Reviewer #1

GENERAL COMMENT: I really enjoyed reading this paper. I think this research is very novel and of high impact. However, authors should revise carefully the results and put more emphasis on the fatty-acids results (table ST1). If they really want to assess the difference between Mud and Sand, then some statistical analyses comparing these two groups should be more evident. I hope that my specific comments (see below) help to resolve these more general issues.
Thank you for taking the time to review our paper. We appreciate your positive feedback and are glad to hear that you found our research novel and of high impact. In light of your comments, we have carefully revised the ms., and tried to give more prominence to the fatty-acids results, particularly highlighting the comparison between the Mud and Sand groups. We have included additional data (chlorophyll a, and fatty acid data) as well as additional statistical analyses to strengthen the assessment of the differences between the two sites. However, the fatty acid table (ST1) is the result of a literature review, and we believe it is more appropriate to present it as supplementary material.

Introduction:

I miss information about fatty acids. Specifically,

1) line 97, which are the fatty acid biomarkers?
We explained the methodology and added some examples.

2) lines 103-105, which ratios to which bacteria or algal groups?
We added some examples.

3) lines 112-115: authors should specify which are the fatty acid biomarkers of diatoms and bacteria
We previously mentioned a few examples of fatty acid (FA) biomarkers and their ratios. However, relying on a single FA as an indicator can be risky and speculative. Therefore, we conducted a thorough literature review to identify the significant, essential, and minor FAs associated with each microorganism. The findings of this review are presented in Table ST1. We believe that discussing specific FAs in the introduction section might be premature, as it could direct readers towards specific FAs without sufficient context. Instead, we propose expanding on the conclusion and linking it to the FAs in the subsequent discussion section.

Materials and methods:

Lines 137-147, please specify the references for the colloidal EPS extraction and for the bound EPS extraction.
References were added

Results and discussion:

Lines 264-267, what about C, N and P from other origins different than bacteria and algae but attached to the EPS (e.g. detritus)? Could they modify the isotopic fractionation?
Our stable isotope analysis focused solely on carbon (C) and nitrogen (N) isotopes, without considering phosphorus (P). However, it is important to acknowledge that C and N from alternative sources can potentially alter the isotopic ratios. This is primarily influenced by the origin of the organic matter (OM) rather than isotopic fractionation. For example, if the majority of OM originates from phytoplankton and exhibits similar C and N isotopic ratios to microphytobenthos, it becomes challenging to determine the EPS origin (whether it originated from phytoplankton or microphytobenthos). These two sources typically have distinct C and N isotopic ratios.
Additionally, the degradation of OM can also modify the original C and N isotopic ratios, making it difficult to trace the origin of the OM. These factors introduce potential biases in the interpretation of results, which is why we approach our conclusions with caution.

Section Elemental EPS compositions:

For me it is not clear the comparison between mud and sand. This section puts a lot of emphasis between Ms1 and Ms2 but what about mud and sand? From Fig.2 b, we can see that the signature 13C is useful to differentiate between bound and colloidal EPS but not between mud and sand environments… variability among sites Ms1, Ms2, Ms3, Ms4, Ss1, Ss2 and Ss3 is higher than differences between the Ms and Ss as grouped factor.

Statistics of Fig.2 are detailed in the GitHub repository https://github.com/Hubas-prog/EPS_FA_CSIA. *In accordance to Fig.2, we preformed Van der Waerden tests across 14 levels that crossed the EPS types and sampling occasions (see lines 127-151 in the gitHub repository: https://github.com/Hubas-prog/EPS_FA_CSIA/blob/main/Identification_EPS_CSIA.R*

By conducting the analysis considering four levels instead of fourteen (i.e., EPS type and sediment type: BoundMud, BoundSand, ColloidalMud, and ColloidalSand), we observed significant differences between these levels. The specific details of these differences are provided below.

