

1 Factors controlling spatiotemporal variability of soil carbon accumulation and stock estimates in a tidal
2 salt marsh

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17

18 **Abstract**

19 Tidal salt marshes are important contributors to soil carbon (C) stocks despite their relatively small land
20 surface area. Although it is well understood that salt marshes have soil C burial rates orders of magnitude
21 greater than those of terrestrial ecosystems, there is a wide range in accrual rates among spatially
22 distributed marshes. In addition, wide ranges in C accrual rates also exist within a single marsh
23 ecosystem. Tidal marshes often contain multiple species of cordgrass due to variations in hydrology and
24 soil biogeochemistry caused by microtopography and distance from tidal creeks, creating distinct subsites.
25 Our overarching objective was to observe how soil C concentration and dissolved organic carbon (DOC)
26 vary across four plant phenophases and across three subsites categorized by unique vegetation and
27 hydrology. We also investigated the dominant biogeochemical controls on the spatiotemporal variability
28 of soil C and DOC concentrations. We hypothesized that subsite biogeochemistry drives spatial
29 heterogeneity in soil C concentration, and this causes variability in total soil C and DOC concentration at
30 the marsh scale. In addition, we hypothesized that soil C concentration and porewater biogeochemistry
31 vary temporally across the four plant phenophases (i.e., senescence, dormancy, green-up, maturity). To
32 test these interrelated hypotheses, we quantified soil C and DOC concentrations in 12 cm sections of soil
33 cores (0-48 cm depth) across time (i.e., phenophase) and space (i.e., subsite), alongside several other
34 porewater biogeochemical variables. Soil C concentration varied significantly ($p < 0.05$) among the three
35 subsites and was significantly greater during plant dormancy. Soil S, porewater sulfide, redox potential,
36 and depth predicted 44% of the variability in soil C concentration. There were also significant spatial
37 differences in the optical characterization properties of DOC across subsites. Our results show that soil C
38 varied spatially across a marsh ecosystem up to 63% and across plant phenophase by 26%, causing
39 variability in soil C accrual rates and stocks depending on where and when samples are taken. This shows
40 that hydrology, biogeochemistry, and plant phenology are major controls on salt marsh C content. It is
41 critical to consider spatiotemporal heterogeneity in soil C concentration and porewater biogeochemistry
42 to account for these sources of uncertainty in C stock estimates. We recommend that multiple locations

43 and sampling timepoints are sampled when conducting blue C assessments to account for ecosystem-scale
44 variability.

45

46 **1 Introduction**

47 Coastal blue carbon (C) cycled in tidal salt marshes is critically important for global soil C
48 sequestration despite the small relative land area (Mcowen et al. 2017). High primary productivity
49 coupled with high sedimentation rates and slowed organic C decomposition due to flooded anoxic soils
50 allow salt marshes to rapidly accrete and preserve soil C (Arias-Ortiz et al. 2018). Soils in such
51 ecosystems retain approximately 15% of their yearly primary productivity in soils compared to just 1%
52 for tropical rainforests (Duarte 2017). Restoring, protecting, and artificially creating salt marshes can
53 facilitate removal of CO₂ from the atmosphere and storage in soils on timescales conducive to climate
54 change mitigation goals. These ecosystems should therefore be included in climate mitigation policy
55 (Ewers Lewis et al. 2019; Serrano et al. 2019). However, a wide range of global salt marsh soil C
56 sequestration rates of ~ 1 to >1100 g C m⁻² year⁻¹ has been reported (Wang et al. 2021). The inclusion of
57 salt marshes in improved climate mitigation policy is, in part, contingent upon improving our
58 understanding of the environmental variables causing wide ranges in marsh soil C concentration and thus
59 soil sequestration rates (Saintilan et al. 2013; Macreadie et al. 2019). Understanding key controls on salt
60 marsh soil C variability will also decrease uncertainty in Earth System Models and inform new policy
61 aimed at protecting these valuable ecosystems.

62 Soil C concentrations in salt marsh ecosystems vary spatially across the globe. Part of this
63 variation is explained by regional environmental controls such as average annual air temperature (Chmura
64 et al. 2003), geomorphic setting (van Ardenne et al. 2018), salinity gradients, inundation frequency (van
65 de Broek et al. 2016; Baustian et al. 2017; Luo et al. 2019), rainfall patterns (Sanders et al. 2016;
66 Negandhi et al. 2019), soil controls such as pH, soil moisture, and soil type, as well as plant controls such
67 as the dominant plant species (Bai et al. 2016; Ford et al. 2019). Soil C accumulation rates also vary based

68 on the age of the marsh and tend to be highest in newly expanding marsh edges (Miller et al. 2022). Other
69 logistical factors contributing to variability and heterogeneity in salt marsh blue C estimates include the
70 type of corer used (Smeaton et al. 2020) and the depth of soil that is integrated into soil C accrual rates
71 (Bai et al. 2016; Van De Broek et al. 2016; Mueller et al. 2019). While understanding global and regional
72 controls on soil C is important for reducing uncertainty in C estimates, understanding site-level factors is
73 also critical because ecosystem-level variability can be just as high as regional- to global-level variability
74 (Ewers Lewis et al. 2018). Belowground biogeochemical heterogeneity can be attributed to the
75 aboveground vegetation and plant controls due to striking zonation of marsh grass species across the
76 marsh platform. This is often associated with small spatial-scale changes in hydrologic patterns (Guimond
77 et al. 2020b, a) based on proximity to the tidal channel that drives unique subsite biogeochemistry
78 (Seyfferth et al. 2020) which subsequently determines the type of vegetation that can survive within a
79 given tidal zone (Davy et al. 2011). While tidal zonation alters vegetation and belowground
80 biogeochemistry, it remains unclear if soil C concentrations are directly or indirectly altered by these
81 coupled plant and soil biogeochemical controls.

82 Primary production rates may partially control soil C concentration and may vary among
83 vegetative zones. For example, the short form of *Spartina alterniflora* has a lower primary production rate
84 than the tall form (Roman and Daiber 1984) and *Phragmites australis* has above and below ground
85 production rates two times that of the shorter *Spartina patens* (Windham 2001). Belowground production
86 of dissolved organic carbon (DOC) can arise from root exudation (Luo et al. 2018) and influence soil C
87 concentration because belowground productivity often exceeds above-ground productivity in these
88 ecosystems (Frasco and Good 1982). Even though DOC exudates are considered to be labile (Yousefi
89 Lalimi et al. 2018), they may contribute to soil C accumulation over time due to microbial transformation
90 (Valle et al. 2018) and association with soil minerals such as Fe oxides (Chen et al. 2014; Chen and
91 Sparks 2015; Sowers et al. 2018a, b, 2019). The optical characterization of DOC of chromophoric
92 dissolved organic carbon (CDOM) can also affect degradability (Clark et al. 2014) and may differ across
93 the marsh platform as a result of differing plant species.

94 Vegetation zones or subsites can have unique biogeochemical signatures based on soil redox
95 conditions and inundation extent and frequency. For example, high marsh areas and areas near tidal
96 channels have soils which are at least periodically oxic to sub-oxic and are dominated by iron (III)
97 reduction, whereas low marsh areas have continuously inundated soils and are dominated by sulfate
98 (SO_4^{2-}) reduction (Seyfferth et al. 2020). While these biogeochemical characteristics can directly
99 influence vegetation (Moffett and Gorelick 2016) and thus indirectly influence soil C concentrations,
100 these heterogeneous biogeochemical characteristics may also directly affect soil C through the
101 interactions of soil C cycling with soil minerals. Fe oxides have an intimate role in the C cycle and C
102 stabilization in soils experiencing dynamic redox fluctuation (Sodano et al. 2017), as previous work has
103 shown that 99% of the dissolved Fe in the ocean is complexed with organic ligands (Whitby et al. 2020)
104 and ~21% of all organic C in marine sediments is bound to reactive Fe species (Lalonde et al. 2012). Fe
105 oxides may play an important role in C stabilization in soils experiencing dynamic redox fluctuation. Fe
106 oxides can protect DOC against microbial degradation through physiochemical protection (Blair and Aller
107 2012; Chen and Sparks 2015; Sodano et al. 2017; Sowers et al. 2018a; Dorau et al. 2019; Wordofa et al.
108 2019), but these organo-mineral assemblages can be dissociated under reducing conditions (Riedel et al.
109 2013; Wordofa et al. 2019; Lacroix et al. 2022; Fettrow et al. 2023a). Therefore, examining the spatial
110 variability in soil biogeochemistry and relating those variables to soil C concentration may elucidate
111 important mechanisms that cause the wide range in salt marsh soil C concentrations.

112 While it is critical to assess spatial heterogeneity in soil C concentration, it is also important to
113 assess temporal variability. The temporal assessment of soil C in salt marshes often considers long-term
114 trends of historic C burial rates (Cusack et al. 2018; McTigue et al. 2019; Breithaupt et al. 2020; Cuellar-
115 Martinez et al. 2020), but variability of salt marsh soil C concentrations may also occur on shorter time
116 scales such as across a single year. Several studies suggest salt marsh soil C does not significantly change
117 across seasons throughout the year (Yu et al. 2014; Zhao et al. 2016), even though major changes in soil
118 biogeochemical variables occur on this timescale (Koretsky et al. 2005; Negrin et al. 2011; Seyfferth et al.
119 2020; Trifunovic et al. 2020; Zhu et al. 2021). While soil C concentration may be stable across seasons, it

120 is unclear if soil C concentration changes based on site-specific plant phenology. The phenophase of a
121 marsh is associated with the greenness index of vegetation (Trifunovic et al. 2020) and is strongly
122 associated with C dynamics in wetland systems (Desai 2010; Kang et al. 2016). Soil C concentration
123 should be measured across plant phenophase to determine if temporal changes in phenology alter soil C
124 concentration, adding to variability in blue C estimates.

