

2022

Benthic Biofilm Potential for Organic Carbon Accumulation in Salt Marsh Sediments

Kendall Valentine

Abbey Hotard

Tracy Elsey-Quirk

Giulio Mariotti

Follow this and additional works at: <https://scholarworks.wm.edu/vimsarticles>



Part of the [Sedimentology Commons](#)

This manuscript has been submitted for publication in Wetlands. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

1 **Benthic biofilm potential for organic carbon accumulation in salt marsh sediments**

2 Kendall Valentine^{1,2*}, Abbey Hotard^{1,3}, Tracy Elsey-Quirk¹, Giulio Mariotti^{1,4}

3 *Corresponding Author: kvalentine@vims.edu, ORCID: 0000-0002-5143-3266, Twitter:
4 @kvalentine_7

5
6 ahotard@tamu.edu, tquirk@lsu.edu, gmariotti@lsu.edu

7
8 ¹ Department of Oceanography and Coastal Sciences, College of the Coast and Environment,
9 Louisiana State University, Baton Rouge, LA, USA.

10 ² Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, VA, USA.

11 ³ Department of Marine and Coastal Environmental Science, Texas A&M Galveston, Galveston,
12 TX, USA.

13 ⁴ Center for Computation and Technology, Louisiana State University, Baton Rouge, Louisiana,
14 USA.

15

16 **Abstract**

17 Coastal salt marshes are productive environments with high potential for carbon accumulation
18 and storage. Even though organic carbon in salt marsh sediment is typically attributed to plant
19 biomass, it can also be produced by benthic photosynthetic biofilms. These biofilms, generally
20 composed of diatoms and their secretions, are known for their high primary productivity and
21 contribution to the basal food web. In this study, we conducted laboratory experiments to test (1)
22 if biofilms can potentially accumulate carbon in marsh soil and (2) how different sedimentation
23 rates affect the amount of carbon accumulation. Containers filled with a settled mud bed were
24 inoculated with natural biofilms collected from a marsh surface and allowed to grow with
25 favorable light exposure, nutrient supply, and absence of grazing. Mud was added weekly in
26 different amounts, resulting in an equivalent sedimentation rate from 12 to 189 mm/yr. After 11
27 weeks, the sediment columns were sampled and analyzed for chlorophyll (chl *a*), loss on ignition
28 (LOI), and total organic carbon (TOC). Chl *a* accumulation rates ranged from 123-534
29 mg/cm²/yr, organic matter accumulation ranged from 86-456 g/m²/yr, and TOC accumulation
30 rates ranged from 31-211 g/m²/yr. All three metrics (chl *a*, organic matter, and TOC) increased
31 with increased sedimentation rate. These results show that biofilms can potentially contribute to
32 carbon accumulation in salt marsh soils. Furthermore, areas with high sedimentation rates have
33 the potential for higher amounts of organic matter from biofilms in the sediment.

34

35 **Keywords (4-6)**

36 Microphytobenthos, blue carbon, sedimentation, laboratory experiment, diatoms

37

38

39 **1. Introduction**

40 Organic carbon (OC), ubiquitous in wetland soils, is important for food web dynamics
41 (rapid carbon dynamics) and carbon sequestration (long-term carbon dynamics). Labile OC
42 serves as the base of the food web, providing nutrients and energy to higher trophic levels (Kwak
43 and Zedler 1997). Additionally, the waterlogged conditions and rapid accumulation of sediments
44 can allow OC, especially recalcitrant carbon, to be buried and stored for significant time periods
45 (Chmura et al. 2003, Dodla et al. 2012, Hopkinson et al. 2012). As a result, coastal salt marshes
46 store up to 1700 g/m²/yr of organic carbon, making them one of the most carbon-rich
47 environments on Earth (McLeod et al. 2011). Half of all marine carbon burial occurs in wetlands,
48 even though wetlands occupy only 0.2% of the area available for marine carbon burial (Duarte et
49 al. 2013). Due to the high amount of stored carbon, coastal marshes are considered a blue carbon
50 ecosystem leading to intense study of marsh carbon burial rates over the past several decades
51 (Chmura et al. 2003, Duarte et al. 2005, McLeod et al. 2011, Ouyang and Lee 2014).

52 Most of the carbon found in salt marsh soils has been attributed to plants (macrophytes)
53 (Chmura et al. 2003, Ouyang and Lee 2014). Belowground biomass, in the form of roots and
54 rhizomes, contributes organic carbon directly to sediments, while above-ground biomass can
55 decay on the surface, be exported by tides, or is buried. Although salt marsh plants are probably
56 the main contributor to this carbon pool, algae may be a significant source of organic carbon in
57 salt marsh sediment. Indeed, stable carbon isotopes values of marsh sediments have indicated
58 that a major source of carbon may be from planktonic or benthic photosynthetic microorganisms
59 (Middelburg et al. 1997). Microphytobenthos or biofilms, have been suggested to be a major
60 contributor to the carbon storage in marsh systems (Connor et al., 2001). Additionally, while

This manuscript has been submitted for publication in *Wetlands*. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

61 marsh productivity is often driven by plants, gross primary production by biofilms can be similar
62 to that of plants. For example, Zedler (1980) found that biofilm net primary production was 0.8
63 to 1.4 times the aboveground production, while Gallagher and Daiber (1974) found that algal
64 production beneath salt marsh vegetation was $\sim 1/3$ of the net production by the plants.

65 Benthic photosynthetic biofilms, primarily composed of diatoms and their extracellular
66 polymeric substances (EPS), are typically found as patchy mats on marsh surfaces and intertidal
67 zones worldwide (Decho 2000). Living biofilms, because of their light requirements, are limited
68 to the top several millimeters of the sediment surface, but have been shown to have some vertical
69 motility (MacIntyre et al. 1996, Kingston 1999).

70 The net primary production of biofilms may be greater than 90% of their gross primary
71 production (Pomeroy 1959), suggesting that most of the carbon biofilms create is not respired,
72 but instead is available for decomposition, transfer to other trophic levels, or burial. Although the
73 organic material produced by biofilms, particularly the EPS, is relatively labile compared to
74 marsh plants (McKew et al. 2011), the sheer volume of carbon produced by the rapid turnover
75 rate of these microorganisms may contribute significantly to the marsh sediment carbon pool. In
76 marshes, biofilms are either decomposed by heterotrophic bacteria, buried, resuspended, or
77 consumed by other organisms (Middelburg et al. 2000). Furthermore, biofilms can be a CO₂ sink
78 on the sediment surface, suggesting that they can accumulate C (Chen et al. 2019). Biofilms that
79 are rapidly buried may decompose slower in an anaerobic environment than at the surface,
80 allowing greater carbon preservation.

81 Biofilms exist in a delicate balance with sediment deposition. If sedimentation rates are
82 too low, biofilms will be exposed to oxic conditions, resulting in more rapid decomposition and
83 less burial of carbon. On the other hand, if sediment deposition rates are too high, biofilms may

This manuscript has been submitted for publication in *Wetlands*. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

84 be buried, unable to reach the surface and photosynthesize, fix carbon, and reproduce (Miller et
85 al. 1996, Jesus et al. 2009, Pivato et al. 2019). The existence of a maximum sedimentation
86 threshold for biofilm survival has been postulated even within the context of stromatolite growth
87 (Grotzinger and Knoll 1999), but it has never been tested experimentally.