C-content

```
$groups
                   score groups
Bound Mud       0.5211049      a
Bound Sand      0.2628307     ab
Colloidal Mud  -0.1865894      b
Colloidal Sand -1.2904864      c
```

N-content

```
$groups
                   score groups
Bound Mud       0.6698236      a
Bound Sand      0.1230631      b
Colloidal Mud  -0.2912639      b
Colloidal Sand -1.2963836      c
```

d13C

```
$groups
                   score groups
Colloidal Mud   0.8789693      a
Colloidal Sand  0.8717543      a
Bound Mud      -0.3631996      b
Bound Sand     -0.8057199      c
```

d15N

```
$groups
                   score groups
Colloidal Mud   1.04658042      a
Bound Mud       0.02189593      b
Colloidal Sand -0.29071285     bc
Bound Sand     -0.72311433      c
```

These stats were added to the manuscript (table 2 of V2.0) and to the R script in our GitHub repository (L153-176)

Lines 304-306, have you analysed the biological composition of your sites? Your both sites (muddy and sand) are diatom-dominated, right? So then you should not expect differences due to bacterial-dominated sites…

This study is part of a broader scientific project where we have measured numerous parameters. In addition to the measurements discussed in this manuscript, we also estimated the microbial community diversity using metabarcoding techniques and determined the taxonomic diversity of diatoms during each sampling event. The study sites are primarily dominated by diatoms, although the presence of cyanobacteria is also significant. Detailed counts, including within low tide variability, will be included in a future paper, which will incorporate metabarcoding and environmental data. We can however provide a qualitative assessment in the current manuscript. For taxonomic identification purpose, the microphytobenthos was sampled using the lens tissues method that captures epipelic (motile) diatoms. The muddy site is predominantly dominated by epipelic diatoms and euglenophytes. The sandy site's lens tissues contained fewer diatoms, as expected, possibly due to the prevalence of non-motile epipsammic diatoms. The absence of epipelic diatoms in the lens tissues suggests that they are not as abundant at the sandy site. We introduced this information as unpubl. obs. In addition to the citation of Meleder et al. 2007 (L.465 + L480-491 of V2.0) to reinforce our findings.

Section EPS isotopic compositions:

Lines 330-333: could differences in 13C between bound and colloidal EPS be also due to deposition/adsorption of organic matter in the EPS?

Deposition of phytoplankton-derived organic matter (marine snow) and local sediment enrichment through macrofauna activities (such as mucus trails) could influence d13C values. But, typically, phytoplankton tends to exhibit depleted δ13C values compared to microphytobenthos, and the values obtained in this study align well with the known δ13C values of microphytobenthos. As for the influence of macrofauna, it is expected to increase variability, potentially leading to outlier values in a few replicates.

Section Carbon isotope ratio of fatty acid classes:

Lines 340-350: if you are aiming to compare muddy sites from sandy sites, why don't you test if there are significant differences between muddy and sandy sites per each fatty acid group separately? (i.e. compare BFA in mud vs. BFA in sand). This would allow the reader to understand better if there are differences between sites.
The main objective of this paper is to identify the primary producers of extracellular polymeric substances (EPS) by comparing the isotopic composition of fatty acids and EPS. In order to achieve this goal, we decided it would be more informative to directly compare these variables separately for each site. This approach allowed us to focus on the δ13C values of different classes of fatty acids and the δ13C values of EPS fractions. We observed distinct patterns in each site, which are further highlighted throughout the manuscript, particularly in Figure 4.
In order to better compare the two sites, we also introduced additional data as suggested by the reviewer. We introduced the chlorophyll a concentration and the relative proportion of fatty acids classes and discussed the main differences between the sites (L290-318 of V2.0). We also provided additional univariate statistics. The new data, allow to better compare muddy sites from sandy sites.

