Seafloor sediment characterization improves estimates of organic carbon standing stocks: an example from the Eastern Shore Islands, Nova Scotia, CanadaSeafloor sediment characterization to improve estimate of organic carbon standing stocks in continental shelves.

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Abstract. Continental shelf sediments contain some of the largest stocks of organic carbon (OC) on Earth and play a vital role in influencing the global carbon cycle. Quantifying how much OC is stored in shelf sediments and determining its residence time is key to assessing how the ocean carbon cycle will be altered by climate change and possibly human activities how human activities can accelerate the process of OC remineralization into carbon dioxide. Spatial variations in terrestrial carbon stocks are well studied and mapped at high resolutions, but our knowledge of the distribution of marine OC in different seafloor settings is still very limited, particularly in the highly dynamic and spatially variable shelf environments. The This lack of knowledge reduces our ability to understand and predict how much and for how long oceans sequester CO<sub>2</sub>. In this study, we use high-resolution multibeam echosounder (MBES) data from the Eastern Shore Islands offshore Nova Scotia (Canada), combined with OC measurements from discrete samples, to assess the distribution of OC content in seafloor sediments. We derive four-three different spatial estimates of organic carbon stock: i) OC density estimates scaled to the entire study region assuming a homogenous seafloor, the organic earbon density stock estimates were sealed to the entire study region; ii) interpolating ion the of organic carbon OC density estimates using an Empirical Bayesian Kriging method; iii) OC density using a high resolution substrate map, the estimates were scaled estimates scaled to areas to the of areas of soft substrate only estimated using a high-resolution classified substrate map, and finally, ivii) Empirical Bayesian Regression Kriging using of OC density within areas of estimated soft sediment Empirical Bayesian Regression Kriging prediction to, carbon stock estimates OC density in the soft substrate only were refined to account for spatial variability in the concentration of OC. These four three distinct spatial models yielded dramatically different estimates of average standing stock of OC in our study area of 223 km<sup>2</sup>,-: 80,9011275, 58,406259, 16,437 and 6,475203 Mt of OC, respectively. Our study demonstrates that high-resolution mapping is critically important for improved estimates of OC stocks on continental shelves, and to the identification of carbon hotspots that need to be considered in seabed management and climate mitigation strategies.

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## 1 Introduction

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## 1.1 Blue Marine Carbon

Blue Carbon has received tremendous interest as a natural option for climate change mitigation due to the fact that some marine habitats can store disproportionate amounts of organic carbon (OC) on an area-by-area basis compared to terrestrial habitats (Hilmi et alet al., 2021). The Intergovernmental Panel on Climate Change (IPCC) defines blue carbon Blue Carbon as: "All biologically driven carbon fluxes and storage in marine systems that are amenable to management." (2019Hilmi et al., 2021). Blue carbonBy this definition, Blue Carbon is therefore often associated with vegetation in coastal zones, such as tidal marshes, mangroves, and seagrasses (McLeod et alet al., 2011Brown et al., 2016; Fourqurean et al., 2012). OC -in mMarine sediments are often not included in Blue Carbon calculations and definitions since these environments do not sequester carbon via photosynthesis (Lovelock et al., 2019-Hunt et al., 2021). However, marine sediments are essential carbon reservoirs and regulate climate change by effectively burying organic carbonOC over thousands to millions of years if left undisturbed (Berner, 2003-; Burdige, 2007-Avelar et al., 2017; Fennel et al., 2019; Atwood et al., 2020). -Studies are therefore beginning to acknowledge marine sediments as an emerging Blue Carbon ecosystem (Howard et al., 2023).

The fate and flux of organic carbon in benthic systems is influenced by a range of factors acting over different timescales (Middelburg, 2018), including natural and anthropogenic-induced processes (Bianchi *et al.*, 2021, and 2023). Recent studies have concluded that, on a global scale, fishing activities such as all bottom trawling and dredging-can disturbs the seafloor with an estimated 1.47 Pg of aqueous CO<sub>2</sub> emissions (Sala et alet al., 2021). These However, these estimates have substantial errors (Epstein et alet al., 2022) and often ignore that the mineralization of benthic carbon stores comes from natural cycles (Hilborn et alet al., 2023). Combined, tThese studies emphasize that further understanding of sediment ocean carbon processes are urgently required to determine if bottom trawling and dredging anthropogenic activities could cause the semi-permanent OC stocks in surficial marine sediments to remineralize back to CO<sub>2</sub> (Bianchi et alet al., 2023). Also, future further studies into new approaches to determining the distribution of OC are essential to locate areas of carbon-rich seabed. Furthermore, this research could expand the definition of that could become Marine Protected Areas (MPAs) to include areas of high OC stock (Oceans North, 2024).

## 65 1.2 Seafloor Substrate

Sediment characteristics, such as mud content, are known to influence the distribution of OC in marine ecosystem (Burdige, 2007; Serrano *et al.*, 2016), with recent studies highlighting that the importance of sediment properties are as important predictors of organic carbon storage in Blue Carbon ecosystems (Dahl *et al.*, 2016; Krause *et al.*,

2022). In shelf environments, where sediment heterogeneity can be high, sediment classification maps may therefore offer a mechanism to determine areas of low and high OC content (Bianchi et al., 2021). Multibeamechosounder (MBES) systems provide information about the environmental characteristics of the seafloor, such as depth, substrate hardness, and sediment characteristics, by collecting bathymetry and backscatter information, which can be used to determine seafloor morphology and as a proxy for seafloor substrate type (Brown et alet al., 2011). Advancements in MBES have allowed us to create spatially continuous high-resolution maps of the ocean floor (Brown et alet al., 2011; Buhl-Mortensen et alet al., 2021; Misiuk and Brown, 20232024), at a horizontal resolutions down to sub-meter scales (depending on water depth and sonar specifications; ) (Mayer et alet al., 2018). Seafloor sediment mapping is defined as using describes the use of geophysical and physical sampling systems to determine the character of the surface sediments, and includes mapping the quantities of clay/silt, sand, gravel, cobble, and boulder using the Wentworth scale- (e.g., Misuik Misiuk et alet al., 2019). The modern Recent methods for producing seabed sediment maps combine high-resolution MBES with ground-truth sampling data using machine learning algorithms (Misuik Misiuk et alet al., 2019). Statistical learning techniques include k-Nearest Neighbour (Lucieer et alet al., 2013; Stephens and Diesing, 2014), Artificial Neural Networks (Huang et alet al., 2012; Stephens and Diesing, 2014), and Bayesian Decision Rules (Simons and Snellen, 2009; Stephens and Diesing, 2014). The most widely used statistical model for substrate classification and regression maps is Random Forest, due to its ease of implementation and a robust capacity for handling complex, non-linear relationships between environmental variables and ground truthing while avoiding overfitting (Stephens and Diesing, 2015; Misiuk and Brown, 20232024).

## 1.3 Benthic Carbon Mapping

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Early marine carbon mapping studies have applied interpolation methods comprising semi-variogram analyses and kriging to spatially predict OC in surficial sediments (Mollenhauer et alet al., 2004; Acharya and Panigrahi, 2016). More recently, soil organic carbon OC has been modeled using multiple methods in terrestrial ecosystems. Mallik et alet al. (2022) compared artificial neural networks (ANN), Empirical Bayesian Regression Kriging (EBRK), and hybrid approaches combining the two, including ANN-OK (ordinary kriging) and ANN-CK (cokriging). They found that the EBRK method outperformed all other models with highest values of  $R^2$  (0.936) (Mallik et alet al., 2022). The EBRK method has been widely used in terrestrial soil carbon models but has still not been explored for marine sediment carbon models. More recent studies have utilized MBES and machine learning algorithms to model and map organic carbonOC at broad spatial scales at the seafloor (Atwood et alet al., 2020; Diesing et alet al., 2017; Smeaton et alet al., 2019). Diesing et alet al (2017) used Random Forest to model particulate organic carbonOC (POC) at the seafloor using POC measurements from physical seafloor samples, and spatially continuous seafloor environmental variables (500 m grid resolution) covering the Northwest European continental shelf. Similarly, Smeaton et alet al. (2019) generated a map of seafloor substrate using the Efolk classification and calculated the OC stock per substrate class (100 m grid resolution). This latter study Smeaton was the only one amongst those listed also the only studyone to utilize MBES data to predict OC stock. (Smeaton et alet al., 2019). Epstein et alet al. (20243) also used applied Random Forest to model organic

105 <u>earbonOC</u> stocks and accumulation rates in surficial sediments of the Canadian continental margin at <u>a coarse</u> resolution (200 m grid resolution) and <u>point out emphasize</u> that ignoring the geographic extent of hard substrate (i.e., bedrock) at <u>these such</u> broad spatial scales could inflate carbon stock estimates. -These studies have been critical to understanding the carbon hotspots at broad spatial scales, as the traditional lower-resolution maps often lead to oversimplification and inconsistency in carbon averaging. However, understanding distributions of sedimentary OC at a higher spatial resolutions <u>are may be</u> required for effective seabed management strategies (Legge <u>et alet al.</u>, 2020), and.

