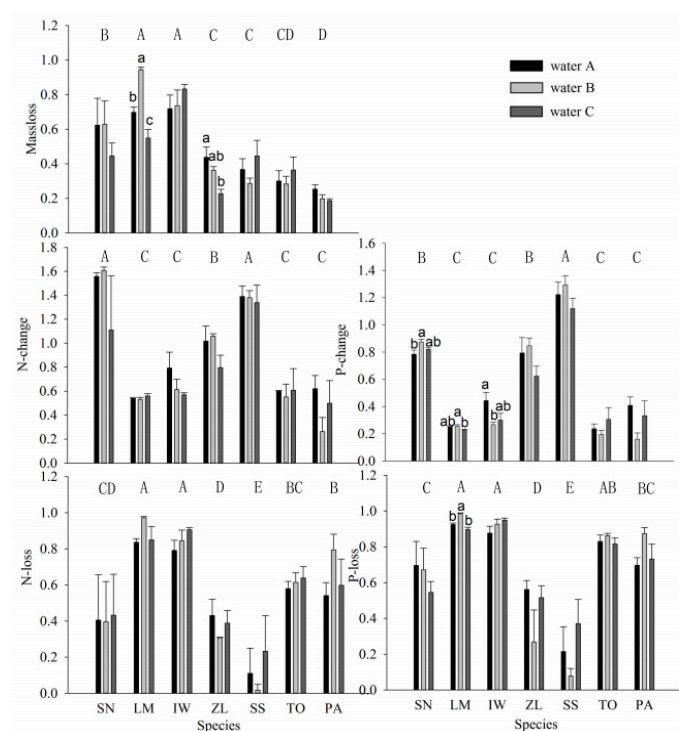
Litter chemical traits were measured both before and after the decomposition experiment. The TN concentration of the litter was analyzed by an automated elemental analyzer. The TP concentration was analyzed by inductively coupled plasma emission spectroscopy (Perkin Elmer Optima 3000 ICP Spectrometer, Waltham, MA, USA). The lignin concentration was measured by the extraction of non-ligneous compounds [34]. Moreover, the ratios of N/Lignin, P/lignin, N/P were calculated. Litter nutrient changes were characterized in two ways—changes in amount and changes in concentration—both of which are considered to be important from an ecological point of view. Litter mass loss was calculated as  $(m_0 - m_1)/m_0$ , where  $m_0$  is the (estimated) oven-dried weight of the initial litter and  $m_1$  refers to the oven-dried weight of the remaining litter.

## 2.4. Statistical Analysis

All data were checked for assumptions of homogeneity of variance and normality before analysis. The effects of water substrates and plant species or plant growth forms on litter mass loss, the TN and TP changes, and the TN and TP losses were analyzed using two-way ANOVA in SPSS Statistics (SPSS, Chicago, IL, USA). The differences in the litter mass loss, the TN and TP changes, and the TN and TP losses among species were compared using multiple comparison. Moreover, the relationship of initial litter quality (lignin concentration, TN concentration and TP concentration) and litter mass loss, TN and TP changes, and TN and TP losses were analyzed using correlation analysis in SPSS Statistics (SPSS, Chicago, IL, USA). Differences between means were tested with Duncan tests; effects were considered significant at  $p < 0.05$ . Note that analyses were conducted using both raw and (log and standardized) transformed data, and the results were consistent. Therefore, the results below were based on the raw data.

