

Dear Dr Michael Weintraub

Thank you and all of the reviewers for the positive comments on our work. We have revised the manuscript accordingly. In the revised manuscript, revised and corrected text (including references) are marked in red. We hope the revisions makes our manuscript more worthy of publication. Our detailed responses are as follows:

Response to Reviewers

Comment 1

L31: Change “application” to “addition”.

Response 1

Changed as suggested, thank you.

Comment 2

L66: Change “SOC pool” to “S-SOCP”.

Response 2

Corrected as suggested, Thank you!

Comment 3

L74: Change “litter” to “leaf litter”.

Response 3

Changed as suggested, thank you very much.

Comment 4

L131: Not “TOC analyser” but “measured with a TOC analyser”

Response 4

Changed as suggested, Thank you!

Comment 5

L 202, 287: Change “-25-cm” to “25 cm”.

Response 5

We are sorry for the carelessness, and changed it as suggested, Thank you!

Comment 6

L257: Change “onset” to “initial”.

Response 6

Revised as suggested, thank you.

Comment 7

Fig. 2b, Fig. 4 and Fig. 5: The error bar should be bolder.

Response 7

The figures had been adjusted as suggested, Thank you very much!

Comment 8

L376-377, L378-380: Please check the reference format.

Response 8

We are sorry for the negligence, and the references were revised in accord with the journal style.

Again, we appreciate the editor and reviewers for taking the time and energy to help us improve the manuscript. We look forward to hearing from you.

Sincerely yours

Lianlian Zhu, on behalf of co-authors

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

4
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18

19 **Abstract.** Litter decomposition plays a vital role in wetland carbon cycling. However, the contribution
20 of 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 the SOC pool at different water
23 levels. The *Carex* genus is ubiquitous to global freshwater wetlands. We sampled this plant leaf litter at
24 -25, 0, and +25 cm relative to the soil surface over 280 days and analysed leaf litter decomposition and
25 its contribution to the SOC pool. The percentage litter dry weight loss and the instantaneous litter dry
26 weight decomposition rate were the highest at +25 cm water level (61.8%, 0.01307d⁻¹), followed by the
27 0 cm water level (49.8%, 0.00908 d⁻¹), and the lowest at -25 cm water level (32.4%, 0.00527 d⁻¹).
28 Significant amounts of litter carbon, nitrogen, and phosphorus were released at all three water levels.
29 Litter input significantly increased the soil microbial biomass and fungal density but had nonsignificant
30 impacts on soil bacteria, actinomycetes, and the fungal/bacterial concentrations at all three water levels.
31 Compared with litter removal, litter **addition** increased the SOC by 16.93%, 9.44%, and 2.51% at the
32 +25 cm, 0 cm, and -25 cm water levels, respectively. Hence, higher water levels facilitate the release of
33 organic carbon from leaf litter into the soil via water leaching. In this way, they increase the soil carbon
34 pool. At lower water levels, soil carbon is lost due to the slower litter decomposition rate and active
35 microbial (actinomycete) respiration. Our results revealed that the water level in natural wetlands
36 influenced litter decomposition mainly by leaching and microbial activity, by extension, and affected
37 the wetland surface carbon pool.

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

39 **1 Introduction**

40 Wetlands are important terrestrial carbon pools. Depending on the definition of “wetland”, they contain
41 between 82 and 158 Pg SOC, (Kayranli et al., 2010; Kochy et al., 2015). The surface soil organic carbon
42 (SOC) pool (S-SOCP) and its turnover are sensitive to climate, topography, and hydrological conditions
43 (Wang et al., 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 (Bowden et al., 2014; Cao et al., 2020). Litter

47 input destabilised carbon storage by stimulating soil mineralisation and increasing labile soil carbon
48 fractions (microbial biomass carbon [MBC], soil dissolved organic carbon [DOC]), and enzyme activity
49 in the freshwater marshland of Northeast China (Song et al., 2014). It also promoted soil carbon loss via
50 CO₂ emissions and microbial activity in alpine and coastal wetlands (Gao et al., 2016; Liu et al., 2017).
51 In contrast, a study has recently found that litter decomposition stabilised the soil carbon pool after
52 processing by soil microbes in the Jiaozhou Bay wetland (Sun et al., 2019).

53 Litter decomposition is a physicochemical process that reduces litter to its elemental chemical
54 constituents (Berg and Mcclaugherty, 2003). Litter decomposition rates are determined mainly by
55 environmental factors (climatic and soil conditions), litter quality (litter composition such as C, N, and
56 lignin content) and decomposer organisms (microorganisms and invertebrates) (Yan et al., 2018; Yu et
57 al., 2020). A previous study showed that regional and global environmental conditions explain > 51% of
58 the variation in litter decomposition rate (Zhang et al., 2019). In wetland ecosystems, the water level
59 ecosystem processes determine soil aerobic and anaerobic conditions which, in turn, affect the microbial
60 decomposition of litter and SOC decomposition (Liu et al., 2017; Yan et al., 2018). An earlier study
61 reported that high soil moisture content and long flooding periods facilitate litter decomposition by
62 promoting leaching, fragmentation, and microbial activity (Van de Moortel et al., 2012). The water level
63 may contribute to soil physicochemical conditions which, in turn, regulate litter decomposition (Xie et
64 al., 2016b). Leaf litter contributes more to soil organic carbon than fine roots (Cao et al., 2020), litter also
65 strongly influences root decomposition rates, particularly near the surface (Hoyos-Santillan et al., 2015).
66 However, the contribution of litter decomposition to the **S-SOCP** pool has seldom been quantified.

