Summer 2018

A Search for Blue Carbon in Central Salish Sea Eelgrass Meadows

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Central Salish Sea Eelgrass Meadows

By

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Accepted in Partial Completion
Of the Requirements for the Degree
Master of Science

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A Thesis
Presented to
The Faculty of
Western Washington University

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August 24, 2018

Abstract

This study quantified eelgrass (*Zostera marina*) sediment organic carbon and carbon sequestration rates in Skagit County,Wa, in sites likely to support organic content and sediment accretion, the key components for carbon sequestration. These data may inform eelgrass-specific projects aimed at mitigating climate change through the protection and restoration of "blue carbon" ecosystems. Blue carbon is the carbon stored or emitted by coastal wetlands, which have the capacity to sequester more organic carbon (OC) than equivalent areas of mature forest. This study follows recent research from a single site within Padilla Bay, Wa. (Padilla transect), reporting OC stock approximately three times lower and sequestration rates nearly five times lower than reported global seagrass (all species) averages. The Padilla transect study sampled in the intertidal zone of one established transect in northern Padilla Bay National Estuarine Research Reserve. This site has minimal terrestrial sediment input and low or negative sediment accretion rates. These limitations inspired a search for higher OC storage and sequestration values by expanding the study area to include two bays with active river distributaries and selecting sites likely to support organic content and sediment accretion *a priori*. Site criteria included location within eelgrass meadows having a combination of similar canopy heights and stem densities but lower elevations, and closer proximities to distributary mouths of active river systems than in the Padilla transect. Study objectives were to: a) quantify OC stocks and sequestration rates in the expanded study area in sites expected to support C sequestration b) compare these values to those from the Padilla transect and to those from widespread *Zostera marina* studies and global seagrass meadow averages, and c) identify site characteristics associated with higher OC where found. We measured stem density, canopy height, depth below mean lower low water (MLLW), and sediment compaction in the field, and analyzed sediment samples for bulk density, carbon concentration by direct C-analysis and loss on ignition (LOI), sediment accretion rate from gamma ray spectroscopy of ^{210}Pb activity levels, and sediment grain size in the lab. Considering site selection in areas expected to support C sequestration, results may be considered within the upper bounds of the ranges found within our study area. The overall mean % OC ± SE over 3 bays was 0.43 ± 0.01 %, with a range of 0.17% to 3.66% (n=20). Overall mean OC density ± SE was 0.0058 ± 0.0001 g cm^{-3} and ranged from 0.0018 to 0.0479 g cm^{-3} (n=20). Organic C stock to 50cm ± SE was 27.10 ± 1.40 Mg ha^{-1}, with a range of 15.23-49.20 Mg ha^{-1} and SE=1.96 (n=20). The mean carbon sequestration rate ± SE was 43.88 ± 9.19 g C m^{-2} yr^{-1}, ranging from 13.90 to 93.04, SE=17.04, (n=7), which should be considered the upper bounds of possible rates in this infauna-rich region. We found higher OC sequestration rates in our study than in the Padilla transect study ($F = 8.41$, $p = 0.01$ on 1 and 10 df) however, there were no differences in % OC ($F = 0.26$, $p = 0.62$ on 1 and 13 df) nor OC stock ($F = 1.86$, $p = 0.20$ on 1 and 13 df) between studies. Like the Padilla transect study, organic C values in our study were also 3-5 times lower than estimated global averages for % OC, OC stock, and OC sequestration rates, but within the range reported by five studies conducted in *Zostera marina* meadows worldwide. Percent sediment pore space explained most of the variation in OC stock. We conclude that within-meadow eelgrass OC increases with environmental factors contributing to the accretion of fine sediments which increase porosity, that *Z. marina* meadows in the Pacific Northwest and elsewhere exhibit far lower OC values than global averages for all seagrass species, which may be due to key factors preventing it from thriving in areas conducive to high C sequestration. We recommend that region- and site-specific C values be considered when valuing restoration projects to avoid underestimation of eelgrass area required to offset emissions "purchased" through the voluntary or compliance (cap and trade) C markets.

Acknowledgements

To John Rybczyk, for estuarine prose and giving enough sage advice to let me explore my questions with abandon but keep me within the guiding tenets of Science. To the scientists of the Cascadian Women in Blue Carbon (CWIBC): Katrina Poppe, for patient guidance in field and lab methods, unfailing responses to blue C ponderings and ideas by email at 10pm on Saturday nights, and for kombucha, chocolate, and inspiration-sharing, in that order; Erin Murray, my partner in coring, for gung-ho navigation and level-headed seawomanship, even in the presence of sharks; and Carolyn Prentice, for cross-border collaboration, R-tutelage, mixed effects modeling support, idea-sharing, and ceaseless good cheer, especially in the company of cheese and chocolate. To Jude Apple of Padilla Bay National Estuarine Research Reserve (PBNERR), for enthusiastic project support and blue carbon methods tutelage. To Nicole Burnett, and Captain Heath Bohlman of PBNERR, and Captain Nate Schwarck of Shannon Point Marine Center and Zoe Zilz, fellow grad, for research vessel support and scientific diving par excellence. To John McLaughlin, for introduction to the Information Theoretic Approach to data analysis. To outstanding interns, Chelsea Johnson, for leading the grainsize analysis efforts; Meriel Kaminsky, for returning to the field sites to quantify effects of pesky bioturbators; and Mason Lanphear, for mad GIS cartographic and spatial analysis skills. To Cristina Villalobos, Martin Chamberlain, and Tristin Munich, for lab and field assistance and precious friendship. To Brian Bingham and Robin Matthews for high caliber research design and analysis methodology. To Dr. Peter Homann, for extraordinary patience in teaching me proper protocol with the CHN analyzer, even as I seemed bent on its demise. To my graduate cohort: Cristina, Matt, Faythe, Lillian, Ryan, Shannon, Sara, Jesse, Krista, and Geoff, for stats emotional support, code-sharing, and the blood, sweat, tears, and laughter essential to masters degree survival. To Sylvia Yang for teaching Scientific Writing, which held me to task in applied Kaizen. To all who supported this endeavor financially, without your belief and support this document and many like it, along with the experiences which they represent, would not have been possible: The Padilla Bay Foundation for the Research Assistantship, the Anacortes Chapters of the American Association of University Women and Soroptimist International, for generous scholarships, the Dean's Fund for Sustainability Studies, Huxley Small Grants, and the Ross Travel Grant, for project and conference funds. To Ed Weber, Rose Kawczynski, Bonnie Drewes, and the faculty of Huxley College of the Environment and the Marine and Estuarine Science Program, you are a student-centered team. To David Hickenbottom, whose unfailing and unconditional love and guidance continues to awaken my spirit to its life purpose. To my father, Herlwyn Lutz, for sharing the names of organisms from ridge crest to seafloor here in Cascadia, for keeping a marine aquarium in his school, for endeavoring to earn his masters whilst supporting a family of 6, and all the while serving as a shining model of selfless service to humanity. And to my mother, Barbara Lutz, for her indomitable will and insatiable quest for the freedom of the wild. To Matt Castle, for the use of the trusty *"R/V Zostera,"* for supporting me and our little family in ways too numerous to count, and for believing in me even more than I believed in myself. And finally, to *Zostera sp*. The joys of wading and diving through your bubbling, green, and waving meadows made all the trials of a thesis worthwhile.

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Introduction

Climate effects from climbing atmospheric greenhouse gas concentrations have spurred research on carbon capture and storage, or *sequestration* (Bertram 2010; IPCC 2014, 2013). Coastal wetlands sequester carbon naturally (Chmura et al. 2003; IPCC 2014, 2013). These are some of the most highly productive ecosystems on Earth, supporting vascular plants, macroalgae, benthic diatoms, and phytoplankton (Odum et al. 1995; Beck et al. 2003; Day 2012; Tiner 2013). Unlike upland systems, it is the high sediment deposition rates in coastal wetlands that bury both autochthonous and allochthonous organic carbon (OC) in hydric sediments, which prevent its return to the atmosphere for millennia. This carbon (C) burying capacity adds C sequestration to the long list of coastal wetland ecosystem services, including habitat, nutrient cycling, wave attenuation, sediment stabilization, and storm surge protection (Nelleman 2009). The OC sequestered from the atmosphere by plants and buried in tidal wetland sediments, specifically those of mangrove, saltmarsh, and seagrass meadows, is termed *blue carbon* (Figure 1). 'Blue carbon' is a reference to these ecosystems being marine counterparts to the United Nation's acronym for reducing emissions from deforestation and forest degradation (REDD), a land-based C-reducing platform (UNFCC 1995). Like forests, blue C ecosystems play an elegant role in climate mitigation. Unlike terrestrial forests, mangroves, salt marsh, and seagrass meadows sequester C at a global rate disproportionately higher than terrestrial ecosystems of similar size (Figure 2) (Duarte et al., 2005; McLeod et al. 2011). High OC sequestration capacity has made blue C the focus of intense interest (Figure 2) (Nelleman 2009; Kennedy et al. 2010; Duarte et al. 2010; McLeod et al. 2011; Fourqurean 2012; Herr and Laffoley 2012; Macreadie et al. 2014) and led to its inclusion in the C market (VCS 2015).

Figure 1: Organic carbon is trapped, buried, and stored long-term in blue carbon ecosystem sediments, including a) mangrove, b) saltmarsh, and c) seagrass meadows. Photos by Getty Images, Puget Sound Regional Council, and IUCN, respectively.

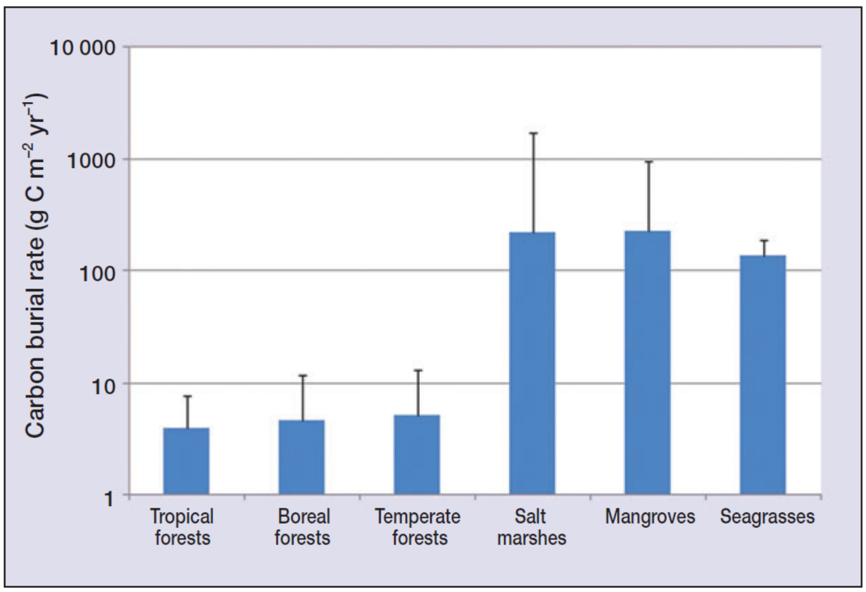


Figure 2: Carbon burial (sequestration) rates in terrestrial and coastal wetland ecosystems compiled from studies around the world (reprinted from McLeod et al. 2011). Note the logarithmic y-axis scale.

Funds paid by industry or individuals to offset emissions through voluntary or compliance C markets can be banked and then traded by brokers and allotted to C-sequestration-enhancing projects (Herr et al. 2015). Coastal wetland restoration projects can qualify for C market funding if accurate C flux accounting verifies a net greenhouse gas (ghg) reduction resulting from the project (VCS 2015). Ecosystems under threat of land-use change can be protected, and those already disturbed can be restored. Successful implementation of C-market-funded restoration in mangrove systems is currently under way (Sutton-Grier et al. 2016). As of 2015 seagrass meadows joined salt marsh and mangroves in their eligibility to receive C market funds due to key qualities for C sequestration (VCS 2015).

Seagrass blue C sequestration results from the following phenomena: high productivity in seagrass meadows provides organic matter (OM) for burial (Gacia and Duarte, 2002; Greene and Short 2003). Aboveground biomass (stems and leaves) slows water velocity, enabling OC-containing particles from both autochthonous and allochthonous C to settle and become buried during tidal inundation (Mcleod et al. 2011). Mats of rhizomes stabilize substrate and enhance sediment retention (Duarte, Sintes, and Marbá 2013). Recalcitrant rhizome mats can extend deep into anoxic sediment, where decomposition is slow, retarding oxidation of C to CO_2. (Enríquez 1997). Finally, high sediment accretion rates can bury C for millennia (Mateo et al. 1997; Nelleman 2009).

The C sequestration rate is the product of sediment OC content (density) and sediment accretion rate. Carbon sequestration rates for seagrasses globally are up to 35 times higher than temperate and tropical forests (McLeod et al. 2011). Alternately, disturbance of seagrass beds at the current rate of loss (1.5% yr^{-1}) is releasing 299 Tg C yr^{-1} back into the atmosphere as CO_2 (Fourqurean et al. 2014). These discoveries launched a widespread effort to quantify the blue C budgets needed to assess seagrass' contribution to the suite of sustainable management, policy, and planning activities that aim to reduce emissions from shoreline conversion and restore coastal carbon sinks (Herr and Lafoley 2012). The issue with applying these C sequestration rates to all seagrass ecosystems worldwide is that they are largely based on subtropical seagrasses, such as *Posidonia oceanica* (Laffoley and Grimsditch 2009; Fourqurean 2012). *Posidonia* has a robust vertical growth habit and recalcitrant rhizomes, which extend more than a meter into the sediment, persist for millennia, and boast C storage from 40–410 kg C m^{-2} in the top meter of soil (Mateo et al. 1997; Duarte et al. 2013; Lavery et al. 2013). Initial studies did not account for extensive region nor species variability in blue C, failing to offer accurate valuation for restoration project funding. Data from the temperate eelgrass meadows of the Pacific Northwest of N. America are particularly scarce, especially within the Salish Sea of Washington State and British Columbia.

An urgency to assess blue C in Pacific Northwest eelgrass ecosystems stems from land-use conversion and multiple warming-induced ecosystem changes that are reducing meadow area rapidly (Orth et al. 2006; Thom et al. 2014). Just two published studies provide eelgrass blue C values in the Salish Sea (Spooner 2015; Poppe and Rybczyk 2018). Both studies report C sequestration rates too low to fully or substantially support restoration projects financially: between 8.2 to 40.2 g C m^{-2} yr^{-1} (Poppe and Rybczyk 2018) and between 0.0 to 20.0 g C m^{-2} yr^{-1} (Spooner 2015). The Poppe and Rybczyk study (Padilla transect) was based on six sediment cores within the intertidal area of one established transect in the Padilla Bay National Estuarine Research Reserve (PBNERR), a bay with little sediment supply. They found all six sites to be erosional environments, which may partially explain the low C sequestration rates in their study. Spooner's study was based on a similar number of sediment cores within the K'ómox Estuary on Vancouver Island, British Columbia. Spooner's cores included depositional areas throughout an active river delta. The K'ómox OC sequestration rates appear slightly lower than the Padilla transect rates, which may be due to their exclusion of the top 20 cm to remove the sediment mixing layer (SML). Results from both studies are still low compared to global averages (Kennedy et al. 2010). Each of these studies provided vital insight into the low blue C values in the Salish Sea, yet left room to explore what the upper bounds of eelgrass blue C here might be.

Research objectives

Here we present the results from our search for *Zostera marina* sediment OC in Skagit County, Washington State to determine whether this species and region has the capacity for higher percent, stock, and accumulation rate ranges than those reported to date (Spooner 2015; Poppe and Rybczyk 2018). Data from this, when considered with concurrent studies in Washington and British Columbia (Pacific Northwest Blue C Working Group, pers. comm.; Postlethwaite et al. 2018; Prentice 2018), may serve to improve global average estimates of seagrass C sequestration and offer site- and region-specific values to managers for realistic assessment of restoration and mitigation requirements (Lavery et al. 2013).

