Response to Anonymous Referee #1 Interactive comment on "Drivers and modelling of blue carbon stock variability" by Carolyn J. Ewers Lewis et al.

We thank Anonymous Referee #1 for their thoughtful comments (see Interactive Comment published on 01 Oct 2019 by Anonymous Referee #1). Below, we have responded to each comment and included changes to the manuscript text based on the feedback from Anonymous Referee #1.

In this paper, the authors look to create a framework for modelling shallow carbon stocks (0-30cm) in vegetated coastal ecosystems. They use a combination of geomorphological, anthropogenic and ecological variables, combined with carbon stocks from a large number of shallow cores (n = 287) to construct the model and estimate carbon stocks for a region in southern Australia. The model could account for $\sim 49\%$ of the variability in shallow carbon stocks, with plant community being the strongest predictor in the model.

Generally this is an interesting paper, but I have some points that should be clarified.

1. Can these shallow carbon stocks be considered "blue carbon"? There is growing evidence that carbon within the surface layers is still highly susceptible to degradation. A typical profile of carbon down the soil profile in these vegetated habitats show a decline with depth until reach a pseudo steady state. Do the authors have some deeper cores to show that carbon density in the top 30 cm is representative of long-term sequestration? Along these same lines — some of the plant communities looked at in this study (e.g. mangroves) can put "new" carbon into depths below 30cm through root production. The combination of these factors may lead to erroneous definition of carbon stocks as "blue carbon".

"Blue Carbon" is broadly defined in the scientific literature as organic carbon captured and stored in ocean ecosystems, especially mangrove forests, seagrass meadows, and salt marshes (Macreadie et al., 2019; Mcleod et al., 2011; Nellemann et al., 2009), and includes carbon pools in the living plant biomass (above- and belowground), dead plant biomass (e.g. leaf litter, dead wood), and sediments (Mcleod et al., 2011). Though carbon stocks associated with sediments are often considered more "permanent" relative to living biomass, both biomass and sediments (including shallow portions of sediments) are defined as "blue carbon".

Across depths, sediments may span a continuum of carbon quality from "labile" to "recalcitrant" or "refractory" based on a number of criteria, which involve more than depth alone (Lovelock et al.). By these criteria, virtually all carbon is susceptible to degradation to some degree. However, this does not negate the fact that carbon present in surface sediments represents the offsetting capacity of blue carbon sediments (potential long-term carbon storage), and is an important measure of carbon that may be more vulnerable to remineralization following disturbance.

To be very clear that we are not referring to the entire sediment C pool, we have altered our wording throughout the entire paper to specify "shallow sediment C stocks" in place of "C stocks" or "sediment C stocks". This includes the title, which has been changed from *Drivers and modeling of blue carbon stock variability* to *Drivers and modeling of blue carbon stock variability in shallow sediments of southeast Australia*, as well as section headers and within the entirety of the text.

We have added text to the methods to explain our rationale for the study design, including the use of 30 cm cores:

Though it is common in the literature to sample to 1 m deep in blue C sediments, the sampling protocol used for collecting these data (Ewers Lewis et al., 2018) was designed to maximize spatial coverage of shallow sediment C samples rather than sample entire sediment profiles (which may extend well beyond 1 meter deep). Greater spatial coverage allowed us to test the relationships between a variety of potential drivers and surface sediment C stocks on both fine and broad scales.

We have also added text to the discussion to explain why the top 30 cm are the most relevant sediments for assessing the impact of contemporary environmental factors on shallow sediment C stocks (please see new discussion text under our response to your second comment, which directly addresses this point).

Thank you for bringing up the points about "new" C at depth and the relationship between shallow and deep C stocks. We recognize that surface stocks may not always represent stocks at depth, and also that processes happening at the surface can impact deeper sediments, and have added text about this topic to the discussion:

Modern-day factors influencing vegetation can also have impacts on C stocks deeper than the sediments we measured. The effects of underground biomass on sediment C stocks can extend beyond the top 30 cm, and in fact new C inputs and active C cycling by microbial communities can occur as deep as underground roots extend (Trumbore, 2009). These new C additions (and fluxes) at depth fall outside the general pattern of sediment C decay down-core in vegetated ecosystems (Trumbore, 2009) which has previously allowed for linear or logarithmic regressions to be used to extrapolate 1-m deep C contents from shallow (e.g. 30-50 cm deep) sediment C data (Macreadie et al., 2017a; Serrano et al., 2019). The activity of underground biomass and microbes at depth, when considered over space and time, may account for large C fluxes. The influence of anthropogenic activities, such as land use changes, on these processes via impacts to vegetation may largely go unnoticed based on current methods (Trumbore, 2009), both in this study and in blue C stock assessments on larger scales. We suggest further research to understand the dynamics of active C cycling at sediment depths traditionally considered stable.

Later in the discussion we have also added:

It is important to emphasize here that total sediment depths in blue C ecosystems can vary greatly, and are commonly deeper than 30 cm. Blue C ecosystems can have sediments up to several meters deep (e.g. Lavery et al., 2013; Scott and Greenberg, 1983), suggesting the estimates of C stocks measured here are conservative. In spite of these limitations, surface sediment C stock estimates give us valuable knowledge about the sediment C pool most vulnerable to disturbance and how it may be impacted by environmental drivers.

2. I wonder how applicable the use of contemporary variables is to the assessment of carbon stocks that are assumedly a function of conditions over the last several decades. This might be worth considering, and could explain the 50% of variability unaccounted for by the model outcomes. For example, community composition is changing in temperate regions such as the study area in this paper. Assuming a sediment accretion rate similar to SLR (\sim 3mm/yr) – the 30cm soil profile used in the model integrates \sim 100 years of environmental, ecological and anthropogenic conditions. Could this discrepancy in the temporal scale used for the predictor variables and carbon stock accumulation be an issue?

Thank you for this comment. We have added paragraphs to the discussion to describe the challenges associated with matching temporal time points in the sediments to contemporary variables and how the depth we chose and regional accretion rates might shine light on some of these issues:

We also aimed to maximize our ability to capture relationships between contemporary drivers and sediment C stocks by utilizing sediment C stock data to only 30 cm deep, a sediment horizon more directly impacted by recent environmental conditions compared to deeper stocks due to age. Based on previously estimated sediment accretion rates in blue C ecosystems in the study region (averaging 2.51 to 2.66 mm year-1 in tidal marshes (Ewers Lewis et al., 2019; Rogers et al., 2006a) and 7.14 mm year-1 in mangroves (Rogers et al., 2006a)), the top 30 cm of sediment represents roughly ~113-120 years of accretion in Victorian tidal marshes and ~42 years of accretion in Victorian mangroves. These time scales suggest sediments depths utilized in this study are more appropriate for assessing the impacts of modern environmental conditions on sediment C stocks compared to meter-deep stocks, which can be thousands of years old (e.g. Ewers Lewis et al., 2019). Using shallow sediment C stocks also allows us to be more confident that the vegetation present now has been there during the time of sediment accretion, unlike deeper sediments that are thousands of years old and for which it is difficult to determine what vegetation, if any, was present at the time of accretion.

The variability in shallow sediment C stocks that could not be explained by our modeling may also be related to the inherent challenges surrounding spatial and temporal matching of driver proxies and sediment C stock measurements; the relationship between shallow sediment C stocks and contemporary environmental settings can be represented more accurately for some variables over others.

Ecosystem type was a relatively powerful predictor of shallow sediment C stock variability in our study and this is likely due, in part, to the direct relationship between vegetation and surface sediments. In most vegetated ecosystems, the majority of underground plant biomass and microbial activity exists within the top 20 cm of soils (Trumbore, 2009). For saltmarsh, it has been demonstrated that the top 30 cm of sediment are directly impacted by current vegetation (Owers et al., 2016). Therefore, using shallow sediment C stock measurements allowed us to take advantage of the direct relationship between vegetation and C stocks to explain variability in surface sediments.

The portion of recently accreted sediments influenced by contemporary anthropogenic drivers is harder to identify than that of ecosystems. Based on estimated accretion rates for this region from the literature (Ewers Lewis et al., 2019; Rogers et al., 2006b), 30 cm deep sediments would have taken an average of ~80 years to accumulate in Victoria (~117 years in tidal marsh and ~42 years in mangroves). Though sedimentation rates vary over time, they are relatively steady in comparison to changes in anthropogenic drivers, such as land use change. This means that modern day maps of land use, though useful for looking at the general impact of various activities, may be more useful for relating to variability in sediment C stocks when the data is assessed at a finer resolution. For example, comparing land use area data across various time periods with C densities in aged bands of sediment could help capture the pulse effects of sudden land use changes in narrower sediment horizons representative of the same time periods. In this study, the effects of land-use change may have been too diluted within the 30-cm horizons to relate to impacts on sediment C stock.

3. It would be good to see some kind of power analysis to assess whether the sample size is appropriate. I note there are R packages to do this for this kind of modelling approach.

Using a simpler modelling approach, a power analysis was conducted on this dataset for a separate study using the SIMR package. This analysis showed that a power of 80% was reached across all ecosystems within the sample sizes of this dataset (Young, M. A., Macreadie, P. I., Duncan, C., Carnell, P. E., Nicholson, E., Serrano, O., Duarte, C. M., Shiell, G., Baldock, J. and Ierodiaconou, D. 2018. Optimal soil carbon sampling designs to achieve cost-effectiveness: a case study in blue carbon ecosystems, Biol. Lett., 14(20180416), doi:10.1098/rsbl.2018.0416).

Though we like the idea of running a power analysis for this more complex modelling approach, we have not been able to find an R package that is specifically compatible with the information theory multimodel approach we have taken. Though there are R packages for doing power analysis on glmm/glmer models created in the R package lme4 (e.g. SIMR), we have not found a package compatible with averaged models that have been generated by dredging and averaging general linear mixed effects models (made with the R package MuMIn).

To clarify why we used the more complex modelling approach (AICc with model averaging), we added the following paragraph to the methods, which explains the better accuracy of predictions generated from averaged models compared to traditional approaches utilizing a single "best" model (e.g. glmer in the lme4 package):

To identify drivers of shallow sediment C stock variability and create the best predictive model of shallow sediment C stocks to 30 cm deep we utilized a multi-step process based on an information theoretic approach and multimodel inference (Figure 3). Traditional approaches have relied on identification of the "best" data-based model; however, information-theoretic approaches allow for more reliable predictions through utilization of multiple models, especially in cases where lower ranked models may be essentially as good as the best (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). Further, information theoretic model selection has been demonstrated to provide significant advantages for explaining phenomena with more complex drivers (Richards et al., 2011). Here, we first looked broadly at our variables of interest by narrowing down to the best models containing all possible variables ("global" models, as explained below) using AICc (Akaike information criteria, corrected for small sample size) to explain the variability observed in the training dataset (70% of total C stock data; Symonds and Moussalli, 2011). From there, we identified which variables within the best global models best explained the observed variability in C stock data in order to remove unnecessary variables from the model equation (through the process of "dredging" and selecting the best subset, explained in detail below). The validity of removing unnecessary variables from the model is supported by the concept of parsimony, which suggests models more complicated than the best model provide little benefit and should be eliminated (Burnham and Anderson, 2002; Richards, 2008). The best subset of models generated from the global models ("dredge products") were selected based on delta AICc<2, which are viewed as essentially interchangeable with the best model (Symonds and Moussalli, 2011). Each subset of best models was used to generate an averaged model, which was tested by generating predictions of C stocks for a reserved (30%) subset of the dataset. The best performing model was used to generate a predictive map of C stocks to 30 cm deep for mapped blue C ecosystems in Victoria.

Minor comments: Title – see comment 1 above, I am not sure the paper really assesses blue carbon due to the shallow sediment profile analysed. Also as this is a regional study, I think it might be appropriate to include something to clarify that in the title.

Thank you for this feedback. We have changed the title to better reflect the specifics of our study by changing it from "Drivers and modelling of blue carbon stock variability" to "Drivers and modelling of blue carbon stock variability in shallow sediments of southeast Australia".

Abstract – Aims 1 and 2 should include the term regional, as the paper doesn't really produce a model that is applicable beyond the region of the study area

We have updated the aims as suggested by adding the phrase "... in southeast Australia" to each of them (in both the abstract and introduction).

Abstract – last sentence. Without testing the validity of the modelling method to other regions, I am not sure this statement can be made. Suggest removing this statement or validating the modelling method elsewhere.

Thank you for this comment. We have modified this sentence to reflect the need to test the validity of using the modelling methods elsewhere.

The sentence previously read:

Globally these methods can be applied to identify relationships between environmental drivers and C stocks to produce predictive C stock models at scales relevant for resource management.

And has been changed to:

We recommend these methods be tested for applicability to other regions of the globe for identifying drivers of C stock variability and producing predictive C stock models at scales relevant for resource management.

Introduction Ln 40-43 Stocks of carbon are not directly related to greenhouse gas inventories which are based on flux rates.

We are aware of this, but also recognize that knowledge of stocks enables for more accurate estimates of fluxes, which are related to stocks (e.g. the maximum amount of carbon that can be remineralized and converted to CO2 if subjected to disturbance is the total stock present). We have added "and fluxes" to this sentence to ensure we convey the importance of fluxes, which relate C stocks and greenhouse gas inventories to one another:

With the current momentum for including blue C ecosystems in global greenhouse gas inventories, there is a need to quantify the magnitude of C stocks and fluxes...

Line 86 – Best not to start a new paragraph with "However" as this is a conjunction for connecting sentences within a paragraph.

This has been removed.

Materials and methods Ln 153-159 Can the authors expand upon the methods used, including accuracy of analytical methods, the number of samples analysed by FT-MIR vs EA, and the results of cross validation between these 2 methods.

Thank you for this request. We have added more details, such as those requested, to make our methods more transparent and easier to understand without referencing the original C stock paper, as follows:

Sediment C stocks to 30 cm deep (referred to throughout the paper as "shallow sediment C stocks") were estimated for 287 sediment cores from 96 blue C ecosystems across Victoria in southeast Australia (Ewers Lewis et al., 2018; Figure 1). Full details of sample collection, laboratory analyses, and calculations of C stocks can be found in Ewers Lewis et al. (2018). Briefly, three replicate sediment cores (5-cm inner diameter) were taken in each ecosystem (n=125 in tidal marsh, n=60 in mangroves, and n=102 in seagrasses). Once back in the laboratory, samples were taken from three depths (0-2, 14-16, 28-30 cm) within each core. Samples were dried at 60°C until a consistent weight was achieved, then ground. Dry bulk density (DBD) was calculated as the dry weight divided by the original volume for all samples.

Based on the protocols by Baldock et al. (2013), a combination of diffuse reflectance Fourier transform mid-infrared (MIR) spectroscopy and elemental analysis via oxidative combustion using a LECO Trumac CN analyzer was used to determine organic C contents of all samples. Previous studies have demonstrated the accuracy of using MIR to estimate organic C stocks of sediments (Baldock et al., 2013; Van De Broek and Govers, 2019; Ewers Lewis et al., 2018). MIR spectra were acquired for all samples, then a subset of 200 representative samples was selected based on a principle components analysis (PCA) of the MIR results utilizing the Kennard-Stone algorithm. Gravimetric contents of organic carbon were measured directly in the laboratory for the 200-sample subset (Baldock et al. 2013). A partial least squares regression (PSLR) was created using a Random Cross Validation Approach (Unscrambler 10.3, CAMO Software AS, Oslo, Norway) and used to build algorithms to predict square root transformed total carbon, total organic carbon, total nitrogen, and inorganic carbon for the entire dataset. The PSLR model was evaluated based on parameters from the chemometric analysis of soil properties (Bellon-Maurel et al., 2010; Bellon-Maurel and McBratney, 2011), and the relationship between measured and predicted values was assessed based on slope, offset, correlation coefficient (r), Rsquared, the root mean square error (RMSE), bias, and the standard error (SE) of calibration (SEC) and validation (SEP; see Ewers Lewis et al., 2018 for full details). R-squared values for all square root transformed variables were ≥ 0.94 .

