



1 **Factors controlling *Carex brevicuspis* leaf litter**  
2 **decomposition and its contribution to surface soil organic**  
3 **carbon pool at different water levels**

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18



19 **Abstract.** Litter decomposition plays a vital role in wetland carbon cycling. However, the contribution of  
20 aboveground litter decomposition to the wetland soil organic carbon (SOC) pool has not yet been  
21 quantified. Here, we conducted a *Carex brevicuspis* leaf litter input experiment to clarify the intrinsic  
22 factors controlling litter decomposition and quantify its contribution to SOC pool at different water  
23 levels. This species is ubiquitous to global freshwater wetlands. We sampled this plant leaf litter at -25,  
24 0, and +25 cm relative to the soil surface over 280 days and analysed leaf litter decomposition and its  
25 contribution to the SOC pool. The mass loss and carbon release rates were the highest at +25 cm water  
26 level, followed by the 0 cm water level. The rates of these parameters were the lowest at -25 cm water  
27 level. Significant amounts of litter carbon, nitrogen, and phosphorus were released at all three water  
28 levels. Litter input significantly increased the soil microbial biomass and fungal density but had  
29 nonsignificant impacts on soil bacteria, actinomycetes, and fungal/bacterial concentrations at all three  
30 water levels. Compared with litter removal, litter application increased the SOC by 25.12%, 9.58%, and  
31 4.98% at the +25 cm, 0 cm, and -25 cm water levels, respectively. Hence, higher water levels facilitate  
32 the release of organic carbon from leaf litter into the soil via water leaching. In this way, they strengthen  
33 the soil carbon pool. At lower water levels, soil carbon is lost as the slower litter decomposition rate and  
34 active microbial (actinomycete) respiration. Our results revealed that the water level in natural wetlands  
35 influences litter decomposition mainly by leaching and microbial activity, by extension, affects wetland  
36 surface carbon pool.

37 **Key words:** *Carex brevicuspis*; decomposition; leaf litter; soil surface organic carbon pool; water level

## 38 **1 Introduction**

39 Wetlands are important terrestrial carbon pools. They contain  $1.5 \times 10^3$  Pg carbon (~35% of the global  
40 carbon supply) and 25–63% of the soil carbon distributed in the 0–30 cm topsoil layer (Whiting and  
41 Chanton, 2001; Means et al., 2016; Cao et al., 2017). The surface soil organic carbon (SOC) pool  
42 (S-SOCP) and its turnover are sensitive to climate, topography, and hydrological condition (Wang et al.,  
43 2016; Zhang et al., 2017; Pinto et al., 2018).

44 Leaf litter decomposition is a major biotic carbon input route from vegetation to S-SOCP in wetland  
45 ecosystems (Whiting and Chanton, 2001; Moriyama et al., 2013). However, the reported impacts of litter  
46 decomposition on the soil carbon pool are highly variable (Busse et al., 2009; Crow et al., 2009). Litter  
47 input destabilised carbon storage by stimulating soil mineralisation and increasing soil labile carbon  
48 fractions (microbial biomass carbon [MBC] and soil dissolved organic carbon [DOC]) and enzyme  
49 activity in the freshwater marshland of Northeast China (Song et al., 2014). It also promoted soil carbon  
50 loss via CO<sub>2</sub> emissions and microbial activity in alpine and coastal wetlands (Gao et al., 2016; Liu et al.,  
51 2017). In contrast, a study has recently found that litter decomposition increased soil active organic  
52 carbon and stabilised the soil carbon pool in the Jiaozhou Bay wetland (Sun et al., 2019).



53 Litter decomposition is a physicochemical processes that reduces litter to its elemental chemical  
54 constituents (Aerts, 1997). Litter decomposition rates are determined mainly by environmental factors  
55 (climatic and soil conditions), litter quality (litter composition such as C, N, and lignin content) and  
56 decomposer organisms (microorganisms and invertebrates) (Aerts, 1997). A previous study showed that  
57 regional and global environmental conditions explain > 51% of the variation in litter decomposition rate  
58 (Zhang et al., 2019). In wetland ecosystems, the water level ecosystem processes as it determines soil  
59 aerobic and anaerobic conditions which, in turn, affect microbial decomposition of litter and SOC  
60 decomposition (Liu et al., 2017; Yan et al., 2018). An earlier study reported that high soil moisture  
61 content and long flooding periods facilitate litter decomposition by promoting leaching, fragmentation,  
62 and microbial activity (Zhang et al., 2019). The water level may contribute to soil physicochemical  
63 conditions which, in turn, regulate litter decomposition (Xie et al., 2016). Aboveground litter  
64 decomposition is the main source of SOC (Upton et al., 2018). However, the contribution of litter  
65 decomposition to the SOC pool has seldom been quantified.