The primary objectives of this study were to 1) Seek out and quantify OC percent, stock and accumulation rates in sites likely to support C sequestration *a priori*, and 2) assess these OC values from this study in the context of a) data from the Padilla transect study (Poppe and Rybczyk 2018), b) other *Zostera marina* studies around the world (Miyajima et al. 2015; Dahl et al. 2016; Rohr et al. 2016; Spooner 2015; Prentice 2018), and c) reported global average seagrass blue C (Kennedy et al. 2010; Fourqurean et al. 2012; Cebrian et al. 1997). A secondary objective which gained our interest during the study was to explore which ecological drivers, including proximity to active riverine sediment input, eelgrass canopy complexity, tidal elevation, and sediment grain size, were most responsible for variability in OC stocks. We selected sites in areas meeting the following deposition-supporting criteria: a) permanently flooded (subtidal) eelgrass meadows, where constant exposure to suspended particulate matter and lack of wave wash enables settlement out of the water column and contributes to OC burial (French and Spencer 1993), b) in interior areas of meadows with a combination of high stem density and canopy height that might provide sloughed OM, slow water, trap suspended particles, and enable particle settlement (Poppe 2016; Oreska et al. 2017), c) near distributary mouths of active river deltas where sediments carried from river banks or beach erosion are settling as their water conveyance slows (DeLaune et al. 1989, Chmura and Hung 2004, Thorne et al. 2014), and d) in sediments composed of high percentage of fine particles, which harbor more OM and where a high particle surface area to volume ratio enables more OC adsorption (Chanton et al. 1983; Cundy et al. 1995; Keil and Mayer 2014;).

We hypothesized that we would find higher OC values in areas meeting the above criteria than found in the Padilla transect due to its lacking a sufficient sediment load to support net accretion (Poppe and Rybczyk 2018). Finally, we hypothesized that tidal elevation would be revealed as the primary driver of OC stock variability, with the highest OC stocks accumulating in the lowest within-meadow elevations. Results from this study are some of the first to report carbon sequestration rates across a variety of eelgrass ecosystems in the central Salish Sea.

Methods

Study site

We measured sediment OC in three adjacent estuaries within Skagit County, Washington State: Samish Bay, Padilla Bay, and Skagit Bay (Figure 3). The bays were selected for their extensive eelgrass meadows and for their freshwater input rates, and therefore differing sediment loads, for exploring effects of river size on sediment accretion rates. Samish Bay is an estuary along the northern-most coastline of Skagit County. It is separated from Padilla Bay to the south by Samish Island (a historic island, connected to the mainland by a wide, dike-induced tombolo for some 100 years) and surrounded by residential and agricultural lands to the east and south and by a steep, forested hillside to the north. Hydrology is influenced by the 40 km Samish River, which drains a 227.4 km^2 watershed at an annual mean of 9.4 m^3 s^{-1} in mid-April (USGS 2017). Padilla Bay is a 4200 ha bay within a National Estuarine Research Reserve north of Puget Sound. Hydrology in the reserve is influenced somewhat by inputs from the Samish River to the north, and Skagit River through the Swinomish Channel to the south, as well as the small, slow-moving Joe Leary and No Name Sloughs, draining just 19.0 km^2 and delivering less than 1.4 m^3 s^{-1} (Bulthuis 2013). Most sediments here were historically delivered directly by the Skagit river, but cut off when the river channel changed, helped by diking for agricultural development in the beginning of the last century, which orphaned this estuary from the river's north fork (Bulthuis 2013). Samish and Padilla Bay together contain roughly 27% of the eelgrass in the Puget Sound basin (Selleck et al. 2005; Goehring et al. 2015). The Skagit River delta has the greatest hydrological input of the three bays, and second largest in Washington State, running 241.4 km from mountains in British Columbia into the Salish Sea between La Conner and Stanwood, Wa, and draining 6,879 km^2 at an annual mean of 424.7 m^3 s^{-1} in mid-April (USGS 2017).

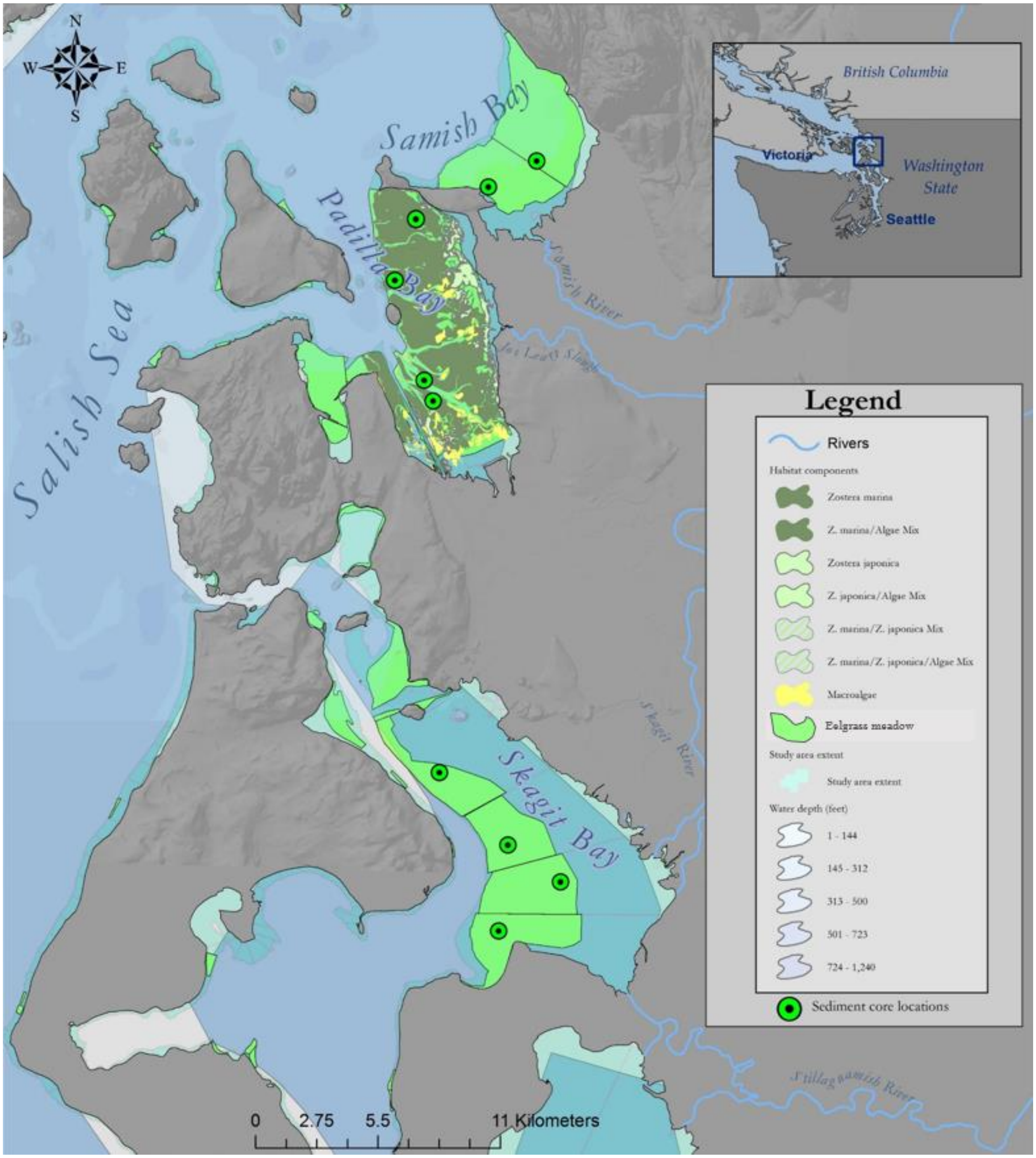


Figure 3: Study area, including, Samish, Padilla, and Skagit Bays. Circles indicate sampling sites. Two cores were taken at each site for a total of 20 cores. All cores used for OC analyses except for sequestration rates, for which n = 7. Vegetation polygons are from Washington Dept. of Natural Resources and geodata from Arcmap (2008).

Sites within bays were selected *a priori* that were likely to be depositional to maximize the potential of documenting carbon sequestration in the area (Figure 3). We chose two sites in Samish Bay, and four sites in each of Padilla and Skagit Bays. Site criteria included a) location within a structurally complex patch (high canopy height and stem density) (Trevathan-Tackett et al. 2015) of a meadow at least 50 m in diameter (Gullström et al. 2017), b) within the lower- or subtidal zone, below approximately -0.4 m mean lower low water (MLLW), and/or c) near a distributary mouth of an active river system where sediment input was visually apparent (WDNR 2012; NOAA 2013; Google Earth Pro 2010-2016).

Field methods

Eelgrass characteristics

We recorded stem density, and canopy height adjacent to each coring location. Stem density was measured by counting stems within a 20 x 20 cm steel square adjacent to the coring hole at each site. Canopy height was measured by taking 3 handfuls of at least 3 stems and measuring from the substrate to the top of the mid-sized leaves.

Tidal elevations

We estimated elevations using the NOAA tide predictions (NOAA 2016) and measuring water depth and time at each coring site. For Skagit Bay sites, tides were based on the gauge at Crescent Harbor, Whidbey Island. Padilla Bay tides were based on NOAA tidal height predictions for the north end of the Swinomish Channel in the south and corrected by adding 30 minutes for sites in the middle and 60 minutes for sites in the north of the bay. Samish tidal heights were based on NOAA tide predictions for Chuckanut Bay. Because of the inaccuracy of tidal predictions due to atmospheric pressure, wind waves, and distance from the tidal gauge, we designated each site as either lower intertidal or subtidal based on our measured depths and tide prediction corrections. Sampling was conducted as close to low tide as possible on each sampling day.

Sediment coring

We collected two cores from each site, one within a structurally complex, vegetated patch, and one in an unvegetated patch with matching elevation nearby, if available (Table 1), for reference. Cores were taken in the approximate center of an eelgrass meadow of at least 50 m in average diameter. This increased the likelihood that the patch had influenced sediment characteristics therein for at least 100 years, based upon lateral patch growth rates between 2-40 cm yr^{-1} (Olesen and Sand-Jensen 1994) and upon *Z. marina* spreading mostly asexually, through rhizome growth, rather than seed dispersal (Yang pers. comm.). One hundred years was selected because it is the age at which lead-210 (^{210}Pb) activity, used here for geochronology, drops to zero, rendering sediments below the 100 year depth unsuitable for dating by this method (Krishnaswami 1971).

Sediment cores were collected by driving 10 cm wide pvc corers into the substrate with a sledge hammer or fence post pounder, as close to 100 cm as possible (Figure 4). Compaction was measured by placing a graduated 3 cm pvc pipe to reach the substrate level within and adjacent to the corer once the final depth was reached and was recorded as the difference between these depths. Compaction was assumed to be linear during sampling and estimations of depth corrections were applied as such (Marbà et al 2015). We collected cores on foot for intertidal sites and by scuba for subtidal sites. We recorded coordinates measured with a GPS to mark each core location. Cores were transferred upright to the lab and frozen for slicing. Freezing facilitated slicing equivalent subsamples without propagation of error in depth measurement and avoided further compaction that can occur when extruding a soft core with a piston and hammer.

Figure 4: Field team, Erin Murray, University of Washington, and Taz, sediment coring with 10 cm x 1.4 m pvc coring tubes, aluminum tripod, and come-along in Skagit Bay, Wa.

Laboratory methods

Bulk density

We sliced 2 cm sections of each core, measured for wet volume, then dried at 105 °C for at least 72 hours (Crooks et al. 2014). We measured the dry weight (DW) of each puck and calculated the bulk density as the ratio of dry weight to wet volume. Half of each puck was kept intact for grain size analysis and visual reference and half ground in a mortar and pestle, followed by a

Thomas Wiley Mini-Mill with #40 screen (0.425 mm mesh). Samples were stored in labeled plastic bags for CN analysis and ^{210}Pb dating (Poppe and Rybczyk 2018).

Sediment composition

We measured organic vs. mineral content by loss on ignition. Twenty-gram subsamples were re-dried for approximately 5 hours prior to weighing, then burned at 500 °C for 6-24 hours in pre-weighed, ceramic crucibles. Percent organic matter by weight was the ratio of the mass lost to initial mass. Percent mineral matter was the difference of 100 % minus organic matter mass %. Percent pore space (porosity) was the difference of 100% minus the sum of organic matter volume % and mineral matter volume %. We calculated volume of organic and mineral matter as the product of the percent by weight and a known organic or mineral particle density of 1.14 and 2.62 g cm^{-3}, respectively (Eq. 1) (Callaway et al. 1996).

$$\text{Eq. 1: } V = \%\, M \times D$$

Where V = organic matter or mineral matter, $\%M$ = percent organic or mineral matter by weight, and D = known organic or mineral particle density (g cm^{-3}).

We conducted a grain size analysis to estimate percent fine sediments (i.e. < 63 µm). Thirty mL portions from homogenized 2 cm sediment slices were re-dried for at least 5 hours before weighing. We soaked these samples for at least 1 hour, periodically shaking and swirling to separate joined clay grains (Keil et al 1994). We then separated sands from fines, rinsing samples through a 63 µm sieve. Sands were rinsed from the sieve into evaporating dishes, dried for 72 hours and re-weighed to calculate % sand and % fines.

Carbon Content

Percent organic carbon

We calculated % OC from loss on ignition (LOI). The OC fraction of total organic matter (OM) lost to LOI was determined from direct C-analysis on representative samples from each core before and after LOI. Three 2-cm sections of each sediment core (n=60) were run in a Thermo Electron Corp. FlashEA 1112 nitrogen and carbon analyzer. We packed ~450 mg subsamples of each slice into tin capsules with adjustments of sediment quantity to ensure the mass of C was sufficient to lie within the range of the carbon calibration curve. A chemical and soil standard were analyzed for quality control at the start of each analyzing period and after every ten samples or as needed. We performed direct C-analysis on post-LOI subsamples of the same core sections to determine % inorganic C, assuming all OC (and no inorganic C) was burned away in LOI. Corresponding OC-content and LOI values were correlated using a Pearson's Correlation. This ratio was then used to determine % OC from OM lost on ignition in every 2 cm sediment core subsection.

Organic carbon density

Organic C density was calculated as the product of % OC and bulk density for each 2 cm core section (Eq. 2).

$$\text{Eq. 2: } \boldsymbol{d} = \frac{\%\,\boldsymbol{C}}{\mathbf{100}\,\%} \times \boldsymbol{D}$$

Where d = OC density, %C = percent OC, and D = sediment bulk density (g cm^{-3}).

Organic carbon stock

The mass of OC in each section calculated as the product of the OC density in that section and the volume of that section (Eq. 3),

$$\text{Eq. 3: } m = d \times V \times 10{,}000$$

where m = OC mass (g m^{-2}), d = OC density (g OC cm^{-3}), V = puck volume (cm^{-3}) and 10,000 is the conversion factor from cm^2 to m^2. We summed OC over 50 cm for each core.

We used inverse distance weighting in ArcMap to interpolate and map spatial variation in OC stocks throughout the study area.

Long term accretion

We used the Constant Initial Concentration (CIC) method of ^{210}Pb radiometric dating to estimate sediment accretion rates in recently-deposited (< 100 years) sediments (Krishnaswami 1971). The naturally-occurring ^{210}Pb emanates to the air from the decay of gaseous radon from the soils then falls to the ground with rain at an assumed constant rate (Nevissi 1985; Crooks et al. 2014) (Figure 5). Lead-210 which did not emanate to the atmosphere occurs in low and unchanging levels in soils everywhere where its concentration is “supported” by the uranium decay series. Recently-deposited sediments contain “unsupported,” or “excess” ^{210}Pb, which accumulates at the sediment surface with rain and overland water flow from the watershed.

