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Benthic biofilm potential for organic carbon accumulation in salt marsh sediments

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Abstract

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

Keywords (4-6)

Microphytobenthos, blue carbon, sedimentation, laboratory experiment, diatoms

1. Introduction

Organic carbon (OC), ubiquitous in wetland soils, is important for food web dynamics (rapid carbon dynamics) and carbon sequestration (long-term carbon dynamics). Labile OC serves as the base of the food web, providing nutrients and energy to higher tropic levels (Kwak and Zedler 1997). Additionally, the waterlogged conditions and rapid accumulation of sediments can allow OC, especially recalcitrant carbon, to be buried and stored for significant time periods (Chmura et al. 2003, Dodla et al. 2012, Hopkinson et al. 2012). As a result, coastal salt marshes store up to 1700 g/m^2/yr of organic carbon, making them one of the most carbon-rich environments on Earth (Mcleod et al. 2011). Half of all marine carbon burial occurs in wetlands, even though wetlands occupy only 0.2% of the area available for marine carbon burial (Duarte et al. 2013). Due to the high amount of stored carbon, coastal marshes are considered a blue carbon ecosystem leading to intense study of marsh carbon burial rates over the past several decades (Chmura et al. 2003, Duarte et al. 2005, McLeod et al. 2011, Ouyang and Lee 2014).

Most of the carbon found in salt marsh soils has been attributed to plants (macrophytes) (Chmura et al. 2003, Ouyang and Lee 2014). Belowground biomass, in the form of roots and rhizomes, contributes organic carbon directly to sediments, while above-ground biomass can decay on the surface, be exported by tides, or is buried. Although salt marsh plants are probably the main contributor to this carbon pool, algae may be a significant source of organic carbon in salt marsh sediment. Indeed, stable carbon isotopes values of marsh sediments have indicated that a major source of carbon may be from planktonic or benthic photosynthetic microorganisms (Middelburg et al. 1997). Microphytobenthos or biofilms, have been suggested to be a major contributor to the carbon storage in marsh systems (Connor et al., 2001). Additionally, while marsh productivity is often driven by plants, gross primary production by biofilms can be similar to that of plants. For example, Zedler (1980) found that biofilm net primary production was 0.8 to 1.4 times the aboveground production, while Gallagher and Daiber (1974) found that algal production beneath salt marsh vegetation was ~1/3 of the net production by the plants.

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

The net primary production of biofilms may be greater than 90% of their gross primary production (Pomeroy 1959), suggesting that most of the carbon biofilms create is not respired, but instead is available for decomposition, transfer to other trophic levels, or burial. Although the organic material produced by biofilms, particularly the EPS, is relatively labile compared to marsh plants (McKew et al. 2011), the sheer volume of carbon produced by the rapid turnover rate of these microorganisms may contribute significantly to the marsh sediment carbon pool. In marshes, biofilms are either decomposed by heterotrophic bacteria, buried, resuspended, or consumed by other organisms (Middelburg et al. 2000). Furthermore, biofilms can be a CO_2 sink on the sediment surface, suggesting that they can accumulate C (Chen et al. 2019). Biofilms that are rapidly buried may decompose slower in an anaerobic environment than at the surface, allowing greater carbon preservation.

Biofilms exist in a delicate balance with sediment deposition. If sedimentation rates are too low, biofilms will be exposed to oxic conditions, resulting in more rapid decomposition and less burial of carbon. On the other hand, if sediment deposition rates are too high, biofilms may be buried, unable to reach the surface and photosynthesize, fix carbon, and reproduce (Miller et al. 1996, Jesus et al. 2009, Pivato et al. 2019). The existence of a maximum sedimentation threshold for biofilm survival has been postulated even within the context of stromatolite growth (Grotzinger and Knoll 1999), but it has never tested experimentally.

