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### THE ROYAL SOCIETY

#### **Conservation biology**

# Optimal soil carbon sampling designs to achieve cost-effectiveness: a case study in blue carbon ecosystems

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Researchers are increasingly studying carbon (C) storage by natural ecosystems for climate mitigation, including coastal 'blue carbon' ecosystems. Unfortunately, little guidance on how to achieve robust, cost-effective estimates of blue C stocks to inform inventories exists. We use existing data (492 cores) to develop recommendations on the sampling effort required to achieve robust estimates of blue C. Using a broad-scale, spatially explicit dataset from Victoria, Australia, we applied multiple spatial methods to provide guidelines for reducing variability in estimates of soil C stocks over large areas. With a separate dataset collected across Australia, we evaluated how many samples are needed to capture variability within soil cores and the best methods for extrapolating C to 1 m soil depth. We found that 40 core samples are optimal for capturing C variance across 1000's of kilometres but higher density sampling is required across finer scales (100-200 km). Accounting for environmental variation can further decrease required sampling. The within core analyses showed that nine samples within a core capture the majority of the variability and log-linear equations can accurately extrapolate C. These recommendations can help develop standardized methods for sampling programmes to quantify soil C stocks at national scales.

#### 1. Introduction

Research into carbon (C) storage by natural ecosystems is growing, propelled by the urgent need to identify effective approaches to address climate change. Within coastal 'blue carbon' environments in particular—which include seagrass meadows, tidal marshes and mangroves — there are major efforts globally to estimate carbon stocks and sequestration rates at local, regional and national scales to inform carbon inventories and guide potential carbon offset initiatives [1,2].

With this heightened interest in blue carbon, there is a need to develop standardized approaches for robustly quantifying blue carbon stocks to identify gains and losses and inform greenhouse gas inventories as well as carbon markets. Several methodologies have been outlined for quantifying carbon stocks [3] (figure 1). However, a weakness of all existing methodologies is that they

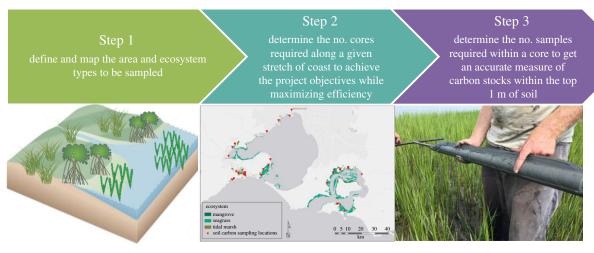
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**Figure 1.** Key steps in a soil coring campaign to develop spatially explicit maps of soil carbon stocks along a given area of the coast. This study focuses on providing explicit guidance relating to steps 2 and 3. (Online version in colour.)

**Table 1.** Results from depth extrapolations of soil carbon down to 1 m using different methods from the literature. The method used is in the first column followed by the equation for extrapolating C values associated with each method, the Spearman rank correlation coefficient (rho), the significance of the correlation (sig), and the mean squared predictive error (MSPE, standardized). C = C content (g), C = C content (g), C = C content (g), C = C density (mg cm<sup>-3</sup>), C = C and C = C density (mg cm<sup>-3</sup>), C = C and C = C density (mg cm<sup>-3</sup>), C = C density (mg cm<sup>-3</sup>),

	equation	three samples			seven samples		
method		rho	sig	MSPE	rho	sig	MSPE
log – log, c <sup>a</sup>	$\log$ (SOCcontent) = $K \log d + I$	0.59	< 0.001	0.485	0.75	< 0.001	0.485
log-linear, c <sup>a,b</sup>	$\log (SOCcontent) = K d + I$	0.6	< 0.001	0.485	0.78	< 0.001	0.485
log—log, d <sup>a</sup>	$\log (SOCdensity) = S \log d + I$	0.59	< 0.001	0.946	0.24	< 0.001	2.952
log-linear, d <sup>a</sup>	$\log (SOCdensity) = S d + I$	0.49	< 0.001	0.496	0.19	< 0.001	3.055
linear, c <sup>c</sup>	$SOCcumulative = K \; d + I$	0.33	< 0.001	0.488	n.a.	n.a.	n.a.
average %C content <sup>d</sup>	average percent C content (%dw) calculated from known depths and multiplied to 1 m	0.61	< 0.001	0.563	0.69	< 0.001	0.558

<sup>&</sup>lt;sup>a</sup>Extrapolation method applied in [9].

lack explicit guidance on the number of soil cores needed within project boundaries and the number of analyses needed per core to achieve robust estimates of blue carbon.