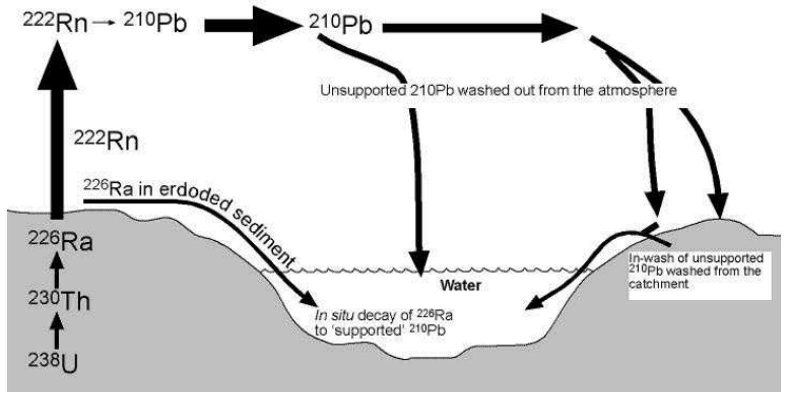


Fig 5 Pathway of ^{210}Pb from uranium-238 in soils to supported (background levels in the soils) and unsupported (washed into the sediments with rain and watershed inputs) ^{210}Pb. From Appleby and Oldfield (1984).

We estimated sediment accretion rates from the exponential decrease in activity of excess ^{210}Pb with depth, with the following assumptions: 1) the sedimentation rate and the flux of initial excess ^{210}Pb to the sediment-water interface is constant, 2) ^{210}Pb loss from the sediment is only from radioactive decay, 3) ^{210}Pb does not migrate within the sediments, 4) sediment mixing only occurs in the sediment mixed layer and is constant with depth, and 5) the decay constant for ^{210}Pb is known, and 6) the activity of ^{210}Pb supported by radium-226 in the sediments is independent of depth (Krishnaswami et al. 1971; Robbins and Edgington 1975).

Excess ^{210}Pb activity was analyzed with a Canberra Germanium Detector (model GL2820R).) Gamma spectrometer measurements yielded gamma emissions at 46 keV (total ^{210}Pb activity) and 351 keV (supported ^{214}Pb activity) and were recorded by Genie 2000 software (Canberra 2001). We calculated excess ^{210}Pb activity in disintegrations per second, or Becquerels (Bq) as the total ^{210}Pb activity detected at 46 keV minus the supported ^{210}Pb activity, detected with ^{214}Pb activity at 351 keV. We analyzed approximately 35 g of sediment in sections of the top 25-32 cm of sediment cores, depending on the depth at which ^{210}Pb activity dropped to zero (Poppe and Rybczyk 2018). Each sample was analyzed for 48 to 72 hours, until the counting error rates for ^{210}Pb and ^{214}Pb dropped below 10%. We analyzed a calibration standard for each core to account for differences in spectrometer counting efficiencies at varying energy levels. We made the standard by adding approximately 0.75 g of pitchblende silica-ore standard (CRM 103-A, New Brunswick Laboratory, USDOE) to a previously analyzed 35 g sediment sample. The vertical distribution of excess 210Pb below the sediment mixing layer follows the exponential curve defined by equation 4 (Kirchner and Ehlers 1998):

$$\text{Eq. 4:} \quad \mathbf{C_x = C_0 \cdot e^{(-\lambda x / S)}}$$

where: x = depth below the sediment surface (cm), C_x = excess ^{210}Pb activity at depth x below the sediment surface (Bq/g), C_0 = is the excess ^{210}Pb activity at the sediment surface (Bq/g), λ = half-life of ^{210}Pb (yr^{-1}), and S = sediment accumulation rate (cm yr^{-1}).

We used a linear regression of the ln of excess ^{210}Pb activity versus depth to determine accretion rate (Eq. 5),

$$\text{Eq. 5:} \quad \boldsymbol{S = -\lambda/s}$$

where λ is the half-life of ^{210}Pb (22.2 yrs) and s is the slope of this regression. Error was calculated for excess ^{210}Pb by propagating error from supported ^{210}Pb and ^{214}Pb counts quadratically, following methods described by Gwozdz (2006). We removed all accretion rates calculated from cores with either insufficient, increasing, or variable ^{210}Pb activity with depth from our dataset (Figure A5).

Lead-210 flux

It was assumed from previous radionuclide accretion rate studies in Padilla Bay that profiles would have mixing due to bioturbation by polychaetes, molluscs, and shrimp (Shull 2001; Kairis 2008). We chose to include the SML in the ^{210}Pb activity profiles to compare data across current blue C literature, which, except for Spooner (2015), elected to retain it. Values reported here are therefore to be considered the upper bounds of accretion rates, and thereby OC accumulation rates, for this region.

Annual flux to the sediment varies regionally with annual rainfall (Appleby 1998). We assessed the relative effect of bioturbation and erosion by comparing ^{210}Pb flux calculated from excess ^{210}Pb inventories for each core in this study to the expected flux from the atmosphere for Seattle, 0.44 Bq cm^{-2} (Nevissi 1985). A flux lower than expected indicates a non-depositional site, while one similar indicates deposition along with bioturbation. Values are reported as a range with minimum flux value being the sum of the area under the curve of the plotted ln of ^{210}Pb density and the maximum value being the integral of the ^{210}Pb density from zero to infinity, assuming one slope.

Organic carbon accumulation rate

We calculated C accumulation rates as the product of the sediment accretion rate and the average carbon density in samples from 0 cm to the depth at which ^{210}Pb activity fell to zero (Eq. 6).

$$\text{Eq 6: } \boldsymbol{a} = \boldsymbol{S} \times \boldsymbol{d} \times \mathbf{10{,}000}$$

Where a = OC accumulation rate (g m^{-2} yr^{-1}), S = sediment accretion rate (cm yr^{-1}), d = OC density (g cm^{-3}) in the top 50 cm, and 10,000 is the conversion factor to m^{2}.

Statistical analyses

We first used one-way ANOVA with the car package in R (Fox and Weisberg 2011) to test the effects of eelgrass presence (vegetated vs. unvegetated coring locations) and of bay (Samish, Padilla, and Skagit) on OC %, stock, and sequestration rate values. We ensured homoscedasticity with a Levene's Test, normality with a Shapiro-Wilk test and by visually inspecting residuals using quantile-quantile (Q-Q) plots.

Model selection for factors affecting organic carbon

We applied a Linear Mixed Effects Model to parse out environmental drivers of variability in OC stock (Burnham and Anderson 2002; Zuur 2010). We first ensured homoscedasticity and normality by visual inspection of residual versus fitted values plots and normal Q-Q plots, respectively (Figure A2). We selected environmental factors likely to have the greatest effect on organic content and sediment deposition, based on findings from previous studies (Poppe 2016; Samper-Villareal et al. 2016; Serrano et al. 2016; Oreska et al. 2017), then eliminated collinear factors (correlation coefficients ≥ 0.3) using Pearson correlation coefficients from pair plots in R (Figure A3) (Lander 2018). Final factors included elevation below MLLW, percent fines, and the product of canopy height and stem density to form a single factor, canopy complexity, which accounted for the combined water-slowing effects of both. The authors recognize that canopy height and stem density can have an inverse correlation, but this was not the case in our study area (correlation coefficient = 0.07, Figure A4). Perhaps light was not limiting to eelgrass in our sampling elevations (-1 to 180 cm below MLLW).

We performed model selection by maximum likelihood estimation with small sample size-corrected Akaike's Information Criteria (lowest AIC_c, or most parsimonious, and highest Δ, or best fit model), using the MuMIn package in R (Bartón 2018). The AIC_c was calculated using $-2\ln(L) + 2K$, where K = the number of parameters and L = the log likelihood of each model. A likelihood ratio test was used to evaluate the statistical significance of the fit of the full model with that of every iteration of reduced model. Uncertainty analysis was performed with the usdm package in R (Naimi 2017).

Our sample size for OC sequestration rates (n = 7) being below the minimum for the number of predictor variables in an LMM (Burnham and Anderson 2003; Zuur 2010), we evaluated the effects of the same environmental factors on OC sequestration rates using a multiple regression analysis (lm, stats package, R Core Team 2016) with a Kendall correlation (rcor.test, ltm package) to ensure factors met multiple regression assumptions. We conducted a power analysis in G Power (Faul et al. 2007) to determine if our small sample size was sufficient to detect an effect.

This study in the context of global blue C

Padilla Bay transect study

We compared average percent (%), stock (kg m^{-2}), and OC accumulation rates (g m^{-2} yr^{-1}) per core in just vegetated sites from all bays in this study (n = 9) to the intertidal data from the Padilla transect (n = 6) using a one-way ANOVA. We checked for homoscedasticity with a Levene's Test. We tested for normality using a Shapiro-Wilk's normality test with the car package in R and visually inspected residuals using Q-Q plots.

Zostera marina studies

We compiled a total of 20 site means from five published studies and created boxplots for visual comparison of our OC values to *Z. marina* blue C studies in temperate locations around the world. Study locations included Finland and Denmark (Rohr et al. 2016), Portugal, Bulgaria, and Sweden (Dahl et al. 2016), Japan's Seto Sea (Miyajima 2015), the British Columbia central coast (Prentice 2018) and SE Vancouver Island (Spooner 2015).

Global seagrass averages

Finally, we plotted our values for percent, stock, and accumulation rates against global seagrass OC medians and ranges (Duarte 2005; Kennedy et al. 2010; McLeod 2011; Fourqurean et al. 2012) and inspected differences visually. Sediment depth over which we averaged percent and calculated OC stocks was reduced to 30 cm, the depth common to all studies, for each comparison.

Due to the premise of our study being to select sites in depositional areas, OC content and accumulation rates presented here should be considered in the upper bounds of what can be estimated in the eelgrass meadows of Skagit Co., WA.

Results

Results from one-way ANOVA revealed no bay effect at the $\alpha = 0.05$ level on % OC by DW ($F = 2.03$ $p = 0.16$ on 2 and 17 df) nor OC stock ($F = 1.65$ $p = 0.22$ on 2 and 17 df), averaged over each core to 50 cm. However, we do present results both as overall means and as means by bay, to consider each meadow as a distinct ecosystem, as might be relevant to an ecosystem-wide economic valuation (VCS 2015). Neither did results of one-way ANOVA reveal any difference in %OC ($F = 2.28$ and $p = 0.14$ on 1 and 18 df) nor OC stock values ($F = 0.59$, $p = 0.45$ on 1 and 18 df) between vegetated and unvegetated patches (Figure A1). All cores were pooled for all analyses, except for comparison to the Padilla Bay transect study (Poppe and Rybczyk 2018) which only reported results from vegetated cores; we used values from vegetated cores alone in this ANOVA.

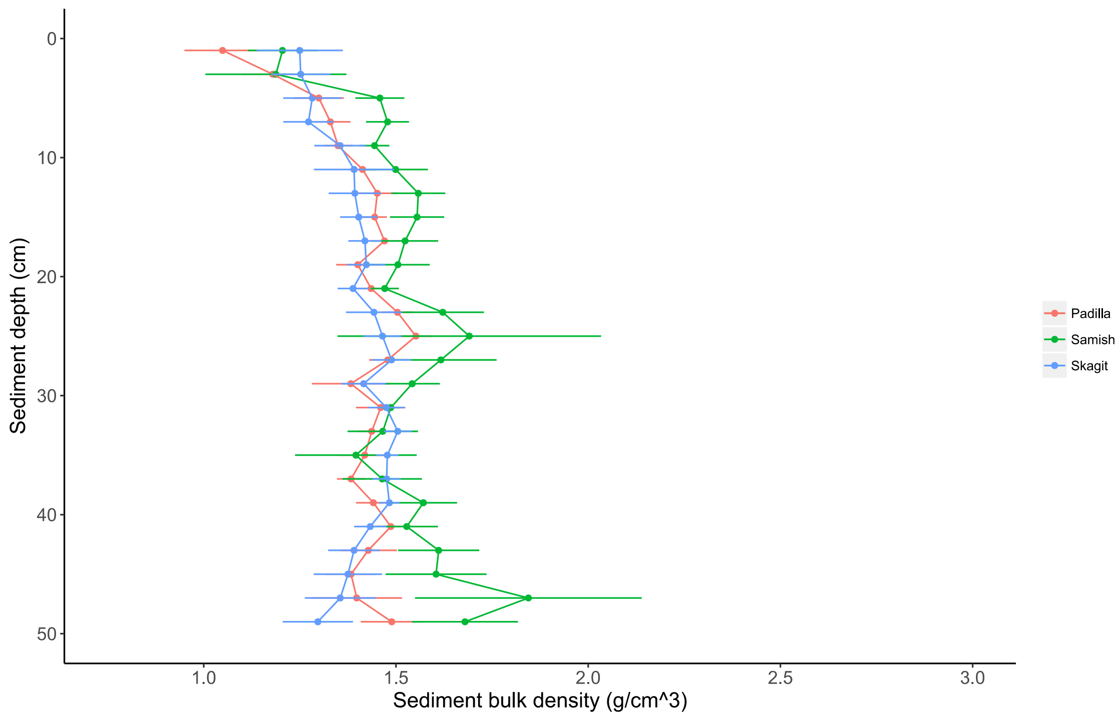
Sediment properties

Mean bulk density ± SE overall was 1.42 ± 0.01 and ranged from 0.35 to 2.68 g cm^{-3} (Table 1, Figure 7). Mean overall % OM by DW ± SE was 1.84 ± 0.04 % and ranged from 0.75% to 7.01%, with highest values in Skagit Bay. One sample in Samish Bay reached over 15%, but contained contents of a whole clam, which caught fire during LOI. Mean % OM for each bay was as follows: Samish 1.49 ± 0.14% SE; Padilla 1.77 ± 0.04% SE; and Skagit 2.08 ± 0.07% SE Mean overall % mineral matter by DW was 98.13 ± 0.04% and ranged from 84.08 to 99.24%. Mean % mineral matter ± SE for each bay for was Samish 98.41± 0.14%, Padilla 98.23 ± 0.04, and Skagit 97.92 ± 0.07%. Mean overall porosity ± SE was 44.81 ± 0.32% and ranged from 8.35 to 86.05 with largest values in Skagit Bay. Mean % porosity ± SE for Samish 42.51 ±; 0.77, Padilla 45.31 ± 0.53, and Skagit 45.28 ± 0.46%. Mean overall % fine sediments <63 µm (%

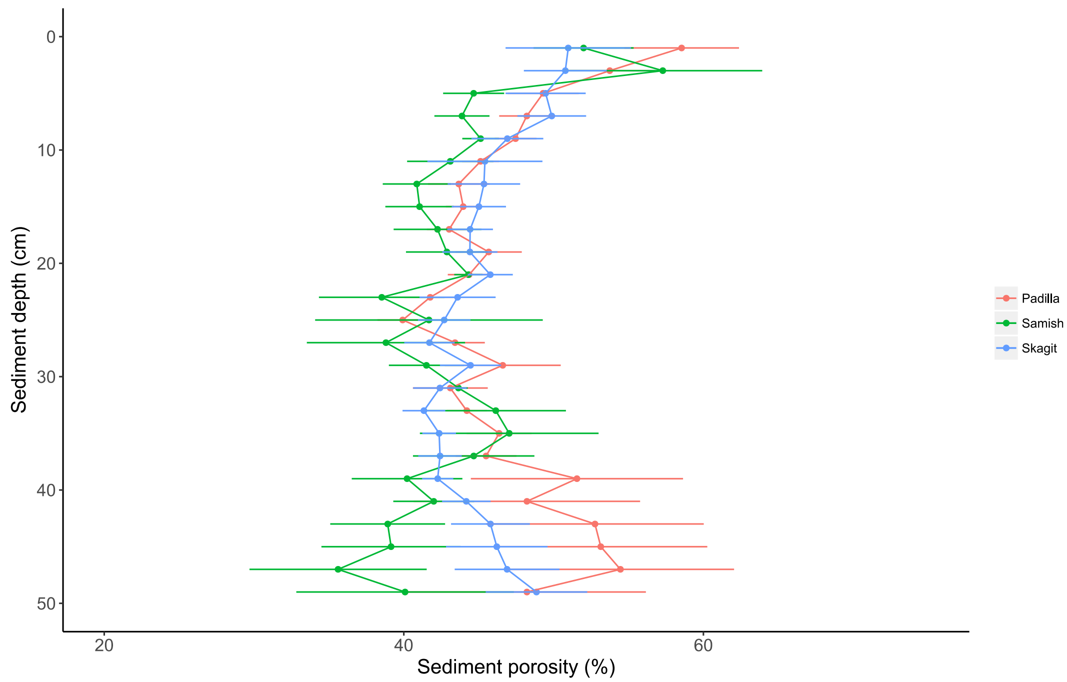
fines) ± SE was 17.49± 0.92% and ranged from 2.96 to 75.66 (Table 1). Mean % fines ± SE for Samish was 7.62 ± 0.84%, Padilla 17.72 ± 1.28%, and Skagit 21.93 ± 1.60%.