Sediment C stocks were calculated based on Howard et al., 2014. Organic C density (mg C cm⁻³) was calculated by multiplying organic C content (mg C g⁻¹) by DBD (g cm⁻³). Linear splines were applied to each core to estimate C density for each 2 cm increment within the 30 cm core, then C densities for each interval (measured and extrapolated) were summed and converted to Mg C ha⁻¹ to estimate total stock down to 30 cm deep for each core location...

Ln 168 – 172 Assigning catchment characteristics to estuarine communities makes sense, but looking at Figure 1 most samples were collected from coastal embayment's. How might the influence from multiple catchments affect the model?

The averaged model that we used to make the C stock prediction maps included ecosystem type, slope, and distance to coast, none of which we would expect to be substantially affected by the influence of multiple catchments. The influence of anthropogenic activities on surface sediment C stocks in locations receiving inputs from multiple locations, on the other hand, would be very difficult to track, and could have affected our ability to identify a relationship between anthropogenic drivers and C stock variability in our models. We have added the following text to the discussion to address this point, as follows:

Spatially, anthropogenic variables are also difficult to assign to particular ecosystem locations or depths. Many blue C ecosystems in Victoria are located on coastal embayments and receive inputs from multiple catchments, making the influence of specific areas of land-use or population changes difficult to track to specific ecosystem locations.

Ln 182-187 What was the vertical accuracy of the DEM? Considering the small elevation gradients across the intertidal zone, this is important.

Thank you for this question. We have added the following text to the methods section description of the elevation data, as follows, to clarify the accuracy of the DEM:

The elevation data are a composite product that integrated terrestrial and bathymetric LIDAR as well as multibeam sonar data. The vertical accuracies of the data varied with sensor setup for acquisition: ± 10 cm at 1 sigma (68% conf. level) in bare ground for topographic LIDAR data (for the majority of our dataset), ± 50 cm for bathymetric LIDAR, and $\pm < 10$ cm for multibeam sonar data.

Ln 210 – Is the 2001 data the most recent, or is this a typo?

This should say 2011 – thank you for bringing this to our attention.

Ln 215 Why was 30cm chosen as the depth representative of blue carbon stocks (see also earlier comment)? Can the authors add a few comments about this?

Thank you for this question. We have added a paragraph in the methods to address this topic and have also added a paragraph about the implications of measuring to 30 cm in our discussion section.

Added to methods:

Though it is common in the literature to sample to 1 m deep in blue C sediments, the sampling protocol used for collecting these data (Ewers Lewis et al., 2018) was designed to maximize spatial coverage of shallow sediment C samples rather than sample entire sediment profiles (which may extend well beyond 1 meter deep). Greater spatial coverage allowed us to test the relationships between a variety of potential drivers and surface sediment C stocks on both fine and broad scales.

Added to discussion:

We also aimed to maximize our ability to capture relationships between contemporary drivers and sediment C stocks by utilizing sediment C stock data to only 30 cm deep, a sediment horizon more directly impacted by recent environmental conditions compared to deeper stocks due to age. Based on previously estimated sediment accretion rates in blue C ecosystems in the study region (averaging 2.51 to 2.66 mm year-1 in tidal marshes (Ewers Lewis et al., 2019; Rogers et al., 2006a) and 7.14 mm year-1 in mangroves (Rogers et al., 2006a)), the top 30 cm of sediment represents roughly ~113-120 years of accretion in Victorian tidal marshes and ~42 years of accretion in Victorian mangroves. These time scales suggest sediments depths utilized in this study are more appropriate for assessing the impacts of modern environmental conditions on sediment C stocks compared to meter-deep stocks, which can be thousands of years old (e.g. Ewers Lewis et al., 2019). Using shallow sediment C stocks also allows us to be more confident that the vegetation present now has been there during the time of sediment accretion, unlike deeper sediments that are thousands of years old and for which it is difficult to determine what vegetation, if any, was present at the time of accretion.

Results Throughout the results and the discussion the error associated with all estimates should be included. This error should combine model error and uncertainty in the spatial coverage of habitat areas.

Model error is presented through model validation in our study. We reserved 30% of our C stock dataset to test the accuracy of the predictions generated by each averaged model (as described in sections 3.2 of the results and displayed in Figure S3). These results showed that our best model explained ~half the observed variability in C stocks (Adj R-sq=0.4881; averaged model 2). We have clarified the errors associated with our model predictions by providing further details on the outputs of our validation step, which utilized the reserved dataset (30% of our original carbon dataset) for assessing the prediction power of each averaged model. Using linear regression, we compared predicted values from each averaged model to actual measured C stock values for reserved dataset. The complete outputs for this analysis have been added to the results section 3.2 ("Model validation"), as follows:

Linear regressions of predicted versus actual measured shallow sediment C values produced the following outputs for each averaged model: averaged model 11, residual standard error (RSE)=38.36 on 84 degrees of freedom (df), adjusted R-squared (R-sq(adj))=0.4868, F-statistic(F-stat)=81.63 on 1 and 84 df, p-value=5.044e-14; averaged model 5, RSE=38.51, R-sq(adj)=0.4829, F-stat=80.39 on 1 and 84 df, p-value=6.953e-14; averaged model 2, RSE=38.32, R-sq(adj)=0.4881, F-stat=82.06 on 1 and 84 df,

p-value=4.517e-14; averaged model 10, RSE=39.67, R-sq(adj)=0.4514, F-stat=70.93 on 1 and 84 df, p-value=8.645e-13; averaged model 4, RSE=39.84, R-sq(adj)=0.4465, F-stat=69.58 on 1 and 84 df, p-value=1.254e-12; averaged model 1; RSE=39.48, R-sq(adj)=0.4566, F-stat=72.43 on 1 and 84 df, p-value=5.73e-13; averaged model 7, RSE=39.29, R-sq(adj)=0.4618, F-stat=73.94 on 1 and 84 df, p-value=3.81e-13.

We have also been more explicit about the error associated with the averaged model used to generate our state-wide shallow sediment C stock predictions by adding text to the results section on modelled shallow sediment blue C stocks (section 3.3) as follows:

We estimated a total of over 2.31 million Mg C stored in the top 30 cm of sediments in the ~68,700 ha of blue C ecosystems across Victoria (Table 4; Figure 5). This estimate is based on predictions from our best averaged model that utilized ecosystem type as the ecological variable (averaged model 7), which explained 46.18% of observed variability in C stock data and had an RSE of 39.29.

Due to the complexity of our multimodel approach, we did not include standard errors in predicted values for the entire region of Victoria due to the combination of using an averaged model and having random effects (there are no compatible R packages, to our knowledge).

For spatial coverage, there is no data available to estimate uncertainty in habitat area that gives the specificity we would need to alter our predictions (i.e. we would have to know the exact locations due to the spatially explicit nature of the model). We used the most recent and complete maps available for each ecosystem for Victoria, which did not include specific locations where coverage was questionable. The main uncertainty stems from the potential changes in habitat area since the time of mapping or errors in mapping, and we cannot measure that error because no more-recent maps are available.

Discussion Ln 434 As with the use of "however" to start a paragraph, "Further" should also be avoided.

We thank you for the suggestion. We have moved this sentence up to be included in the previous paragraph, and moved the remainder of the paragraph to an earlier section of the text to improve flow.

452-454 See earlier comment regarding applicability of this modelling framework to global assessments.

We understand your point and have edited the text to reflect that this framework needs testing in other regions for applicability.

The text previously read:

Globally, these methods are applicable for identifying relationships between potential environmental drivers and C stocks for creating predictive C stock models in blue C ecosystems at scales relevant for resource management applications.

And has been changed to:

We recommend these methods be tested in other areas of the globe to determine whether they may be applicable for identifying relationships between potential environmental drivers and C stocks for creating predictive C stock models in blue C ecosystems at scales relevant for resource management applications in other regions.

Data availability – I would like to see all of the underlying data made publically available rather than just the model outputs. These data can easily be attached as a supplementary.

To improve transparency and accessibility of the data both utilized and produced in this study we have added the following table to the supplements (below) with a reference to it in the methods section:

Complete details of data availability for inputs and outputs of our models can be found in supplementary Table S10.

Any modifications made to these data for producing our models are described in the methods section of this manuscript.

Please also note the data produced in this study (R code and model prediction rasters) will be uploaded to an online repository upon acceptance of this manuscript for publication, due to both the large size of the raster files (making them too large for supplementary information) and the intellectual property associated with this work as part of the first author's Ph.D. dissertation. We have created a project Dataverse to host the data in the Harvard Dataverse open access repository (https://dataverse.harvard.edu/) which will be published upon manuscript acceptance and accessible through a digital object identifier (DOI) that we will add to the manuscript (both in Table S10 and in the "Data Availability" section of the manuscript).

Table S10. Data availability

| Data Item | Description | Data Source & Location |
|----------------|--|---|
| Carbon Stock | Percent organic carbon and dry bulk density | Ewers Lewis et al. 2018; Deakin Research |
| Dataset | data for sediment sampled to 30 cm deep in | Online |
| | 96 blue carbon ecosystems (saltmarshes, | Deakin University's Data Repository |
| | mangrove forests, and seagrass meadows) | https://dro.deakin.edu.au/view/DU:300934 |
| | across Victoria, Australia. | <u>05</u> |
| Ecosystem | 1. Mangrove areal extent in Victoria, | 1. Boon et al. 2001; OzCoasts Australian |
| Extent Vectors | Australia; saltmarsh areal extent and | Online Coastal Information, Victorian |
| | ecological vegetation classes in Victoria, | Saltmarsh and Mangrove Vegetation Maps |
| | Australia. | https://ozcoasts.org.au/geom_geol/vic/Salt |
| | 2. Seagrass areal extent in the major bays and | marsh/Master |
| | estuaries of Victoria, Australia. | 2. Available from: |
| | a. Port Phillip Bay | a. Ball et al., 2014; Blake and Ball, 2001a |
| | b. Western Port Bay | https://discover.data.vic.gov.au/dataset/por |
| | c. Corner Inlet and Nooramunga | -phillip-bay-1-25-000-seagrass-2000 |
| | d. Gippsland Lakes | b. Blake and Ball, 2001b |
| | e. Minor Inlets of Victoria | Distribution of Seagrass in Western Port |
| | | in 1999 |
| | | https://discover.data.vic.gov.au/dataset/dist |
| | | ribution-of-seagrass-in-western-port-in- |
| | | <u>1999</u> |
| | | c. Roob et al., 1998 |
| | | Corner Inlet Seagrass 1998 |
| | | https://discover.data.vic.gov.au/dataset/cor |
| | | ner-inlet-seagrass-1998 |
| | | d. Roob and Ball, 1997 |
| | | Gippsland Lakes Seagrass 1997 |
| | | https://discover.data.vic.gov.au/dataset/gip |
| | | psland-lakes-seagrass-1997 |
| | | e. Blake et al., 2000 |
| | | Anderson Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/and |
| | | erson-inlet-seagrass-1999 |
| | | Tamboon Inlet Seagrass 1999 |

| | 1 | 1.0 //12 1.0 //12 |
|--------------|---|---|
| | | https://discover.data.vic.gov.au/dataset/tam |
| | | boon-inlet-seagrass-1999 |
| | | Wingan Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/win |
| | | gan-inlet-seagrass-1999 |
| | | Shallow Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/shal |
| | | low-inlet-seagrass-1999 |
| | | Mallacoota Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/mal |
| | | lacoota-inlet-seagrass-1999 |
| | | Sydenham Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/syd |
| | | enham-inlet-seagrass-1999 |
| Elevation | A gap free digital elevation model (DEM) for | Victorian Coastal Digital Elevation Model |
| Raster | the coastal region of Victoria, Australia, that | (VCDEM 2017) |
| Kastei | combines 2.5 m and 10 m DEMs. | , |
| | combines 2.5 m and 10 m DEIVIS. | https://vmdp.deakin.edu.au/geonetwork/srv |
| | | /eng/metadata.show?uuid=8d3ccf63-ee85- |
| | | 41cd-917e-933624a50b2e |
| Freshwater | Location of channels and other freshwater | Vicmap Hydro 1:25,000 |
| Vectors | objects in Victoria, Australia. | Victorian Government Data portal |
| | | https://discover.data.vic.gov.au/dataset/vic |
| | | <u>map-hydro-1-25-000</u> |
| Coastline | Line delineating the coastline of Victoria, | Victorian Coastline 2008 |
| Vector | Australia. | Victorian Government Data portal |
| | | https://discover.data.vic.gov.au/dataset/vict |
| | | orian-coastline-2008 |
| Lithology | Rock types across Victoria, Australia. | Geomorphology of Victoria |
| Vectors | , | Victorian Government Data portal |
| , 551515 | | https://discover.data.vic.gov.au/dataset/geo |
| | | morphology-of-victoria |
| | | morphology of victoria |
| Land Use | Primary land use designations for land | Victorian Land Use Information System |
| Vectors | parcels in Victoria, Australia. | 2014/2015 |
| VECTOIS | parceis iii victoria, Australia. | |
| | | Victorian Government Data portal |
| | | https://discover.data.vic.gov.au/dataset/vict |
| | | orian-land-use-information-system-2014- |
| D 1.1 | | 2015 |
| Population | Human population data for Victoria, | Australian Population Grid, 2011 |
| Raster | Australia. | Australian Bureau of Statistics |
| | | https://www.abs.gov.au/AUSSTATS/abs@ |
| | | .nsf/Lookup/1270.0.55.007Main+Features1 |
| | | 2011?OpenDocument |
| R Code | R code used to identify drivers and model | This study. |
| | carbon shallow sediment carbon stocks. | DOI: |
| | | (TBD upon acceptance for publication) |
| | | |
| Model Output | Shallow sediment (to 30 cm deep) carbon | This study. |
| Raster | stock predictions in blue carbon ecosystems | DOI: |
| | (seagrass meadows, mangrove forests, and | (TBD upon acceptance for publication) |
| | saltmarshes) in Victoria, Australia | (122 spon seceptance for paonession) |
| | baranaronco, in victoria, Austrana | |

Response to Anonymous Referee #2 Interactive comment on "Drivers and modelling of blue carbon stock variability" by Carolyn J. Ewers Lewis et al.

We thank anonymous referee #2 for their kind words and helpful insights on the present manuscript (see Interactive Comment published on 25 October 2019 by Anonymous Referee #2). Below, we respond to each comment individually and describe how this feedback has been used to improve the manuscript.

The manuscript 'Drivers and modelling of blue carbon stock variability' reports on the main drivers of sedimentary OC stocks (ecosystem – geomorphological – anthropogenic) in the top 30 cm of blue C ecosystems (tidal marshes, mangroves and sea grass meadows) across the state of Victoria (Australia). In addition, the authors used different general linear mixed-effects models to predict the spatial distribution of topsoil OC stocks of tidal wetlands in this region. The authors used a dataset they previously constructed (Ewers Lewis et al. 2018) of 287 sediment cores down to 30 cm depth to perform their analyses. The main results of the study show that ecological drivers (i.e. ecosystem type and dominant vegetation species) best explain the variability in C stocks, better than geomorphological and anthropogenic drivers. In addition, the authors calculate the regional topsoil C stock in tidal wetlands in Victoria to be 2.31 million ton C while identifying 'regions of interest', storing a substantial portion of total C in the studied region.