67 Peng et al. reported that the organic carbon density in Dongting Lake wetland soil at 1 m depth was 127.3
68 ± 36.1 t hm⁻² and the carbon density in the 0–30 cm topsoil was 46.5 ± 19.7 t hm⁻² (Peng et al., 2005).
69 *Carex brevicuspis* is a dominant species in the Dongting Lake wetland and has large carbon reserves
70 (~6.5 × 10⁶ t y⁻¹) (Kang et al., 2009). However, due to the dam construction upstream of Dongting Lake,
71 the water regime varies considerably (early water withdrawal and decline of groundwater in non-flood
72 season) in recent years, leading to a significant carbon loss in this floodplain wetland (Hu et al., 2018;
73 Deng et al., 2018).

74 Here, we investigated *C. brevicuspis* **leaf litter** decomposition and its contribution to the SOC pool at
75 three water levels (-25 cm, 0 cm, and +25 cm relative to the soil surface) to find the factors controlling
76 *C. brevicuspis* leaf litter decomposition and quantify the contribution of litter decomposition to the SOC

77 pool. We tested the following hypotheses. Firstly, the water level has a significant effect on litter
78 decomposition. Secondly, the intrinsic factors that control litter decomposition rate at three water levels
79 are different. Thirdly, the contribution of leaf decomposition to S-SOCP is relatively higher at the +25
80 cm water level.

81 **2 Materials and methods**

82 **2.1 Soil core collection and leaf litter preparation**

83 Dongting Lake (28°30'–30°20' N, 111°40'–113°10' E) is the second-largest freshwater lake in China. It
84 is connected to the Yangtze River via tributaries. Dongting Lake wetlands are characterised by large
85 seasonal fluctuations in water level (≤ 15 m) and are completely flooded during June–October and
86 exposed during November–May (Chen et al., 2016). Soil cores (40 cm diameter \times 50 cm length) were
87 taken from the wetland. Leaf litter was collected in May 2017 from an undisturbed *Carex brevicuspis*
88 community at the sampling site (29°27'2.02" N, 112°47'32.28" E) of the Dongting Lake Station for
89 Wetland Ecosystem Research, which is part of the China Ecosystem Research Network. The litter was
90 cleaned with distilled water, oven-dried at 60 °C to a constant weight, and cut into pieces 5–10 cm long.
91 Pre-weighed litter samples (5 g; 10.73 ± 0.28 g kg⁻¹ N, 0.89 ± 0.04 g kg⁻¹ P, $40.23 \pm 2.6\%$ organic C, and
92 $17.83 \pm 0.25\%$ lignin) were placed into 10 cm \times 15 cm 1 mm mesh nylon bags. This mesh size excluded
93 macroinvertebrates but permitted microbial colonisation and litter fragment leaching (Xie et al., 2016a).

94 **2.2 Experimental design**

95 There were three water level treatments (-25 cm, 0 cm, and +25 cm relative to the soil surface) nested by
96 two litter treatments (input vs removal) and three replicates. The experiment was conducted in nine
97 cement ponds (2 m \times 2 m \times 1 m) at the Dongting Lake Station for Wetland Ecosystem Research. For the
98 -25 cm treatment, the water level was 25 cm below the soil surface. For the 0 cm treatment, the soil was
99 fully wetted with belowground water (the belowground water was extracted from the well in the
100 experiment site by a water pump) but without surface pooling. For the +25 cm treatment, the water level
101 was 25 cm above the soil surface. Water levels were adjusted weekly using belowground water (TOC:
102 3.44 mg L⁻¹; TN: 0.001 mg L⁻¹; TP: 0.018 mg L⁻¹). Three soil core sets were placed in each pond. One
103 was designated the litter removal control (S), the second was distributed on the soil surface with 15 litter

104 bags to observe the effects of leaf litter input on soil carbon pool (L), and the third was distributed on the
105 soil surface with 15 litter bags to monitor the litter decomposition rate and process (D) (Fig. 1). Litter
106 bags were laid flat on the surface of the soil. Each litter bag was not filled, and there are a little overlap
107 between the litter bags where there is no litter. All the litter bags were fixed to the soil surface with
108 bamboo sticks. The experiment started on 20 August 2017 and lasted 280 d. By that time, no further
109 significant change in litter dry weight was observed. Before incubation, three litter and three soil samples
110 (SOC: 63.32 g kg⁻¹) were collected to determine their initial quality. Litter bags were randomly collected
111 from treatment D after 20 d, 40 d, 60 d, 80 d, 100 d, 130 d, 160 d, 190 d, 220 d, 250 d, and 280 d. After
112 collection, the litter samples were separated, cleaned with distilled water, and oven-dried at 60 °C to a
113 constant weight (± 0.01 g). All samples were pulverised and passed through a 0.5-mm mesh screen for
114 litter quality analysis. At the end of incubation, the surface soil (0–5 cm, ~600 g FW) was collected to
115 eliminate the influences of root decomposition on the soil organic pool. The soil samples were placed in
116 aseptic sealed plastic bags and transported to the laboratory. The samples were sieved (< 2 mm),
117 thoroughly mixed, and divided into three subsamples. The first subsample (~150 g) was stored at -20 °C
118 and freeze-dried for phospholipid fatty acid (PLFA) analysis. The second one (~150 g) was stored at 4 °C
119 for MBC and DOC measurements. The third subsample (~300 g) was air-dried for physicochemical
120 analysis.