Table 1: **Eelgrass sediment characteristics and meadow variables in three study areas within the central Salish Sea in Washington State.** Percent OC is presented as means of the top 50 cm, while OC stock (Mg C ha^{-1}) is the total mass in the top 50 cm of sediment. Sediment accretion rates are estimated using gamma ray spectroscopy of ^{210}Pb activity in sediment, and should be considered the upper bounds in highly mixed estuarine sediments.

	Eelgrass meadow sediment characteristics									Environmental factors		
Bay (river discharge) and core ID	**Bulk Density (g cm^{-3})**	**Porosity (% by vol.)**	**Grain size (% fines <63µm)**	**Organic Matter (% by DW)**	**OC Density (g cm^{-3})**	**Percent OC (% by DW)**	**OC Stock (Mg C ha^{-1})**	**Accretion Rate (cm yr^{-1})**	**C accum. Rate (g m^{-2} yr^{-1})**	**Elevation (tidal zonation)**	**Eelgrass canopy height (cm)**	**Stem density (shoots m^{-2})**
Samish (250 m^3 s^{-1})												
S1a	1.67 (0.11)	36.93 (4.48)	7.20 (1.20)	1.43 (0.10)	0.0056 (0.0006)	0.33 (0.02)	23.18	0.26 (0.10)	13.90	lower	70 (10)	300
S1b	1.54 (0.04)	40.71 (1.43)	5.32 (0.53)	1.28 (0.06)	0.0045 (0.0002)	0.29 (0.01)	19.73	-	-	lower	-	-
S2a	1.45 (0.03)	43.38 (1.20)	9.28 (1.35)	2.09 (0.50)	0.0072 (0.0018)	0.48 (0.19)	32.33	-	-	sub	145 (20)	375
S2b	1.42 (0.04)	45.98 (1.01)	5.54 (2.53)	1.49 (0.04)	0.0048 (0.0001)	0.34 (0.01)	23.18	-	-	sub	145 (18)	386
MEAN	**1.49 (0.02)**	**42.28 (0.87)**	**7.62 (0.84)**	**1.49 (0.14)**	**0.0053 (0.0004)**	**0.37 (0.0004)**	**24.60 (2.70)**	**0.26 (0.10)**	**13.90**		**120 (25)**	**354 (27)**
Padilla (< 1.5 m^3 s^{-1})												
PB1a	1.32 (0.03)	48.56 (1.27)	21.41 (2.82)	2.11 (0.12)	0.0061 (0.0002)	0.49 (0.03)	27.29	-	-	mid	100 (15)	120
PB1b	0.29 (0.05)	49.58 (1.89)	23.11 (3.24)	1.94 (0.18)	0.0054 (0.0002)	0.45 (0.04)	26.00	-	-	mid	100 (12)	126
PB2a	1.46 (0.06)	43.18 (2.38)	10.30 (0.77)	1.56 (0.06)	0.0052 (0.0002)	0.36 (0.01)	32.11	0.57 (0.01)	29.45	lower	80 (16)	145
PB2b	1.45 (0.05)	43.54 (2.09)	13.30 (1.95)	1.69 (0.07)	0.0056 (0.0002)	0.39 (0.02)	25.32	-	-	lower	80 (16)	134
PB3a	0.70 (0.11)	48.53 (1.34)	12.04 (1.01)	1.63 (0.06)	0.0048 (0.0004)	0.38 (0.01)	23.61	0.84 (0.01)	40.14	sub	244 (10)	100
PB3b	1.52 (0.04)	40.70 (0.99)	11.44 (0.93)	1.59 (0.06)	0.0058 (0.0002)	0.37 (0.01)	26.15	0.7 (0.004)	40.95	sub	242 (33)	100
PB4a	1.35 (0.04)	45.52 (0.93)	17.09 (2.97)	2.03 (0.17)	0.0044 (0.0002)	0.47 (0.04)	21.07	-	-	upper	30 (12)	1700*
PB4b	1.46 (0.02)	44.23 (0.83)	30.98 (4.78)	1.53 (0.06)	0.0056 (0.0002)	0.35 (0.01)	25.62	-	-	upper	25 (6)	125
MEAN	**1.19 (0.03)**	**45.31 (0.53)**	**17.72 (1.28)**	**1.77 (0.04)**	**0.0058 (0.0001)**	**0.41 (0.01)**	**25.90 (1.11)**	**0.7 (0.006)**	**36.85 (3.71)**		**114 (30)**	**121 (6)** PB4a excluded from avg
Skagit (17,000 m^3 s^{-1})												
Sk1a	1.33 (0.06)	48.56 (1.77)	34.21 (4.32)	2.20 (0.17)	0.0062 (0.0004)	0.51 (0.04)	29.53	0.64 (0.08)	44.44	lower	105 (5)	425
Sk1b	1.41 (0.03)	43.66 (0.94)	18.16 (2.38)	1.75 (0.07)	0.0049 (0.000)	0.36 (0.01)	22.02	0.93 (0.02)	45.27	lower	-	-
Sk2a	1.35 (0.06)	48.82 (2.35)	22.60 (4.31)	3.45 (0.38)	0.0097 (0.0019)	0.79 (0.09)	49.20	).95 (0.006)	93.04	lower	168 (3)	150
Sk2b	1.48 (0.03)	43.29 (1.26)	17.78 (3.03)	1.66 (0.18)	0.0051 (0.0008)	0.38 (0.04)	23.61	-	-	lower	-	-
Sk3a	1.40 (0.02)	45.27 (0.84)	12.55 (1.68)	1.84 (0.05)	0.0058 (0.0002)	0.42 (0.01)	27.13	-	-	upper	83 (6)	183
Sk3b	1.40 (0.04)	44.84 (1.01)	28.52 (8.26)	1.91 (0.17)	0.0056 (0.0005)	0.44 (0.04)	26.31	-	-	upper	-	-
Sk4a	1.38 (0.01)	46.05 (0.64)	23.92 (3.59)	1.94 (0.04)	0.0059 (0.0001)	0.45 (0.01)	27.95	-	-	sub	65 (5)	150
Sk4b	1.46 (0.02)	42.26 (1.17)	18.51 (3.76)	2.00 (0.06)	0.0067 (0.0003)	0.46 (0.01)	30.74	-	-	sub	230 (15)	50
MEAN	**1.42 (0.02)**	**45.28 (0.46)**	**21.93 (1.60)**	**2.08 (0.07)**	**0.0063 (0.0001)**	**0.48 (0.02)**	**29.56 (2.98)**	**0.86 (0.08)**	**60.91 (16.06)**	**-0.30 MLLW**	**130 (30)**	**192 (62)**
MEAN OVERALL	**1.42 (0.01)**	**44.77 (0.33)**	**17.49 (0.92)**	**1.87 (0.04)**	**0.0058 (0.0001)**	**0.43 (0.01)**	**27.10 (1.40)**	**0.56 (0.16)**	**43.88 (9.19)**		**127 (22)**	**175 (29)**

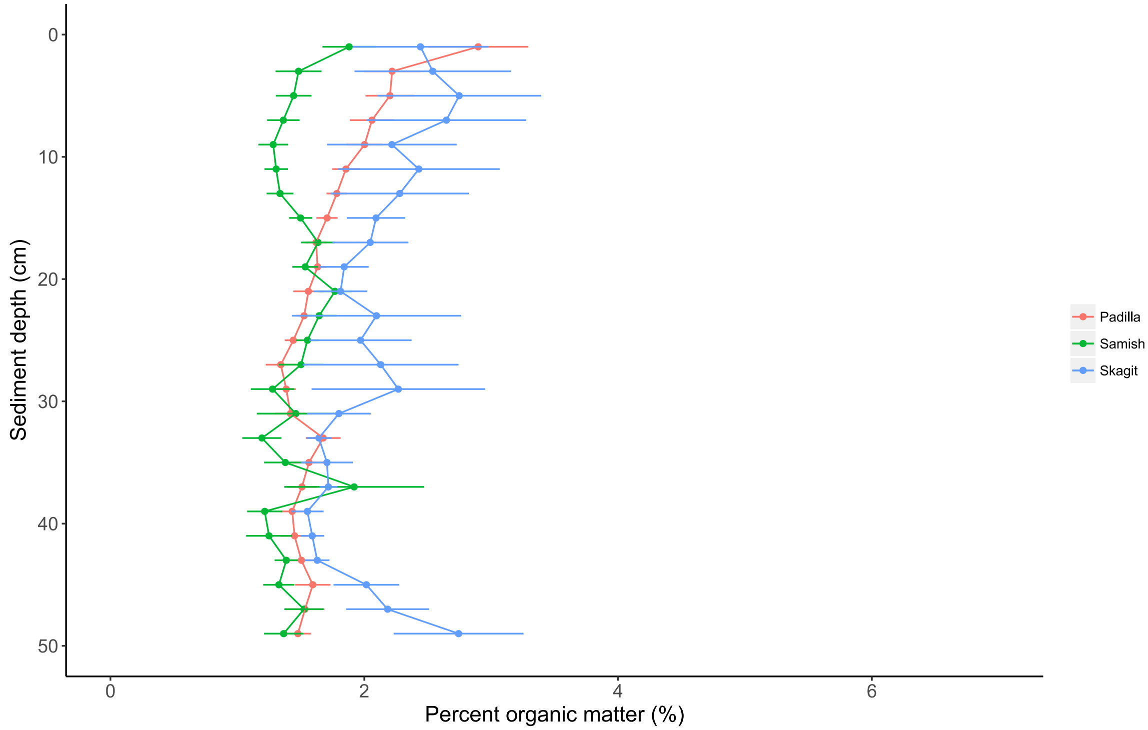


a)



b)

Figure 6: Depth profiles of a) bulk density, b) porosity, and c) percent organic matter (next pg) to 50cm for Samish (n = 4), Padilla (n = 8), and Skagit (n = 8) Bays. Error bars represent SE.



c)

Figure 6 ctd: Depth profiles of a) bulk density, b) porosity, and c) percent organic matter to 50cm for Samish (n = 4), Padilla (n = 8), and Skagit (n = 8) Bays. Error bars represent SE.

Carbon content

Results of direct C analysis of a subset of samples from each core (n = 45) before and after LOI yielded an OC:OM ratio of 0.23 ± 0.02 SE (Figure 8). This was used to calculate % OC from % OM estimated from LOI. Mean % inorganic C ± SE was 0.06 ± 0.02% of sediment DW, ranging from 0.0009% to 0.85%, with most values near the mean, and highest values in Samish Bay in areas of high shell hash.

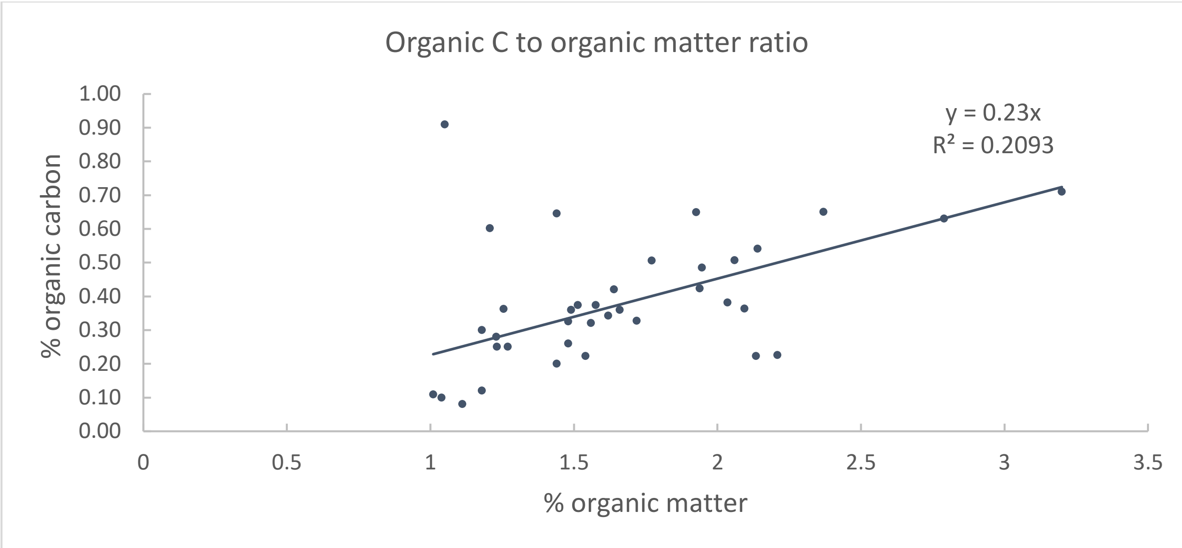


Figure 7: Organic carbon to organic matter ratio determined by direct carbon analysis before and after loss on ignition (LOI) at 550°C for at least 6 hours. N=44.

Organic carbon percent and density

Overall mean % OC was 0.43 ± 0.01 % DW and ranged from 0.17-3.66%. Mean % OC ± SE by bay was as follows: Samish 0.37 ± 0.03%; Padilla 0.41 ± 0.01%; and Skagit 0.48 ± 0.02% (Table 1; Figures 8, 9, A5, and A6). Overall mean OC density ± SE was 0.0058 ± 0.0001 g cm^{-3}, ranging from 0.0018-0.0479 g cm^{-3}. Mean OC density ± SE by bay was as follows: Samish 0.0053 ± 0.0004 g cm^{-3}; Padilla 0.0058 ± 0.0001 g cm^{-3}; and Skagit 0.0063 ± 0.0001 g cm^{-3}.

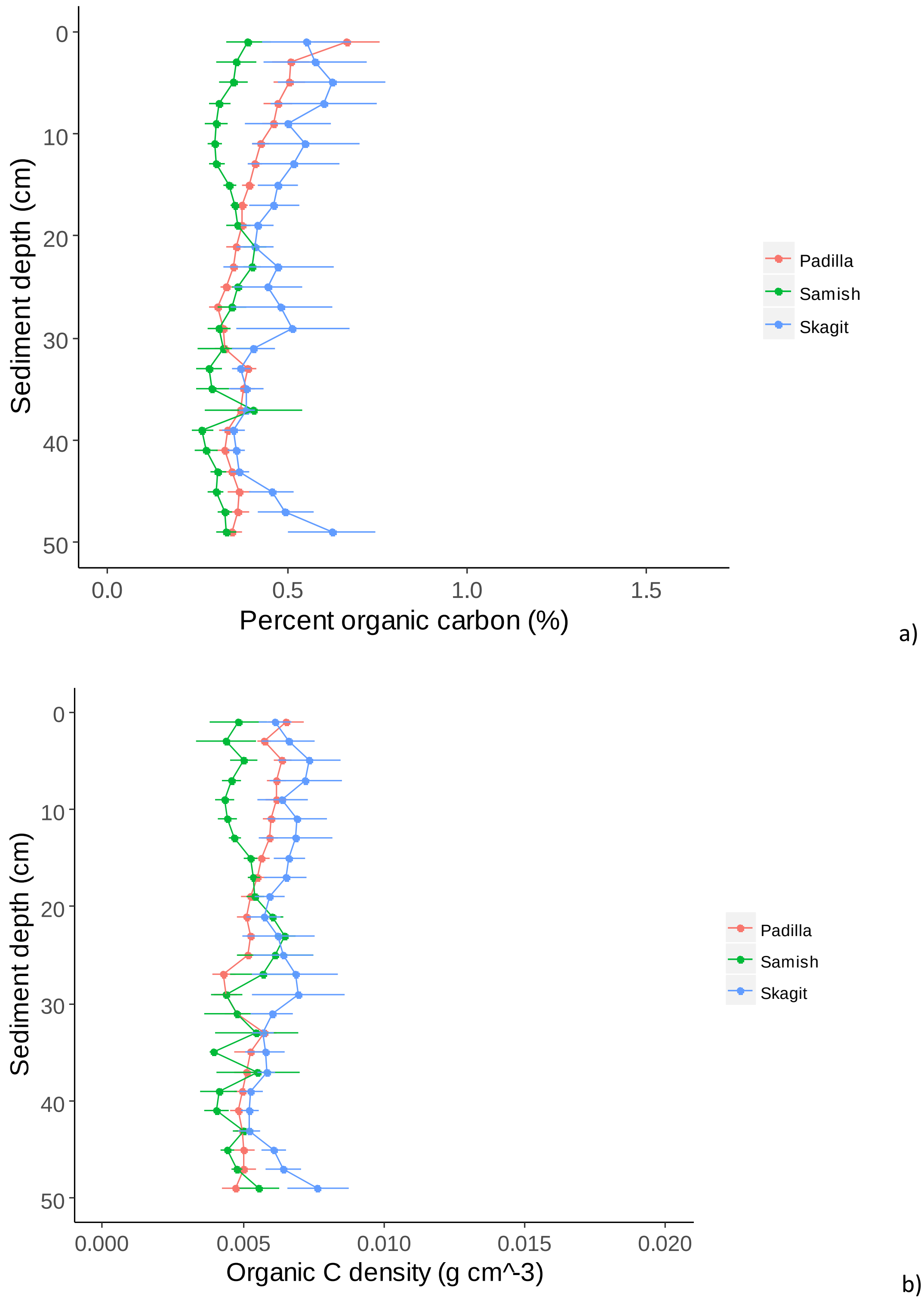


Figure 8: Depth profiles of a) mean % organic C and b) organic C densities (g cm^{-3}) (N=500) in Samish, Padilla, and Skagit Bays, WA. N=8, 4, and 8, respectively. Error bars represent SE.