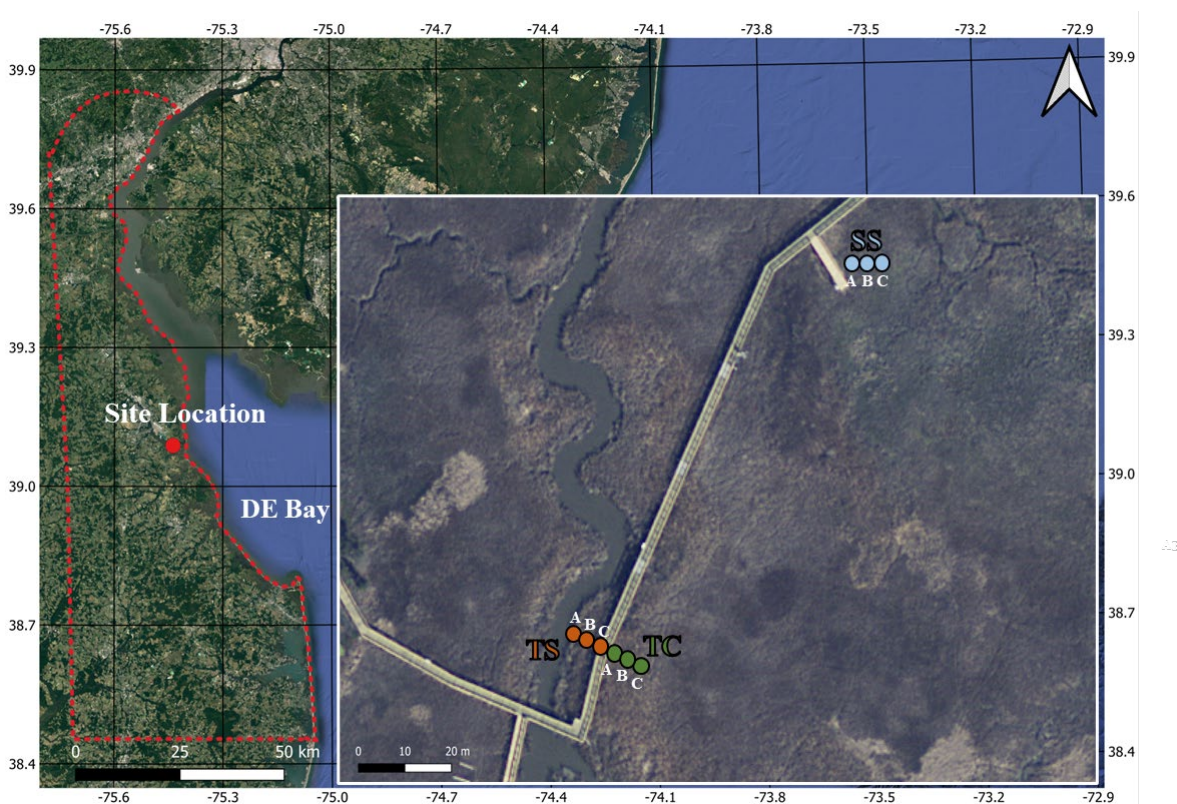
125 To address these knowledge gaps, we conducted a year-long study of a temperate tidal salt marsh
126 to assess how soil C concentration and porewater biogeochemistry change in space (subsite) and time
127 (phenophase). Our overarching research objectives were to understand how soil C and porewater DOC
128 concentration and properties change across spatial and temporal scales and to investigate key
129 biogeochemical drivers of these C concentrations at the ecosystem level. We hypothesized that subsites
130 would contain significantly different concentrations of soil C due to differences in soil biogeochemistry
131 across the marsh platform. We further hypothesized that soil C concentration and associated porewater
132 DOC and biogeochemistry would significantly differ across plant phenophase. Our results improve
133 understanding of mechanistic controls on salt marsh soil C with implications for characterizing and
134 reducing uncertainty in C sequestration estimates, while also adding to the body of literature that shows
135 tidal salt marshes are critical reservoirs of sequestered C.

136 **2.0 Methods and Materials**

137 **2.1 Field Site**

138 This study was conducted at the St. Jones National Estuarine Research Reserve located in Dover,
139 Delaware (Figure 1). The ecosystem is classified as a temperate mesohaline tidal salt marsh with a tidal
140 creek salinity ranging from 5 to 18 ppt (Capooci et al. 2019). Three separate subsites were previously
141 identified at this site, each with a different vegetation type and hydrology (Guimond et al. 2020a; Seyfferth
142 et al. 2020). The subsite nearest the channel is primarily colonized by the tall form of *Spartina alterniflora*
143 and has semidiurnal tidal oscillation. This subsite is hereafter referred to as Tall Spartina (TS). Farther from
144 the tidal channel, the elevation is slightly higher due to a natural levee and flooding of the upper 25 cm of
145 soil occurs only during spring tides; this location has the larger cordgrass *S. cynosuroides* and is hereafter

146 referred to as Tall Cordgrass (TC). The third subsite is farthest from the tidal channel, lowest in elevation,
147 and is primarily colonized by the short form of *S. alterniflora* due to near continuous inundation; this subsite
148 is hereafter referred to as Short Spartina (SS). These subsites have distinct hydro-biogeochemistry and
149 vegetation that varies across small spatial scales and thus provides an ideal setting to understand site-level
150 variability in soil C concentration, porewater biogeochemistry and their relationships.



151
152 **Figure 1.** Map of the field site located at the St Jones Reserve near Dover, DE. Three unique subsites (TS,
153 TC and SS) have been characterized based on previous studies at this field site showing subsite specific
154 hydrology, vegetation, and biogeochemistry based on distance from the tidal creek (Guimond et al., 2020a;
155 Seyfferth et al., 2020). The coring locations were sampled in triplicate (Core A, B and C), with core A
156 starting closest to the creek and each subsequent core in each subsite being ~30 cm from one another. The
157 base layer for the map was obtained from public base layers in QGIS (© Google Maps).

158
159
160 **2.2 Soil Sampling and Analysis**

161 Soil cores were obtained from each of the three subsites (TS, TC, SS) in triplicate during each sampling
162 event. Replicates were taken approximately 30 cm from one another and are labeled cores A, B, and C
163 based on distance to the tidal channel with A being closest to the channel and C the farthest (Figure 1).

164 Sampling events occurred at four separate times of the year to coincide with each of the phenophases (i.e.,
165 senescence on 10/3/2019, dormancy on 12/3/2019, green-up on 4/29/2020, maturity on 8/13/2020), which
166 were previously determined using the Greenness Index (Trifunovic et al. 2020). Cores were obtained at
167 the same tidal inundation cycle each season to ensure consistent saturation during each campaign. Each
168 sampling campaign resulted in 36 total cores (or 144 core sections, see below) that we used to understand
169 spatiotemporal variability; unfortunately, we could not obtain more cores due to conditions of the strict
170 soil coring permit at the estuarine preserve. Soil cores (6 cm x 48 cm) were extracted using a gouge auger
171 that has been shown to be an effective coring technique for reducing compaction in soft marsh soils
172 (Smeaton et al. 2020). Soil cores were quickly sectioned in the field into 12 cm increments (0-12 cm, 12-
173 24 cm, 24-36 cm and 36-48 cm relative to the soil surface) and preserved under anoxic conditions
174 following previous methods (Seyfferth et al. 2020). For reference, the rooting zone of *Spartina* grasses is
175 between 8-20 cm (Muench and Elsey-Quirk 2019), so the upper two sections likely include C from fresh
176 root exudates. The 12 cm increments were chosen because many soil C stock papers use increments
177 between 10-15 cm and there tends to be little variation across the ~10 cm increment in a variety of
178 wetland soils (Baustian et al. 2017). Briefly, the soil sections were placed into 250 ml HDPE bottles
179 which were left uncapped in gas-impermeable bags that contained oxygen scrubbers (AneroPack-Anero,
180 Mitsubishi), and the bags were vacuum-sealed in the field. The soil samples were placed on ice during
181 transport back to the lab. Once back in the lab, the soil sections in the gas-impermeable bags were
182 immediately placed inside an anoxic glove bag containing ~5% hydrogen and ~95% nitrogen. A
183 subsample of soil was dried, ground, sieved (2 mm), and powdered for analysis of total C and S (Vario
184 EL Cube, Elementar). We clarify that we did not separate inorganic versus organic soil C and report only total
185 soil C. Soil C and S are reported as % C (= 100% * g C/g soil dry wt.) and % S (= 100% * g S/g soil dry
186 wt.). We used soil C % to calculate soil C stocks using previously obtained bulk density measurements at our field
187 site (Wilson and Smith 2015), and we calculated soil C accrual rates using previously obtained
188 sedimentation rate values (Tucker 2016). The remaining field-moist soil was left inside the HDPE vial,
189 capped inside the glove bag and centrifuged for extraction of residual porewater. The amount of porewater

190 we obtained was a function of soil saturation that was consistent during each campaign because we sampled
191 at the same tidal cycle each season. After centrifugation, the remaining soil sample was further dried inside
192 of the glove bag. While this drying procedure could have introduced artificial H₂-fueled metabolism, this
193 should be negligible because the soils were rapidly dried within the glove bag with freshly replaced
194 desiccant and because the saturated sample was only minimally in contact with the H₂ atmosphere.

195 .

196 **2.3 Porewater Extraction and Analysis**

197 Porewater was extracted from each 12-cm soil section by centrifugation for 2 minutes under an
198 anoxic atmosphere at 2,500 rpm. A portion of the porewater was filtered with 0.45µm PTFE syringe
199 filters while the rest was vacuum filtered using glass fiber filters (0.7µm). The 0.45µm PTFE filtered
200 porewater was immediately analyzed for Fe²⁺ using the ferrozine colorimetric method (Stookey 1970),
201 S²⁻ using the methylene blue method (Cline 1969), redox potential with a 220 mV offset, pH, and
202 conductivity using calibrated probes (Orion Ross Ultra pH/ATC Triode, Orion 9179E Triode, Orion
203 DuraProbe Conductivity Cell), and the remaining sample was acidified to 2% HNO₃ for elemental
204 analysis using an ICP-OES. The porewater filtered with glass fiber (0.7 µm) was acidified with HCl and
205 analyzed for DOC (Vario TOC Analyzer, Elementar). To characterize the DOC, unacidified DOC
206 samples from the plant maturity sampling event were analyzed via ultraviolet-visible (UV-VIS)/
207 excitation-emission matrix spectroscopy (EEMs) (Aqualog Spectrophotometer, Horiba). The Aqualog
208 was zeroed with double deionized water blanks, checked using the manufacturer's excitation check,
209 corrected for inner filter effects, applied first and second order Rayleigh masking and data were
210 normalized using the average Raman area (Gao et al. 2011; Clark et al. 2014). Measurements were taken
211 over the wavelengths of 200-730nm with 2nm steps. Fluorescence and absorbance peaks and indices were
212 calculated using previously established equations (Table S1).

