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Effects of Increased Sedimentation on Carbon Accumulation of Salt Marsh Benthic Biofilms

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Effects of Increased Sedimentation on Carbon Accumulation of Salt Marsh Benthic Biofilms

by

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I. Abstract

A comprehensive understanding of the processes of carbon accumulation, storage, and eventual sequestration is required to look for ways to capture and remove carbon from the atmosphere and mitigate effects of climate change. Coastal wetlands store significant amounts of carbon as they accrete vertically, accumulating mineral and organic sediments. Most studies of salt marsh carbon storage attribute organic carbon production entirely to plants and exclude the unique processes associated with photosynthetic microbial communities. These typically diatom-based benthic photosynthetic microbial communities, or biofilms, contribute to the total carbon fixation within these ecosystems, but the magnitude of this is unknown. This project attempts to quantify the ability of biofilms to fix atmospheric carbon dioxide into organic carbon under varying rates of sedimentation. We hypothesize that biofilm carbon accumulation increases with increased sedimentation up to a point in which sedimentation is too high for the biofilm to maintain production. Artificial mud beds were inoculated with microbes sampled from a salt marsh in Cocodrie, Louisiana. The microbes were grown under ideal nutrient conditions and were subjected to varying rates of weekly sedimentation to analyze the effect of burial on carbon accumulation. After three months, the mud surface was extracted in layers, homogenized, and analyzed for chlorophyll-a, organic matter, and total organic carbon accumulated in the sediments. The amount of organic carbon produced in the mud was higher in trials subjected to higher rates of sedimentation up to extremely high rates of sedimentation.

II. Introduction

The current concentration of atmospheric carbon dioxide is higher than the concentrations inferred using proxies for the past 800,000 years. Atmospheric carbon dioxide has been increasing rapidly and steadily since 1958 due to land use change and increased

industrialization ("The Keeling Curve," 2017). As international treaties seek to mitigate the effects of global climate change, there have been increased efforts to understand and ultimately maximize carbon storage in terrestrial systems (Watson et al., 2000). While different ecosystems have varying ranges of carbon storage potential, coastal salt marshes have one of the highest ranges with rates as high as 1,700 $g/m^2/yr$ (Mcleod et al., 2011). This makes salt marshes a high priority for research of carbon cycling and storage.

The high productivity of salt marsh plants, benthic microbial communities, and phytoplankton fixes carbon dioxide into organic compounds which can accumulate within the sediments over time. The natural sedimentation and vertical accretion of salt marshes allows for the burial of organic carbon produced. This burial protects the carbon from consumption by deposit feeders and slows its rate of decomposition by microbes allowing for carbon accumulation and storage over time (Connor et al, 2001). A study published by Connor et al. (2001) evaluating the spatial and temporal patterns of carbon accumulation in Bay of Fundy salt marshes noted that carbon accumulation increased with higher levels of suspended sediment supply and thus the local sedimentation. The authors specifically acknowledged the role of benthic microalgal communities in the increased carbon accumulation and in marsh surfaces before burial. Our research project aims to explore this further by testing the effects of sedimentation rate on the carbon accumulation of benthic microbes.

Salt marsh microphytobenthos, referred to as "biofilms" in this paper, are typically found as patchy mats on the mud surface composed of cyanobacteria, diatoms, and extracellular polymeric secretions. These organisms are photosynthetic and therefore primarily in the top several millimeters of the mud surface with limited but present vertical motility (MacIntyre et al., 1996). The net primary production of biofilms may be greater than 90% of their gross primary

production (Pomroy, 1959). Although the organic carbon produced by biofilms is overturned relatively quickly compared to marsh grasses, the sheer amount may be able to contribute greatly to the carbon accumulation and burial within salt marsh sediments (Connor et al., 2001).

III. Research Questions

This research has two main objectives: 1) confirm the carbon accumulation ability of benthic biofilms under ideal conditions and 2) test if the rate of this carbon accumulation depends on the rate of mineral sedimentation. Here we assume that ideal conditions include sufficient nutrients and light, constant temperature, regular sedimentation, and no predation. We hypothesize that the increased sedimentation rates will increase the organic carbon stored in the sediments by biofilms. However, we also hypothesize that there exists a maximum sedimentation rate at which this trend would reverse due to the inability of the biofilms to grow through the new sediments. Ultimately there may be a rate of sedimentation that would prevent biofilm growth and therefore prevent organic carbon accumulation within the sediments. To test the second part of this hypothesis, we use a relatively wide range of mineral sedimentation rates (0.786-12.574 $g/cm^2/yr$) for our treatments.