High-resolution maps (6 m grid resolution) of OC have been produced at a local scale using 48 m resolution backscatter from MBES surveys as a predictor (Hunt et alet al., 2020; Hunt et alet al., 2021). Backscatter can be predictive of seabed sediment properties, and was hetermined problemsized to be a proxy for OC based on observed due to extrapolating empirical relationships between sediment-grain size and OC, and also potentially other additional sedimentary properties that influence backscatter reflectance (Hunt et alet al., 2020) and between sedimentary properties and backscatter reflectance. Backscatter data may thus be valuable This method could be practical in studies where there are scarce sediment data are scarce. Hunt et alet al. (2020) indicated that the backscatter data-reliably captured information regarding the spatial heterogeneity of the seabed, and that OC correlated strongly with the MBES backscatter signal as a function of sediment composition. However, a more recent study suggested that backscatter distinguishes between coarse and fine sediments (low and high OC) but struggled to differentiate fine-scale variability within finer-grained sediments (Hunt et alet al., 2021). Differences in results between these studies could be due to the different geographical setting of the studies, limited and asynchronous data, sediment mobility over time, and complex environmental processing of OC in shelf sediments (Hunt et al., 2021).

The studies in the North-West European continental margin (Diesing et alet al., 2017, 2021; Hunt et alet al., 2020; Hunt et alet al., 2021; Legge et alet al., 2020; Smeaton et alet al., 2021) have shown promising early results. Other studies of carbon stocks have been conducted in the North American Coastal region but without spatially explicit estimates (Fennel et alet al., 2019; Najjar et alet al., 2018). Overall, spatially mapping OC at the seabed has only been attempted at a few locations globally, and there is an urgent need to establish robust approaches to conducting obtaining spatial estimates of OC at the seafloor. Furthermore, conducting hHigh resolution OC mapping can may additionally help to improve current estimates of seafloor OC stocks and provide insight on marine sediments as an emerging Blue Carbon ecosystem. Our study region, the Eastern Shore islands, is an ideal location since it is an a conservationAs a conservation -Area of Interest (AOI) for the Canadian government, the Eastern Shore Islands (ESI) is an ideal location to test emergent OC mapping methods; it and comprises a heterogenous seabed, which that can may provide insight on the effectiveness of various baseline sediment OC estimates of sediment organic carbonOC in complex seafloor typesion and mapping methodologies.

This study addresses three key questions:

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- 140 1. What is the spatial distribution of seafloor sediment types in the Eastern Shore Islands ESI area?
  - 2. Are seafloor sediments a good high-resolution proxy that enable more accurate estimation of organic earbonOC stocks?
  - 3. Does the spatial heterogeneity of substrate type and carbon content influence estimates of <u>earbon\_OC</u> stock?

## 145 2 Study Area

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The study region is located within the Eastern Shore Islands (ESI), an area of interest (AOI) for conservation objectives, approximately 60 km northeast of Halifax (Nova Scotia, Canada, Figure 1). The site stretches from Lower West Jeddore to Fern Hill and extends approximately 25 km from the mainland in the Scotian Shelf region with an area of approximately 223 km² (Fisheries and Oceans Canada, 2019) (Jeffery, 2020)(Figure 1). The Eastern Shore Islands ESI is an conservation AOI for the Canadian government due to its unique coastal habitat and significant quantities of kelp beds and eelgrass. Also, tThe estuaries and rivers that drain into the site are considered important habitats for endangered species like Atlantic Ssalmon and juvenile Atlantic cCod. Furthermore, the hundreds of islands have been identified as an Ecologically and Biologically Significant Area (EBSA), that which providess essential nesting and foraging ground for many colonial seabirds and shorebirds, including h purple sandpiper, and Proseate tern, which are endangered according to the Species at Risk Act (Fisheries and Oceans Canada, 2019).

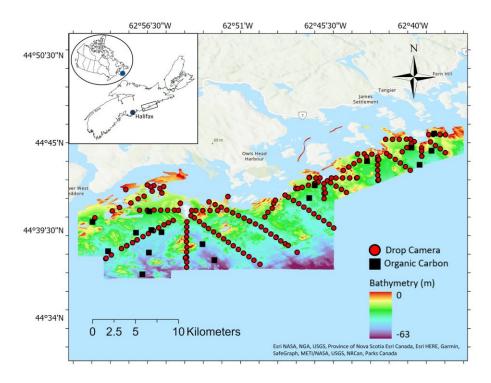


Figure 1. Seafloor MBES bathymetry <u>and sample locations outlining the geographical extent</u> for the survey area <u>within at</u> the Eastern Shore Islands, <u>on the east coast of</u> Nova Scotia, Canada <u>(inset)</u>. <u>The drop camera</u>

imagery is signified by red points and the blue points are the organic carbon OC samples. The inset map shows the location in the context of Nova Scotia. (Basemap: Esri NASA, NGA, USGS, Province of Nova Scotia Esri Canada, Esri HERE, Garmin, SafeGraph, METI/NASA, USGS, NRCan, Parks Canada)

The study area has a water depth between 31 and 63 m. The seafloor is characterized by bedrock, at places covered 165 by a layer of sediments of varying grain size (from elay to boulders) and thickness (King, 2018). The surficial geology of the -ESI surficial geology has is high spatially variability and is heterogeneous, with bedrock overlaid by mud, sand, gravel, cobble, and boulder substrates. The seabed character of the ESI is primarily bedrock since it continues from the geomorphology of the land (King, 2018). The bedrock topography is an extension of the terrestrial geomorphology and heavily influences the type and distribution of the surficial deposits. The glacial 170 imprint is substantial in the area, depositing having deposited a sequence of till and glaciomarine mud, which lie directly on the bedrock (King, 2018). There is also a thin layer of wave-modified sand and gravel, and more recent deposits of estuarine mud derived from coastal erosion (Fisheries and Oceans Canada, 2019). The ESI surficial geology has high spatial variability and is heterogeneous, with bedrock overlaid by mud, sand, gravel, cobble, and boulder substrates. Ocean surface temperatures in the ESI are around 1° C in winter for the 0-100 m depth range and increase in the summer with some stratification leading to surface temperatures exceeding 15° C (Fisheries 175 and Oceans Canada, 2019) (Jeffery, 2020). By the fall, mixing deepens this warm layer. Ocean currents run predominantly southwestwards on the ESI, with some fluctuation around the coast (Feng et alet al., 2022). The combination of upwelling, currents, and wind allows for the mixing of nutrients, acting as an essential component of the marine food web in the region (Fisheries and Oceans Canada, 2019) (Jeffery, 2020).- Nutrients are derived 180 from river, coastal runoff and mixing. They are depleted in the spring due to phytoplankton blooms and replenished in the fall when upwelling is predominant (Fisheries and Oceans Canada, 2019) (Jeffery, 2020). Major human<del>anthropocentric</del> activities in this area include lobster fishing, recreational fishing, and boating, but the human impact is low due to low population density and reduced coastal development compared to nearby Halifax and St. Margaret's Bay (Fisheries and Oceans Canada, 2019). (Jeffery, 2020).