213 **2.4 Statistical Analysis**

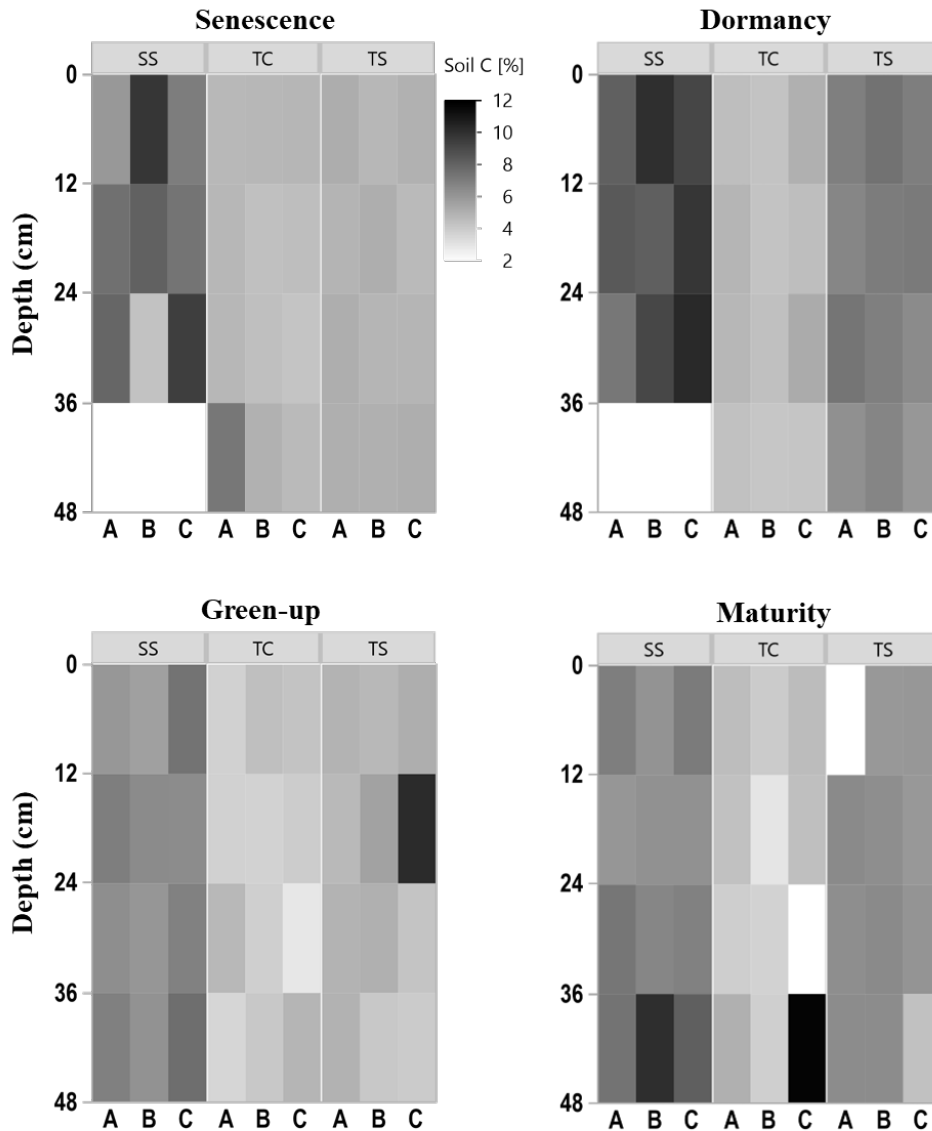
214 A three-way analysis of variance (ANOVA) was performed to understand significant interactions
215 between factors of subsite, depth, and phenophase on soil and porewater variables. Subsequently,

216 statistical differences between subsites and phenophase were analyzed using repeated measures ANOVA
217 with a post-hoc Tukey-HSD analysis to determine differences between individual subsites and
218 phenophases. Assumptions of ANOVA were met by assessing for normality with QQ plots prior to analysis and
219 transforming when necessary. Equal variance was tested to ensure homogeneity of variance between subgroups using
220 Levene's test. Correlations with depth were analyzed using linear regression and only the significant
221 ($p < 0.05$) relationships are reported. Relationships among all measured variables were assessed using
222 principal components analysis. In addition, a stepwise regression model was built to determine variables
223 that significantly predict soil C concentration. This was done by maximizing the R^2 -value of the model while
224 using the least amount of variables to explain the variance. All statistical analyses were conducted in JMP
225 (Version 16.2).

226 **3.0 Results**

227 **3.1 Soil Carbon and Sulfur**

228 To explore the spatiotemporal heterogeneity of soil carbon (C) and sulfur (S) at each subsite,
229 subsamples of each collected soil increment were combusted for soil C and S concentration.
230 Concentrations of soil C were highly variable among subsites, phenophase, depth, and replicate cores
231 (Figure 2), indicating several spatiotemporal sources of variability in marsh soil C estimates. SS appeared
232 to have higher soil C concentrations, as illustrated by darker colors in the heat map, compared to both TS
233 and TC. Soil C also appeared higher at TS than TC, illustrated by relatively darker colors in the heat map.
234 For all subsites, soil C concentrations changed throughout the year, appearing higher during plant
235 dormancy and lower during green-up. However, variability across individual replicates A, B, and C and
236 with depth complicated generalities across time and space. For example, at subsite SS from 24-36 cm
237 during senescence, core A is ~5% soil C while core C is ~10% soil C, a factor of 2 difference between
238 replicates. Large ranges among replicates were also observed during green-up at TS from 12-24 cm and
239 during maturity at TC from 36-48 cm. This exemplifies the high spatial and temporal heterogeneity
240 inherent in marsh soils, and indicates several sources of uncertainty in marsh soil C estimates.



241

242 **Figure 2.** Heat maps of soil C concentration with depth at the three subsites (SS, TC, and TS), four
 243 phenophases, and for each replicate core (A (closest to channel), B, and C (farthest from channel)). No
 244 measurement was able to be obtained for some 12 cm sections as shown by white rectangles.

245

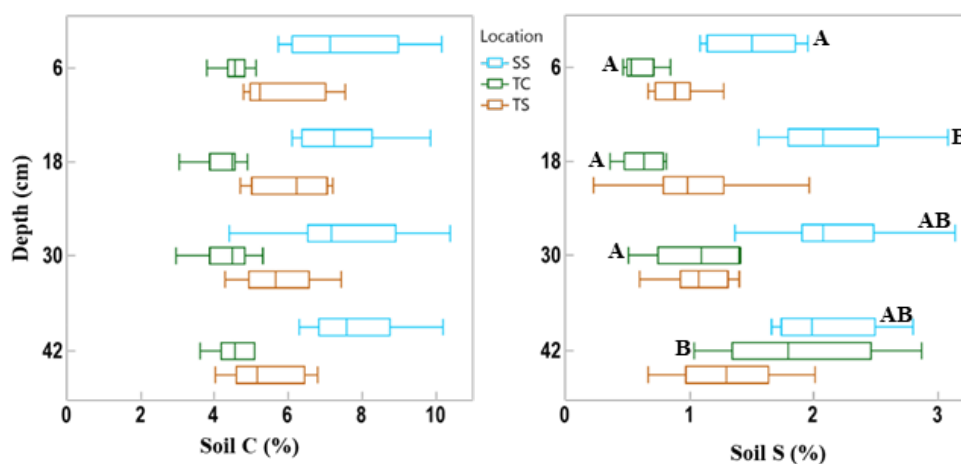
246

247 There was also variability in soil C concentration with depth (Figure 3). Subsite SS had the
 248 highest mean soil C concentration at all four depths, as well as the largest range in values. TS had the
 249 second highest mean soil C values at all four depths as well as the second largest range in values. TC had
 250 the lowest mean soil C at all four depths as well as the smallest range in values at each depth. It is clear
 251 from this graph that SS contains higher overall concentrations of soil C, followed by TS and then TC.
 When observing linear trends with depth, soil C at TS during dormancy significantly decreased with

252 depth ($R^2=0.44$, $p=0.02$) and soil C at SS during maturity significantly increased with depth ($R^2=0.41$,
 253 $p=0.02$). No other linear correlations in soil C existed with depth.

254 We also assessed differences in soil C and S with depth by averaging by phenology and subsite
 255 replicates (Figure 3). These results showed that there were no significant differences in soil C with depth For
 256 soil S, only the first and second depths were significantly different from one another at site SS and at TC,
 257 the deepest cores had significantly more soil S than all other depths.

