Dear Dr Michael Weintraub

Thank you very much for giving us the opportunity to revise our manuscript entitled "Factors controlling *Carex brevicuspis* leaf litter decomposition and its contribution to surface soil organic carbon pool at different water levels". We carefully considered all the comments from the two anonymous reviewers. These comments are very valuable and provide great help for us to revise and improve the clarity and rigour of the presentation of our work. We have read all the comments carefully, responded point-by point, and revised the manuscript accordingly.

In the revised manuscript, revised and corrected contents (including references) are marked in red. We hope the revisions makes our manuscript more worthy of publication.

Our detailed responses to the comments are as follows:

Response to Reviewer #1

Comment 1.1

General comments

Zhu et al. not only identified the major factor controlling leaf litter decomposition as water level, but also revealed its working approach in natural freshwater wetlands. The systematic and scientifically sound design delivered new insights into wetland leaf litter decomposition processes and consequences. I recommend to be accepted after revision.

Response 1.1

We appreciate the positive evaluations from the reviewer on our work and are grateful for the reviewer for recognizing the potential impact of our work.

Comment 1.2

Specific comments

Abstract: L25-27: The key rate values should be added.

Response 1.2

Thank you very much for your detailed suggestion. L25-27 has been changed to:

*The percentage litter dry weight loss and the instantaneous litter dry weight decomposition rate were the highest at +25 cm water level (61.8%, 0.01307*d^{-1}*), followed by the 0 cm water level (49.8%, 0.00908 *d^{-1}*), and the lowest at -25 cm water level(32.4%, 0.00527 *d^{-1}*).* See Line 25-27 in the revised manuscript.

Comment 1.3

L33: Change “strengthen” to “increase”.

Response 1.3

Changed as suggested, Thank you!

Comment 1.4

L35: Change “influences” to “influenced”.

Response 1.4

Changed as suggested, Thank you!

Comment 1.5

L36: Change “affects” to “and affected”.

Response 1.5

Changed as suggested, Thank you!

Comment 1.6

Introduction: L40: Change “25” to “25%”.

Response 1.6

Thank you for your suggestion, but after all things considered, we deleted the sentence.

Comment 1.7

L66-69: Move to M & M.

Response 1.7

Thank you for the suggestion. We have moved *“Dongting Lake (28°30’–30°20’N, 111°40’–113°10’E) is the second largest freshwater lake in China. It is connected to the Yangtze River via tributaries. Dongting Lake wetlands are characterized by large seasonal fluctuations in water level (≤ 15 m) and are completely flooded during June–October and exposed during November–May (Chen et al., 2016).”* to “Materials and methods” section as suggested. Please see L83-L86 in the revised manuscript.

Comment 1.8

L71: Species is not a vegetation

Response 1.8

Thank you for the reminder. This sentence has been rephrased as “*Carex brevicuspis* is a dominant species in the Dongting Lake wetland”. Please see L69 in the revised manuscript.

Comment 1.9

L82: Unclear. “decomposition controls differs”?

Response 1.9

We are sorry for the ambiguity. It means that the intrinsic control factors are different at different water levels. This sentence has been rephrased as "*the intrinsic factors that control litter decomposition rate at three water levels are different*" to avoid confusing. Please see L78-79.

Comment 1.10

L100: Move "which is …" to L91.

Response 1.10

Thank you for the comment. This sentence has been moved to line 88-89 in the revised manuscript as suggested.

Comment 1.11

L101: What's the source of the belowground water?

Response 1.11

We are very sorry for our negligence of detailed description about the belowground water. The belowground water is extracted from the well in the experiment site by the water pump. We have added this information in M&M part. Please see L98-100 in the revised manuscript.

Comment 1.12

L105: How to arrange the 15 litterbags (10 cm × 15 cm) within each soil cores (40 cm diameter)?

Response 1.12

We are very sorry for our negligence. Litter bags were laid flat on the surface of the soil. Each litter bag was not filled, and there are a little overlap between the litter bags where there is no litter. Please see L104-105 in the revised manuscript.

Comment 1.13

L170: Multiple regression method should be added.

Response 1.13

Thank you for the suggestion. We have added the following sentence in the statistical analyses section.

The intrinsic litter decomposition rate-limiting factors were analyzed by stepwise regression method in a multiple regression model. Please see L178-179 in the revised manuscript.

Comment 1.14

L198-201, Table 1: Why not choose the same variables in every regression model? Please explain or give the methodology basis.

Response 1.14

We are very sorry for our negligence. Stepwise regression is used to calculate the regression model, the variables were the result of Stepwise regression model filtering, so the variables were different. The regression model methodology has been added in section 2.6, in L178-179.

Comment 1.15

Figure 1: The full words of S, L and D should be added in the caption.

Response 1.15

Thank you for the detailed suggestion. The following paragraph has been added in the Figure 1 caption.

L represents litter which was distributed on the soil surface in 15 litter bags to observe the effects of leaf litter input on soil carbon pool; S represents soil which was designated the litter removal control; D represents decomposition which was distributed on the soil surface in 15 litter bags to monitor the litter decomposition rate and process. Please see L437-441 in the revised manuscript.

Comment 1.16

L227: K-value should be kept consistent with k occurred in M & M.

Response 1.16

We are very sorry for our negligence. K-value in L227 in the original manuscript has been changed to the instantaneous litter dry mass decay rate (*k*) that was kept consistent with k occurred in M & M. Please see L233-235 in the revised manuscript.

Comment 1.17

L230: Please specify which results.

Response 1.17

These results are that the percentage litter dry weight loss and the decomposition rate increased with water level supported our first hypothesis.

"Hence, the percentage litter dry weight loss and the decomposition rate increased with water level. These results supported our first hypothesis." has been rephrased as following: *"Hence, the percentage litter dry weight loss and the decomposition rate increased with water level, which supported our first hypothesis."* Please see L235-237.

Comment 1.18

L232-233, 244-245: Not always the truth. Water will inhibit most decomposition as well for lack of oxygen.

Response 1.18

We are sorry for the ambiguity. The purpose of quoting this sentence is to show that there are existing studies supporting the results in our study, and to provide a scientific and reasonable explanation for my research results. The sentences have been changed to: *Related research showed that the wetland water level strongly affects litter leaching and microbial decomposition (Peltoniemi et al., 2012). Molles et al (1995) also found that compared with the terrestrial environment, in wetland, water promotes litter leaching and microbial metabolism, thereby accelerating litter decomposition. Moreover, water infiltration into litter also increases relative leaching loss (Molles et al., 1995).* Please see L238-242 in the revised manuscript.

Comment 1.19

L251-259: It's more interesting to discuss why the same litter subject to various water levels were mainly controlled by different factor?

Response 1.19

Thank you for the suggestion. This is mainly because that in different water levels, the rates of N lost were different. *At the 0 cm and +25 cm water level, N is rapidly lost and the L/N ratio significantly increases. Thus, L/N is the main internal limiting factor at the 0 cm and +25 cm water level.* Please see L265-267 in the revised manuscript.

Comment 1.20

L279-280? Any references?

Response 1.20

References (Gao et al., 2016;Chen et al., 2018) have been added in the text. Please see L290-291.

Comment 1.21

L285-286: Repeated from Abstract.

Response 1.21

We are sorry for the mistake. The conclusion has been rephrased as "*In this study, we quantified the contribution of leaf litter decomposition on soil surface organic carbon pools (S-SOCPs) under different water level conditions. Appropriate flooding (+25 cm water level treatment in our study) can significantly promote the decomposition of litter and contribute about 13.75% organic carbon to S-SOCPs. Under waterlogging condition (0 cm water level), litter decomposition, which mainly controlled by microbial activity, contributed 4.73% organic carbon to S-SOCP. However, under relative drought conditions (-25 cm water level treatment in our study), litter decomposition only contributes about 2.51% organic carbon to S-SOCP, which is largely ascribed to the slower decomposition rate and soil carbon lost by microbe metabolism (i.e., actinomycetes). We also found that lignin or lignin/N content were intrinsic factors controlling the litter decomposition rate in Carex brevicuspis. In Dongting Lake floodplain, the groundwater decline due to climate change and human disturbance would slow down the return rate of organic carbon from leaf litter to the soil, and facilitate the S-SOCP loss.*" Please see L299-310 in the revised manuscript.

Comment 1.22

L291-293: Beyond the support of this study.

