

**We thank the reviewer for their valuable feedback and constructive criticism. We have addressed each comment individually (in bold).**

Mundanett et al. present improved methods to detect glycans in marine sediments, and demonstrate that fucoidan, a long-chain sulfated polysaccharide typically associated with brown algae, persists in sediment on at least Holocene time scales in a core collected off the coast of Antarctica. The topic is well within the scope of Biogeosciences, and the manuscript is generally clear and presents interesting results. I think some aspects of the manuscript organization could be improved, the motivation and implications of the study could be clarified, and some aspects of the results could be placed in a bit more context. There were also several minor grammatical errors, some of which I detailed below.

Specific comments:

Line 19: I would briefly define fucoidans here.

**In accordance with a comment from reviewer #1, we have included a definition at multiple instances in the manuscript. We have added the following definition to the abstract: “We operationally defined fucoidans as the glycans recovered using anion-exchange chromatography, which contain deoxyhexoses, and are reactive to fucoidan-specific monoclonal antibody BAM1.”**

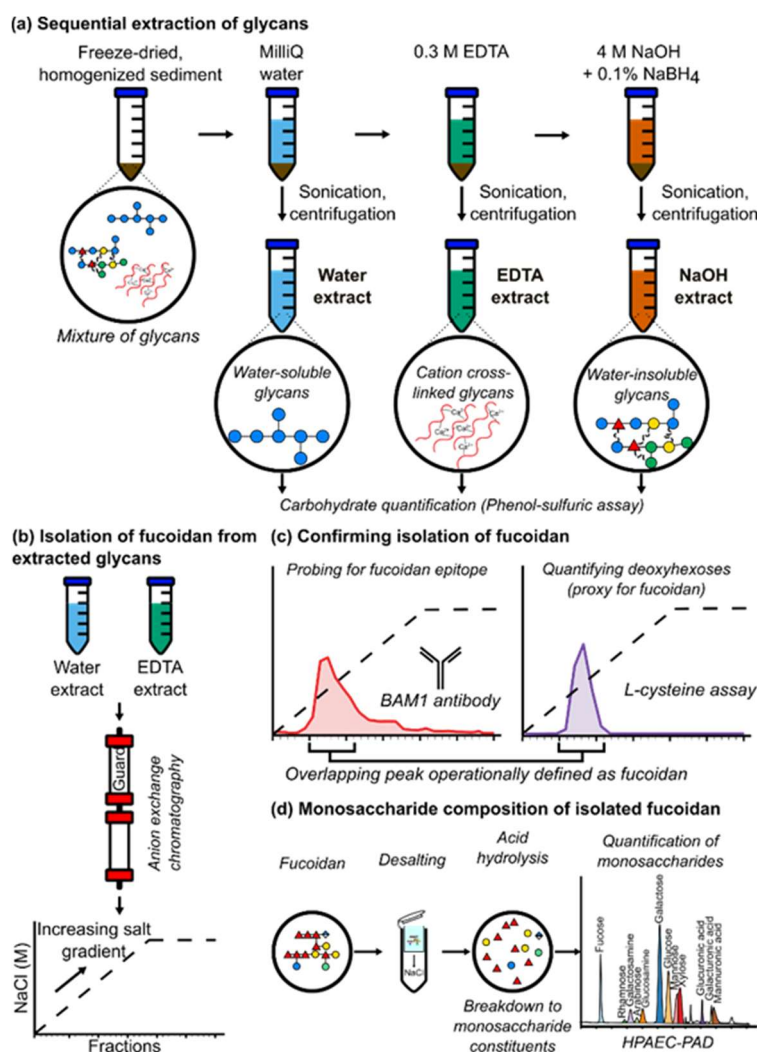
Lines 158-166, lines 179-188: A lot of this information seems out of place for the results section. I think it might fit better in the methods, and you can just report the results here. There are other isolated sentences in the results that are describing/justifying methods. I think in general these are not needed in the results section. Reviewer 1’s suggestion to add a schematic of the analytical workflow to the methods section is a good one, and would help readers be able to place your results into the context of the work that was done, without writing out so many methods in the results section.