### 185 3 Materials and Methods

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To quantify OC stock in the ESI, sediment samples were collected, and OC content and sediment grain size were measured. OC density was calculated for each sample, and four OC stock estimations were generated. The first assumed a homogenous seafloor by scaling up the average OC density to the entire study area. The second also assumed a homogeneous seafloor, but used Empirical Bayesian Kriging (EBK) to derive the spatial variability of OC density for the study area. Both scenario 1 and 2 were conducted to evaluate OC estimates when no high-resolution mapping data is available. To further refine the OC stock estimates, a substrate classification map was developed by combining high-resolution seafloor predictor variables (derived from multibeam sonar data – see below) and subsea camera imagery of the seabed. The substrate classification map partitioned the study area into hard and soft substrates. The third OC stock estimate utilized the sediment classification and scaled the average

OC density to the area of the soft substrate. The final OC stock estimate also utilized the sediment classification map but used <u>and an EBRK regression</u> Empirical Bayesian Regression Kriging (EBRK) prediction to assess incorporate the spatial variability of the OC density within the soft substrate only. Scenarios 3 and 4 determine OC estimates when sediment information and high-resolution mapping data is available. The general An overview of the data processing analysis workflow is shown in Figure 2.

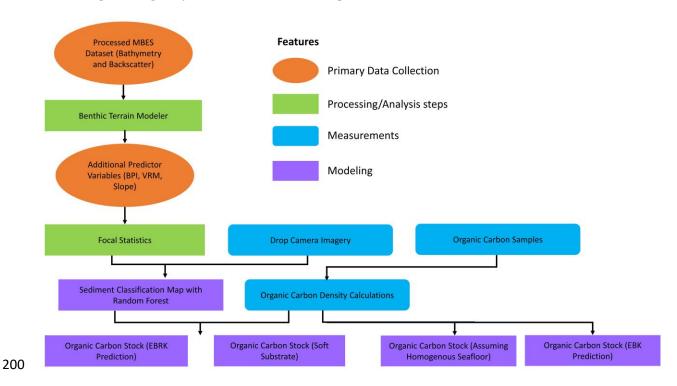


Figure 2. Flow chart outlining the geoprocessing General analysis steps used to estimate organic carbon determine the carbon estimates for all—for four scenarios.—Chart shows data inputs and outputs, processing steps, measurements, and modelling.

# 205 3.1 Hydrographic Datasets

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MBES data were collected by the Canadian Hydrographic Service over two separate surveys (20 June – 29 July 2019; 17 August – 05 September 2020) (Bondt, 2019, Bondt, 2020). Three launches were used to complete this survey – the CSL Kestrel, CSL Tern and CSL Pelican. The survey launch CSL Kestrel was equipped with an R2Sonic 2022 multibeam echosounder. The survey launches CLS Tern and CSL Pelican were outfitted with Kongsberg EM2040C and EM2040C Dual Head echo sounders, respectively. All surveys were conducted at MBES operating frequencies of 200-400kHz. Vessel position and orientation were corrected in real time by Trimble/Applanix POSMV V5 motion compensation systems. Echosounder data was corrected for sound velocity in real time using Applied Microsystems Limited sound velocity sensors. The vessel position was recorded in real-time using the CANNET RTK NTRIP connected directly through the POSMV. Raw position and orientation data

from the POSMV were logged throughout the survey for further post-processing where required. Bathymetry and backscatter data were processed using the QPS software suite. Bathymetry data were processed in QIMERA Qimera 2.5.3 to generate a bathymetric digital elevation model (DEM) for the survey area. Backscatter data were processed in FMGT 7.10.2 to generate backscatter mosaics for each of the data sets. Backscatter data were not calibrated; the different survey data sets were harmonised using bulk shift methods (Misiuk et alet al., 2020, 2021;
 Haar et alet al, 2023) from areas of overlap between the survey data sets to generate a corrected backscatter mosaic for the entire study area.

Seafloor morphology features were derived from the primary bathymetric datasets to provide additional predictor variables for the sediment classification modelling. These were selected based on literature review, expert suggestions, and access to data, and were calculated using ArcGIS Pro 3.1.2 and using the Benthic Terrain Modeler (BTM) 3.0 Toolbox. The terrain features included slope, bathymetric position index (BPI) and vector ruggedness measure (VRM), which are considered successful useful predictors in previous for seabed substrate classification studies (Stephens and Diesing, 2015; Misiuk et alet al, 2019) (Table 1) (Figure 32). The Focal Statistics tool was used to obtain the mean value for each for all the predictor variables over and calculated for each input cell location ausing statistics of the values within a specified neighbourhood (ArcGIS Pro, 2023). In this study, a rectangle neighbourhood type of a 20 by 20 pixels neighbourhood ealculated the mean pixel value, which helped smooth the surface and to reduce noise in the predictor variables. These The predictor variables were then used in both the substrate map and the organic carbon OC model. The environmental variables were determined based on literature review, expert suggestions, and access to data.

Table 1. Description of predictor variables used to model sediment type, including their units and description.

Environmental Variables	Description	Resolution	Units
Bathymetry	Depth of the seafloor	2 m	meters
Backscatter	Measure of intensity of acoustic signal from MBES and indicator of bottom hardness	2 m	relative dB
Slope	Measures maximum change in elevation (steepness)	2 m	degrees
Vector Ruggedness Measure (VRM)	Measures terrain ruggedness of grid cells within a neighbourhood	2 m	meters
Broad-Bathymetric Position Index (BPI)	Differences in values of centre cell to mean of surrounding cells.	2 m	meters

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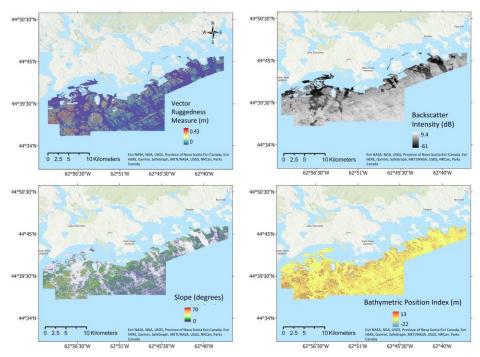


Figure 32. Backscatter, Slope, VRM and BPI data mapped in the Eastern Shore Islands study area. (Basemaps: Esri NASA, NGA, USGS, Province of Nova Scotia Esri Canada, Esri HERE, Garmin, SafeGraph, METI/NASA, USGS, NRCan, Parks Canada)

# 3.2 Seabed Seediment Seampling

Sampling surveys for OC and grain size were conducted between 9 - 27 May 2022 from the MV Island Venture. A stratified random sampling technique was used Sampling locations were randomly placed in regions of low MBES backscatter-based on the backscatter mosaic, which indicate softer, unconsolidated sediments where grab sampling by grab sampler would should be achievable-successful -(Figure 32). Acoustic backscatter was used to select sampling locations since it is agan be as a good proxy of for sediment grain size and is commonly used in substrate classification routines (Goff et al., 2000; Sutherland et al., 2007; Collier et al., 2014; Hunt et alet al., 2020). A 0.1 m<sup>2</sup> Van Veen grab sampler sampling to a maximum depth of 20cm was fitted with a GoPro camera was operated to collect sediment samples and drop camera imagery at each sample location, with the grab penetrating up to ~10 cm depth into the substrate. The GPS position of the research vessel was recorded at the point of contact on at the seabed at each grab station. A total of 17 gGrabs deployments were done only successful in areas of soft substrate where it was possible to retrieve a sample. Generally, it is difficult to sample a coarser sediment matrix successfully, and these sediment types are often under-represented in sedimentary carbon studies (Hunt et alet al., 2020). After thoroughly mixing the sediment in the Van Veen grab, 0.907 kg subsamples of sediment were taken from the grabs, and each placed in a 32-oz plastic container for organic earbonOC analysis. Following collection, these samples were stored in a cooler during the day and put into a freezer in the evening.