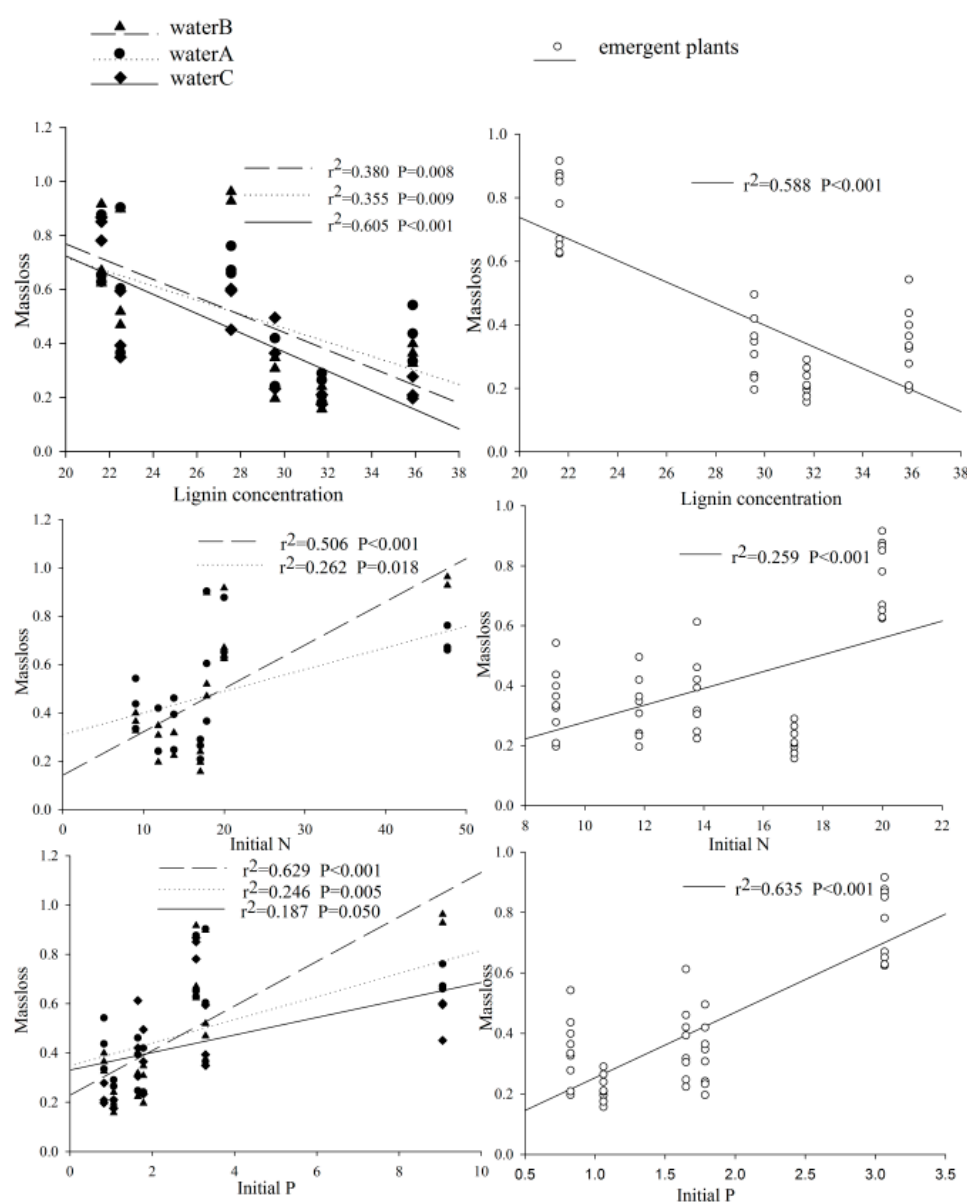
## 3. Results

Plant species had significant effects on litter mass loss (Table 1:  $F_6 = 28.416$ ,  $p < 0.01$ ), TN change (Table 1:  $F_6 = 23.852$ ,  $p < 0.01$ ), TP change (Table 1:  $F_6 = 116.654$ ,  $p < 0.01$ ), TN loss (Table 1:  $F_6 = 14.689$ ,  $p < 0.01$ ) and TP loss (Table 1:  $F_6 = 32.037$ ,  $p < 0.01$ ), but plant growth form only had a significant effect on litter mass loss (Table 1:  $F_1 = 15.751$ ,  $p < 0.01$ ). Water substrate had no effect on any of those decomposition variables above (Table 1), but for individual plant species, there were significant effects of water substrates on the mass losses of LM and ZL, the TP changes of SN, LM and IW, and the TP losses of LM (Figure 2). Moreover, there were interactive effects between plant species and water substrates on litter mass losses and TP changes (Table 1:  $F_{12} = 2.164$ ,  $p = 0.033$ ;  $F_{12} = 2.020$ ,  $p = 0.047$ ).



