

We thank the reviewer for their valuable feedback and constructive criticism. We will address each comment individually (in bold).

General comments

This manuscript presents an interesting method for detecting fucoidan-like glycans in marine sediments. The authors extract glycans and separate the water- and EDTA-extractable material by anion-exchange chromatography. They then examine the extracts and chromatographic fractions using three methods with different roles. First, BAM1-based ELISA detects an epitope related to fucoidan. Second, the L-cysteine assay estimates the amount of deoxyhexoses, including fucose. Third, HPAEC-PAD shows the relative monosaccharide composition after acid hydrolysis.

When the BAM1 signal and the deoxyhexose peak occur in the same chromatographic fractions, and the monosaccharide composition is also consistent with fucoidan, the evidence for fucoidan is stronger than that from only one analysis. However, none of these methods alone can prove that the detected material is fucoidan. The experiments in this study were carefully designed, and I highly appreciate this point.

I am generally positive about publication after revision. My main concern is that the method enriches and detects material consistent with fucoidan, but some parts of the Results and Conclusions appear to describe the measured values as concentrations of total fucoidan. The authors should clearly state that the material extracted and measured by this method is operationally defined as fucoidan in this study.

Most of issues can be addressed by clearly defining the measured material, explaining the calculations and analytical limits in more detail, and using more careful wording in the Conclusions. I do not consider extensive additional experiments using the limited Bransfield Strait samples necessary for the present manuscript.

Major comments

1. Define “fucoidan”

Please explain early in the manuscript which chemical features are used to define fucoidan. The extraction and analytical procedure used in this study enriches an acidic glycan fraction that contains deoxyhexoses and reacts with BAM1. However, these properties alone cannot prove that all the measured material is fucoidan. In addition, the water and EDTA extractions measure only an extractable fraction, not total fucoidan in the sediment. However, it would be difficult for readers if the authors repeated “putative fucoidan fraction” throughout the manuscript. I suggest providing one clear operational definition near the end of the Introduction or at the beginning of the Methods. For example:

“In this study, the amount of material extracted with water and EDTA, reactive with BAM1, containing deoxyhexoses, and recovered in the selected anion-exchange fractions is operationally reported as the fucoidan concentration.”

After this definition, the shorter terms “fucoidan” and “fucoidan concentration” may be used. However, the Abstract, main conclusions, and figure and table captions should remind readers that these values are operationally defined and do not represent total fucoidan in the sediment.

We have incorporated the suggestions to operationally define fucoidans in the text.

In the Abstract (lines 17-19, original manuscript), we have changed

“Here, we combined anion exchange chromatography with glycan-specific monoclonal antibodies to isolate and detect fucoidans from marine sediments. Applying this method to a sediment core from the Bransfield Strait in the Southern Ocean revealed that fucoidan was buried and persisted for the extent of the Holocene (11.8 ka).”

to

“Here, we combined anion exchange chromatography with other analytical methods to isolate and detect fucoidan from marine sediments. We operationally defined fucoidans as the glycans recovered using anion-exchange chromatography, which contain deoxyhexoses, and are reactive to fucoidan-specific monoclonal antibody BAM1. Applying this method to a sediment core from the Bransfield Strait in the Southern Ocean revealed that the operationally defined fucoidan was buried and persisted for the extent of the Holocene (11.8 ka).”

In Section 2.10 Overview of analytical workflow (new section, see below) we have included “The BAM1 reactive material containing deoxyhexoses was henceforth defined as fucoidan.”

To the sentence in lines 187-188 (original manuscript) i.e. “The concentrations of deoxyhexoses and the antibody signals both peaked in the same elution window (see Fig. 2).”, we have now added “This peak was henceforth operationally defined as fucoidan.”

In Figure 3 caption (Fig. 2 in original manuscript) “This region signifies the fucoidans isolated from the corresponding depth.” was changed to “The glycans in the fractions in this region is operationally defined as fucoidans isolated from the corresponding depth.”

In Figure 4 caption (Fig. 3 in original manuscript) we have changed “Relative abundances of monosaccharides before (left) and after (right) anion exchange chromatography are compared.” to “Relative abundances of monosaccharides before anion exchange chromatography (left) and the operationally defined fucoidans i.e. after anion exchange chromatography (right) are compared.”

We have changed lines 218-219 (original manuscript) in the Discussion from

“In this study, we estimated the contribution of fucoidans towards carbon sequestration in millennia-old marine sediments.”

to

“In this study, we estimated the contribution of the operationally defined fucoidan towards carbon sequestration in millennia-old marine sediments.”

