

Reviewer 2

1. The authors provide a very useful review of the literature. However, having done so, I am left wondering what we do not understand, and thus why another study is required? I suggest the authors explain the novelty of their work.

Thank you for reviewing our work and your appreciation of the literature review. The development of a robust methodology and protocol for the application and impacts of extracted EPS in flume facilities provides the novelty of our work (this is explicitly mentioned and has been given emphasis on P5, lines 14-17). Indeed, earlier studies have investigated natural diatom-biofilm behaviour (e.g. Gerbersdorf and Wieprecht, 2015) and also work was done on extracted EPS already (e.g. Tolhurst 2002). Our study builds on this work and explicitly relates the sediment stabilising ability of natural diatom-biofilms to that of extracted EPS. A unique aspect of our study is that we use the same sediment for the natural diatom-biofilms and extracted EPS tests so we can compare the results directly (this is explicitly mentioned on P6, line 1 and P6, lines 11-13). In addition, we expand the existing knowledge on the application and impacts of extracted EPS by testing four different EPS for a range of environmental conditions. Such knowledge is currently lacking and has led to costly and time-consuming trial-and-error approaches in a variety of different modelling facilities. Our findings present a systematic methodology and protocol for a range of commercially available EPS and are therefore expected to inform future studies seeking to introduce biological cohesion in a rapid and controlled manner (the importance for preparation time and experimental duration are emphasised on P5 line 6-9).

2. I was disappointed the Introduction and Methods section did not make it clear what type of freshwater system is investigated. Which system are the scaled flume experiments trying to represent? I think this is especially important because we are told that one of the motivations for this study is that there has been a lot of work on biostabilisation in coastal settings but not in freshwater systems, and yet the studies biofilms are common in coastal zones. Furthermore, how do the studied conditions (e.g. slopes, depth:width, relative roughness, grain size, Reynolds number) pertain to those found in the natural system and match the conditions commonly found where these biofilms grow? Likewise, the authors should comment on how closely the Cohesive Strength Meter systems mimic erosion processes in the natural system? Furthermore little detail is provided on the setup of the small-scale synthetic EPS experiments. For example, I have read over the paper several times and I still cannot establish whether these tests were performed in a flume.

We use brackish water in our experiment. This experimental condition is explicitly mentioned on P7, lines 15-16. The brackish water setting is representative of estuarine, mangrove and deltaic settings within the fluvial-to-marine transition zone. In our literature review, we indeed mention that the role of EPS in freshwater systems is not as well understood as in marine systems (P2, lines 16-20) but our experiment was not aimed at gaining a better understanding of the EPS behaviour in freshwater conditions.

The experimental design conditions are in approximate agreement with natural reference systems. Please note that the experiment was not setup to replicate a specific natural system but rather a collection of shallow brackish environments. The channels had no initial gradient (but the flow may have

created a self-formed gradient in the substrate during the experiment), a width-to-depth ratio of 5, 110 microns sand and a Reynolds number indicating turbulent flow ($Re = 5000 - 10000$). We added this information to the Methods section 2.1.1. But most importantly, these experimental conditions resulted in a thriving biofilm with a species composition that was consistent with species commonly seen in brackish coastal environments (P13, lines 6-15).

The Cohesive Strength Meter employs a vertical jet to measure the erosion shear stress of sediments. This approach differs from natural erosion processes, which predominantly generate a horizontal shear. Based on a series of systematic tests, Tolhurst et al (1999) provides a calibration of the vertical jet to an equivalent critical erosion shear stress. A full discussion on the strengths and weaknesses of the CSM erosion device as well as the development history and relation to other erosion devices is provided in Tolhurst et al (1999) and we refer the reviewer to this document for full details. In our study, we applied the calibration of the vertical jet to an equivalent critical erosion shear stress, and we would also like to stress that the CSM provides one of the few erosion devices allowing workers to make quantitative and repeat measurements of sediment stability.