258



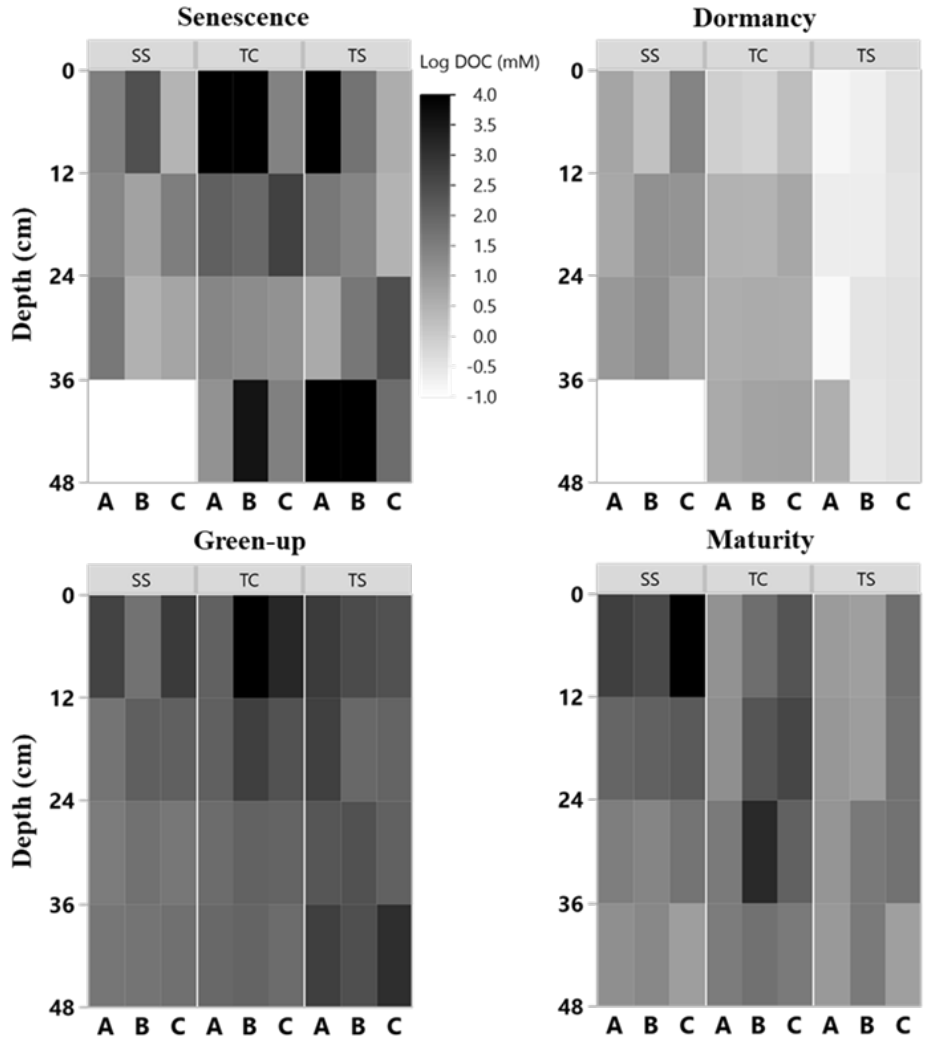
259 **Figure 3.** Box and whisker plot of soil C and S concentrations across the three subsites and separated by
 260 the four sampling depths. This indicates the difference in soil C and S variability among subsites and with
 261 depth. Whiskers indicate the minimum and maximum values, and the box indicates the upper and lower
 262 quartiles. The line in the box indicates the median. Letters with significant differences ($p<0.05$) with depth
 263 for each subsite are shown by different letters; subsites and depths with no letters are statistically similar.
 264

265 SS had the highest mean soil S concentration at each depth, and the range of values initially
 266 increased with depth. TS has a higher mean concentration than TC at all depths except at the bottom core
 267 section. The range of soil S values increased with depth at TC while the range was more consistent with
 268 depth at TS, except for the wide range of values measured at the 18 cm depth interval. Soil S at SS during
 269 maturity significantly increased with depth ($R^2=0.50$, $p=0.01$), as did TC during dormancy ($R^2=0.88$,
 270 $p<0.0001$), green-up ($R^2=0.51$, $p=0.01$), and senescence ($R^2=0.42$, $p=0.02$).
 271

272 3.2 Porewater Data

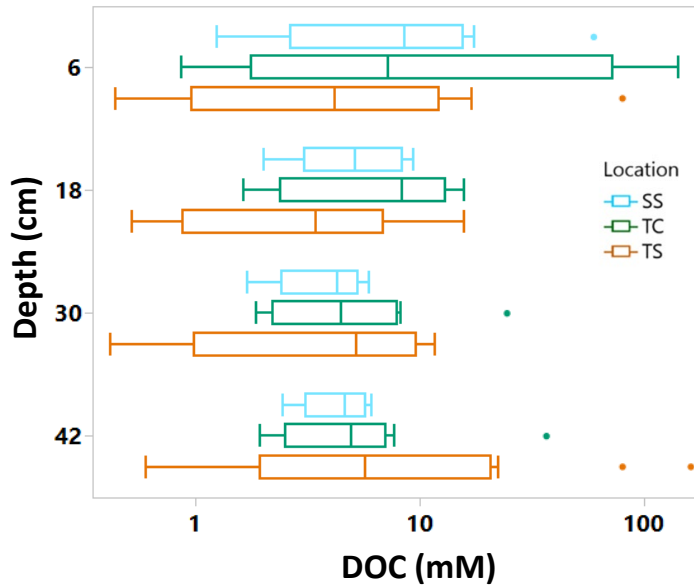
273 3.2.1 Porewater DOC and Characterization

274 Porewater DOC was highly variable across subsites, phenophase, depth, and replicate cores (Figure 4).
275 Note that the data in Figure 4 have been log transformed (natural log) due to large ranges in values across
276 the one-year sampling campaign. Unlike soil C, which was relatively consistent with depth, DOC
277 concentrations were highly variable with depth and even more so among replicate cores. Some of the
278 highest individual concentrations of DOC were detected nearest the surface and rooting zone, which can
279 extend to 20 cm below the surface (Muench and Elsey-Quirk 2019), but also at depth at SS during
280 senescence. DOC concentrations decreased with depth at SS during green-up ($R^2=0.44$, $p=0.02$) and
281 maturity ($R^2=0.37$, $p=0.03$) and increased with depth at TC during dormancy ($R^2=0.76$, $p=0.0002$). These
282 results indicate the highly variable nature of porewater DOC concentrations, possibly leading to
283 additional and complexity in marsh soil C estimates. In addition, we summarized DOC concentrations
284 across depths and subsite (Figure 5) to better understand variability with depth. The top depth increment
285 at 6 cm appeared to contain the greatest variability, particularly at subsite TC. Variability at TC decreased
286 with depth, as did variability at SS. This is apparent because the range tends to decrease with depth at
287 both TC and SS. Overall, TC seems to contain the most variability followed by TS and SS appears to
288 contain the least amount of variability at each depth increment.
289



290

291 **Figure 4.** Heat maps of porewater DOC (natural log) concentration with depth at the three subsites (SS,
 292 TC, and TS), four phenophases, and for each replicate core (A (closest to channel), B, and C (farthest
 293 from channel)). No measurement was able to be obtained for some 12 cm sections as shown by white
 294 rectangles.
 295

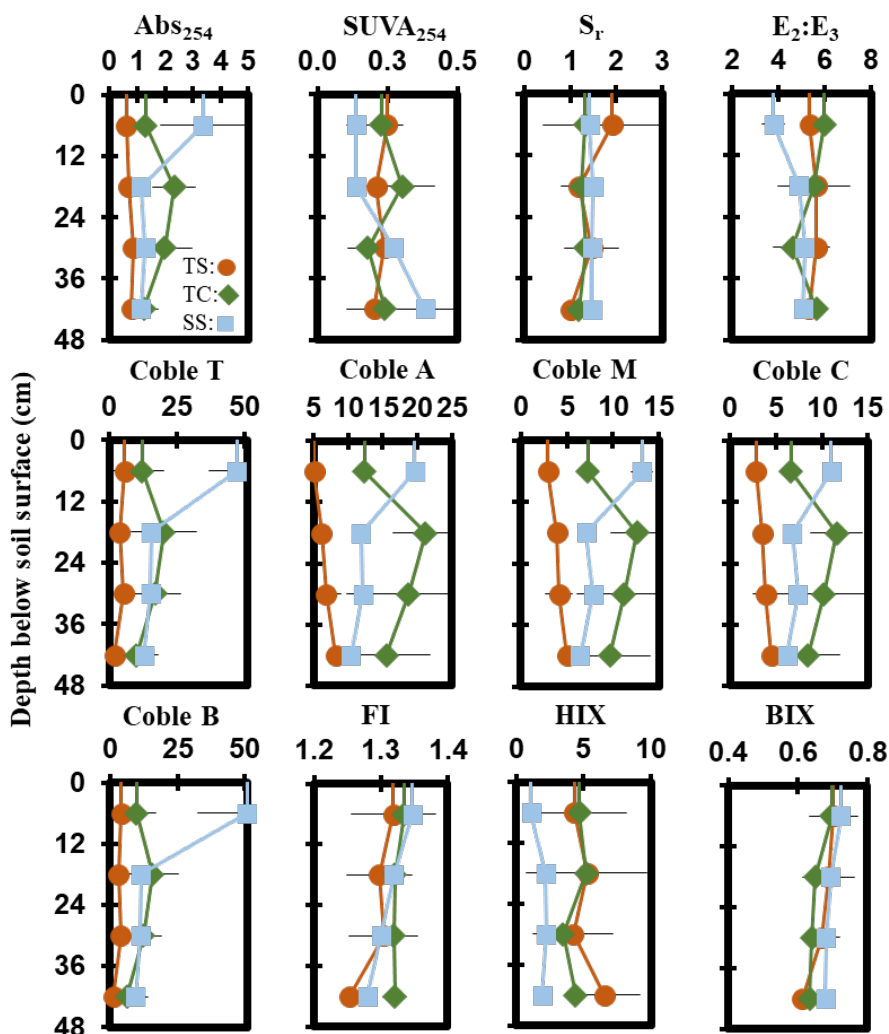


296

297 **Figure 5.** Box and whisker plot of porewater DOC concentrations plotted on log scale across the three
 298 subsites at all phenophases and separated by the four sampling depths. Whiskers indicate the minimum
 299 and maximum values, and the box indicates the upper and lower quartiles. Points outside the boxes indicate
 300 outliers. Due to large variability present across depths, there are no statistically significant differences
 301 between depths.
 302