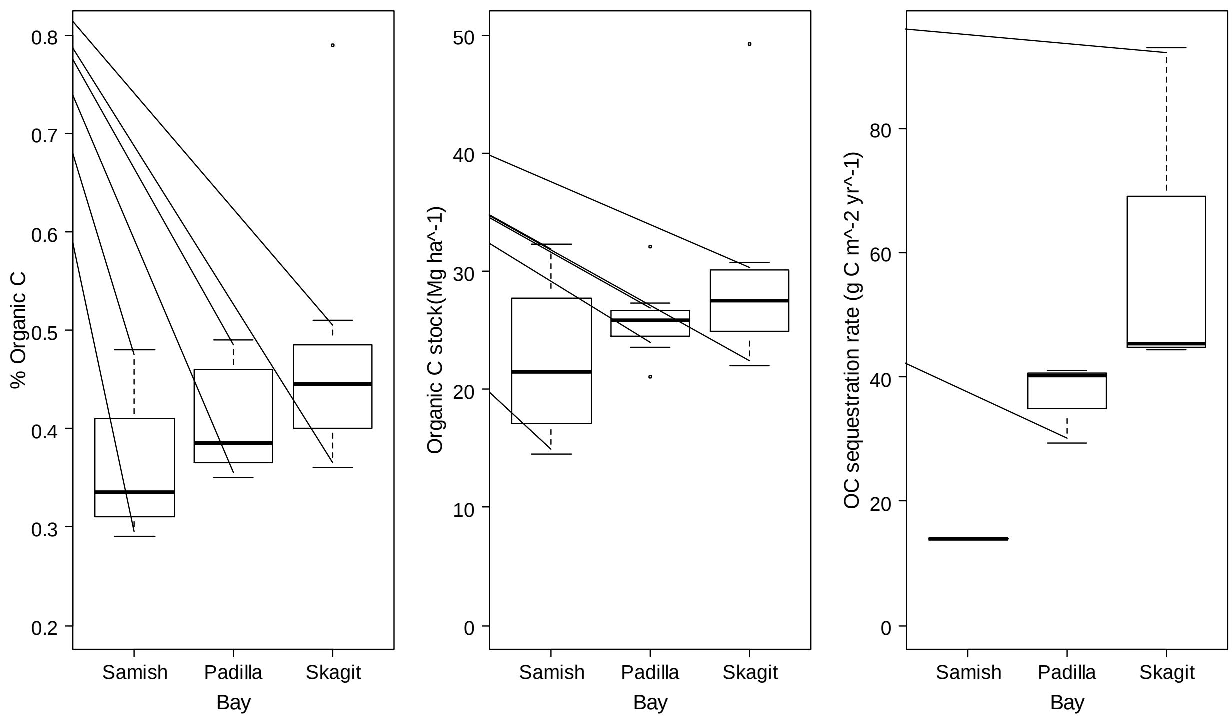


Figure 9: Box and whisker plots of percent organic C and organic C stock (n=20) to 50 cm, and C sequestration rates (n = 6) to 27-33 cm, from this study by bay.

Organic carbon stock

We found an overall mean OC stock of 27.10 ± 1.40 Mg ha^{-1}, ranging from 19.73 to 49.20 Mg ha^{-1} over 50 cm of sediment depth (Table 1, Figure 9). Mean OC stock ± SE by bay over 50 cm was as follows: Samish 24.60 ± 2.70 Mg C ha^{-1}; Padilla 25.90 ± 1.11 Mg C $ha^{-}1$; and Skagit 29.56 ± 2.98 Mg C ha^{-1}.

Organic C accumulation rates

The estimated overall mean OC sequestration rate ± SE was 43.88 ± 9.19 g C m^2 yr^{-1} and ranged from 13.90 to 93.04 g C m^2 yr^{-1} (n = 7, Table 1, Figure 10). Mean OC sequestration rate ± SE by bay was as follows: Samish 13.90 g C m^{-2} yr^{-1}; Padilla 36.85 ± 3.71 g C m^{-2} yr^{-1}; and Skagit 60.91 ± 16.06 g C m^{-2} yr^{-1}. Site Sk2a experienced a punctuated deposition event when the N fork of the Skagit main stem changed direction over the past five years (Google Earth Pro 2010-2016). I chose to retain this core in the dataset, as it is representative of events occurring regularly in a large, tumultuous delta system such as the Skagit. Five cores had ^{210}Pb depth profiles which showed evidence of mixing or insufficient levels of ^{210}Pb, failing CIC assumptions, and were discarded (Figure A7).

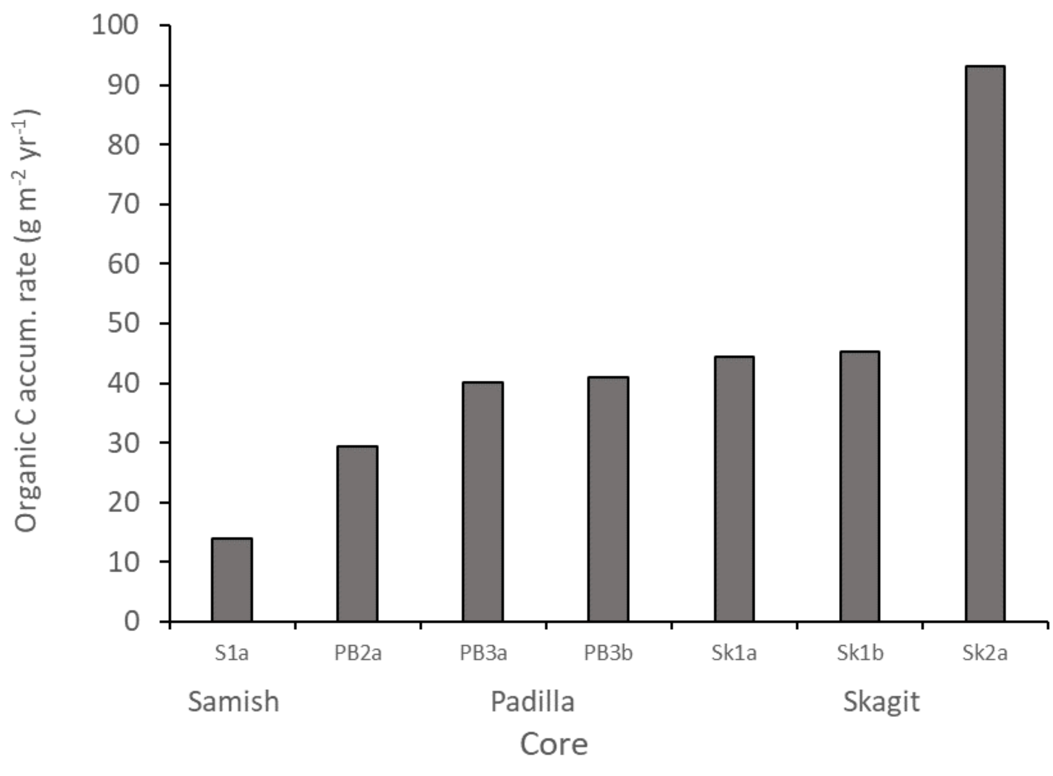


Figure 10: Organic C sequestration rates estimated by the CIC method of ^{210}Pb geochronology. PB = Padilla Bay, S = Samish Bay, and Sk = Skagit Bay cores.

^{210}Pb flux values for cores from Samish 1a, Skagit 1a, Skagit 1b, and Skagit 2a had all or part of their range below the expected flux to the sediment for the region, 0.44 disintegrations per minute (dpm) (Nevissi 1985), suggesting that the positive accretion rates found may be due to bioturbation and therefore inflated (Table 2). Values reported here should be considered the maximum possible in this area.

Table 2: **^{210}Pb flux values (dpm cm^{-2} yr^{-1}) and estimated accretion rates (cm yr^{-1}) in selected cores**. The expected flux for the Puget Sound region is 0.44 dpm cm^{-2} yr^{-1} (Nevissi 1985). Flux values < 0.44 cm^{-2} yr^{-1} indicate an erosional site with inflated accretion rates due to mixing from bioturbation or other surficial turbulence. Values = 0.44 dpm cm^{-2} yr^{-1} indicate accretion along with sediment mixing. Values > 0.44 Bq cm^{-2} indicate a greater than expected flux of ^{210}Pb from the atmosphere, but the dominant transport process is still likely mixing.

Core ID	Samish 1a	Padilla 2a	Padilla 3a	Padilla 3b	Skagit 1a	Skagit 1b	Skagit 2a
^{210}Pb flux (dpm cm^{-2})	0.37 - 0.39	1.19 - 1.26	0.66 - 1.28	0.96 - 1.35	0.40-0.78	0.42-0.52	0.41 - 1.03
Est. accretion rate (cm yr^{-1}) ± SE	0.26±0.10	0.25±0.01	0.84 ± 0.01	0.66 ± 0.004	0.65 ± 0.07	0.93 ± 0.02	0.95 ± 0.006

Factors driving organic C variability

Of canopy complexity, tidal elevation, and % porosity as potential explanatory factors, the best-fit, most parsimonious model from a Linear Mixed Effects Model included sediment porosity alone; porosity explained the most variability and had a positive effect on OC stock. (χ^2=4.87, $p < 0.0001$, weight=0.496) (Table 3, Figure 11). It should be noted that percent fine sediments (grainsize < 63 µm), covaried with porosity (R^2=0.3 p = 0.01), and so was removed from the set of candidate models, but had a similarly positive effect on OC stock (Figure A8). While the importance of the effects of porosity and percent fine sediments on OC content should not be overlooked, it should be noted that there was little variability in OC content within and among our *Z. marina sites* to be explained (Figure 9 and A1). The inclusion of the random effect, site, did not improve model fit (marginal R^2 = conditional R^2).

Table 3: **Strength of evidence for environmental factors affecting OC stock**. Models are ranked using Akaike Information Criterion corrected for small sample size (AICc). The model containing the environmental factors with the highest strength of evidence for explaining the variation in OC in this study (in gray) is distinguished from those below it by lowest AICc (most parsimonious) and ΔAICc (best fit), and highest weight (K-L distance) and Chi^2 (Burnham and Anderson 2002). Models 2-4 were ranked nearest the top model and are shown for comparison only.

Model selection results

Model	df	ΔAICc	AICc Weight	log likelihood	Chi^2	Pr (>Chi^2)
1) Organic C stock ~ % pore space	5	0	0.496	-63.77	4.87	<0.0001
2) Organic C stock ~ % pore space + elevation	5	1.26	0.263	-63.21	4.75	<0.0001
3) Organic C stock ~ %pore space + eelgrass	5	3.90	0.070	-63.63	0.00	1
4) Organic C stock ~ null model	4	4.16	0.062	-66.76		

Final Model Structure for factors affecting OC stock (OC stock ~ % pore space)
Fixed effects

Environmental factor	Value	SE	T	p
Intercept	26.67	1.31	20.33	< 0 0001
Porosity (% pore space)	3.17	1.35	2.35	< 0.0001

Random effects
Coring site nested in bay

Marginal R^2 (variance explained by fixed effects) 23.6%

Conditional R^2 (variance explained by full model) 23.6%

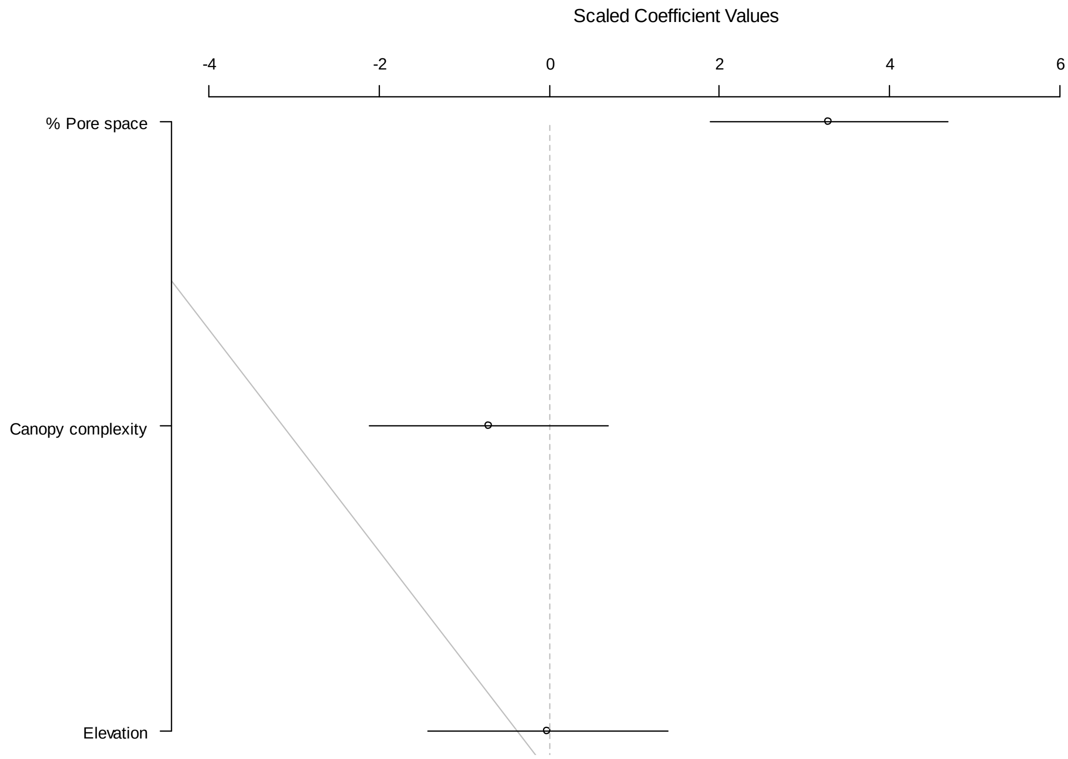


Figure 11: Scaled coefficient plot showing the relative strength of evidence for environmental factors affecting organic carbon stock in eelgrass sediments across three bays in Skagit County, WA. Points are scaled coefficient values and lines represent 95% confidence intervals (CI) for the three factors of interest in the Linear Mixed Effects Model. Porosity had a positive effect on organic C stock, while effects of elevation and canopy complexity were not different from zero.

Results of a multiple regression showed no effect of grainsize, eelgrass canopy complexity, elevation, nor bay on sequestration rates (F = 2.76 on 4 and 1 df; p=0.42). A power analysis in Gpower revealed that low power may be preventing detection of an effect, if one should exist ($n = 6$, $\alpha = 0.05$, power = 0.055, $F_{crit} = 224.6$).