66 Dongting Lake (28°30'–30°20' N, 111°40'–113°10' E) is the second largest freshwater lake in China. It  
67 is connected to the Yangtze River via tributaries. Dongting Lake wetlands are characterised by large  
68 seasonal fluctuations in water level ( $\leq 15$  m) and are completely flooded during June–October and  
69 exposed during November–May (Chen et al., 2016). Peng et al. reported that the organic carbon density  
70 in Dongting Lake wetland soil at 1 m depth was  $127.3 \pm 36.1$  t  $\text{hm}^{-2}$  and the carbon density in the 0–30 cm  
71 topsoil was  $46.5 \pm 19.7$  t  $\text{hm}^{-2}$  (Peng et al., 2005). *Carex brevicuspis* is a dominant vegetation in the  
72 Dongting Lake wetland and has large carbon reserves ( $\sim 6.5 \times 10^6$  t  $\text{y}^{-1}$ ) (Kang et al., 2009). However, due  
73 to the dam construction in the upstream of Dongting Lake, the water regime varies remarkably (early  
74 water withdrawn and declining of groundwater in non-flood season) in recent years, leading a significant  
75 carbon loss in this floodplain wetland (Hu et al., 2018; Deng et al., 2018).

76 Here, we investigated *C. brevicuspis* litter decomposition and its contribution to the SOC pool at three  
77 water levels (-25 cm, 0 cm, and +25 cm relative to the soil surface) to find the factors controlling *C.*  
78 *brevicuspis* leaf litter decomposition and quantify the contribution of litter decomposition to the SOC  
79 pool. We tested the following hypotheses. First, the litter decomposition rate at the +25 cm water level is  
80 faster than that at the 0 and -25 cm water levels because of leaching, fragmentation, and microbial  
81 activity. Second, the rates of litter carbon, nitrogen and phosphorus release are the highest at the +25 cm  
82 water level, and the intrinsic litter decomposition controls differs among the three water levels because of



83 the differences among them regarding water infiltration. Third, the S-SOCP is relatively higher at the  
84 +25-cm water level because of substantial carbon accumulation resulting from significant litter  
85 decomposition. In contrast, it is comparatively lower at the -25 cm water level owing to carbon loss  
86 because of slow litter decomposition.

## 87 **2 Materials and methods**

### 88 **2.1 Soil core collection and leaf litter preparation**

89 Soil cores (40 cm diameter × 50 cm length) and leaf litter were collected in May 2017 from an  
90 undisturbed *Carex brevicuspis* community at the sampling site (29°27'2.02" N, 112°47'32.28" E) of the  
91 Dongting Lake station for wetland ecosystem research. The litter was cleaned with distilled water,  
92 oven-dried at 60 °C to a constant weight, and cut into pieces 5–10 cm long. Preweighed litter samples (5  
93 g; 10.73 ± 0.28 g kg<sup>-1</sup> N, 0.89 ± 0.04 g kg<sup>-1</sup> P, 40.23 ± 2.6% organic C, and 17.83±0.25% lignin) were  
94 placed into 10 cm × 15 cm 1mm mesh nylon bags. This mesh size excluded macroinvertebrates but  
95 permitted microbial colonisation and litter fragment leaching (Xie et al., 2016).

### 96 **2.2 Experimental design**

97 There were three water level treatments (-25 cm, 0 cm, and +25 cm relative to the soil surface) nested by  
98 two litter treatments (input vs. removal) and three replicates. The experiment was conducted in nine  
99 cement ponds (2 m × 2 m × 1 m) at the Dongting Lake station for wetland ecosystem research which is  
100 part of the China Ecosystem Research Network. For the -25 cm treatment, the water level was 25 cm  
101 below the soil surface. For the 0 cm treatment, the soil was fully wetted with belowground water but  
102 without surface pooling. For the +25 cm treatment, the water level was 25 cm above the soil surface.  
103 Water levels were adjusted weekly using belowground water (TOC: 3.44 mg L<sup>-1</sup>; TN: 0.001 mg L<sup>-1</sup>; TP:  
104 0.018 mg L<sup>-1</sup>). Three soil core sets were placed in each pond. One was designated the litter removal  
105 control (S), the second was distributed on the soil surface in 15 litter bags to observe the effects of leaf  
106 litter input on soil carbon pool (L), and the third was distributed on the soil surface in 15 litter bags to  
107 monitor the litter decomposition rate and process (D) (Fig. 1). The experiment started on 20 August 2017  
108 and lasted 280 d. By that time, no further significant change in litter dry weight was observed. Before  
109 incubation, three litter and three soil samples were collected to determine their initial quality. Litter bags



110 were randomly collected from treatment D after 20 d, 40 d, 60 d, 80 d, 100 d, 130 d, 160 d, 190 d, 220 d,  
111 250 d, and 280 d. After collection, the litter samples were separated, cleaned with distilled water, and  
112 oven-dried at 60 °C to a constant weight ( $\pm 0.01$  g). All samples were pulverised and passed through a  
113 0.5-mm mesh screen for litter quality analysis. At the end of incubation, the surface soil (0–5 cm, ~600 g  
114 FW) was collected to eliminate the influences of root decomposition on the soil organic pool. The soil  
115 samples were placed in aseptic sealed plastic bags and transported to the laboratory. The samples were  
116 sieved ( $< 2$  mm), thoroughly mixed, and divided into three subsamples. The first subsample (~150 g) was  
117 stored at -20 °C and freeze-dried for phospholipid fatty acid (PLFA) analysis. The second one (~150 g)  
118 was stored at 4 °C for MBC and DOC measurements. The third subsample (~300 g) was air-dried for  
119 physicochemical analysis.