## 3.3 Processing of Seediment Ggrab Seamples

Prior to sediment grain size and OC analysis, the samples were dried from frozen in the oven at 60° C overnight and kept in a dark dry cabinet. The sSedimentary OC from the grab samples was quantified using an elemental analyzer (EA, Elementar Microcube microcube) with a detection limit of 0.03 mg. Based on the method from of Verardo et alet al. (1990), a section of the grab samples (five-5 gramsg) were dried in the oven at 60° C overnight and were was ground using a mortar and pestle to form a homogenous powder. Coarse grained sediments (above >2 mm diameter) were excluded since they are too large for elemental analysis. Two samples (ES-31, ES-35) had contained significant concentrations of sediment grains coarser than 2 mm notable amounts of course-grained sediments (around 30% of sample). These sand grains were removed using mesh sieves prior to grinding and EA analysis, but final sedimentary OC concentrations were adjusted to total sample weight following EA analysis. The \_coarse fraction was removed from these samples using a mesh sieve and the % OC adjusted accordingly. Silver capsules were used to weigh the initial mass (0.5-0.7 mg), and acid fumigation was performed by exposing the samples to 37% hydrochloric acid (HCl) to remove any inorganic carbon (IC). It is significant to note that an acid wash could also potentially remove some OC content, which could alter the results (Verardo et al., 1990). These capsules were then placed in an oven overnight at 60° C before undergoing analysis.

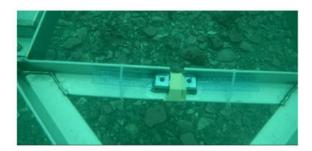
The remaining section of the grab samples was used for sSediment grain size analysis, <u>and\_was conducted using following</u> the protocol <u>derived frombyof</u>. Mason (2011). The sediment was first split into pebble/cobble (>4000 μm), gravel (>2000 μm) and fine sediment (<2000 μm) material using mesh sieves. The fraction <2000 μm was evaluated using a Beckman Coulter's LS 13 320 particle size analyzer at the Bedford Institute of Oceanography. Following the guidance <u>by of Mason</u> (2011), the samples were not treated with acid or hydrogen peroxide because the samples had relatively low organic contentbased on the OC analysis, of the samples had relatively low organic content for each sample the different sediment types (supplementary material). It should be noted that dry bulk density was unable to be not measured directly in this study and but was instead calculated (see section 3.6).

### 3.4 Subsea Vvideo Ssurveys

A total of 174 drop camera stations videos were conducted collected by Fisheries and Oceans Canada (DFO) over 13 days during September and October 2017 aboard the vessel RV Sigma-T (Fisheries and Oceans Canada, 2019). (Jeffery et al., 2020) (Figure 1). An HD subsea video camera (SV-HD SDI) was used with which recorded camera time and positional information using recorded using a video overlay streamed from the chart plotter (Proteus II), where it received positional and time/date stamp data from the chart plotter (Vandermeulen, 2018). The overlay sent tThe completed video feed with overlay outputted to a direct-to-disk HD recorder and a standard low-power LED TV. The GPS antenna for the navigation system was mounted on the roof of the wheelhouse approximately 10 m distant from the drop camera when deployed off the stern gallows. In this manner, all positional information in the video overlay would be was offset by at least 10 m and This offset was adjusted when reviewing the

dataduring post-processing-accounted for in subsequent analyses. Drop camera targets from the GIS were labelled and embedded into the RV Sigma T navigation computer. Approximately three minutes of moving video was recorded at each drop camera location with the camera light turned on. Due to the camera moving, the location would change in small increments, therefore the location in the The centre middle of each video drift was recorded as the station location. All the drop camera sites were occurred at depths greater than 10 m. Additionally, tThe GoPro camera imagery of the seafloor collected from the GoPro camera fitted to with the grab sampler during the sampling of organic carbon OC sampling in 2022 (see sediment sampling section above) was also additionally incorporated with the drop camera imagery conducted by DFO for subsequent analysis (Figure 1).

From each video station, a presence (1) and absence (0) of different sediment types were recorded in post processing. The data was classified into two sediment types: <u>h</u>Hard substrate (rock, boulder, cobbles, pebbles, and gravel), and soft substrate (mud and sand) (Figure 43) (supplementary material).



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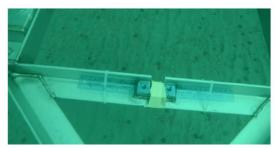


Figure 43. Example of seafloor imagery from each of the two substrate classes: hHard Substrate (left); soft substrate (right). Photos from a GoPro camera mounted on the van Veen grab. Width of images are approximately 0.5 m, with the frame of the grab providing scale for classification of substrata.

### 3.5 Sediment Modelling Sediment Classification Model

Random Forest has been used in previous carbon mapping studies due to its high predictive accuracy, capacity to manage many predictor variables, and unbiased internal validation (Diesing et alet al., 2017). In our study, Random Forest was used to model the sediment grain size typeclass to inform, which would then provide insight into areas with high OC content estimation. Random Forest was performed here using R version 4.3.1 with the randomForest package (Liaw and Wiener, 2002). The model was initially trained with default hyperparameters (ntree = 500, mtry = 2, nodesize = 1) using the substrate classification observations and all predictor variables (bathymetry, backscatter, BPI, VRM and slope). Random Forest is an ensemble modelling approach comprising many individual classification trees, each grown on a bootstrapped version of the dataset. The observations not selected for a given tree are termed the "out-of-bag" (OOB) observations. Given enough trees, each response observation will be represented in the OOB sample multiple times. By predicting the OOB values for each individual tree during model training, the results can be aggregated over all trees to provide a useful set of validation predictions that were not used to inform traininghave not been exposed to training observations. The OOB observations were used here to estimate predictor variable importance by permuting the predictor values

and measuring the resulting increase in OOB error (Liaw and Wiener, 2002). Random Forest is generally considered robust to the use of correlated predictors and estimates of importance additionally suggested contribution to the model by all variables, which were thus retained. Informal trials suggested that a model of 100 trees (i.e., ntree = 100) provided sufficient predictive capacity but improved computational speed. After training the final model with these parameters, a confusion matrix was generated using the OOB observations and predictions to evaluate the map accuracy, and the model was then predicted across the full map extent using the predictor variable rasters. The performance-kappa statistic was used to evaluate the Random Forest-model was Kappapredictions, which indicates indicating how much-well observers predictions agree with observations beyond the level of agreement that could be expected by chance: and is calculated from:

$$k = \frac{\rho_0 - \rho_e}{1 - \rho_e} \tag{1}$$

where  $\kappa$  is the value of kappa between -1 and 1, po is the proportion correctly classified and pe is the proportion correctly classified due to chance, based on the frequency of observations and predictions of each class (Misiuk, 2019). A kKappa =score of 0.00 should be taken as representing is considered "poor" agreement, between while values in the range 0.000 and to 0.20 as are often considered "slight" agreement, 0.21  $\pm$ to 0.40 as "fair"

agreement, 0.41 ±to 0.60 as "moderate" agreement, 0.61 ±to 0.80 as "substantial" agreement, and 0.81 ±to 0.99 as "almost perfect" agreement. A kappa coefficient of 1 represents perfect agreement (McHugh, 2012).

# 3.6 Estimation of Total Standing Stock of Organic Carbon Standing Stock of Organic Carbon

The elemental analyser reports OC value as a proportion (weight %). Previous studies have stated that an arcsine transformation on the OC value (X) is advisable since it can make the variance constant and the data appears normally distributed (Sokal and Rohlf, 1981; Diesing et algt al., 2017):

$$Y = \arcsin\sqrt{X}$$
 (1)

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Dry bulk density was not measured directly in this study but calculated from estimated porosity and density. Porosity  $(\Phi)$  is was calculated from predicted mud content (dimensionless fraction), which is a combination of clay and silt from the grain size distribution measurements using the equation (2) derived from Jenkins (2005).

$$\Phi = 0.3805 * C_{mud} + 0.42071_{2} \tag{2}$$

where  $\Phi$  and  $C_{mud}$  (mud content) are dimensionless fractions. The equation was derived based on data from the Mississippi-Alabama-Florida shelf, and it is assumed that the equation is not site-specific (e.g., Diesing et alet al., 2017).