The manuscript is well-written and reads smoothly. The introduction provides a good overview of different factors controlling sedimentary OC stocks in vegetated coastal ecosystems that have been identified in literature. The material and methods section gives a clear overview of the study site, the data used and how the different models have been constructed, aided by a figure visualizing the workflow. The results section describes the most important results in a concise way and the discussion section frames the results with respect to existing literature. Overall, this manuscript provides an interesting approach to calculating sedimentary C stocks in blue C ecosystems at a large spatial scale and is well-worth publishing.

Thank you very much.

General comments

My main concern with the current manuscript is that it addresses topsoil C stocks, while it reports on 'blue C stocks' throughout the manuscript, without referring to the topsoil aspect. Emphasizing this aspect is, however, important: it is well known that sampling depth can have a large effect on conclusions drawn on relative differences in C stocks between coastal sediments at different locations or in different ecosystems. For example, depending on ecosystem-specific conditions, C stocks are known to decrease substantially with depth below the surface in certain ecosystems, while in others C stocks remain relatively constant with depth. Therefore, I would invite the authors to stress this aspect more throughout the manuscript: (i) the title would be more informative by including that the study concerns topsoil C stocks and (ii) the discussion should include a section where the implications of only considering topsoil samples is discussed.

We appreciate this point and agree that it is important we make it more clear that we are referring to the top 30 cm of sediments. We have done this throughout the manuscript. First, the title has been changed from *Drivers and modeling of blue carbon stock variability* to *Drivers and modeling of blue carbon stock*

variability in shallow sediments of southeast Australia. Next, we have altered our wording throughout the entire text and in the section headings to specify "shallow sediment C stocks" in place of "C stocks" or "sediment C stocks". We have clarified this point in the methods also:

Sediment C stocks to 30 cm deep (referred to throughout the paper as "shallow sediment C stocks")...

We have also added a rationale for our 30 cm measurements in the methods:

Though it is common in the literature to sample to 1 m deep in blue C sediments, the sampling protocol used for collecting these data (Ewers Lewis et al., 2018) was designed to maximize spatial coverage of shallow sediment C samples rather than sample entire sediment profiles (which may extend well beyond 1 meter deep). Greater spatial coverage allowed us to test the relationships between a variety of potential drivers and surface sediment C stocks on both fine and broad scales.

We have added a discussion of the implications of only measuring the top 30 cm of sediments to the discussion section:

We also aimed to maximize our ability to capture relationships between contemporary drivers and sediment C stocks by utilizing sediment C stock data to only 30 cm deep, a sediment horizon more directly impacted by recent environmental conditions compared to deeper stocks due to age. Based on previously estimated sediment accretion rates in blue C ecosystems in the study region (averaging 2.51 to 2.66 mm year-1 in tidal marshes (Ewers Lewis et al., 2019; Rogers et al., 2006a) and 7.14 mm year-1 in mangroves (Rogers et al., 2006a)), the top 30 cm of sediment represents roughly ~113-120 years of accretion in Victorian tidal marshes and ~42 years of accretion in Victorian mangroves. These time scales suggest sediments depths utilized in this study are more appropriate for assessing the impacts of modern environmental conditions on sediment C stocks compared to meter-deep stocks, which can be thousands of years old (e.g. Ewers Lewis et al., 2019). Using shallow sediment C stocks also allows us to be more confident that the vegetation present now has been there during the time of sediment accretion, unlike deeper sediments that are thousands of years old and for which it is difficult to determine what vegetation, if any, was present at the time of accretion.

The variability in shallow sediment C stocks that could not be explained by our modeling may also be related to the inherent challenges surrounding spatial and temporal matching of driver proxies and sediment C stock measurements; the relationship between shallow sediment C stocks and contemporary environmental settings can be represented more accurately for some variables over others.

Ecosystem type was a relatively powerful predictor of shallow sediment C stock variability in our study and this is likely due, in part, to the direct relationship between vegetation and surface sediments. In most vegetated ecosystems, the majority of underground plant biomass and microbial activity exists within the top 20 cm of soils (Trumbore, 2009). For saltmarsh, it has been demonstrated that the top 30 cm of sediment are directly impacted by current vegetation (Owers et al., 2016). Therefore, using shallow sediment C stock measurements allowed us to take advantage of the direct relationship between vegetation and C stocks to explain variability in surface sediments...

. . .

...Modern-day factors influencing vegetation can also have impacts on C stocks deeper than the sediments we measured. The effects of underground biomass on sediment C stocks can extend beyond the top 30 cm, and in fact new C inputs and active C cycling by microbial communities can occur as deep as underground roots extend (Trumbore, 2009). These new C additions (and fluxes) at depth fall outside the general pattern of sediment C decay down-core in vegetated ecosystems (Trumbore, 2009) which has previously allowed for linear or logarithmic regressions to be used to extrapolate 1-m deep C contents

from shallow (e.g. 30-50 cm deep) sediment C data (Macreadie et al., 2017a; Serrano et al., 2019). The activity of underground biomass and microbes at depth, when considered over space and time, may account for large C fluxes. The influence of anthropogenic activities, such as land use changes, on these processes via impacts to vegetation may largely go unnoticed based on current methods (Trumbore, 2009), both in this study and in blue C stock assessments on larger scales. We suggest further research to understand the dynamics of active C cycling at sediment depths traditionally considered stable...

. . .

...It is important to emphasize here that total sediment depths in blue C ecosystems can vary greatly, and are commonly deeper than 30 cm. Blue C ecosystems can have sediments up to several meters deep (e.g. Lavery et al., 2013; Scott and Greenberg, 1983), suggesting the estimates of C stocks measured here are conservative. In spite of these limitations, surface sediment C stock estimates give us valuable knowledge about the sediment C pool most vulnerable to disturbance and how it may be impacted by environmental drivers.

Although I greatly appreciate that the authors have provided a statement that data is available upon request, I would like to ask the authors to consider publishing the data together with the manuscript, or making it available through an online repository, so references to the data can be made. Open data is becoming increasingly important and has the potential to greatly advance the field of wetland biogeochemistry.

Thank you for this suggestion. We have created a project Dataverse to host the data in the Harvard Dataverse open access repository (https://dataverse.harvard.edu/) which will be published upon manuscript acceptance and accessible through a digital object identifier (DOI) that we will add to the manuscript (both in Table S10 and in the "Data Availability" section of the manuscript).

Additionally, to improve transparency and accessibility of the data both utilized and produced in this study, we have added the following table to the supplements (below) with a reference to it in the methods section:

Complete details of data availability for inputs and outputs of our models can be found in supplementary *Table S10*.

Any modifications made to these data for producing our models are described in the methods section of this manuscript. Please note the reference to the data produced in this study (R code and model prediction rasters) will be updated in this table. Due to both the large size of the raster files and the intellectual property associated with this work as part of the first author's Ph.D. dissertation, the data will be hosted in an online repository (rather than as a supplement) at the time of publication.

Table S10. Data availability

| Data Item | Description | Data Source & Location |
|----------------|---------------------------------------|---|
| Carbon Stock | Percent organic carbon and dry bulk | Ewers Lewis et al. 2018; Deakin Research Online |
| Dataset | density data for sediment sampled to | Deakin University's Data Repository |
| | 30 cm deep in 96 blue carbon | https://dro.deakin.edu.au/view/DU:30093405 |
| | ecosystems (saltmarshes, mangrove | |
| | forests, and seagrass meadows) across | |
| | Victoria, Australia. | |
| Ecosystem | 1. Mangrove areal extent in Victoria, | 1. Boon et al. 2001; OzCoasts Australian Online |
| Extent Vectors | Australia; saltmarsh areal extent and | Coastal Information, Victorian Saltmarsh and |

| | 1 1 1 - 4 2 1 1 | M V V |
|------------|--|--|
| | ecological vegetation classes in | Mangrove Vegetation Maps |
| | Victoria, Australia. | https://ozcoasts.org.au/geom_geol/vic/Saltmarsh/ |
| | 2. Seagrass areal extent in the major | Master |
| | bays and estuaries of Victoria, | 2. Available from: |
| | Australia. | a. Ball et al., 2014; Blake and Ball, 2001a |
| | a. Port Phillip Bay | https://discover.data.vic.gov.au/dataset/port- |
| | b. Western Port Bay | phillip-bay-1-25-000-seagrass-2000 |
| | c. Corner Inlet and Nooramunga | b. Blake and Ball, 2001b |
| | d. Gippsland Lakes | Distribution of Seagrass in Western Port |
| | e. Minor Inlets of Victoria | in 1999 |
| | | https://discover.data.vic.gov.au/dataset/distributio |
| | | n-of-seagrass-in-western-port-in-1999 |
| | | c. Roob et al., 1998 |
| | | Corner Inlet Seagrass 1998 |
| | | https://discover.data.vic.gov.au/dataset/corner- |
| | | inlet-seagrass-1998 |
| | | d. Roob and Ball, 1997 |
| | | Gippsland Lakes Seagrass 1997 |
| | | https://discover.data.vic.gov.au/dataset/gippsland- |
| | | lakes-seagrass-1997 |
| | | e. Blake et al., 2000 |
| | | Anderson Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/anderson- |
| | | inlet-seagrass-1999 |
| | | |
| | | Tamboon Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/tamboon- |
| | | inlet-seagrass-1999 |
| | | Wingan Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/wingan- |
| | | inlet-seagrass-1999 |
| | | Shallow Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/shallow- |
| | | inlet-seagrass-1999 |
| | | Mallacoota Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/mallacoot |
| | | a-inlet-seagrass-1999 |
| | | Sydenham Inlet Seagrass 1999 |
| | | https://discover.data.vic.gov.au/dataset/sydenham |
| | | -inlet-seagrass-1999 |
| Elevation | A gap free digital elevation model | Victorian Coastal Digital Elevation Model |
| Raster | (DEM) for the coastal region of | (VCDEM 2017) |
| | Victoria, Australia, that combines 2.5 | https://vmdp.deakin.edu.au/geonetwork/srv/eng/m |
| | m and 10 m DEMs. | etadata.show?uuid=8d3ccf63-ee85-41cd-917e- |
| | | <u>933624a50b2e</u> |
| Freshwater | Location of channels and other | Vicmap Hydro 1:25,000 |
| Vectors | freshwater objects in Victoria, | Victorian Government Data portal |
| | Australia. | https://discover.data.vic.gov.au/dataset/vicmap- |
| | | hydro-1-25-000 |
| Coastline | Line delineating the coastline of | Victorian Coastline 2008 |
| Vector | Victoria, Australia. | Victorian Government Data portal |
| | , . | https://discover.data.vic.gov.au/dataset/victorian- |
| | | coastline-2008 |
| Lithology | Rock types across Victoria, Australia. | Geomorphology of Victoria |
| Vectors | Teor types across victoria, rustiana. | Victorian Government Data portal |
| | | https://discover.data.vic.gov.au/dataset/geomorph |
| | | ology-of-victoria |
| | | ology-ol-victolia |

| Land Use | Primary land use designations for land | Victorian Land Use Information System |
|--------------|--|---|
| Vectors | parcels in Victoria, Australia. | 2014/2015 |
| | | Victorian Government Data portal |
| | | https://discover.data.vic.gov.au/dataset/victorian- |
| | | land-use-information-system-2014-2015 |
| Population | Human population data for Victoria, | Australian Population Grid, 2011 |
| Raster | Australia. | Australian Bureau of Statistics |
| | | https://www.abs.gov.au/AUSSTATS/abs@.nsf/L |
| | | ookup/1270.0.55.007Main+Features12011?Open |
| | | <u>Document</u> |
| R Code | R code used to identify drivers and | This study. |
| | model carbon shallow sediment | DOI: |
| | carbon stocks. | (TBD upon acceptance for publication) |
| | | |
| Model Output | Shallow sediment (to 30 cm deep) | This study. |
| Raster | carbon stock predictions in blue | DOI: |
| | carbon ecosystems (seagrass | (TBD upon acceptance for publication) |
| | meadows, mangrove forests, and | , |
| | saltmarshes) in Victoria, Australia | |

Specific comments

L83: allochthonous C can also come from terrestrial or estuarine sources

Thank you we have altered the wording to reflect that although we are referring to C transported into the ecosystem via marine tidal flooding, we recognize that C could have originally come from other sources (including, but not limited to terrestrial or estuarine sources), as follows:

In higher elevations tidal flooding is less frequent, providing less opportunity for particles and C to settle out of the water column, resulting in a lower contribution of allochthonous C from marine or other sources compared to lower, more frequently inundated marshes...

L149-150: would be good to provide a justification of why only the top 30 cm has been sampled Thank you for this suggestion. We agree and have added a paragraph here in the methods to address this point, as follows:

Though it is common in the literature to sample to 1 m deep in blue C sediments, the sampling protocol used for collecting these data (Ewers Lewis et al., 2018) was designed to maximize spatial coverage of shallow sediment C samples rather than sample entire sediment profiles (which may extend well beyond 1 meter deep). Greater spatial coverage allowed us to test the relationships between a variety of potential drivers and surface sediment C stocks on both fine and broad scales.

L154-155: would be good to report on the uncertainty associated with the use of spectroscopic techniques to estimate the 'C contents' of the samples. Any idea about the magnitude of this uncertainty? How were C stocks calculated? Were depth profiles of bulk density collected as well? Please briefly explain this, as this is important for the interpretation of the uncertainty on your results.

Thank you, we appreciate this reminder to include a brief explanation here (in addition to referencing the original C stock data paper) to aid in the interpretation of our modelling results. We have added the following text to ensure transparency of this information in the present manuscript:

Sediment C stocks to 30 cm deep (referred to throughout the paper as "shallow sediment C stocks") were estimated for 287 sediment cores from 96 blue C ecosystems across Victoria in southeast Australia (Ewers Lewis et al., 2018; Figure 1). Full details of sample collection, laboratory analyses, and

calculations of C stocks can be found in Ewers Lewis et al. (2018). Briefly, three replicate sediment cores (5-cm inner diameter) were taken in each ecosystem (n=125 in tidal marsh, n=60 in mangroves, and n=102 in seagrasses). Once back in the laboratory, samples were taken from three depths (0-2, 14-16, 28-30 cm) within each core. Samples were dried at 60°C until a consistent weight was achieved, then ground. Dry bulk density (DBD) was calculated as the dry weight divided by the original volume for all samples.

Based on the protocols by Baldock et al. (2013), a combination of diffuse reflectance Fourier transform mid-infrared (MIR) spectroscopy and elemental analysis via oxidative combustion using a LECO Trumac CN analyzer was used to determine organic C contents of all samples. Previous studies have demonstrated the accuracy of using MIR to estimate organic C stocks of sediments (Baldock et al., 2013; Van De Broek and Govers, 2019; Ewers Lewis et al., 2018). MIR spectra were acquired for all samples, then a subset of 200 representative samples was selected based on a principle components analysis (PCA) of the MIR results utilizing the Kennard-Stone algorithm. Gravimetric contents of organic carbon were measured directly in the laboratory for the 200-sample subset (Baldock et al. 2013). A partial least squares regression (PSLR) was created using a Random Cross Validation Approach (Unscrambler 10.3, CAMO Software AS, Oslo, Norway) and used to build algorithms to predict square root transformed total carbon, total organic carbon, total nitrogen, and inorganic carbon for the entire dataset. The PSLR model was evaluated based on parameters from the chemometric analysis of soil properties (Bellon-Maurel et al., 2010; Bellon-Maurel and McBratney, 2011), and the relationship between measured and predicted values was assessed based on slope, offset, correlation coefficient (r), Rsquared, the root mean square error (RMSE), bias, and the standard error (SE) of calibration (SEC) and validation (SEP; see Ewers Lewis et al., 2018 for full details). R-squared values for all square root transformed variables were ≥ 0.94 .

