Response to Anonymous Referee #1

We gratefully thank the referee for his/her constructive comments and have revised the manuscript accordingly. In our response below, referee comments are shown in italicized *blue*, our response in **black.** Please note that the line and page numbers in our responses refer to the revised version of the manuscript.

General Comments:

This study examines microbial and hydrodynamic mechanisms for soil organic carbon (SOC) in rivers based on a series of incubation experiments. The topic is both timely and important. The authors found that amending incubations with aquatic microbes drove a significant increase in SOC decomposition, whereas shaking the incubations did not have a significant impact on respiration rates.

In general the manuscript is well-written. The discussion section can be improved with a bit more referencing in parts, and also a bit deeper discussion of factors that may lead to the observed results. The introduction could also be improved by adding some nuance to the discussion about SOC. The authors overly simplify that SOC mobilized into rivers is 1) generally old and 2) assume that all soil-derived OC enters rivers in the particulate phase. In terms of data, it would be useful to describe the starting conditions of the incubations. Specifically, the DO and nutrient levels are not mentioned, both of which could significantly influence interpretations of the results. Specific comments are given below.

Reply: We thank the reviewer for the encouraging words and constructive comments, which have been very helpful to improve the quality of the manuscript. We have revised the manuscript according to the comments given above.

i) We agree that the Discussion section could be improved with a deeper discussion and more thorough referencing. In doing so we have improved the Discussion section by further scrutinizing the results and connecting them with the previous studies. For more detailed information, see **Line 225-227, Line 237-245, Page 7; Line 305-309, Line 312-315, Page 9**.

ii) As for the introduction section, we agree that by only focusing on the particulate phase, we oversimplified the SOC mobilization in river systems. We have added discussion on the SOC including both the particulate and the dissolved phase and the variability among rivers (see **Line 27-31, Page 1)**. However, our experiments were specifically designed to examine the fate of POC in riverine environment.

iii) For the starting conditions of the incubations, we have added the initial DO concentration of the river water (varied between 7.58-10.67 mg L^{-1}) in the Results section (see **Line 146, Page 5**). As for the nutrient data, we have compiled relevant data on inorganic nutrients in the section of the Dijle River where the samples were taken (Table 2, data available online from the Flanders Environment Agency)–based on these data we do not expect that DO or inorganic nutrient concentrations would have been a limiting during our incubations (**Table 2)**. This has been discussed in the Discussion section, see **Line 312-315, Page 9.**

Table 2. Nutrient concentrations in the Dijle river water

Year	Number of measurements	NH_4^+ (µmol L^{-1})	NO_3^- (µmol L^{-1})	PO_4^{3-} (µmol L^{-1})
2020	3	24 ± 7	717 ± 55	no data
2016	6	48 ± 30	410 ± 15	6 ± 1
2014	6	26 ± 11	537 ± 94	6 ± 1

Note: values are Mean ± SD.

Line 29: In general, the statement that much of the SOC transported into rivers is old should be made with more nuance. For example, the Mayorga reference shows that DOM in the Amazon and DIC are both predominantly modern in age. The point that tropical, temperate, and high latitude rivers behave differently is important to make.

Likewise, when reading the intro I get the impression that the authors refer to SOC as being in the river in the particulate phase only. Soil organic carbon is also leached into rivers in the dissolved phase, and most studies indicate that DOM is the main substrate fueling respiration.

Reply: Agreed, this part has been improved as outlined above.

i) We have added more discussion on the SOC including both the particulate and the dissolved phase and the variability among rivers, see **Line 27-31, Page 1**.

ii) Data on the ^{14}C age has been added to the description of the soils used in our experiments (**Table 3**).

iii) In addition, we reanalysed soil texture, and the results have been corrected in the revised manuscript (**Table 3**).

Table 3. Characteristics of the two soils used in this study. Texture was determined using laser diffraction (Coulter LS 13 320).

Soil samples	soil texture		OC content (%)	C/N (weight/weight)	$\delta^{13}C$ (‰)	^{14}C age (yr BP)
arable land	Sand %	23	2.40	9.9	–29.4	267 ± 21
	Loam %	69				
	Clay %	8				
forest	Sand %	39	5.20	17.6	–28.6	334 ± 22
	Loam %	56				
	Clay %	5				

Line 90: Please provide more detail. Were soil cores collected or surface soils? How deep were the soils collected from? Do the authors suspect that the SOC used was old or modern per my comment on Line 29?