2. Overview of the analytical workflow

A short overview of the analytical workflow would also help readers. Please briefly explain the purpose of each main step. Sequential extraction separates different extractable glycan pools. The water and EDTA extracts are combined for further analysis. Anion-exchange chromatography enriches acidic glycans and separates them from part of the sediment matrix. BAM1-based ELISA and the L-cysteine assay provide complementary measurements of the collected fractions. Silica treatment removes salts before HPAEC-PAD. Acid hydrolysis releases monosaccharides for compositional analysis. Please also make clear that ELISA, the deoxyhexose assay, and HPAEC-PAD are complementary analyses. They are not successive extraction steps.

We agree that an overview will help the readers, hence we have now written a new section at the end of the Materials and Methods. Additionally, in accordance with Reviewer 2's comments we have modified and moved the parts describing the methods from the Results section to the new sections; namely from lines 158-166 (original manuscript); and lines 179-188 (original manuscript) (please refer response to Reviewer 2). The new section is now written 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 neutralisation 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.

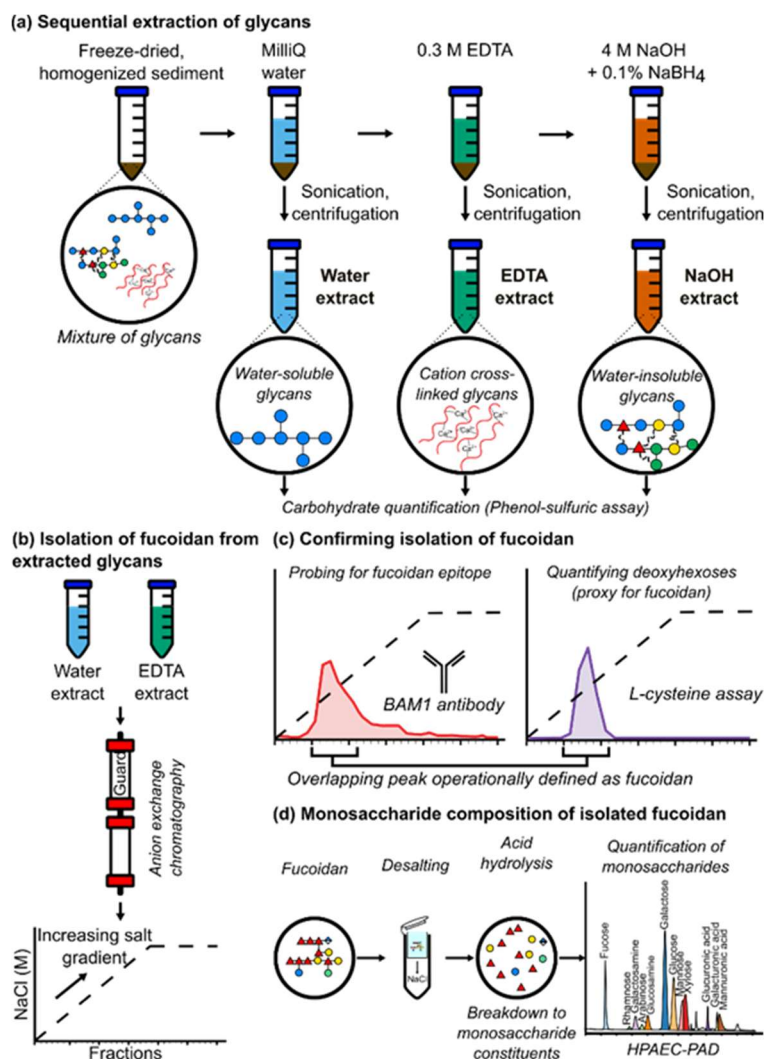


Figure 2: Schematic overview of methodology. (a) Glycans were sequentially extracted from the sediment using MilliQ water, 0.3 M EDTA and 4 M NaOH+0.1% NaBH₄ which target water-soluble glycans, cation cross-linked glycans and water-insoluble glycans respectively. **(b)** Water and EDTA extracts were 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).

3. Clarify the apparent recovery and matrix effects

The apparent recovery of 200% at 192 cm indicates an unresolved matrix effect or analytical interference. This value should be described as quantitatively uncertain and should preferably not be used for quantitative interpretation. This issue does not invalidate the qualitative detection of BAM1-reactive material. However, it limits the comparison of absolute concentrations among different depths. For future quantitative validation, a whole-procedure spike-recovery test would be useful. An available and well-characterized BAM1-reactive fucoidan could be added to an alternative natural sediment before extraction. For example, the Wadden Sea sediment already used in this study could be considered. I do not request this additional experiment for the present manuscript. However, I suggest discussing it as an important topic for future work.