88 Here we hypothesize that at some intermediate sediment deposition rate, the burial of
89 biofilm OC is maximized. The purpose of this study is twofold. First, we test whether benthic
90 biofilms can accumulate carbon in muddy sediments under favorable conditions (light exposure,
91 nutrient supply, and in the absence of grazing). Second, we test how sedimentation rate affects
92 the rate of biofilm carbon accumulation.

93 **2. Methods**

94 *2.1 Laboratory Set Up*

95 A homogenized bentonite-mud slurry (125 g/L bentonite, 35 psu Instant Ocean seawater)
96 was poured into plastic cylinders (height = 20 cm, diameter = 9.5 cm; Figure 1). The cylinders
97 were placed on orbital shakers (orbital diameter = 0.5 cm, 100 RPM) and allowed to settle to
98 create a sediment bed ~10 cm thick with an overlying water column of ~10 cm. The water
99 column was then exchanged weekly using a peristaltic pump to avoid disturbing the bed surface.
100 The replacement medium was a solution of DI water, Instant Ocean salts (to achieve a salinity of
101 35 psu), and a diluted f/2 medium (Bigelow Laboratory), which provided the necessary nitrogen
102 (10 μM, same order of magnitude as world rivers (Sprague et al. 2011)), phosphorus, silica,
103 vitamins and trace metals for growth (N:P:Si = 24.4:1:2.9). Each cylinder was inoculated with a
104 sample of biofilm scraped from the surface of a salt marsh in Cocodrie, Louisiana (USA). Once
105 inoculated, the cylinders were exposed to a 12-hour light/dark cycle using grow lights
106 (Agrobrite, 120V, 60 Hz high output fluorescent lighting system). The sides of the containers

This manuscript has been submitted for publication in *Wetlands*. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

107 were covered in dark paper to ensure light came only from the provided source. Control
108 containers did not receive the inoculum, were treated with 150 uL of bleach, and kept in the dark
109 to prevent biofilm growth. The cylinders were kept on the orbital shaker, which provided a
110 gentle agitation and promoted vertical mixing of the water column.

111 The sedimentation experiment began after the observed colonization of the sediment
112 surface by biofilms (two weeks of growth). A slurry of bentonite clay mixed with the medium
113 was added according to five sedimentation rates (Table 1), ranging from 12 to 189 mm/yr. These
114 rates represent very high mineral deposition rates compared to field measurements and represent
115 areas such as newly-forming deltas (Shields et al. 2017). Biofilm growth was monitored using a
116 pulse-amplitude modulation (PAM) fluorometer throughout the duration of the experiment. PAM
117 fluorescence values have been used as a proxy for chl *a* and biomass of biofilms in previous
118 studies (Honeywill et al. 2002, Jesus et al. 2005, Murphy et al. 2009, Orvain et al. 2014), and has
119 the advantage of being not destructive. Thirteen points were measured using PAM fluorescence
120 over a regular grid. The fluorescence values demonstrate relative growth within the experiment,
121 not biomass values. Bed heights were also measured and recorded throughout the experiment.

122 *2.2 Sampling and Analyses*

123 After 11 weeks, i.e., one week following the last sedimentation event, the sediment in
124 each cylinder was analyzed to calculate the total amount of organic matter, organic carbon, and
125 chl *a* accumulated throughout the sediment column. Operationally, these measurements were
126 made by separating the top six centimeters of the sediment column – which encompassed the
127 whole layer in which biofilm grew – into two layers (0-3 and 3-6 cm). Each layer was then
128 homogenized and subsampled for bulk density and water content, chl *a* analysis (EPA Method
129 445.0), loss on ignition (LOI), and total organic carbon (TOC) (Ramnarine et al. 2011). For LOI

This manuscript has been submitted for publication in *Wetlands*. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

130 analysis, the samples were burned at 550 °C (Dean 1974). As bentonite clay has high structural
131 water content (Hoogsteen et al. 2015) and our samples had relatively low amounts of organic
132 matter, the mass lost in the control samples was subtracted from all samples to account for the
133 loss of this structural water during the LOI procedure. The total amount of chl *a*, organic matter,
134 and carbon in each layer was then summed together and divided by the duration of the
135 experiment and the surface area, thus obtaining accumulation rates per unit of area.

136 The LOI and TOC data were fit according to the form:

$$137 \quad C_{acc} = C_{max} \left(1 - \exp\left(-\frac{D}{a}\right) \right) \quad \text{(Equation 1)}$$

138 where C_{acc} is the accumulation rate of LOI or TOC, C_{max} is the maximum rate of accumulation
139 of OM or C mediated by sediment deposition, D is the deposition rate, and a is a fitting
140 parameter.

141 **3. Results**

142 *3.1 PAM Fluorescence and Vertical Accretion*

143 Fluorescence values increased approximately two weeks following inoculation in all
144 experiments (Figure 2A). The fluorescence values were variable between containers and over
145 time; however, all containers with inoculum had similar values indicating that biofilm was able
146 to grow in all experiments in a replicable way.

147 The height of the sediment-water interface in each container demonstrated that the
148 addition of bentonite increased the height of the sediment column and the rate of height increase
149 depended on the amount of sediment added (Figure 2B). The height of the containers increased
150 by 4 mm to 45 mm, for the lowest and highest mineral sedimentation rate respectively over the
151 11-week experiment. Following each sediment addition, there was an initial increase in bed
152 height and then a slight decrease due to the consolidation of the added sediment.

153 3.2 *Chlorophyll-a*

154 Sediment chl *a* accumulation rate increased with increasing vertical accretion (Figure
155 3A). The containers with the lowest vertical accretion contained on average 123 mg/cm²/yr C
156 and the containers with the highest vertical accretion rate contained on average 534 mg/cm²/yr C.

157 3.3 *LOI and TOC*

158 As sedimentation rate increased, more organic matter was stored in the sediments (Figure
159 3B). The average amount of organic matter for the highest vertical accretion rate was 456
160 g/m²/yr, which is over five times the average amount of organic matter measured in the
161 containers with the lowest vertical accretion rates (86 g/m²/yr). The sedimentation rate was 16
162 times higher in the treatment with the highest vertical accretion rate compared to the lowest.
163 Similarly, the amount of carbon increased with increasing rates of vertical accretion (Figure 3C).
164 The containers with the lowest accretion rates contained 31 g/m²/yr C, while those with the
165 highest accretion rate contained 211 g/m²/yr C.

166 We fit the exponential model to the LOI and TOC datasets (Equation 1, Figure 3) with
167 the assumption that there is little to no accumulation of OM or C from biofilms without sediment
168 deposition, as without burial the labile OM from biofilms will decompose and will not to
169 contribute to OM/C accumulation. As accumulation rates increase, the rates of C production
170 increase decreases (Figure 3B, 3C). For LOI, we found that the maximum amount of OM
171 accumulated, C_{max} , was 534 g/m²/yr. In terms of TOC, C_{max} was determined to be 201 g/m²/yr
172 C.

173 4. Discussion

174 4.1 *The potential for biofilm carbon accumulation*

175 The carbon accretion rates (CAR) from this study are comparable with those observed in
176 marshes worldwide. We found rates of 100-200 g/m²/yr C with moderate to high accretion rates,
177 while worldwide rates for marshes range from 100-300 g/m²/yr C, depending on the latitude and
178 vegetation type, amongst other variables (Ouyang and Lee 2014). Our results demonstrate that
179 under favorable conditions (light, nutrients, no grazing or competition), biofilms have the
180 potential to produce soil carbon at the same order of magnitude of what is observed in marshes
181 worldwide.