Response 1.22

We accept this comment and this part had been deleted. Our conclusion has been rephrased as follows: *In this study, we quantified the contribution of leaf litter decomposition on soil surface organic carbon pools (S-SOCPs) under different water level conditions. Appropriate flooding (+25 cm water level treatment in our study) can significantly promote the decomposition of litter and contribute about 13.75% organic carbon to S-SOCPs. Under waterlogging condition (0 cm water level), litter decomposition, which mainly controlled by microbial activity, contributed 4.73% organic carbon to S-SOCP. However, under relative drought conditions (-25 cm water level treatment in our study), litter decomposition only contributes about 2.51% organic carbon to S-SOCP, which is largely ascribed to the slower decomposition rate and soil carbon lost by microbe metabolism (i.e., actinomycetes). We also found that lignin or lignin/N content were intrinsic factors controlling the litter decomposition rate in Carex brevicuspis. In Dongting Lake floodplain, the groundwater decline due to climate change and human disturbance would slow down the return rate of organic carbon from leaf litter to the soil, and facilitate the S-SOCP loss.* Please see L299-310 in the revised manuscript.

The references in the responses were listed as follows:

Chen, H. Y., Zou, J. Y., Cui, J., Nie, M., and Fang, C. M.: Wetland drying increases the temperature sensitivity of soil respiration, Soil Biology & Biochemistry, 120, 24-

27, 10.1016/j.soilbio.2018.01.035, 2018.

Gao, J. Q., Feng, J., Zhang, X. W., Yu, F. H., Xu, X. L., and Kuzyakov, Y.: Drying-rewetting cycles alter carbon and nitrogen mineralization in litter-amended alpine wetland soil, Catena, 145, 285-290, 10.1016/j.catena.2016.06.026, 2016.

Response to Reviewer #2

Comment 2.1

Line 20 and 46: I recommend including (Cao et al. 2020), and references therein, that address aboveground litter decomposition on SOC pools.

Response 2.1

Thank you for your recommendation, we had cited (Bowden et al., 2014; Cao et al., 2020) in the revised manuscript in L45-46.

Comment 2.2

Line 30: The SOC increase due to litter application (Figure 5d) appears to be calculated from Figure 5a, but I could not reconcile the value for -25 cm. While Figure 5 does seem to support that litter increases SOC, I have two concerns about this presentation. First, the differences in Figure 5a for the 0 cm water levels is labelled as significant, but the error bars clearly overlap. Please clarify. Second, and a potential fundamental flaw in the data presentation, is that the baseline SOC is not provided and there is no way to know if SOC changed throughout the course of the experiment. It appears based on Equation 4 you did measure baseline SOC for each core (_18 g kg-1)? Please clarify. In either case, a significant observation, which is not discussed, is that the no-litter treatments resulted in very large SOC increases and adding litter resulted in a small additional increase.

Response 2.2

For the first concern, we are sorry for the mistake of the error bars. Figure 5a has been reconstructed.

For the second concern, the soil cores were collected from the same site, and the baseline SOC was 63.32g kg^{-1}. The aim of this study was to clarify the impact of litter addition on SOC, so we did not present the SOC baseline. To avoid confusion, we have added the baseline SOC (63.32g kg^{-1}) in section 2.2 in L109-110 in the revised manuscript.

We are sorry for the negligence. The SOC differences among three water levels were caused by different soil mineralization in different environments. Soil mineralization in aerobic environment (-25 cm) was significantly higher than that in the flooded environment (0 cm, +25 cm) (Qiu et al., 2018), so the SOC at -25 cm water level was lower than the other two water levels. We had added the sentences "*In wetlands, water level fluctuations could readily cause carbon loss (Gao et al., 2016; Chen et al., 2018). The SOC differences among three water levels were caused by different soil mineralization in different environments. Soil mineralization in aerobic environment (-25 cm) was significantly higher than that in the flooded environment (0 cm, +25 cm) (Qiu et al., 2018), so the SOC at -25 cm water level was lower than the other two water levels"* in the revised manuscript in L290-294.

Comment 2.3

Line 40: References for this statement are inappropriate, or incorrectly cited. Means et al. 2016 does not discuss global carbon pools. Whiting and Chanton (2001) is an accurate source for the value you used for wetland carbon stocks, but they cite Schlesinger 1991 as their source, and there are more up-to-date carbon stock estimates, such as (Köchy, Hiederer, and Freibauer 2015). Cao et al., 2017, is a secondary reference, like Whiting and Chanton (2001), and neither reflect the range of wetland soil carbon (25– 63%) you provide. The value in Whiting and Chanton (2001) is 3 – 68% (secondary references) and the value in Cao et al. (2017) is 12 – 15% (also a secondary reference). Use of the most up-to-date sources and an accurate reflection of those sources adds value to the manuscript. I recommend adding a citation such as (Kayranli et al. 2010), which could also be useful in your discussion considering what happens to the SOC after it is leached from the litter into the soil.

Response 2.3

We are sorry for the mistake and thank the reviewer very much for the commendation and suggestion. We have add the value into the manuscript, and cited the references (Kayranli et al., 2010;Kochy et al., 2015). The sentences have been rephrased as follows:

Wetlands are important terrestrial carbon pools. They contain between 82 and 158 Pg SOC, which depending on the definition of "wetland" (Kayranli et al., 2010; Kochy et al., 2015). Please see L40-41 in the revised manuscript.

Comment 2.4

Line 52: The Sun et al., 2019 study does not support the statement that litter decomposition stabilized the soil organic carbon pool. Litter decomposition made

DOC more mobile and labile, which the authors suggested could lead to SOC stability after processing by soil microbes.

Response 2.4

We are sorry for the mistake. The sentences have been rephrased as: *In contrast, a recent study found that litter decomposition stabilized the soil carbon pool after processing by soil microbes in the Jiaozhou Bay wetland (Sun et al., 2019).* Please see L51-52 in the revised manuscript.

Comment 2.5

Lines 54-56: Aerts (1997) addresses litter decomposition in non-wetland sites where shredder invertebrates (detritovores) are important, but their role in wetland settings is more uncertain (Inkley, Wissinger, and Baros 2008). Shredding would be an important physio-chemical control on DOC leaching.

Response 2.5

We thank the reviewer very much for the commendation and suggestion. The references have been changed, and the sentences have been rephrased as follows: *Litter decomposition is a physicochemical processes that reduces litter to its elemental chemical constituents (Berg and McClaugherty, 2003). Litter decomposition rates are determined mainly by environmental factors (climatic and soil conditions), litter quality (litter composition such as C, N, and lignin content) and decomposer organisms (microorganisms and invertebrates) (Yu et al., 2020;Yan et al., 2018).* Please see L53-57.

Comment 2.6

Line 62: Zhang 2019 supports the statement that water levels affected microbial activity, but leaching and fragmentation were only discussed, not measured.

Response 2.6

We are sorry for the ambiguity. The references have been changed to (Van de Moortel et al., 2012), which designed a leaching experiment to clarify the leaching process of litter decomposition. Please see L60-62 in the revised manuscript.

Comment 2.7

Line 64: This is a mischaracterization of the Upton, 2018 study. Perhaps a better reference is (Hoyos-Santillan et al. 2015). However, clarification is needed because Hoyos-Santillan states that roots (not litter) are the main source of SOC in peatlands, but litter strongly influences root decomposition rates, particularly near the surface.

Response 2.7

We are sorry for our carelessness. The sentence has been rephrased as follows: *Leaf litter contributes more to soil organic carbon than fine roots (Cao et al., 2020), litter also strongly influences root decomposition rates, particularly near the soil surface (Hoyos-Santillan et al., 2015).* Please see Line 64-66.

Comment 2.8

Line 151. The Olson (1963) simple decay model assumes constant k, which you demonstrated is not a constant. Although use of this decay model is common in the literature, it is an oversimplification. This does not adversely affect your comparative analysis, but the paper would be strengthened with a more sophisticated analysis, such as a double exponential decay model (Berg 2014 or Wider and Lang 1982).

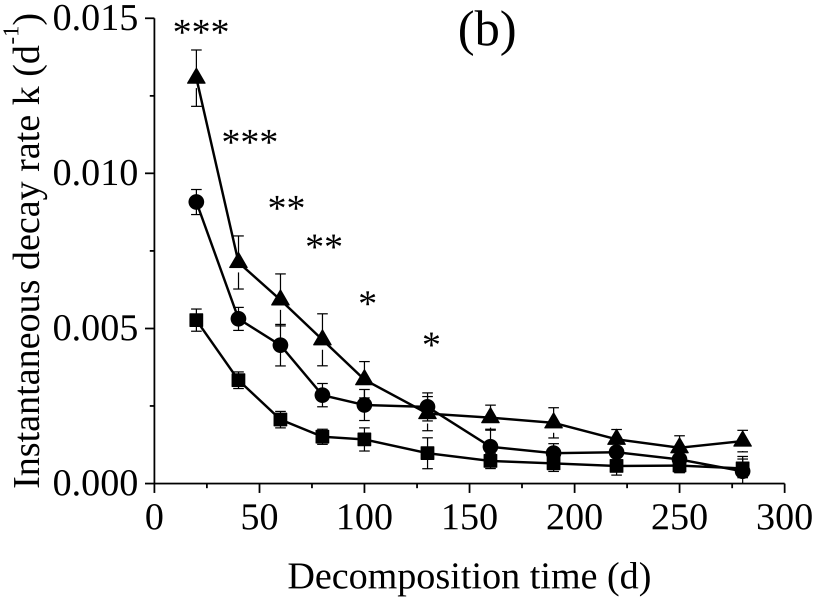
Response 2.8

Thank you for the constructive suggestions. We have modified the model based on your suggestion to highlight the instantaneous rate variation of litter decomposition. The model is:

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

Where M_{t_n} is the litter dry matter weight at *n*th sampling (g), $M_{t_{n-1}}$ is the litter dry matter weight at (n-1)th sampling (g), $t_n - t_{n-1}$ is the time between the *n*th and (n-1)th sampling, k_n is the instantaneous decomposition rate at the *n*th sampling. (Please see line 154-159 in the revised manuscript.

This model would be more accurate. The result was as following: The instantaneous litter decomposition rate was highest at initial and slowly decreased and stabilized at all three water levels. The maximum decomposition rates at the -25 cm, 0 cm, and +25 cm water levels were 0.00527 d^{-1}, 0.00908 d^{-1}, and 0.01307 d^{-1}, respectively (Fig. 2b). Please see L192-196 in the revised manuscript.



Based on the change of the instantaneous decomposition rate, we recalculated the multiple regression model which was used to analyze the intrinsic litter decomposition rate-limiting factor. The models are as follows (Table 1):

Water level (cm)	Multiple regression model	*F*	R^2	*P*
-25	$R = \mathbf{-0.715L} - 0.443C + 0.033$	5.738	0.727	0.006
0	$R = \mathbf{-928LN} - 0.233CN + 0.023$	5.928	0.927	< 0.001
+25	$R = \mathbf{-0.717LN} + 0.016$	9.543	0.793	0.002

The multiple regression model of the instantaneous litter decomposition rate and the litter properties showed that at the -25 cm, the main decomposition rate-limiting factor was the lignin concentration, whilst at 0 cm and +25 cm water level, the main litter decomposition rate-limiting factor was the lignin/N ratio. Please see L204-207 in the revised manuscript.

Comment 2.9

Line 266: Doesn’t your argument imply C. brevicuspis, due to it’s lower lignin content, would return less carbon to the soil compared to the other plants you cited? The manuscript may be strengthened, and have a wider inference, if you listed and compared decomposition rates and lignin contents of wetland plants including C. brevicuspis.

Response 2.9

Thank you for the suggestion. But we just intended to clarify that the lignin content of *C. brevicuspis* leaf litter was lower than the other plants, so the *C. brevicuspis* leaf litter was more easily to be leached and then contributed more to the SOC pool. Due to the different environment, the litter decomposition rates were different, so we didn't compared decomposition rates. On the other hand, the aim of our study was to explore the contribution of litter decomposition to SOC pool, instead of the relationship between lignin content and litter decomposition rate. Taking all these into account, we didn't list and compare decomposition rates and lignin contents of wetland plants.

Comment 2.10

Line 284: Your conclusion contains a significant amount of new and largely unsupported discussion material. Conclusions should stick to what you were able to show in your experiments.

Response 2.10

Thank you very much for the comment. The conclusion part has been rephrased as follows: *In this study, we quantified the contribution of leaf litter decomposition on soil surface organic carbon pools (S-SOCPs) under different water level conditions. Appropriate flooding (+25 cm water level treatment in our study) can significantly promote the decomposition of litters and contributed about 16.93% organic carbon to S-SOCPs. Under waterlogging condition (0 cm water level), litter decomposition, which mainly controlled by microbial activity, contributed 9.44% organic carbon to S-SOCP. However, under relatively drought condition (-25 cm water level treatment* *in our study), litter decomposition only contribute about 2.51% organic carbon to S-SOCP, which is largely ascribe to the slower decomposition rate and soil carbon lost by metabolism of the microbes (i.e. actinomycete). We also found that lignin and/or lignin/N content were intrinsic factors controlling litter decomposition rate in Carex brevicuspis. In Dongting Lake floodplain, the groundwater decline which was caused by the climate change and human disturbance would slowdown the return rate of organic carbon from leaf litter to soil, and facilitate the S-SOCP loss.* Please see L299-310 in the revised manuscript. We hope this modification can meet the requirements.

Comment 2.11

Table 1: Is the lignin content (L) in the regression model the initial lignin content?

Response 2.11

Sorry we didn’t clearly define the model indictors. The regression model is used to analyse the intrinsic litter decomposition rate-limiting factor. We added “All indicators used to analyse the model was refered to the content at each time point” in L432-433 in the revised manuscript.

Comment 2.12

Figure 3d (LRRI%) is nearly identical to Figure 2a (litter dry weight loss, %), which seems counter-intuitive unless lignin were the sole material being mineralized during the decomposition process. Did you measure lignin content at each time point?

Response 2.12

We measured lignin content at each time point. In fact, the results of stepwise regression analysis showed that lignin content is the main intrinsic litter decomposition rate-limiting factor, which is consistent with the figure 3d.

Technical corrections:

Comment 2.13

Line 23: Carex brevicuspis may be ubiquitous to wetlands in China; however, is this true globally?

Response 2.13

We are sorry for the ambiguity. This sentence has been rephrased as: The *Carex* genus is ubiquitous to global freshwater wetlands. Please see L23 in the revised manuscript.

Comment 2.14

Line 25: Is “mass loss” = “carbon release”? If so, one of these phrases is redundant.

Response 2.14

Thank you for remind us. In our opinion, mass loss includes not only carbon release but also other elements release, such as N, P. but because of the high proportion of carbon release, the trend of mass loss and carbon release are similar. Mass loss reflected the whole process of litter decomposition, while carbon release reflected the process of carbon release.

Comment 2.15

Line 82: The way you stated your hypotheses imply you have tested causal factors, which you did not. Specifically, leaching, fragmentation and infiltration.

Response 2.15

Thank you for reminding us. We have rephrased the hypotheses as follows: First, water level has a significant effect on litter decomposition. Second, the intrinsic limiting factors may be different among three water levels. Third, the contribution of leaf decomposition to S-SOCP was relatively higher at the +25 cm water level. Please see L77-80 in the revised manuscript.

Comment 2.16

Line 102: Did the litter bags float? Did you need to pin them in contact with the soil surface?

Response 2.16

We are sorry for the negligence. All litter bags were fixed to the soil surface with bamboo sticks. And the sentence has been added in section 2.2 in L107-108.

Comment 2.17

Line 105 - 107: Clarify how many soil cores were used in each pond for each purpose and how they were prepared (e.g. were soils blended prior to starting the experiment). The text is confusing.

Response 2.17

We have clarified that all the soil cores were undisturbed soil. The experiment was conducted in nine cement ponds (2 m × 2 m × 1 m) (please see L107-108 in revised manuscript). Three soil core sets were placed in each pond. One was designated the litter removal control (S), the second was distributed on the soil surface in 15 litter bags to observe the effects of leaf litter input on soil carbon pool (L), and the third was distributed on the soil surface in 15 litter bags to monitor the litter decomposition rate and process (D) in L102-108 in revised manuscript.

Comment 2.18

Line 183: You use capital letters (Fig 2A) in text references, but lower-case letters in the Figures.

Response 2.18

We are sorry for the mistake. The capital letters in the text references have been changed into lower-case letters.

Comment 2.19

Line 187/188 and Line 247: I would not interpret your data that decomposition rates “rapidly increased” – the decomposition rate at time t=0 is undefined.

Response 2.19

We are sorry for our obscure writing. The sentences have been rephrased as: *The instantaneous litter dry weight decomposition rate was highest at initial and slowly decreased and stabilized at all three water levels*. Please see L193-194 in revised manuscript.

Comment 2.20

Figure 4: Are these figures reporting mass? The units are nmol g-1.