### 120 **2.3 Litter quality analyses**

121 Litter organic carbon content was analysed by the  $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$  heat method. Litter nitrogen was  
122 extracted by Kjeldahl digestion and quantified with a flow injection analyser (AA3; Seal Analysisten  
123 GmbH, Langenselbold, Germany) (Xie et al., 2017). Litter phosphorus content was quantified by the  
124 molybdenum-antimony anti-spectrophotometric method. The lignin content was measured by hydrolysis  
125 (72%  $\text{H}_2\text{SO}_4$ ) (Graça et al., 2005; Xie et al., 2017).

### 126 **2.4 Soil quality analyses**

#### 127 **2.4.1 Soil chemical analyses**

128 SOC was determined by wet oxidation with  $\text{KCr}_2\text{O}_7 + \text{H}_2\text{SO}_4$  and titration with  $\text{FeSO}_4$  (Xie et al., 2017).  
129 Soil DOC was extracted with  $\text{K}_2\text{SO}_4$  and measured with a TOC analyser (TOC-VWP; Shimadzu Corp.,  
130 Kyoto, Japan). MBC was analysed by chloroform fumigation,  $\text{K}_2\text{SO}_4$  extraction, and TOC analyser  
131 (TOC-VWP, Shimadzu Corp., Kyoto, Japan) (Tong et al., 2017).

#### 132 **2.4.2 Soil microbial composition**

133 The total and specific microbial group biomass values and the microbial community structure were  
134 estimated by phospholipid fatty acid (PLFA) analysis. The PFLAs were extracted from 8 g of  
135 freeze-dried soil and analysed as previously described (Zhao et al., 2015). The concentrations of each  
136 PLFA was calculated relative to that of the methyl nonadecanoate (19:0) internal standard. The PLFAs



137 for the following groups were determined: (a) bacterial biomass, sum of i15:0, a15:0, 15:0, i16:0, 16:1u7,  
138 i17:0, a17:0, 17:0, cy17:0, and cy19:0; actinomycete biomass, sum of 10 Me 16:0, 10 Me17:0, and 10 Me  
139 18:0; and fungal biomass, 18:2 ω6 and 18:1 ω9. The total microbial biomass was represented by the sum  
140 of the bacterial, fungal, and actinomycete biomass values. The ratios of fungal to bacterial lipids (F/B)  
141 were used to evaluate the microbial community structure ((Bossio and Scow, 1998; Wilkinson et al.,  
142 2002; Zhao et al., 2015).

## 143 2.5 Data processing

### 144 2.5.1 Litter decomposition rate

145 The percentage of litter dry weight loss was calculated as follows (Zhang et al., 2019):

$$146 L_t = \frac{M_0 - M_t}{M_0} \times 100\% \quad (1)$$

147 where  $L_t$  is the percentage litter dry weight loss at time  $t$  (%),  $M_t$  is the litter dry matter weight at the time  
148  $t$  (g), and  $M_0$  is the initial dry matter weight (g).

149 The instantaneous litter dry mass decay rate ( $k$ ) was calculated using the Olson negative exponential  
150 attenuation model (Olson, 1963):

$$151 M_t = M_0 e^{-kt} \quad (2)$$

152 where  $M_t$  is the litter dry matter weight at time  $t$  (g),  $M_0$  is the initial dry matter weight (g),  $k$  is the  
153 instantaneous litter decay rate at time  $t$ , and  $e$  is the natural base number.  $M_t$  increased with litter  
154 decomposition rate.

### 155 2.5.2 Relative release index

156 The relative release indices (RRIs) of C, N, and P from the plant litter were calculated as follows (Zhang  
157 et al., 2019):

$$158 RRI_t = \frac{M_0 \times C_0 - M_t \times C_t}{M_0 \times C_0} \times 100\% \quad (3)$$

159 where  $C_t$  is the concentration of an element in the litter at time  $t$ ,  $C_0$  is the initial concentration of an  
160 element in the litter, and  $M_t$  is the litter dry matter weight at time  $t$  (g). CRRi, NRRi, PRRI, and LRRi  
161 represent the carbon, nitrogen, phosphorus, and lignin RRIs, respectively. A positive RRI indicates a net  
162 release of the element during litter decomposition whilst a negative RRI indicates a net accumulation of  
163 the element during litter decomposition.