**We have moved statements about the justification and description to a new overview section at the end of the Materials and Methods as follows:**

#### **“2.10 Overview of the analytical workflow**

**Glycans were sequentially extracted from freeze-dried and homogenized sediment samples. The extraction method was previously found suitable to extract different algae- and plant-derived types of complex glycans from marine sediments (Vidal-Melgosa et al., 2022, Hellige et al., 2026). MilliQ water extracts water-soluble glycans like laminarin. EDTA chelates cations cross-linked to anionic glycans such as pectin and other uronic acid-containing glycans. NaOH is required to extract crystalline, insoluble glycans such as cellulose. Anion exchange chromatography was then used to isolate fucoidans from the glycans extracted (MilliQ and EDTA extracts) from the sediment. NaOH extracts were not used as the high pH or high salt concentration after neutralization is not suitable for anion exchange chromatography. Epitope detection chromatography is a method described in previous studies which uses specific monoclonal antibodies for the detection of glycan epitopes after anion exchange purification (Cornuault et al., 2014; Vidal-Melgosa et al., 2021). Similarly, we tested the chromatographic fractions for fucoidans using the specific monoclonal antibody BAM1. Additionally, the deoxyhexose concentrations (as an estimate for L-fucose) in the fractions were measured as a proxy for fucoidans. The BAM1 reactive material containing deoxyhexoses was henceforth operationally defined as fucoidan.”**

Additionally, we have included a figure showing the schematic overview of the analytical methods.



**Figure 2: Schematic overview of methodology.** (a) Glycans were sequentially extracted from the sediment using MilliQ water, 0.3 M EDTA and 4 M NaOH+0.1% NaBH<sub>4</sub> which target water-soluble glycans, cation cross-linked glycans and water-insoluble glycans respectively. (b) Water and EDTA extracts were loaded onto anion-exchange columns and fucoidans were separated using an increasing salt gradient. (c) The anion exchange fractions were probed for fucoidan using a fucoidan-specific monoclonal antibody BAM1, and deoxyhexoses were quantified using L-cysteine assay as a proxy for fucoidan. Fractions containing overlapping signals in these complementary analyses were defined as fucoidan. (d) To determine monosaccharide composition, the fucoidan-containing fractions were pooled, desalted using silica columns, and then acid hydrolysed to monosaccharide constituents, and analysed using high performance anion exchange chromatography coupled with a pulsed amperometric detector (HPAEC-PAD).

Lines 206-207: Rhamnose seems like it is not even present in the isolated fucoidans? I would comment on that, rather than saying it is less abundant

We have made changes to lines 206-207 (original manuscript): “Arabinose, and glucosamine show lower relative abundances, while rhamnose was not detected in the isolated fucoidans.”

We have also changed figure 4 caption (Fig. 3 in original manuscript) from “Monosaccharides like rhamnose and arabinose, however, have a lower abundance indicating selection of certain glycans.” to

**“Monosaccharides like glucosamine and arabinose have a lower abundance, while rhamnose is absent, indicating selection of certain glycans and the omission of the rest.”**

Lines 208-210, 232-234: What does this selection/omission imply for the reliability of the anion exchange chromatography for evaluating the role of glycans in long-term C-sequestration?

**We cannot use this method for all glycans, as anion-exchange chromatography selects for only anionic glycans, which was the purpose of our study. Therefore, the selection/omission of certain monosaccharides is expected and wanted, and it increases confidence to evaluate the role of specific glycans (fucoidans) in carbon sequestration.**

**We have now changed lines 208-210 for clarifying this: “The differences in monosaccharide compositions before and after anion exchange chromatography indicates anionic glycans, such as fucoidans were successfully enriched relative to neutral and weakly acidic glycans, such as rhamnose-containing glycans.”**

Lines 241-242: It would be helpful to explain why it is important to determine the source of fucoidans

**For future studies it would be beneficial to more clearly resolve the origin of the fucoidan for example using MS fingerprinting with purified fucoidan standards from known macro- and microalgal sources. This way the contribution of these algal types to fucoidan carbon sequestration could be determined. The changes made to reflect this point are addressed to the comment below.**

Lines 264-271: The content of the outlook section seems out of place to me. The motivation to understand carbon sequestration potential of brown algae seems like an underlying reason to do this type of investigation, and should be presented in the introduction.

This would also help the reader understand why we should even care about which algae were responsible for producing sedimentary fucoidans (see above comment). I would rather finish with a concluding section that briefly summarizes the key findings of the study and explains why they are important in a broader context. I think there would be some scope within the discussion to outline remaining uncertainties in this approach and/or identify goals for follow-up work.