Dry bulk density ( $p_d$ ) of the sediment was estimated using the porosity and sand grain density ( $p_s$ = 2650 kg m<sup>-3</sup>) (Diesing et alet al., 2017; Hunt et alet al., 2020):

$$p_d = (1 - \Phi) p_s \tag{3}$$

The organic carbon density (kg m<sup>-3</sup>) was calculated by multiplying the %OC (Y) (expressed as a decimal proportion) by the sediment dry bulk density ( $p_{d\tau}$ ). Following prior studies that quantified marine sedimentary organic carbonOC (e.g., Diesing 2017, Hunt 2021), the standing stock of organic carbon\_standing stock of organic carbon\_( $m_{OC}$ ) per grid cell ( $m_{OC}$ ) was estimated by-multiplying the average OC density by the transformed organic carbon% OC (expressed as a decimal proportion) concentration (Y), dry bulk density ( $p_d$ ), the average sampling depth of the Van Veen grab (d = 0.1 m), and area of mapped grid cell ( $A = 4 \text{ m}^2$ ) and converted to metric tonnes (divided by 1000) using the equation (4) below:

$$m_{0C} = (Y * p_d * d * A - )/1000$$
 (4)

Finally, the total standing stock was the <u>moc</u> multiplied by the total pixels in the study site (scenarios 1 and 2) or the total pixels in the soft substrate (scenarios 3 and 4).

# 3.7 Spatial Interpolation of Organic Carbon (no substrate map - No Substrate)

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After the *moc* was calculated for each sample, EBK was used to spatially interpolate *moc* within the entire study site. EBK is a geostatistical interpolation method that builds a valid-kriging model by subsetting the study area, coupled with multiple simulations to obtain the best fit (Krivoruchko and Gribov, 2019). This process finally creates several simulated semi-variograms, and each semi-variogram-each of which is an estimate of the true semi-variogram for the subset (Pellicone *et al.*, 2018). EBK differs from other kriging methods since it considers the uncertainty in the semi-variogram estimation step, which provides providing an accurate estimate of the prediction standard errors-of prediction. An exponential semi-variogram and an empirical transformation were selected The data were transformed empirically and EBK was executed in the geostatistical wizard in ESRI ArcGIS Pro 3.1.

Also, an empirical transformation was applied to lower the error and cross-validation results.

## 3.87 Spatial Interpolation of Organic Carbon Density - Soft Substrate in the soft substrate

-EBRK was used for the spatial interpolation of OC density moe and estimation of values at unknown locations within the extent of the soft substrate. EBRK is a geostatistical interpolation method that combines ordinary least square regression and kriging to provide accurate predictions of non-stationary data at a local scale (Giustini et alet al., 2019). An exponential semi-variogram model and an empirical transformation were selected for the EBRK model was performed since it provided the lowest error in the cross validation results, which was EBRK was performed in the geostatistical wizard in ESRI ArcGIS Pro 3.1. The EBRK model was evaluated using leave-one-out cross-validation (Mallik et alet al., 2022). The EBRK method is different from EBK since you can add in that predictor variable information is s-into-accommodated by including their principal components as regression variables prior to the kriging stepthe model. Thus, all the predictor variables from the substrate classification map (bathymetry, backscatter, bpi, vrm and slope) were masked to the soft substrate area using the extract by mask tool in ESRI ArcGIS Pro 3.1 and added-included in the EBRK to the-model to improve estimation of OC density

390 <u>estimations.</u> The model was validated according to the mean error (ME) and the root mean square error (RMSE).

ME is the average of the cross validation errors, measures model bias and should have a value close to zero (Acharya and Panigrahi, 2016). RMSE is defined as the square root of the average squared prediction errors and measures prediction accuracy.

## 395 3.9 Cross Validation Methods

To evaluate the prediction accuracy of the eross validation method for the EBK and EBRK predictions, the mean error (ME) and the root-mean-square error (RMSE) were calculated. ME is the average of the cross-validation errors, measures model bias and should have a value close to zero (Acharya and Panigrahi, 2016).

$$ME = \frac{1}{n} \sum_{i=1}^{n} \{ z(xi) - \hat{z}(xi) \}$$
 (5)

400 RMSE measures the difference between the predicted and the observed values and estimates the standard deviation of the residuals (Boumpoulis *et al.*, 2023). A small root mean square error (RMSE) indicates that the model has performed well and can predict the data accurately.

$$RMSE = \left[\frac{1}{n}\sum_{i=1}^{n} \{z(xi) - \hat{z}(xi)\}^{2}\right]^{1/2}$$
 (6)

The z(xi) is the observed OC and  $\hat{z}$  (xi) is the prediction of OC at location xi, and n is the number of observations.

405 Statistical parameters of the These cross-validation error parameters were calculated using within the Geostatistical Wizard Tool in the ESRI ArcGIS Pro 3.1-software.

### 4 Results

## 4.1 Grain size Distributions, Sediment Properties, and Organic Carbon Content Concentrations

Van Veen grab samples provided grain size and organic carbon OC measurements at each station (Table 2). It is important to note that silt and clay were merged into a single mud class to estimate the OC stock (Burdige, 2007; Hedges &and Keil, 1995).

Table 2. Raw data from grab samples including grain size and organic carbon OC measurements.

Station >4000 >2000	Sand	<u>Silt</u>	Clay	Porosity	Dry Bulk	Organic		
	um (%) um (%)	Content	Content	Content		Density		
			(%)	<u>(%)</u>	(%)	(k	$(kg/m^3)$	Content
								(%)
ES-02	0.27	0.08	54.3	38.4	7.13	0.59	1077.6	1.22

ES-03	0.003	0.06	90.8	<u>7.11</u>	2.03	0.46	1443.0	0.12
ES-04	0.33	0.01	93.7	4.28	1.69	0.44	1475.1	0.13
ES-07	0.00	0.00	24.4	65.2	10.3	0.71	773.0	1.85
ES-15	0.59	0.11	94.6	<u>3.44</u>	1.29	0.44	1487.4	0.06
ES-17	2.07	0.30	63.7	<u>30.5</u>	4.18	0.55	1185.1	0.10
ES-18	0.60	0.04	80.2	<u>17.4</u>	1.93	0.49	1340.5	0.23
ES-19	0.00	0.04	96.7	<u>2.15</u>	1.12	0.43	1502.2	0.08
ES-21	0.14	0.10	91.4	<u>7.23</u>	1.17	0.45	1450.4	0.06
ES-23	0.08	0.21	93.8	4.68	1.27	0.44	1475.1	0.07
ES-25	0.006	0.01	95.4	3.50	1.05	0.44	1489.3	0.05
ES-27	0.04	0.05	85.0	13.3	1.67	0.48	1384.7	0.07
ES-28	0.00	0.05	85.2	<u>13.2</u>	1.63	0.48	1385.8	0.08
ES-29	0.00	0.02	86.7	<u>11.4</u>	1.87	0.48	1401.4	0.08
ES-31	21.42	9.97	45.8	<u>22.2</u>	<u>1.11</u> 4 <del>.78</del>	0.51	1199.2	0.57
ES-34	2.15	0.71	52.4	<u>39.9</u>	6.15	0.59	1071.1	0.61
ES-35	34.00	0.37	17.3	<u>44.0</u>	<u>4.26</u> 8.83	0.61	793.01	0.62

# 4.2 Relationship between Grain Ssize and Organic Carbon -Content

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A <u>linear regression</u> ordinal least square regression (OLS) was performed to examine the relationship between OC <u>content</u> (%) and the percentage grain size composition of mud. There was a significant positive relationship between OC <u>content</u> and percent mud (p<0.001; R<sup>2</sup>=0.81) (Figure 54), suggesting that, here, % mud content sediment type may be useful as a proxy for OC <u>content</u>, as also observed at many other sites (Burdige, 2007; Hedges & and Keil, 1995; C. A. Hunt et alet al., 2021).