121 **2.3 Litter quality analyses**

122 Litter organic carbon content was analysed by the H₂SO₄-K₂Cr₂O₇ heat method. Litter nitrogen was
123 extracted by Kjeldahl digestion and quantified with a flow injection analyser (AA3; Seal Analysisten
124 GmbH, Langenselbold, Germany) (Xie et al., 2017). Litter phosphorus content was quantified by the
125 molybdenum-antimony anti-spectrophotometric method. The lignin content was measured by hydrolysis
126 (72% H₂SO₄) (Graça et al., 2005; Xie et al., 2017).

127 **2.4 Soil quality analyses**

128 **2.4.1 Soil chemical analyses**

129 SOC was determined by wet oxidation with KCr₂O₇ + H₂SO₄ and titration with FeSO₄ (Xie et al., 2017).

130 Soil DOC was extracted with K₂SO₄ and measured with a TOC analyser (TOC-VWP; Shimadzu Corp.,
131 Kyoto, Japan). MBC was analysed by chloroform fumigation, K₂SO₄ extraction, and measured with a
132 TOC analyser (TOC-VWP, Shimadzu Corp., Kyoto, Japan) (Tong et al., 2017).

133 2.4.2 Soil microbial composition

134 The total and specific microbial group biomass values and the microbial community structure were
135 estimated by phospholipid fatty acid (PLFA) analysis. The PLFAs were extracted from 8 g of freeze-
136 dried soil and analysed as previously described (Zhao et al., 2015). The concentrations of each PLFA was
137 calculated relative to that of the methyl nonadecanoate (19:0) internal standard. The PLFAs for the
138 following groups were determined: bacterial biomass, sum of i15:0, a15:0, 15:0, i16:0, 16:1u7, i17:0,
139 a17:0, 17:0, cy17:0, and cy19:0; actinomycete biomass, sum of 10 Me 16:0, 10 Me17:0, and 10 Me 18:0;
140 and fungal biomass, 18:2 ω6 and 18:1 ω9. The total microbial biomass was represented by the sum of the
141 bacterial, fungal, and actinomycete biomass values. The ratios of fungal to bacterial lipids (F/B) were
142 used to evaluate the microbial community structure (Bossio and Scow, 1998; Wilkinson et al., 2002;
143 Zhao et al., 2015). We calculated PLFA mass content first, PLFA (ng g⁻¹ dry soil) = (Response of PLFA
144 / Response of 19:0 internal standard) × concentration of 19:0 internal standard × (volume of sample /
145 mass of soil). Concentration of 19:0 internal standard: 5 μg ml⁻¹, volume of sample: 200 μl, mass of soil:
146 8g dry soil. And then we calculated PLFA molar mass concentration, PLFA (n mol g⁻¹ dry soil) = PLFA
147 (ng g⁻¹ dry soil)/ relative molecular mass.

148 2.5 Data processing

149 2.5.1 Litter decomposition rate

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

$$151 L_t = \frac{M_0 - M_t}{M_0} \times 100\% (1)$$

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

154 The instantaneous litter dry mass decay rate (k) was calculated based on the Olson negative exponential
155 attenuation model and double exponential decay model (Olson, 1963; Berg, 2014):

156 $M_{t_n} = M_{t_{n-1}} e^{-k_n(t_n - t_{n-1})}$ (2)

157 where M_{t_n} is the litter dry matter weight at nth sampling (g), $M_{t_{n-1}}$ is the litter dry matter weight at
158 (n-1)th sampling (g), $t_n - t_{n-1}$ is the time between the nth and (n-1)th sampling, k_n is the instantaneous
159 decomposition rate at the nth sampling.

160 **2.5.2 Relative release index**

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

163
$$RRI_t = \frac{M_0 \times C_0 - M_t \times C_t}{M_0 \times C_0} \times 100\% \quad (3)$$

164 where C_t is the concentration of an element in the litter at time t , C_0 is the initial concentration of an
165 element in the litter, and M_t is the litter dry matter weight at time t (g). CRRI, NRRI, PRRI, and LRRI
166 represent the carbon, nitrogen, phosphorus, and lignin RRIs, respectively. A positive RRI indicates a net
167 release of the element during litter decomposition whilst a negative RRI indicates a net accumulation of
168 the element during litter decomposition.

169 **2.5.3 Contribution of litter-C input to the SOC pool**

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

171
$$LC = \frac{SOC_L - SOC_S}{SOC_i} \times 100\% \quad (4)$$

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

175 **2.6 Statistical analyses**

176 The percentage of litter dry weight losses and the instantaneous decomposition rates were compared
177 among the three water levels by repeated ANOVA analyses. The water level was the main factor, and
178 time was the repeated factor. The intrinsic litter decomposition rate-limiting factor was analysed by the
179 stepwise regression method in a multiple regression model. The surface soil chemical components and
180 the microbial community structure were compared by two-way ANOVA. Treatment (with or without
181 litter input) and water level were the main factors. The percentage differences in litter dry weight loss,

182 the instantaneous decomposition rates, the soil chemical components, and the microbial community
183 structure were evaluated by LSD at the 0.05 significance level. The data were expressed as means \pm
184 standard error. All statistical analyses were performed in SPSS 21 (IBM Corp., Armonk, NY, USA).