**We now end the introduction as follows: “By quantifying fucoidan in marine sediments, the contribution of macro- and microalgae to carbon sequestration, for example via macroalgae farming, could potentially be resolved at higher molecular resolution than is currently achievable.”.**

**We now begin the paragraph (line 235 in original manuscript) as follows: “Resolving the origin of fucoidans will help determine the contribution of various algal types to carbon sequestration.”**

**We end the paragraph by stating “This is important to predict the future evolution and magnitude of glycan carbon sequestration under changing algal abundances driven by climate change and other stressors (Filbee-Dexter et al., 2026).”**

**According to the reviewer’s suggestions, we write a conclusion (instead of the outlook) summarizing the results and highlighting what should be carried out in future work.**

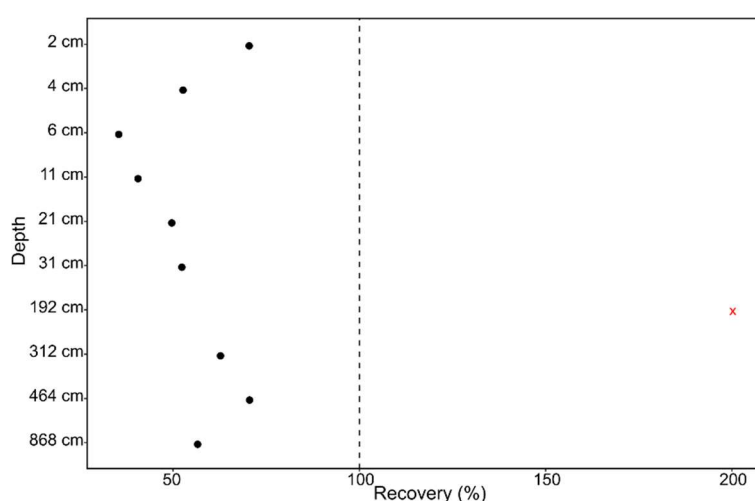
The conclusion now reads: “In this study, we were able to isolate and detect fucoidans that had been buried for millennia in Bransfield Strait sediments. The methodological framework developed in our study for the quantification of fucoidans and potentially other algal glycans in sediments, provides a foundation for their use as proxies to assess carbon sequestration. In the future, this approach therefore may facilitate the long-term monitoring of brown algal carbon sequestration via algal farming or from natural populations beyond the immediate vicinity of cultivation or natural occurrence sites. Future work should include species-specific fucoidan analysis so that these molecules can be unambiguously assigned to their algal source and geographical origin in the surface ocean. Through such an approach carbon accounting might become more realisable and therefore feasible.”

Figure S4: What is going on with the value at 192 cm? Why would matrix effects have such a big impact on this sample, but not any of the others? Seems like something could have been messed up in the calculations or in the lab handling of this sample

We have verified the calculation but the sample from 192 cm remains an outlier. Hence, we have now changed lines 191-192 in the original manuscript from “The apparent recovery of 200% at 192 cm indicates an unresolved matrix effect, analytical interference, or other operational error. Thus, this sample was not used for quantitative interpretation. Conducting spike-recovery experiments in the future could help identify potential matrix effects and improve the reliability of the analytical results.”

We have also indicated the values in the table with an asterisk (\*) next to the values and provided an explanation in the note: “\*Either due to matrix effects or lab handling errors, the value after AEX was twice than before AEX. Hence, these values were not used for quantitative interpretation”

We have also changed the Fig. S4 to the following:



**Figure S4: Recovery of deoxyhexoses after anion exchange chromatography.** Except at 192 cm depth, 35-70% of the deoxyhexoses in the MilliQ and EDTA extracts were captured using anion exchange chromatography. At 192 cm depth there was a 200% recovery (red x), indicating underestimation in the extracts due to matrix effects or an overestimation in the purified samples. This sample was therefore excluded from quantitative interpretation. The vertical dotted line indicates a 100% recovery.

Typos and minor corrections (not an exhaustive list, please proofread the revised manuscript carefully):

After proofreading we have made minor corrections throughout the manuscript.

We have corrected the heading of the table 1 from “Contribution of carbohydrate carbon to the sedimentary organic carbon pool.” to “Contribution of water and EDTA extracted carbohydrate carbon to the sedimentary organic carbon pool.”

Line 25: add “the” before “form”

**This has been changed.**

Line 39: delete “for”

**This has been changed.**

Line 81: “was” should be “were”

**This has been changed.**

Line 118: add “the” before “dark”

**This has been changed.**

Line 130: add “The” before “protocol”

**This has been changed.**

Line 162: delete the comma after both

**This has been changed.**

Figure 3: Have you checked that the color scheme here is appropriate for color blind readers?

We had previously used the “paired” colour palette from the R package RcolorBrewer. While this palette is considered not fully colour-blind friendly (some colours blended together for people with red-green blindness), the colours were distinguishable based on the order used in the plot. However, now we have changed the palette to “Safe” from the R package “rcartocolor”. The colours are based on the recommendations of Paul Tol. We also have verified this using the colour-blindness simulator <https://www.color-blindness.com/coblis-color-blindness-simulator/>. The figure is now changed:

