**Reviewer #1's general comment:** The manuscripts presents a study on the development of a method adapted to the determination of alginate in sediments. The interest of this modified protocol is well justified, both from a scientific and from practical approaches. The work planning is well presented and the results are clearly presented and discussed.The authors used the protocol to characterize samples from different locations.

**Response to the Reviewer #1's general comment:** We sincerely appreciate the time you devoted to reviewing our manuscript and for providing constructive and insightful comments. We are also grateful for your positive evaluation of the protocol from both scientific and practical perspectives. Based on your valuable suggestions, we have prepared detailed responses to each of your comments below. We hope that our explanations adequately address your concerns, and we thank you again for your thoughtful feedback.

**Reviewer #1's comment 1:** Unify the format of units, w/w or $w \cdot w^{-1}$

**Response to the reviewer #1's comment 1:** To address this comment, we have unified the formatting of concentration units throughout the manuscript. Specifically, all solution concentrations previously expressed as "% (w/v or v/v)" have been revised to "g $L^{-1}$" or "mL $L^{-1}$," as appropriate. We have also replaced the descriptive ratio-style expression for alginate in sediments, "mg per 300 g (alginate/wet weight of sediment)," with the standardized notation "mg 300 $g^{-1}$".

**Reviewer #1's comment 2:** Why yield is expressed per 300 g ?

**Response to the reviewer #1's comment 2:** We originally expressed alginate yield as "mg per 300 g" to indicate the amount of alginate extracted from each sediment sample, whose wet weight was fixed at 300 g. In response to your comment, we have standardized this notation to "mg 300 $g^{-1}$" throughout the manuscript.

The rationale for selecting 300 g as the sediment sample weight has also been clarified in Section 2.3, where we added the statement: "The weight of 300 g corresponds approximately to the amount of sediment collected from the upper 0–1 cm layer using a grab sampler."

To further improve clarity, we have added a note indicating "Sediment sample weight = 300 g (wet)" in Tables 2, 5, and 8, where alginate yield (mg 300 $g^{-1}$) is reported. These revisions improve consistency and readability in the presentation of the alginate yield data.

**Reviewer #1's comment 3:** Latin names in References should be in italics

**Response to the reviewer #1's comment 3:** Thank you for pointing this out. All Latin names appearing in the references have been converted to italic font in accordance with the journal's formatting requirements.

**Reviewer #2's general comment:** This is a useful paper, its main contribution is methodological. The authors show that the original food-chemistry protocol performs poorly in sediment, and that their modified protocol improves recovery substantially. That is the strongest result in the paper, and it should be the main message.

**Response to the Reviewer #2's general comment:** We would like to express our sincere gratitude for your thoughtful comments and constructive suggestions, which have significantly improved the clarity of our manuscript. Based on the valuable feedback provided, we have addressed each point in detail. The following section details the points raised by the reviewer and the corresponding revisions made to the manuscript. Additionally, the text highlighted in yellow represents new sentences added to provide further clarification based on your suggestions. We hope these explanations and the subsequent revisions sufficiently address the concerns raised. We once again thank you for your time and insightful contributions.

**Reviewer #2's comment 1:** My main concern is overstatement. The paper often sounds as if it has cleanly measured brown-algal carbon in sediment. But the evidence presented is more limited. The method measures uronic acids. The authors themselves show some remaining non-alginate signal, and the enzyme experiment only supports that at least part of the measured material is alginate. The paper is much stronger if framed as a promising sediment method with a first field application, not as definitive proof of brown-algal carbon sequestration. The paper should center its novelty more clearly. The real novelty is not the broad blue-carbon context. It is that the authors adapted an existing alginate method to a much harder matrix, marine sediment, and identified two practical changes that matter: adding EDTA and removing ethanol precipitation.

**Response to the reviewer #2's comment 1:** The Abstract and Conclusion now clearly state the primary objectives of this study—developing a novel analytical method for alginate in marine sediments and applying it to natural samples—while avoiding overstatement regarding the relationship between our findings and carbon sequestration. Key methodological improvements, specifically the addition of EDTA-2Na and the omission of ethanol precipitation, have also been clarified in the revised manuscript.

Page 1, Line 8–20: In this study, we developed a novel extraction method for alginate, a major organic component derived from brown algae, in marine sediments and evaluated its applicability for quantitative analysis. Alginate analytical methods have been established in food chemistry: we modified these techniques to apply them to marine sediments, which are characterized by the cation composition (e.g., Ca, Mg, Fe) and humic substance-like high-molecular-weight organic compounds. By modifying the protocol through the addition of EDTA-2Na and the omission of ethanol

precipitation, we improved the extraction efficiency of alginate from marine sediments, as demonstrated by spike recovery tests where recoveries of $Na^+$-Alg significantly increased from 38.7 % with the conventional method to 64.7–82.6 %. Enzymatic degradation tests using alginate lyase confirmed that a portion of the extracted uronic acids from marine sediments (at least 34 %) was derived from alginate, verifying its presence in the natural samples. Using this modified method, alginate contents in sediment samples from coastal waters around Hokkaido, Japan, were quantified as 6.11–26.0 mg $m^{-2}$ in Funka Bay, 39.0–41.3 mg $m^{-2}$ in Hakodate Bay, 11.8–14.7 mg $m^{-2}$ off Cape Esan, and 58.3–74.1 mg $m^{-2}$ off Muroran. However, these values may be overestimated due to the presence of other uronic acids; therefore, they should be interpreted with caution rather than being directly equated with absolute alginate content.

Page 17, Line 642–657: In this study, we developed a novel extraction protocol for alginate in marine sediments, based on a conventional method used in food chemistry, and evaluated its applicability for quantitative analysis. We modified two key aspects—the addition of EDTA-2Na and the omission of ethanol precipitation—to improve the extraction efficiency of alginate from marine sediments. Spike recovery tests with $Na^+$-Alg (200, 100, and 50 mg) demonstrated substantially higher recoveries (64.7–82.6 %) by using the modified protocol than with the conventional method (38.7 %). To assess potential interference from non-alginate polyuronic acids, such as those derived from phytoplankton, phytoplankton aggregates were subjected to the modified protocol. Although most of the interference was effectively eliminated, the minor amounts of uronic acids were detected, corresponding to 23 % of the estimated alginate content in marine sediments at the same site. To confirm that the uronic acids in marine sediments extracts originated from alginate, an enzymatic degradation test using alginate lyase was performed on the natural extracts. The uronic acid concentration decreased following enzymatic degradation, confirming that a portion of the uronic acids (at least 34 %) was derived from alginate. Finally, the alginate content of marine sediments collected around southern Hokkaido, Japan, was quantified using the modified protocol. Alginate concentrations were 6.11–26.0 mg $m^{-2}$ in Funka Bay (August–December 2024), 39.0–41.3 mg $m^{-2}$ in Hakodate Bay (June 2024), 11.8–14.7 mg $m^{-2}$ off Cape Esan (June 2024), and 58.3–74.1 mg $m^{-2}$ off Muroran (February 2025). These findings demonstrate the establishment of a quantification method for alginate in marine sediments and provide an initial application to the surface sediments of the target area.

**Reviewer #2's comment 2:** The claims about specificity should be softened. The phytoplankton test still gives a residual signal equivalent to 23% of the sediment signal, which is not trivial. The enzyme test also leaves a large residual signal, and the authors conclude only that alginate accounts for at least one-third of the uronic acids detected. That supports the presence of alginate, but not a clean one-to-

one conversion from color signal to alginate alone. The wording should reflect that more carefully throughout.

**Response to the reviewer #2's comment 2:** In the Sect. 3.5, we have explicitly noted the potential interference from phytoplankton-derived uronic acids and the fact that alginate accounted for approximately 34% of the total uronic acids in the natural samples. Consequently, we concluded that the detected values of uronic acids from marine sediments should not be directly equated with absolute alginate content. Furthermore, in both the Abstract and Conclusion, we have clarified that quantified alginate values in marine sediments may represent an overestimation.

Page 16, Line 622–624: However, given that the extract solution may contain a fraction of phytoplankton-derived uronic acids and that confirmed alginate accounted for at least 34 % of the total, these detected values should be interpreted with caution rather than being directly equated with absolute alginate content.

Page 1, Line 8–20; Page 17, Line 642–657: Described in Response to the comment 1.

**Reviewer #2's comment 3:** The conclusion goes a bit too far. The manuscript currently says the method provides "quantitative and direct evidence" of brown-algal carbon in ocean sediments, and later links the findings to coastal carbon sequestration. That feels stronger than the data support. The study shows alginate-like material in surface sediments. It does not directly show long-term burial or sequestration. I would recommend more cautious language here.