182 Previous experiments have shown that much of the carbon from biofilms is in the form of
183 extra-polymeric substances (EPS), and that this material is rapidly degraded (Guarini et al. 2000,
184 de Brouwer and Stal 2001). These experiments looked at the surface biofilm and the associated
185 carbon, and not at the biofilm carbon with time or depth. Our experiment did not show the ability
186 to store carbon over decadal to centennial time scales due to logistical restraints. Yet, recent
187 studies (Unger et al. 2016) showed that even labile carbon can be stored at depth and for greater
188 than 50 years in marsh sediment, enhanced by high sedimentation rates.

189 4.2 Sedimentation rate increases carbon accumulation

190 Our experiment clearly shows that the rate of chl *a* and carbon accumulation increases
191 with the rate of sedimentation. A possible explanation for this trend is that sedimentation
192 stimulates biofilm production by providing additional nutrients. However, this hypothesis is not
193 likely given the abundance of nutrients in the water column; none of these experiments were
194 nutrient limited and therefore a small increase in nutrients from the addition of bentonite should
195 not have increased carbon production significantly.

196 Another explanation for the increase in OC accumulation with sedimentation rate is that
197 sedimentation could provide additional space (volume) that the biofilms are able to fill as they

198 grow upward towards the light source. Sedimentation necessitates vertical movement by the
199 photosynthetic organisms, and thus causes an increase in organic matter production (Pinckney
200 and Zingmark 1993). Diatoms have been shown to migrate in sediments in short time frames,
201 largely as a response to light (Paterson 1989, Underwood and Kromkamp 1999). As a
202 mechanism of migration, diatoms use their organic secretions (EPS) to aid in their vertical
203 movement (Underwood et al. 1995, Smith and Underwood 1998). With higher sedimentation
204 rates, the diatoms need to migrate further and therefore secrete more organic material.
205 Furthermore, as diatoms migrate, dead cells remain scattered through the sediment (Debenay et
206 al. 2007); with increased sedimentation and increased migration, the amount of carbon from dead
207 cells would also increase. Ultimately, the more volume of sediment present for biofilms to grow
208 upon leads to higher amounts of organic matter production by the biofilms.

209 Furthermore, sedimentation may affect the “age” of the biofilm, and therefore change the
210 rate of production. The physiological state of biofilm changes over time (Sutherland et al. 1998),
211 with lower rates of photosynthesis (Serodio et al. 2005) and higher EPS production for more
212 mature biofilms (Orvain et al. 2003). We find that early in the experiment (days 20-50),
213 fluorescence measurements (i.e. rates of photosynthesis) are equal across sedimentation rates, but
214 late in the experiment (days 50-98), fluorescence values are linearly related to sedimentation rate
215 (Figure 4). In fact, at low sedimentation rates, fluorescence values are lower during the later
216 stage of the experiment, supporting the hypothesis of decreased rates of photosynthesis with time
217 (Serodio et al. 2005). Conversely, with high sedimentation rates, fluorescence rates remain high.
218 Our results suggest that sedimentation may constantly “reset” the biofilm age and allow it to
219 grow as in the early stage of development, allowing for the production of more carbon and
220 increased carbon in the sediments.

221 High rates of carbon accumulation have been related to high mineral suspended sediment
222 supply (Connor et al. 2001), and therefore increased marsh accretion rates (Kirwan and
223 Megonigal 2013). While Connor and others (2001) are reporting CARs from all C sources, they
224 suggest that at low elevations, where sediment accretion rates are higher, biofilms may be a
225 factor influencing carbon accumulation. We find in our experiments that OM from biofilms
226 agree with the relationship between high suspended sediment, high sedimentation rates, and high
227 rates of OC burial.

228 *4.3 Limits on C accumulation by biofilms*

229 The consistent trend in all metrics of biofilm growth (chl *a*, LOI and TOC) confirm that
230 biofilm grown under favorable conditions can maintain itself and even thrive under
231 sedimentation rates nearly 16 times the natural rate along the Gulf Coast (Cahoon et al. 2010).
232 Although our results suggest that a constant level of organic carbon accumulation can be reached
233 for arbitrary high sedimentation rates, this is likely not the case. We expect that there is a
234 sedimentation maximum which the biofilms would not be able to recover from (Grotzinger and
235 Knoll 1999), thus limiting its ability to accumulate carbon. Ultimately, at some deposition rate,
236 the biofilms would not be able to reach the sediment surface, or not be able to colonize, grow and
237 reproduce quickly enough on the surface to contribute to carbon accumulation. At very high
238 sedimentation rates, OM and C accretion rates would likely decline quickly as less and less of
239 the biofilm is able to reestablish on the sediment surface.

240 The limited number of samples and replicates in this experiment make it difficult to draw
241 any statistical conclusions. However, the trend present in all three methods of estimating the
242 productivity of biofilms (chl *a*, LOI, and TOC) suggests that higher sedimentation rates do allow
243 for more biofilm growth, more organic carbon, and more organic matter.

244 An unexpected result of this experiment was that the biofilms were incredibly resilient
245 and able to grow despite large sedimentation rates. Following each sedimentation event, the
246 biofilms colonized the new sediment-water interface very quickly, within 24-48 hours. Indeed,
247 PAM fluorescence (Figure 2A) did not decrease following the sedimentation events, even though
248 these measurements were taken 24-48 hours following such an event. The mineral sedimentation
249 rates tested in this experiment exceed most sedimentation rates for coastlines worldwide and
250 were done episodically. As the biofilms were able to grow in these extreme conditions, biofilms
251 in nature would likely be able to withstand normal sedimentation, as well as sedimentation from
252 storm events.

253 *4.4 Consequences for natural systems*

254 The importance of increased sedimentation rates on the productivity of salt marsh biofilm
255 is particularly relevant for coastal restoration projects. Some methods of marsh restoration
256 projects, including sediment diversions (e.g.: Elsey-Quirk et al. 2019) and thin-layer sediment
257 deposition (e.g.: Ford et al. 1999), involve the introduction of high rates of sedimentation to
258 marshes. For example, in a restored marsh in the Bay of Fundy, high sedimentation rates and
259 high carbon accumulation rates were measured prior to the establishment of marsh vegetation
260 (Wollenberg et al. 2018). Wollenberg and others (2018) suggest that the high C accumulation
261 prior to vegetation is allochthonous. However, given the results of our experiment, biofilm
262 productivity could explain high rates of carbon accumulation prior to the establishment of marsh
263 vegetation.

264 While in this study, we focus on the role of biofilm OM in salt marsh sediments, biofilms
265 can also be an important source of C in tidal flats. There are substantial data gaps in our
266 understanding of how much carbon is stored in tidal flats (Lovelock and Reef 2020), and it is

This manuscript has been submitted for publication in *Wetlands*. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

267 possible that these systems may play a large role in coastal carbon storage (Lovelock and Duarte
268 2019). As there is no vascular vegetation, the primary autochthonous C in tidal flats is biofilms.
269 Thus, quantifying the amount of C in tidal flats from biofilms will improve our understanding of
270 this potential carbon sink.

271 *4.5 Future directions*

272 Future studies should improve the ability to individuate the source of the carbon in marsh
273 sediments (Macreadie et al. 2019). This could help to quantify the impact of biofilms in terms of
274 OC in nature and reconcile our laboratory results with field results. A combination of
275 approaches, including stable isotopes (Choi et al. 2001, Gebrehiwet et al. 2008, Galvan et al.
276 2008, Tanner et al. 2010), organic biomarkers (Spohn and Giani 2012, Johnson et al. 2019), and
277 environmental DNA (Reef et al. 2017) will yield a better understanding of the source of carbon
278 in marsh sediments (Geraldi et al. 2019). For example, studies that have used an increased suite
279 of isotopic signatures were more successful in identifying biofilms (Moncreiff and Sullivan
280 2001, Hondula and Pace 2014, Duarte et al. 2018). These tools have been primarily used to map
281 out food webs, but expanding their use to identify carbon sources can help quantify the
282 contribution of biofilms to salt marsh carbon in the field.