We have now changed lines 191-192 in the original manuscript from “At depth 192 cm, a 200% recovery was observed, indicating possible matrix effects causing underestimation of the crude extracts or overestimation in the purified samples.” to “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:

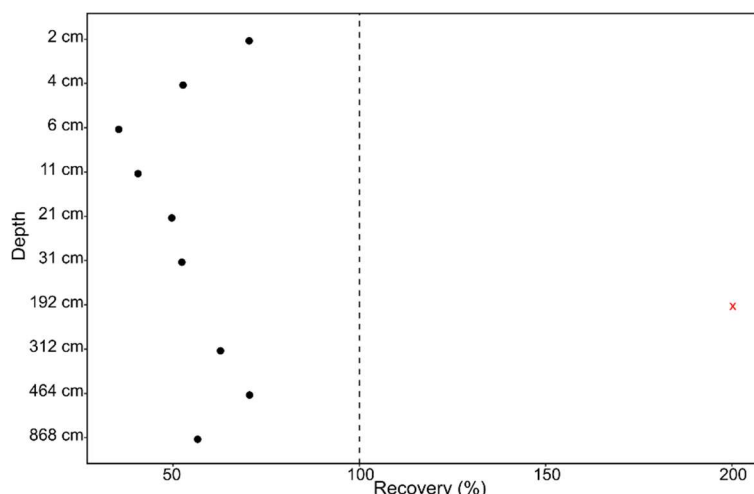


Figure S4: Recovery of deoxyhexoses after anion exchange chromatography. Except at 192 cm depth, 35-70% of the deoxyhexoses in the MilliQ and EDTA extracts were captured using anion exchange chromatography. At 192 cm depth there was a 200% recovery (red x), indicating underestimation in the extracts 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.

4. Revise the interpretation of the 30% value

The 2 cm sample is the uppermost sediment sample. However, it does not represent material immediately after deposition from the water column. A 2 cm sediment interval contains material accumulated over a certain period, for example. It may represent at least several months and possibly several years. Fucoidan may already have been degraded during this period. Therefore, the value at 2 cm does not represent the amount produced at the sea surface, the amount that started to sink, or the amount that first reached the seafloor.

We have removed the 30% statement regarding the first 2 cm due to the mentioned concerns. We have now changed the sentence to “At least 0.005 mgC. g_{dw}⁻¹ of fucoidan remained buried for the entire extent of the Holocene (11.8 ka).”

5. Discuss possible inhibition of BAM1 by humic-like substances

Marine sediments contain large amounts of humic-like organic matter. Humic-like substances may bind to glycans or antibodies and reduce the access of BAM1 to its target. They may also interfere with adsorption to the ELISA plate or increase the background colour. The purification procedure used in this study may reduce these effects. However, the manuscript does not clearly show that these effects were sufficiently removed or were similar at all

depths. Please explain how possible inhibition by humic-like substances was evaluated and how it may affect the results. If inhibition is stronger in deeper samples, a lower BAM1 signal may indicate not only a lower fucoidan concentration but also stronger inhibition. I understand that it may be difficult to remove the effects of humic-like substances completely. However, this limitation should be clearly stated.

We acknowledge that humic substances may interfere with antibody binding, and that this effect could be reduced by the purification procedure. Sichert et al., 2021 (see references in original manuscript) showed that brown phenolic compounds bind tightly to the anion exchange column thereby removing the contaminants from the analytes. We have also observed binding of the dark brown contaminants to the column, which are released only partially after a thorough NaOH washing. As the ELISA was done after chromatography, we can assume that the interference is reduced.

However, we have now included BAM1 signals in water and EDTA extracts of different depths in the supplementary (Figure S5). This is in corroboration with previous studies namely, Vidal-Melgosa et al., 2022 (bioRxiv); Sálmean et al., 2022; and Hellige et al., 2026. This shows us that BAM1 can still be used to detect fucoidan epitopes in samples containing humic substances.

We have now addressed this in lines 224 (original manuscript, before the sentence “While other glycans...”): “While BAM1 signals were also observed in the water and EDTA extracts (see supplementary figure S5), anion exchange chromatography may have reduced interference from humic substances (Sichert et al., 2021).”

Specific and minor comments

Figure 2: The x-axis is labelled only as “Chromatographic fractions.” Please provide the x-axis unit, fraction numbers, or other information needed to identify the fractions.

The fraction numbers are now indicated on the x-axis.

Line 81: “Samples of known weight was” should be changed to “Samples of known weight were.”

This has been changed.

Lines 250–251: “The binding ... also suggest” should be changed to “The binding ... also suggests.”

This has been changed.