Response 2.20

We are sorry for that we didn’t clearly define the calculation method about microbial community structure. These figures were used to report the PLFA molar mass concentration. This is a common way to show the microbial content (Zhao et al., 2015). We calculated PLFA mass content first, PLFA (ng g^{-1} dry soil) = (Response of

PLFA/Response of 19:0 internal standard) × concentration of 19:0 internal standard × (volume of sample / mass of soil). Concentration of 19:0 internal standard: 5 μg ml^{-1}, volume of sample: 200μl， mass of soil: 8g dry soil. And then we calculated PLFA molar mass concentration, PLFA (n mol g^{-1} dry soil) = PLFA (ng g^{-1} dry soil)/ relative molecular mass. Please see L143-147 in revised manuscript.

The references in the responses were listed as follows:

Cao, J. B., He, X. X., Chen, Y. Q., Chen, Y. P., Zhang, Y. J., Yu, S. Q., Zhou, L. X., Liu, Z. F., Zhang, C. L., and Fu, S. L.: Leaf litter contributes more to soil organic carbon than fine roots in two 10-year-old subtropical plantations, Science of the Total Environment, 704, 8, 10.1016/j.scitotenv.2019.135341, 2020.

Hoyos-Santillan, J., Lomax, B. H., Large, D., Turner, B. L., Boom, A., Lopez, O. R., and Sjogersten, S.: Getting to the root of the problem: litter decomposition and peat formation in lowland Neotropical peatlands, Biogeochemistry, 126, 115-129, 10.1007/s10533-015-0147-7, 2015.

Kayranli, B., Scholz, M., Mustafa, A., and Hedmark, A.: Carbon Storage and Fluxes within Freshwater Wetlands: a Critical Review, Wetlands, 30, 111-124, 10.1007/s13157-009-0003-4, 2010.

Kochy, M., Hiederer, R., and Freibauer, A.: Global distribution of soil organic carbon - Part 1: Masses and frequency distributions of SOC stocks for the tropics, permafrost regions, wetlands, and the world, Soil, 1, 351-365, 10.5194/soil-1-351-2015, 2015.

Qiu, H. S., Ge, T. D., Liu, J. Y., Chen, X. B., Hu, Y. J., Wu, J. S., Su, Y. R., and Kuzyakov, Y.: Effects of biotic and abiotic factors on soil organic matter mineralization: Experiments and structural modeling analysis, Eur. J. Soil Biol., 84, 27-34, 10.1016/j.ejsobi.2017.12.003, 2018.

Yan, J. F., Wang, L., Hu, Y., Tsang, Y. F., Zhang, Y. N., Wu, J. H., Fu, X. H., and Sun, Y.: Plant litter composition selects different soil microbial structures and in turn drives different litter decomposition pattern and soil carbon sequestration capability, Geoderma, 319, 194-203, 10.1016/j.geoderma.2018.01.009, 2018.

Yu, X. F., Ding, S. S., Lin, Q. X., Wang, G. P., Wang, C. L., Zheng, S. J., and Zou, Y. C.: Wetland plant litter decomposition occurring during the freeze season under disparate flooded conditions, Science of the Total Environment, 706, 9, 10.1016/j.scitotenv.2019.136091, 2020.

Zhao, J., Zeng, Z. X., He, X. Y., Chen, H. S., and Wang, K. L.: Effects of monoculture and mixed culture of grass and legume forage species on soil microbial community structure under different levels of nitrogen fertilization, Eur. J. Soil Biol., 68, 61-68, 10.1016/j.ejsobi.2015.03.008, 2015.

Again, we greatly appreciate the editor and reviewers for all the insightful comments. We worked hard to be responsive to them. We sincere thank 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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Factors controlling *Carex brevicuspis* leaf litter decomposition and its contribution to surface soil organic carbon pool at different water levels

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Abstract. Litter decomposition plays a vital role in wetland carbon cycling. However, the contribution of aboveground litter decomposition to the wetland soil organic carbon (SOC) pool has not yet been quantified. Here, we conducted a *Carex brevicuspis* leaf litter input experiment to clarify the intrinsic factors controlling litter decomposition and quantify its contribution to the SOC pool at different water levels. The *Carex* genus is ubiquitous to global freshwater wetlands. We sampled this plant leaf litter at -25, 0, and +25 cm relative to the soil surface over 280 days and analysed leaf litter decomposition and its contribution to the SOC pool. The percentage litter dry weight loss and the instantaneous litter dry weight decomposition rate were the highest at +25 cm water level (61.8%, 0.01307d^{-1}), followed by the 0 cm water level (49.8%, 0.00908 d^{-1}), and the lowest at -25 cm water level (32.4%, 0.00527 d^{-1}). Significant amounts of litter carbon, nitrogen, and phosphorus were released at all three water levels. Litter input significantly increased the soil microbial biomass and fungal density but had nonsignificant impacts on soil bacteria, actinomycetes, and the fungal/bacterial concentrations at all three water levels. Compared with litter removal, litter application increased the SOC by 16.93%, 9.44%, and 2.51% at the +25 cm, 0 cm, and -25 cm water levels, respectively. Hence, higher water levels facilitate the release of organic carbon from leaf litter into the soil via water leaching. In this way, they increase the soil carbon pool. At lower water levels, soil carbon is lost due to the slower litter decomposition rate and active microbial (actinomycete) respiration. Our results revealed that the water level in natural wetlands influenced litter decomposition mainly by leaching and microbial activity, by extension, and affected the wetland surface carbon pool.

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

1 Introduction

Wetlands are important terrestrial carbon pools. Depending on the definition of “wetland”, they contain between 82 and 158 Pg SOC, (Kayranli et al., 2010; Kochy et al., 2015). The surface soil organic carbon (SOC) pool (S-SOCP) and its turnover are sensitive to climate, topography, and hydrological conditions (Wang et al., 2016; Zhang et al., 2017; Pinto et al., 2018).

Leaf litter decomposition is a major biotic carbon input route from vegetation to S-SOCP in wetland ecosystems (Whiting and Chanton, 2001; Moriyama et al., 2013). However, the reported impacts of litter decomposition on the soil carbon pool are highly variable (Bowden et al., 2014; Cao et al., 2020). Litter input destabilised carbon storage by stimulating soil mineralisation and increasing labile soil carbon fractions (microbial biomass carbon [MBC], soil dissolved organic carbon [DOC]), and enzyme activity in the freshwater marshland of Northeast China (Song et al., 2014). It also promoted soil carbon loss via CO_2 emissions and microbial activity in alpine and coastal wetlands (Gao et al., 2016; Liu et al., 2017). In contrast, a study has recently found that litter decomposition stabilised the soil carbon pool after processing by soil microbes in the Jiaozhou Bay wetland (Sun et al., 2019).

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

Peng et al. reported that the organic carbon density in Dongting Lake wetland soil at 1 m depth was 127.3 ± 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). *Carex brevicuspis* is a dominant species in the Dongting Lake wetland and has large carbon reserves (~6.5 × 10^{6} t y^{-1}) (Kang et al., 2009). However, due to the dam construction upstream of Dongting Lake, the water regime varies considerably (early water withdrawal and decline of groundwater in non-flood season) in recent years, leading to a significant carbon loss in this floodplain wetland (Hu et al., 2018; Deng et al., 2018).

Here, we investigated *C. brevicuspis* litter decomposition and its contribution to the SOC pool at three water levels (-25 cm, 0 cm, and +25 cm relative to the soil surface) to find the factors controlling *C. brevicuspis* leaf litter decomposition and quantify the contribution of litter decomposition to the SOC pool. We tested the following hypotheses. Firstly, the water level has a significant effect on litter decomposition. Secondly, the intrinsic factors that control litter decomposition rate at three water levels are different. Thirdly, the contribution of leaf decomposition to S-SOCP is relatively higher at the +25 cm water level.