Reply: Details about soil collection have been added in the Materials & Methods part, and data on the ^{14}C age has been added in the revised version (**Table 3**). In this study, we collected surface soils: for arable land soils, we collected soil from a depth of 0-20 cm depth; for forest soil, we first removed the top litter layer, then collected soil from a depth of 0-20 cm depth. For more detailed information, see **Line 93-94, Page 3**.

Line 93: Why was this concentration chosen? That is a rather high POC concentration. Was the intention to mimic conditions you might find in the rivers being studied?

Reply: Our intention was to use realistic concentrations, but we also had to ensure that effects could be detected. Before starting these incubations, we set up a series of trial experiments with different POC concentrations (2 mg L^{-1}, 4 mg L^{-1}, 7 mg L^{-1}, 12 mg L^{-1}) and measured the DO consumption continuously for 7 days. The results showed that, in order to reliably detect POC mineralisation a minimum POC concentration of 12 mg L^{-1} was more sensitive. We therefore decide to conduct further experiments with a POC concentration of around 10–12 mg L^{-1}.

Furthermore, the concentrations we used are well within the range of conditions we may find in the Dijle river during high water stages. We collected river water samples throughout a year, and POC concentrations ranged from 0.5-18.0 mg L^{-1}, the higher POC concentrations did not occur frequently but were observed several times during or after large rainfall events (when the inputs of soil-derived organic carbon can be assumed to dominate). These concentrations thus fall within the range of those observed in our study area and are not uncommon in various other (turbid) river systems.

Line 105: What was the DO measurement frequency?

Reply: DO was measured using an optical oxygen meter (FireStingO_2) with a measurement frequency of 10 s. This information has been included in the revised manuscript, see **Line 110, Page 4**.

Line 180: Is there any nutrient data available for the experiments and/or the river water that was used? For example, more N was presumably added for the arable soils since the C:N ratio is lower than the forest soils. Nutrient limitation could be one important factor, but I am unable to evaluate this.

Reply: See reply to the previous suggestions: data on inorganic nutrients (NH_4^+, NO_3^-, and phosphate) in the river water are collected by the Flanders Environment Agency, and available from a publicly accessible dataportal. We have summarized these data and added it as **Table 2** in the revised manuscript. Given these concentrations, we assume that nutrient limitations are unlikely. This has been added to the Discussion section, for more detailed information, see **Line 312-315, Page 9.**

Line 193: Use consistent units

Reply: This has been checked and corrected as suggested, see **Line 197, Page 6**.

Line 212: Perhaps you could expand on this discussion a bit more. The Ward 2018 experiment was fundamentally different for many reasons, so it's not surprising that the results did not show the same thing. The biggest factor is that they used raw (unfiltered) water, which means the abundance and composition of POC, DOC, microbes was the same as ambient conditions, whereas this present study used an inoculum and manipulated soil additions. Another difference to mention is that the Ward experiment took place in a tropical river known for its high respiration rates as opposed to this study taking place in a temperate environment. This present study also added ~2-3 times more SOC than is present in the turbid Amazon River and also added beads to the incubation. Do POC concentrations in the Dilje River ever get that high, i.e. were the manipulations realistic? How full of beads were the containers? This particle surface, could allow microbes to be active throughout the entire bottle even when stationary, e.g. the hypothesis by Ward 2018 was that "The relationship between rotational velocity and respiration rates exists because of the importance of interactions between suspended particles, dissolved constituents, and free-living and particle-bound microbes in driving aquatic metabolism." In contrast, this present study hypothesizes that the physical breakdown of SOC particles by disturbance is what should cause higher respiration rates.

Reply: We agree that the differences in experimental conditions might indeed influence POC decomposition rates, and we have brought this aspect into the discussion in the revised version. However, these differences are *as such* not a reason to expect that shaking would not play a role here: the factors described above as a rational for shaking to be important are also present in our experiments. Thus, there must be an additional factor. Our hypothesis is that soil/aggregate strength is also crucial. For more detailed information, see **Line 225-227, Page 7.**

Another important point is context about nutrient conditions. How do we know that nutrients weren't limiting?