In this study, we provide guidelines to optimize effort allocation within cores and across landscapes in blue carbon projects, with the aim of obtaining robust estimates in soil organic C while maximizing benefit—cost ratios of projects. By capitalizing on one of the world's largest blue carbon databases encompassing landscape variability, we demonstrate spatial variability in organic C estimates at a range of scales and show how this can affect estimates of carbon storage at regional levels.

#### 2. Material and methods

#### (a) Soil sampling and carbon analysis

To develop sampling recommendations, we used two soil C datasets collected in blue carbon ecosystems [4]: 287 cores from 96 sites across Victoria, Australia for the spatially explicit analyses (VIC; electronic supplementary material, figure S1), and 220 up to 1 m-long soil cores from Australian blue carbon ecosystems for the within core analyses (AUS). More details on sampling programmes are provided in the electronic supplementary material along with detailed methods.

#### (b) Spatial sampling variability analyses

To determine how many samples are required to capture C variability over a broad landscape, we used a bootstrapping approach [5] within and across all ecosystems, using the depth-averaged C density (g cm $^{-3}$ ) value per core across two scales: statewide = 2000 km and CMA (Catchment Management Authority) = 100–200 km. To determine how far apart to sample, we used spline correlograms [6] to assess spatial correlation of C (depth-averaged C density) values across the sampling extent in Victoria within and across blue carbon ecosystems. To assess the number of cores required to reach a desired power of 80% for precisely estimating soil C stocks across a landscape [7], we used power analyses in the *R* package *simr* [8] to compare how many cores are needed if/if not accounting for environmental variability (elevation, aspect, slope, topographic position, land use; electronic supplementary material, table S1).

<sup>&</sup>lt;sup>b</sup>Extrapolation method applied in [10].

<sup>&</sup>lt;sup>c</sup>Extrapolation method applied in [11].

<sup>&</sup>lt;sup>d</sup>Extrapolation method applied in [12].

**Table 2.** Results from the generalized linear mixed models (GLMMs) associating depth-averaged soil carbon stock values with landscape scale environmental variables.

fixed effects	coefficient	abs (z-score)	<i>p</i> -value	
saltmarsh GLMM				
(intercept)	7.180	79.8	< 0.001	
slope	0.069	37.4	< 0.001	
disturbance (within 5 km radius)	-0.068	23.0	<0.001	
elevation	-0.082	22.4	< 0.001	
topographic position index (1000 m scale)	0.01	2.4	0.016	
mangrove GLMM				
(intercept)	7.356	39.2	< 0.001	
cosine of aspect (northness)	<b>−0.144</b>	35.2	< 0.001	
bare land (within 2 km radius)	<b>−2.736</b>	19.6	< 0.001	
elevation	-0.132	18.9	< 0.001	
topographic position index (2500 m scale)	0.085	18.6	< 0.001	
sine of aspect (eastness)	-0.027	6.5	< 0.001	
seagrass GLMM				
(intercept)	7.712	214.0	< 0.001	
disturbance (within 5 km radius)	<b>−</b> 0.167	17.6	< 0.001	
slope	0.020	7.8	< 0.001	
topographic position index (1000 m scale)	-0.017	5.3	< 0.001	

# (c) Within core sampling effort and depth extrapolations

Using the AUS dataset, we assessed the number of samples needed to reduce within-core variation by running a Markov chain Monte Carlo (MCMC) procedure in Excel. Applying multiple equations for a mix of organic and non-organic soils, we extrapolated C values from the varied soil types in the AUS dataset down to 1 m depth (table 1). We compared the predicted C values from the extrapolations to the actual values by running Spearman's rank correlation analyses and calculating the mean squared predictive error (MSPE).

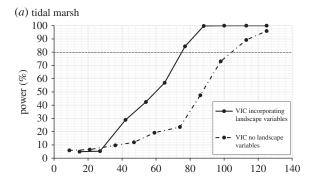
#### 3. Results and discussion

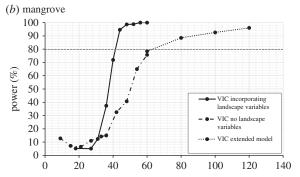
#### (a) Spatial sampling variability analyses

Results from the bootstrapping analyses revealed that increasing sampling intensity decreased the unexplained variance of C density estimates (electronic supplementary material, figure S2) with a significant decrease in variation after 40 cores. The variability in tidal marsh and seagrass ecosystems decreased significantly with 40 cores and with 30 cores for mangroves. The bootstrapping analyses across all ecosystems at the scale of the individual CMA areas showed notable but not significant drops in variation at sample sizes of 30-60 soil cores (electronic supplementary material, figure S2b). These results demonstrate that substantial variability can exist across core samples at broad scales and Victoria experiences a known range of different biotic and abiotic factors shown to influence C storage [13,14]. Based on the VIC dataset, we recommend sampling a minimum of 40 cores to robustly estimate soil C stocks across broad areas (e.g. Victoria) with a similar number of cores required over smaller areas (e.g. CMAs).