185 **3 Results**

186 **3.1 litter decomposition process**

187 The percentage of litter dry weight loss was the highest for the +25 cm water level treatment through the
188 entire litter decomposition period followed by the 0 cm water level treatment. The percentage of litter
189 dry weight loss was the lowest for the -25 cm water level treatment ($P < 0.01$; Fig. 2a). After 280 d
190 decomposition, the percentage litter dry weight loss values under the +25 cm, 0 cm, and -25 cm water
191 level treatments were 61.8%, 49.8% and 32.4%, respectively.

192 The instantaneous decomposition rate at each measurement time point was calculated based on the Olson
193 negative exponential attenuation model and double exponential decay model. The instantaneous
194 decomposition rate was highest at initial and slowly decreased and stabilised for all three water levels.
195 The maximum decomposition rates for the -25 cm, 0 cm, and +25 cm water levels were 0.00527 d^{-1} ,
196 0.00908 d^{-1} , and 0.01307 d^{-1} , respectively (Fig. 2b).

197 **3.2 Intrinsic litter decomposition rate-limiting factor**

198 During the entire decomposition process, CRRI, NRRI, PRRI, and LRRI significantly increased with the
199 water level. Litter carbon and lignin were always released at all three water levels whilst at -25 cm,
200 nitrogen and phosphorus enrichment appeared in the middle stage (Fig. 3a–3d). At the start of the
201 experiment, neither the C/N nor the lignin/N ratio significantly differed at the three water levels. At the
202 middle stage, however, both the C/N and lignin/N ratios were significantly lower at the -25 cm water
203 level than they were at the 0 cm and -25 cm water levels (Fig. 3e–3f).

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

208 3.3 Soil surface microbial community structure

209 Under both litter input and litter removal conditions, the bacterial, fungal, and microbial biomass levels
210 were the highest under the 0 cm water level treatment; however, these parameters showed nonsignificant
211 differences between +25 cm above and below water level treatments ($P > 0.05$; Fig. 4a, 4b, and 4f). The
212 actinomycete biomass was the highest under the -25 cm water level treatment, followed by that under the
213 0 cm water level treatment. It was the lowest under the +25 cm water level treatment (Fig. 4c). Litter
214 input significantly stimulated fungal and microbial biomass at all three water levels but only significantly
215 stimulated bacterial and actinomycete biomass at the -25 cm water level ($P < 0.05$; Fig. 4a–4c and 4e).
216 Under litter input conditions, the fungal/bacteria ratio was the highest at the 0 cm water level, followed
217 by the +25 cm water level. It was the lowest under the -25 cm water level treatment. Under litter removal
218 conditions, however, the fungal/bacteria ratio was significantly higher under the -25 cm water level
219 treatment than it was under the 0 cm and +25 cm water level treatments ($P < 0.05$; Fig. 4d).

220 3.4 Contribution of leaf decomposition to the soil surface carbon pool

221 The SOC, MBC, and DOC concentrations were significantly affected by the water level. The SOC and
222 MBC were the highest at the 0 cm water level and the lowest at the -25 cm water level ($P < 0.01$; Fig. 5a
223 and 5b). The DOC was the highest at the -25 cm water level and the lowest at the +25 cm water level (P
224 < 0.01 ; Fig. 5c).

225 Compared with the litter removal group, the SOC concentrations were significantly higher for the litter
226 input group at the +25 cm and 0 cm water levels. Relative to the litter removal group, the DOC
227 concentrations were significantly higher for the litter input group at the 0 cm- and -25 cm water levels
228 ($P < 0.001$; Fig. 5a and 5c). The contribution of the litter-C input to the S-SOCP was the highest for the
229 +25 cm water level treatment (16.93%), intermediate for the 0 cm water level treatment (9.44%), and the
230 lowest for the -25 cm water level treatment (2.51%) ($P < 0.001$; Fig. 5d).

231 4 Discussion

232 4.1 Environmental control of litter decomposition

233 The water level significantly influenced *C. brevicuspis* leaf litter decomposition ($P < 0.001$). The

234 instantaneous decomposition rates (k) were the highest for the +25 cm water level treatment, intermediate
235 for the 0 cm water level treatment, and the lowest for the -25 cm water level treatment (Fig. 2b). Hence,
236 the percentage litter dry weight loss and the decomposition rate increased with the water level, which
237 supported our first hypothesis. The wetland water level strongly affects litter leaching and microbial
238 decomposition (Peltoniemi et al., 2012). Related research showed that the wetland water level strongly
239 affects litter leaching and microbial decomposition (Peltoniemi et al., 2012). Molles et al. (1995) also
240 found that compared with the terrestrial environment, in wetland, water promotes litter leaching and
241 microbial metabolism, thereby accelerating litter decomposition. Moreover, water infiltration into litter
242 also increases relative leaching loss (Molles et al., 1995). Here, the high litter decomposition rate
243 measured for the +25 cm water level treatment may be explained primarily by litter leaching. This finding
244 was consistent with results reported for *Carex cinerascens* litter decomposition in Poyang Lake (Zhang
245 et al., 2019) and *Calamagrostis angustifolia* litter decomposition on the Sanjiang Plain (Sun et al., 2012).
246 The high soil total microbial, bacterial and fungal biomass levels at the 0 cm water level could account
247 for the rapid litter decomposition observed there. Certain microorganisms are vital to the decomposition
248 process (Yarwood, 2018). Fungi are primary litter decomposers as they fragment dead plant tissues by
249 breaking down lignin and cellulose. Bacteria are secondary decomposers that utilise the simpler
250 compounds generated by fungal activity (de Boer et al., 2005; Bani et al., 2019). Microbial decomposers
251 generally flourish in humid environments. At the 0 cm water level, microbial activity explains most of
252 the litter decomposition. While at the -25 cm water level, there are comparatively few microbial
253 decomposers, and decomposition is very slow.