This study in the context of local and global seagrass blue C

One-way ANOVA revealed higher OC sequestration rates in this study than in the Padilla transect (F=8.42 on 1 and 10 df, p = 0.02) (Figure 12). There was no difference in % OC nor OC stock between studies (F = 0.26 and p = 0.62 on 1 and 13 df, and F = 1.86 and p = 0.20 on 1 and 13 df, respectively).

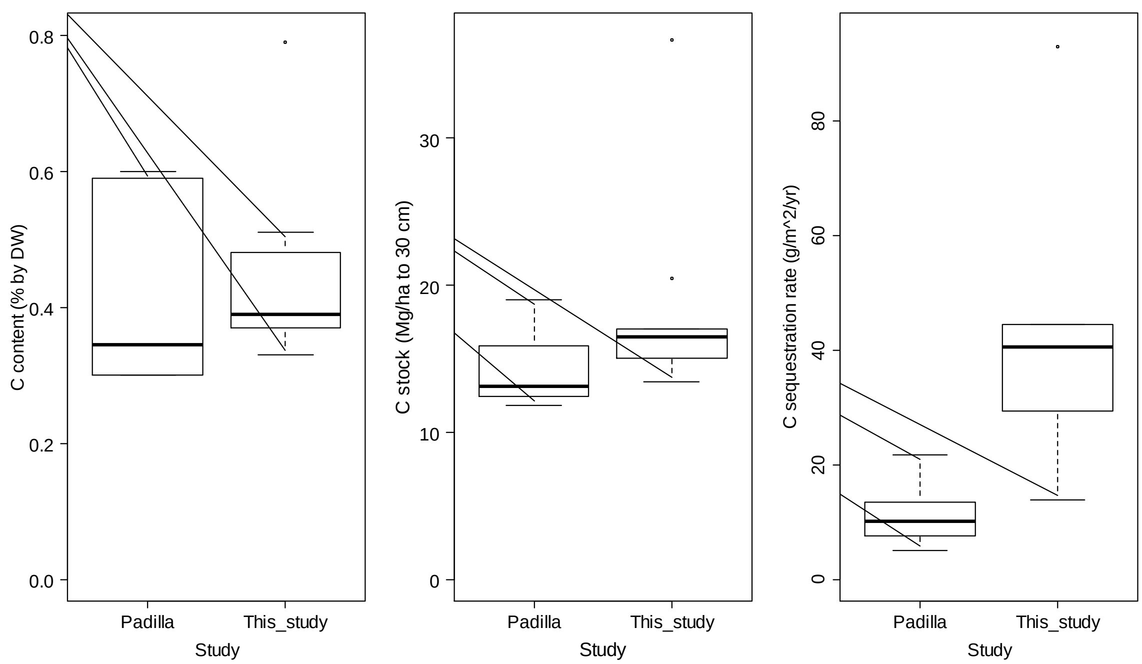


Figure 12: Boxplots of mean % OC, OC stock, and OC sequestration rates from vegetated sites from this study compared to those from the Padilla transect (all vegetated) (Poppe and Rybczyk 2018).

Comparing blue C values visually from this study (n=20) to site means of four *Z. marina* studies in Finland and Denmark (Rohr et al. 2016), Portugal, Bulgaria, and Sweden (Dahl et al. 2016), Japan's Seto Sea (Miyajima 2015), the British Columbia central coast (Prentice 2018) and SE Vancouver Island (Spooner 2016) combined (n=20) suggests higher accumulation rates in this study than combined *Z. marina* studies, but no difference in percent OC nor OC stock (Figure 13).

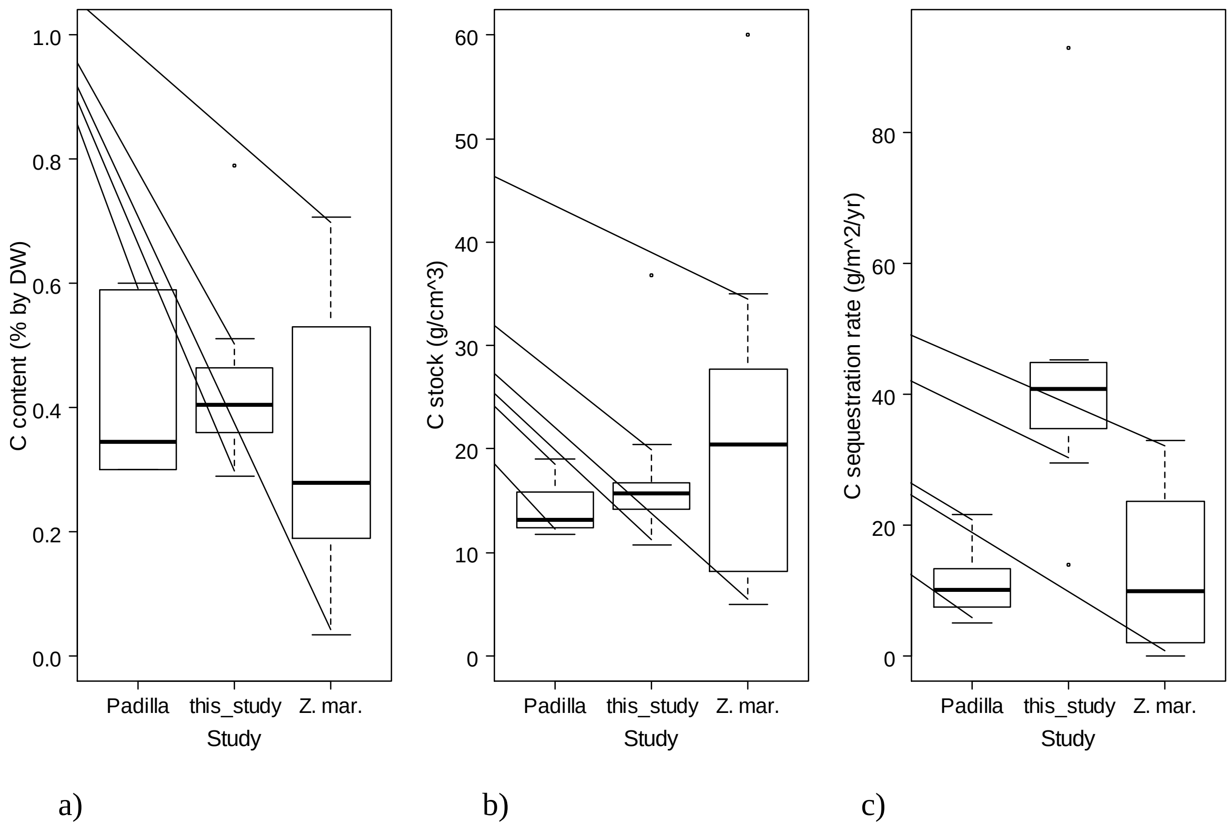


Figure 13: Boxplot comparison of a) percent organic C (%), b) organic C stock (Mg ha^{-1}), and c) OC sequestration rates (g m^{-2} yr^{-1}) for the Padilla transect study (Poppe and Rybczyk 2018), this study, and site means from global *Z. marina* meadow studies (Miyajima et al. 2015; Dahl et al. 2016; Rohr et al. 2016; Spooner et al. 2015; Prentice 2018).

Both this study and the current *Z. marina* blue C studies are lower in all parameters than global seagrass averages (Duarte et al. 2005; Duarte et al. 2010; Kennedy et al. 2010; Fourqurean et al. 2012; McLeod et al. 2011) (Figure 14).

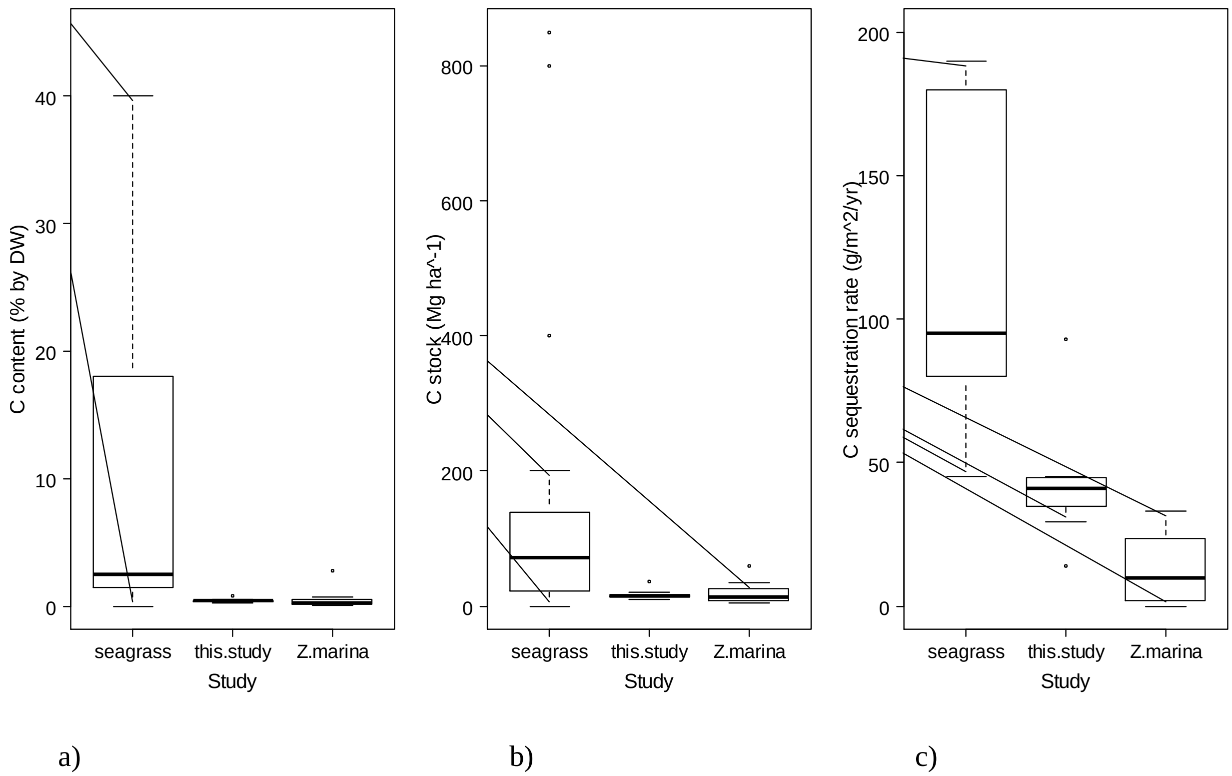


Figure 14: Boxplot comparison of a) percent OC, b) OC stock over 30 cm, and c) OC sequestration rates between reported global averages for all seagrass species combined (seagrass) (Kennedy et al. 2010; Fourqurean et al. 2012; Cebrian et al. 1997), this study, and global *Zostera marina* blue carbon studies to date (Miyajima 2015; Dahl et al. 2016; Rohr et al. 2016; Spooner 2016; Poppe and Rybczyk 2018; Prentice 2018). Values adjusted to 30 cm, the sediment depth common to all studies.

Discussion

We searched for evidence of OC storage and sequestration in three bays in Skagit County, Wa by selecting sites expected *a priori* to be depositional, with criteria including being subtidal, near a distributary mouth of an active river delta, and with high eelgrass canopy complexity (stem density and canopy height). We set out to answer whether OC values found in selected sites were higher than those reported from one intertidal transect in Padilla Bay (within our study area), and how both of our results compared to OC values from *Z. marina* studies and to reported averages for all seagrass species combined. Additionally, we attempted to identify the main environmental driver/s of OC variability in our sites. These data help to fill a gap in the rapidly-expanding blue C literature, which heretofore largely neglected the Salish Sea of the Pacific Northwest.

Our main findings were as follows. Our hypothesis that OC is being sequestered at higher rates in areas which we expected to be depositional than in those along the intertidal area of the established biomonitoring transect in Padilla Bay was supported. Mean OC accumulation rates from vegetated sites in this study were nearly four times greater than those from the Padilla transect (Figure 12 c). Sediment OC content (% and stock), however, did not differ, indicating that differing sediment accretion rates drove the difference in OC accumulation rates between these two studies. Sites with more depositional potential, i.e. located near a distributary mouth of a river, showed more accretion overall. Samish Bay accretion was the lowest here, but this datum was the result of one core from the side of the bay, away from distributary mouths, and may not be representative of the whole bay. We discovered that OC values from this study and from Poppe and Rybczyk (2018) are within the range of *Z. marina* values reported in widespread, temperate areas of the world and that all *Z. marina* values are lower than those from reported global seagrass averages. Finally, our results caused us to reject our hypothesis that elevation is the main driver of variability in OC stock; sediment porosity explained most of the variation in stock.

Zostera marina **and low OC**

It is important to consider that on the scale of global seagrass blue C, and even more so that of salt marsh and mangrove systems (Crooks et al. 2014; Day et al. 2013), there is relatively little OC in this system. Note that, as we sought out depositional areas in the meadows, our OC values are likely in the upper range for this region. In this light, there is also relatively little variability in any OC parameter. Percent OC in our study averaged nearly 5 times less than the global seagrass average (Kennedy et al. 2010) (Figure 14). Extrapolated to 1m for comparison (assuming uniform OC content below 20 cm) OC stocks in our study averaged nearly 4 times less than the global mean of 165 Mg ha^{-1} (Fourqurean et al. 2012). The mean sequestration rate in this study is nearly 3 times lower than the global mean of 138 ± 38 g C m^{-2} yr^{-1} (Kennedy et al.2010). Since the start of our project in 2015, several new *Z. marina* blue carbon studies have been published, adding to those in Virginia (Greiner et al. 2013), Padilla Bay, WA, (Poppe and Rybczyk 2018), and the K'ómox Estuary, British Columbia (Spooner 2015), and enabling broader spatial comparison. Our study yielded %OC, stock and sequestration rate values similar to those from multiple sites in the Baltic Sea (Dahl et al. 2016; Jankowska 2016; Rohr et al. 2016), Faro, Portugal (Dahl et al. 2016), the Seto Inland Sea, Japan (Miyajima et al. 2015), and the British Columbia central coast (Prentice 2018). The studies varied in elevation, stem density, canopy height, and sediment characteristics, but, like ours, were all substantially lower in OC than the initial reports of global seagrass blue C averages (Figure 14) (Kennedy et al. 2010; Fourqurean et al. 2012; Cebrian et al. 1997). Organic C sequestration has not been identified as a notable quality of *Z. marina*. Results from this study are similar to other *Z. marina* findings and point to the need to acknowledge seagrass species variability in carbon sequestration (Poppe and Rybczyk 2018; Prentice 2018).

Fate of *Z. marina* OC

Low sediment OC in our study area is not for lack of productivity; mean annual net primary productivity (NPP) in Puget Sound eelgrass is 351 g C m^{-2} yr^{-1} (3.51 Mg C ha^{-1} yr^{-1}) (Thom et al. 1988; Thom 1990; Thom et al. 2008; Bulthuis 2013). While this is lower than salt marshes and especially lower than mangroves (Day et al. 2013), this level of productivity is within the range for seagrasses (Greene and Short 2003). Yet productivity of these meadows does not translate to high rates of burial nor storage. Currents and waves transport eelgrass OC to beaches (Figure 15) and possibly to deeper water detrital food webs, where it is exposed to decomposers and remineralized. Duarte and Krause-Jensen (2017) reported that 24.3% of seagrass NPP is exported to the deep sea, where it can be considered sequestered. Here it may be confined by pycnoclines that prevent return to surface waters for thousands of years, limiting cycling to microbial loops (Libes 2009; Miller and Wheeler 2012).

FIGURE 15: Eelgrass composting en masse on the beaches of Padilla Bay National Estuarine Research Reserve, WA. and hearty colleague, Cristina Villalobos, WWU, still smiling even after a coring expedition 1 mile from shore.