Sediment C stocks were calculated based on Howard et al., 2014. Organic C density (mg C cm⁻³) was calculated by multiplying organic C content (mg C g⁻¹) by DBD (g cm⁻³). Linear splines were applied to each core to estimate C density for each 2 cm increment within the 30 cm core, then C densities for each interval (measured and extrapolated) were summed and converted to Mg C ha⁻¹ to estimate total stock down to 30 cm deep for each core location...

L237: I would refer to table S4 here; this will help the reader to understand how the models were constructed

Thank you, we have added a reference to table S4 here as suggested.

L244: it's not clear from the text how the 'averaged models' were obtained and what these exactly are, please explain this in more detail

Thank you for this suggestion. We want to make sure the generation of the averaged models is very clear, so we have added text to the following portion of the methods section:

The dredge products of each global model (i.e. models created from "dredging") were ranked using AICc and the best models (delta AICc <2) were used to produce averaged models (named based on the global model they were generated from, e.g. global model 7 -> dredged and averaged -> averaged model 7). Averaged models were produced using the model.avg function ('MuMIn' package v. 1.42.1; Barton, 2018). The parameter estimates for each averaged model represent the average of that parameter's values from the models in which the variable appeared (from within the subset AICc<2).

To help clarify the rationale for the modelling approach we used, which is better for generating robust predictions when complex predictors are involved but cannot utilize standard methods for generating standard errors, we have added the following text to the beginning of section 2.3:

To identify drivers of shallow sediment C stock variability and create the best predictive model of shallow sediment C stocks to 30 cm deep we utilized a multi-step process based on an information theoretic approach and multimodel inference (Figure 3). Traditional approaches have relied on identification of the "best" data-based model; however, information-theoretic approaches allow for more reliable predictions through utilization of multiple models, especially in cases where lower ranked models may be essentially as good as the best (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). Further, information theoretic model selection has been demonstrated to provide significant advantages for explaining phenomena with more complex drivers (Richards et al., 2011). Here, we first looked broadly at our variables of interest by narrowing down to the best models containing all possible variables ("global" models, as explained below) using AICc (Akaike information criteria, corrected for small sample size) to explain the variability observed in the training dataset (70% of total C stock data; Symonds and Moussalli, 2011). From there, we identified which variables within the best global models best explained the observed variability in C stock data in order to remove unnecessary variables from the model equation (through the process of "dredging" and selecting the best subset, explained in detail below). The validity of removing unnecessary variables from the model is supported by the concept of parsimony, which suggests models more complicated than the best model provide little benefit and should be eliminated (Burnham and Anderson, 2002; Richards, 2008). The best subset of models generated from the global models ("dredge products") were selected based on delta AICc<2, which are viewed as essentially interchangeable with the best model (Symonds and Moussalli, 2011). Each subset of best models was used to generate an averaged model, which was tested by generating predictions of C stocks for a reserved (30%) subset of the dataset. The best performing model was used to generate a predictive map of C stocks to 30 cm deep for mapped blue C ecosystems in Victoria.

L298: it is not clear what you mean with 'intercept'

Thank you for this comment. We have clarified the definition of 'intercept' and added some text to clarify the meaning of other model outputs:

Parameter estimates from averaged models suggests dominant species/EVC was the most important predictor of shallow sediment C stocks, and was the only variable for which the 95% confidence interval of the estimates did not cross zero (Tables 2 and S7), suggesting a true effect of the variable on observed C stock variability (an estimate that included zero means there is potentially no impact of the variable on C stocks). Specifically, seagrasses P. australis, R. megacarpa, Z. muelleri, and Z. nigricaulis had shallow sediment C stocks significantly different than those of coastal tussock saltmarsh (assigned as the intercept in the model, or baseline dominant species/EVC for which to compare the effect of other dominant species/EVCs on C stocks), while all other tidal marsh EVCs, mangroves, and seagrass L. marina did not. This was confirmed by the ANOVA and Tukey's pairwise comparisons...

L333: Would be good to provide a measure of uncertainty on the total calculated C stock, similar to the standard deviations you report on the calculated numbers further down in this paragraph. Thank you for this suggestion. The standard deviation estimates you are referring to in the text were calculated by taking the average of the predicted C values for all individual cells overlapping with each ecosystem's areal extent, then taking the standard deviation. The model predictions are spatially explicit; i.e. a predicted C value is generated for each individual raster cell based on the unique characteristics (i.e.

combination of spatial data) of that cell that were included in the averaged model. Therefore, it was not possible to predict a single standard deviation or standard error for the total C stock for the entire state of Victoria in the same way because it was a sum.

Instead, the uncertainty of our predicted C stock estimates is represented in our validation step (e.g. Figure S3). We reserved 30% of our C stock dataset to test the accuracy of the predictions generated by each averaged model (as described in sections 3.2 of the results and displayed in Figure S3). These results showed that our best model explained ~half the observed variability in C stocks (Adj R-sq=0.4881; averaged model 2). We have clarified the errors associated with our model predictions by providing further details on the outputs of our validation step, which utilized the reserved dataset (30% of our original carbon dataset) for assessing the prediction power of each averaged model. Using linear regression, we compared predicted values from each averaged model to actual measured C stock values for reserved dataset. The complete outputs for this analysis have been added to the results section 3.2 ("Model validation"), as follows:

Linear regressions of predicted versus actual measured shallow sediment C values produced the following outputs for each averaged model: averaged model 11, residual standard error (RSE)=38.36 on 84 degrees of freedom (df), adjusted R-squared (R-sq(adj))=0.4868, F-statistic(F-stat)=81.63 on 1 and 84 df, p-value=5.044e-14; averaged model 5, RSE=38.51, R-sq(adj)=0.4829, F-stat=80.39 on 1 and 84 df, p-value=6.953e-14; averaged model 2, RSE=38.32, R-sq(adj)=0.4881, F-stat=82.06 on 1 and 84 df, p-value=4.517e-14; averaged model 10, RSE=39.67, R-sq(adj)=0.4514, F-stat=70.93 on 1 and 84 df, p-value=8.645e-13; averaged model 4, RSE=39.84, R-sq(adj)=0.4465, F-stat=69.58 on 1 and 84 df, p-value=1.254e-12; averaged model 1; RSE=39.48, R-sq(adj)=0.4566, F-stat=72.43 on 1 and 84 df, p-value=5.73e-13; averaged model 7, RSE=39.29, R-sq(adj)=0.4618, F-stat=73.94 on 1 and 84 df, p-value=3.81e-13.

We have also been more explicit about the error associated with the averaged model used to generate our state-wide shallow sediment C stock predictions by adding text to the results section on modelled shallow sediment blue C stocks (section 3.3) as follows:

We estimated a total of over 2.31 million Mg C stored in the top 30 cm of sediments in the ~68,700 ha of blue C ecosystems across Victoria (Table 4; Figure 5). This estimate is based on predictions from our best averaged model that utilized ecosystem type as the ecological variable (averaged model 7), which explained 46.18% of observed variability in C stock data and had an RSE of 39.29.

Due to the complexity of our multimodel approach, we did not include standard errors in predicted values for the entire region of Victoria due to the combination of using an averaged model and having random effects (there are no compatible R packages, to our knowledge).

L336: Please briefly explain how the standard deviations were calculated. What do they exactly represent? Only the spatial variation within these ecosystems, or also uncertainties related to the model procedures used?

The standard deviations here refer to those related to the mean of predicted C stock values for every raster cell of each ecosystem's mapped areal extent. We have updated the text, as follows, to clarify this:

Mean predicted C stocks (\pm SD) to 30 cm deep for each ecosystem type were 57.96 (\pm 2.90) Mg C ha⁻¹ for tidal marsh, 50.64 (\pm 1.35) Mg C ha⁻¹ to mangroves, and 23.48 (\pm 0.57) Mg C ha⁻¹ for seagrass based on predicted C stock values in all raster cells of each ecosystem's mapped areal extent in Victoria. These C

stock values ranged from 23.33 - 291.18, 23.34 - 77.81, and 23.33 - 73.42 Mg C ha⁻¹ for tidal marsh, mangroves, and seagrass, respectively.

L411: I would suggest changing this title to 'Modelled topsoil blue C stocks'

Thank you, we have changed it to "Modelled **shallow sediment** blue C stocks" here and in the results to be consistent with the wording in the text body and to be transparent about the depth of sediments considered in the study.

Tables and figures

Table 2 and 3: it would be good to refer to where the description of the global models can be found (Table S4) in the caption (after '(global model 11, 5, 2 and 8)')

Thank you for this suggestion, we have added a reference to the supplementary Table S4 in each of the two table captions (Tables 2 and 3) as you have suggested.

Table 4: I would change the caption to: '[...] and calculated C stocks [...]' We have changed this as suggested, thank you.

Table 5: I would change the caption to: 'Calculated blue C stocks [...]' We have changed this as suggested, thank you.

Figure 4: Please explain in the caption what the error bars represent (standard deviation?) and how they should be interpreted.

Thank you for pointing out that this information was missing from the caption. We have updated the Figure 4 caption to more clearly describe how the figure should be interpreted, including the meaning of the error bars, as follows:

Figure 4. Measured C stocks (Mg C ha⁻¹; average \pm standard error) in the top 30 cm of sediment by dominant species/ecological vegetation class (EVC). Bars are color-coded by ecosystem type: red = tidal marsh, green = mangrove, blue = seagrass. C stocks differed significantly by dominant species/EVC, with higher C stocks in coastal tussock saltmarsh, wet saltmarsh herbland, wet saltmarsh shrubland, mangroves A. marina, and seagrass L. marina (group a) compared to seagrasses P. australis, Z. nigricaulis, and Z. muelleri (group b; ANOVA and Tukey pairwise comparison, $F_{8,284}$ = 34.80, p < 0.001, R-sq(adj) = 48.77 %). Error bars represent one standard error of the mean.

Technical corrections

L40: remove 'our'

We have corrected this, thank you.

L250: variable => variables

We have corrected this, thank you.

Drivers and modelling of blue carbon stock variability in shallow sediments of southeast Australia

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Abstract. Tidal marshes, mangrove forests, and seagrass meadows are important global carbon (C) sinks, commonly referred to as coastal 'blue carbon'. However, these ecosystems are rapidly declining with little understanding of what drives the magnitude and variability of C associated with them, making strategic and effective management of blue C stocks challenging. In this study, our aims were threefold: 1) identify ecological, geomorphological, and anthropogenic variables associated with shallow sediment C stock variability in blue C ecosystems in southeast Australia; 2) create a predictive model of shallow sediment blue C stocks in southeast Australia; and, 3) map regional shallow sediment blue C stock magnitude and variability. We had the unique opportunity of using a high-spatial-density C stock dataset of sediments down to 30 cm deep from 96 blue C ecosystems across the state of Victoria, Australia, integrated with spatially explicit environmental data to reach these aims. We used an information theoretic approach to create, average, validate, and select the best averaged general linear mixed effects model for predicting C stocks across the state. Ecological drivers (i.e. ecosystem type or dominant species/ecological vegetation class) best explained variability in C stocks, relative to geomorphological and anthropogenic drivers. Of the geomorphological variables, distance to coast, distance to freshwater, and slope best explained C stock variability. Anthropogenic variables were of least importance. Our model explained 46% of the variability in shallow sediment C stocks and Www estimated over 2.31 million Mg C stored in the top 30 cm of sediments in coastal blue C ecosystems in Victoria, 88% of which was contained within four major coastal areas due to the extent of blue C ecosystems (~87% of total blue C ecosystem area). Regionally, these data can inform conservation management, paired with assessment of other ecosystem services, by enabling identification of hotspots for protection and key locations for restoration efforts. We recommend Globally, these methods be tested for applicability to other regions of the globe for identifying drivers of shallow sediment C stock variability and producing can be applied to identify relationships between environmental drivers and C stocks to produce predictive C stock models at scales relevant for resource management.

1 Introduction

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Vegetated coastal wetlands – particularly tidal marshes, mangrove forests and seagrass meadows – serve as valuable organic carbon (C) sinks, earning them the term 'blue carbon' (Nellemann et al., 2009). Still, an

increasing proportion of these ecosystems are being degraded and converted, and with pressures associated with human population growth the competition for land use in-our coastal zones continues to increase. With the current momentum for including blue C ecosystems in global greenhouse gas inventories, there is a need to quantify the magnitude of Cthese stocks and fluxes, especially in the sediments where the majority of the long-term C pool persists (Mcleod et al., 2011). However, global and regional assessments of blue C reveal large variability in sediment C stocks, both on small and large scales (Ewers Lewis et al., 2018; Liu et al., 2017; Macreadie et al., 2017a; Ricart et al., 2015; Sanderman et al., 2018). Identification of environmental variables driving differences in sediment C stocks in blue C ecosystems has become a key objective in blue C science and a necessary next step for quantifying C storage as an ecosystem service. Knowledge of such drivers is also important for coastal blue C management, including identification of hotspots to prioritize for conservation, as well as maximization of C gains through strategic restoration efforts.

Drivers of sediment C stock variability are innately difficult to identify in that the stocks represent the net result of many complex processes acting simultaneously, simplified as: 1) production of autochthonous C; 2) trapping and burial of autochthonous and allochthonous C, and; 3) remineralization and preservation of buried and surface C. Spatial variability in sediment blue C stocks resulting from these processes exists in hierarchical levels across global, regional, local, and ecosystem patch level scales (Ewers Lewis et al., 2018; Sanderman et al., 2018) and may be influenced by climatic, ecological, geomorphological, and anthropogenic factors (Osland et al., 2018; Rovai et al., 2018; Twilley et al., 2018).

At the global scale, climatic parameters appear to drive broad-scale variability in C stocks through effects on C sequestration (Chmura et al., 2003). Mangroves in the tropics have higher C stocks compared to subtropical and temperate mangroves, with rainfall being the single greatest predictor; when modelled, a combination of temperature, tidal range, latitude, and annual rainfall explained 86% of the variability in global mangrove forest C (Sanders et al., 2016). Sanderman et al. (2018) found large-scale factors driving soil formation (e.g. parent material, vegetation, climate, relief) were four times more important than local drivers for predicting mangrove sediment C stock density; but still, localized covariates were necessary for modelling the variability of sediment C stocks at finer spatial scales.

Differences in sediment stocks have also been observed across blue C ecosystem types, with meter-deep C stocks being highest in tidal marshes (389.6 Mg C ha⁻¹), followed by mangroves (319.6 Mg C ha⁻¹), then seagrass (69.9 Mg C ha⁻¹; Siikamäki et al. 2013). In southeast Australia this trend was observed on a regional scale, where an assessment of 96 blue C ecosystems revealed sediment C stocks to 30 cm deep were highest in tidal marshes (87.1 \pm 4.9 Mg C ha⁻¹) and mangroves (65.6 \pm 4.2 Mg C ha⁻¹), followed by seagrasses (24.3 \pm 1.8 Mg C ha⁻¹; Ewers Lewis et al. 2018).

Considerable variability in sediment C stocks has also been observed across species of vegetation. Lavery et al. (2013) compared 17 Australian seagrass habitats encompassing 10 species and found an 18-fold difference in sediment C stocks across them. Similarly, saltmarsh species differ not only in <u>magnitudequantity</u> of C stocks, but also in their capacity to retain allochthonous C (Sousa et al. 2010a). Species richness within an ecosystem type may also play a role in sediment C stock variability. In a global assessment, mangrove stands with five genera had 70-90% higher sediment C stocks per unit area compared to other richness levels (1-7 species stands; Atwood et al., 2017).

Beyond vegetation type, geomorphological factors appear to be most important when considering fine spatial scale sediment C stock variability (Sanderman et al., 2018). Elevation is likely an important driver of C stock variability in blue C ecosystems. Generally, the majority of the variability in C sequestration rates is linked to differences in sediment supply and inundation (Chmura et al. 2003). In lower elevations, faster sediment deposition may aid in C sequestration by trapping organic matter from macrophytes and microbes growing on soil surfaces (Connor et al. 2001). In higher elevations tidal flooding is less frequent, providing less opportunity for particles and C to settle out of the water column, resulting in a lower contribution of allochthonous C from marine or other sources compared to lower, more frequently inundated marshes (Chen et al., 2015; Chmura et al., 2003; Chmura and Hung, 2004).

However, tThe relative importance of elevation on sediment C stocks may vary depending on the contributions of autochthonous and allochthonous C. In ecosystems where the majority of the sediment C pool is autochthonous, elevation may be less important. Large variations in the origin of organic C can occur in mangroves, often with high C stocks being associated with autochthonous C and lower C stocks being associated with imported allochthonous C from marine and estuarine sources; similar variability in C origin has been observed in temperate tidal marshes (Bouillon et al. 2003). Higher C accumulation rates have been observed for upper tidal marsh assemblages that included rush (*Juncus*) compared to succulent (*Sarcocornia*) and grass (*Sporobolus*) tidal marsh assemblages located lower in the tidal frame (Kelleway et al., 2017). Rushes had high autochthonous C inputs, while sedimentation in succulents and grasses were mainly mineral.

Evidence is mounting that blue C ecosystems higher up in catchments (i.e. primarily fluvially influenced) maintain larger sediment C stocks than ecosystems further down in catchments (i.e. primarily marine influenced). For example, in southeast Australia, tidal marshes in brackish fluvial environments had sediment C stocks two times higher than those in marine tidal settings (Kelleway et al., 2016; Macreadie et al., 2017a). The deeper, stable C stores of tidal marshes are also higher in fluvial vs. marine-influenced settings, aiding long-term preservation of C (Van De Broek et al., 2016; Saintilan et al., 2013). The influence of fluvial inputs on sediment C stocks appears to be linked to three possible mechanisms: 1) fluvial environments are usually associated with smaller grain size sediments (silts and muds), which can enhance C preservation by reducing sediment aeration compared to sandy sediments (Kelleway et al., 2016; Saintilan et al., 2013); 2) higher freshwater input may lead to higher plant biomass and therefore autochthonous C inputs (Kelleway et al., 2016); and, 3) there is a greater contribution of terrestrial sediments via suspended particulate organic C and suspended sediment concentration higher up in the catchment compared to near the coast (Van De Broek et al., 2016).

Along with position in an estuary or catchment, proximity to freshwater inputs may drive differences in sediment C stocks among and within ecosystem patches. Tidal marsh accretion rates, which have been positively correlated (87%) with organic matter inventory, tend to decrease with distance from freshwater channels (Chmura and Hung, 2004), suggesting sediment C stocks may be higher closer to channels. Distance to freshwater is positively correlated with surface elevation, suggesting areas further from channels are inundated less frequently so have less sedimentation and slower accretion rates (Chmura and Hung, 2004).

It is important to note that high sedimentation rates do not necessarily result in high C sequestration rates or stocks if inorganic sediments make up a substantial portion of new sediment composition. Finer particles have higher surface area to volume ratios and tend to bind more organic molecules than coarse particles (Mayer, 1994). In seagrasses, high mud content is correlated with high sediment organic C content, except when large

autochthonous inputs (e.g. seagrass detritus from large species such as those of *Posidonia* and *Amphibolis* genera) disrupt this correlation (Serrano et al., 2016a).

Anthropogenic activities may also influence the C sink capacity of blue C ecosystems, even when the sediments are not directly disturbed (Lovelock et al., 2017). Land use, particularly greater area of farmland and urbanization, has been associated with worsening of seagrass condition, including abundance and species richness (Quiros et al., 2017), which may result in impacts to sediment C stocks. Nutrient additions resulting from agriculture and urbanization may increase primary productivity in nutrient limited areas (Armitage and Fourqurean, 2016). However, reduced nutrient inputs to coastal ecosystems could benefit C sequestration, as nutrient additions can result in net C loss through plant mortality, erosion, efflux, and remineralization via enhanced microbial activity (Macreadie et al., 2017b). Further, excess N has been linked to enhanced decomposition and an overall increase in tidal marsh ecosystem respiration due to shifts in microbial communities (Kearns et al., 2018).

Land use and human population may also impact blue C sediment stocks through erosion of terrestrial soils. Human activities causing erosion on land can result in increased sediment loads to coastal areas, including fine particles with a high affinity for C (Mazarrasa et al., 2017; Serrano et al., 2016b). An average of 60% of global soil erosion has been tied to human activities, particularly population density, agriculture, and deforestation (Yang et al., 2003). Export of fine sediments to coastal ecosystems from eroded terrestrial soils may encourage trapping and preservation of C within the sediments of blue C ecosystems.

Assessments of the drivers of blue C stock variability are often completed at global scales (Atwood et al., 2017; Rovai et al., 2018). Given the variability of sediment C stocks at finer spatial scales, and that coastal resources are managed on finer scales, we wanted to investigate drivers influencing regional blue C sediment stock variability. Here, we had the opportunity to exclude comparisons between temperate and tropical climates or effects of latitude by working on a stretch of coastline that spans approximately 1500 km west to east. We tested the relationship between ecological, geomorphological, and anthropogenic variables and sediment blue C stocks in the mineral-dominated sediments of southeast Australia. By identifying drivers of small-scale variability in sediment C stocks, across and within ecosystem patches, we created a predictive model for estimating C stocks on a scale relevant to coastal resource management. Our specific objectives were to: 1) identify ecological, geomorphological, and anthropogenic factors driving variability in shallow sediment blue C stocks within and across ecosystem patches in southeast Australia, 2) produce a spatially explicit model of current shallow sediment blue C stocks based on the relative importance of environmental drivers in southeast Australia, and, 3) map regional shallow sediment blue C stock magnitude and variability.

2 Materials and Methods

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2.1 Shallow sediment C stock dataestimates

Sediment C stocks to 30 cm deep (referred to throughout the paper as "shallow sediment C stocks") were estimated for 287 sediment cores from 96 blue C ecosystems across Victoria in southeast Australia (Ewers Lewis et al., 2018; Figure 1). Full details of sample collection, laboratory analyses, and calculations of C stocks can be found in Ewers Lewis et al. (2018). Briefly,

Tthree replicate sediment cores (5-cm inner diameter) were taken in each ecosystem (n=125 in tidal marsh, n=60 in mangroves, and n=102 in seagrasses), and oOnce back in the laboratory, samples were taken from three depths (0-2, 14-16, 28-30 cm) within each core. Samples were dried at 60°C until a consistent weight was achieved, then ground. Dry bulk density (DBD) was calculated as the dry weight divided by the original volume for all samples.

Based on the protocols by Baldock et al. (2013), a combination of diffuse reflectance Fourier transform mid-infrared (MIR) spectroscopy and elemental analysis via oxidative combustion <u>using a LECO Trumac CN</u> analyzer <u>waswere</u> used to determine organic C contents of all samples.-Previous studies have demonstrated the accuracy of using MIR to estimate organic C stocks of sediments (Baldock et al., 2013; Van De Broek and Govers, 2019; Ewers Lewis et al., 2018). MIR spectra were acquired for all samples, then a subset of 200 representative samples was selected based on a principle components analysis (PCA) of the MIR results utilizing the Kennard-Stone algorithm. Gravimetric contents of organic carbon were measured directly in the laboratory for the 200-sample subset (Baldock et al. 2013). A partial least squares regression (PSLR) was created using a Random Cross Validation Approach (Unscrambler 10.3, CAMO Software AS, Oslo, Norway) and used to build algorithms to predict square root transformed total carbon, total organic carbon, total nitrogen, and inorganic carbon for the entire dataset. The PSLR model was evaluated based on parameters from the chemometric analysis of soil properties (Bellon-Maurel et al., 2010; Bellon-Maurel and McBratney, 2011), and the relationship between measured and predicted values was assessed based on slope, offset, correlation coefficient (r), R-squared, the root mean square error (RMSE), bias, and the standard error (SE) of calibration (SEC) and validation (SEP; see (Ewers Lewis et al., 2018) for full details). R-squared values for all square root transformed variables were ≥0.94.

Sediment C stocks were calculated based on (Howard et al., 2014). Organic C density (mg C cm⁻³) was calculated by multiplying organic C content (mg C g⁻¹) by DBD (g cm⁻³). Linear splines were applied to each core to estimate C density for each 2 cm increment within the 30 cm core, then C densities for each interval (measured and extrapolated) were summed and converted to Mg C ha⁻¹ to estimate total stock down to 30 cm deep for each core location.summed and converted to estimate total sediment C stock to 30 cm depth based on each core, a depth for which sediment C stocks have been linked to vegetation structure (Owers et al., 2016). Full details of sample collection, laboratory analyses, and calculations of C stocks can be found in Ewers Lewis et al. (2018).

Though it is common in the literature to sample to 1 m deep in blue C sediments, the sampling protocol used for collecting these data (Ewers Lewis et al., 2018) was designed to maximize spatial coverage of shallow sediment C samples rather than sample entire sediment profiles (which may extend well beyond 1 meter deep). Greater spatial coverage allowed us to test the relationships between a variety of potential drivers and surface sediment C stocks on both fine and broad scales.

3 a depth for which sediment C stocks have been linked to vegetation structure (Owers et al., 2016).

2.2 Generation of predictor variables

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Our general approach to identifying potential drivers of <u>shallow</u> sediment C stock variability was to develop a predictive model based on spatially explicit environmental factors associated with our high spatial density of sediment C sampling. For clarity, we have grouped predictor variables into three categories – ecological, anthropogenic, and geomorphological – though the processes impacting C storage for each may span all three categories (Table 1; Table S1).

Values of predictor variables for each core were determined from spatial data either as the collective value representing activities within the catchment or based on the exact location of sample collection, dependent on the variable. Geographical boundaries for catchments in Victoria were derived using high resolution elevation data and flow accumulation models to define the spatial extents influencing fluvial and estuarine catchments (J. Barton, Pope, Quinn, & Sherwood, 2008; Figure S1). In some instances, seagrass locations sampled were beyond fluvial and estuarine catchments defined, thus we allocated characteristics of the nearest catchment region to characterize catchment influences at these locations.

Plant community was defined in two ways. First, more generally as 'ecosystem' (mangrove forest, tidal marsh, or seagrass meadow) based on the plant cover where the sample was taken. Second, plant communities were further defined by either dominant species (for seagrasses, for which most were monotypic beds) or ecological vegetation class (EVC; for tidal marshes). Dominant species/EVC were determined for each sampling location based on % cover of 1-m² quadrat photos taken during sample collection. Tidal marsh EVCs sampled included coastal tussock saltmarsh, wet saltmarsh herbland, and wet saltmarsh shrubland, as described by Boon et al., 2011. Only one mangrove species is present in Victoria (the grey mangrove, *Avicennia marina*), therefore further classification of this ecosystem was not used. Seagrass species sampled included *Lepilaena marina*, *Posidonia australis*, *Ruppia megacarpa*, *Zostera muelleri*, and *Zostera nigricaulis*.

Topographical variables for each sample location included elevation and slope. Elevation data was obtained from the Victorian Coastal Digital Elevation Model 2017 (VCDEM 2017) from the Cooperative Research Centre for Spatial Information. Elevation data at 2.5 m spatial resolution were used where available. Where not available (for 2.8% of cores), 10 m spatial resolution elevation data were used to fill in the gaps. Slope was calculated from these data using the Slope tool in ArcMap (v. 10.2.2 for desktop). Examples of spatial data used to develop models can be seen in Figure 2. The elevation data are a composite product that integrated terrestrial and bathymetric LIDAR as well as multibeam sonar data. The vertical accuracies of the data varied with sensor setup for acquisition: ±10 cm at 1 sigma (68% conf. level) in bare ground for topographic LIDAR data (for the majority of our dataset), ±50 cm for bathymetric LIDAR, and ±<10 cm for multibeam sonar data. Examples of spatial data used to develop models can be seen in Figure 2.

Geomorphological setting was represented for each sample location using two proxies: distance to coast and distance to freshwater channel. For each, continuous Euclidean distance rasters at 10 m resolution were created for the feature of interest using the Euclidean Distance tool in ArcMap. Coastline and freshwater channel data came from the State of Victoria, Department of Environment, Land, Water & Planning 2018 (Victorian Coastline 2008 and Vicmap Hydro shapefiles, respectively). The Extract Values to Points tool in ArcMap was used to extract raster values to each sample location.

Primary lithology (rock type, i.e. potential sediment parent material) was defined as the rock type covering the greatest proportion of catchment area intersecting with sample locations. To calculate area of each lithology, the Tabulate Area tool was used in ArcMap based on the catchment region polygons. From the total area of each lithology in each catchment, the one with the greatest proportion was identified and input into a new field from which a new primary lithology raster was created. The Extract Values to Points tool in ArcMap was used to extract primary lithology raster values to each sample location. In total, 21 lithologies were identified in the dataset, 17 of which were identified as primary lithologies of the coastal catchments (Table S2).

Variables to assess the influence of anthropogenic processes on <u>shallow sediment</u> blue C-<u>sediment</u> stocks included three relating to land use and one relating to human population. Primary land use for the catchment was first defined as the primary land use (based on land use in individual polygons) covering the greatest proportion of catchment area. Land use spatial data was obtained from the Victorian Land Use Information System (2014/2015) from the Victoria State Government, Department of Economic Development, Jobs, Transport and Resources 2018. In total, nine general primary land use categories were identified in the dataset, all of which were identified as primary land uses of the coastal catchments (Table S3). The nine land use categories were pooled into three simplified categories: urbanized, agricultural, and natural. Then the areas of each within the catchment were summed and divided by total catchment area to provide the proportion of each catchment associated with those categories.

Human population densities were calculated for each catchment based on $20\underline{1}0$ Australian census data, which were the most recent data available (Table S1). Population density was calculated for each district by dividing the population of the district by the area; this was then converted to a raster (100 m² resolution) to calculate the mean population density for the area of each catchment.

Complete details of data availability for inputs and outputs of our models can be found in supplementary Table S10.

2.3 Model generation, selection, averaging, and validation

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To identify drivers of shallow sediment C stock variability and create the best predictive model of shallow sediment C stocks to 30 cm deep we utilized a multi-step process based on an information theoretic approach and multimodel inferencemodel averaging (Figure 3). Traditional approaches have relied on identification of the "best" data-based model; however, information-theoretic approaches allow for more reliable predictions through utilization of multiple models, especially in cases where lower ranked models may be essentially as good as the best (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). Further, information theoretic model selection has been demonstrated to provide significant advantages for explaining phenomena with more complex drivers (Richards et al., 2011). Here, we first looked broadly at our variables of interest by narrowing down to the best models containing all possible variables ("global" models, as explained below) using AICc (Akaike information criteria, corrected for small sample size) to explain the variability observed in the training dataset (70% of total C stock data; (Symonds and Moussalli, 2011). From there, we identified which variables within the best global models best explained the observed variability in C stock data in order to remove unnecessary variables from the model equation (through the process of "dredging" and selecting the best subset, explained in detail below). The validity of removing unnecessary variables from the model is supported by the concept of parsimony, which suggests models more complicated than the best model provide little benefit and should be eliminated (Burnham and Anderson, 2002; Richards, 2008). The best subset of models generated from the global models ("dredge products") were selected based on delta AICc<2, which are viewed as essentially interchangeable with the best model (Symonds and Moussalli, 2011). Each subset of best models was used to generate an averaged model, which was tested by generating predictions of C stocks for a reserved (30%) subset of the dataset. The best performing model was used to generate a predictive map of C stocks to 30 cm deep for mapped blue C ecosystems in Victoria.