### 164 2.5.3 Contribution of litter-C input to the SOC pool

165 The contribution of litter-C input to the SOC pool was calculated as follows (Lv and Wang, 2017):

$$166 LC = \frac{SOC_L - SOC_S}{SOC_i} \times 100\% \quad (4)$$

167 where  $LC$  is the contribution of the litter-C input to SOC pool,  $SOC_L$  is the SOC concentration for the  
168 litter input treatment,  $SOC_S$  is the SOC concentration for the treatment without litter input, and  $SOC_i$  is  
169 the initial SOC content before the experimental treatments.

### 170 2.6 Statistical analyses

171 The percentage litter dry weight losses and the instantaneous decomposition rates were compared among  
172 the three water levels by repeated ANOVA. Water level was the main factor and time was the repeated  
173 factor. The surface soil chemical components and the microbial community structure were compared by  
174 two-way ANOVA. Treatment (with or without litter input) and water level were the main factors. The  
175 percentage differences in litter dry weight loss, the instantaneous decomposition rates, the soil chemical  
176 components, and the microbial community structure were evaluated by LSD at the 0.05 significance  
177 level. The data were expressed as means  $\pm$  standard error. All statistical analyses were performed in  
178 SPSS 21 (IBM Corp., Armonk, NY, USA).

## 179 3 Results

### 180 3.1 litter decomposition process

181 The percentage litter dry weight loss was the highest for the +25 cm water level treatment through the  
182 entire litter decomposition period followed by the 0 cm water level treatment. The percentage litter dry  
183 weight loss was the lowest for the -25 cm water level treatment ( $P < 0.01$ ; Fig. 2A). After 280 d  
184 decomposition, the percentage litter dry weight loss values under the +25 cm, 0 cm, and -25 cm water  
185 level treatments were 61.8%, 49.8% and 32.4%, respectively.

186 The instantaneous decomposition rate at each measurement time point was calculated using the Olson  
187 negative exponential decay model. The instantaneous litter dry weight decomposition rate rapidly  
188 increased and then slowly decreased and stabilised for all three water levels. The maximum  
189 decomposition rates for the -25 cm, 0 cm, and +25 cm water levels were  $0.0069631 \text{ d}^{-1}$ ,  $0.008990 \text{ d}^{-1}$ , and  
190  $0.012954 \text{ d}^{-1}$ , respectively (Fig. 2B).



### 191 3.2 Intrinsic litter decomposition rate-limiting factor

192 During the entire decomposition process, CRR1, NRRI, PRRI, and LRRI significantly increased with  
193 water level. Litter carbon and lignin were always released at all three water levels whilst at -25 cm,  
194 nitrogen and phosphorus enrichment appeared in the middle stage (Fig. 3A–3D). At the start of the  
195 experiment, neither the C/N nor the lignin/N ratio significantly differed at the three water levels. At the  
196 middle stage, however, both the C/N and lignin/N ratios were significantly lower at the -25-cm water  
197 level than they were at the 0 cm and +25 cm water levels (Fig. 3E–3F).

198 The multiple regression model of the instantaneous litter decomposition rate and the litter properties  
199 showed that at the -25 cm and 0 cm water levels, the main decomposition rate-limiting factor was the  
200 lignin concentration whilst at the +25 cm water level, the main litter decomposition rate-limiting factor  
201 was the lignin/N ratio (Table 1).

### 202 3.3 Soil surface microbial community structure

203 Under both litter input and litter removal conditions, the bacterial, fungal, and microbial biomass levels  
204 were the highest under the 0 cm water level treatment; however, these parameters showed nonsignificant  
205 differences between 25 cm above and below water level treatments ( $P > 0.05$ ; Fig. 4A, 4B, and 4F). The  
206 actinomycete biomass was the highest under the -25 cm water level treatment, followed by that under the  
207 0 cm water level treatment. It was the lowest under the +25 cm water level treatment (Fig. 4C). Litter  
208 input significantly stimulated fungal and microbial biomass at all three water levels but only significantly  
209 stimulated bacterial and actinomycete biomass at the -25 cm water level ( $P < 0.05$ ; Fig. 4A–4C and 4E).

210 Under litter input conditions, the fungal/bacteria ratio was the highest at the 0 cm water level, followed  
211 by the +25 cm water level. It was the lowest under the -25 cm water level treatment. Under litter removal  
212 conditions, however, the fungal/bacteria ratio was significantly higher under the -25 cm water level  
213 treatment than it was under the 0 cm and +25 cm water level treatments ( $P < 0.05$ ; Fig. 4D).

### 214 3.4 Contribution of leaf decomposition to the soil surface carbon pool

215 The SOC, MBC, and DOC concentrations were significantly affected by the water level. SOC and MBC  
216 were the highest at the 0 cm water level and the lowest at the -25 cm water level ( $P < 0.01$ ; Fig. 5A and  
217 5B). DOC was the highest at the -25 cm water level and the lowest at the +25 cm water level ( $P < 0.01$ ;  
218 Fig. 5C).