**Figure 2.** Effects of water substrate (A: polluted water; B: purified water by a constructed wetland; C: natural tap water) and plant species on the litter mass loss, the TN and TP changes, and the TN and TP losses. Error bars show SE. Upper letters (A, B, C, D) show the results of multiple comparisons among plant species; lower letters (a, b, c and no symbol) show the results of multiple comparisons among different water substrates. Species are: SN—*Salvinia natans*; LM—*Lemna minor*; IW—*Iris wilsonii*; ZL—*Zizania latifolia*; SS—*Sparganium stoloniferum*; TO—*Typha orientalis*; PA—*Phragmites australis*.

Among all those species, the litter of LM and IM had the highest losses of total mass, TN and TP compared to the other plant species litter (Figure 2). Litter of PA had the slowest mass loss, and litter of SS had the slowest losses of TN and TP (Figure 2). For nutrient changes, litter of SN and SS had the highest changes of TN, and litter of SS had the highest TP. The changes of TN and TP were similar among LM, IW, TO and PA. Overall, there were significantly negative relationships between litter mass loss and litter lignin, but significantly positive relationships between litter mass loss and initial litter concentrations of TN and TP. Moreover, for emergent plant species, these relationships remained ( $r^2 = 0.588, p < 0.001$ ;  $r^2 = 0.259, p < 0.001$ ;  $r^2 = 0.635, p < 0.001$ ), but not for the floating plant species. In addition, the relationship between litter mass loss and initial litter TN concentration disappeared under water C ( $r^2 = 0.1354, p = 0.102$ ), but the other significant relationships remained (Figure 3).



**Figure 3.** Relationship between initial litter quality (lignin concentration, TN and TP) and litter mass loss. Data points are shaped by water substrates or plant growth forms (A: polluted water, black circle; B: purified water by a constructed wetland, black triangle; C: natural tap water, black diamond; Emergent plants, white circle). Note that floating plants were not shown in the growth form figure due to the limited data points.



**Table 1.** ANOVAs for effects of water substrates (W) and plant species (S) or growth form (G) on the litter mass loss, the TN and TP changes, and the TN and TP losses, Values where  $p < 0.05$  are in bold.

	<i>df</i>	Mass Loss		TN Change		TP Change		TN Loss		TP Loss	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Group 1:											
Water (W)	2	1.348	0.271	1.862	0.169	1.654	0.204	0.316	0.731	0.155	0.857
Species (S)	6	<b>28.416</b>	<b>&lt;0.01</b>	<b>23.852</b>	<b>&lt;0.01</b>	<b>116.654</b>	<b>&lt;0.01</b>	<b>14.689</b>	<b>&lt;0.01</b>	<b>32.037</b>	<b>&lt;0.01</b>
W*S	12	<b>2.164</b>	<b>0.033</b>	0.856	0.595	<b>2.020</b>	<b>0.047</b>	0.417	0.948	1.681	0.107
Group 2:											
Water (W)	2	1.231	0.300	0.396	0.675	0.043	0.958	0.070	0.933	0.040	0.961
Growth form (G)	1	<b>15.751</b>	<b>&lt;0.01</b>	2.507	0.119	0.064	0.801	1.304	0.258	<i>2.866</i>	<i>0.096</i>
W*G	2	2.004	0.144	0.206	0.814	0.145	0.865	0.021	0.979	0.341	0.713

#### 4. Discussion

In the present study, the litter decomposition process happened in water throughout the whole experiment. Decomposition in water is a unique feature of constructed wetland ecosystems compared to other terrestrial ecosystems. The results showed that there was an average of 50% mass loss during a 2-month decomposition, and for some species, the litter mass losses reached more than 90%. The fast decomposition rates in such a short period of time might be attributable to either the strong leaching processes or the decomposers. However, in the greenhouse experiment there was no sediment in the water, and the soil decomposers might have been much fewer than in natural wetland ecosystems. These results might indicate that leaching processes and water microbes together have a great contribution to litter mass loss, and submerging litter in water might strengthen the relative importance of leaching in the decomposition processes.