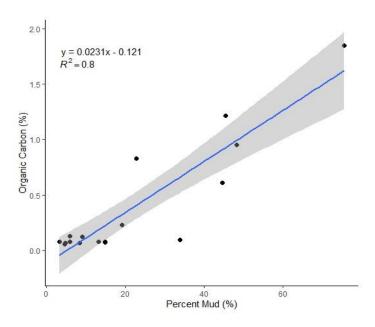


Figure  $\underline{54}$ . Linear regression indicating the relationship between OC and percent mud- $\underline{\text{(top right)}}$ . The grey area represents a 95 percent confidence interval for the slope of the regression line.

# 425 4.3 Substrate Classification Map

Outputs from Random Forest indicated that bathymetry, backscatter, vector ruggedness measure (VRM) and slope were all important for the sediment classification. Figure 65 shows the relative significance importance of the five variables tin the modelo prediction accuracy. Backscatter was the most important variable for predicting sediment type, followed by VRM, slope, bathymetry, and BPI.

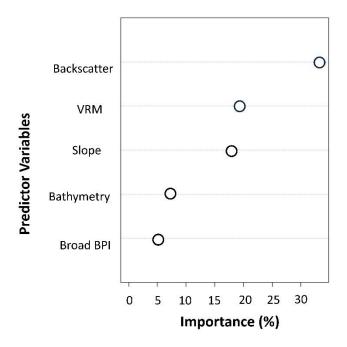


Figure <u>65</u>. Variable <u>ilmportance scores</u>. The importance of predictor variables as <u>indicated by the estimated using</u> Random Forest <u>algorithm</u>. The y axis indicates the variables of the final model, the x-axis indicates the relative percent importance.

The confusion matrix <u>calculated using the OOB observations</u> is presented in Table 3. The A kKappa score was of 0.69 and F1 was 0.91 (Table 4). This ese statistics indicates indicates that there was substantial agreement between observations and predictions of each class, the OOB samples were predicted with high accuracy (87%) and precision (89%), suggesting that the model was able to successfully differentiate soft and hard substrates within the study area.

Table 3. Confusion Matrix of sediment map\_substrate type predictions to assess the level of agreement between the observed drop camera imagery values and the mapped sediment type.

# **Observed Drop Camera Imagery Actual**

#### **Values**

Soft Substrate

		Tiara Saostrate	Soft Substitute
Predicted	Hard Substrate	129	16
<b>Values</b> Mapped Predicted	Soft Substrate	9	44
Sediment Type			

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Hard Substrate

Substrate Classification	<del>Kappa</del>
	0.6909

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The sediment classification map revealed that the hard substrate was the most spatially extensive (178 km<sup>2</sup>) whereas the soft substrate class was smaller, covering approximately 45 km<sup>2</sup> of the study area, corresponding with contiguous patches of relatively low relief seafloor (Figure 8). Sediment grain size from the grab samples revealed grain size average percentiles  $d_{10}=17$  um,  $d_{50}=147$  um and  $d_{90}=1822$  um. This suggests predominantly sandy that most sediments samples were represented by sand, with varying smaller proportions of silt and clay (Figure 7). Two samples were comprised of around 30% coarse substrategravel (>2000 um) (Figure 7).

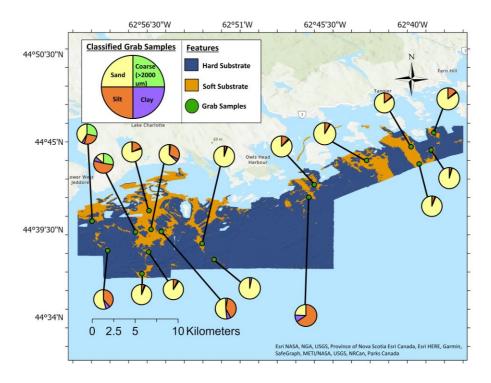


Figure 7. Sediment classification map indicating predicted <u>soft hard-(orange)</u> and <u>hardsoft (blue) substrates substrates</u> (<u>bluepurple</u>). Pie charts depict ratios of sand (yellow), silt (orange red), clay (purple), <u>pebble/cobble (blue)</u>, and <u>coarse</u>

455 <u>gravel (green) found in for each sediment sample collected. (Basemap: Esri NASA, NGA, USGS, Province of Nova Scotia Esri Canada, Esri HERE, Garmin, SafeGraph, METI/NASA, USGS, NRCan, Parks Canada)</u>

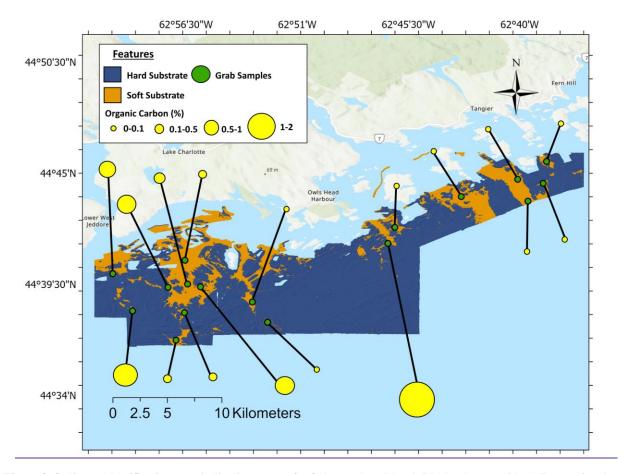


Figure 8. Sediment classification map indicating areas of soft (orange) and hard (blue) substrate (blue). Proportional
symbols of OC indicate the sampled percentage (yellow). (Basemap: Esri NASA, NGA, USGS, Province of Nova Scotia
Esri Canada, Esri HERE, Garmin, SafeGraph, METI/NASA, USGS, NRCan, Parks Canada)

# 4.4 Organic Carbon Density Prediction Maps

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Predicted OC density was high on the west part of the study site near lower west Jeddore and in the middle of the study area near Owls Head Harbour (Figure 9). Cross-validation of the EBK model indicates the accuracy of the OC density predictions in the study site.were Results had ME=-0.27 kg/m³-, and RMSE=4.21 kg/m³ (Table 5), suggesting low bias but also that the magnitude of prediction error was substantial compared to the range of the observed data (e.g., Figure 9).

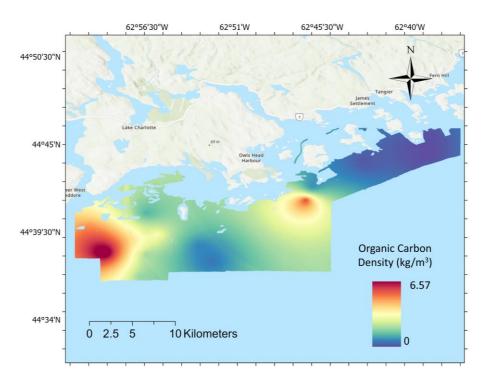


Figure 9. Spatial interpolation of OC using an EBK-method. Low quantities of OC are represented in blue and the high quantities in red. (Basemap: Esri NASA, NGA, USGS, Province of 335 Nova Scotia Esri Canada, Esri HERE, Garmin, SafeGraph, METI/NASA, USGS, NRCan, Parks Canada).

The EBRK model prediction demonstrates suggested that there are high amounts of OC density found in the west and south-west of the study area. There is also a A significant quantity of OC density was predicted slightly eastward near Owls Head Harbour (Figure 10). The lowest amount of OC density is was predicted at the eastern part of the study area with quantities close to zero.