254 **4.2 Intrinsic factors controlling litter decomposition**

255 The instantaneous decomposition rate was highest at initial and slowly decreased and stabilised for all
256 three water levels. (Fig. 2b). Water-soluble components and non-lignin carbohydrates are preferentially
257 and quickly decomposed at the **initial** of decomposition (Davis et al., 2003). Here, a multiple regression
258 model of the instantaneous litter decomposition rate and litter properties showed that the internal limiting
259 factors affecting the rate of *C. brevicuspis* leaf litter decomposition varied with the water level. The lignin
260 concentration determined the litter decomposition rate for the -25 cm water level treatment whilst the
261 lignin/N ratio regulated the litter decomposition rate for the 0 cm and +25 cm water level treatment. This

262 discovery upheld our second hypothesis and was consistent with the findings of Zhang et al. who reported
263 that wetland ecosystems decomposed *Carex cinerascens* lignin much earlier and faster than terrestrial
264 ecosystems (Zhang et al., 2019). Here, we found that the lignin content was the major internal limiting
265 factor of the *C. brevicuspis* leaf litter decomposition rate at -25 cm water level. At the 0 cm and +25 cm
266 water level, N is rapidly lost, and the L/N ratio significantly increases. Thus, L/N is the main internal
267 limiting factor at the 0 cm and +25 cm water levels. A few studies have shown that the lignin content is
268 a key factor limiting terrestrial plant and hygrophyte litter decomposition (Yue et al., 2016; Zhang et al.,
269 2018). Therefore, the amount of carbon that the litter can return to the ecosystem is closely associated
270 with the plant lignin content. The lignin content of *C. brevicuspis* leaf litters is ~10% less than that of
271 other wetland plants such as *Miscanthus sacchariflorus* (~30%) (Xie et al., 2016), *Spartina alterniflora*
272 (~40%) (Yan et al., 2019), and terrestrial plants such as willow (~25%), larch (~38%), and cypress (~28%)
273 (Yue et al., 2016), so the *C. brevicuspis* leaf litter is more easily leached and then contributes more to the
274 SOC pool. Furthermore, in Dongting lake wetland, the *Carex* genus covers a large area (~23,950 hm²)
275 and generates abundant litter (~36,547 t) (Kang et al., 2009). Thus, *C. brevicuspis* litter may potentially
276 return large amounts of carbon to the soil.

277 **4.3 Contribution of leaf decomposition to the soil surface carbon pool**

278 Litter decomposition is the main pathway by which nutrients are transferred from the plants to the soil.
279 Litter affects the SOC, the stabilisation of which affects other soil properties such as sorption, nutrient
280 availability, pH, and water holding capacity (Brady and Weil, 2008). The results of this study showed
281 that litter addition increases SOC in a manner that varies with the water level. The contribution of litter-
282 C input to the S-SOCP was the highest under the +25 cm water level treatment (16.93%), intermediate
283 under the 0 cm water level treatment (9.44%), and the lowest under the -25 cm water level treatment
284 (2.51%). For this reason, flooding conditions are conducive to litter carbon input into the soil. These
285 findings corroborated our third hypothesis. In addition, litter input had a similar effect on soil DOC at
286 the 0 cm and -25 cm water levels. Therefore, litter decomposition contributes mainly soluble carbon to
287 the soil (Zhou et al., 2015). However, this DOC is also readily lost and decomposed (Sokol and Bradford,
288 2019; Gomez-Casanovas et al., 2020). This fact accounts for the significantly lower relative DOC under
289 the +25 cm water level treatment here. Wetlands have comparatively larger but also more unstable S-

290 SOCPs than terrestrial environments. In wetlands, water level fluctuations could readily cause carbon
291 loss (Gao et al., 2016; Chen et al., 2018). The SOC differences among three water levels were caused by
292 different soil mineralization in different environments. Soil mineralization in aerobic environment (-25
293 cm) was significantly higher than that in the flooded environment (0 cm, +25 cm) (Qiu et al., 2018), so
294 the SOC at -25 cm water level was lower than the other two water levels. Nevertheless, we considered
295 mainly aboveground litter in this experiment. Hence, the influence of underground litter (root)
296 decomposition on the SOC pool should be investigated in future research (Sokol and Bradford, 2019;
297 Lyu et al., 2019).