We concur with the suggestion by Poppe and Rybczyk (2018) that *Z. marina* is lacking some key features that lend species like *Posidonia* their OC storing super-capacity. The first is that *Z. marina* rhizomes spread along the sediment surface, mostly from 1-4 cm, but reach up to 20 cm deep (Phillips 1984). Though their rhizomes do provide some sediment stabilization (Cheap et al. 1985), their shallow hold enables uprooting by brant grazing (Pacific Flyway Council 2002), bioturbation (Bulthuis 2013), and orbital wave motion, which has been found to penetrate the canopy, inducing wave-enhanced bottom shear stress (Hansen and Reidenbach 2013). *Zostera marina* can have little effect on sediment erodability, especially in muddy sediments (Widdows et al. 2008). A second shortcoming is that, though the canopy can exceed 2 m in our study area

(Bulthuis 2013), the leaves are thin and supple and high currents can lay them quite flat near the sediment surface, almost eliminating their particle-trapping function (Prentice 2018). Third, *Z. marina* has shown decreased growth and survival where sediment OC is high, leading to increased sulfides, which are toxic to *Z. marina* (Walser and Shull 2013; Kaldy 2014). High OM and structurally complex *Z. marina* may be mutually exclusive occurrences. Finally, turbidity inhibits *Z. marina* growth (Thom 2008). The OC-bearing particles in suspension, key ingredients for OC sequestration, may prevent *Z. marina* growth or cause die-offs with levels that decrease irradiance to < 50 µmol quanta m^{-2} s^{-1} in Puget Sound eelgrass meadows (Thom 2008).

Sediment porosity and grain size

Porosity is inversely proportional to grain size (Fleming 2018) by the relationship in Eq. 7.

$$\text{Eq. 7: } P = \frac{V_T - V_G}{V_T} \times 100$$

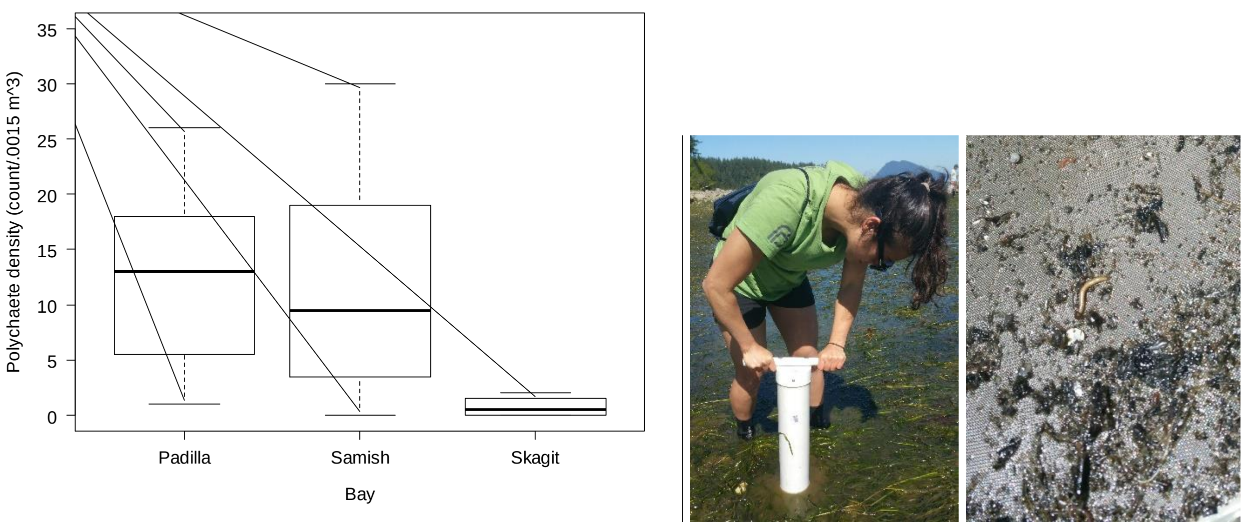
where P is % porosity, V_T is the total volume of sediment, V_G is the total volume of grains within the total volume of sediment (Urumović and Urumović Sr. 2014). Unsurprisingly, we found these two factors to be positively correlated. The greater surface area to volume ratio of fine particles supports more C adsorption onto the sediment particle surface (Mayer 1994). Fine sediments are responsible for increased porosity, and likely the actual driver of variance in these data. Many studies have positively correlated organic matter with silt + clay fraction (Calvert et al. 1995; Lin et al. 2002). Accordingly, fine sediments have a positive effect on OC stocks (Miyajima et al. 2015; Dahl et al. 2016; Rohr et al. 2016; Samper-Villareal et al. 2016; Serrano et al. 2016; Oreska et al. 2017). Serrano et al. (2016) even suggest that mud content may be used as a proxy for estimating soil OC content, explaining 34-91% of the variability. Existing literature reports increased blue C where environmental factors result in deposition of high concentrations of fine sediments: low water motion (Fonseca and Bell 1998; Hansen and Reidenbach 2012; Samper-Villareal 2016; Prentice, in submission), high seagrass structural complexity (Samper-Villareal 2016), spatial proximity to the meadow center of large meadows (Miyajima 2015; Oreska et al. 2017; Prentice et 2018), high turbidity levels (Samper-Villareal et al. 2016), and low density and high porosity of the sediments (Dahl et al. 2016). While porosity alone was revealed as a driver of OC variability in this study, it is likely that the combination of environmental factors contributed to fine sediment deposition and so increased porosity.

We were not able to conclude that the presence of eelgrass was an important factor in OC sequestration (Figure A1). This result corroborated other *Z. marina* studies (Mellors et al. 2002; Dahl et al. 2016). However, our unvegetated cores were from bare patches within the eelgrass meadows and could still have had influence from the presence of surrounding vegetation, though it was at least 30 m away. Dahl et al. found a positive correlation between seagrass-associated variables and %OC, but all seagrass variables had weaker correlations to %OC than any of the sediment characteristics. Oreska et al. (2017) reported higher OC concentration and stock in the interior of *Z. marina* meadows, but sampled to a depth of just 12 cm, where OC will likely be oxidized by mixing processes, not stored long-term (Shull 2001).

There has been much debate recently regarding the sediment mixing layer (SML) and the proportion of OC that is truly being stored long-term, as opposed to being remineralized by mixing processes (Johannessen and Macdonald 2016 and 2018). We agree that measuring below the SML would prevent overestimation of long-term storage of OC. At present this is only possible in areas of high sediment deposition rates when using ^{210}Pb as a radionuclide, due to its short half-life (22.3 years). In our study area we reached undetectable activity within the top 20-33 cm, representing approximately 100 years of deposition.

Bioturbation and low OM limit usefulness of ^{210}Pb geochronology

Obtaining valid sediment accretion rates in eelgrass meadows using the ^{210}Pb method of geochronology presented challenges due to mixing. Our study area, possibly with the exception of Skagit Bay, is rich in benthic infauna, especially polychaetes. We confirmed this through counts after sorting 20 cm deep x 10 cm fresh sediment cores through a 500 µm sieve at most coring sites (Figure 16).



a. b. c.

Figure 16: a) Boxplots showing showing polychaetes (>500 µm) counts per 20 x 10 cm core of fresh sediment. N = 12 for Samish, 15 for Padilla, and 16 for Skagit Bay, b) Meriel Kaminsky, WWU, searching for infauna in Padilla Bay, and c) sample of sieve contents.

Polychaetes constantly rework surficial sediments and complicate radiometric dating efforts (Carpenter et al. 1985). Based on ^{210}Pb activity depth profiles, in half of our cores, infauna and/or channel shifting mixed sediments down to > 30 cm. These cores were removed from the dataset. In the areas of lowest accretion rates, as in Padilla Bay, the age of sediments at 20-30 cm deep was determined to be roughly 100 years, also the maximum age for detectable ^{210}Pb due to its half-life of 22.3 years (Kairis 2008; Johannessen 2016). Additionally, our study area has comparatively low OM content. This prevents retention of radionuclides even when mixing is minimal, further confounding accretion rate results (Ritchie and McHenry 1990). Lead-210 dating can be further confounded by loss of sediments, deposition of material remobilized from the nearshore during storm surges, and changes in sedimentation rates (Kirchner and Ehlers 1998). Except when using sediment elevation tables, which accurately measure net sediment surface elevation change after installation (Cahoon et al. 1995; Calloway et al. 2013; Crooks et al. 2014), we recommend sediment accretion rate measurements in highly mobile estuarine sediments be presented as the uppermost bounds, and with the understanding that sites may be either accretionary or erosional (Carpenter 1985; Shull 2001; Crusius 2004).

Value

It is unlikely that the OC stocks and sequestration rates in our study area would qualify for funding to substantially support restoration projects should these sites be disturbed (VCS 2015). However, though sediment OC values in this study are lower than global averages, they still represent an important C sink that, if disturbed, will oxidize and return to the atmosphere. At the upper end of the range, as estimated in the scope of this study, OC stored in the top 50 cm of eelgrass meadow sediments in Skagit County represents over 300 Mg in the top 50 cm, with the potential to become over one million metric tons of CO_2 if released (Table 4). While of no monetary value while left in place (VCS 2015), in the face of habitat conversion, Skagit County eelgrass meadow sediments have potential impacts on the "social cost of carbon" (Natl. Academies of Sciences, Engineering, and Medicine 2017).

Table 4: Meadow-wide Organic C stock and CO_2 equivalents, and the approximate number of passenger cars driving for one year to produce the same amount of emissions (EPA 2018).

Bay	Eelgrass extent (ha)	OC Stock to 50 cm (Mg C ha^{-1})	OC stock meadow-wide total to 50 cm (Mg C)	OC stock in tons CO_2 equivalents (tCO2e)	Passenger cars driving for 1 yr (count)
Padilla Bay	4,604.05	25.90	119244.90	437231.28	93,626
Samish Bay	2,502.05	24.60	61550.43	225684.91	44,103
Skagit Bay	4206.72	29.56	124350.64	455952.36	97,634

Conclusions

Data from this study suggest variability in seagrass blue C on the species and meadow levels. Our results, having sought out sites most likely to support C sequestration, should be considered the upper bounds of OC content and accumulation rates in this study area. Even so, these results are far below what is reported as global averages for all seagrass species; central Salish Sea eelgrass meadow blue C is underrepresented in global averages. As our results are similar to those from widespread *Z. marina* blue C studies, we surmise that *Z. marina* as a species may not be capable of notable OC sequestration. Considering this variability between species and sites within meadows, seagrass blue C accounting for restoration projects should include site-specific assessment of OC sequestration rates. Carbon storage, in addition to dozens of other ecosystem services which eelgrass provides (Beck et al. 2003; Greene and Short 2003; Orth et al. 2016), warrants careful conservation of Salish Sea *Z. marina* meadows.

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Appendix

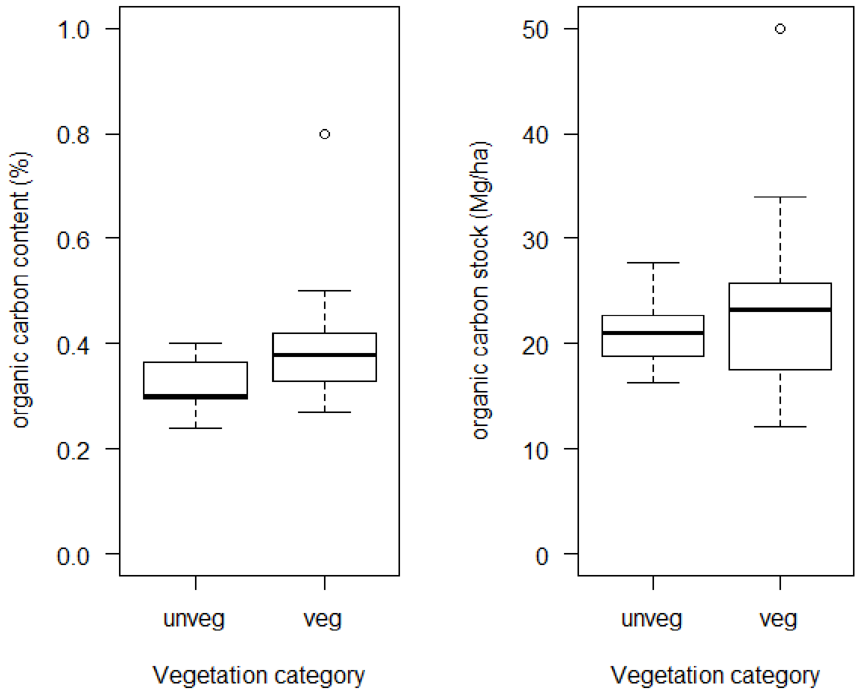

Figure A1: Percent organic C in unvegetated (n = 5) vs. vegetated (n = 15) patches within the same coring site (within 30 m in the same meadow).

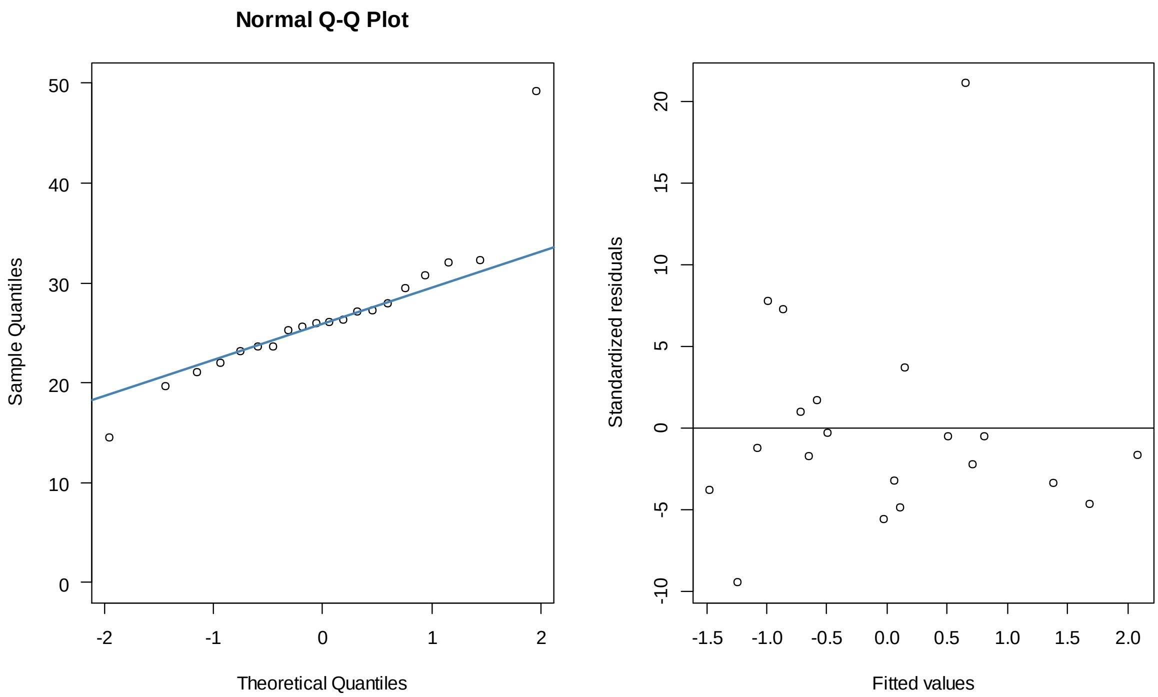

Figure A2: Quantile-quantile (QQ) plot and residual plot for final linear mixed effects model validation for factors affecting OC stock. The QQ plot indicates that organic C stock data are normally distributed, and the residual plot reveals no distinct pattern, indicating homogeneous variance.