Cross-validation <u>validation</u> indicated of the EBRK model indicated the accuracy of <u>OC density OC (mac)</u> predictions in the soft sediment areas. Results had ME= <u>-0.310.16</u> kg/m<sup>3</sup> and and RMSE= <u>3.527.93</u> kg/m<sup>3</sup>, suggesting slightly higher bias than the EBK model, yet -more accurate predictions (Table 5). These results imply that the ME was close to 0 and the RMSE is small indicating a minimal biased prediction.

Table 6. Outputs from the EBRK model predicting OC density within the soft substrate. Model performance results are given by the ME= Mean error and RMSE= Root Mean Squared Error.

### 485 5. EBRK model details

No of simulation	Model selected	No of variables	ME	RMSE
100	Exponential	5	<u>-0.31</u>	<u>3.52</u>

After calculating the porosity, dry bulk density and measuring percent OC, the predicted mass of OC per unit area (moc) was determined and spatially interpolated. The spatial distribution of moc values is shown in Figure 8. The highest amount of OC concentration was found in the west of the study area along with a small patch of high concentration near Owls Head Harbour.

The EBRK model prediction demonstrates that there are high amounts of OC density found in the west and southwest of the study area. There is also a significant quantity of OC density slightly eastward near Owls Head Harbour (Figure 10). The lowest amount of OC density is the east part of the study area with quantities close to zero.

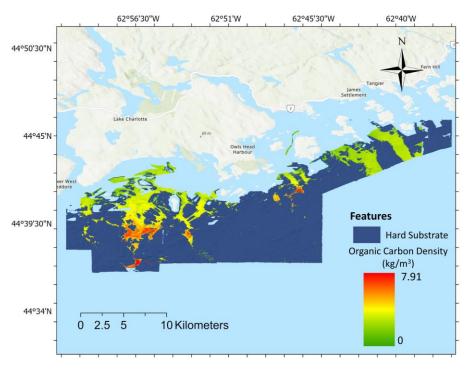


Figure 108. Spatial interpolation of organic carbon OC using the EBRK method. Low quantities of organic carbon OC are represented by green and the high quantities by red. (Basemap: Esri NASA, NGA, USGS, Province of Nova Scotin Esri Canada, Esri HERE, Garmin, SafeGraph, METI/NASA, USGS, NRCan, Parks Canada).

<u>Table 56.</u> Outputs from the EBRK model predicting OC density within the soft substrate. Model performance results are given by the ME= Mean error and RMSE= Root Mean Squared Error.

No of simulation	Model selected	No of variables	<u>ME</u>	RMSE
<u>100</u>	<u>Exponential</u>	<u>5</u>	<u>-0.31</u>	3.52

# 4.5 Organic Carbon Estimates

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Estimates of <u>average OC density</u>, OC stock per pixel and total OC stock <u>were were calculated from for all four three-scenarios (Table 6).</u> : 1) assuming a homogeneous seafloor across the study area (i.e. lacking high resolution

seafloor mapping data); 2) averaging OC across the soft sediment area; 3) estimating spatial heterogeneity of OC across the soft sediment area (Table 6) (supplementary material). In scenario 1, the OC samples were scaled up to the entire study area with an average estimate of 1275 Mt. In scenario 2, when using the high resolution substrate map (assuming negligible OC in the hard substrate regions), the average moc for the study area was calculated at 259 Mt. In scenario 3, after determining the average moc found in the spatially interpolated soft sediment areas, the quantity was estimated at 203 Mt of OC (Table 6). The second two estimations assume that there is no OC within the coarse substrata and is based on the spatial extent of the soft substrate class.

Table  $\underline{6}$ 6. Calculations used to determine the total stock of OC in the mud/sand sediment type and the total stock of OC in the entire study area.

Maps	Average  densitystock of organic carbonOC  per grid cell  (kg/m³)	Average OC stock  per grid cell (kg  per m²)	Total grid cells	Total stock of organic earbonOC in study area (Mt)
Scenario 1: <u>a</u> Assuming homogenous seabed (entire study site)	3.62 (0.804 to 14.31)	1.45 (0.322 to 5.72)	5.58E+07	80,901 (17,949 to 319, 335)
Scenario 2: EBK  mMethod (entire study site)	2.62 (1.08 to 6.57)	1.05 (0.432 to 2.63)		58,406 (24,092 to 146,560)
Scenario 3: Aassuming heterogenous seabed (Soft soft substrate) sediments	3.62 (0.804 to 14.31)	1.45 (0.322 to 5.72)	1.13E+07	16,437 (3,647 to 68,882)
Scenario 4: EBRK  Mmethod (soft substrate) organie earbonOC map	1.45 (0 to 7.91)	0.57 (0 to 3.16)		6,475 (0 to 35,850)

## 515 5 Discussion

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Our study explores how high-resolution spatial models can improve carbon budget estimates. We have described a quantitative spatial model of hard and soft substrate on in a continental shelf environment and determined fourthree estimates of OC-stocktotal moc in the surficial sediments (top 10 cm) of sediments: scaling to the entire study area (scenario 1), interpolating OC density using an EBK model (scenario 2), scaling to only the soft

substrate (scenario 32), and the refininged moc moc within the soft substrate estimated from the an EBRK model (scenario 43). The results demonstrate that as spatial models become more detailed, the accuracy of OC stock estimation increases in accuracy but decreases the overall predicted OC stock. moc estimates increases, while the estimates of organic carbon OC stock decrease substantially.

# 5.1 Evaluation of Sediment Map

The sediment map effectively classified the hard and soft substrate (<a href="kappa=0.69">kappa=0.69</a>)F1=0.91</a>) and significantly refined our understanding of the detailed distribution of the <a href="https://organic.carbon\_OC">organic carbon\_OC</a>. Previous studies have applied similar machine learning modelling approaches with success (Stephens and Diesing et alet al., 2015; Misiuk et alet al., 2019; Mitchell et alet al., 2019; Epstein et alet al. 2023). Our results further demonstrate that this approach is suitable for mapping benthic substrates where high-resolution MBES data sets and suitable sediment ground-truthing are available. Other studies have found the highest POC concentrations are associated with gravelly mud, mud, and sandy mud that the highest mass of POC was associated with gravelly mud, and sandy mud areas (Diesing et alet al., 2017). This agrees with our linear regression OLS analysis that areas of increased OC have a high mud content (Figure 4). The empirical relationship observed between mud content sediment grain size and OC strongly suggests the importance of using substrate maps to precisely estimate the stock of OC.

# 5.2 Variability in Organic Carbon Stocks

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Differences in estimated OC stock suggest that the substrate map was an essential component to this study. Smeaton et al. (2021) has stated note that the seafloor is commonly assumed homogenous (There is commonly an assumption in benthic OC studies studies that the seafloor is homogenous (Smeaton et al., 2021). Since sShelf environments are inherently heterogeneous, and scaling up OC measurements the approach applied here where high\_resolution mapping data are available offers an effective way of obtaining accurate estimates of OC in shelf environments these areas (Snelgrove et alet al., 2018). To improve estimates and better identify how the ocean carbon cycle will be altered by climate change and possibly human activities, carbon studies should embrace the full complexity of the seafloor (Snelgrove et alet al., 2018; Epstein et alet al., 2023). Our study also emphasizes the benefits of high resolution MBES data for such applications, and the need for additional coverage and collection of seafloor mapping data sets in coastal waters where coverage is currently limited (Mayer et alet al., 2018).

The difference in the total  $m_{OC}$  calculated based on the substrate map ( $\underline{16,437}$  and  $\underline{6,475259}$  Mt of OC) versus estimates in the absence of a map ( $\underline{80,901}$  and  $\underline{58,4061275}$  Mt of OC) emphasizes that a spatial component to OC estimations is essential for carbon system models. This difference demonstrates the need to understand the presence of hard substrate at the seabed when calculating carbon stocks as <u>emphasized suggested</u> in recent broadscale carbon modelling studies (Epstein  $\underline{et}$  al., 2023). Currently, global carbon models are oversimplifying carbon processes due to a <u>our</u> lack of information and data on<del>understanding of</del> the complexity of the marine carbon

cycle. For instance, previous studies have examined carbon quantity at the surface of the oceans by analyzing phytoplankton activity using satellite imagery, since there is an assumption that carbon at the surface of the oceans correlates with areas of high carbon storage at the seafloor (Chase et alet al., 2022). This assumption ignores the complexity of carbon moving through the pelagic and benthic regions. Spatially continuous seafloor mapping data are is a step towards improving accuracy in our estimation, which will enhance the ongoing investigations into the marine carbon cycle.