2 Materials and methods

2.1 Soil core collection and leaf litter preparation

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

2.2 Experimental design

There were three water level treatments (-25 cm, 0 cm, and +25 cm relative to the soil surface) nested by two litter treatments (input vs removal) and three replicates. The experiment was conducted in nine cement ponds (2 m × 2 m × 1 m) at the Dongting Lake Station for Wetland Ecosystem Research. For the -25 cm treatment, the water level was 25 cm below the soil surface. For the 0 cm treatment, the soil was fully wetted with belowground water (the belowground water was extracted from the well in the experiment site by a water pump) but without surface pooling. For the +25 cm treatment, the water level was 25 cm above the soil surface. Water levels were adjusted weekly using belowground water (TOC: 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 was designated the litter removal control (S), the second was distributed on the soil surface with 15 litter bags to observe the effects of leaf litter input on soil carbon pool (L), and the third was distributed on the soil surface with 15 litter bags to monitor the litter decomposition rate and process (D) (Fig. 1). Litter bags were laid flat on the surface of the soil. Each litter bag was not filled, and there are a little overlap between the litter bags where there is no litter. All the litter bags were fixed to the soil surface with bamboo sticks. The experiment started on 20 August 2017 and lasted 280 d. By that time, no further significant change in litter dry weight was observed. Before incubation, three litter and three soil samples (SOC: 63.32 g kg^{-1}) were collected to determine their initial quality. Litter bags 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, 250 d, and 280 d. After collection, the litter samples were separated, cleaned with distilled water, and oven-dried at 60 °C to a constant weight (±0.01 g). All samples were pulverised and passed through a 0.5-mm mesh screen for litter quality analysis. At the end of incubation, the surface soil (0–5 cm, ~600 g FW) was collected to eliminate the influences of root decomposition on the soil organic pool. The soil samples were placed in aseptic sealed plastic bags and transported to the laboratory. The samples were sieved (< 2 mm), thoroughly mixed, and divided into three subsamples. The first subsample (~150 g) was stored at -20 °C and freeze-dried for phospholipid fatty acid (PLFA) analysis. The second one (~150 g) was stored at 4 °C for MBC and DOC measurements. The third subsample (~300 g) was air-dried for physicochemical analysis.

2.3 Litter quality analyses

Litter organic carbon content was analysed by the H_2SO_4-$K_2Cr_2O_7$ heat method. Litter nitrogen was extracted by Kjeldahl digestion and quantified with a flow injection analyser (AA3; Seal Analysisten GmbH, Langenselbold, Germany) (Xie et al., 2017). Litter phosphorus content was quantified by the molybdenum-antimony anti-spectrophotometric method. The lignin content was measured by hydrolysis (72% H_2SO_4) (Graça et al., 2005; Xie et al., 2017).

2.4 Soil quality analyses

2.4.1 Soil chemical analyses

SOC was determined by wet oxidation with KCr_2O_7 + H_2SO_4 and titration with $FeSO_4$ (Xie et al., 2017).

Soil DOC was extracted with K_2SO_4 and measured with a TOC analyser (TOC-VWP; Shimadzu Corp., Kyoto, Japan). MBC was analysed by chloroform fumigation, K_2SO_4 extraction, and TOC analyser (TOC-VWP, Shimadzu Corp., Kyoto, Japan) (Tong et al., 2017).

2.4.2 Soil microbial composition

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

2.5 Data processing

2.5.1 Litter decomposition rate

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

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

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

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

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

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 (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 decomposition rate at the nth sampling.

2.5.2 Relative release index

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

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

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

2.5.3 Contribution of litter-C input to the SOC pool

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

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

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

2.6 Statistical analyses

The percentage of litter dry weight losses and the instantaneous decomposition rates were compared among the three water levels by repeated ANOVA analyses. The water level was the main factor, and time was the repeated factor. The intrinsic litter decomposition rate-limiting factor was analysed by the stepwise regression method in a multiple regression model. The surface soil chemical components and the microbial community structure were compared by two-way ANOVA. Treatment (with or without litter input) and water level were the main factors. The percentage differences in litter dry weight loss, the instantaneous decomposition rates, the soil chemical components, and the microbial community structure were evaluated by LSD at the 0.05 significance level. The data were expressed as means ± standard error. All statistical analyses were performed in SPSS 21 (IBM Corp., Armonk, NY, USA).

3 Results

3.1 litter decomposition process

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

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

3.2 Intrinsic litter decomposition rate-limiting factor

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

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

3.3 Soil surface microbial community structure

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

3.4 Contribution of leaf decomposition to the soil surface carbon pool

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

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

4 Discussion

4.1 Environmental control of litter decomposition

The water level significantly influenced *C. brevicuspis* leaf litter decomposition ($P < 0.001$). The instantaneous decomposition rates (*k*) were the highest for the +25 cm water level treatment, intermediate for the 0 cm water level treatment, and the lowest for the -25 cm water level treatment (Fig. 2b). Hence, the percentage litter dry weight loss and the decomposition rate increased with the water level, which supported our first hypothesis. The wetland water level strongly affects litter leaching and microbial decomposition (Peltoniemi et al., 2012). Related research showed that the wetland water level strongly affects litter leaching and microbial decomposition (Peltoniemi et al., 2012). Molles et al. (1995) also found that compared with the terrestrial environment, in wetland, water promotes litter leaching and microbial metabolism, thereby accelerating litter decomposition. Moreover, water infiltration into litter also increases relative leaching loss (Molles et al., 1995). Here, the high litter decomposition rate measured for the +25 cm water level treatment may be explained primarily by litter leaching. This finding was consistent with results reported for *Carex cinerascens* litter decomposition in Poyang Lake (Zhang et al., 2019) and *Calamagrostis angustifolia* litter decomposition on the Sanjiang Plain (Sun et al., 2012). The high soil total microbial, bacterial and fungal biomass levels at the 0 cm water level could account for the rapid litter decomposition observed there. Certain microorganisms are vital to the decomposition process (Yarwood, 2018). Fungi are primary litter decomposers as they fragment dead plant tissues by breaking down lignin and cellulose. Bacteria are secondary decomposers that utilise the simpler compounds generated by fungal activity (de Boer et al., 2005; Bani et al., 2019). Microbial decomposers generally flourish in humid environments. At the 0 cm water level, microbial activity explains most of the litter decomposition. While at the -25 cm water level, there are comparatively few microbial decomposers, and decomposition is very slow.

4.2 Intrinsic factors controlling litter decomposition

The instantaneous decomposition rate was highest at initial and slowly decreased and stabilised for all three water levels. (Fig. 2b). Water-soluble components and non-lignin carbohydrates are preferentially and quickly decomposed at the onset of decomposition (Davis et al., 2003). Here, a multiple regression model of the instantaneous litter decomposition rate and litter properties showed that the internal limiting factors affecting the rate of *C. brevicuspis* leaf litter decomposition varied with the water level. The lignin concentration determined the litter decomposition rate for the -25 cm water level treatment whilst the lignin/N ratio regulated the litter decomposition rate for the 0 cm and +25 cm water level treatment. This discovery upheld our second hypothesis and was consistent with the findings of Zhang et al. who reported that wetland ecosystems decomposed *Carex cinerascens* lignin much earlier and faster than terrestrial ecosystems (Zhang et al., 2019). Here, we found that the lignin content was the major internal limiting factor of the *C. brevicuspis* leaf litter decomposition rate at -25 cm water level. At the 0 cm and +25 cm water level, N is rapidly lost, and the L/N ratio significantly increases. Thus, L/N is the main internal limiting factor at the 0 cm and +25 cm water levels. A few studies have shown that the lignin content is a key factor limiting terrestrial plant and hygrophyte litter decomposition (Yue et al., 2016; Zhang et al., 2018). Therefore, the amount of carbon that the litter can return to the ecosystem is closely associated with the plant lignin content. The lignin content of *C. brevicuspis* leaf litters is ~10% less than that of other wetland plants such as *Miscanthus sacchariflorus* (~30%) (Xie et al., 2016), *Spartina alterniflora* (~40%) (Yan et al., 2019), and terrestrial plants such as willow (~25%), larch (~38%), and cypress (~28%) (Yue et al., 2016), so the *C. brevicuspis* leaf litter is more easily leached and then contributes more to the SOC pool. Furthermore, in Dongting lake wetland, the *Carex* genus covers a large area (~23,950 hm^2) and generates abundant litter (~36,547 t) (Kang et al., 2009). Thus, *C. brevicuspis* litter may potentially return large amounts of carbon to the soil.

4.3 Contribution of leaf decomposition to the soil surface carbon pool

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

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

5 Conclusion

In this study, we quantified the contribution of leaf litter decomposition on soil surface organic carbon pools (S-SOCPs) under different water level conditions. Appropriate flooding (+25 cm water level treatment in our study) can significantly promote the decomposition of litter and contribute about 13.75% organic carbon to S-SOCPs. Under waterlogging condition (0 cm water level), litter decomposition, which mainly controlled by microbial activity, contributed 4.73% organic carbon to S-SOCP. However, under relative drought conditions (-25 cm water level treatment in our study), litter decomposition only contributes about 2.51% organic carbon to S-SOCP, which is largely ascribed to the slower decomposition rate and soil carbon lost by microbe metabolism (i.e., actinomycetes). We also found that lignin or lignin/N content were intrinsic factors controlling the litter decomposition rate in *Carex brevicuspis*. In Dongting Lake floodplain, the groundwater decline due to climate change and human disturbance would slow down the return rate of organic carbon from leaf litter to the soil, and facilitate the S-SOCP loss.