283 Furthermore, there is a need to conduct more laboratory experiments including additional
284 factors, such as grazing. Biofilms are an important component of the diet of grazing macrofauna
285 in coastal ecosystems (Daggers et al. 2020). However, while we demonstrate that high
286 sedimentation promotes biofilm C accumulation, little work has been done on how sedimentation
287 rate affects grazers. In sediment-addition restoration projects, snail growth rates were highest
288 with intermediate sediment addition (Stagg and Mendelsohn 2012). It is unclear whether the
289 higher sedimentation rates will allow more of the biofilms to be buried and protected from

This manuscript has been submitted for publication in *Wetlands*. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

290 grazing, or if bioturbation could increase and overall grazing may increase. The strength and
291 direction of this feedback will impact how much biofilm carbon is able to be stored in salt marsh
292 sediments in real settings.

293 Another important aspect to investigate is the fate of resuspended biofilms. Previous studies
294 have focused on the transfer of biofilm OM to consumers in the water column and adjacent
295 habitats from consumers (Carlton and Hodder 2003) or resuspension events (Ubertini et al. 2012,
296 Savelli et al. 2019). While it is clear that biofilm resuspension dynamics are important, the
297 ultimate fate of the resuspended biofilm carbon is not well understood. Much of the resuspended
298 biofilm OM is likely consumed or decomposed, but some of the biofilm may be redeposited and
299 subsequently buried and stored in the sediments. For example, recent flume experiments (Chen
300 et al. 2019) found that resuspended biofilms allowed for faster biofilms recovery and suggested
301 that repeated erosion redistributed surface biofilms deeper in the bed. They argued that this is
302 important for sediment stabilization, but we posit that it would also be important for C storage.

303 **5 Conclusions**

304 Benthic biofilms in coastal environments are resilient and able to flourish under high
305 sedimentation rates, given ample nutrients and light. These experiments clearly demonstrate that
306 biofilms have the potential to contribute to carbon accumulation in salt marsh sediments. Based
307 on the results presented here, biofilms have the potential to accumulate as much carbon in soils
308 as what is typically measured in salt marshes. While this carbon is labile and may not be stored
309 on a centennial to millennial timescale, it likely plays an important role in the carbon cycle in the
310 marsh.

311 All analyses validate our hypothesis that higher sedimentation rates increase biofilm C
312 accumulation. A sedimentation threshold above which biofilms cease to grow and to accumulate

This manuscript has been submitted for publication in Wetlands. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

313 carbon might still exist, but it would be relatively high (i.e., >20 mm/yr). The results of this
314 experiment represent the upper bounds of organic carbon accumulation by biofilms, as they were
315 grown under favorable conditions over a short timescale. Further experiments should quantify
316 the role of grazing in limiting biofilm C accumulation, and how this effect changes as a function
317 of the sedimentation rate.

318

319 **Declarations**

320 **Funding**

321 KV was funded by the Louisiana Board of Regents.

322 **Conflicts of interest**

323 The authors declare that they have no conflicts of interest

324 **Availability of data and material**

325 All data generated or analysed during this study are included in this published article [and its
326 supplementary information files].

327 **Code availability**

328 Not applicable

329 **Authors' contributions**

330 KV, AH, and GM designed the experiment. KV and AH conducted the experiment. KV and AH
331 performed the majority of the analysis. GM and TE-Q provided feedback and comments. AH
332 wrote the initial draft, KV wrote all subsequent drafts.

333

334 **Acknowledgements**

This manuscript has been submitted for publication in Wetlands. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

335 Many thanks to T. Blanchard, S. Gay, M. Kondrat'yev and A. Cole for laboratory assistance. We
336 would also like to thank K. Moran and A. Cole for their help in editing the manuscript.

337

338 6 **References**

- 339 Cahoon, D.R., 2010, Sea-Level rise impact on salt marsh processes in the Northeast region, Sea-
340 Level Rise and Salt Marsh Restoration Workshop, NOAA Restoration Center,
341 Gloucester, MA, 14 September 2010.
- 342 Carlton, J.T., and J. Hodder, 2003, Maritime mammals: terrestrial mammals as consumers in
343 marine intertidal communities, *Marine Ecology Progress Series* 256: 271-286.
- 344 Chen, S., Chmura, G.L., Wang, Y., Yu, D., Ou, D., Chen, B., Ye, Y., and G. Chen, 2019,
345 Benthic microalgae offset the sediment carbon dioxide emission in subtropical mangrove
346 in cold seasons, *Limnology and Oceanography* 64(3): 1297-1308.
- 347 Chen, X., Zhang, C., Paterson, D.M., Townend, I.H., Jin, C., Zhou, Z., Gong, Z., and Q. Feng,
348 2019, The effect of cyclic variation of shear stress on non-cohesive sediment stabilization
349 by microbial biofilms: the role of 'biofilm precursors', *Earth Surface Processes and*
350 *Landforms* 44(7): 1471-1481.
- 351 Chmura, G.L., Anisfeld, S.C., Cahoon, D.R., and J.C. Lynch, 2003, Global carbon sequestration
352 in tidal, saline wetland soils, *Global Biogeochemical Cycles* 17(4): 1111.
353 doi:10.1029/2002GB001917
- 354 Choi, Y., Wang, Y., Hsieh, Y-P, and L. Robinson, 2001, Vegetation succession and carbon
355 sequestration in a coastal wetland in northwest Florida: evidence from carbon isotopes,
356 *Global Biogeochemical Cycles* 15(2): 311-319.
- 357 Connor, R.F., Chmura, G.L, and C.B. Beecher, 2001, Carbon accumulation in Bay of Fundy salt
358 marshes: Implications for restoration of reclaimed marshes, *Global Biogeochemical*
359 *Cycles*, 15: 943-954.
- 360 Dagers, T.D., van Oevelen, D., Herman, P.M.J., Boschker, H.T.S., and D. van der Wal, 2020,
361 Spatial variability in macrofaunal diet composition and grazing pressure on
362 microphytobenthos in intertidal areas, *Limnology and Oceanography* 65(11): 2819:2834.
- 363 De Brouwer, J.F.C., and L.J. Stal, 2001, Short-term dynamics in microphytobenthos distribution
364 and associated extracellular carbohydrates in surface sediments of an intertidal mudflat,
- 365 Dean, W.E., 1974, Determination of carbonate and organic matter in calcareous sediment and
366 sedimentary rocks by loss on ignition: comparison with other methods, *Journal of*
367 *Sedimentary Petrology* 44(1): 242-248.
- 368 Debenay, J-P., Jouanneau, J-M., Sylvestre, F., Weber, O., and D. Guiral, Biological origin of
369 rhythmites in muddy sediments of French Guiana, *Journal of Coastal Research* 236:
370 1431-1442.
- 371 Decho, A.W., 2000, Microbial biofilms in intertidal systems: an overview, *Continental Shelf*
372 *Research* 20(10-11): 1257-1273.
- 373 Dodla, S.K., Wang, J.J., DeLaune, R.D., 2012. Characterization of labile organic carbon in
374 coastal wetland soils of the Mississippi River deltaic plain: Relationships to carbon
375 functionalities. *Science of The Total Environment* 435-436, 151-158.