There were significant differences in the litter mass losses between emergent plants and floating plants. Litter of floating plant species had faster mass losses than that of emergent plant species. This could be, consistent with Chimney [11] (2006) and Lan [19] (2012), because the floating plant species had higher initial TN and TP concentrations, although the results did not show significantly differences in initial litter TN and TP (Appendix B), and/or the fact that wetland floating plants grow completely in the water, thus having a large area of contact with the water surface compared to emergent plants. However, there were no significant differences between floating plant species and emergent plant species in litter nutrient changes or losses (TN and TP). These results might be due to the leaching process having little effect on TN and TP, although other nutrients are more impacted by the leaching process. These results might indicate that submerging the litter of different plant growth forms may have similar effects on the water TN and TP, and hence have similar effects on the increase of eutrophication [35]. However, previous studies have indicated that the submergence of floating plants might be more negative than that of emergent plants [36–38]. This might be because floating plant decomposition benefits the denitrification process in wetland ecosystems [3].

Certain researchers have argued that the initial litter traits are more important than environmental factors in the decomposition process [39–41]. The traits of plant litter include ingredients that are easy to break down and some tissue structures that are difficult to break down, such as lignin [42,43]. Litter decomposition rates depend strongly on the contents of refractory substances (e.g., lignin). The data showed that there were negative relationships between initial litter lignin concentrations and litter mass losses, and some other nutrient release variables (Appendix A), which is consistent with previous findings [44]. Moreover, the initial litter TN and TP concentrations and N/P ratios are commonly believed to be important traits that determine litter decomposition rates or nutrient release [45]. The results showed that there were positive relationships between initial litter TN, TP and litter mass losses, and some nutrient release variables (Appendix A). The strong positive relationships between decomposition rates and the chemical composition of the litter have been documented in

other studies [46,47]. These findings may indicate that higher concentrations of initial litter TN and TP might facilitate the litter decomposition rates and nutrient release by promoting water microbes.

A great deal of the inconsistency in decomposition rates among wetland plant litter can be attributed to the interface of several dynamic physiological and environmental variables [11]. The present study showed that both the chemical composition of the plant litter (e.g., TN and TP) and environmental variables (trophic conditions of the wetland or nutrient availability) significantly affected the decomposition rates of plant litter in constructed wetland ecosystems. Nutrient availability in water is often considered to be an important factor in controlling decomposition rate. One reason is that nutrient demands, associated with decomposer activity, often exceed nutrient supply from litter [17,48,49]. In the present study, there was no significant difference in the decomposition rates of wetland plant litter among the three water substrates because the difference among the initial water qualities was small. But individual species with water substrates exhibited in mass loss and TP change and loss. This study is in agreement with previous findings, which also observed faster plant litter decomposition in nutrient-rich systems. In conclusion, water substrates, together with plant litter traits (higher TN concentration), significantly affected decomposition [50,51]. The results of the present study confirm that plants with higher TN and TP decompose faster, since these nutrients form the base media for microbial colonization.

## 5. Conclusions

As it is understood that constructed wetlands are mostly nutrient-rich ecosystems that are linked with numerous human services and livelihoods, due management of wetland plant-litter decomposition is warranted for appropriate inclusive nutrient management. Our results showed that the interspecific variation of decomposition rates and nutrient release was large, and certain species, especially floating plants, released nitrogen and phosphorus faster in the water, which might lead to serious eutrophication or even pollution [38]. Therefore, we suggest that the CW manager make it a priority to clean up the litter of floating plants such as LM, in order to minimize the negative effects of plant litter on the water quality of constructed wetlands. Additionally, examining the decomposition rates and the nutrient release of plant litter is an indirect way of testing the effects of plant litter on the ecosystem services of CWs, e.g., water purification. Plant litter decomposition might be of great importance in CWs in a positive way. For example, plant litter can be used as a carbon source to facilitate denitrification processes in the water [38]. We suggest that future research is needed to both directly and indirectly examine the effects of plant litter and their interactions with soil sediments on the water quality in CWs [52].

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**Author Contributions:** Y.P., X.P. and L.C. conceived and designed the experiments; Y.P., X.P. and W.L. executed the experiments; J.W. and J.Z. helped to measure the data; Y.P. and X.P. analyzed the data, and made the figures; L.C. and Y.L. contributed to writing and editing the manuscript; Y.P. and X.P. wrote the first draft.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

**Table A1.** Correlation Analysis between Initial Litter Quality (Lignin Concentration, TN and TP) and Litter Mass Loss, the Changes and Losses the TN, TP Losses. E represents emergent plants; F represents floating plants.