To begin this processFirst, potential ecological, geomorphological, and anthropogenic drivers were identified from the literature and relevant proxies were extracted from available spatial data using ArcMap (Table 1; Table S1). Predictor variable values derived from spatial data (along with our response variable values of C stocks) were compiled into a master data table in ArcMap. Sample rows were randomly assigned as either "training" data to build the model (70% of the data) or "evaluating" data with which to validate the model (the remaining 30% of the data). The training dataset was imported into R (R Core Team, 2018) for further analysis.

Covariates were tested for correlation before composing the global models. From our 11 covariates of interest, covariate pairs were considered correlated and not used together in modelling based on a threshold value of ~≥0.4 correlation. The exception to this was covariate pairs that had a correlation value <0.4 but were still considered correlated by definition and therefore were not used together in modelling (e.g. proportion of catchment area urbanized and proportion agricultural, Figure S2). This resulted in four variables that did not correlate with other covariates and could be used together in all models (slope, distance to coast, distance to freshwater, and primary lithology – hereafter referred to as 'geomorphological covariates'), along with correlating covariates that fell into one of two groupings: 1) ecosystem, dominant species/EVC, and elevation were correlated (hereafter referred to as 'ecological covariates'; and 2) mean population density, proportion urbanized, proportion agricultural, and proportion natural land use were correlated (hereafter referred to as 'anthropogenic covariates').

As a first step, we aimed to identify which models that included all (non-correlated) variables were best for explaining the variability in C stock data. 12-"Gglobal" models (i.e. containing all possible variables) were created and ranked to identify the most important drivers of C stock variability. General linear mixed-effects models (GLMMs) were generated (family = gamma because our data were right-skewed; 'lme4' package v. 1.1-17; Bates et al. 2015) using all geomorphological covariates, along with one covariate each from the ecological and anthropogenic variable groups, resulting in 12 global models containing 6 covariates each (Table S4). Continuous covariates were scaled in R. Site (i.e. a single sampling area that contained from one to all three ecosystems) was used as a random effect in all models to account for spatial autocorrelation observed at ~78 km.

The 12 global models were ranked using AICc ((Akaike information criteria, corrected for small sample size; 'AICcmodavg' package v. 2.1-1; Mazerolle, 2017; Table S5). The four best global models were chosen for further analysis based on delta AICc $\leq \sim 5.0$ compared to > 30 for all other models. Because the top four global models all used dominant species/EVC as the ecological variable, this process was repeated for the next four best models – those that included "ecosystem" as the ecological predictor – to create averaged models that could be tested and used for predictions when more specific, spatially-explicit plant community data (i.e. dominant species/EVC) were not available.

The eight global models were "dredged" ('MuMIn' package v. 1.42.1; Barton, 2018) to assess the relative importance of covariates included in each model. In this context, "dredging" refers to the generation of a set of models that includes all possible combinations of fixed effects from the global model, containing from six to one variables (i.e. all combinations of five variables, all combinations of four variables, and so on). The dredge products of each global model (i.e. models created from "dredging") were ranked using AICc and the best models (delta AICc <2) were used to produce averaged models (named based on the global model they were generated from, e.g. global model 7 -> dredged and averaged -> averaged model 7). Averaged models were produced using the model.avg function ('MuMIn' package v. 1.42.1; Barton, 2018). The parameter estimates for each averaged

model represent the average of that parameter's values from the models in which the variable appeared (from within the subset AICc<2).

Averaged models were validated using the 30% evaluation dataset. Due to the limitations of using cross validation and bootstrapping on models with random effects (Colby and Bair, 2013), a direct comparison was done between predicted and actual values of the reserved dataset. The predict function in R was used to generate predicted C stock values <u>for shallow sediments</u> using each of the eight averaged models on the reserved dataset. Each set of predicted values was compared to measured <u>shallow sediment</u> C stock values using a linear model to compute R-squared (adjusted) values. The models with the highest R-sq (adj) value from each set (one for "ecosystem" based models and one for "dominant species/EVC" based models) were applied to generate C stock predictions.

To test for differences in <u>shallow sediment</u> C stocks among species and EVCs, C stocks were log transformed to meet assumptions of normality and equal variances (log(Mg C ha⁻¹)) and a one-way analysis of variance (ANOVA) was run using dominant species/EVC as the factor. A Tukey's post-hoc analysis was used to distinguish groupings.

2.4 Prediction of shallow sediment blue Cearbon stocks

Spatial data relevant to the best ecosystem model were compiled for prediction of current ecosystem extent shallow sediment C stocks, and included rasters for total current ecosystem extent across Victoria (all mapped tidal marsh, mangrove, and seagrass), Euclidean distance to coast, and slope. Details and source information for all spatial data can be found in Table S1. All rasters were 10 m resolution and cut to the same extent using the Extract by Mask tool in ArcMap. The rasters were brought into R and processed using the raster package (Hijmans, 2017). Continuous variables were scaled to match the scaled variables of the model. Rasters were then compiled into a list, stacked, and used to generate a predictive raster map (*.tif file) of shallow sediment C stocks using the predict function. The C stock prediction raster (10 m resolution) was brought into ArcMap and resampled to 5 m resolution to better align to ecosystem extents. Shallow sediment C stock values for each ecosystem extent were extracted to separate rasters and used to generate zonal statistics tables for estimating shallow sediment C stock sums and means. Rasters used for calculating C sums were converted to proper units to match map resolution using the Map Algebra tool (e.g. Mg C ha⁻¹ converted to Mg C per 25 m² raster cell). Shallow sediment C stocks were summed for each ecosystem by catchment region, regions of interest, and the entire state. Regions of interest were identified visually as bays or estuaries hosting a substantial fraction of the state's blue C ecosystem distribution.

3 Results

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3.1 Drivers of shallow sediment blue C stock variability

Ranking of the 12 global models using AICc suggesteds the ecological variable wais the most important for determining model quality (Table S4 and S5). The top four models all contained dominant species/EVC as the ecological variable, with the following four containing ecosystem, and the remaining four containing elevation. The top four models fell within a delta AICc value of ~5.0 and under, compared to the remaining models having delta AICc values of ~35 or more, suggesting the top four models using dominant species/EVC were much better

at explaining <u>shallow sediment</u> C stock variability than the remaining models. Within rankings for each ecological variable, anthropogenic variables in the top eight models ranked as follows, from highest to lowest importance: proportion catchment land use that is natural, proportion urbanized, mean population density, and proportion agricultural.

Dredging the top four global models and averaging the best dredge products (delta AICc<2; Table S6) resulted in only three unique sets of model-averaged parameters (Table 2; full output can be seen in Table S7). The anthropogenic variables of mean population density and proportion agricultural land use did not appear in the best models produced from dredging global models 2 and 8, respectively. Therefore, both resulted in averaged models containing the same ecological and geomorphological variables, with no anthropogenic variable, and will hereafter be referred to as averaged model 2.

Parameter estimates from averaged models suggests dominant species/EVC was the most important predictor of shallow sediment C stocks, and was the only variable to have levels for which the 95% confidence interval of the estimates did not cross zero (Tables 2 and S7), suggesting a true effect of the variable on observed C stock variability (an estimate that included zero means there is potentially no impact of the variable on C stocks). Specifically, seagrasses P. australis, R. megacarpa, Z. muelleri, and Z. nigricaulis had an effect onshallow sediment C stocks significantly different than those to that of coastal tussock saltmarsh (assigned as the intercept in the model, or baseline dominant species/EVC for which to compare the effect of other dominant species/EVCs on C stocks), while all other tidal marsh EVCs, mangroves, and seagrass L. marina did not. This was confirmed by the ANOVA and Tukey's pairwise comparisons; there was a significant difference in shallow sediment C stocks based on dominant species/EVC ($F_{8,284} = 34.80$, p < 0.001, R-sq(adj) = 48.77 %); tidal marsh, mangrove, and seagrass L. marina had significantly higher C stocks than seagrasses P. australis, P0. nigricaulis, and P1. muelleri (Figure 4).

Across all three dominant species/EVC averaged models, distance to coast was the next most important geomorphological predictor, ranging from 50-51% relative importance compared to dominant species/EVC, followed by distance to freshwater (23-29% relative importance to dominant species/EVC), then slope (19-24% relative importance to dominant species/EVC). Of the two anthropogenic variables included, proportion urbanized land use was 47% relative importance compared to dominant species/EVC (averaged model 5) and proportion natural land use was 21% relative importance compared to dominant species/EVC (averaged model 11), suggesting proportion urbanized better explains variability in shallow sediment C stocks. The factor lithology did not appear in any of the best dredged models from the four global models.

For the next four averaged models, the ecological variable, ecosystem, was again the most important covariate (relative importance = 1.00; Table 3; Tables S8 and S9). Seagrasses impacted shallow sediment C stocks differently than tidal marshes (the intercept), as evidenced by the seagrass confidence intervals not crossing zero, while mangroves were no different than tidal marshes. However, in these averaged models, anthropogenic variables had greater relative importance than geomorphological predictors, unlike the models using dominant species/EVC as the ecological covariate. Proportion urbanization was still the most important anthropogenic variable, followed by proportion natural, but both had much higher relative importance (0.87 and 0.82, respectively) to the ecological variable compared to in the dominant species/EVC models. Additionally, mean population density appeared in one of the averaged models, though it did not appear in any of the dominant species/EVC models. Geomorphological variables, on the other hand, appeared less important in the ecosystem

models than the dominant species/EVC models. Relative importance of distance to coast and slope were both lower than in the previous models, and distance to freshwater channels did not appear in the top dredged models with ecosystem at all.

3.2 Model validation

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Comparison of shallow sediment C stock predictions from averaged models to actual measured shallow sediment C stock values in the 30% evaluation dataset show that our models accounted for ~44-49% of the observed variability in shallow sediment C stock values (Figure S3). Linear regressions of predicted versus actual measured shallow sediment C values produced the following outputs for each averaged model: averaged model 11, residual standard error (RSE)=38.36 on 84 degrees of freedom (df), adjusted R-squared (R-sq(adj))=0.4868, F-statistic(F-stat)=81.63 on 1 and 84 df, p-value=5.044e-14; averaged model 5, RSE=38.51, R-sq(adj)=0.4829, F-stat=80.39 on 1 and 84 df, p-value=6.953e-14; averaged model 2, RSE=38.32, R-sq(adj)=0.4881, F-stat=82.06 on 1 and 84 df, p-value=4.517e-14; averaged model 10, RSE=39.67, R-sq(adj)=0.4514, F-stat=70.93 on 1 and 84 df, p-value=8.645e-13; averaged model 4, RSE=39.84, R-sq(adj)=0.4465, F-stat=69.58 on 1 and 84 df, pvalue=1.254e-12; averaged model 1; RSE=39.48, R-sq(adj)=0.4566, F-stat=72.43 on 1 and 84 df, p-value=5.73e-13; averaged model 7, RSE=39.29, R-sq(adj)=0.4618, F-stat=73.94 on 1 and 84 df, p-value=3.81e-13. The best threefour averaged models, using dominant species/EVC as the ecological predictor (averaged models 11, 5, and 2, had very similar-adjusted R-sq(adj) values (ranging 0.4829-0.4881), with the best model (averaged model 2) being the one that did not include any anthropogenic variables. The same was true when comparing models using ecosystem as the ecological variable (averaged models 10, 4, 1, and 7) – the best R-sq(adj) was for the model with no anthropogenic variable (averaged model 7; 0.4618 compared to 0.4514, 0.4465, and 0.4566; Figure S3).

3.3 Modelled shallow sediment blue C stocks

We estimated a total of over 2.31 million Mg C stored in the top 30 cm of sediments in the ~68,700 ha of blue C ecosystems across Victoria (Table 4; Figure 5). This estimate is based on predictions from our best averaged model that utilized ecosystem type as the ecological variable (averaged model 7), which explained 46.18% of observed variability in C stock data and had an RSE of 39.29. Tidal marshes stored 48.2 %, mangroves stored 11.0 %, and seagrasses stored 40.8 % of total predicted shallow sediment C stocks. Mean predicted shallow sediment C stocks densities (±SD) to 30 cm deep for each ecosystem type were 57.96 (±2.90) Mg C ha⁻¹ for tidal marsh, 50.64 (±1.35) Mg C ha⁻¹ to mangroves, and 23.48 (±0.57) Mg C ha⁻¹ for seagrass based on predicted C stock values in all raster cells of each ecosystem's mapped areal extent in Victoria. These shallow sediment C stock values ranged from 23.33 – 291.18, 23.34 – 77.81, and 23.33 – 73.42 Mg C ha⁻¹ for tidal marsh, mangroves, and seagrass, respectively.

Fourteen areas of the coast were identified as regions of interest (ROIs) and contained over 99.5% of Victoria's total <u>shallow</u> sediment blue C stocks (Table 5) in 95.6% of the state's blue C ecosystem area (~65,700 ha). Of these regions, four of them contained over 87.6% of total estimated <u>shallow sediment C</u> stocks in 86.5% (~59,410 ha) of the state's blue C ecosystem area. Listed from highest to lowest <u>shallow sediment</u> C stocks, they were: Corner Inlet, Westernport Bay, Gippsland Lakes, and Port Phillip Bay.

4 Discussion

4.1 Drivers of **shallow sediment** blue C stock variability

Our best model explained 48.8% of the observed variability in shallow sediment C stocks, with the ecological variable, i.e. plant community, being the greatest predictor of C stock variability in all of the models. Plant community is related to C stocks both directly and indirectly through correlation with other variables driving C stock variability. Plant morphology may directly influence shallow sediment C stocks through the magnitude of plant biomass contributed to autochthonous C stocks and through an interaction with hydrodynamics. For example, higher C stock values in larger seagrass species, such as *P. australis*, are thought to be linked to both higher inputs of autochthonous C (larger rhizomes with more refractory C), and better particle trapping via a deeper canopy, which reduces water velocities and resuspension (Lavery et al., 2013). Under similar hydroperiods, saltmarsh grasses have been shown to have better sediment trapping abilities compared to mangrove trees (Chen et al., 2018), further suggesting plant traits (e.g. productivity and morphology) are an important driver of C stocks, rather than indirect impacts of inundation regimes alone.

Plant community is correlated with a number of other variables that may influence C storage, such as inundation regimes. Within and among similar ecosystems, elevation is a proxy for inundation regimes and can drive differences in C stocks. For example, in southeast Australia, tidal marshes in the upper intertidal zone had lower C accumulation rates than mangroves, with the cause hypothesized to be that the tidal inundation was shallower, less frequent, and for shorter durations, limiting the amount of allochthonous C accumulation (Saintilan et al., 2013). This appeared to be a more important driver in C accumulation variability than the difference in biomass production between the two ecosystems (Saintilan et al., 2013), highlighting the importance of elevation in determining C stocks. In our study, elevation was correlated to ecosystem and dominant species/EVC, so the differing effects of elevation compared to vegetation community could not be teased apart without violating assumptions of non-collinearity in our models. However, the higher ranking of global models with dominant species/EVC or ecosystem above those with elevation in our study suggests plant community itself is a better predictor of shallow sediment C stocks than simply position in the tidal frame.