303 Porewater ultraviolet-visible (UV-VIS) and excitation emission matrices (EEMs) data were
 304 collected only from the maturity sampling event to further characterize DOC molecular properties (Figure
 305 5). Optical properties (i.e., peaks, indices) from spectroscopic data were calculated and interpreted
 306 following previous studies cited in the supplemental table (Table S1). These data show significant trends
 307 with depth at SS. At SS, coble peak intensities T ($R^2=0.55$, $p=0.01$), B ($R^2=0.49$, $p=0.01$), A ($R^2=0.57$,
 308 $p=0.004$), M ($R^2=0.55$, $p=0.01$) and C ($R^2=0.49$, $p=0.01$) all significantly decreased with depth, as did the
 309 fluorescence index (FI) ($R^2=0.79$, $p=0.0001$), the biological index (BIX) ($R^2=0.50$, $p<0.01$) and
 310 absorbance at 254nm (Abs_{254}) ($R^2=0.36$, $p=0.04$), indicating decreases in CDOM with depth. To ensure
 311 the coble peaks represented changes in CDOM properties and not DOC concentration, they were
 312 normalized to DOC concentration and the relationships remained significant ($p<0.05$), except for the
 313 Coble B peak ($R^2=0.11$, $p=0.20$). The $E_2:E_3$ ($R^2=0.50$, $p=0.01$) and $SUVA_{254}$ ($R^2=0.53$, $p=0.007$)
 314 significantly increased with depth at SS, indicating a decrease in molecular weight and an increase in

315 aromaticity with depth. No significant trends with depth were present at TC or TS. Differences in DOC
 316 molecular properties among subsites are apparent for many of the calculated indices and peaks.

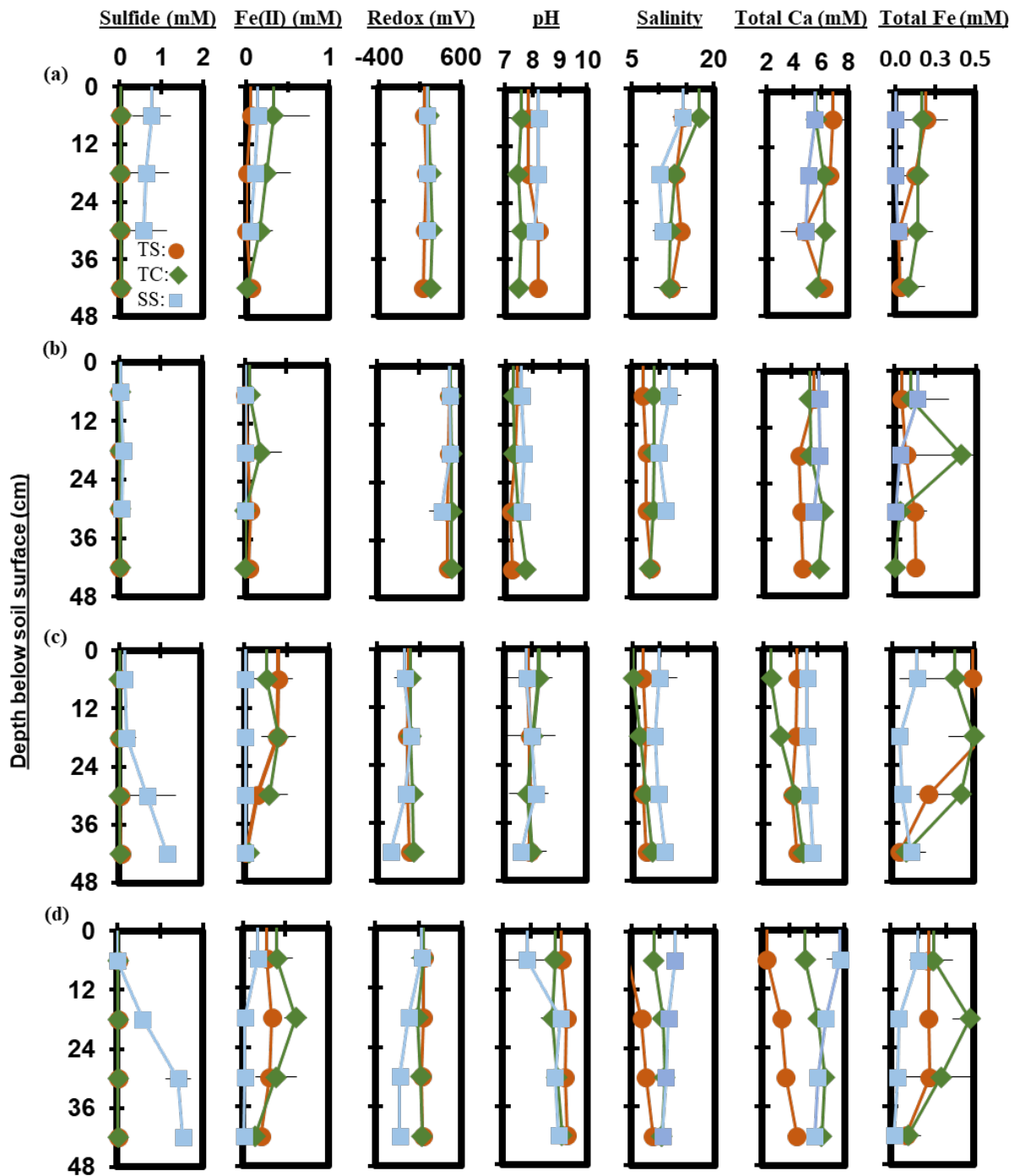


317
 318 **Figure 6.** Depth profiles of porewater EEMs/ UV-VIS peaks and indices down to 48cm taken during the
 319 maturity sampling event. Each point represents the mean between replicates (n=3) with error lines
 320 indicating the standard deviation (± 1 SD).
 321

322 3.2.2 Porewater Chemistry

323 Measured porewater biogeochemistry was variable across subsites, phenophase, and depth
 324 (Figure 6). Porewater redox potentials showed minimal trends with depth, except for a significant
 325 decrease with depth at SS during maturity ($R^2=0.58$, $p=0.004$), though redox showed variability between
 326 replicates (Figure S2). The pH was relatively consistent with depth, except for a significant increase with

327 depth at TC during dormancy ($R^2=0.42$, $p=0.02$), and a significant decrease with depth at TS during
 328 dormancy ($R^2=0.56$, $p=0.005$). Redox potential and pH formed a significant but weak negative correlation
 329 ($R^2=0.12$, $p<0.0001$) across the entire 1-year dataset.



330

331 **Figure 7.** Depth profiles of porewater chemistry variables down to 48 cm for sampling events that
332 occurred during plant (a) senescence, (b) dormancy, (c) green-up and (d) maturity. Each point represents
333 the mean between replicates (n=3) with error lines indicating the standard deviation (± 1 SD).
334

335 Porewater S^{2-} varied significantly with depth. S^{2-} increased significantly with depth across the
336 entire 1-year dataset ($R^2=0.04$, $p=0.03$). S^{2-} increased significantly with depth at SS during green-up
337 ($R^2=0.51$, $p=0.01$) and maturity ($R^2=0.86$, $p<0.0001$). TS S^{2-} increased significantly during green-up
338 ($R^2=0.46$, $p=0.02$) while TC S^{2-} increased significantly during maturity ($R^2=0.36$, $p=0.04$). Porewater Fe^{2+}
339 trended negatively with S^{2-} ($R^2=0.06$, $p=0.004$) and decreased with depth ($p=0.01$, $R^2=0.05$) across the
340 entire 1-year dataset. Significant decreases were observed at TS during green-up ($R^2=0.68$, $p=0.001$), and
341 at SS during maturity ($R^2=0.41$, $p=0.02$). Total Fe concentration followed similar depth trends to Fe^{2+} ,
342 with a significant decrease with depth across the entire 1-year experiment ($R^2=0.06$, $p=0.01$). Total Fe
343 decreased with depth at TS during senescence ($R^2=0.41$, $p=0.03$) and green-up ($R^2=0.58$, $p=0.004$), and at
344 SS during maturity ($R^2=0.57$, $p=0.01$).

345 Porewater salinity formed varying relationships with depth. Salinity significantly decreased with
346 depth at TC during senescence ($R^2=0.52$, $p=0.01$), and at SS during maturity ($R^2=0.62$, $p=0.002$) while
347 salinity significantly increased with depth at TC during green-up ($R^2=0.69$, $p=0.001$) and at TS during
348 maturity ($R^2=0.87$, $p<0.0001$). Salinity and total Ca generally increased together ($p>0.0001$, $R^2=0.42$)
349 across the entire 1-year experiment. Total Ca increased significantly with depth at TC during green-up
350 ($R^2=0.86$, $p<0.0001$) and at TS ($R^2=0.80$, $p<0.0001$) and TC ($R^2=0.47$, $p=0.01$) during maturity. SS total
351 Ca significantly decreased with depth during maturity ($R^2=0.60$, $p=0.005$).

352 **3.3 Analysis of Variance (ANOVA) Among Subsite, Depth, and Phenophase**

353 A three-way ANOVA was run to assess the interaction between the three factors of phenology,
354 subsite, and depth and to understand which factors are the most predictive for each variable (Table 1). Of
355 the measured variables, only porewater DOC, sulfide, and salinity had significant interactions between all
356 three factors; for these, one-way ANOVAs were performed. These analyses showed that DOC was
357 significantly higher during senescence at TS and TC in the surface than the other depths, subsites, and
358 phenophases, and that salinity was highest in the surface at all three subsites during senescence. In

359 contrast, sulfide was highest during maturity at SS in the deepest core section compared to the other
 360 depths, subsites, and phenophases. For the variables without significant interaction, the three-way
 361 ANOVA showed that subsite was highly significant for soil C while phenology and depth were not
 362 significant. In contrast, phenology was only significant for porewater pH and Fe(II) while depth and
 363 subsite were not significant, and depth was only significant for soil S and sulfide (Table 1).