One finding in the Ward et al 2019 paper that was cited was that respiration rates varied in response to the proportion of turbid vs clearwater river water added to incubations. There was an optimal mixture that resulted in the highest rates, and in those experiments, there wasn't always a significant difference between stationary and spinning chambers. That could perhaps be something to bring up here, e.g. perhaps you would have seen different results with lower POC concentrations more similar to what you'd observe in situ. And likewise, removing the beads could have made the rotation treatment more important.

Reply: As outlined above, we have discussed the potential effect of the different experimental settings on POC decomposition rates in the revised manuscript. However, we still believe these could not fully explain the minor effect of rotation in our study, which leads us to hypothesized that soil/aggregate strength might play an important role.

As for the nutrient conditions, we have compiled the inorganic nutrient in the section of the Dijle River where the samples were taken as outlined above (**Table 2**). The nutrient status has been discussed in the revised manuscript, see **Line 312-315, Page 9.**

Line 298: How do you know the SOC in this experiment was old?

Reply: As presented in **Table 3**, the radiocarbon age of the surface soil samples used in our incubation were 267-334 yr BP, which were relatively young compared to that found in other aquatic ecosystems (in the range of 1000–5000 y B.P.) (Marwick et al., 2015; Raymond and Bauer, 2001; McCallister and Del Giorgio, 2012). Given the potential variability of the radiocarbon age of SOC among rivers, we have improved our manuscript without highlighting old SOC in the revised manuscript, see **Line 7, Line 32, Page 1; Line 255, Page 7; Line 327, Page 9**.

Reference

Marwick, T. R., Tamooh, F., Teodoru, C. R., Borges, A. V, Darchambeau, F. and Bouillon, S.: The age of river-transported carbon: a global perspective, Global Biogeochem. Cycles, 29, 122–137, doi:10.1002/2014GB004911.Received, 2015.

McCallister, S. L. and Del Giorgio, P. A.: Evidence for the respiration of ancient terrestrial organic C in northern temperate lakes and streams, Proc. Natl. Acad. Sci. U. S. A., 109(42), 16963–16968, doi:10.1073/pnas.1207305109, 2012.

Raymond, P. A. and Bauer, J. E.: Riverine export of aged terrestrial organic matter to the North Atlantic Ocean, Nature, 409(6819), 497–500, doi:10.1038/35054034, 2001.

Lines 225-280: This section could use more references and literature comparisons.

Reply: As outlined in the former reply to the general comments, we have reworked the Discussion section by further exploring the results and connecting them with previous studies. For more detailed information, see **Line 225-227, Line 237-245, Page 7; Line 305-309, Line 312-315, Page 9.**

Table 3: The caption is a little confusing. By "weight" do you mean the mass of C added?

Reply: Yes, by "weight" we mean the mass of C added in the beginning and recovered from the final sample. "weight" has been replaced by "the mass of C" in the caption of Table 4, see **Line 472-473, Page 17**.

Response to Anonymous Referee #2

We thank Referee #2 for his/her constructive comments, which are addressed as explained below. In our response below, referee comments are shown in italicized *blue*, our response in **black**.

General comments

In the present study, the authors aim to understand the mechanisms of the SOC decomposition in river systems. Their study is based on 2 hypotheses (e.g., (i) in the river water, SOC is exposed to an aquatic microbial community which may be able to metabolize SOC much more quickly than the soil microbial community, and (ii) SOC decomposition in rivers is facilitated due to the hydrodynamic disturbance of sediment) for which they will investigate their weight through an incubation experiment. The paper is interesting and the study is well designed. However, before acceptance, I would suggest addressing my comments.

My main concern is related to the discussion. I think the authors could improve the discussion with a deeper exploration of their results. Some parts look weak and not necessarily well supported by the literature (see my specific comments). Then, some other parts are the opposite. I would also suggest adding sub-sections to the Discussion to give a framework to the discussion.