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

2. Methods

2.1 Laboratory Set Up

A homogenized bentonite-mud slurry (125 g/L bentonite, 35 psu Instant Ocean seawater) was poured into plastic cylinders (height = 20 cm, diameter = 9.5 cm; Figure 1). The cylinders were placed on orbital shakers (orbital diameter = 0.5 cm, 100 RPM) and allowed to settle to create a sediment bed ~10 cm thick with and overlying water column of ~10 cm. The water column was then exchanged weekly using a peristaltic pump to avoid disturbing the bed surface. The replacement medium was a solution of DI water, Instant Ocean salts (to achieve a salinity of 35 psu), and a diluted f/2 medium (Bigelow Laboratory), which provided the necessary nitrogen (10uM, same order of magnitude as world rivers (Sprague et al. 2011)), phosphorus, silica, vitamins and trace metals for growth (N:P:Si = 24.4:1:2.9). Each cylinder was inoculated with a sample of biofilm scraped from the surface of a salt marsh in Cocodrie, Louisiana (USA). Once inoculated, the cylinders were exposed to a 12-hour light/dark cycle using grow lights (Agrobrite, 120V, 60 Hz high output fluorescent lighting system). The sides of the containers were covered in dark paper to ensure light came only from the provided source. Control containers did not receive the inoculum, were treated with 150 uL of bleach, and kept in the dark to prevent biofilm growth. The cylinders were kept on the orbital shaker, which provided a gentle agitation and promoted vertical mixing of the water column.

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

2.2 Sampling and Analyses

After 11 weeks, i.e., one week following the last sedimentation event, the sediment in each cylinder was analyzed to calculate the total amount of organic matter, organic carbon, and chl *a* accumulated throughout the sediment column. Operationally, these measurements were made by separating the top six centimeters of the sediment column – which encompassed the whole layer in which biofilm grew – into two layers (0-3 and 3-6 cm). Each layer was then homogenized and subsampled for bulk density and water content, chl *a* analysis (EPA Method 445.0), loss on ignition (LOI), and total organic carbon (TOC) (Ramnarine et al. 2011). For LOI analysis, the samples were burned at 550 °C (Dean 1974). As bentonite clay has high structural water content (Hoogsteen et al. 2015) and our samples had relatively low amounts of organic matter, the mass lost in the control samples was subtracted from all samples to account for the loss of this structural water during the LOI procedure. The total amount of chl a, organic matter, and carbon in each layer was then summed together and divided by the duration of the experiment and the surface area, thus obtaining accumulation rates per unit of area.

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

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

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

3. Results

3.1 PAM Fluorescence and Vertical Accretion

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

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

3.2 Chlorophyll-a

Sediment chl *a* accumulation rate increased with increasing vertical accretion (Figure 3A). The containers with the lowest vertical accretion contained on average 123 mg/cm^2/yr C and the containers with the highest vertical accretion rate contained on average 534 mg/cm^2/yr C.

3.3 LOI and TOC

As sedimentation rate increased, more organic matter was stored in the sediments (Figure 3B). The average amount of organic matter for the highest vertical accretion rate was 456 g/m^2/yr, which is over five times the average amount of organic matter measured in the containers with the lowest vertical accretion rates (86 g/m^2/yr). The sedimentation rate was 16 times higher in the treatment with the highest vertical accretion rate compared to the lowest. Similarly, the amount of carbon increased with increasing rates of vertical accretion (Figure 3C). The containers with the lowest accretion rates contained 31 g/m^2/yr C, while those with the highest accretion rate contained 211 g/m^2/yr C.

We fit the exponential model to the LOI and TOC datasets (Equation 1, Figure 3) with the assumption that there is little to no accumulation of OM or C from biofilms without sediment deposition, as without burial the labile OM from biofilms will decompose and will not to contribute to OM/C accumulation. As accumulation rates increase, the rates of C production increase decreases (Figure 3B, 3C). For LOI, we found that the maximum amount of OM accumulated, C_{max}, was 534 g/m^2/yr. In terms of TOC, C_{max} was determined to be 201 g/m^2/yr C.

4. Discussion

4.1 *The potential for biofilm carbon accumulation*

The carbon accretion rates (CAR) from this study are comparable with those observed in marshes worldwide. We found rates of 100-200 g/m^2/yr C with moderate to high accretion rates, while worldwide rates for marshes range from 100-300 g/m^2/yr C, depending on the latitude and vegetation type, amongst other variables (Ouyang and Lee 2014). Our results demonstrate that under favorable conditions (light, nutrients, no grazing or competition), biofilms have the potential to produce soil carbon at the same order of magnitude of what is observed in marshes worldwide.