Data availability

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

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

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

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

Acknowledgement

We are immensely thankful to the teachers at the Dongting Lake Station and Key Laboratory of Agro-ecological Processes in Subtropical Region for their help in soil sample collection and chemical analysis. We would like to thank Editage [www.editage.cn] for English language editing. The data used in this paper are available in the supplementary material.

Financial support

This study was financially supported by the Hunan Innovative Province Construction Projection (Hunan Key Research and Development Project, 2019NK2011), the National Natural Science Foundation of China (41401290), Training Program for Excellent Young Innovators of Changsha (kq1905048), and the Natural Science Foundation of Hunan province (2020JJ4101).

References

Berg, B., and Mcclaugherty, C.: Plant litter: decomposition, humus formation, carbon sequestration, 2003.

Berg, B.: Decomposition patterns for foliar litter - A theory for influencing factors, Soil Biology & Biochemistry, 78, 222-232, 10.1016/j.soilbio.2014.08.005, 2014.

Bossio, D. A., and Scow, K. M.: Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns, Microb. Ecol., 35, 265-278, 10.1007/s002489900082, 1998.

Bowden, R. D., Deem, L., Plante, A. F., Peltre, C., Nadelhoffer, K., and Lajtha, K.: Litter Input Controls on Soil Carbon in a Temperate Deciduous Forest, Soil Science Society of America Journal, 78, S66-S75, 10.2136/sssaj2013.09.0413nafsc, 2014.

Cao, J. B., He, X. X., Chen, Y. Q., Chen, Y. P., Zhang, Y. J., Yu, S. Q., Zhou, L. X., Liu, Z. F., Zhang, C. L., and Fu, S. L.: Leaf litter contributes more to soil organic carbon than fine roots in two 10-year-old subtropical plantations, Science of the Total Environment, 704, 8, 10.1016/j.scitotenv.2019.135341, 2020.

Chen, H. Y., Zou, J. Y., Cui, J., Nie, M., and Fang, C. M.: Wetland drying increases the temperature sensitivity of soil respiration, Soil Biology & Biochemistry, 120, 24-27, 10.1016/j.soilbio.2018.01.035, 2018.

Chen, X.-S., Deng, Z.-M., Xie, Y.-H., Li, F., Hou, Z.-Y., and Wu, C.: Consequences of Repeated Defoliation on Belowground Bud Banks of Carex brevicuspis (Cyperaceae) in the Dongting Lake Wetlands, China, Frontiers in Plant Science, 7, 10.3389/fpls.2016.01119, 2016.

Deng, Z. M., Li, Y. Z., Xie, Y. H., Peng, C. H., Chen, X. S., Li, F., Ren, Y. J., Pan, B. H., and Zhang, C. Y.: Hydrologic and Edaphic Controls on Soil Carbon Emission in Dongting Lake Floodplain, China, J. Geophys. Res.-Biogeosci., 123, 3088-3097, 10.1029/2018jg004515, 2018.

Gao, J. Q., Feng, J., Zhang, X. W., Yu, F. H., Xu, X. L., and Kuzyakov, Y.: Drying-rewetting cycles alter carbon and nitrogen mineralization in litter-amended alpine wetland soil, Catena, 145, 285-290, 10.1016/j.catena.2016.06.026, 2016.

Graça, M. A. S., Bärlocher, F., and Gessner, M. O.: Methods to Study Litter Decomposition, Springer Netherlands, 2005.

Hoyos-Santillan, J., Lomax, B. H., Large, D., Turner, B. L., Boom, A., Lopez, O. R., and Sjogersten, S.: Getting to the root of the problem: litter decomposition and peat formation in lowland Neotropical peatlands, Biogeochemistry, 126, 115-129, 10.1007/s10533-015-0147-7, 2015.

Hu, J. Y., Xie, Y. H., Tang, Y., Li, F., and Zou, Y. A.: Changes of Vegetation Distribution in the East Dongting Lake After the Operation of the Three Gorges Dam, China, Frontiers in Plant Science, 9, 9, 10.3389/fpls.2018.00582, 2018.

Kang, W. X., Tian, H., Jie-Nan, H. E., Hong-Zheng, X. I., Cui, S. S., and Yan-Ping, H. U.: Carbon Storage of the Wetland Vegetation Ecosystem and Its Distribution in Dongting Lake, Journal of Soil & Water Conservation, 2009.

Kayranli, B., Scholz, M., Mustafa, A., and Hedmark, A.: Carbon Storage and Fluxes within Freshwater Wetlands: a Critical Review, Wetlands, 30, 111-124, 10.1007/s13157-009-0003-4, 2010.

Kochy, M., Hiederer, R., and Freibauer, A.: Global distribution of soil organic carbon - Part 1: Masses and frequency distributions of SOC stocks for the tropics, permafrost regions, wetlands, and the world, Soil, 1, 351-365, 10.5194/soil-1-351-2015, 2015.

Liu, S. L., Jiang, Z. J., Deng, Y. Q., Wu, Y. C., Zhao, C. Y., Zhang, J. P., Shen, Y., and Huang, X. P.: Effects of seagrass leaf litter decomposition on sediment organic carbon composition and the key transformation processes, Sci. China-Earth Sci., 60, 2108-2117, 10.1007/s11430-017-9147-4, 2017.

Lv, F., and Wang, X.: Contribution of Litters to Soil Respiration : A Review, Soils, 49, 225-231, 2017.

Moriyama, A., Yonemura, S., Kawashima, S., Du, M. Y., and Tang, Y. H.: Environmental indicators for estimating the potential soil respiration rate in alpine zone, Ecol. Indic., 32, 245-252, 10.1016/j.ecolind.2013.03.032, 2013.

Olson, J. S.: ENERGY-STORAGE AND BALANCE OF PRODUCERS AND DECOMPOSERS IN ECOLOGICAL-SYSTEMS, Ecology, 44, 322-&, 10.2307/1932179, 1963.

Peng, P., Zhang, W., Tong, C., Qiu, S., and Zhang, W.: Soil C, N and P contents and their relationships with soil physical properties in wetlands of Dongting Lake floodplain, Ying yong sheng tai xue bao = The journal of applied ecology, 16, 1872-1878, 2005.

Pinto, O. B., Vourlitis, G. L., Carneiro, E. M. D., Dias, M. D., Hentz, C., and Nogueira, J. D.: Interactions between Vegetation, Hydrology, and Litter Inputs on Decomposition and Soil CO_2 Efflux of Tropical Forests in the Brazilian Pantanal, Forests, 9, 17, 10.3390/f9050281, 2018.

Qiu, H. S., Ge, T. D., Liu, J. Y., Chen, X. B., Hu, Y. J., Wu, J. S., Su, Y. R., and Kuzyakov, Y.: Effects of biotic and abiotic factors on soil organic matter mineralization: Experiments and struc tural modeling analysis, Eur. J. Soil Biol., 84, 27 34, 10.1016/j.ejsobi.2017.12.003, 2018.

Song, Y. Y., Song, C. C., Tao, B. X., Wang, J. Y., Zhu, X. Y., and Wang, X. W.: Short-term responses of soil enzyme activities and carbon mineralization to added nitrogen and litter in a freshwater marsh of Northeast China, Eur. J. Soil Biol., 61, 72-79, 10.1016/j.ejsobi.2014.02.001, 2014.

Sun, X. L., Kong, F. L., Li, Y., Di, L. Y., and Xi, M.: Effects of litter decomposition on contents and three-dimensional fluorescence spectroscopy characteristics of soil labile organic carbon in coastal wetlands of Jiaozhou Bay, China, Ying yong sheng tai xue bao = The journal of applied ecology, 30, 563-572, 10.13287/j.1001-9332.201902.036, 2019.

Tong, C., Cadillo-Quiroz, H., Zeng, Z. H., She, C. X., Yang, P., and Huang, J. F.: Changes of community structure and abundance of methanogens in soils along a freshwater-brackish water gradient in subtropical estuarine marshes, Geoderma, 299, 101-110, 10.1016/j.geoderma.2017.03.026, 2017.

Van de Moortel, A. M. K., Du Laing, G., De Pauw, N., and Tack, F. M. G.: The role of the litter compartment in a constructed floating wetland, Ecol. Eng., 39, 71-80, 10.1016/j.ecoleng.2011.11.003, 2012.

Wang, X. L., Xu, L. G., and Wan, R. R.: Comparison on soil organic carbon within two typical wetland areas along the vegetation gradient of Poyang Lake, China, Hydrol. Res., 47, 261-277, 10.2166/nh.2016.218, 2016.