		MS		TN—Change		TP—Change		TN—Loss		TP—Loss	
		<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Lignin	All	−0.653	<b>&lt;0.001</b>	−0.256	0.067	−0.012	0.935	−0.269	0.054	−0.435	<b>&lt;0.001</b>
	F	0.354	0.164	−0.813	<b>&lt;0.001</b>	−0.992	<b>&lt;0.001</b>	0.682	<b>0.003</b>	0.757	<b>&lt;0.001</b>
	E	−0.767	<b>&lt;0.001</b>	0.225	0.194	0.481	<b>0.003</b>	−0.759	<b>&lt;0.001</b>	−0.713	<b>&lt;0.001</b>
	water A	−0.596	<b>0.009</b>	−0.297	0.231	0.065	0.797	−0.264	0.291	−0.466	0.051
	water B	−0.617	<b>0.008</b>	−0.342	0.179	−0.069	0.792	−0.173	0.521	−0.488	<b>0.047</b>
	water C	−0.778	<b>&lt;0.001</b>	−0.137	0.600	−0.038	0.880	−0.361	0.141	−0.372	0.129
N	All	0.520	<b>&lt;0.001</b>	−0.281	<b>0.028</b>	−0.391	<b>&lt;0.001</b>	0.490	<b>&lt;0.001</b>	0.453	<b>&lt;0.001</b>
	F	0.354	0.165	−0.813	<b>&lt;0.001</b>	−0.992	<b>&lt;0.001</b>	0.682	<b>0.003</b>	0.757	<b>&lt;0.001</b>
	E	0.509	<b>&lt;0.001</b>	−0.311	<b>0.040</b>	−0.331	<b>0.028</b>	0.523	<b>&lt;0.001</b>	0.467	<b>0.001</b>
	water A	0.512	<b>0.018</b>	−0.373	0.096	−0.453	<b>0.049</b>	0.503	<b>0.020</b>	0.459	<b>0.036</b>
	water B	0.711	<b>&lt;0.001</b>	−0.287	0.220	−0.342	0.140	0.526	<b>0.021</b>	0.470	<b>0.036</b>
	water C	0.367	0.102	−0.203	0.390	−0.413	0.063	0.463	<b>0.034</b>	0.468	<b>0.032</b>
P	All	0.594	<b>&lt;0.001</b>	−0.173	0.184	−0.329	<b>&lt;0.001</b>	0.447	<b>&lt;0.01</b>	0.430	<b>&lt;0.001</b>
	F	0.354	0.164	−0.813	<b>&lt;0.001</b>	−0.992	<b>&lt;0.001</b>	0.682	<b>0.003</b>	0.757	<b>&lt;0.001</b>
	E	0.797	<b>&lt;0.001</b>	−0.095	0.542	−0.228	0.136	0.454	<b>0.002</b>	0.432	<b>0.003</b>
	water A	0.588	<b>0.005</b>	−0.274	0.229	−0.397	0.074	0.483	<b>0.026</b>	0.469	<b>0.032</b>
	water B	0.793	<b>&lt;0.001</b>	−0.145	0.542	−0.261	0.267	0.436	0.062	0.424	0.063
	water C	0.432	0.050	−0.124	0.603	−0.350	0.120	0.437	<b>0.047</b>	0.437	<b>0.048</b>

## Appendix B

**Table A2.** The initial litter traits of different plan used in the study.

Latin Name	Initial TN (g/kg)	Initial TP (g/kg)	Lignin (%)
<i>Salvinia natans</i>	17.83	3.30	22.50
<i>Lemna minor</i>	2.41	5.11	27.57
<i>Iris wilsonii</i>	19.99	3.07	21.63
<i>Zizania latifolia</i>	9.03	0.83	35.88
<i>Sparganium stoloniferum</i>	13.77	1.65	-
<i>Typha orientalis</i>	11.82	1.79	29.57
<i>Phragmites australis</i>	17.04	1.06	31.72

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