219 Compared with the litter removal group, the SOC concentrations were significantly higher for the litter  
220 input group at the +25 cm and 0 cm water levels. Relative to the litter removal group, the DOC  
221 concentrations were significantly higher for the litter input group at the 0 cm- and -25 cm water levels ( $P$   
222  $< 0.001$ ; Fig. 5A and 5C). The contribution of the litter-C input to the S-SOCP was the highest for the +25  
223 cm water level treatment (13.75%), intermediate for the 0 cm water level treatment (4.73%), and the  
224 lowest for the -25 cm water level treatment (2.51%) ( $P < 0.001$ ; Fig. 5D).

## 225 4 Discussion

### 226 4.1 Environmental control of litter decomposition

227 Water level significant influenced *C. brevicuspis* leaf litters decomposition ( $P < 0.001$ ). The  $K$ -values  
228 were the highest for the +25 cm water level treatment, intermediate for the 0 cm water level treatment,  
229 and the lowest for the -25 cm water level treatment (Fig. 2B). Hence, the percentage litter dry weight loss  
230 and the decomposition rate increased with water level. These results supported our first hypothesis. The  
231 wetland water level strongly affect litter leaching and microbial decomposition (Peltoniemi et al., 2012).  
232 Compared with the terrestrial environment, water promotes litter leaching and microbial metabolism,  
233 thereby accelerating litter decomposition. Moreover, water infiltration into litter also increases relative  
234 leaching loss (Molles et al., 1995). Here, the high litter decomposition rate measured for the +25 cm  
235 water level treatment may be explained primarily by litter leaching. This finding was consistent with  
236 those reported for *Carex cinerascens* litter decomposition in Poyang Lake (Zhang et al., 2019) and  
237 *Calamagrostis angustifolia* litter decomposition on the Sanjiang Plain (Sun et al., 2012).

238 The high soil total microbial, bacterial and fungal biomass levels at the 0 cm water level could account  
239 for the rapid litter decomposition observed there. Certain microorganisms are vital to the decomposition  
240 process (Yarwood, 2018). Fungi are primary litter decomposers as they fragment dead plant tissues by  
241 breaking down lignin and cellulose. Bacteria are secondary decomposers that utilise the simpler  
242 compounds generated by fungal activity (de Boer et al., 2005; Bani et al., 2019). Microbial decomposers  
243 generally flourish in humid environments. At the 0-cm water level, microbial activity explains most of  
244 the litter decomposition. At the -25-cm water level, the soil surface is dry, there are comparatively few  
245 microbial decomposers, and decomposition is very slow.



#### 246 4.2 Intrinsic factors controlling litter decomposition

247 Decomposition rapidly increased to reach a maximum by 20 d. Thereafter, it slowly decreased and then  
248 stabilised (Fig. 2B). Water-soluble components and non-lignin carbohydrates are preferentially and  
249 quickly decomposed at the onset of decomposition (Davis et al., 2003). Here, a multiple regression model  
250 of the instantaneous litter decomposition rate and litter properties showed that the internal limiting  
251 factors affecting the rate of *C. brevicuspis* leaf litter decomposition varied with water level. The lignin  
252 concentration determined the litter decomposition rate for the -25 cm and 0 cm water level treatments  
253 whilst the lignin/N ratio regulated the litter decomposition rate for the +25 cm water level treatment. This  
254 discovery upheld our second hypothesis and was consistent with the findings of Zhang et al. who  
255 reported that wetland ecosystems decomposed *Carex cinerascens* lignin much earlier faster than did  
256 terrestrial ecosystems (Zhang et al., 2019). Here, we found that the lignin content was the major internal  
257 limiting factor of the *C. brevicuspis* leaf litter decomposition rate. At the +25 cm water level, N is rapidly  
258 lost and the L/N ratio significantly increases. Thus, L/N is the main internal limiting factor at the +25 cm  
259 water level. A few studies showed that the lignin content is a key factor limiting terrestrial plant and  
260 hygrophyte litter decomposition (Yue et al., 2016; Zhang et al., 2018). Therefore, the amount of carbon  
261 that the litter can return to the ecosystem is closely associated with the plant lignin content. The lignin  
262 content of *C. brevicuspis* leaf litters is ~10% less than that of other wetland plants such as *Miscanthus*  
263 *sacchariflorus* (~30%) (Xie et al., 2016), *Spartina alterniflor* (~40%) (Yan et al., 2019), and terrestrial  
264 plants such as willow (~25%), larch (~38%), and cypress (~28%) (Yue et al., 2016). Furthermore, *C.*  
265 *brevicuspis* covers a large area (~23,950 hm<sup>2</sup>) and generates abundant litter (~36,547 t) (Kang et al.,  
266 2009). Thus, *C. brevicuspis* litter may potentially return large amounts of carbon to the soil.