Whiting, G. J., and Chanton, J. P.: Greenhouse carbon balance of wetlands: methane emission versus carbon sequestration, Tellus Ser. B-Chem. Phys. Meteorol., 53, 521-528, 10.1034/j.1600-0889.2001.530501.x, 2001.

Wilkinson, S. C., Anderson, J. M., Scardelis, S. P., Tisiafouli, M., Taylor, A., and Wolters, V.: PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress, Soil Biology & Biochemistry, 34, 189-200, 2002.

Xie, Y., Xie, Y., Chen, X., Li, F., Hou, Z., and Li, X.: Non-additive effects of water availability and litter quality on decomposition of litter mixtures, Journal of Freshwater Ecology, 31, 153-168, 10.1080/02705060.2015.1079559, 2016a.

Xie, Y. J., Xie, Y. H., Hu, C., Chen, X. S., and Li, F.: Interaction between litter quality and simulated water depths on decomposition of two emergent macrophytes, J. Limnol., 75, 36-43, 10.4081/jlimnol.2015.1119, 2016b.

Xie, Y. J., Xie, Y. H., Xiao, H. Y., Chen, X. S., and Li, F.: Controls on Litter Decomposition of Emergent Macrophyte in Dongting Lake Wetlands, Ecosystems, 20, 1383-1389, 10.1007/s10021-017-0119-y, 2017.

Yan, J. F., Wang, L., Hu, Y., Tsang, Y. F., Zhang, Y. N., Wu, J. H., Fu, X. H., and Sun, Y.: Plant litter composition selects different soil microbial structures and in turn drives different litter decomposition pattern and soil carbon sequestration capability, Geoderma, 319, 194-203, 10.1016/j.geoderma.2018.01.009, 2018.

Yu, X. F., Ding, S. S., Lin, Q. X., Wang, G. P., Wang, C. L., Zheng, S. J., and Zou, Y. C.: Wetland plant litter decomposition occurring during the freeze season under disparate flooded conditions, Science of the Total Environment, 706, 9, 10.1016/j.scitotenv.2019.136091, 2020.

Zhang, L., Zhou, G. S., Ji, Y. H., and Bai, Y. F.: Grassland Carbon Budget and Its Driving Factors of the Subtropical and Tropical Monsoon Region in China During 1961 to 2013, Scientific Reports, 7, 11, 10.1038/s41598-017-15296-7, 2017.

Zhang, Q. J., Zhang, G. S., Yu, X. B., Liu, Y., Xia, S. X., Ya, L., Hu, B. H., and Wan, S. X.: Effect of ground water level on the release of carbon, nitrogen and phosphorus during decomposition of Carex. cinerascens Kukenth in the typical seasonal floodplain in dry season, Journal of Freshwater Ecology, 34, 305-322, 10.1080/02705060.2019.1584128, 2019.

Zhao, J., Zeng, Z. X., He, X. Y., Chen, H. S., and Wang, K. L.: Effects of monoculture and mixed culture of grass and legume forage species on soil microbial community structure under different levels of nitrogen fertilization, Eur. J. Soil Biol., 68, 61-68, 10.1016/j.ejsobi.2015.03.008, 2015.

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

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

where *R* is the litter instantaneous decomposition rate, L is the lignin concentration, CN is the carbon-to-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 the model was refered to the content at each time point.

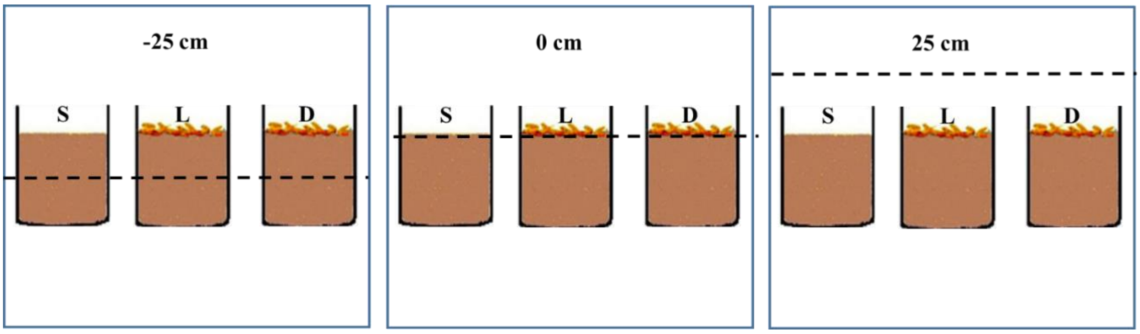

Figure 1: Schematic diagram of the experimental setup. The dotted line represents the water level. L represents litter which was distributed on the soil surface in 15 litter bags to observe the effects of leaf litter input on soil carbon pool; S represents soil which was designated the litter removal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

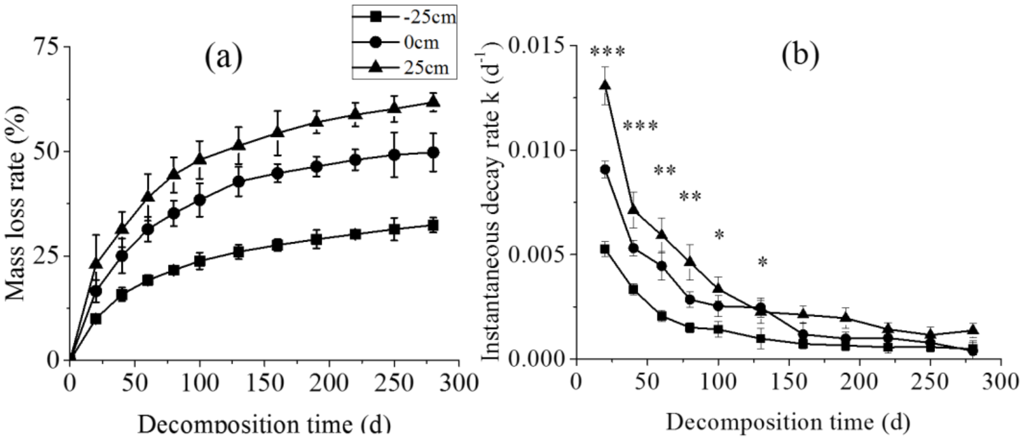

Figure 2: Percentage litter dry weight loss and decomposition rate during *C. brevicuspis* decomposition at three water levels (-25 cm, 0 cm, and +25 cm). *, **, and * represent significant differences of the litter instantaneous decay rate among the three water levels at the 0.05, 0.01, and 0.001 significance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