Section Biomarkers revealed contrasting EPS producers between sites:

Explain the link between fatty acids and bacteria, and between fatty acids and diatoms. This section is very interesting but the core of the article is explained in the supplementary information… I would suggest to include a detailed explanation about fatty acids and EPS producers, otherwise a lot of information is missing.
This is because a portion of the rationale was previously presented in the discussion section (between lines 262 and 279 of V1.0), where the concept of isotopic fractionation was discussed. To address this concern, we have decided to move that particular section to the section titled "Section Biomarkers revealed contrasting EPS producers between sites." This modification enhances the clarity of the text and ensures that the rationale is presented in a more organized and coherent manner.

Section Epipelic and epipsammic diatoms contributed differently to the EPS chemistry:

Have you characterized them? Somewhere in the paper you should include the characterization of these biofilms (at least density of bacteria and chl-a) to reinforce your idea that they are different.
As mentioned earlier, the study sites we sampled have been previously analyzed in terms of taxonomic composition, specifically regarding the presence of epipsammic and epipelic communities. We also conducted additional measurements to validate the previous conclusions. According to reviewer suggestion, we included a new figure in the supp. Material (Fig. SF1 of V2.0) that shows a comparison of biomass indicators and general composition of the microphytobenthos (i.e. 16:0/16:1w7 ratio, chlorophyll a concentration and fatty acid classes proportion) between the two study sites. We thanks the reviewer for the suggestion. We have made a deliberate decision not to include detailed taxonomic analyses in this manuscript in order to maintain clarity and readability. Instead, we plan to communicate these findings in a separate paper dedicated to taxonomic analysis.

Lines 405 – 416: what about the other fatty acids that show an alignment between their 13C and bound and colloidal EPS 13C in mud and sand (fig. 4)? E.g.:

15:0anteiso, 15:0iso and 24:0 in sand EPS bound

17:0iso in Mud EPS colloidal
24:0 is discussed separately in the discussion (L363 and 380 of V1.0) because of extreme and unusually negative d13C values (−66.89 ± 35.84‰ and −59.24 ± 71.82‰) indicative of a methane-oxidizers metabolism. It also sometimes showed a plurimodal distribution which indicate that 24:0 had likely varied bacterial origins.
Branched fatty acids (15:0anteiso, 15:0iso, 17:0iso) are characteristic of bacteria (as stated L469 & 487 of V1.0 and in table ST1) thus their case is duly debated and the role of bacteria in EPS production is also outlined (L470-471, L497-498, V1.0).

17:0, 18:1n-9 in Mud EPS bound
The fatty acid 17:0 is a saturated fatty acid that is challenging to associate with a specific microorganism type. Additionally, in our study, 18:1n-9 coelutes with 18:3n-3, making it difficult to distinguish between cyanobacteria and chlorophyta. Therefore, we exercise caution in interpreting the contribution of these two fatty acids, and we consistently present the contribution of cyanobacteria and chlorophyta together. However, based on the metabarcoding data, we can likely attribute the signal to cyanobacteria in this particular case.

Lines 446-448: in several times in the discussion the author points towards the difference in composition between epipsammic (i.e. diatoms, cyanobacteria, green algae and bacteria) and epipelic biofilms. In this line, it could be very useful if the authors can provide some results (even if they are semi-quantitative) regarding these different biological groups between mud and sand study sites.
As mentioned earlier, the taxonomic composition of the study sites we sampled has been extensively studied, specifically with regards to the presence of epipsammic and epipelic communities. We further conducted additional measurements to validate these previous conclusions. We have a high level of confidence in the epipsammic and epipelic nature of our biofilms. However, we are unsure about the best approach to present the results in a simplified manner that would effectively support and reinforce our conclusions. We already referred to previously published works, and introduced that this was also a personal unpublished observation of the present study but we are open to suggestions on how to effectively strengthen our findings and make them easily understandable to readers.