#### 267 4.3 Contribution of leaf decomposition to the soil surface carbon pool

268 Litter decomposition is the main pathway by which nutrients are transferred from the plants to the soil.  
269 Litter affects SOC whose stabilisation affects other soil properties such as sorption, nutrient availability,  
270 pH, and water holding capacity (Brady and Weil, 2008). The results of this study showed that litter  
271 addition increases SOC in a manner that varies with water level. The contribution of litter-C input to the  
272 S-SOCP was the highest under the +25 cm water level treatment (13.75%), intermediate under the 0 cm  
273 water level treatment (4.73%), and the lowest under the -25 -cm water level treatment (2.51%). For this  
274 reason, flooding conditions are conducive to litter carbon input into the soil. These findings corroborated



275 our third hypothesis. In addition, litter input had a similar effect on soil DOC at the 0 cm and -25 cm  
276 water levels. Therefore, litter decomposition contributes mainly soluble carbon to the soil (Zhou et al.,  
277 2015). However, this DOC is also readily lost and decomposed (Sokol and Bradford,  
278 2019; Gomez-Casanovas et al., 2020). This fact accounts for the significantly lower relative DOC under  
279 the +25 cm water level treatment here. Wetlands have comparatively larger but also more unstable  
280 S-SOCs than terrestrial environments. In wetlands, water level fluctuations could readily cause carbon  
281 loss (Gao et al., 2016; Chen et al., 2018). Nevertheless, we considered mainly aboveground litters in this  
282 experiment. Hence, the influences of underground litter (root) decomposition on the SOC pool should be  
283 investigated in future research (Sokol and Bradford, 2019; Lyu et al., 2019).

## 284 **5 Conclusion**

285 The water level in natural wetlands influences litter decomposition mainly by leaching and microbial  
286 activity, by extension, affects wetland surface carbon pool. Higher water levels facilitate the release of  
287 organic carbon from leaf litter into the soil via water leaching, and thus strengthened soil carbon pool. At  
288 lower water levels, wetland soil carbon is lost as the litter decomposition is lower, but active microbial  
289 (actinomycete) respiration rates is comparatively higher. The groundwater decline which was caused by  
290 the climate change and human disturbance in Dongting Lake floodplain would slowdown the return  
291 rate of organic carbon from leaf litter to soil, and facilitate the S-SOCP loss. Therefore, in wetland  
292 management and restoration practices, the construction of microhabitat with prolonged flooding period  
293 and relatively higher water level are essential ways to improve the carbon sequestration potential.

## 294 **Data availability**

295 The data used in this paper are stored in the open-access online database Figshare and can be accessed  
296 using the following link: <https://doi.org/10.6084/m9.figshare.12758387.v1> (Zhu et al. 2020).

## 297 **Conflict of interest**

298 The authors declare that they have no conflict of interest.



299 **Author contributions**

300 Lianlian Zhu designed experiments, collected samples, acquired, analyzed, interpreted data, and wrote  
301 the manuscript. Zhengmiao Deng designed experiments, interpreted data and revised the manuscript.  
302 Yonghong Xie designed experiments and revised the manuscript. Xu Li, Feng Li, Xinsheng Chen and  
303 Yeai Zou collected samples and revised the manuscript. Chengyi Zhang and Wei Wang interpreted data  
304 and revised the manuscript.

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- 455



456 **Table 1: Multiple regression model of instantaneous litter decomposition rate and litter properties**

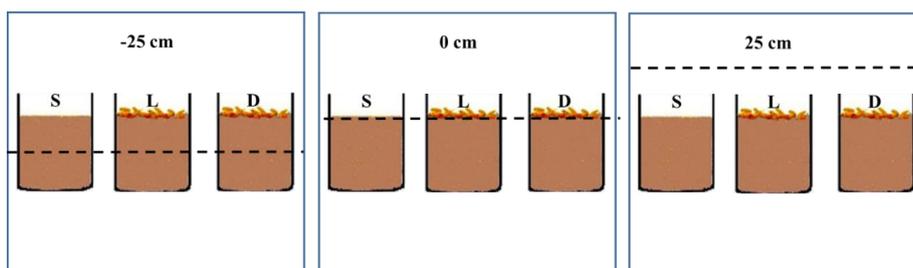
Water level (cm)	Multiple regression model	<i>F</i>	<i>R</i> <sup>2</sup>	<i>P</i>
-25	$R = -0.861L - 0.404C + 0.239CN + 2.586$	27.264	0.887	< 0.001
0	$R = -1.131L - 0.390P - 0.218LN + 2.124$	48.330	0.934	< 0.001
+25	$R = -0.739LN - 0.636N + 4.162$	19.465	0.787	< 0.001

457 where *R* is the litter instantaneous decomposition rate, *L* is the lignin concentration, *C* is the carbon  
 458 concentration, *N* is the nitrogen concentration, *P* is the phosphorus concentration, *CN* is the  
 459 carbon-to-nitrogen ratio (*C/N*, g g<sup>-1</sup>), and *LN* is the lignin-to-nitrogen ratio (lignin/*N*, g g<sup>-1</sup>).