The spline correlograms, used to assess spatial patterns in C densities, showed positive spatial autocorrelation in the depth-averaged density of soil C (g cm<sup>-3</sup>) regardless of ecosystem type (electronic supplementary material, figure S4). The positive correlation decreased with distances ranging from 67 to 154 km, which is similar to the mean distance across CMAs in Victoria. This threshold may indicate that catchment-specific differences are driving the observed spatial pattern and sampling should be stratified to take into account this clustering. Differences in environmental settings (e.g. soil texture and mineralogy, geomorphology) among the CMAs studied may explain changes in the number of cores required. However, given the narrow latitudinal range (less than 1.5° latitude) of the Victorian coast, it is less likely that climatic conditions may be influential [15]. There can also be significant small-scale variation on carbon stocks, depending on where cores have been taken, which has likely contributed to some of the unexplained variability [16]. The recommendations provided here are only indicative and further studies are required to provide guidelines for contrasting environmental and geomorphic contexts, especially across the latitudinal range of mangroves.

The power analyses to determine the sample size required to reach a desired power of 80% for precisely estimating C densities, showed variation among ecosystems across Victoria with and without incorporating landscape structure (table 2). To reach a power of 80%, tidal marsh and mangrove ecosystems required fewer core samples (70 and 40, respectively) when incorporating landscape variation (table 2 and figure 2) compared to 100 and 60 when excluding landscape variables (figure 2*a*,*b*). However, seagrass ecosystems had an opposite pattern with the model not including landscape variables requiring fewer samples (figure 2*c*). The opposite pattern in seagrass is likely due to the inability of the topographic





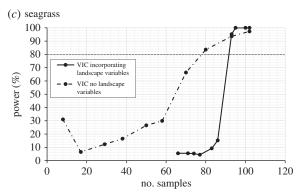


Figure 2. Results from the power analyses to determine the power associated with different samples sizes within tidal marsh (a), mangrove (b) and seagrass (c) ecosystems.

variables (i.e. elevation, slope) to capture landscape information important to soil C densities in seagrass ecosystems [14]. The models could potentially be improved with the incorporation of more variables related to C sequestration (e.g. duration of saturation, rainfall, tidal influence, estuary condition, species composition) [14,17]. See electronic supplementary material, figure S6 for a comparison of these methods applied to an independent dataset along the New South Wales coastline for mangrove and tidal marsh ecosystems.

#### (b) Within core sampling effort and depth extrapolations

The results from the within core MCMC simulations to assess how many samples within a core are needed to capture variability in soil C, showed a significant decrease in mean SE after nine samples were taken from within the cores (electronic supplementary material, figures S2 and S3). Based on these results, we conclude that at least nine samples are needed to capture variability in C throughout 1 m-long soil cores and these samples should be stratified by depth to capture variability as noted by Chimner et al. [18], with higher density of sampling in the shallower half of the 1 m core. Approaches such as homogenizing large portions of a whole core and measuring C may also provide a cost-effective method [3].

The different methods for extrapolating C sampled within the 0-30 cm depth range of the core down to 1 m varied in their accuracy when compared to actual C values sampled within those cores (table 1). Results from the depth extrapolation analyses show that the log-linear C content extrapolation using seven samples had the highest correlation with observed C content values down to 1 m ( $\rho = 0.78$ ) and one of the lowest MSPE (table 1). Generally, the predictions for C content (Cg<sup>-1</sup> soil) were more accurate than for C density (g cm<sup>-3</sup>) and accuracy tended to increase with samples and the use of log transformations. Owing to bias toward seagrass in the AUS dataset, further studies will need to determine if this pattern holds across more variability in mangrove/tidal marsh ecosystems but we still feel that the estimates are reasonable because extrapolation predictions had similar accuracy across all ecosystems.

Overall, we were able to devise recommendations for optimally sampling soil C across broad landscapes and within soil cores including: 40 cores are optimal to capture C variability in this region with higher density coring required at finer scales, mangrove ecosystems may require more sampling due to higher variability, incorporating environmental variability can decrease sampling effort, nine samples are optimal for capturing variation within cores and log-linear extrapolation methods perform best when extrapolating cores to 1 m depth. These can help scientists and managers design sampling programmes to characterize blue carbon stocks for assessing the C storage potential of these ecosystems.

Ethics. Research was conducted under a Department of Environment and Primary Industries research permit (10007248).