364

365 **Table 1** Three-way ANOVA results for all variables, with interaction results of subsite, phenology
 366 and depth. Bolded p-values indicate significance ($p \leq 0.05$).

367

296

Variable	Phenology	Subsite	Depth	Phenology*Subsite*Depth
Soil C (%)	0.06	<0.0001	0.95	0.96
Soil S (%)	0.99	0.89	0.01	0.99
DOC (mM)	.17	.91	.02	.004
Redox (mV)	.07	.31	.36	.77
pH	<0.0001	0.43	0.77	0.92
Fe ²⁺ (mM)	<0.0001	0.06	0.39	0.91
Sulfide (mM)	0.80	0.91	0.01	0.05
Salinity (ppt)	<0.0001	<0.0001	0.99	0.003
Total Fe (mM)	0.98	0.27	0.21	0.75
Total Ca (mM)	0.0001	0.003	0.41	0.37

368

369 In addition to the three-way ANOVA, we also averaged variables by phenophase, subsite, or
 370 depth and performed one-way ANOVAs with post-hoc Tukey tests (Tables 2, 3, and Supplementary Table 2).

371 When averaged by subsite, all subsites contained significantly different average concentrations of soil C,
 372 with SS having the highest average (7.5 % C), followed by TS (5.8 % C) and TC (4.6 % C) (Table 2).
 373 This indicates that on average, subsite SS contains ~29 % more soil C than TS and 63 % more soil C than
 374 TC. Site SS also had higher soil S, sulfide, and salinity and lower redox potential and Fe(II) than the other
 375 subsites. When grouped by phenophase, plant dormancy contained significantly more soil C than plant
 376 green-up (Table 3). In addition, plant dormancy had significantly higher redox potential and the lowest Fe(II)
 377 and DOC than the other plant phenophases (Table 3). When averaged by depth, soil S was nearly 2x higher at
 378 the deepest depth (36-48 cm) than the surface (0-12 cm) (Supplementary Table 2).

379
 380 **Table 2.** ANOVA and Post-hoc Tukey results for all assessed soil and porewater biogeochemical
 381 variables. Mean values represent average values for each subsite for subsamples from all depths and
 382 phenophases. The mean is reported (\pm SD) along with a connecting letter report. Means with letters that
 383 differ are significantly ($p < 0.05$) different.

Variable	Tall Spartina (TS)	Tall Cordgrass (TC)	Short Spartina (SS)
Soil C (%)	5.8 \pm (1.2) ^B	4.6 \pm (1.3) ^C	7.5 \pm (1.4) ^A
Soil S (%)	1.1 \pm (0.5) ^B	1.0 \pm (0.6) ^B	2.0 \pm (0.7) ^A
DOC (mM)	11.9 \pm (27) ^A	13.6 \pm (27) ^A	7 \pm (9) ^A
Redox (mV)	179 \pm (176) ^{AB}	211 \pm (185) ^A	93 \pm (235) ^B
pH	8.12 \pm (0.8) ^A	7.99 \pm (0.7) ^A	8.13 \pm (0.6) ^A
Fe ²⁺ (mM)	0.15 \pm (0.1) ^A	0.22 \pm (0.3) ^A	0.04 \pm (0.1) ^B
Sulfide (mM)	0.02 \pm (0.01) ^B	0.02 \pm (0.01) ^B	0.6 \pm (0.6) ^A
Salinity (ppt)	8.8 \pm (3.1) ^B	9.7 \pm (3) ^{AB}	11 \pm (2) ^A
Total Fe (mM)	0.21 \pm (0.2) ^A	0.26 \pm (0.3) ^A	0.08 \pm (0.1) ^B
Total Ca (mM)	4.7 \pm (1.3) ^B	5.4 \pm (1.2) ^A	5.8 \pm (0.8) ^A

385
 386

387 **Table 3.** ANOVA and post-hoc Tukey results for all assessed soil and porewater biogeochemical
 388 variables. Mean values represent average values for each phenophase for subsamples from all depths and
 389 subsites. The mean is reported (\pm SD) along with a connecting letter report. Means with letters differ are
 390 significantly ($p < 0.05$) different.

Variable	Senescence	Dormancy	Green-up	Maturity
Soil C (%)	5.7 \pm (1.5) ^{AB}	6.7 \pm (1.1) ^A	5.3 \pm (1.5) ^B	6.1 \pm (1.8) ^{AB}
Soil S (%)	1.4 \pm (0.7) ^A	1.4 \pm (0.9) ^A	1.4 \pm (0.7) ^A	1.3 \pm (0.7) ^A
DOC (mM)	22.2 \pm (42) ^A	1.6 \pm (1) ^B	12.3 \pm (14) ^{AB}	7.9 \pm (10) ^B
Redox (mV)	193 \pm (60) ^B	453 \pm (58) ^A	-42 \pm (98) ^D	83 \pm (111) ^C
pH	7.89 \pm (0.4) ^B	7.45 \pm (0.2) ^C	7.96 \pm (0.6) ^B	8.94 \pm (0.5) ^A
Fe²⁺ (mM)	0.1 \pm (0.2) ^{BC}	0.03 \pm (0.1) ^C	0.2 \pm (0.2) ^{AB}	0.2 \pm (0.2) ^A
Sulfide (mM)	0.2 \pm (0.4) ^{AB}	0.04 \pm (0.04) ^B	0.2 \pm (0.4) ^{AB}	0.3 \pm (0.6) ^A
Salinity (ppt)	12.9 \pm (2.4) ^A	9.0 \pm (1.8) ^{BC}	8.0 \pm (2.1) ^C	9.6 \pm (2.4) ^B
Total Fe (mM)	0.1 \pm (0.1) ^B	0.1 \pm (0.2) ^B	0.3 \pm (0.2) ^A	0.3 \pm (0.2) ^A
Total Ca (mM)	5.8 \pm (1.0) ^A	5.5 \pm (0.7) ^A	4.5 \pm (0.9) ^B	5.3 \pm (1.6) ^A

391
 392 DOC concentration also varied among subsites (Table 2) and phenology (Table 3). The average
 393 DOC concentration at SS was approximately half of that found at TS and TC, but these results are not
 394 statistically significant due to large variability and ranges in concentration observed across the 1-year
 395 experiment. This large variability is exemplified by standard deviations that are larger than the means. In
 396 addition, DOC also varied across phenophases. Dormancy had the lowest mean DOC concentration and
 397 was significantly lower than senescence by an order of magnitude. Maturity and green-up did not have
 398 statistically different DOC concentrations. The EEMs/ UV-VIS dataset from plant maturity was analyzed
 399 based on subsites (Table 4). There were significant differences in peaks and indices between subsites.
 400 Coble peaks T, A, M, C and Abs₂₅₄ were significantly lower at TS than at both TC and SS by at least a
 401 factor of two which is in line with the lower DOC concentrations observed for TS at maturity. Subsite SS
 402 had a significantly lower HIX and E₂:E₃ than both TS and TC suggesting it to have DOM with less

403 relative humic content and higher average molecule weight. These results indicate significantly different
 404 DOC molecular characteristics across subsites. EEMs/ UV-VIS data could not be assessed across
 405 phenology since these data were collected only during plant maturity.

406 **Table 4.** One-way ANOVA results for UV-VIS EEMs during the plant maturity phenophase. Mean
 407 values represent average values for each subsite for subsamples from all depths. The mean is reported (\pm
 408 SD) along with a connecting letter report. Means with letters that differ are significantly ($p < 0.05$)
 409 different.

Parameter	Tall Spartina (TS)	Tall Cordgrass (TC)	Short Spartina (SS)
Abs₂₅₄	0.7 \pm (0.2) ^B	1.7 \pm (0.9) ^A	1.7 \pm (1.3) ^A
SUVA₂₅₄	0.2 \pm (0.1) ^A	0.2 \pm (0.1) ^A	0.2 \pm (0.1) ^A
s_r	1.39 \pm (0.95) ^A	1.27 \pm (0.33) ^A	1.46 \pm (0.28) ^A
E₂:E₃	5.5(0.4) ^A	5.4 \pm (1.1) ^A	4.7 \pm (0.7) ^B
Coble T	4.1 \pm (3.8) ^B	14.7 \pm (10.3) ^A	22.6 \pm (16.2) ^A
Coble A	6.6 \pm (2.1) ^B	16.9 \pm (7.02) ^A	13.5 \pm (4.2) ^A
Coble M	4.0 \pm (1.4) ^B	10.2 \pm (4.4) ^A	8.6 \pm (3.1) ^A
Coble C	3.7 \pm (1.2) ^B	9.2 \pm (4.0) ^A	7.8 \pm (2.3) ^A
FI	1.3 \pm (0.6) ^A	1.3 \pm (0.02) ^A	1.3 \pm (0.03) ^A
HIX	5.1 \pm (3.0) ^A	4.4 \pm (3.1) ^A	1.9 \pm (0.6) ^B
BIX	0.7 \pm (0.7) ^A	0.7 \pm (0.03) ^A	0.7 \pm (0.02) ^A

410
 411 Differences in porewater chemistry among subsites (Table 2) and phenophase (Table 3) were also
 412 significant. SS had the lowest average redox potential and was significantly different from TC which had
 413 the highest, while TS was not significantly different from either SS or TC. Redox potentials were even
 414 more variable between phenophase where all four phases had significantly different means. The highest
 415 mean was measured during dormancy and decreased significantly in the order senescence, maturity and
 416 green-up. The pH was not significantly different across any of the subsites but did change significantly

417 with phenology. Dormancy had the lowest pH which was significantly different from all other
418 phenophases. Senescence and green-up had a statistically similar mean pH values that were higher than
419 dormancy, and the porewater pH during maturity was statistically higher than all other phenophases.