460



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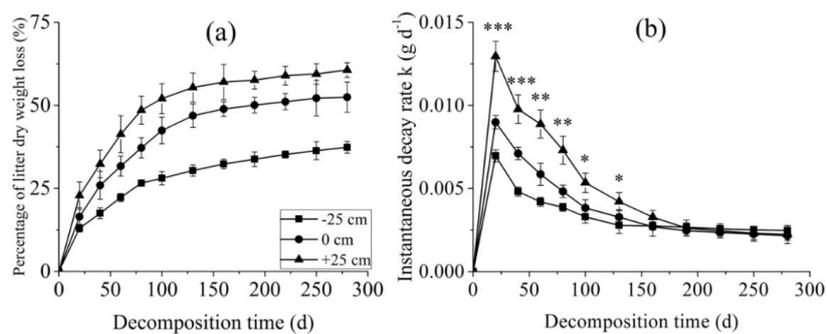


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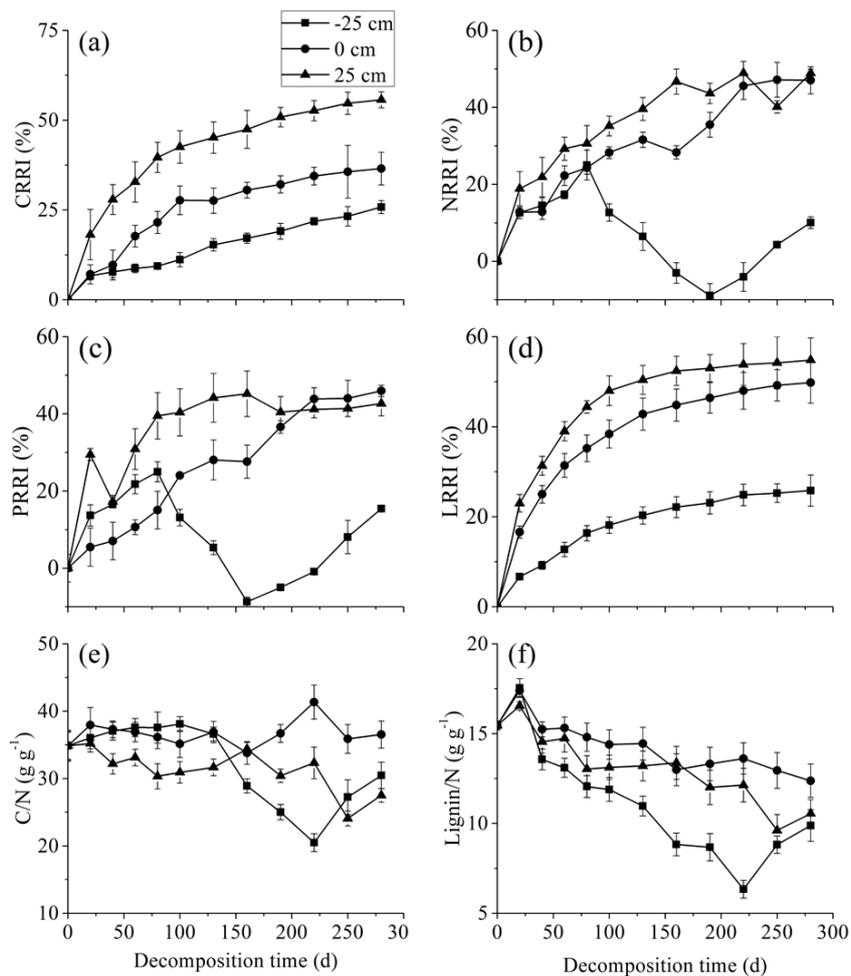
Figure 1: Schematic diagram of the experimental setup. The dotted line represents the water level.

