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.**

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 made a point-to-point response to the comments given above.

- We agree that the Discussion section could be improved with a deeper discussion and more thorough referencing. In doing so we will improve the Discussion section by further scrutinizing the results and connecting them with the previous studies.
- As for the introduction section, we agree that by only focusing on the particulate phase, we oversimplified the SOC mobilization in river systems. We will add more discussion on the SOC including both the particulate and the dissolved phase and the variability among rivers in different climate zones. However, we would also like to point out that the paper does indeed focus on the fate of POC in riverine environments.
- For the starting conditions of the incubations, we will add the DO concentrations for both soil types in the Result section. 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 1, data available online from the Flanders Environment Agency), and will discuss the nutrient status in the revised version – based on these data we do not expect that inorganic nutrient concentrations would have been a limiting during our incubations.

Table 1 The inorganic nutrients (NH_4, NO_3, PO_4) concentrations in the water sampling site of the Dijle river

Year	Number of measurements	NH_4 (µmol L^{-1})	NO_3 (µmol L^{-1})	PO_4 (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 will be improved as outlined above. Both soil samples are currently being sent out for ^{14}C dating, these results will be added to the description of the soils used in our experiments, and we will highlight the role of climate and other environmental conditions as controls on (i) the amount and nature of POC in a river and (ii) rates of mineralisation.

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 soils collection will be added in the Materials & Methods part - data on the ^{14}C age will be added in the revised version (see above).

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 leaves layer, then collected soil from a depth of 0-20 cm depth.

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 make sure that effects could be detected. Before starting these incubations, we set up a series of test 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 advisable. Therefore, in order to have a detectable DO consumption from POC, POC concentration was controlled at 10–12 mg L^{-1}.

Furthermore, the concentrations we used mimic 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. 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 will be included in the revised manuscript.

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 will summarize these data in the revised manuscript (Table 1).

Line 193: Use consistent units

Reply: Consistent units will be used in the revised manuscript.

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, we will bring 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 the reasons why shaking can 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.

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: We agree that POC concentrations and the beads additions may play a role when exam the difference between stationary and rotation condition, we will discuss the potential effect of these factors on POC decomposition rates in the revised manuscript. However, as outlined above, we still believe these could not fully explain the minor effect of rotation in our study, which lead 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. We will discuss the nutrient status in the revised manuscript.

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

Reply: See earlier replies: the 14C age of the bulk organic C in our two soils is currently being analysed, we will report these results in the revised version.

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

Reply: As outlined above, we will seriously rework on the Discussion section by further exploring the results and connecting them with the previous studies.

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" will be replaced by "the mass of C" in the revised manuscript.