420 S^{2-} also varied significantly among subsites. SS contained on average more than an order of
421 magnitude greater S^{2-} than both TS and TC. S^{2-} is lowest during dormancy but is only significantly
422 different than maturity which has the highest S^{2-} mean. Variability in Fe^{2+} between subsites was opposite
423 of S^{2-} . While TS and TC had low concentrations of S^{2-} , they had high concentrations of Fe^{2+} , which were
424 more than double and significantly higher than Fe^{2+} at SS. Fe^{2+} concentrations varied with phenology
425 similar to S^{2-} where dormancy had the lowest mean which was significantly different only from maturity
426 when the highest levels of Fe^{2+} were detected. Differences between subsite total Fe followed the same
427 trend as Fe^{2+} , where SS was significantly lower than both TS and TC. Total Fe was lowest during
428 dormancy and senescence, which were both statistically similar, but different from green-up and maturity.

429 SS had the highest mean salinity and was significantly different only from TS which had the
430 lowest mean salinity. Green-up had a significantly lower mean salinity than all other phenophases except
431 dormancy. Dormancy was only significantly different from senescence, which had the highest mean
432 salinity. Subsite differences in Ca were similar to salinity where SS had a significantly higher mean Ca
433 concentration than TS, but not TC. Green-up had the lowest mean Ca concentration which was
434 significantly different from all other phenophases.

435 **3.4 Stepwise Regression Model Results**

436 A stepwise regression model was run across the entire 1-year experiment to determine the most
437 important biogeochemical predictors of soil C concentration in our dataset (Table 5). The model results
438 indicate that depth, redox potential, soil S, and sulfide are the best predictors of soil C concentration. The
439 model R^2 value of 0.44 indicates that these variables explain 44% of the variability in our soil C
440 concentration data and the model is highly significant ($p < 0.0001$). Sulfide, redox potential, and soil S
441 each have positive estimates, meaning that these variables increase as soil C increases while depth had a

442 negative estimate, meaning that soil C tends to decrease with depth across the entire dataset. Each
443 individual predictor variable is also significant ($p < 0.05$).

444

445 **Table 5.** Stepwise regression results for predicting soil carbon.

Parameter	Estimate	P-Value	Model R ²	Model P-Value
Depth	-0.03	0.003	0.44	<0.0001
Sulfide	0.96	0.04		
Redox	0.002	0.002		
Soil S%	1.3	<0.0001		

446

447 **4.0 Discussion**

448 **4.1 Subsite Differences in Soil C and Biogeochemistry**

449 We hypothesized that soil C concentration and soil biogeochemistry would differ across our
450 subsite locations. Our results support this hypothesis and suggest significant differences in both soil C
451 concentration and porewater biogeochemistry among subsites, which is consistent with prior work at this
452 field site (Seyfferth et al. 2020; Guimond et al. 2020a). This finding illustrates the importance of
453 considering multiple sampling locations when conducting blue C assessments to account for ecosystem-
454 scale variability. At SS, average soil C concentrations were 63% higher than at TC and 29% higher than
455 at TS. Even though these subsites are several to tens of meters from one another, they each had
456 statistically different mean soil C concentrations. Higher soil C at SS is not related to higher primary
457 productivity because the *Spartina alterniflora* at SS are stunted. The short form of *S. alterniflora* is
458 generally less productive than the tall form (Roman and Daiber 1984) and likely exudes less DOC from
459 the smaller root mass. This is supported by a lower average DOC concentration at SS. Also, the
460 chromophoric dissolved organic matter (CDOM) properties at SS were different than at the other subsites.
461 SS CDOM had a significantly lower E₂:E₃ than TS and TC, indicative of higher molecular weight DOC at

462 SS. In addition, the humification index (HIX) was significantly lower at SS indicating that the DOC at SS
463 has been reworked by microbes less than it has been at TS and TC. Furthermore, SS consistently had
464 lower porewater redox potentials than the other subsites; while our data represent a snapshot in time for
465 each phenophase and subsite location, they are consistent with prior work of higher resolution porewater
466 over time that shows SS being more strongly reducing than areas closer to the tidal channel (Guimond et
467 al., 2020a; Seyfferth et al. 2020). Redox potentials at SS were low enough to support sulfate reduction.
468 This is confirmed by our elevated S^{2-} porewater concentrations measured at SS. Therefore, the greatest
469 controls on soil C concentration at SS is slower microbial oxidation of C due to strongly reducing
470 conditions caused by nearly constant inundation and limited flushing of oxygenated surface water
471 (Guimond et al. 2020b, a; Seyfferth et al. 2020). These conditions lead to CDOM that is less affected by
472 microbial degradation (i.e., low HIX, low $E_2:E_3$) and a less energetically favorable metabolism (i.e.,
473 sulfate reduction) resulting in more soil C accrual. This has important implications for soil C stock
474 uncertainty because a greater amount of the area at St Jones is composed of subsite SS (Seyfferth et al.
475 2020). Sampling only near the tidal creek (TS and TC) could significantly underestimate soil C stocks,
476 while sampling only in the marsh interior could lead to an oversimplification of soil biogeochemistry and
477 DOC molecular properties in salt marsh ecosystems.

478 In contrast to SS, soil redox potentials were significantly higher at TC and soil C was
479 significantly lower. This is likely due to TC having a slightly higher elevation on a natural levee and less
480 reducing surface soils (Seyfferth et al. 2020). The redox potential is not low enough to support sulfate
481 reduction but is low enough to support Fe reduction. This is supported by the abundant amount of Fe^{2+}
482 measured in the porewater at TC. A higher redox potential and more energetically favorable electron
483 acceptor (Fe^{3+}) likely leads to higher rates of C mineralization and explains the lower soil C concentration
484 at TC. On the other hand, we found some of the highest concentrations of DOC at TC, particularly closer
485 to the surface near the rooting zone. This can be explained by a greater root mass and correspondingly
486 higher root exudation rate of the taller *S. cynosuroides* coupled with porewater flushing occurring only on
487 a spring-neap pattern, which allows DOC to build up in porewater over time (Guimond et al. 2020a, b). A

488 higher concentration of freshly produced DOC and a lower concentration of soil C is also consistent with
489 the priming effect which posits that high concentrations of freshly produced and microbially labile DOC
490 can stimulate microbial growth leading to the degradation of older, more stable soil C (Textor et al. 2019;
491 Zhang et al. 2021). In addition, TC CDOM fluorescence peaks (Coble, A, M, C, T), were similar to SS,
492 indicating that SS and TC have strong sources of fluorescent CDOM.

493 Though TS and TC are biogeochemically more similar than SS, TS had significantly higher soil C
494 than TC likely due to different dominant vegetation and hydrology. TS is lower in elevation and
495 experiences diurnal tidal oscillations with slightly lower average porewater redox values than TC (Table
496 1), which experiences tidal oscillations on a spring-neap cycle (Guimond et al. 2020a). These differences
497 in hydrology may cause soil C to accumulate more so under slightly stronger reducing conditions at TS
498 compared to TC. Another unique attribute of subsite TS is the CDOM signature. The coble peaks (A, T,
499 C, and M) and Abs₂₅₄ were significantly lower at TS than both TC and SS, which indicates a decreased
500 concentration of terrestrially-derived CDOM. This is likely because TS is nearest the tidal creek and
501 therefore porewater solutes are exported to the tidal channel twice daily during ebb tide (Fetrow et al.,
502 2023b), decreasing the marsh grass derived terrestrial CDOM signature in the near-channel porewater.

503 **4.2 Phenophase Differences in Soil C and Biogeochemistry**

504 We further hypothesized that soil C concentration and biogeochemistry would vary across plant
505 phenophase, and our data support this hypothesis. Soil C was greatest during plant dormancy and was on
506 average 26% higher than green-up, 18% higher than senescence, and 10% higher than maturity. This
507 highlights the importance of considering the time of year soil samples are taken when conducting a blue C
508 assessment. Likewise, many of the biogeochemical variables also changed with phenophase. The redox
509 potential of all four phenophases were significantly different from one another, with the highest average
510 redox potential occurring during dormancy. Higher redox potentials during dormancy are associated with
511 significantly lower porewater Fe²⁺ and S²⁻, indicating that microbial reduction is likely suppressed during
512 the winter months when labile DOC produced from root exudation is less available. Dormancy also had
513 the highest soil C concentration. We suggest this may be related to a suppressed priming affect due to low

514 porewater DOC concentrations and to Fe oxide formation during the high redox potential of dormancy,
515 allowing any remaining porewater C to be pulled out of solution and into the solid phase with oxidized Fe
516 minerals (Riedel et al. 2013; Sodano et al. 2017; ThomasArrigo et al. 2019).

517 We found that DOC concentrations are higher during senescence and significantly lower during
518 plant maturity. High porewater DOC during senescence agrees with previous work showing higher
519 belowground allocation of biomass in *Spartina* before the winter (Crosby et al. 2015). Belowground
520 allocation of C in *S. alterniflora* has been shown to increase late into the growing season (Lytle and Hull
521 1980) while concentrations of soil organics have been shown to decrease during the summer months due
522 to higher temperatures and higher rates of soil respiration (Caçador et al. 2004). Higher rates of
523 belowground C allocation during senescence are further supported by the higher rates of soil respiration
524 during senescence (Vázquez-Lule and Vargas 2021) due to increased labile DOC availability and
525 associated microbial activity previously reported at this field site.

526 **4.3 Biogeochemical Controls on Soil C**

527 Our data reveal important biogeochemical controls on soil C concentration across space and time.
528 The results of the stepwise regression model suggest that soil C concentrations are predicted by sulfide,
529 soil S, redox potential, and depth. Soil C increased significantly with increasing sulfide and soil S
530 concentration, indicated by the positive model estimate (Table 5). This is likely associated with the lower
531 elevation, and redox potential and greater accumulation of sulphate at SS due to less tidal flushing. This
532 may also be a result of sulfurization where inorganic sulfur, namely sulfide, may interact with organic
533 matter via abiotic reactions (Alperin et al. 1994). Evidence suggests that this interaction can help preserve
534 and stabilize soil C (Tegelaar et al. 1989), though spectroscopic evidence would be required to determine
535 if this is an important process at this study site.

