MS No.: egusphere-2025-4143

Stability of stream biofilm community composition to transient shifts in dissolved organic carbon characteristics

Dear editor and reviewer,

We thank the reviewer for their constructive comments, our manuscript has improved from the feedback provided. Our responses to each comment are provided in purple. We have followed the format 1) reviewer comment, 2) our response and 3) proposed change(s) to the manuscript (given in ‘’) where provided.

Overall assessment

The authors present a very clear and well-structured study that includes an impressive array of streamwater chemistry and biofilm responses across entire storm events. Given the comprehensive characterisation of storm event carbon quantity and quality, and of biofilm community responses, this work makes and excellent contribution to the generally understudied topic of benthic responses to hydrological events. I therefore suggest only a few minor revisions (see below) before acceptance.

We thank the reviewer for their constructive feedback and positive recommendation of our paper for acceptance after minor revisions. We appreciate the inclusion of line numbers as this helped us to quickly address the feedback given.

Please note all referenced line numbers refer to the preprint version of the manuscript.

Detailed comments

109-110: How was storm event duration defined in more detail, i.e., which criteria were used to define the end of events? Note that SE1 would be classified as two storms with some commonly used criteria (e.g., returning to <100% baseflow).

Our criterium for a storm pulse was a rain event with at least 20mm of precipitation over a 24hours period. It is true that our preference would have been for both SE1 and SE2 to be isolated (single) events. In the manuscript we clearly describe the rain events and hydrological responses for both SE1 and SE2 and note that SE1 occurred as a double ‘spate’. The more complex hydrology of SE1 does not detract at all from the main findings of this work that the biofilm communities are extremely resilient to short-term pulse events.

138: Firstly, pH, EC, and DO are physicochemical parameters, not physical. Secondly, could the authors clarify what is meant by triplicate sampling of these parameters? I suspect that what the authors mean is that the water samples were collected in triplicates.

Thank you for the correction of terminology. We have updated physical to physicochemical when we are reporting on pH or EC. On the second point, we took three readings of each measurement while in the field. We have updated line 138 to better reflect this.

Update to section 2.3 line 138:

'On each water sampling occasion, three readings of each physicochemical parameter were taken at each site while in the field'.

140: I can not find any DO results reported, what is the reason for this? Also, if not included in results it can be removed from the methods section.

Thank you for pointing out %DO. We had measured %DO for the associated hydrological study, but it was not used in this study. We have removed %DO from the methods.

Update to section 2.4 line 140:

'The physicochemical water parameters measured at each site were: pH (Metrohm 826 mobile coupled to Metrohm temperature compensated 'Aquatrode' pH electrode, Metrohm AG, Switzerland) and electrical conductivity (EC) (Hach HQ40d coupled with a CDC401 probe for EC; Hach, USA).'

146-147: When were subsampling and filtration carried out? This is important for assessing the quality of the subsequent analysis.

Thank you for this. We collected, transported and filtered our samples within 6 hours of sampling. This has been added to line 146-147.

Update to section 2.4 line 146-147:

'All water subsamples were filtered (0.45 μm cellulose-acetate; Bonnet Equipment) and collected in 30 mL polycarbonate tubes (Sarstedt, Germany) within 6 hours of sampling.'

148-149: I suggest clarifying this as "Subsamples collected for optical characterisation of DOC by spectroscopic analysis", to not confuse these with subsamples used for other DOC characterisation.

Thank you for this clarification, we have adopted this suggestion.

Update to section 2.4 line 148:

'Subsamples collected for optical characterisation of DOC by spectroscopic analysis were wrapped in foil, stored at 4°C and were analysed within 48 hours of collection. All other samples were frozen at -20°C until analysis.'

158: Could the authors provide more details for how planar projections were achieved?

Planar projections were achieved by tracing the scrubbed area using black pen and 1 cm^2 gridded transparency paper to determine the surface area sampled. Traces were scanned and saved as images and area (cm^2) was calculated using ImageJ (version 1.52a, Wayne Rasband, National institutes of Health, USA).

168: Organic nitrogen detection (OND) was not included in the analysis, thus the method used is LC-OCD.

Thank you for this correction, we have adopted it and removed OND from the description.

170: Correct to “HORIBA” in all further mentions.

Thank you for the correction, we have adopted this feedback.

208-212: Given that biofilm biomass and chl-a were expressed per area, was eventual differences in biofilm thickness in situ accounted for?

No, differences in biofilm thickness were not accounted for, the values represent an average per unit area for the sample. The sample collected for biofilm biomass and chl-a measurements was homogenised, weighed and the slurry was subsampled to measure chl-a and biomass. For biomass, we calculated the ratio of dry material to the wet material in the subsample, then scaled this value to the whole sample. The whole sample was divided by the area the biofilm was collected from to get mg/cm^2. Similarly, for chl-a we measured the absorbance value of the subsample, corrected for the optical measurement factors, scaled this to the whole sample and divided by the area the biofilm was sampled from. We assumed that where there is greater mass, the biofilm was thicker. To clarify this, we have updated section 2.6.1.