Our global models specifying dominant species (for seagrass meadows) or EVC (for tidal marshes) ranked higher in our model selection than those that only specified the ecosystem (i.e. tidal marsh, mangrove, or seagrass). This ranking was supported by our model validation, in which our averaged model that best explained shallow sediment C stock variability included dominant species/EVC and accounted for 48.8% of the variability observed (Figure S3). Still, the best averaged model containing ecosystem as the ecological predictor performed nearly as well, and explained 46.2% of the variability. These results suggest that even when specific data on species composition are not available, shallow sediment C stocks can be estimated with a similar degree of confidence based on ecosystem type, which is often a much more readily available form of data and therefore favorable for calculating shallow sediment C stocks in data-deficient areas.

Geomorphological variables were more important than most anthropogenic variables in our models (Tables 2 and 3). Though lithology was not part of our averaged models, it is possible that its exclusion was due mostly to scale (catchment) and it may be important when accounted for on a more local scale. Distance to coast, distance to freshwater channels, and slope all appeared in the averaged models using dominant species/EVC, with distance to coast being most important. However, in models using ecosystem, distance to freshwater channels was no longer important enough to appear in the averaged models, and the anthropogenic variables, proportion urbanized and proportion natural, were more important than any of the geomorphological variables. Model

validation revealed that the best predictions for either set of models (those using dominant species/EVC and those using ecosystem as the ecological variable) came from the model that did not include any anthropogenic variables.

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Although our models suggest anthropogenic variables have little impact on shallow sediment C stocks, it is more likely that anthropogenic variables are impacting processes we could not measure. For example, excess nutrients resulting from certain land uses may stress plants to the point of affecting survival and therefore sediment stability (Macreadie et al., 2017b); without measuring changes to ecosystem distribution or sediment thickness (i.e. erosion) we could not pick up on these sediment C losses. Similarly, though enhanced sedimentation rates may increase C burial in catchments with certain land uses (e.g. high population density or high area of agriculture; Yang, Kanae, Oki, Koike, & Musiake, 2003), this addition to C stocks would be reflected in sequestration rate, which we did not measure in this study.

Additionally, pProxies for the drivers of sediment C stock variability can be quantified and described for modelling in numerous ways. Though we maximized our ability to choose variables representing meaningful relationships with shallow sediment C stocks by alternating the forms of the anthropogenic variables tested in our models (i.e. proportion urban vs. proportion agriculture vs. proportion natural v. mean population density), it may be beneficial to incorporate more direct measures of anthropogenic impacts in C stock modeling, such as nutrients and suspended particulate organic matter coming from catchments.

We also aimed to maximize our ability to capture relationships between contemporary drivers and sediment C stocks by utilizing sediment C stock data to only 30 cm deep, a sediment horizon more directly impacted by recent environmental conditions compared to deeper stocks due to age. Based on previously estimated sediment accretion rates in blue C ecosystems in the study region (averaging 2.51 to 2.66 mm year-1 in tidal marshes (Ewers Lewis et al., 2019; Rogers et al., 2006a) and 7.14 mm year-1 in mangroves (Rogers et al., 2006a)), the top 30 cm of sediment represents roughly ~113-120 years of accretion in Victorian tidal marshes and ~42 years of accretion in Victorian mangroves. These time scales suggest sediments depths utilized in this study are more appropriate for assessing the impacts of modern environmental conditions on sediment C stocks compared to meter-deep stocks, which can be thousands of years old (e.g. Ewers Lewis et al., 2019). Using shallow sediment C stocks also allows us to be more confident that the vegetation present now has been there during the time of sediment accretion, unlike deeper sediments that are thousands of years old and for which it is difficult to determine what vegetation, if any, was present at the time of accretion.

The variability in shallow sediment C stocks that could not be explained by our modeling may also be related to the inherent challenges surrounding spatial and temporal matching of driver proxies and sediment C stock measurements; the relationship between shallow sediment C stocks and contemporary environmental settings can be represented more accurately for some variables over others.

Ecosystem type was a relatively powerful predictor of shallow sediment C stock variability in our study and this is likely due, in part, to the direct relationship between vegetation and surface sediments. In most vegetated ecosystems, the majority of underground plant biomass and microbial activity exists within the top 20 cm of soils (Trumbore, 2009). For saltmarsh, it has been demonstrated that the top 30 cm of sediment are directly impacted by current vegetation (Owers et al., 2016). Therefore, using shallow sediment C stock measurements allowed us to take advantage of the direct relationship between vegetation and C stocks to explain variability in surface sediments.

The portion of recently accreted sediments influenced by contemporary anthropogenic drivers is harder to identify than that of ecosystems. Based on estimated accretion rates for this region from the literature (Ewers Lewis et al., 2019; Rogers et al., 2006b), 30 cm deep sediments would have taken an average of ~80 years to accumulate in Victoria (~117 years in tidal marsh and ~42 years in mangroves). Though sedimentation rates vary over time, they are relatively steady in comparison to changes in anthropogenic drivers, such as land use change. This means that modern day maps of land use, though useful for looking at the general impact of various activities, may be more useful for relating to variability in sediment C stocks when the data is assessed at a finer resolution. For example, comparing land use area data across various time periods with C densities in aged bands of sediment could help capture the pulse effects of sudden land use changes in narrower sediment horizons representative of the same time periods. In this study, the effects of land-use change may have been too diluted within the 30-cm horizons to relate to impacts on sediment C stock.

Spatially, anthropogenic variables are also difficult to assign to particular ecosystem locations or depths. Many blue C ecosystems in Victoria are located on coastal embayments and receive inputs from multiple catchments, making the influence of specific areas of land-use or population changes difficult to track to specific ecosystem locations. Modern-day factors influencing vegetation can also have impacts on C stocks deeper than the sediments we measured. The effects of underground biomass on sediment C stocks can extend beyond the top 30 cm, and in fact new C inputs and active C cycling by microbial communities can occur as deep as underground roots extend (Trumbore, 2009). These new C additions (and fluxes) at depth fall outside the general pattern of sediment C decay down-core in vegetated ecosystems (Trumbore, 2009) which has previously allowed for linear or logarithmic regressions to be used to extrapolate 1-m deep C contents from shallow (e.g. 30-50 cm deep) sediment C data (Macreadie et al., 2017a; Serrano et al., 2019). The activity of underground biomass and microbes at depth, when considered over space and time, may account for large C fluxes. The influence of anthropogenic activities, such as land use changes, on these processes via impacts to vegetation may largely go unnoticed based on current methods (Trumbore, 2009), both in this study and in blue C stock assessments on larger scales. We suggest further research to understand the dynamics of active C cycling at sediment depths traditionally considered stable.

Another limitation to C stock modelling , including our study, is knowledge of environmental features that may be important in influencing C storage, but are generally not monitored. For example, the maturity of a blue C ecosystem can affect C storage and composition (Kelleway et al., 2015). Within a single saltmarsh species, the maturity of the system is a major factor determining the role of the marsh as a C sink. Mature systems of *Spartina maritime* have higher C retention—via higher belowground production, slower decomposition rate, and higher C content in sediments—than younger *S. maritime* marsh systems (Sousa et al. 2010b). Mature marshes have also been observed to have greater contributions of allochthonous C storage over time, while younger marshes predominantly have autochthonous organic matter signatures (Chen et al. 2015, Tu et al. 2015). Long-term mapping of blue C ecosystems could be beneficial for tracking maturity of vegetation for C stock modelling as well as reduce the error in C stock measurements associated with changes to blue C ecosystem area.

<u>Finally, Wwe</u> suggest future studies examine the relationship between the drivers we have described and individual blue C ecosystem types in order to further refine shallow sediment blue C stock modelling. With a large dataset from a single ecosystem, relationships may be identified that were overshadowed in this study by the inclusion of all three ecosystems. For example, because elevation correlated with our two ecological variables it

was not included in our best models. However, within a single ecosystem, elevation may be an important driver of shallow sediment C stock variability due to its relationship with inundation regimes (Chen et al., 2015; Chmura et al., 2003; Chmura and Hung, 2004).

4.2 Modelled shallow sediment blue C stocks

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Our estimate of 2.31 million Mg C stored in the top 30 cm of sediment in all blue C ecosystems in Victoria was about 20% lower than that of Ewers Lewis et al. (2018), who estimated 2.91 million Mg C based on the same C stock data, but calculated total stocks based on average C stock values and ecosystem extent in each of the five coastal catchments. These results suggest that modelling shallow sediment C stocks based on environmental drivers may reduce the chances of overestimating sediment C stocks by better accounting for fine-scale variability in C stocks. Our modelled shallow sediment C stocks support our earlier findings that tidal marshes store more C than any other blue C ecosystem in Victoria. Our estimates are now refined in that modelled stocks suggest tidal marshes store closer to 48% (rather than 53%) and seagrasses store closer to 41% (rather than 36%) of total blue C stocks (Ewers Lewis et al., 2018). Our original estimate of mangrove contribution to total blue C was supported by our modelling – by either method we estimated mangroves to store 11% of Victoria's blue C stocks.

It is important to emphasize here that total sediment depths in blue C ecosystems can vary greatly, and are commonly deeper than 30 cm. Blue C ecosystems can have sediments up to several meters deep (e.g. Lavery et al., 2013; Scott and Greenberg, 1983), suggesting the estimates of C stocks measured here are conservative. In spite of these limitations, surface sediment C stock estimates give us valuable knowledge about the sediment C pool most vulnerable to disturbance and how it may be impacted by environmental drivers.

In examining C stocks within ROIs, i.e. areas of the coast containing substantial distributions of blue C ecosystems, we found that just four of the 14 ROIs housed nearly 88% of shallow-sediment blue C stocks in the state, a direct reflection of the large proportion of blue C ecosystem area in these regions (nearly 87% of the state's total blue C area). This trend appears to be driven by the presence of large seagrass shallow-sediment C stocks (Table 5) in these four regions, accompanied by large tidal marsh shallow-sediment C stocks. This result has important implications for management of coastal blue C. In cases where resources are limited, identification of areas housing major blue C sinks, in conjunction with evaluation of other ecosystem services, can help provide insight to guide conservation strategies. For example, strategies to conserve tidal marshes in the four major ROIs could serve the additional purpose of helping to preserve the adjacent seagrass meadows via facilitation; tidal marshes serve as filters of excess nutrients coming down from the catchment (Nelson and Zavaleta, 2012) that may otherwise cause a loss of seagrass beds due to light reduction resulting from the growth of algal epiphytes, macroalgae, and phytoplankton (Burkholder et al., 2007).

Further, our mapping of within-ecosystem-patch variability in <u>shallow sediment</u> C stocks is an important output for facilitating management actions on an applicable level, allowing priority of particular parts of an ecosystem patch for conservation when necessary. We suggest future studies examine the relationship between the drivers we have described and individual blue C ecosystem types in order to further refine blue C stock modelling. With a large dataset from a single ecosystem, relationships may be identified that were overshadowed in this study by the inclusion of all three ecosystems. For example, because elevation correlated with our two ecological variables

it was not included in our best models. However, within a single ecosystem, elevation may be an important driver of C stock variability due to its relationship with inundation regimes (Chen et al., 2015; Chmura et al., 2003; Chmura and Hung, 2004).

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5 Conclusions

In this study, we had the unique opportunity to assess a large regional dataset of shallow sediment blue C stocks to explore the influence of ecological, geomorphological, and anthropogenic variables in driving shallow sediment blue C stock variability. Because of the high spatial resolution of sampling within similar latitudes we were able to focus on variables driving differences in shallow sediment C stocks within catchments. We found that plant community was most important for determining shallow sediment C stocks and that combining this variable with geomorphological variables relating to position in the catchment allowed us to model shallow sediment C stocks at a fine spatial resolution. Identification and mapping of these dense shallow sediment blue C sinks in Victoria, in conjunction with evaluation of other ecosystem services, will be useful for conservation management regionally, for example through such as the identification of hotspots for protection and key locations for restoration efforts. We recommend Globally, these methods be tested in other areas of the globe to determine whether they may beare applicable for identifying relationships between potential environmental drivers and shallow sediment C stocks for creating predictive shallow sediment C stock models in blue C systems at scales relevant for resource management applications in other regions.

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Competing Interests. The authors declare that they have no conflict of interest.

Data Availability. The data associated with this article are accessible through the following dataverses [ELC(1]:

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hosted by Harvard Dataverse (https://dataverse.harvard.edu/).

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Table 1. Hypothesized drivers of sediment blue C stock variability. Drivers were grouped into three categories:

1) ecological (ecosystem type and dominant species/ecological vegetation class), 2) geomorphological (elevation, slope, distance to freshwater channel, distance to coast, and lithology), and 3) anthropogenic (land use and population). A more detailed explanation of driver rationale, along with literature and spatial data references, can be found in Table S1.

| Driver | Hypothesis and rationale |
|---|--|
| Ecological | |
| ECOSYSTEM TYPE | Ecosystem is the dominant driver of C stock variability C stocks differ by ecosystem type due to: 1) differences in position in the tidal frame, and 2) differences in morphology, which influence settling and trapping of suspended particles, as well as production of autochthonous C inputs. |
| DOMINANT SPECIES OR ECOLOGICAL VEGETATION CLASS | Species composition better explains C stock variability than ecosystem alone C stocks vary across species and community composition, as well as elevations. |
| Geomorphological | |
| ELEVATION | Lower elevations are correlated with higher C stocks Lower elevations have higher sedimentation rates, aiding the trapping of organic C, and are inundated more often, providing more opportunity for contribution of allochthonous C. |
| SLOPE | Shallower slopes are correlated with higher C stocks Steeper slopes are more vulnerable to erosion and less conducive to sedimentation and particle trapping than shallower slopes. |
| DISTANCE TO FRESHWATER CHANNEL | Distance to freshwater channel is negatively correlated with C stocks Being in close proximity to freshwater inputs may increase plant growth via freshwater and nutrient inputs, and enhance C preservation through delivery of smaller grain size particles. |
| DISTANCE TO COAST | C stocks are greater higher up in the catchment Greater inputs of organic C from terrestrial sources higher in the catchment result in higher sediment C stocks |
| LITHOLOGY | C stocks vary with terrestrial parent material of sediments Rock type may influence grain size and mineral content of sediments exported from catchments; smaller grain sizes and certain minerals enhance C stocks and preservation. |
| Anthropogenic | |
| LAND USE | C stocks vary based on land use activities in the catchment Export of terrestrial C, nutrients, and sediments varies by land use, especially when comparing urbanized, agricultural, and natural land uses. |
| POPULATION DENSITY | C stocks differ across population levels due to a correlation with land use Increases in population size lead to increases in urbanisation and competition for land use. |

Table 2. Parameter estimates for averaged models containing dominant species/ecological vegetation class (EVC) as the ecological variable. Parameter estimates were calculated based on averaging the best model products (delta AICc<2) resulting from dredging the top four dominant species/EVC global models (global model 11, 5, 2, and 8; descriptions of global models can be found in Table S4). Note that averaged model 2 and 8 are the same because neither of the anthropogenic covariates from the global models (mean population density and proportion agricultural land use for global models 2 and 8, respectively) appeared in the best dredge model products. DSE = dominant species/EVC; DSE are color-coded by ecosystem type: red = tidal marsh, green = mangrove, blue = seagrass; Adj SE = adjust standard error; RI = relative importance. N/A = the parameter was not included in the averaged model.