298 **5 Conclusion**

299 In this study, we quantified the contribution of leaf litter decomposition on soil surface organic carbon
300 pools (S-SOCPs) under different water level conditions. Appropriate flooding (+25 cm water level
301 treatment in our study) can significantly promote the decomposition of litter and contribute about 16.93%
302 organic carbon to S-SOCPs. Under waterlogging condition (0 cm water level), litter decomposition,
303 which mainly controlled by microbial activity, contributed 9.44% organic carbon to S-SOCP. However,
304 under relative drought conditions (-25 cm water level treatment in our study), litter decomposition only
305 contributes about 2.51% organic carbon to S-SOCP, which is largely ascribed to the slower
306 decomposition rate and soil carbon lost by microbe metabolism (i.e., actinomycetes). We also found that
307 lignin or lignin/N content were intrinsic factors controlling the litter decomposition rate in *Carex*
308 *brevicuspis*. In Dongting Lake floodplain, the groundwater decline due to climate change and human
309 disturbance would slow down the return rate of organic carbon from leaf litter to the soil, and facilitate
310 the S-SOCP loss.

311 **Data availability**

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

314 **Conflict of interest**

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

316 **Author contributions**

317 Lianlian Zhu designed experiments, collected samples, acquired, analysed, interpreted data, and wrote
318 the manuscript. Zhengmiao Deng designed experiments, interpreted data, and revised the manuscript.
319 Yonghong Xie designed experiments and revised the manuscript. Xu Li, Feng Li, Xincheng Chen and

320 Yeai Zou collected samples and revised the manuscript. Chengyi Zhang and Wei Wang interpreted data
321 and revised the manuscript.

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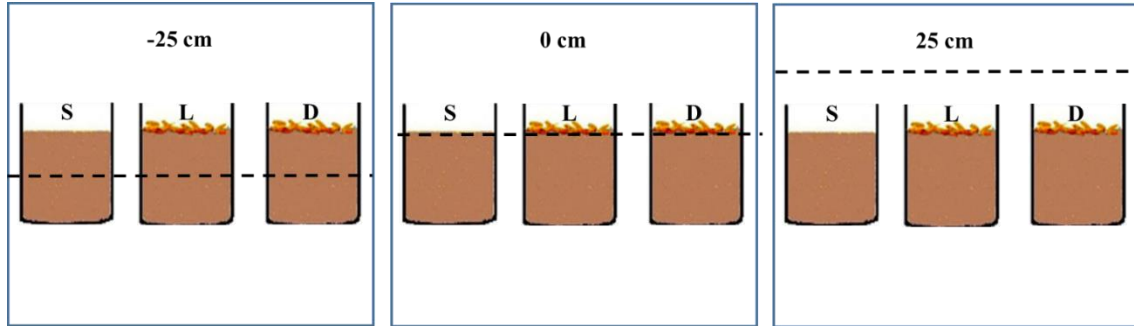
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443

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

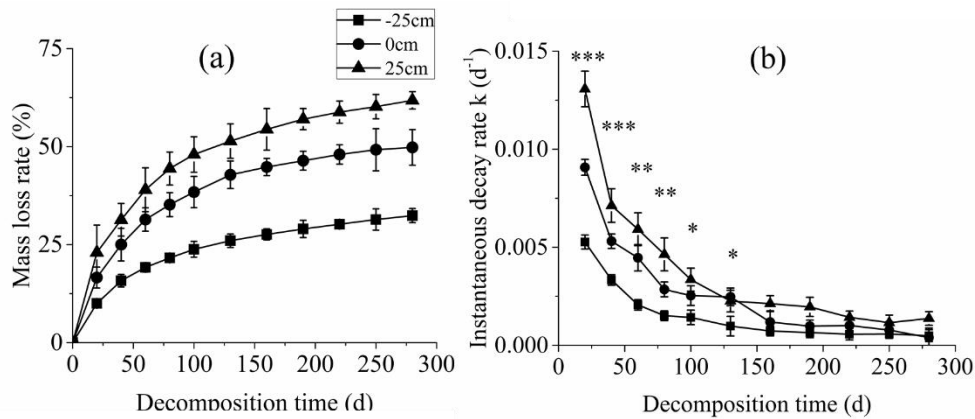
Water level (cm)	Multiple regression model	<i>F</i>	<i>R</i> ²	<i>P</i>
-25	$R = -0.715L - 0.443C + 0.033$	5.738	0.727	0.006
0	$R = -0.928LN - 0.233CN + 0.023$	5.928	0.927	< 0.001
+25	$R = -0.717LN + 0.016$	9.543	0.793	0.002

446 where *R* is the litter instantaneous decomposition rate, *L* is the lignin concentration, *CN* is the carbon-to-
 447 nitrogen ratio (*C/N*, g g⁻¹), and *LN* is the lignin-to-nitrogen ratio (lignin/*N*, g g⁻¹). All indicators used to analyse
 448 the model was referred to the content at each time point.