Update to section 2.6.1 line 211:

‘[…] Both biomass and chl-*a* measurements represent an average per unit area, where higher biomass values are assumed to indicate a thicker and/or denser biofilm. Measurements of […]’

253: Suggest clarification as “Seven missing samples corresponded to an exclusion of 55 datapoints”

Thank you for this clarification, we have adopted this suggestion.

Update to section 2.7.1 line 253:

‘[…] Seven missing water samples corresponded to an exclusion of 55 datapoints from the data set. Missing datapoints were […]’

359: Responses in EC is technically not a salt pulse, it is a pulse of charged ions.

Thank you for this feedback, we agree and have updated and clarified the writing to better reflect this difference.

Update to section 3.2 line 359:

'[…] were characterised by increases in electrical conductivity (pulse of ionic components), bulk DOC concentration (DOC pulse) and higher […]'

546-567: I fully agree that the shorter DOC exposure time, given limited storm durations but also short solute residence times, is a convincing reason for community composition stability. Previous studies that are discussed in this paragraph are appropriate in the way that they investigated effects of DOC concentration and quality on community composition, however, none of these studies have been carried out during storm events. Thus, the authors could consider to also discuss other studies that do include storm event responses, e.g., of whole-stream metabolism: https://doi.org/10.1029/2019WR025025, https://doi.org/10.5194/bg-19-1111-2022, https://doi.org/10.1029/2024JG008308.

We thank the reviewer for these excellent references; they are greatly appreciated. We have added these references to our discussion within Section 4.2 within the revised paragraph 1 (please see responses to Reviewer 1) and to paragraph 2. We have added to the second paragraph section 4.2 from line 563.

Update to section 4.2 line 563:

'[…] Studies investigating the response of whole stream metabolism to changes in discharge (including storm events) found that stream metabolism transiently responded to changes in stream flow (O'Donnell and Hotchkiss 2019; O'Donnell and Hotchkiss 2022; Hallberg et al., 2024). Importantly, O'Donnell and Hotchkiss (2022), found that stream gross primary production transiently decreased following a storm event while ecosystem respiration was more resilient. This could be attributed to differences in biological demand for carbon and energy between autotrophs and heterotrophs at the time of the event (Hallberg et al., 2024). Building on this, if biological demand for carbon or energy is low at the time of a DOC pulse, biofilm communities may need to be exposed the altered DOC composition for longer before showing a response either metabolically or compositionally. Coupled together, the short residency time of water and rapidly changing composition of DOC observed in the present study, may have […]'

595-600: The occurrence of scouring, and potentially also burial, may be more unambiguously interpreted from combined changes in chl-a content and biomass. Chl-a could more directly indicate scouring/deposition, circumventing confounding effects of detrital deposition in biomass changes.

Thank you for this guidance to consider the effects of chl-a and biomass together. We reviewed supplementary data 1 (which contains the biomass and chl-a content data) to look at the changes in both biomass and chl-a between biofilms collected before and after a storm event and re-confirm our findings. There were no clear patterns between which biofilms may have experienced scouring and displayed a shift in community composition. We have updated line 596 to more clearly state the relationship between chl-a and biomass to indicate scouring.

While reviewing, we also noticed and corrected some mistakes in section 3.3.2; chl-*a* content *decreased* at Site A and *increased* at all other sites following SE1. Following SE2, chl-*a* content *decreased* at sites A, B and D and *increased* at Site C. We apologise for this mistake in wording. Additionally, we found we had reported the second lowest average biomass value, 0.29, when it was 0.22. We greatly apologise for this error, and we have thoroughly checked all other biomass and chl-a values reported for accuracy (all others were correct).

Update to section 4.2 line 596:

'It is also possible that at sites C and D the magnitude and/or timing of storm event was enough to shift community composition such as through shearing or scouring (Sousa, 1979). Although, this was difficult to determine in this study as not all biofilms that may have undergone scouring (i.e., displayed an increase in chl-*a* content and decrease in biomass, or a reduction in both) experienced a significant shift in community composition following a storm event (Supplementary Data 1). This could be explained by natural biofilms accumulating variable amounts of externally derived organic (detrital) material within the matrix, that will contribute to differences in biomass weight estimates but are unrelated to biofilm community composition.'

Correction to 3.3.2 line 400-401:

'Average biofilm biomass (normalised to area) ranged between 0.22 (±0.06) to 4.65 (±2.35) mg/cm^2 across all samples (Supplementary Data 1). Average biofilm biomass decreased at sites A and B and increased at sites C and D following SE1 and decreased at all sites following SE2. Average biofilm chl-*a* content (normalised to area) ranged between 0.21 (±0.08) to 6.43 (±2.43) µg/cm^2 (Supplementary Data 1). Biofilm chl-*a* content decreased at Site A and increased at all other sites following SE1, and decreased at sites A, B and D and increased at Site C following SE2.'