The small-scale tests with extracted EPS are performed in petri dishes. This is for example explicitly written on P11, line 11-12 ('The sand-EPS mixture was then poured into plastic petri dishes') and lines 12-13 ('therefore care was taken to create a level surface by tapping the side of the petri dishes before testing'). We also refer to the protocol used in Tolhurst et al (2002) and mention that we follow a similar protocol. To make it more explicit that these small-scale tests were performed in petri dishes, we added this information to section heading 3.1 (Petri dish sediment sample tests with extracted EPS) as well as in referring to the protocol used in Tolhurst et al (2002) on P11, lines 5-6.

3. authors state that synthetic EPS is able "to replicate the sediment stabilising capacity of natural biofilms". However the authors have found that three times more synthetic EPS concentration is needed to replicate the same stabilising effect of natural biofilms, suggesting the capacity is much higher for natural biofilms.

Our findings indeed indicate that extracted EPS can replicate the sediment stabilising capacity of natural biofilms as seen from the biostabilisation index (Table 2). In contrast to the reviewer's suggestion, we do not think that the natural biofilms have a higher stabilizing capacity than observed in our study. The biostabilisation index values are consistent with earlier studies on the sediment stabilising capacity of natural biofilms (Paterson 1989; Dade et al. 1990; Amos et al. 1998; Tolhurst et al. 1999; Tolhurst et al. 2003; Friend et al. 2003; Friend, Collins, and Holligan 2003; Droppo et al. 2007; Righetti and Lucarelli 2007; Vignaga, Haynes, and Sloan 2012; Graba et al. 2013; Thom et al. 2015). It is therefore unlikely that the capacity is much higher for natural biofilms, although the observations in our study indicate a substantial internal variation (Figure 2). Rather, the explanation for the different EPS concentration in the extracted EPS tests and natural biofilm experiment must be sought in the determination of the EPS concentration in the natural biofilm experiment. We provide two explanations for the lower EPS concentrations in the biofilm experiment (P27, lines 6 to P28, line 7) and both may explain the difference with the applied extracted EPS concentrations in the small-scale petri dish tests.

4. The calibration curve in equation 1 is important for gaining an accurate estimate of the critical shear stress. To allow readers to have confidence in their estimates, the authors should present a graph showing how this curve has been derived, and the predictive performance of this curve. Small deviations from the curve are likely to produce larger discrepancies in critical shear stress estimates due to the non-linear relationship between critical shear stress and the applied jet force. For example, Figure 4 has error bars to represent the range in estimates from repeats, but hypothetically speaking, how much large would the error bar be if the uncertainty in the estimates themselves was incorporated?

We refer to Tolhurst et al (2002) for details on the how the calibration curve is derived. This article provides a detailed explanation of the performed tests and the quality of the calibration curve. As shown in Tolhurst et al (2002), the uncertainty in the calibration curve is typically in the order of 0.1-0.2 N/m², which suggests that the error bars would be 0.4 N/m² when, hypothetically, taking this effect into account.

Minor amendments:

P4, lines 14-21: There appears to be mismatch between this paragraph and the approach/results. If the prediction of the potential impacts of climate change on aquatic environments and the application of bioengineering adaptation strategies is important, how does this paper address these needs?

The sentences on P4 (lines 18-21) and P5 (lines 1-9) are included to signal the need for including biological processes in sediment transport predictions. The understanding of these bio-physical relations is currently limited and the relationships may also be different under different climatic conditions. We consider flume experiments a primary tool of researchers to address these bio-physical relationships but the developments are hampered because experiments including real biota are time-consuming and costly, with also some questions raised about the degree of natural behaviour of biota in flume facilities.

Our paper provides the first step to overcome the aforementioned issues by the development of a robust methodology and protocol for the application and resultant impacts of extracted EPS to introduce biological cohesion in a rapid and controlled manner. So although the current paper does not directly address the potential impacts of climate change on aquatic environments, the work described provides an important step in facilitating future work that will do so. We believe that providing this context is helpful to the reader and have checked the manuscript to make sure that the implications of our work are well represented, also with reference to studying the impacts of climate change.

P9, line 16: What is routine S7?

Routine S7 is one of the CSM test routines. To further clarify this, we now explicitly link the use of S7 in the manuscript to the word 'CSM'. See P10, lines 7-12.

Inconsistencies in the use of et al and author names in citations should be corrected.

Done.