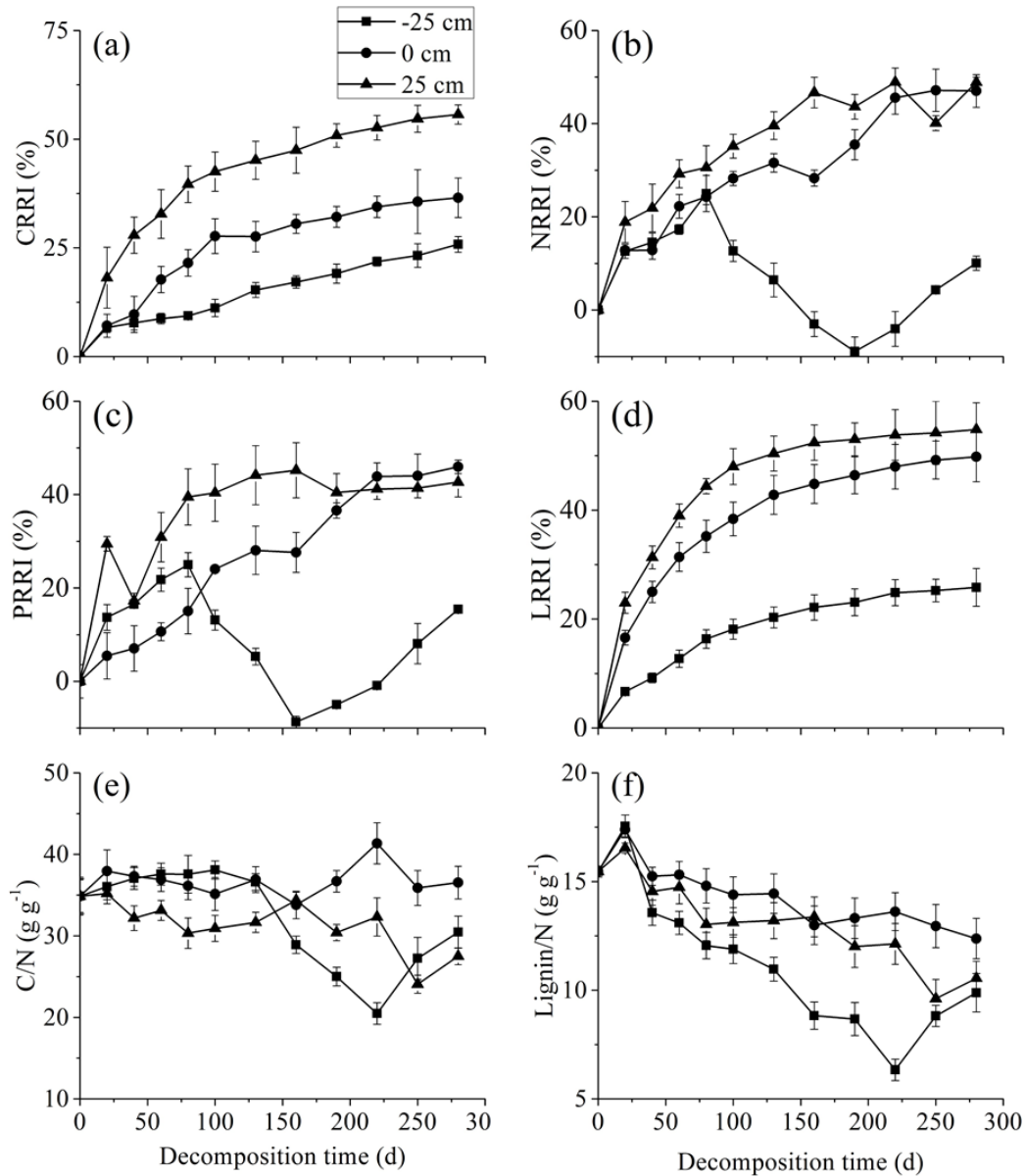


449
 450 **Figure 1: Schematic diagram of the experimental setup. The dotted line represents the water level.**
 451 **L** represents litter which was distributed on the soil surface in 15 litter bags to observe the effects of leaf litter
 452 input on soil carbon pool; **S** represents soil which was designated the litter removal control; **D** represents
 453 decomposition which was distributed on the soil surface in 15 litter bags to monitor the litter decomposition
 454 rate and process.