- 376 Duarte, C.M., Delgado-Huertas, A., Anton, A., Carriollo-de-Albornoz, P., Lopez-Sandoval,
377 D.C., Agusti, S., et al., 2018, Stable isotope (d13C, d15N, d18O, dD) composition and
378 nutrient concentration of red sea primary producers, *Frontiers in Marine Science* 5: 298.
- 379 Duarte, C.M., Losada, I.J., Hendriks, I.E., Mazarrasa, I., and N. Marba, 2013, The role of coastal
380 plant communities for climate change mitigation and adaptation, *Nature Climate Change*
381 3: 961-968.
- 382 Duarte, C.M., Middelburg, J.J., and N. Caraco, 2005, Major role of marine vegetation on the
383 oceanic carbon cycle, *Biogeosciences* 2: 1-8.
- 384 Elsey-Quirk, T., Graham, S.A., Mendelssohn, I.A., Snedden, G., Day, J.W., Twilley, R.R.,
385 Shaffer, G., Sharp, L.A., Pahl, J, and R.R. Lane, 2019, Mississippi river sediment
386 diversions and coastal wetland sustainability: synthesis of responses to freshwater,
387 sediment, and nutrient inputs, *Estuarine, Coastal and Shelf Science* 221: 170-183.
- 388 Ford, MA, Cahoon, D.R., and J.C. Lynch, 1999, Restoring marsh elevation in a rapidly subsiding
389 salt marsh by thin-layer deposition of dredged material, *Ecological Engineering* 12(3-4):
390 189-205.
- 391 Gallagher, J.L. and F.C. Daiber, 1974, Primary production of edaphic algal communities in a
392 Delaware salt marsh, *Limnology and Oceanography* 19(3): 390-395.
- 393 Galvan, K., Fleeger, J.W., and B. Fry, 2008, Stable isotope addition reveals dietary importance
394 of phytoplankton and microphytobenthos to saltmarsh infauna, *Marine Ecology Progress
395 Series* 359: 37-49.
- 396 Gebrehiwet, T., Koretsky, C.M., and R.V. Krishnamurthy, 2008, Influence of *Spartina* and
397 *Juncus* on saltmarsh sediments. III. Organic geochemistry, *Chemical Geology* 225(1-2):
398 114-119.
- 399 Geraldi, N.R., Ortega, A., Serrano, O., Macreadie, P.I., Lovelock, C.E., Krause-Jensen, D.,
400 Kennedy, H., Lavery, P.S., Pace, M.L., Kaal, J., and C.M. Duarte, 2019, Fingerprinting
401 blue carbon: rationale and tools to determine the source of organic carbon in marine
402 depositional environments.
- 403 Grotzinger, J.P. and A.H. Knoll, 1999, Stromatolites in Precambrian carbonates: evolutionary
404 mileposts or environmental dipsticks?, *Annu. Rev. Earth Planet. Sci.* 27: 313-358.
- 405 Guarini, J.M., Blanchard, G.F., Gros, P., Gouleau, D., and C. Bacher, 2000, Dynamic model of
406 the short-term variability of microphytobenthos biomass on temperate intertidal mudflats,
407 *Marine Ecology Progress Series* 291: 291-303.
- 408 Hondula, K.L.. and M.L. Pace, 2014, Macroalgal support of cultured hard clams in a low
409 nitrogen coastal lagoon, *Marine Ecological Progress Series* 498: 187-201.
- 410 Hoogsteen, M.J.J, Lantinga, E.A., Bakker, E.J., Groot, J.C.J., and P.A. Tittonell, 2015,
411 Estimating soil organic carbon through loss on ignition: effects of ignition conditions and
412 structural water loss, *Ear J. Soil Sci* 66:320-328.
- 413 Honeywill, C., Paterson, D.M., and S.E. Hagerthey, 2002, Determination of microphytobenthic
414 biomass using pulse-amplitude modulated minimum fluorescence, *European Journal of
415 Phycology* 37: 485-492.
- 416 Hopkinson, C., Cai, W.-J., & Hu, X. (2012). Carbon sequestration in wetland dominated coastal
417 systems—A global sink of rapidly diminishing magnitude. *Current Opinion in
418 Environmental Sustainability*, 4(2), 186– 194.
- 419 Jesus, B., Brotas, V., Marani, M., and D.M. Paterson, 2005, Spatial dynamics of
420 microphytobenthos determined by PAM fluorescence, *Estuarine, Coastal and Shelf
421 Science*,60: 30-42.

- 422 Jesus, B., Brotas, V., Ribeiro, L., Mendes, C.R., Cartaxana, P., and D.M. Paterson, 2009,
423 Adaptations of microphytobenthos assemblages to sediment type and tidal position,
424 *Continental Shelf Research* 29(13): 1634-1634.
- 425 Johnson, J.J., Olin, J.A., and M.J. Polito, 2019, A multi-biomarker approach supports the use of
426 compound-specific stable isotope analysis of amino acids to quantify basal carbon source
427 use in a salt marsh consumer, *Rapid Communication in Mass Spectrometry* 33: 1781-
428 1791.
- 429 Kingston, M.B., 1999, Effect of light on vertical migration and photosynthesis of *Euglena*
430 *proximo* (Euglenophyta), *Journal of Phycology* 35: 245-253.
- 431 Kirwan, M.L., and J.P. Megonigal, 2013, Tidal wetland stability in the face of human impacts and
432 sea-level rise, *Nature* 504: 53-60.
- 433 Kwak, T.J., and J.B. Zedler, 1997, Food web analysis of southern California coastal wetlands
434 using multiple stable isotopes. *Oecologia* 110: 262-277.
- 435 Lovelock, C.E., and C.M. Duarte, 2019, Dimensions of blue carbon and emerging perspectives,
436 *Biol. Lett.* 15: 20180781.
- 437 Lovelock, C.E., and R. Reef, 2020, Variable impacts of climate change on blue carbon, *One*
438 *Earth* 3(2): 195-211.
- 439 MacIntyre, H.L., Geider, R.J., and D.C. Miller, 1996, Microphytobenthos: The ecological role of
440 the "secret garden" of unvegetated, shallow-water marine habitats. I. Distribution,
441 abundance, and primary production, *Estuaries* 19: 186-201.
- 442 Macreadie, P.I., et al., 2019, The future of blue carbon science, *Nature communications* 10(1): 1-
443 13.
- 444 McKew, B.A., Taylor, J.D., McGenity, T.J., and G.J.C. Underwood, 2011, Resistance and
445 resilience of benthic biofilm communities from temperate saltmarsh to desiccation and
446 rewetting, *IMSE* 5(1): 30.
- 447 Mcleod, E., Chmura, G.L., Bouillon, S., Salm, R., Bjork, M., Duarte, C.M., Lovelock, C.E.,
448 Schlesinger, W.H., and B.R. Silliman, 2011, A blueprint for blue carbon: toward an
449 improved understanding of the role of vegetated coastal habitats in sequestering CO₂,
450 *Frontiers in Ecology and the Environment* 9: 552-560.
- 451 Middelburg, J.J., Barranguet, C., Boschket, H.T.S., Herman, P.M.J., Moens, T., and Heip,
452 C.H.R., 2000, The fate of intertidal microphytobenthos carbon: an in situ ¹³C-labeling
453 study, *Limnol. Oceanography* 45(6): 1224-1234.
- 454 Middelburg, J.J., Nieuwenhuize, J., Lubberts, R.K., and O. van der Plassche, 1997, Organic
455 carbon isotope systematics of coastal marshes, *Estuarine Coastal Shelf Science* 45: 681-
456 687.
- 457 Miller, D.C., Geider, R.J., and H.L. MacIntyre, 1996, Microphytobenthos: the ecological role of
458 the "secret garden" of unvegetated, shallow-water marine habitats. II. Role in sediment
459 stability and shallow-water food webs, *Estuaries* 19: 202-212.
- 460 Moncreiff, C.A. and M.J. Sullivan, 2001, Trophic importance of epiphytic algae in subtropical
461 seagrass beds: evidence from multiple stable isotope analysis, *Marine Ecological*
462 *Progress Series* 215: 93-106.
- 463 Murphy, R.J., Tolhurst, T.J., Chapman, M.G., and A.J. Underwood, 2009, Seasonal distribution
464 of chlorophyll on mudflats in New South Wales, Australia measured by field
465 spectrometry and PAM fluorometry, *Estuarine, Coastal, and Shelf Science* 84: 108-118.
- 466 Orvain, F., De Crignis, M., Guizien, K., Lefebvre, S., Mallet, C., Takahashi, E., and C. Dupuy,
467 2014, Tidal and seasonal effects on the short-term temporal patterns of bacteria,