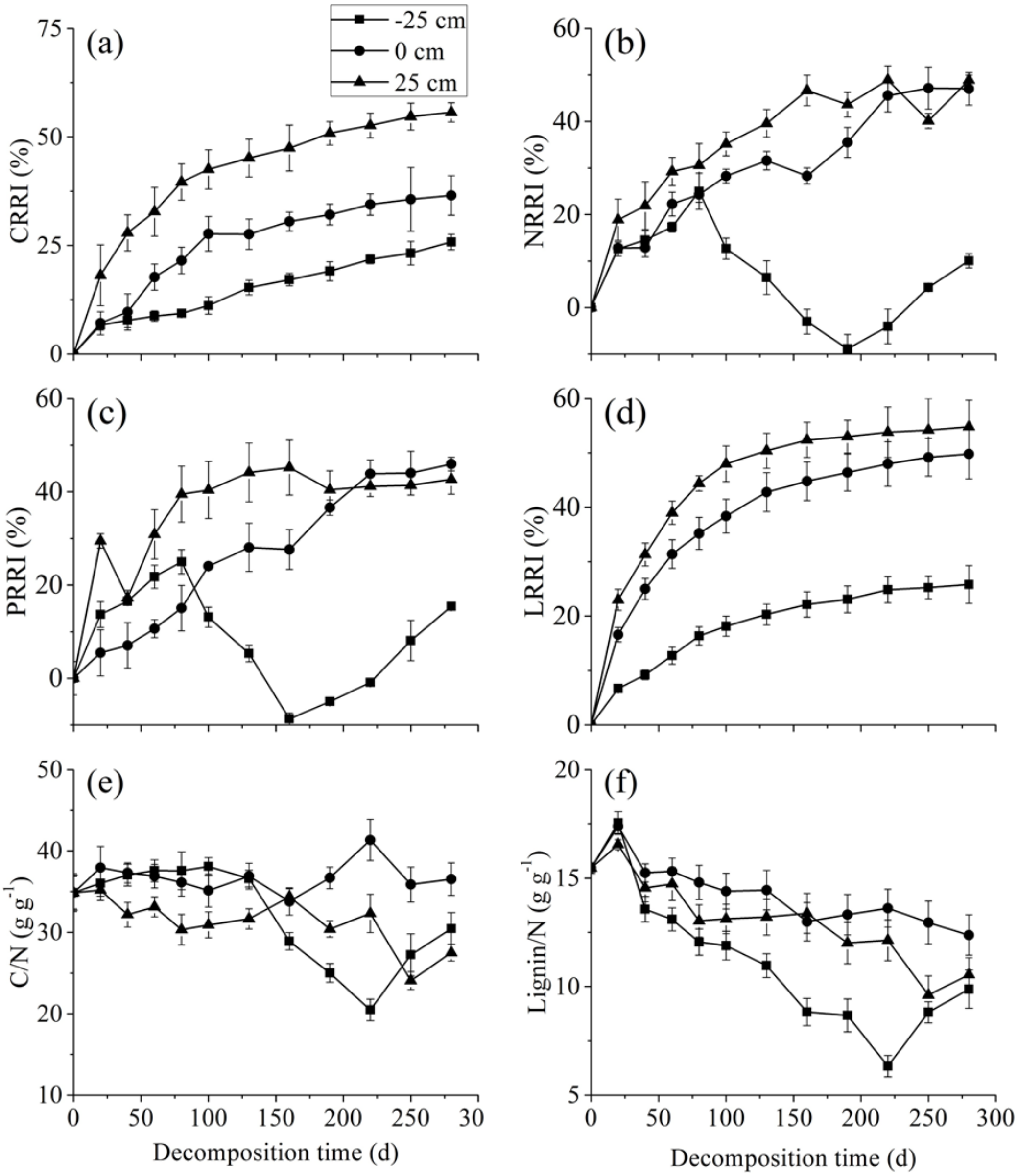

Figure 3: Percentage (mean ± SE) of carbon relative release index (CRRI), nitrogen relative release index (NRRI), phosphorus relative release index (PRRI), lignin relative release index (LRRI), C/N ratio, and lignin/N ratio at three water levels (-25 cm, 0 cm, and +25 cm).

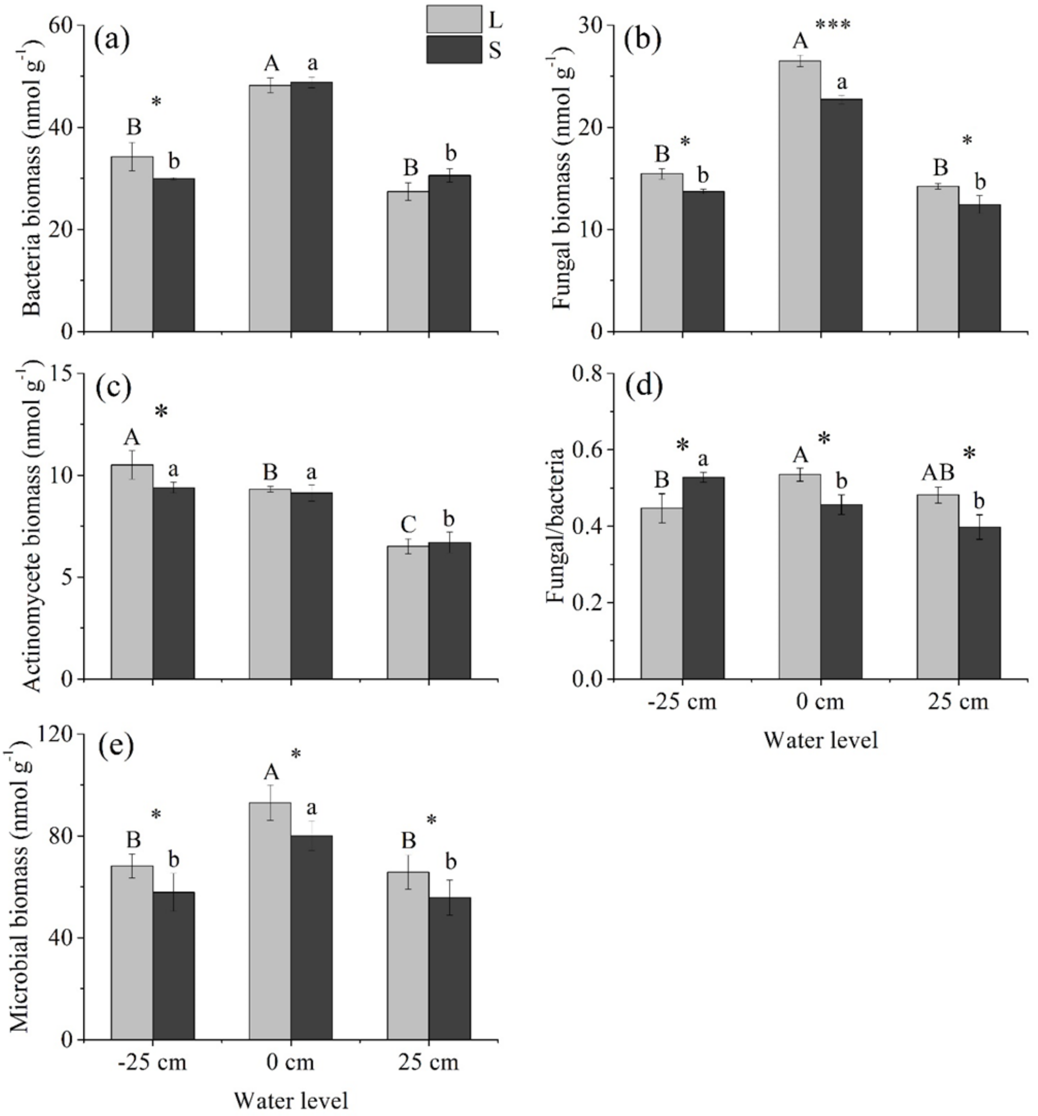

Figure 4: Microbial community structure under litter input and litter removal at three water levels. Different uppercase letters among vertical bars indicate significant differences among the three water levels in the litter input (L) group. Different lowercase letters indicate significant differences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

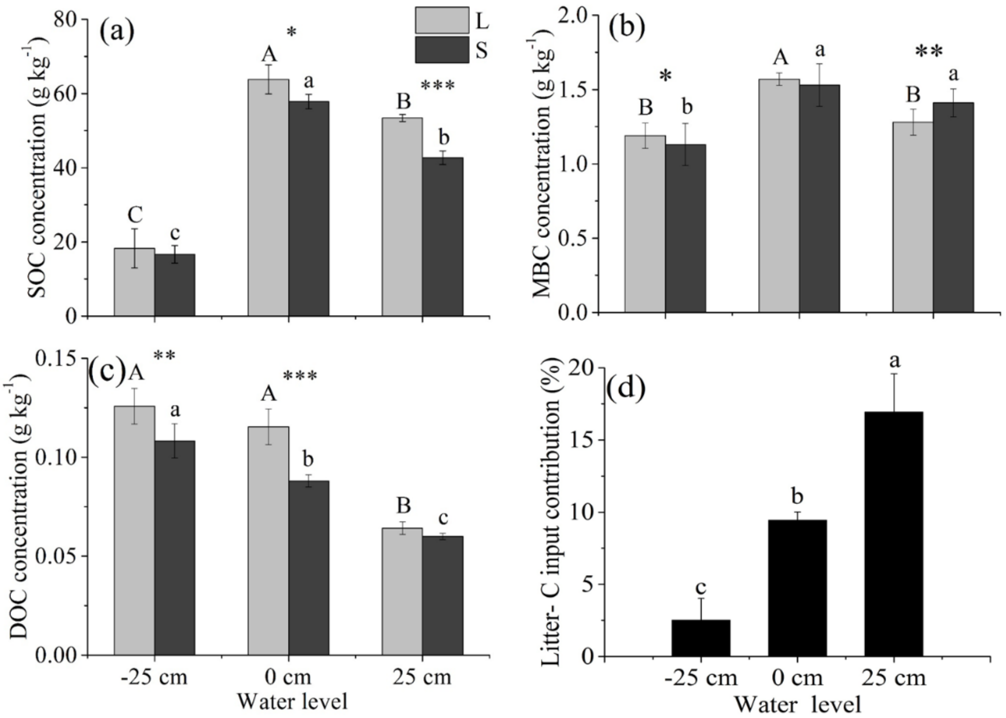

Figure 5: Concentrations of SOC (a), MBC (b), DOC (c) between the litter input (L) and litter removal (S) groups and the litter-C input contribution (d) under three water levels at the end of the experiment. Different uppercase letters among vertical bars indicate significant differences among the three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