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

4.2 Sedimentation rate increases carbon accumulation

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

Another explanation for the increase in OC accumulation with sedimentation rate is that sedimentation could provide additional space (volume) that the biofilms are able to fill as they grow upward towards the light source. Sedimentation necessitates vertical movement by the photosynthetic organisms, and thus causes an increase in organic matter production (Pinckney and Zingmark 1993). Diatoms have been shown to migrate in sediments in short time frames, largely as a response to light (Paterson 1989, Underwood and Kromkamp 1999). As a mechanism of migration, diatoms use their organic secretions (EPS) to aid in their vertical movement (Underwood et al. 1995, Smith and Underwood 1998). With higher sedimentation rates, the diatoms need to migrate further and therefore secrete more organic material. Furthermore, as diatoms migrate, dead cells remain scattered through the sediment (Debenay et al. 2007); with increased sedimentation and increased migration, the amount of carbon from dead cells would also increase. Ultimately, the more volume of sediment present for biofilms to grow upon leads to higher amounts of organic matter production by the biofilms.

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

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

4.3 Limits on C accumulation by biofilms

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

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

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

4.4 Consequences for natural systems

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

While in this study, we focus on the role of biofilm OM in salt marsh sediments, biofilms can also be an important source of C in tidal flats. There are substantial data gaps in our understanding of how much carbon is stored in tidal flats (Lovelock and Reef 2020), and it is possible that these systems may play a large role in coastal carbon storage (Lovelock and Duarte 2019). As there is no vascular vegetation, the primary autochthonous C in tidal flats is biofilms. Thus, quantifying the amount of C in tidal flats from biofilms will improve our understanding of this potential carbon sink.

4.5 Future directions

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

Furthermore, there is a need to conduct more laboratory experiments including additional factors, such as grazing. Biofilms are an important component of the diet of grazing macrofauna in coastal ecosystems (Daggers et al. 2020). However, while we demonstrate that high sedimentation promotes biofilm C accumulation, little work has been done on how sedimentation rate affects grazers. In sediment-addition restoration projects, snail growth rates were highest with intermediate sediment addition (Stagg and Mendelssohn 2012). It is unclear whether the higher sedimentation rates will allow more of the biofilms to be buried and protected from grazing, or if bioturbation could increase and overall grazing may increase. The strength and direction of this feedback will impact how much biofilm carbon is able to be stored in salt marsh sediments in real settings.

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

5 Conclusions

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

All analyses validate our hypothesis that higher sedimentation rates increase biofilm C accumulation. A sedimentation threshold above which biofilms cease to grow and to accumulate carbon might still exist, but it would be relatively high (i.e., >20 mm/yr). The results of this experiment represent the upper bounds of organic carbon accumulation by biofilms, as they were grown under favorable conditions over a short timescale. Further experiments should quantify the role of grazing in limiting biofilm C accumulation, and how this effect changes as a function of the sedimentation rate.

Declarations

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Conflicts of interest

The authors declare that they have no conflicts of interest

Availability of data and material

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

Code availability

Not applicable

Authors' contributions

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

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Figure Captions:

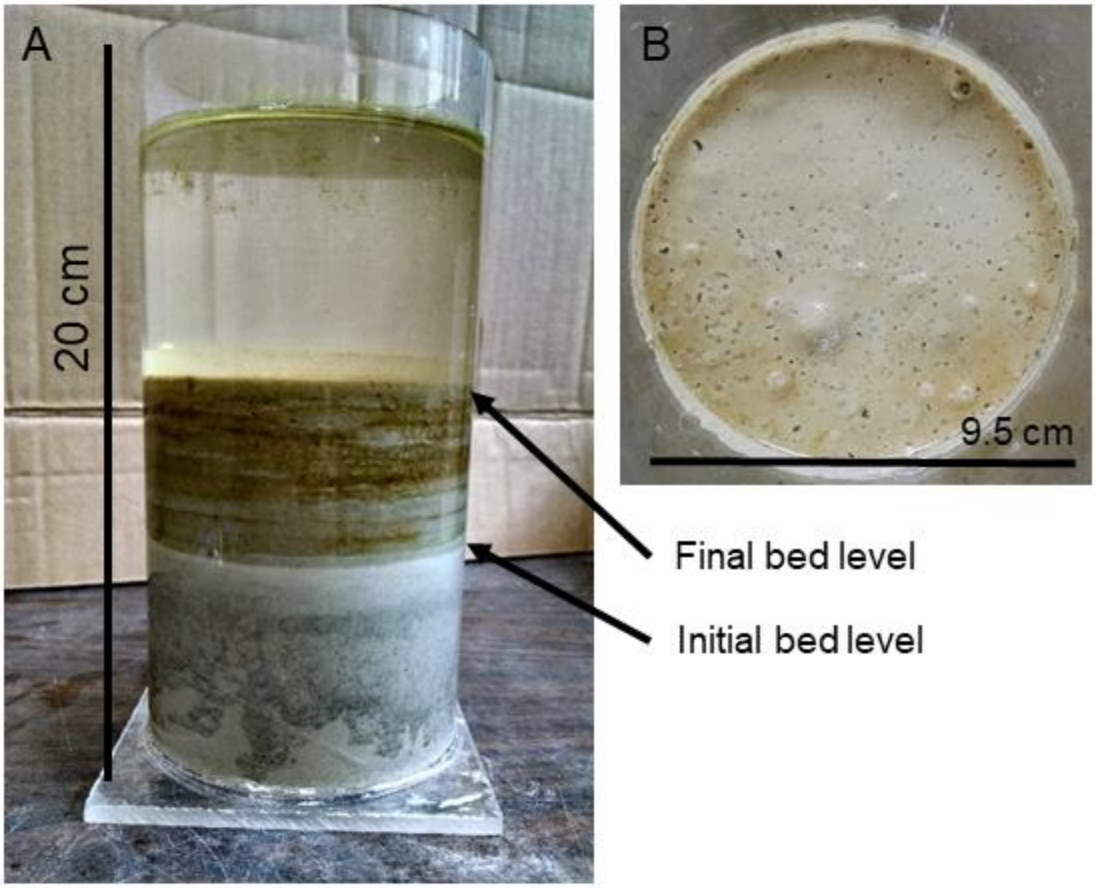

Figure 1: Plastic cylinders used for the experiment after 11 weeks of growth. (A) Side view showing the vertical accumulation of sediments. The parallel layers in the sediment, starting at about half of the sediment column, are from individual sedimentation events and subsequent growth of biofilm. (B)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