- 468 microphytobenthos, and exopolymers in natural intertidal biofilms (Brouage, France),
469 *Journal of Sea Research* 92: 6-18.
- 470 Orvain, F., Galois, R., and C. Barnard, 2003, Carbohydrate production in relation to
471 microphytobenthic biofilm development: an integrated approach in a tidal mesocosm,
472 *Microb. Ecol.* 45: 237-251.
- 473 Ouyang, X., and S.Y. Lee, 2014, Updated estimates of carbon accumulation rates in coastal
474 marsh sediments, *Biogeosciences* 11: 5057-5071.
- 475 Paterson, D.M., 1989, Short-term changes in the erodibility of intertidal cohesive sediments
476 related to the migratory behavior of epipellic diatoms, *Limnology and Oceanography*
477 34(1): 223-234.
- 478 Pinckney, J., and R.G. Zingmark, 1993, Biomass and production of benthic microalgal
479 communities in estuarine habitats, *Estuaries* 16: 887-897.
- 480 Pivato, M., Carniello, L., Moro, I., and P. D'Odorico, 2019, On the feedback between water
481 turbidity and microphytobenthos growth in shallow tidal environments, *Earth Surface*
482 *Processes and Landforms* 44(5): 1192-1206.
- 483 Pomeroy, L., 1969, Algal productivity in salt marshes of Georgia, *Limnology Oceanography* 4:
484 386-397.
- 485 Ramnarine, R., Voroney, R.B., Wagner-Riddle, C., and K.E. Dunfield, 2011, Carbonate removal
486 by acid fumigation for measuring the C of soil organic carbon, *Can. J. Soil Sci.*, 91: 247-
487 250.
- 488 Reef, R., Atwood, T.B., Samper-Villarreal, J., Adame, M.F., Sampayo, E.M., and C.E. Lovelock,
489 2017, Using eDNA to determine the source of organic carbon in seagrass meadows,
490 *Limno. Oceanogr.* 62: 1254-1265.
- 491 Savelli, R., Bertin, X., Orvain, F., Gernez, P., Dale, A., Coulombier, T., Pineau, P., Lachaussee,
492 N., Polsenaere, P., Dupuy, C., and V. Le Fouest, 2019, Impact of chronic and massive
493 resuspension mechanisms on the microphytobenthos dynamics in a temperate intertidal
494 mudflat, *JGR Biogeosciences* 124(12): 3752-3777.
- 495 Serodio, J., Vieira, S., Cruz, S., and F. Barroso, 2005, Short-term variability in the
496 photosynthetic activity of microphytobenthos as detected by measuring rapid light curves
497 using variable fluorescence, *Mar. Biol.* 146: 903-914.
- 498 Shields, M.R., Bianchi, T.S., Mohrig, D., Hutchings, J.A., Kenney, W.F., Kolker, A.S., and J.H.
499 Curtis, 2017, Carbon storage in the Mississippi River delta enhanced by environmental
500 engineering, *Nature Geoscience* 10: 846-851.
- 501 Smith, D.J., and G.J.C. Underwood, 1998, Exopolymer production by intertidal epipellic diatoms,
502 *Limnol. Oceanogr.* 43: 1578-1591.
- 503 Spohn, M. and L. Giani, 2012, Carbohydrates, carbon and nitrogen in soils of a marine and a
504 brackish marsh as influenced by inundation frequency, *Estuarine, Coastal and Shelf*
505 *Science* 107: 89-96.
- 506 Sprague, L.A., Hirsch, R.M., and B.T. Aulenbach, 2011, Nitrate in the Mississippi River and its
507 tributaries, 1980 to 2008: are we making progress?, *Enviorn. Sci. Technol.* 45(17): 7209-
508 7216.
- 509 Stagg, C.L. and I.A. Mendelssohn, 2012, *Littoraria irrorata* growth and survival in a sediment-
510 restored salt marsh, *Wetlands* 32: 643-652.
- 511 Sutherland, T.F., Amos, C.L., and J. Grant, 1998, The effect of carbohydrate production by the
512 diatom *Nitzschia curvilineata* on the erodibility of sediment, *Limnol. Oceanogr.* 43: 65-
513 72.

This manuscript has been submitted for publication in Wetlands. This manuscript has not yet undergone peer review and is a preprint. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the 'Peer-reviewed Publication DOI' link on the right-hand side of this webpage.

- 514 Tanner, B.R., Uhle, M.E., Mora, C.I., Kelley, J.T., Schuneman, P.J., Lane, C.S., and E.S. Allen,
515 2010, Comparison of bulk and compound-specific $\delta^{13}\text{C}$ analyses and determination of
516 carbon sources to salt marsh sediment using *n*-alkane distributions (Maine, USA),
517 *Estuarine, Coastal, and Shelf Science* 86(2): 283-291.
- 518 Ubertini, M., Lefebvre, S., Gangnery, A., Grangere, K., Le Gendre, R., and F. Orvain, 2012,
519 Spatial variability of benthic-pelagic coupling in an estuary ecosystem: consequences for
520 microphytobenthos resuspension phenomenon, *PloS one* 7(8): e44155.
- 521 Underwood, G.J.C., and J. Kromkamp, 1999, Primary production by phytoplankton and
522 microphytobenthos in estuaries, *Adv. Ecol. Res.* 29: 93-153.
- 523 Underwood, G.J.C., Paterson, D.M., and R.J. Parkes, 1995, The measurement of microbial
524 carbohydrate exopolymers from intertidal sediments, *Limnology and Oceanography*
525 40(7): 1243-1253.
- 526 Unger, V., Elsey-Quirk, T., Sommerfield, C., and D. Velinsky, 2016, Stability of organic carbon
527 accumulation in *Spartina alterniflora*-dominated salt marshes of the Mid-Atlantic U.S.,
528 *Estuarine, Coastal, and Shelf Science* 182: 179-189.
- 529 Wollenberg, J.T., Ollerhead, J., and G.L. Chmura, 2018, Rapid carbon accumulation following
530 managed realignment on the Bay of Fundy, *PloS One* 13(3): e0193930.
- 531 Zedler, J.B., 1980, Algal mat productivity: comparisons in a salt marsh, *Estuaries* 3(2): 122-131.