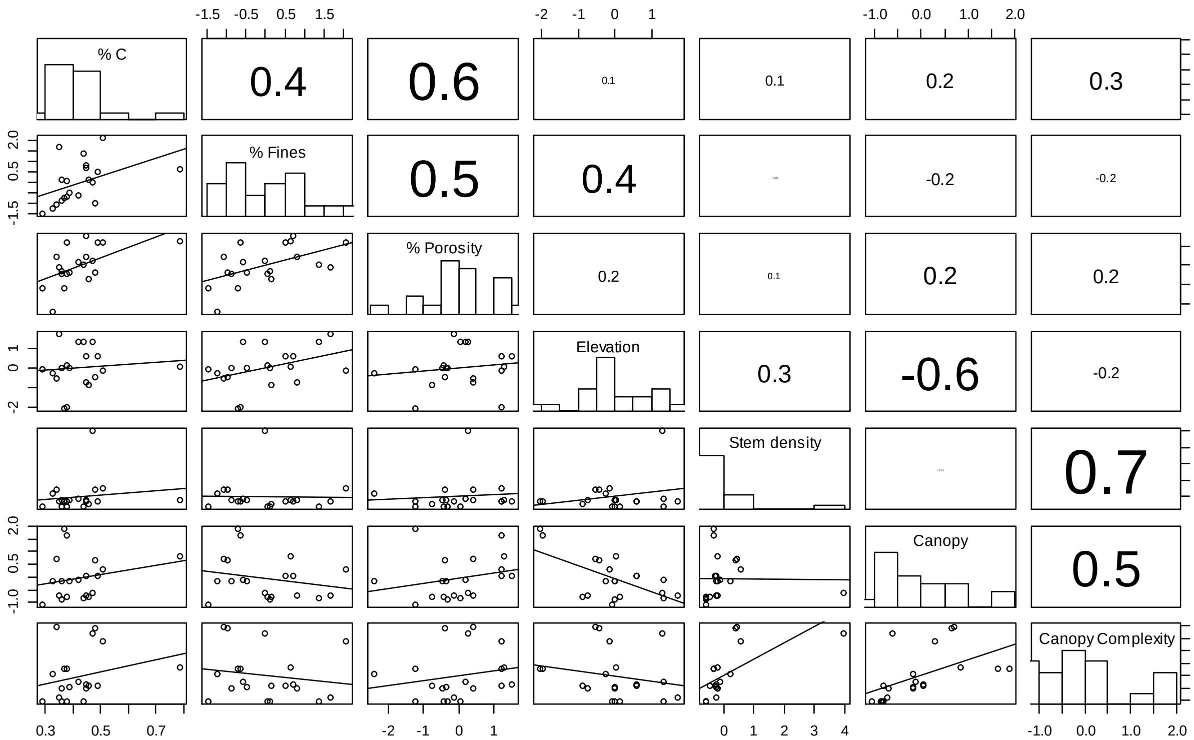

Figure A3: Pair plots of fixed factors of interest and the response variable percent organic C to identify collinear factors that would confound Linear Mixed Effects Model output (Zuur et al 2010, 2013). Barplots are histograms of each factor. Scatterplots show the linear relationship between variables above and to the right of each plot. Numerical fields are correlation coefficients placed at the intersection of the row and column of variables of interest. Numerical font size is proportional to the absolute value of the estimated correlation coefficient. As all correlation coefficients are < 0.3, no collinear relationships exist, and all factors may be included in the mixed model.

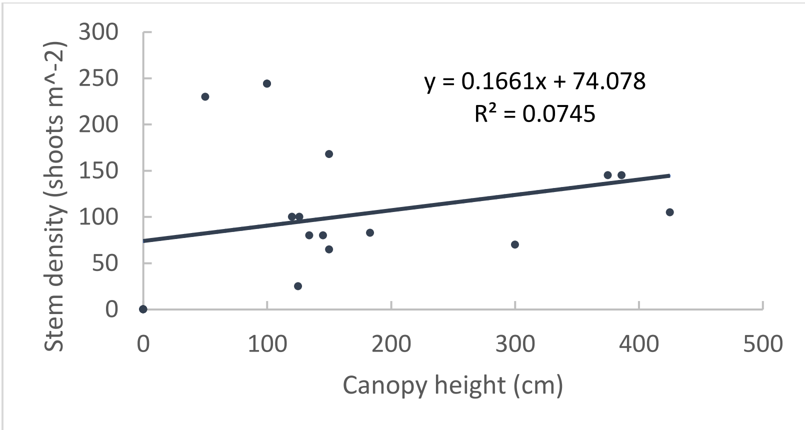

Figure A4: Eelgrass stem densities (shoots m^{-2}) plotted against canopy heights (cm) measured at each sampling site, showing no correlation between these two factors.

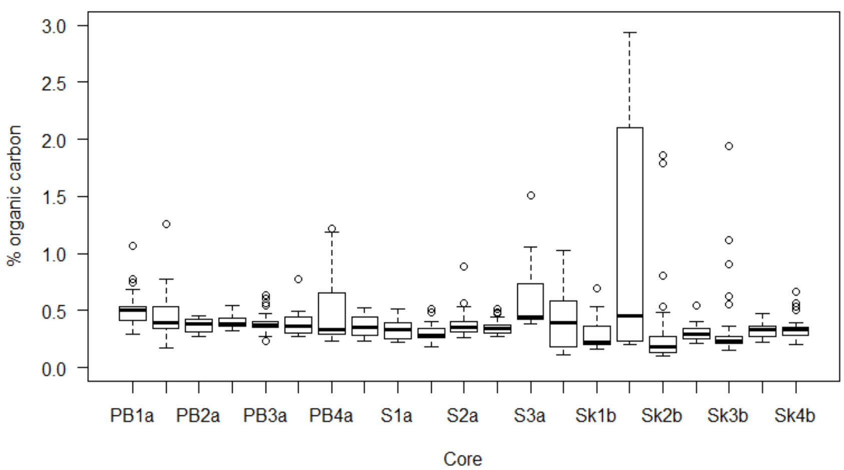

Figure A5: Box and whisker plot of % organic C by core. PB=Padilla Bay cores, S=Samish Bay cores, and Sk=Skagit Bay cores. Cores labeled ‘a’ are from the approximate center of eelgrass meadows at least 50 m in diameter and ‘b’ from bare patches at least 50 m in diameter, except those in PB1-3 and S2, where no bare patches occurred nearby. Outlying C values are from surface slices (0-6cm).

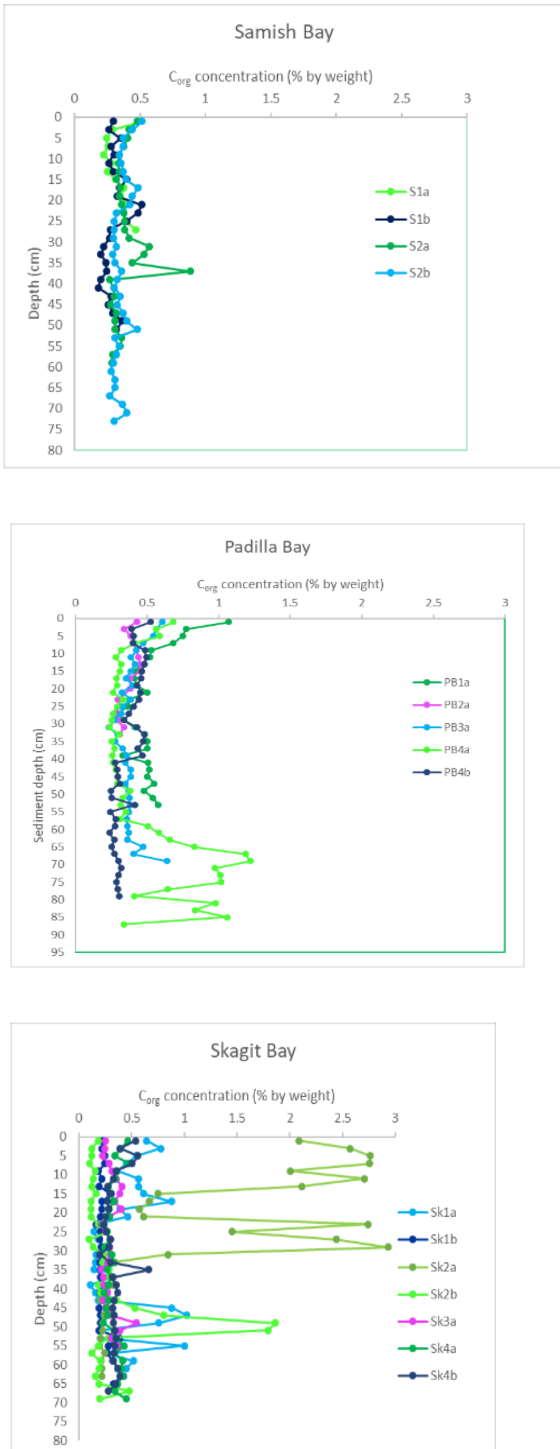

Figure A6: Percent OC depth profiles by core in a) Samish, b) Padilla, and c) Skagit Bays.

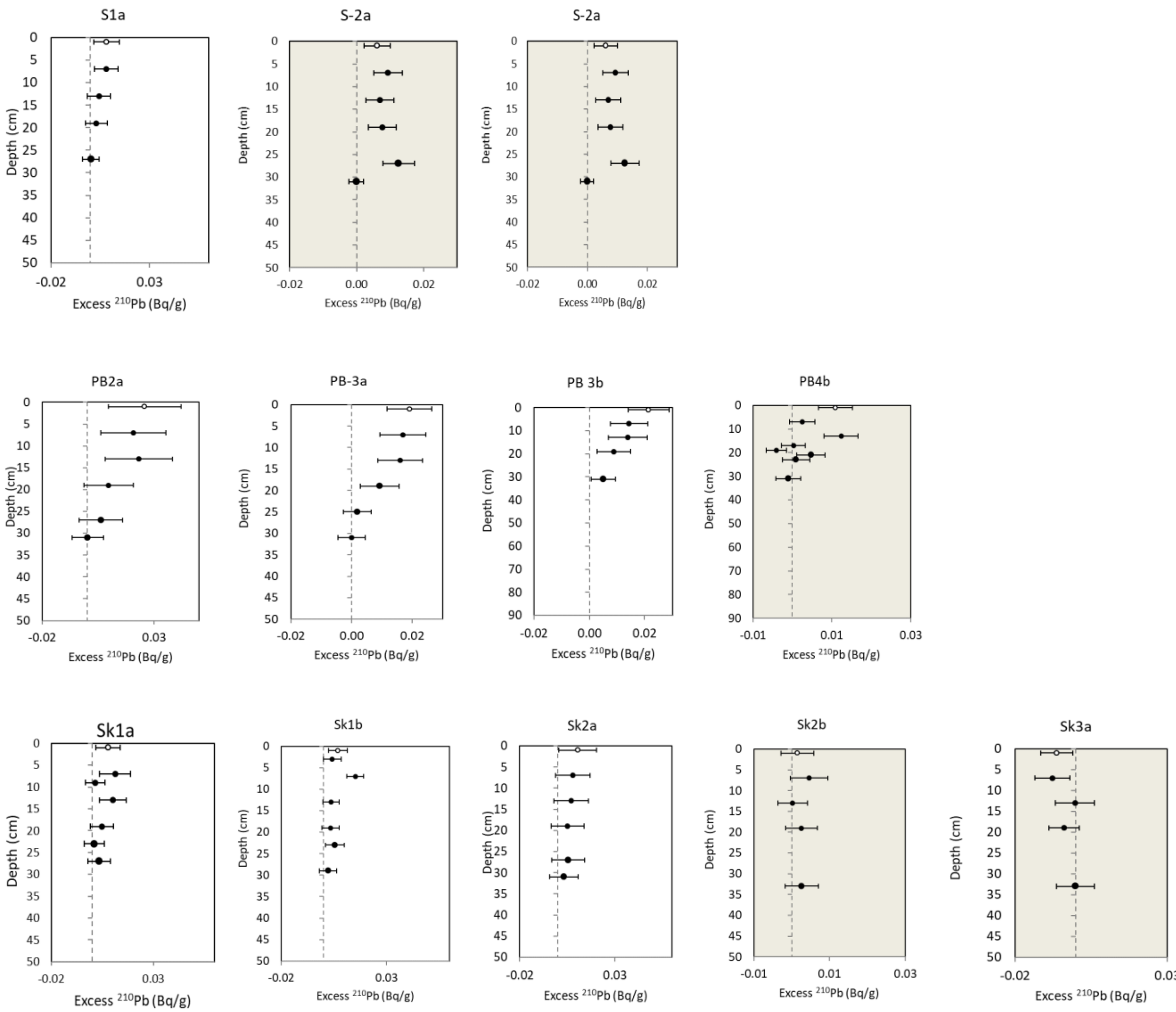

Figure A7: ^{210}Pb activity depth profiles. Five profiles, shown in gray, were excluded from the analysis due to visual evidence of mixing and/or insufficient concentration of ^{210}Pb.

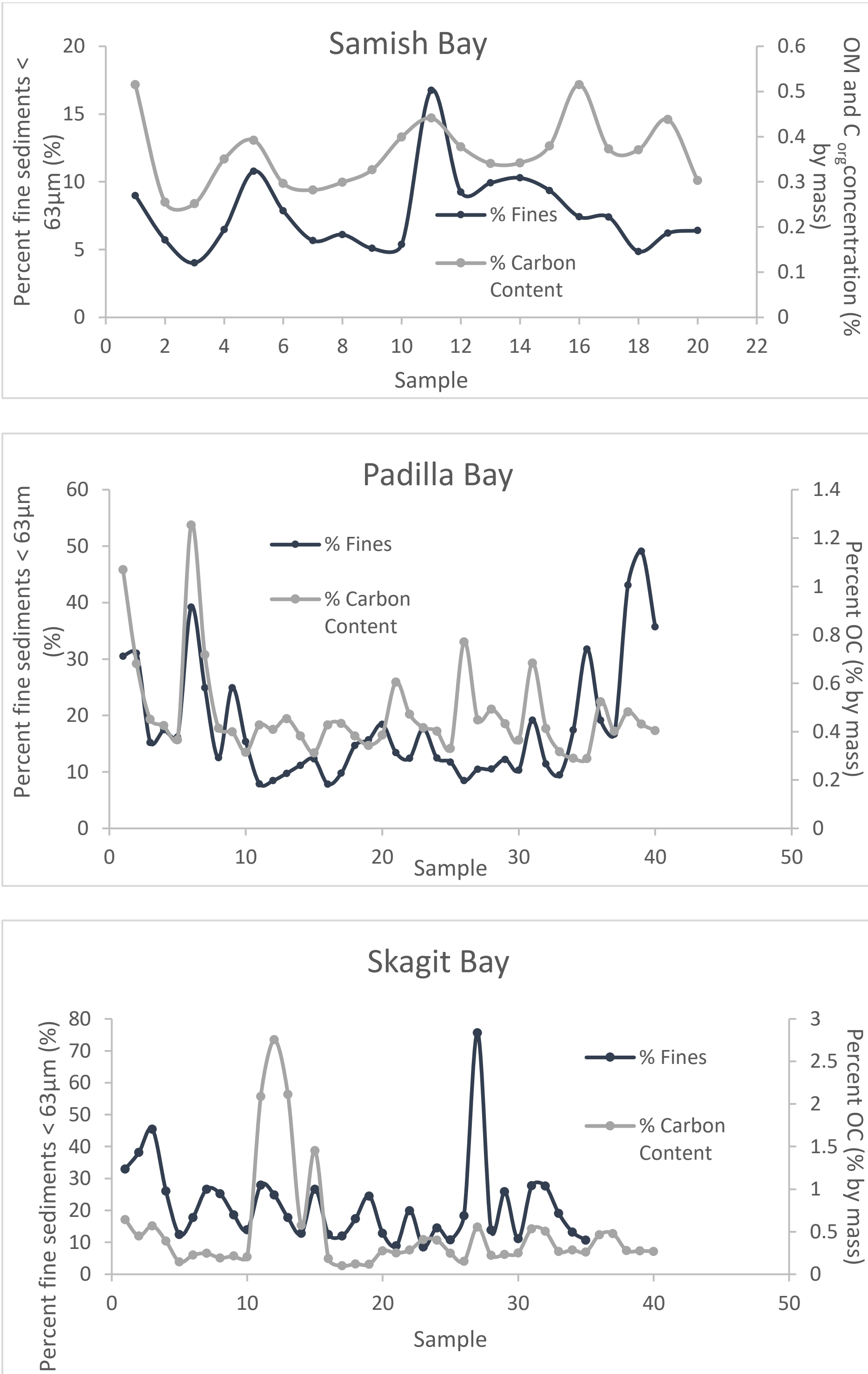

Figure A8: Percent fine sediments and percent organic C by dry weight for Samish, Padilla, and Skagit Bays.