**Response to the reviewer #2's comment 3:** In the Conclusion, we have removed the claim that our results provide "quantitative and direct evidence" of brown-algal carbon sequestration to adopt a more cautious tone and maintain objectivity. Instead, we now conclude that the primary contributions of this study are the establishment of a novel quantification method for alginate in marine sediments and its initial application to surface sediments in the target areas.

Page 17, Line 642–657: Described in Response to the comment 1.

**Reviewer #2's comment 4:** The introduction is longer than needed. It spends too much time on general uronic-acid chemistry before getting to the actual gap. I would shorten that part and move faster to the practical problem: no established method exists for alginate in marine sediment, where cations, low concentrations, and other uronic-acid sources complicate the analysis.

**Response to the reviewer #2's comment 4:** We have streamlined the Introduction by removing redundant descriptions of the chemical structures of uronic acids and polyuronic acids (alginate and

pectin). This allows for a more rapid transition to the core analytical gaps. Specifically, we have focused the text on the three primary challenges of quantifying alginate in marine sediments, providing a more detailed discussion of how complex cation compositions interfere with the analysis.

Page 2, Line 37–60: Uronic acids are monosaccharides characterized by a terminal carboxyl group (–COOH); four primary types with distinct stereochemical configurations—glucuronic, galacturonic, mannuronic, and guluronic acids—are commonly reported in the marine environment (Bergamaschi et al., 1999), where they form various polysaccharides through glycosidic linkages. Alginate is a linear polysaccharide composed of two epimeric uronic acid residues, β-D-mannuronic acid [M] and α-L-guluronic acid [G], which form various block structures (MM, GG, and MG blocks) (Yang et al., 2011). Although also produced by certain bacteria, alginate is primarily derived from the cell walls and intercellular matrix of brown algae (Szekalska et al., 2016), which is considered to be predominant source in the marine environment from a biomass perspective. Another major uronic-acid-containing polysaccharide is pectin, primarily composed of polymerized galacturonic acid (Gerschenson, 2017). Pectin is produced by a wide range of terrestrial and marine plants, including several types of phytoplankton that contribute significantly to marine primary production (Domozych et al., 2007). Both alginate and pectin form gels through ionic interactions between their carboxy groups and polyvalent cations ($Ca^{2+}$, $Cu^{2+}$, and $Fe^{3+}$), whereas monovalent cations such as $Na^{+}$ and $K^{+}$ promote solubilization. In particular, alginate forms significantly more rigid and mechanically stable gels through the "egg-box" model compared to the weaker structures formed by pectin (Grant et al., 1973; Fang et al., 2008).

In brown algae cells, alginate primarily exists as various alginate salts (e.g., $Ca^{2+}$, $Na^{+}$, $Mg^{2+}$, and $K^{+}$), with these cations derived from seawater (Usov and Zelinsky, 2013). While brown algae are considered a significant source of organic carbon, direct evidence of their contribution to carbon transport into the deep ocean remains limited. Although analytical methods for alginate have been established for food samples (Kawasaki et al., 1998), their application to marine sediments can be hampered by the complex matrix, including the cation composition, the trace levels of alginate present, and the coexistence of other polyuronic acids like pectin. Particularly in the pore water of ocean sediments, high concentrations of iron ions interact with alginate to form stable, insoluble iron (III) alginate ($Fe^{3+}$-Alg) gel, which can significantly inhibit extraction efficiency (Klinkhammer, 1980; Menakbi et al., 2016). In this study, our aim was to develop a novel method for quantifying alginate in marine sediment, based on conventional food analysis techniques, and to apply this method to samples collected from the continental shelf to the shelf slope.

**Reviewer #2's comment 5:** There is also a wording inconsistency that should be fixed. The abstract

calls alginate “unique to brown algae,” but the introduction later states that alginate is also produced by some bacteria. The authors should use one careful formulation throughout.

**Response to the reviewer #2’s comment 5:** To ensure consistency throughout the manuscript, we have removed the phrase “unique to brown algae” and now consistently refer to alginate as a “major organic component derived from brown algae.” This phrasing acknowledges its significance in brown algae while remaining scientifically accurate regarding its potential bacterial origins.

Please note that these changes will be incorporated into the revised manuscript.