Additionally, the resolution of the seafloor mapping data is important when modeling OC. For instance, by using a 2m-by-2m grid resolution we can interpolate the carbon within the soft substrate using EBRK models. Through the EBRK interpolation of carbon, the <u>carbon stock</u> total moc (6,389203 Mt of OC) was less than the estimates that assume a homogenous soft substrate. The EBRK method indicates that high resolution interpolated models of OC can help to further refine standing stock estimates and provide insight into where the carbon hotspots are within the study area.

The estimates from our study were compared to the paper by Epstein et al. (2024) since they evaluated organic carbon stock in the entire Canadian continental margin, which included our study area. To compare these estimates, we clipped their OC density map to our study site and found that the mean OC density was 7.12 kg/m³ (3.89 kg/m³ to 11.6 kg/m³). This mean OC density is within the range of scenarios 1, 3 (0.804 kg/m³ to 14,31 kg/m³), and 4 (0 kg/m³ to 7.91 kg/m³) presented here. To compare the total OC stock for the study region, we adjusted the depth used by Epstein et al. (2024) from 0.3 m to 0.1 m by dividing their OC stock estimates by 3. The OC stock was 161,552 t (88,183 t to 263,076 t), which is within the range of scenario 1 (17,949 t to 319, 335 t). One reason for the higher estimates in the study by Epstein et al. (2024) could be that no OC measurements within the study region were available in Epstein et al. (2024). Therefore, their model relied on OC data outside this area, which could lead to error. Furthermore, an underrepresentation of zero values in the response data could lead to an overestimation of organic carbon standing stocks in their study, as zero values are unlikely to be predicted from model outputs. The comparison between both studies highlights the importance of sediment classification maps when estimating sedimentary OC stock; knowing the extent of bedrock can reduce the overestimation of OC content substantially.

# **5.3 Organic Carbon Maps**

When comparing the EBK and the EBRK carbon maps, there were some similarities and differences. Both maps indicate a hotspot near Owls Head Harbour and low OC density on the eastern side of the study sitearea. Yet, the EBK map shows a large area of high OC density on the west side of the study area, whereas the EBRK model has a smaller area slightly east of that location. These differences between the models emphasize that the EBK model could have some inaccurate interpolation due to the limited sediment samples in the study sitearea. In contrast,

the EBRK model was performed in the soft substrate, where all the samples were distributed, with fewer data gaps.- The EBK model indicates that without high-resolution seafloor mapping data, you can obtain a general understanding of OC hotspots. However, the EBRK model can provide a more precise understanding of the spatial variability of OC density in the study site.

Both maps suggest high OC densities associated with locations further offshore (Figures 9 & 10) and within sediments containing increased amounts of silt and sand (Figures 7 & 8). Based on previous evaluations of the study area, inshore sediment is often comprised of bedrock with patchy sand and gravel, whereas further offshore, there is thick glacial marine mud over bedrock (Fisheries and Oceans Canada, 2019). This geomorphology could be whythe cause of there is higher OC density further offshore. The cause of increased OC content near Owls Head Harbour remains uncertain, lacking any aquaculture or substantial runoff from nearby agriculture. However, the ESI has substantial kelp and eelgrass beds; and-future research may explore relationships between these environments and OC.

#### 5.4 Limitations of the Study

The lack of dry bulk density measurements for the OC stock calculations was a major limitation of this study. The use of a dry bulk density equation derived from a previous study could introduce error into calculations based on regional geological differences. Only two seabed sediment classes were mapped here, which does not represent the actual complexity of substrate types within the ESI. Preliminary Random Forest model runs that incorporated additional sediment classes, showed high error and poor performance, likely due to the difficulty in accurately determining sediment types from a small number of subsea video samples. We emphasizes challenges associated with differentiating complex substrate classes that have been noted in previous similar studies (e.g., Diesing et al., 2020).

We have also assumed here that there is no OC in the hard substrate. The hard substrate class included more than bedrock, with regions of mixed sediment such as gravelly mud, visible in the subsea video which could contain some OC content. Thus, improving the sediment classification map to include more complex substrates could improve the OC stock estimates further. The limited number of OC samples may have skewed the interpolation of OC density since the data points were not uniformly distributed within the areas of soft substrate. We therefore recommend higher sampling densities for, future OC studies.

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These limitations highlight the challenges of carbon modelling on the seafloor and the need for further research into evaluating the correct procedure for utilizing sediment classification maps when predicting OC stock. Furthermore, there is persistent uncertainty surrounding how much surface particulate OC (POC) reaches the seafloor and and-the spatial distribution of the sinks of this material. Thus, future carbon studies should evaluate benthic-pelagic -coupling and the impact it has on OC stocks.

## 5.51 Future Implications of Organic Carbon Mmodels

Marine spatial planners are trying to manage the seabed in a sustainable manner and high resolution regional-scale OC mapping data could be a practical option to help identify vulnerable C stores and hotspots, and to determine how these areas may be altered due to environmental change and anthropocentric anthropogenic activities (Hunt et alet al., 2021). Marine protected areasMPAs have been defined as regions that conserve marine resources, ecosystem services or cultural heritage (Mayr et alet al., 2010). High-resolution seafloor OC models could help redefine MPAs and allow them to incorporate areas of high carbon stocksequestration. It is important to recognize sediments as long-term carbon sinks that provide climate regulation services.

It is challenging to measure how <u>human-anthropocentric</u> activities like bottom trawling are impacting the seabed and how they influence OC without understanding of the natural processes of marine carbon cycling. Studies that examine OC spatially and its connections to seafloor composition are a crucial component to piecing together the natural marine carbon cycle, which can help determine if the amount of remineralization occurring from human activities will have a substantial impact on climate. Even with a relatively limited number of OC samples, this study demonstrates that high-resolution seafloor substrate maps and spatial OC models are critical to understanding the spatial heterogeneity of <u>organic carbonOC</u> on the seafloor.

## **6 Conclusions**

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In this study, we generated a high-resolution sediment map that accurately captured the spatial complexity and distribution of broad sediment types in the ESI area. Through the four scenarios for estimating OC stocks, we demonstrated that seafloor sediments are a good high-resolution proxy that enable accurate estimation of OC stock in the area, and that information (or lack of information) regarding the spatial heterogeneity of the seafloor substrata substantially influences estimates of OC stock (ranging from 80,901 - 6,475 t of OC). These results emphasize that further research should explore high-resolution multibeam echosounder data in determining OC rich hotspots to improve our understanding of the role that benthic systems play as global carbon stores, and how management of these systems can contribute towards climate change management strategies and marine climate policy. We have presented a method that utilizes high resolution sedimentary maps and spatial models to quantify OC estimates. We show that examining the spatial heterogeneity of carbon content within the soft substrate can improve the estimates of organic carbon content. These results emphasize that further research should explore high resolution multibeam echosounder data in determining OC rich hotspots to help support management measures. In this study, additional ground truthing is necessary to create more precise measurements of OC and to further evaluate which sediment type is most significant for OC storage. Despite the limited dataset, highresolution sediment classification maps are necessary to improve our understanding of spatial patterns of OC. Additional surficial sediment OC distribution studies are necessary to improve seabed management and marine policy.

## **Data Availability**

Bathymetry data was obtained from the Canadian Hydrographic Service (CHS) NONNA Portal - <a href="https://data.chs-shc.ca/login">https://data.chs-shc.ca/login</a>. All other data used in this study is in the supplementary material or available upon reasonable request.

# **Competing Interests**

One of the authors is a members of the editorial board of *Biogeosciences*.

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