Data accessibility. Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.qj472r2 [4].

Authors' contributions. All authors conceived this study, conducted analyses, contributed to manuscript writing and revision, approved the final version and agreed to be accountable for the content.

Competing interests. We declare we have no competing interests.

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#### References

1. Duarte CM, Losada IJ, Hendriks IE, Mazarrasa I, Marbà N. 2013 The role of coastal plant communities for climate

change mitigation and adaptation. Nat. Clim. Change 3, 961-968. (doi:10.1038/ nclimate1970)

Macreadie PI et al. 2017 Can we manage coastal ecosystems to sequester more blue carbon? Front. Ecol. Environ. 15, 206-213. (doi:10.1002/fee.1484)

- 3. Howard J, Hoyt S, Isensee K, Pidgeon E, Telszewski M. 2014 Coastal blue carbon: methods for assessing carbon stocks and emissions factors in mangroves, tidal salt marshes, and seagrasses. Arlington, VA: Conservation International, Intergovernmental Oceanographic Commission of UNESCO, International Union for Conservation
- Young MA et al. 2018 Data from: Optimal soil carbon sampling designs to achieve costeffectiveness: a case study in blue carbon ecosystems. Dryad Digital Repository. (doi:10.5061/ dryad.qj472r2)
- Efron B, Tibshirani R. 1986 Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. Stat. Sci. 1, 54-75. (doi:10.1214/ss/1177013815)
- Bjornstad ON, Falck W. 2001 Nonparametric spatial covariance functions: estimation and testing. Enviorn. Ecol. Stat. 8, 53-70. (doi:10.1023/ A:1009601932481)
- 7. Field SA, O'Connor PJ, Tyre AJ, Possingham HP. 2007 Making monitoring meaningful. Austral. Ecol. **32**, 485 – 491. (doi:10.1111/j.1442-9993.2007. 01715.x)

- Green P, MacLeod CJ, Nakagawa S. 2016 SIMR: an R package for power analysis of generalized linear mixed models by simulation. Methods Ecol. Evol. 7, 493 - 498. (doi:10.1111/2041-210x.12504)
- 9. Jobbagy E, Jackson RB. 2000 The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol. Appl. 10, 423-436. (doi:10.1890/1051-0761(2000)010[0423:TVD0S0]2. 0.C0;2)
- 10. Fourqurean JW et al. 2012 Seagrass ecosystems as a globally significant carbon stock. Nat. Geosci. 5, 505 – 509. (doi:10.1038/ngeo1477)
- 11. Macreadie PI et al. 2017 Carbon sequestration by Australian tidal marshes. Sci. Rep. 7, 44071. (doi:10. 1038/srep44071)
- 12. Atwood TB et al. 2017 Global patterns in mangrove soil carbon stocks and losses. Nat. Clim. Change 7, 523 – 528. (doi:10.1038/nclimate3326)
- 13. Kelleway JJ, Saintilan N, Macreadie PI, Ralph PJ. 2016 Sedimentary factors are key predictors of carbon storage in SE Australian saltmarshes. Ecosystems 19, 865-880. (doi:10.1007/s10021-016-9972-3)
- 14. Serrano O, Lavery PS, Duarte CM, Kendrick GA, Calafat A, York PH, Steven A, Macreadie Pl. 2016

- Can mud (silt and clay) concentration be used to predict soil organic carbon content within seagrass ecosystems? Biogeosciences 13, 4915-4926. (doi:10.5194/bg-13-4915-2016)
- 15. Twilley RR, Chen RH, Hargis T. 1992 Carbon sinks in mangroves and their implications to carbon budget of tropical coastal ecosystems. Water Air Soil Pollut. **64**, 265-288. (doi:10.1007/ BF00477106)
- 16. Ricart AM, York PH, Rasheed MA, Perez M, Romero J, Bryant CV, Macreadie Pl. 2015 Variability of sedimentary organic carbon in patchy seagrass landscapes. Mar. Pollut. Bull. **100**, 476 – 482. (doi:10.1016/j.marpolbul.2015.
- 17. Kelleway JJ, Saintilan N, Macreadie PI, Baldock JA, Heijnis H, Zawadzki A, Gadd P, Jacobsen G, Ralph PJ. 2017 Geochemical analyses reveal the importance of environmental history for blue carbon sequestration. J. Geophys. Res. Biogeosci. 122, 1789 – 1805. (doi:10.1002/2017jg003775)
- 18. Chimner RA, Ott CA, Perry CH, Kolka RK. 2014 Developing and evaluating rapid field methods to estimate peat carbon. Wetlands 34, 1241-1246. (doi:10.1007/s13157-014-0574-6)