Reply: We thank the reviewer for the constructive comments on the Discussion section, which have been addressed accordingly in the revised manuscript. We have seriously reworked the discussion by further exploring the results and connecting them with previous studies which hopefully further facilitate the reader's understanding. For more detailed information, see **Line 225-227, Line 237-245, Page 7; Line 305-309, Line 312-315, Page 9.**

Specific comments

Line 65-103: I would suggest re-organizing these three sub-sections. For example, in the first sub-section, you present the site but you also include extra information in subsection 3. Then, in reading the sub-section 2, several questions came up in my mind. But I could find the answers only on sub-section 3. In the current form, it is a bit confusing and the readers need to go back and forth to gather all the information.

Reply: Thank you for this constructive comment, we have reorganized the subsections to better describe the approach in the revised version. For more detailed information, see **Line 70, Page 2; Line 89, Line 100-102, Page 3**.

Figures 6 and 7: I would suggest changing the scale of the Y-axis and/or use colour. In particular, Figure 7 a and c or even d are not easily readable.

Reply: Agreed, we have changed the scale of the Y-axis and have also used the colour to better present our results in the revised manuscript (see **Figure 6, Figure 7**).

Line 172-174: Does this "increase" really mean something?

Reply: Yes, this increase was consistent in all replicates, we think that the increase of $\delta^{13}C_{POC}$ values during the first 24–48 hours suggests that during this period an isotopically lighter POC fraction was preferentially mineralised. This resulted in the POC in the aquatic environment becoming enriched in ^{13}C compared to the POC in the original soil sample. While this shift in $\delta^{13}C$ values is relatively small, we do feel it is significant given that it is consistent in both experiments, and larger than the analytical error, However, we are careful in our discussion and as to avoid any overinterpretations on this.

Line 205-207: This needs to be supported by the literature. Please refer to Ward et al., 2019, Wu et al., 2018, etc.

Reply: Agreed, this part have been improved following the suggestions made, see **Line 220, Page 7**.

Line 244: What about the combined effect of AMO and rotation?

Reply: To identify the combined effect of AMO and rotation on the C decomposition rates, two-way ANOVA with the presence of AMO and disturbance conditions as the main factors was employed for the two soil types, separately. Results showed that the presence of AMO and rotation had no significant combined effect on the C decomposition rates for both soil types in our study (*arable land: p=0.430; forest: p=0.683*). This is not surprising, as we proposed in the conceptual model, the mere immersion of soil particles in water might be sufficient to destroy most of the soil particles which were loess derived with the low structure stability. Therefore, further disturbance did not significantly increase the interactions between soil particles and microbial organisms. This combined effect might be more evident for SOC with strong physical protection. This insignificant combined effect has been added in the Result and Discussion section. For more detailed information, see **Line 159-160, Page 5; Line 305-309, Page 9.**

Line 273: After 160h of incubation, can we expect a significant shift of the δ13CPOC? This needs to be discussed.

Reply: If this mineralization does not selectively affect specific fractions of the POC pool, the $\delta^{13}C_{POC}$ values can be expected to remain more or less constant throughout the incubation period. This has already been discussed in the manuscript, see **Line 281-285, Page 8**.

Line 278-290: You never discuss the combined effect of the occurrence of aquatic microbial organisms and physical disturbance. The discussion needs to be improved regarding this point.

Reply: The combined effect of the occurrence of aquatic microbial organisms and physical disturbance has been outlined above. We agreed that the combined effect of the occurrence of AMO and physical disturbance would be an interesting point to be further studied, and we have brought this into discussion in the revised manuscript. For more detailed information, see **Line 305-309, Page 9.**

In addition, the statistical test to identify the combined effect of AMO and physical disturbance on the C decomposition rates has been added in the sub-section "2.3 Statistical analysis", see **Line 140-142, Page 4**.

Line 184-290: I have observed specific behaviour for each type of soils. I think this point needs to be highlighted and also discussed. How do you explain these variations?

Reply: We thank the referee for their constructive comment on comparison of the two soil types. We argue that the different SOC content and the nature of the SOC (derived from agricultural crops versus forest litter) could offer a likely explanation for the observed difference in decomposition behaviour. This has been discussed in the Discussion section to compare the decomposition behaviour, and we have also linked this to the ^{14}C ages data. For more detailed information, see **Line 237-245, Page 7.**