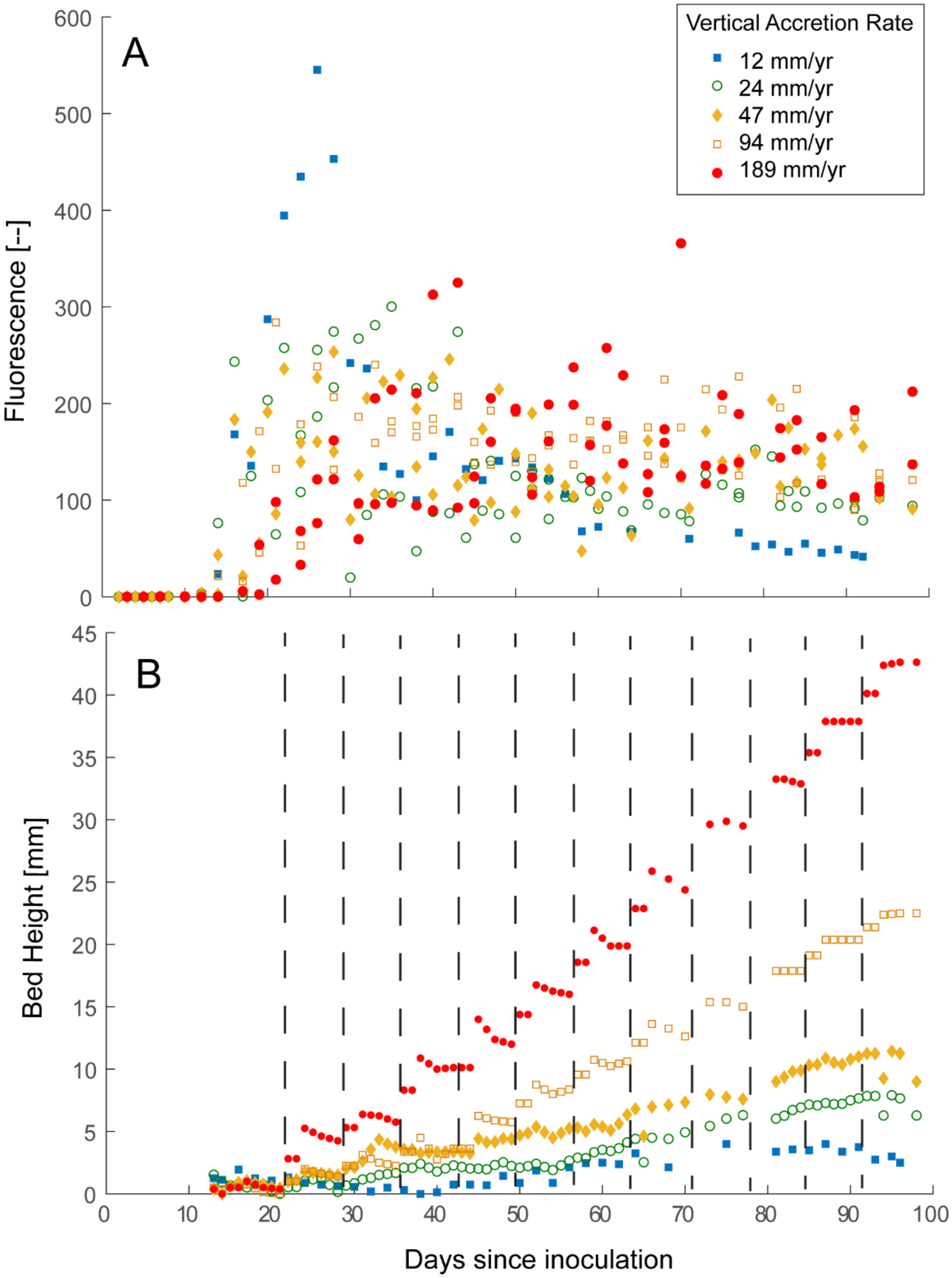

Figure 2: Monitoring of fluorescence (A) and bed height (B) over the 11-week experiment. Colors represent the five different sedimentation rates (see Table 1). Fluorescence measurements are consistent across treatments. Bed height measurements were corrected for the consolidation of the initial bed over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