ABSTRACT

Line 9: the authors have not analysed EPS degradation, so this concept should not be here.
removed

Line 10: “very different communities in muddy and sandy sediments”. The authors have not shown the communities in muddy and sandy sediments, so there are not results to know if the communities are really different. What the author could say is that “are supported by different fatty acid composition suggesting different communities”.
corrected

Line 11: “EPS sources are more diverse in the sand”. I do not agree. What I see in the results (table ST1) is that EPS colloidal sources are more diverse in the sand and that EPS bound sources are more diverse in the mud.
The carbon and nitrogen isotopic ratios of both EPS fractions, shows that the standard deviation is consistently higher in the sandy sediments. This suggests that the origin of EPS can arise from diatoms, bacteria, or potentially different species within the same microbial phylum. The larger standard deviation truly reflects the variability in the sources of EPS, even when the diverse EPS producers belong to the same microbial phylum. This was brought at L316-318 of V1.0.

Lines 12-15: I do not agree with the description of these results, they are not in agreement with table ST1. Revise please.
This is true, we modified the text to better describe the results of the study

Reviewer #2 :

Title: Identification of microbial exopolymer producers in sandy and muddy intertidal sediments by compound-specific isotope analysis
Author: Cédric Hubas, Julie Gaubert-Boussarie, An-Sofie D'Hondt, Bruno Jesus, Dominique Lamy, Vona Meleder, Antoine Prins, Philippe Rosa, Willem Stock, and Koen Sabbe

General Comments:
The work is well done, and although a good deal of literature is available on the EPS in intertidal, investigation of precise composition is limited and much needed. That said, the work is of good quality, and the paper is well-presented, so I only have minor comments.

Specific comment
Line 36: What are the "improvement of the engineering effects."
The concept of cooperating ecosystem engineer is presented in Passarelli et al 2014. The term describe a situation where two species interact in a way which enhances habitat suitability as a result of a combined engineering effect.

Line 38: What are the classifications, and why are they important?
There is unfortunately no clear classification of EPS type in the literature. As stated In the ms. (L41-66 of V1.0) they are commonly divided in 3 categories but generally named after the extraction procedure. A classification would be interesting to identify potential overlap between EPS types that presently have different names but may be similar in terms of structural and/or chemical diversity.

Line 41: Remove "As a general rule".
removed

Line 41-45: This information is unnecessary I would have thought.
We modified this section to make it more readable

Line 137: Please add appropriate references used in this section.
This was a mistake, references were added

Line 139: How many reps were used?
corrected

Fig: 1: What does "s" in Ss and Ms stand for?
In this context, the lowercase "s" represents the sampling occasions, specifically denoted as "s1" to "s4" in the figure caption. This was better explained in the new version.

Line 232: Please add appropriate references used in this section.
Reference added

Line 285: Ms 1 indicates the sampling site of time/date of sampling. Perhaps you could add a letter "e or r" to show ebbing or rising.
Initially, we considered using lowercase "e" and "r" to denote ebb and rising tides, respectively. However, after careful consideration, we determined that it would not be worthwhile to include this factor in our analysis. The primary reason behind this decision is the limited number of replicates available for testing (i.e., insufficient samples for each phase of the tide at each sampling occasion). As a result, we opted not to pursue the original idea and instead focused our discussion on the sampling occasions themselves. While we briefly mention the difference between the two tides, it is important to note that no significant conclusions were drawn from this observation.

Line 296: Any reference to support this statement?
Apart from Hanlon et al. We did not find any other references. We have drawn this hypothesis (as outlined L292 of V1.0) by analogy but we cannot cite any other work mainly because it is surprisingly the first time that C and N content of EPS are measured.

Line 330-339: In Ms1 and 2, as there were differences in the sampling time, would this matter for all sampling dates to be grouped together?
This question was also raised by reviewer #1. By conducting the analysis considering four levels instead of fourteen (i.e., EPS type and sediment type: BoundMud, BoundSand, ColloidalMud, and ColloidalSand), we observed significant differences between these levels. The results are now available in the ms., (see reveiwer #1) and the stats were also added to the R script in our GitHub repository.

Line 420: Could light availability (sampling time) influence the EPS contribution?
Yes! light availability could influence EPS contribution potentially trough secretion of bound EPS for motility purpose.