455

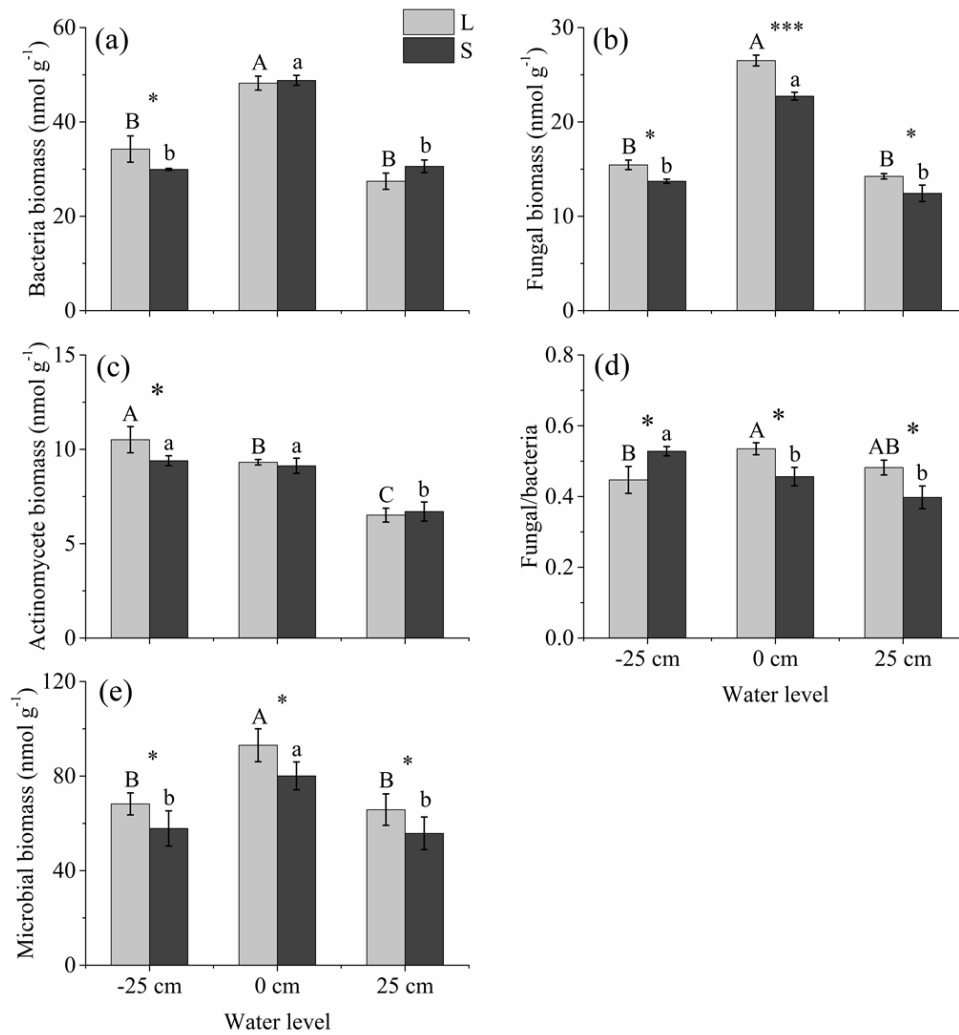


456
 457 **Figure 2: Percentage litter dry weight loss and decomposition rate during *C. brevicuspis* decomposition at**
 458 **three water levels (-25 cm, 0 cm, and +25 cm). *, **, and *** represent significant differences of the litter**
 459 **instantaneous decay rate among the three water levels at the 0.05, 0.01, and 0.001 significance levels,**
 460 **respectively.**



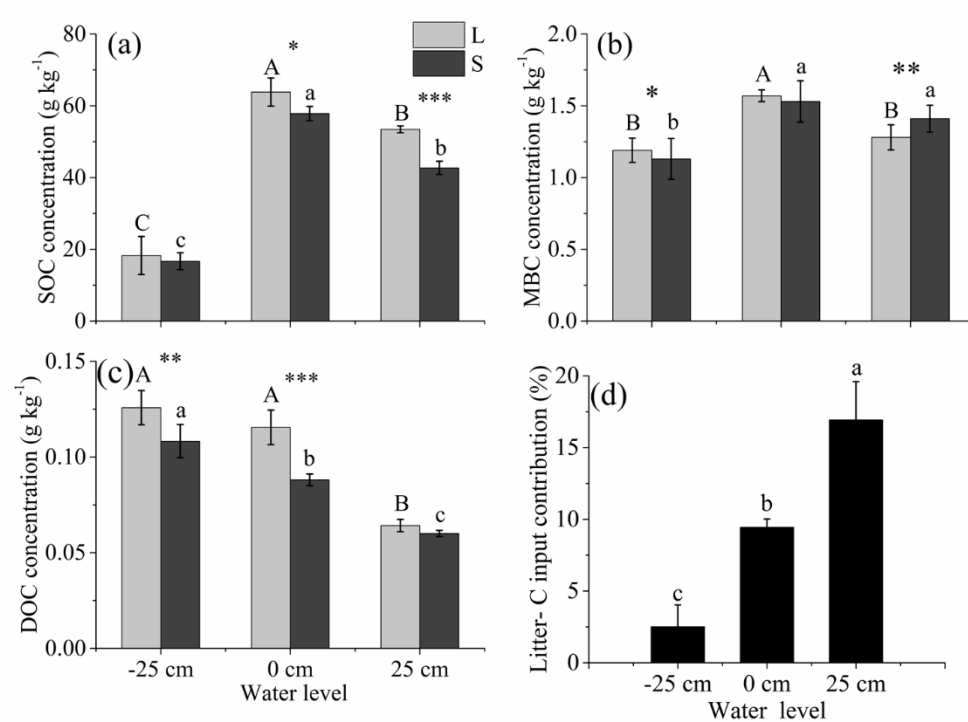
461

462 **Figure 3: Percentage (mean \pm SE) of carbon relative release index (CRRRI), nitrogen relative release index**
 463 **(NRRRI), phosphorus relative release index (PRRI), lignin relative release index (LRRRI), C/N ratio, and**
 464 **lignin/N ratio at three water levels (-25 cm, 0 cm, and +25 cm).**



465

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



472

473 **Figure 5: Concentrations of SOC (a), MBC (b), DOC (c) between the litter input (L) and litter removal (S)**
 474 **groups and the litter-C input contribution (d) under three water levels at the end of the experiment. Different**
 475 **uppercase letters among vertical bars indicate significant differences among the three water levels in the litter**
 476 **input (L) group. Different lowercase letters indicate significant differences among the three water levels in**
 477 **the litter removal (S) group. The significance level is $\alpha = 0.05$. *, **, and *** represent significant differences**
 478 **between the litter input (L) and litter removal (S) groups at the 0.05, 0.01, and 0.001**
 479 **significance levels, respectively.**

480