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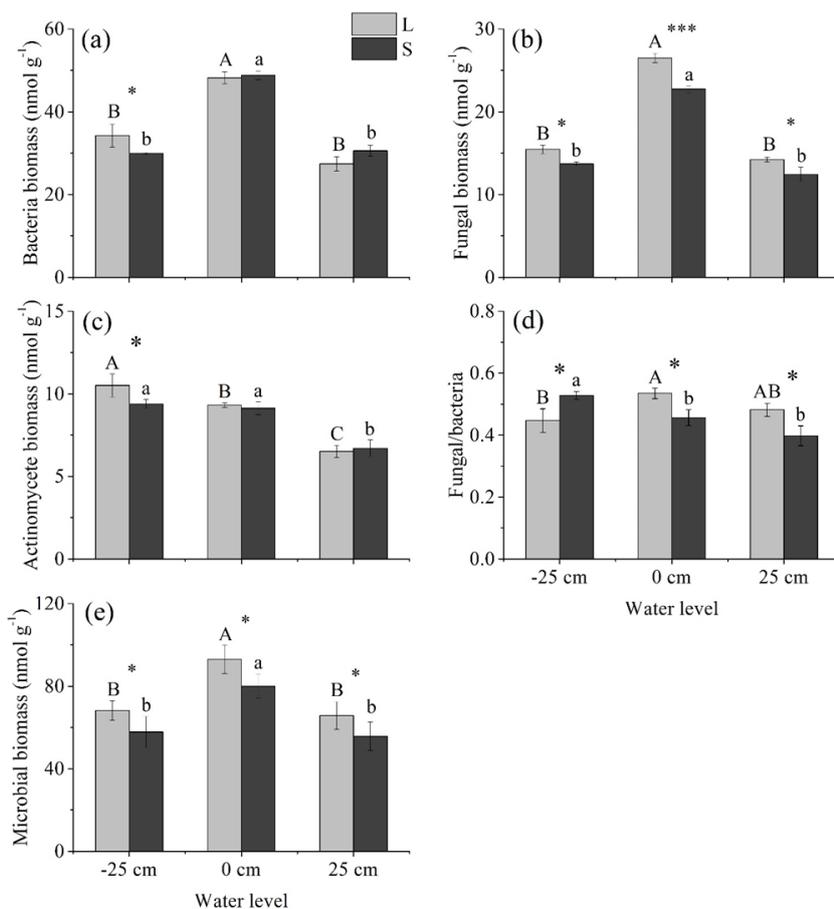
465

466 Figure 2: Percentage litter dry weight loss and decomposition rate during *C. brevicuspis* decomposition at  
467 three water levels (-25 cm, 0 cm, and +25 cm). \*, \*\*, and \*\*\* represent significant differences of the litter  
468 instantaneous decay rate among the three water levels at the 0.05, 0.01, and 0.001 significance levels,  
469 respectively.



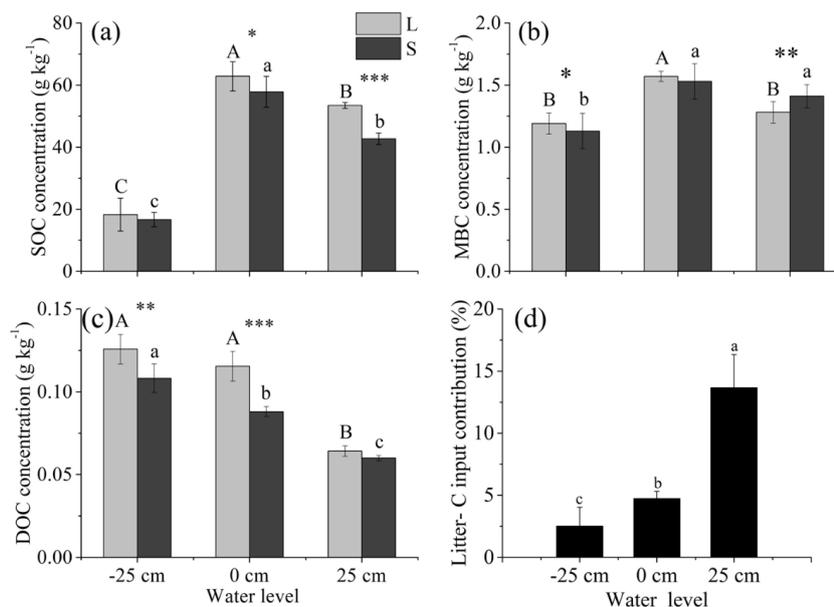
470

471 **Figure 3: Percentage (mean ± SE) of carbon relative release index (CRRRI), nitrogen relative release index**  
472 **(NRRRI), phosphorus relative release index (PRRI), lignin relative release index (LRRI), C/N ratio, and**  
473 **lignin/N ratio at three water levels (-25 cm, 0 cm, and +25 cm).**



474

475 **Figure 4: Microbial community structure under litter input and litter removal at three water levels. Different**  
 476 **uppercase letters among vertical bars indicate significant differences among the three water levels in the**  
 477 **litter input (L) group. Different lowercase letters indicate significant differences among the three water levels**  
 478 **in the litter removal (S) group. The significance level is  $\alpha = 0.05$ . \*, \*\*, and \*\*\* represent significant**  
 479 **differences between the litter input (L) and litter removal (S) groups at the 0.05, 0.01,**  
 480 **and 0.001 significance levels, respectively.**



481

482 **Figure 5: Concentrations of SOC (A), MBC (B), DOC (C) between the litter input (L) and litter removal (S)**  
483 **groups and the litter-C input contribution (D) under three water levels at the end of the experiment. Different**  
484 **uppercase letters among vertical bars indicate significant differences among the three water levels in the**  
485 **litter input (L) group. Different lowercase letters indicate significant differences among the three water levels**  
486 **in the litter removal (S) group. The significance level is  $\alpha = 0.05$ . \*, \*\*, and \*\*\* represent significant**  
487 **differences between the litter input (L) and litter removal (S) groups at the three water levels at the 0.05, 0.01,**  
488 **and 0.001 significance levels, respectively.**