| | Ave | d Model 11 | Ave | ed Model 5 | | Averaged Model 2 | | | | | | |
|--|----------|------------|--------|------------|----------|------------------|--------|------|----------|---|--------|------|
| Parameter | Estimate | ± | Adj SE | RI | Estimate | ± | Adj SE | RI | Estimate | ± | Adj SE | RI |
| Intercept DSE: coastal tussock saltmarsh | 0.0177 | ± | 0.0043 | | 0.0171 | ± | 0.0042 | | 0.0176 | ± | 0.0042 | |
| DSE: wet saltmarsh herbland | 0.0012 | ± | 0.0041 | 1.00 | 0.0013 | ± | 0.0040 | 1.00 | 0.0011 | ± | 0.0041 | 1.00 |
| DSE: wet saltmarsh shrubland | -0.0027 | ± | 0.0042 | = | -0.0023 | ± | 0.0042 | " | -0.0028 | ± | 0.0042 | " |
| DSE: A. marina | 0.0011 | ± | 0.0041 | " | 0.0015 | ± | 0.0041 | " | 0.0011 | ± | 0.0041 | " |
| DSE: L. marina | -0.0024 | ± | 0.0051 | = | -0.0020 | ± | 0.0051 | " | -0.0024 | ± | 0.0051 | " |
| DSE: P. australis | 0.0394 | ± | 0.0179 | = | 0.0405 | ± | 0.0179 | " | 0.0412 | ± | 0.0180 | " |
| DSE: R. megacarpa | 0.0903 | ± | 0.0313 | = | 0.0908 | ± | 0.0314 | " | 0.0909 | ± | 0.0313 | " |
| DSE: Z. muelleri | 0.0291 | ± | 0.0047 | = | 0.0295 | ± | 0.0047 | " | 0.0292 | ± | 0.0047 | " |
| DSE: Z. nigricaulis | 0.0397 | ± | 0.0172 | = | 0.0389 | ± | 0.0172 | " | 0.0398 | ± | 0.0172 | " |
| Distance to coast | -0.0011 | ± | 0.0015 | 0.51 | -0.0011 | ± | 0.0015 | 0.51 | -0.0011 | ± | 0.0015 | 0.50 |
| Distance to freshwater | -0.0005 | ± | 0.0014 | 0.23 | -0.0006 | ± | 0.0015 | 0.29 | -0.0007 | ± | 0.0015 | 0.29 |
| Slope | -0.0001 | ± | 0.0004 | 0.19 | -0.0002 | ± | 0.0005 | 0.23 | -0.0002 | ± | 0.0005 | 0.24 |
| Proportion natural | 0.0003 | ± | 0.0009 | 0.21 | N/A | | N/A | N/A | N/A | | N/A | N/A |
| Proportion urbanized | N/A | | N/A | N/A | -0.0010 | ± | 0.0014 | 0.47 | N/A | | N/A | N/A |

Table 3. Parameter estimates for averaged models containing ecosystem as the ecological variable. Parameter estimates were calculated based on averaging the best model products (delta AICc<2) resulting from dredging the four global models that used ecosystem as the ecological variable (global models 10, 4, 1, and 7: descriptions of global models can be found in Table S4), combined with geomorphological and anthropogenic variables as specified. Ecosystems are color-coded for consistency: red = tidal marsh, green = mangrove, blue = seagrass; Adj SE = adjust standard error; RI = relative importance. N/A = the parameter was not included in the averaged model.

| | Averaged Model 10 | | | Averaged Model 4 | | | Averaged Model 1 | | | Averaged Model 7 | | | | | | |
|--|-------------------|-------|-----------|------------------|----------|---|------------------|------|----------|------------------|-----------|------|----------|---|-----------|------|
| Parameter | Estimate = | | Adj SE | RI | Estimate | ± | Adj SE | RI | Estimate | ± | Adj SE | RI | Estimate | ± | Adj SE | RI |
| Intercept Ecosystem: tidal marsh | 0.0178 = | ± 0.0 | 0020 | | 0.0166 | ± | 0.0018 | | 0.0174 | ± | 0.0020 | | 0.0174 | ± | 0.0020 | |
| Ecosystem: mangrove | 0.0022 | ± 0.0 | 0013 | 1.00 | 0.0024 | ± | 0.0013 | 1.00 | 0.0022 | ± | 0.0013 | 1.00 | 0.0022 | ± | 0.0013 | 1.00 |
| Ecosystem: seagrass | 0.0244 = | ± 0.0 | 0026 | " | 0.0254 | ± | 0.0025 | " | 0.0252 | ± | 0.0025 | ıı. | 0.0252 | ± | 0.0025 | " |
| Distance to coast | -0.0009 | ± 0.0 | 0014 | 0.45 | -0.0006 | ± | 0.0010 | 0.39 | -0.0003 | ± | 0.0008 | 0.22 | -0.0003 | ± | 0.0008 | 0.27 |
| Slope | -0.0002 | ± 0.0 | 0006 | 0.30 | -0.0002 | ± | 0.0005 | 0.29 | -0.0002 | ± | 0.0005 | 0.21 | -0.0002 | ± | 0.0005 | 0.26 |
| Proportion natural | 0.0022 = | ± 0.0 | 0017 | 0.82 | N/A | ± | N/A | N/A | N/A | ± | N/A | N/A | N/A | ± | N/A | N/A |
| Proportion urbanized | N/A = | ±] | N/A | N/A | -0.0024 | ± | 0.0015 | 0.87 | N/A | ± | N/A | N/A | N/A | ± | N/A | N/A |
| Mean population density | N/A = | ±] | N/A | N/A | N/A | ± | N/A | N/A | -0.0001 | ± | 0.0004 | 0.18 | N/A | ± | N/A | N/A |

Table 4. Blue C ecosystem area (ha) and calculated C stocks (Mg C) to 30 cm depth by catchment region and total across the state (Victoria, Australia).

| | <u>Tic</u> | lal Marsh | <u>M</u> | langrove | <u>s</u> | <u>Seagrass</u> | All Blue C Ecosystems in Victoria | | |
|---------------------------------|------------|-----------------|-----------|-----------------|-----------|-----------------|-----------------------------------|------------------------------|--|
| Catchment Region | Area (ha) | C stocks (Mg C) | Area (ha) | C stocks (Mg C) | Area (ha) | C stocks (Mg C) | Total area (ha) | Total blue C stock (Mg C) | |
| Glenelg Hopkins | 138 | 6,828 | 0 | N/A | 32 | N/A | 170 | 6,828 | |
| Corangamite | 3,010 | 187,943 | 58 | 3,022 | 5,355 | 128,117 | 8,423 | 319,083 | |
| Port Phillip & Westernport Bays | 3,108 | 158,604 | 1,828 | 90,359 | 14,457 | 328,725 | 19,393 | 577,688 | |
| West Gippsland | 13,038 | 711,083 | 3,301 | 161,652 | 17,508 | 413,642 | 33,847 | 1,286,377 | |
| East Gippsland | 1,332 | 50,504 | 0 | N/A | 5,552 | 72,873 | 6,884 | 123,377 | |
| Total | 20,626 | 1,114,961 | 5,187 | 255,034 | 42,903 | 943,357 | 68,715 | 2,313,352 | |

Table 5. Calculated bBlue C stocks (Mg C) to 30 cm depth by region of interest (ROI; listed from West to East). N/A = Ecosystem does not occur in ROI.

| | C stocks | (Mg C) by Ecosyst | | |
|--------------------------------------|-------------|-------------------|----------|------------------------------|
| Region of Interest | Tidal Marsh | Mangrove | Seagrass | All Blue C Ecosystems in ROI |
| Breamlea | 18,650 | N/A | N/A | 18,650 |
| Lake Connewarre/Barwon Heads | 101,218 | 2,890 | N/A | 104,109 |
| Port Phillip Bay | 105,169 | 243 | 156,824 | 262,236 |
| Westernport Bay | 120,827 | 90,248 | 300,420 | 511,495 |
| Andersons Inlet | 18,992 | 7,455 | 890 | 27,337 |
| Shallow Inlet | 9,384 | N/A | 19,778 | 29,162 |
| Corner Inlet | 253,367 | 154,198 | 346,317 | 753,882 |
| Jack Smith Lake | 73,839 | N/A | N/A | 73,839 |
| Lake Denison | 7,353 | N/A | N/A | 7,353 |
| Gippsland Lakes | 391,023 | N/A | 99,267 | 490,291 |
| Lake Corringle | 3,449 | N/A | N/A | 3,449 |
| Bemm River region | N/A | N/A | 7,806 | 7,806 |
| Tamboon Inlet | N/A | N/A | 2,563 | 2,563 |
| Wallagaraugh River/Mallacoota region | 3,180 | N/A | 8,117 | 11,296 |
| Total | 1,106,452 | 255,034 | 941,982 | 2,303,468 |

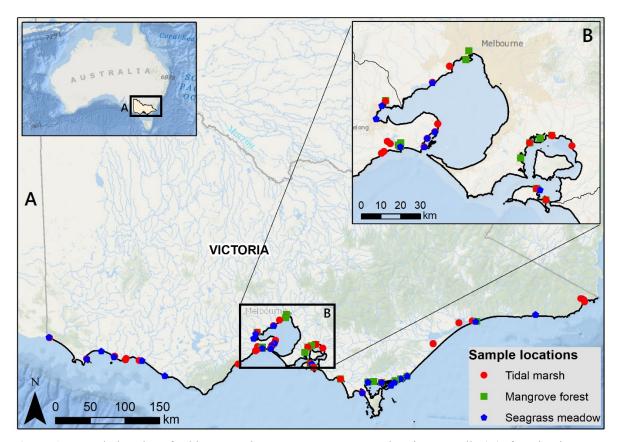


Figure 1. Sample locations for blue C stock measurements across Victoria, Australia (A), focusing in on Port Phillip and Westernport Bays. Service Layer Credits: Esri, Garmin, GEBCO, NOAA NGDC, and other contributors. Adapted from Ewers Lewis et al., 2018.

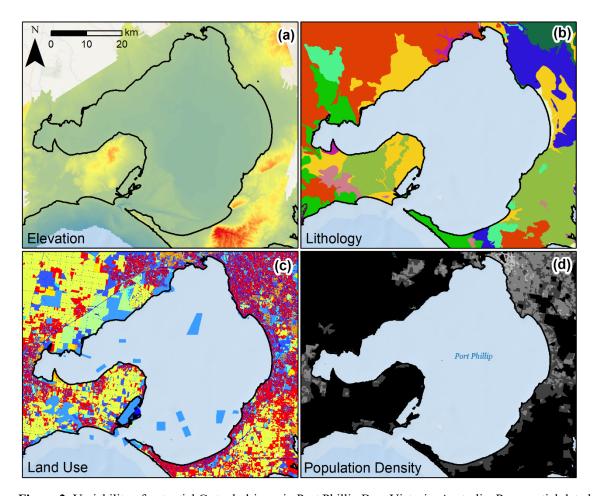


Figure 2. Variability of potential C stock drivers in Port Phillip Bay, Victoria, Australia. Raw spatial data layers were processed to define covariate values at each sample location or for the catchment of the sample location. Pictured layers include: (a) elevation raster at 10 m resolution, (b) lithology polygons, (c) land use polygons, and (d) and population density polygons.

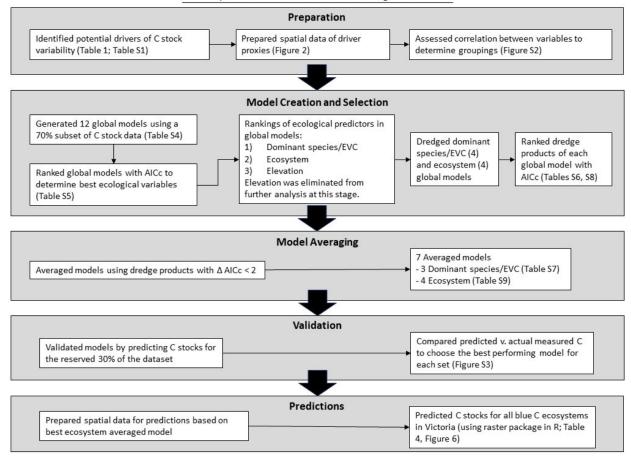


Figure 3. Conceptual workflow of C stock modelling methods: preparation, model creation and selection, model averaging, validation, and predictions.

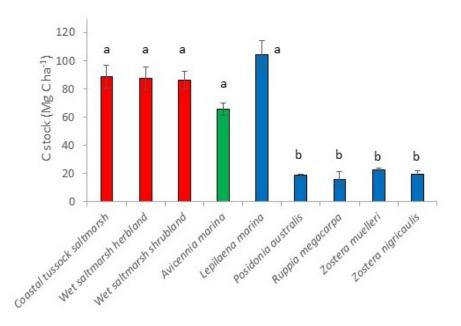


Figure 4. Measured C stocks (Mg C ha⁻¹); average \pm standard error) in the top 30 cm of sediment by dominant species/ecological vegetation class (EVC). Bars are color-coded by ecosystem type: red = tidal marsh, green = mangrove, blue = seagrass. C stocks differed significantly by dominant species/EVC, with higher C stocks in All measured tidal marsh ecological vegetation classes (coastal tussock saltmarsh, wet saltmarsh herbland, and wet saltmarsh shrubland), mangroves (A. marina), and seagrass L. marina (group a) compared to had significantly higher C stocks than seagrasses P. australis, Z. nigricaulis, and Z. muelleri (group b; ANOVA and Tukey pairwise comparison, $F_{8,284} = 34.80$, p < 0.001, R-sq(adj) = 48.77 %). Bars are color-coded by ecosystem type: red = tidal marsh, green = mangrove, blue = seagrass. Error bars represent one standard error of the mean.

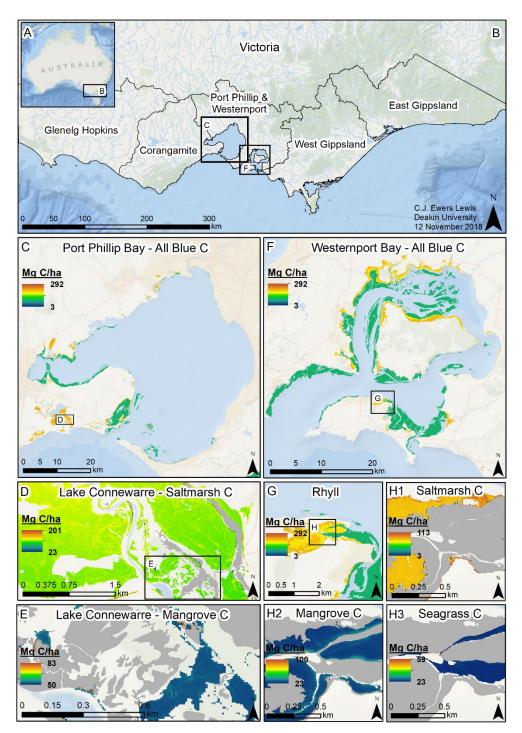


Figure 5. Modelled sediment blue C stocks for Victoria, Australia. Location of Victoria in Australia (A), coastal catchment regions of Victoria (B), modelled C stocks for all blue C ecosystems in Port Phillip Bay (C), modelled saltmarsh C stocks in Lake Connewarre (D); modelled mangrove C stocks in subsection of Lake Connewarre (E); modelled C stocks for all blue C ecosystems in Westernport Bay (F); modelled C stocks for all blue C ecosystems in Rhyll (Phillip Island) (G); and modelled saltmarsh C stocks (H1), mangrove C stocks (H2), and seagrass C stocks (H3) in subsection of Rhyll. Basemap service layer credits: Esri, Garmin, GEBCO, NOAA NGDC, and other contributors.