532

533

534

535

536

537

538

539

540

541

542

543

544

545

546

547

548

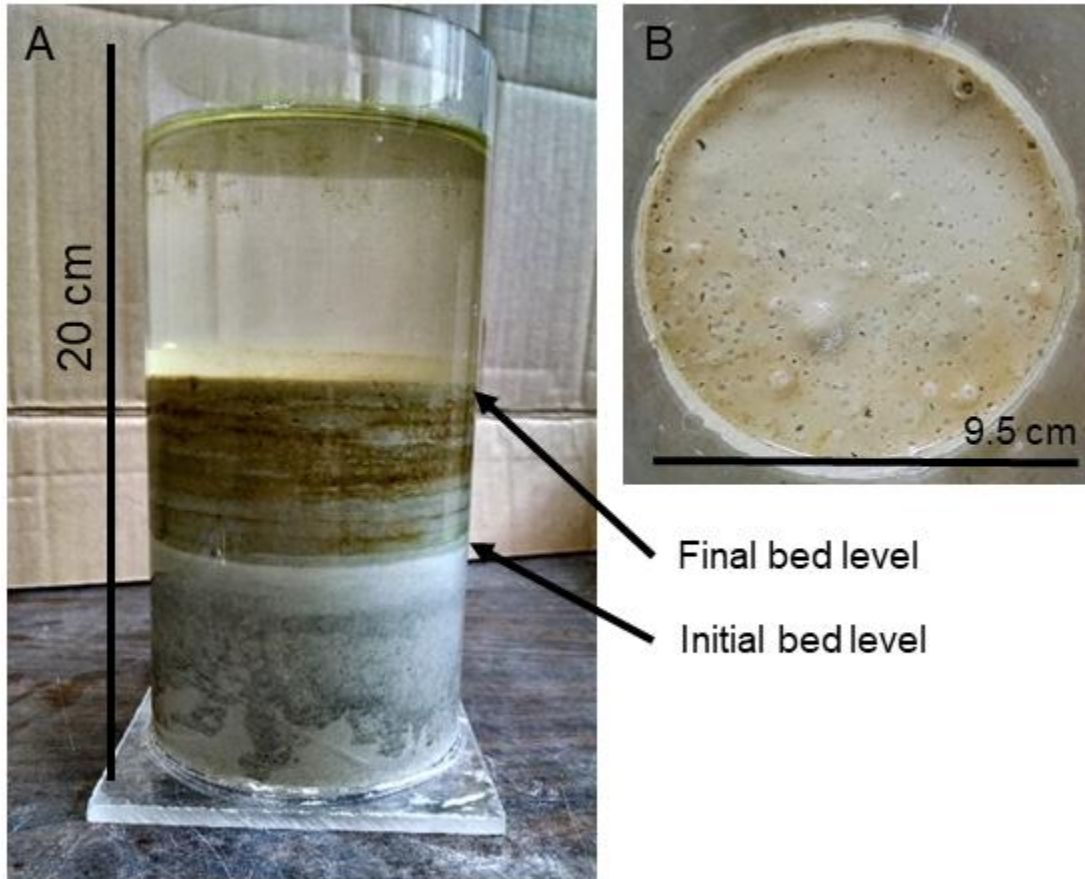
549

550

551

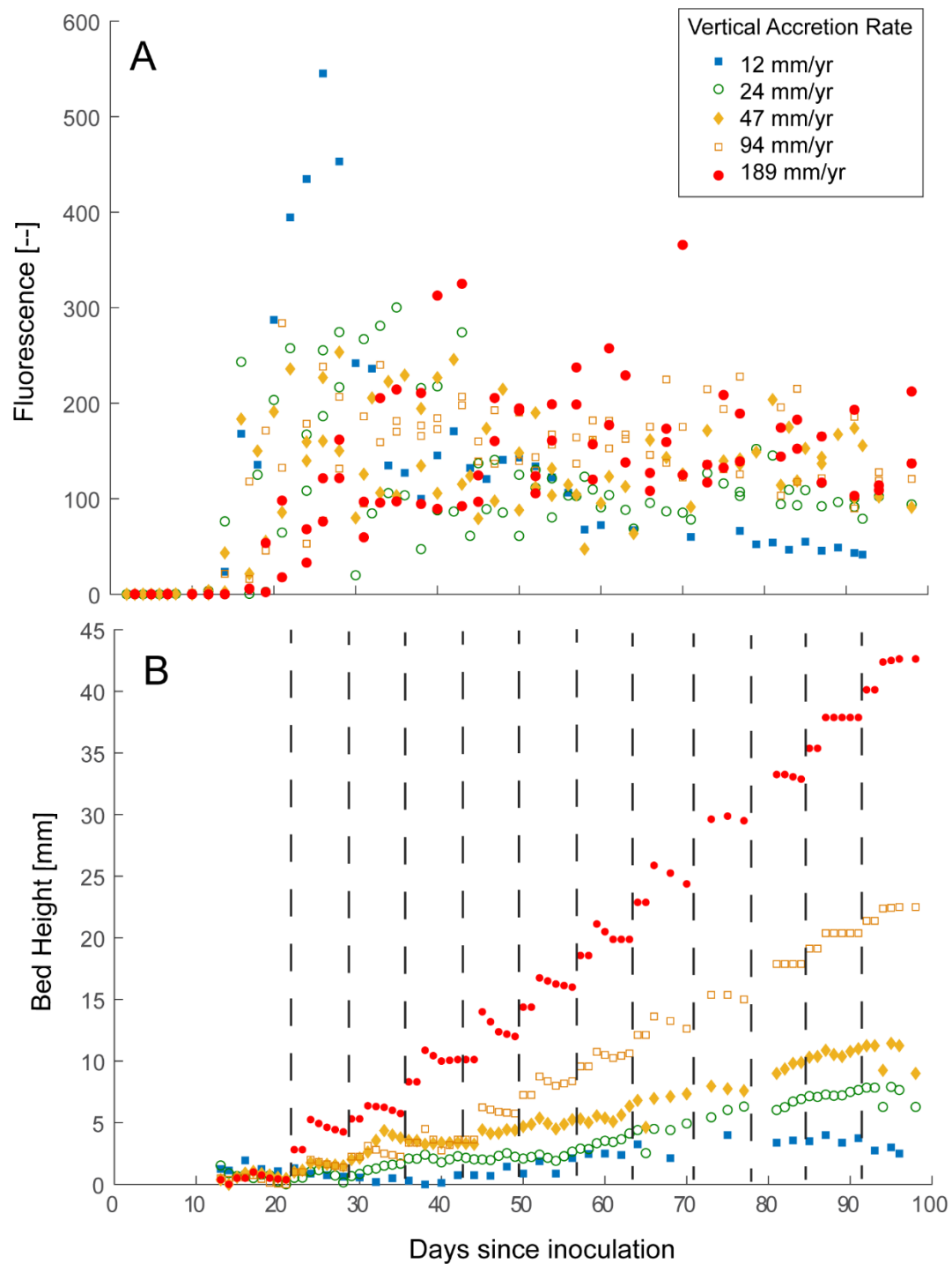
552
553
554
555
556
557

Figure Captions:



558
559
560
561
562
563
564

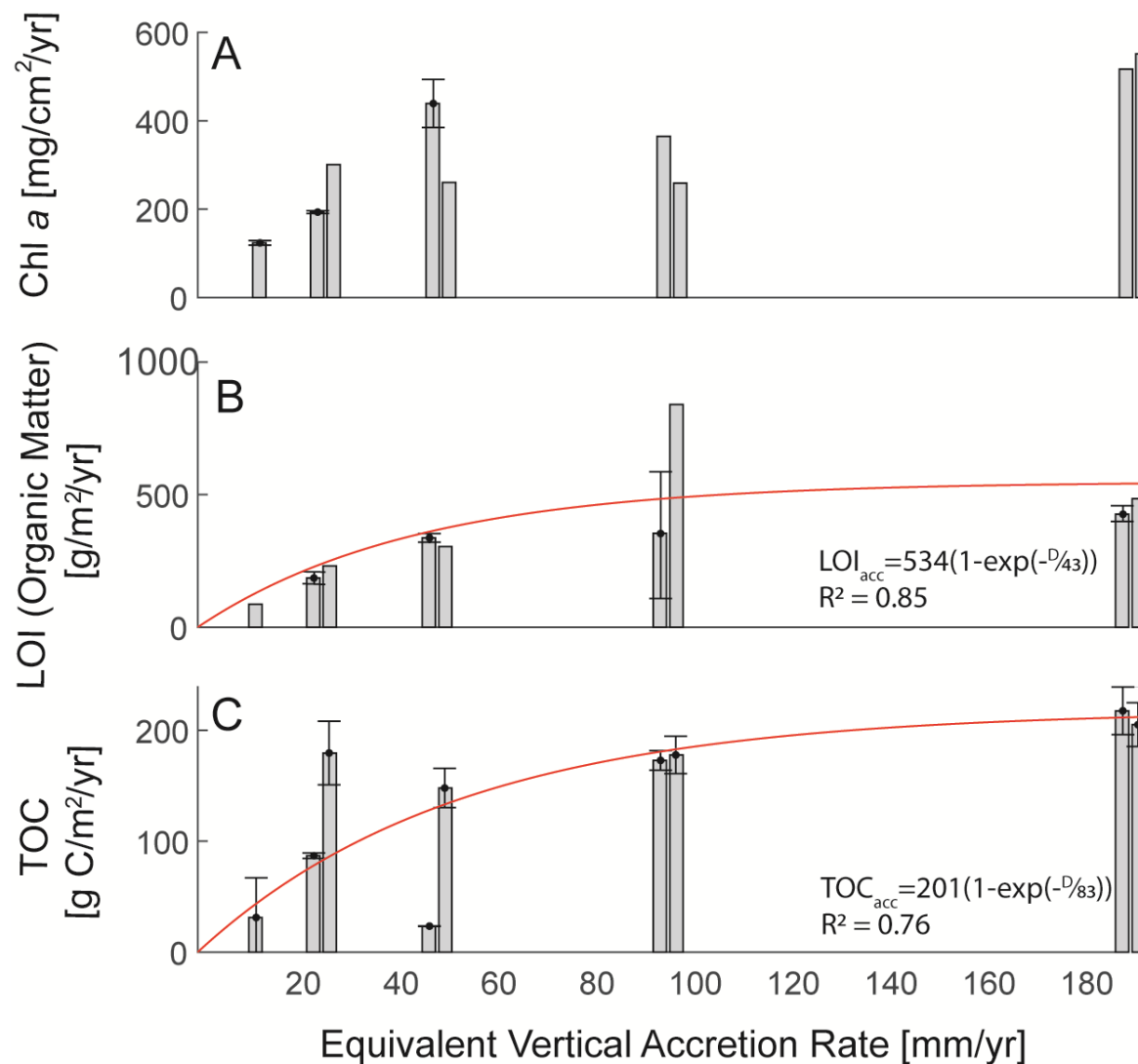
Figure 1: Plastic cylinders used for the experiment after 11 weeks of growth. (A) Side view showing the vertical accumulation of sediments. The parallel layers in the sediment, starting at about half of the sediment column, are from individual sedimentation events and subsequent growth of biofilm. (B) Plane-view of growth experiment. Light brown color is indicative of diatom-based biofilm.



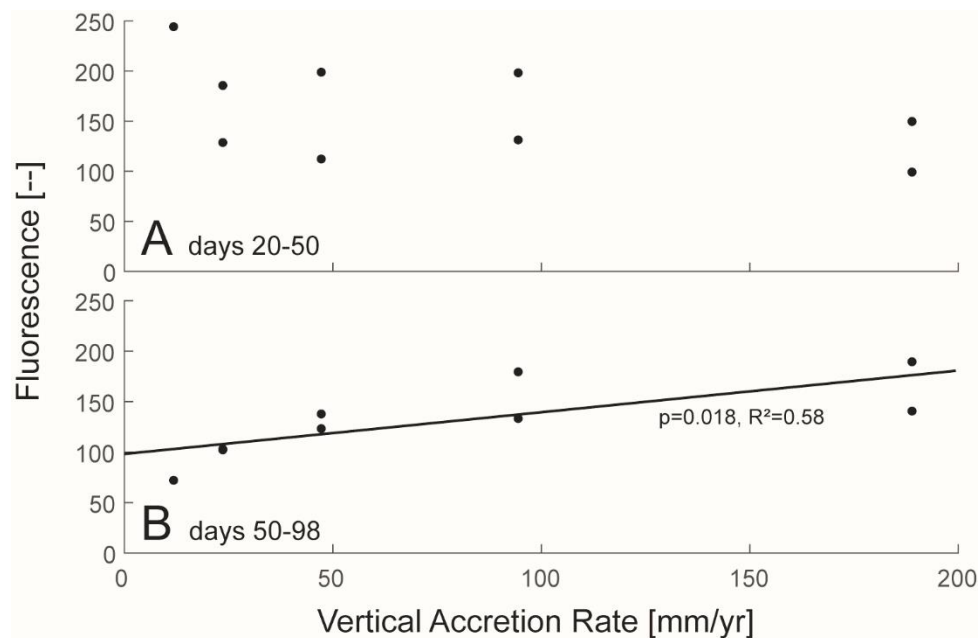
565

566 Figure 2: Monitoring of fluorescence (A) and bed height (B) over the 11-week experiment.
567 Colors represent the five different sedimentation rates (see Table 1). Fluorescence measurements
568 are consistent across treatments. Bed height measurements were corrected for the consolidation
569 of the initial bed over time. Vertical lines in panel B indicate when sediment was added to the
570 experiment.

571



572
573 Figure 3: Chl *a* (A), LOI (B), and TOC (C) values for the content of the containers following the
574 11-week growth experiment for each of the five growth rates. All three metrics show an increase
575 with equivalent vertical accretion rate. Duplicate bars indicate separate trials, standard deviations
576 show measurement variability. Lines in (B) and (C) show best fit to equation 1. (B) $R^2 = 0.85$
577 and (C) $R^2 = 0.76$.
578



579
 580 Figure 4: Average PAM fluorescence value by sedimentation rate for days 20-50 (A) and 50-98
 581 (B). There was no statistically significant relationship between fluorescence and sedimentation
 582 rate in the beginning of the experiments, but in days 50-98, there was a significant linear
 583 relationship ($y=0.41x+98, R^2 = 0.5, p=0.018$).
 584
 585
 586
 587

588 Table 1: List of treatments, or sedimentation rates used in this experiment.

Sedimentation Rank	Mass added each week (g)	Sedimentation Rate ($\text{g}/\text{cm}^2/\text{yr}$)	Equivalent Vertical Accretion Rate (mm/yr)
1	1.069	0.786	11.811
2	2.137	1.572	23.612
3	4.273	3.144	47.222
4	8.547	6.287	94.444
5	17.093	12.574	188.889

589

590