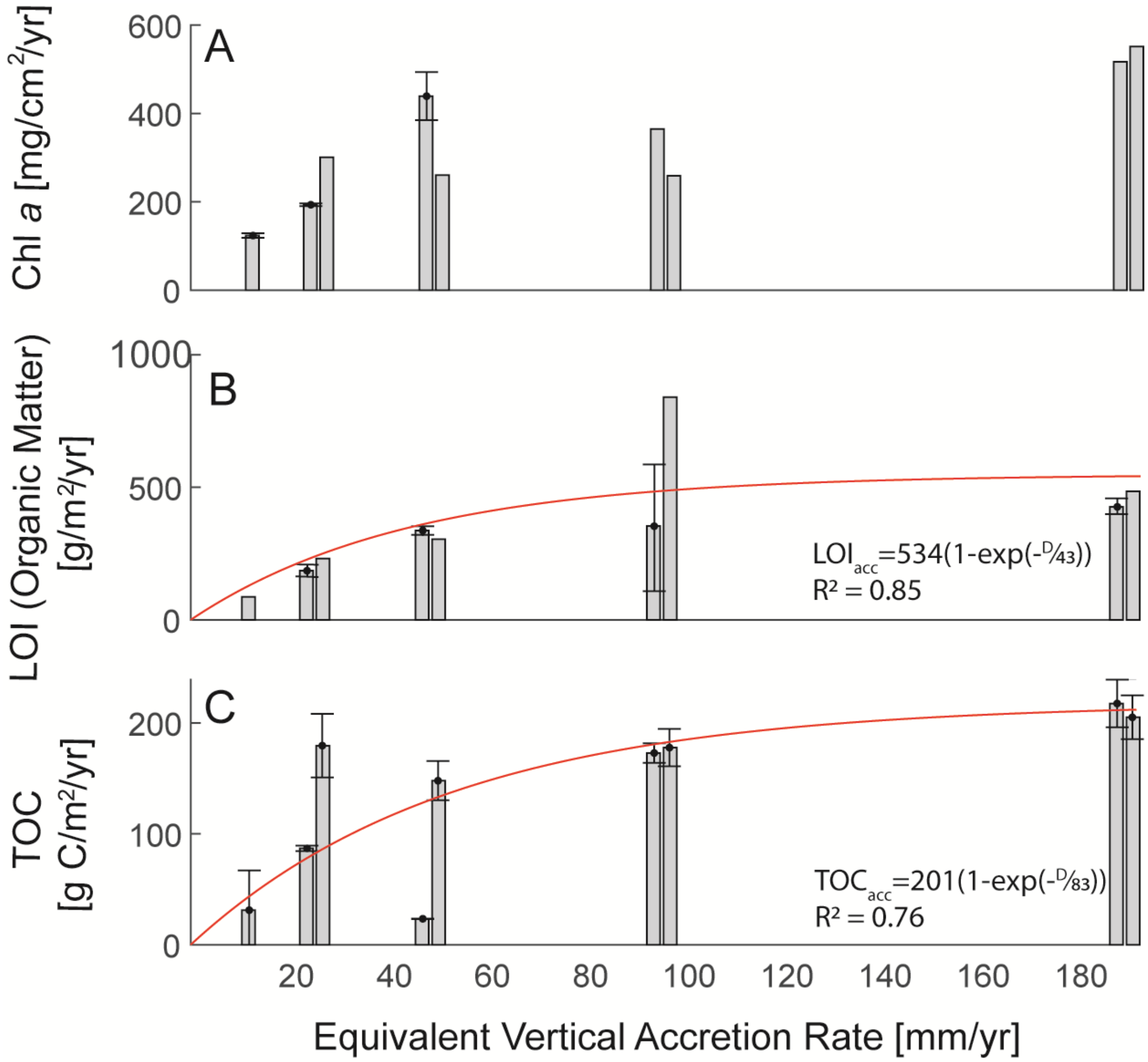

Figure 3: Chl *a* (A), LOI (B), and TOC (C) values for the content of the containers following the 11-week growth experiment for each of the five growth rates. All three metrics show an increase with equivalent vertical accretion rate. Duplicate bars indicate separate trials, standard deviations show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

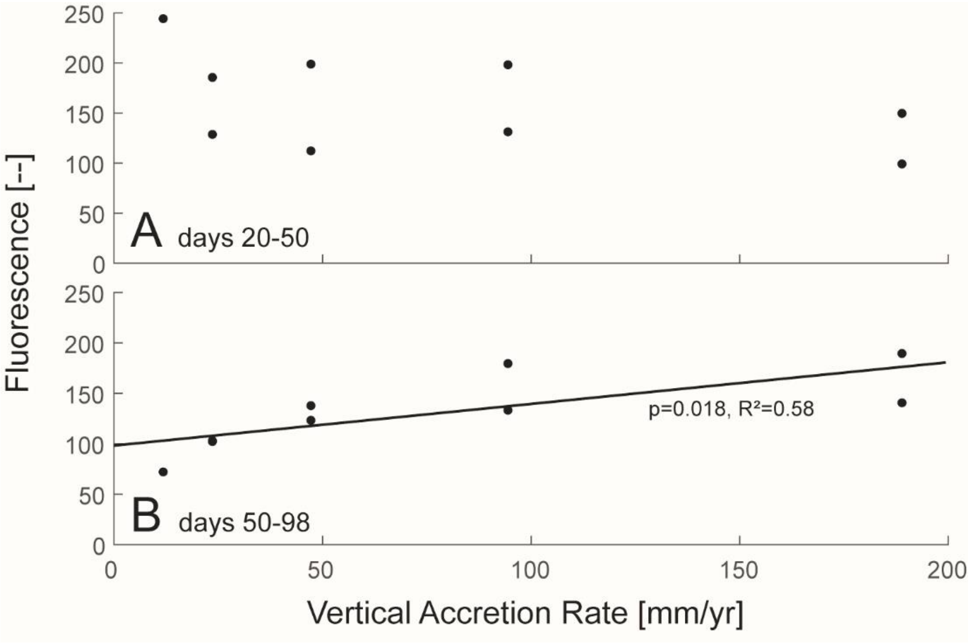

Figure 4: Average PAM fluorescence value by sedimentation rate for days 20-50 (A) and 50-98 (B). There was no statistically significant relationship between fluorescence and sedimentation rate in the beginning of the experiments, but in days 50-98, there was a significant linear relationship (y=0.41x+98,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

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

Sedimentation Rank	Mass added each week (g)	Sedimentation Rate (g/cm^2/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