536 Depth also has an important control on soil C concentration and the estimate was negative,
537 indicating that soil C decreases with depth. This is consistent with the literature suggesting higher soil C
538 concentration at the surface and decreasing with depth in coastal salt marshes (Bai et al. 2016). While
539 depth was an important predictor of soil C from the stepwise regression model, our depth profiles (Figure

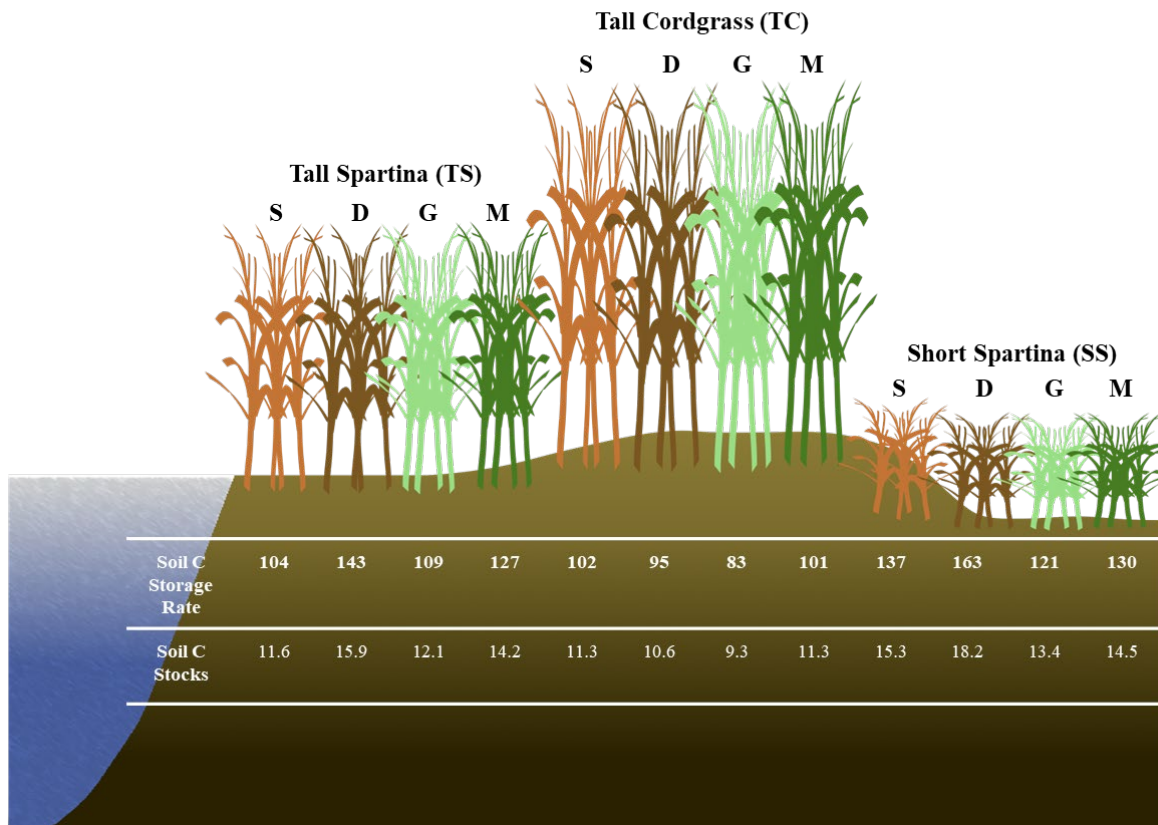
540 4) indicate only small changes with depth. This may be a result of only sampling to 48 cm and integrating
541 across 12 cm increments, or it may be a result of our method design of extracting porewater from the soils
542 and running porewater DOC as a separate fraction of C from the solid phase soil C. Because our
543 porewater DOC results indicate higher concentrations near the surface, the removal of porewater DOC
544 prior to soil C analysis may lead to lower concentrations of soil C at the surface because in most studies,
545 porewater DOC is typically incorporated into the bulk soil C measurements upon soil drying and not
546 extracted as a separate fraction of C (i.e., porewater DOC). We suggest future studies consider porewater
547 DOC as a separate component of the overall soil C concentration, particularly because the variability with
548 depth is much higher for porewater DOC than soil C and porewater DOC is presumed to be more labile
549 and mobile than particulate OC. Therefore, when porewater is extracted from the soil, the measured soil C
550 concentration may appear less variable with depth and time leading to more consistent estimates of the
551 more stable solid-phase soil C.

552 Redox potential was the final significant predictor in the stepwise regression model and increased
553 significantly with soil C. We expected to see a negative relationship between soil C and redox potential
554 due to higher C preservation under reducing conditions, but an overall positive relationship between
555 redox potential and soil C in the model indicates an additional and possibly more important mechanism
556 related to shifting biogeochemistry throughout the year. We observed more oxic conditions at all subsites
557 during plant dormancy in the winter, probably due to the cold winter conditions that allow for the higher
558 dissolved oxygen concentrations in water and porewaters observed previously (Trifunovic et al. 2020).
559 Despite more oxygenated conditions and higher redox potentials in winter, the microbial activity likely
560 decreased during winter, allowing elevated soil C during the winter months when plants were dormant. In
561 addition, the less reducing and more oxygenated conditions in winter likely promoted the formation of Fe
562 oxides that incorporated solution-phase C into the solid phase via coprecipitation. While there is an
563 abundance of evidence showing the importance of Fe oxides in soil C storage in non-wetland ecosystems
564 (Lalonde et al. 2012; Riedel et al. 2013; Sowers et al. 2018a, b, 2019; Adhikari et al. 2019), recent studies
565 have shown the important role of Fe oxides in C cycling in tidal salt marshes (Seyfferth et al. 2020;

566 Fettrow et al. 2023a), but few studies track C cycling during the cool winter months. Variations in Fe
567 oxide complexation with C due to phenological phase should be further investigated.

568 **4.4 Variability in Soil C Accrual Rates and Soil C Stocks**

569 Based on soil accretion rates obtained from a previous study near our core locations (Tucker
570 2016), bulk density at each of the three subsites previously obtained (Wilson and Smith 2015), and our
571 mean soil C concentrations averaged across depth for each subsite within phenophases, we calculated the
572 soil C accumulation rates and soil C stocks at each of the three subsites within each of the four
573 phenophases (Figure 7). These accumulation rates are in range of previously reported values for
574 mesohaline tidal salt marshes (Chmura et al. 2003; Lovelock et al. 2014; Ye et al. 2015; Mcleod et al.
575 2016; Macreadie et al. 2017, 2020), as are the soil C stock estimates (Zhao et al. 2016; Ewers Lewis et al.
576 2018; van Ardenne et al. 2018; Ouyang and Lee 2020; Gorham et al. 2021). These results further illustrate
577 that soil C accrual rates and soil C stocks are highly dynamic and change based on time and space within
578 a single ecosystem. The largest difference between rates and stocks occurred between SS dormancy and
579 TC green-up, in which the average accrual rates varied by 75% and the average stocks varied by 96%.
580 Therefore, within the same ecosystem and between phenophases, soil C accrual rates and stocks can vary
581 substantially, leading to variability and uncertainty. To account for spatial and temporal heterogeneity in
582 soil C accrual rates and stocks, we suggest taking soil cores across multiple vegetation zones (if they
583 exist) and across both the growing and non-growing seasons. Our recommendation follows Howard et al.
584 (2014), who suggest linear plot selection when an obvious feature (i.e., tidal creek) is present and a
585 feature that likely has a strong control on local environmental conditions based on distance from this
586 feature. But we also point out that selecting plot locations based on variation in vegetation is also
587 important, since changing aboveground vegetation is often a sign of changing belowground
588 biogeochemical conditions in tidal systems. This way, the source of variability can be accounted for,
589 leading to less uncertainty in blue C estimates.



590

591 **Figure 8.** Conceptual diagram illustrating the spatial and temporal variability of soil C accrual rates ($\text{g C m}^{-2} \text{yr}^{-1}$) and soil C stocks (kg C m^{-2}) based on subsites by phenophase. Soil C stocks are 0 to 48 cm
 592 $\text{m}^{-2} \text{yr}^{-1}$) and soil C stocks (kg C m^{-2}) based on subsites by phenophase. Soil C stocks are 0 to 48 cm
 593 depth. S= senescence, D= dormancy, G= green-up, M= maturity.

594

595 5.0 Conclusion

596 Our results highlight the variability in soil C in time and space at the site level. We found that
 597 some level of uncertainty in estimates of stocks and accumulation rates is likely related to spatial and
 598 temporal variability of soil C and biogeochemistry at the marsh scale. Subsites that were only a few
 599 meters from one another contained significantly different soil C concentrations, likely using different
 600 metabolic pathways for C mineralization, contained significantly different porewater CDOM molecular
 601 properties and led to considerable variation in soil C accrual rates and soil C stock estimates. The
 602 biogeochemical controls that were best correlated with soil C concentration were redox potential, soil S,
 603 sulfide, and depth, indicating that the redox potential and sulfur content of the soils are critical in

604 controlling how much soil C accumulates in coastal marsh ecosystems. We also found that soil C
605 concentration and thus soil C accrual rates and soil C stock estimates, varies significantly across the
606 phenophases of the marsh grasses. Plant dormancy contained the highest mean soil C concentration,
607 possibly a result of high redox potential during winter months that causes remaining porewater DOC to be
608 incorporated into the solid phase with oxidized minerals such as Fe oxides and lower microbial activity.
609 These results demonstrate the importance of considering marsh-scale spatial and temporal heterogeneity
610 when conducting a blue C assessment. Based on these results, we suggest taking soil cores from multiple
611 locations within a marsh and in replicate, particularly if multiple vegetation types are present, and at
612 different seasons to account for both spatial and temporal variability. These recommendations may help
613 lead to less uncertainty in blue C estimates.

614

615 **Statements and Declarations**

616 **Competing Interests:** The authors have no relevant financial or non-financial interests to disclose.

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620 authors commented on previous versions of the manuscript. All authors read and approved the final
621 manuscript

622 **Data Availability Statement**

623 Data is available on Figshare (DOI: 10.6084/m9.figshare.24274417)

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