IV. Methods

A. Laboratory Setup

This experiment was carried out over two different three month trials to test the effects of five different sedimentation rates. Artificial sediment beds were constructed by settling a homogenized bentonite mud slurry (125 g/L seawater) into plastic jars that were 20 centimeters tall with an inner diameter of 4.75 centimeters (Figure 1). Three jars were used for each sedimentation rate (except for the lowest rate for which we used only two) and two jars were used to monitor mud consolidation over a three-month period for a total of 16 jars over the

course of the entire experiment. The jars were placed on orbital shakers (orbital diameter of 0.5 cm and 100 RPM) for the duration of each trial to aid in settling, gas exchange, and water mixing.

After 48 hours of settling, 90% of the water column was exchanged using a peristaltic pump. This water motion was not strong enough to resuspend sediments. The replacement water was enriched with nutrients based on the f/2 Medium (Bigelow Laboratory for Ocean Sciences). We used a diluted f/2 Medium, a common enriched seawater medium designed for growing coastal marine diatoms, to scale our nutrient concentrations to 10 μM N. We then inoculated each jar with a one milliliter solution of diluted field sample of biofilm collected from a salt marsh in Cocodrie, Louisiana. The jars were placed under growth lights on a 12-hour light/dark cycle. As a control, one jar for each sedimentation rate and the consolidation jars were all treated with three drops of bleach and kept dark to prevent any biofilm growth in those jars. The water column of each jar was exchanged with new water medium on the same day of every week during each trail.

Two weeks after inoculating the jars with biofilm, we began adding inorganic (bentonite) sediments to the jars during the weekly water exchange. The weekly mass of sediment used for our five different rates of sedimentation is shown in Table 1. Each sedimentation rate uses double the mass of sediment from the next lowest rate. The sediment weighed out for each jar was mixed with about 100 mL of the water medium and added dropwise to the respective jars during the regular water exchange with the peristaltic pump.

B. Monitoring

Biofilm growth was monitored using a pulse-amplitude-modulation (PAM) fluorometer aimed 0.5 cm above the mud surface as a measurement of photosynthetic potential of the surface

microbial mat. Thirteen measurements were taken in each jar and were averaged. The measurements were taken several times each week and were used to monitor overall biofilm health. The PAM measurements show that the jars experienced relatively similar and consistent growth patterns between all jars throughout the duration of each trial (Figure 3).

The change bed height was also monitored throughout the trials. The distance from the bottom of the jar to the surface of the mud was measured on four equidistant points around each jar. These points were then averaged for each jar to provide a value for of the mud surface height for each jar. The change in the heights of the mud beds was corrected for consolidation using the average of the height measurements of the consolidation jars. The relative change in height from initial height before sedimentation to the end of the experiment for each sedimentation rate is shown as measured vertical accretion rate (mm/yr) in Figure 4. We calculated the bulk density of the new sediment layers based on the change in vertical height of the mud to then determine an equivalent yearly vertical sedimentation rates (mm/yr) for each of the mass-based sedimentation rates (Table 1). The difference between the measured vertical accretion and equivalent yearly sedimentation rates reflects the range of error in the sedimentation method of the experiment.

C. Sampling and Analysis

The experiments were concluded after 11 weeks of sedimentation. Eight days following last sedimentation, the sediment in each jar was separated into two three-centimeter layers (0-3 cm and 3-6 cm) by measuring from the surface of the mud bed (Figure 2). Each layer was extracted separately, homogenized, and subsampled for analysis. One milliliter mud samples were used to measure for Chlorophyll-a content (EPA Method 445.0). In the first trial, samples were run in triplicate; in the second experiment one sample was used for each sediment layer.

To determine organic matter and carbon content of each layer of each jar, we used loss on ignition (LOI) and total organic carbon (TOC) methods, respectively. To measure LOI and bulk density of each layer of each jar, we measured out and dried approximately 23 mL of mud from each layer and dried the samples in an oven at 60 ℃ for 48 hours. The samples were crushed with a mortar and pestle, dried for another 24 hours, and weighed. The crushed samples were then burned in a furnace at 550 ℃ and weighed to calculate the change in mass. The LOI for samples without biofilm was relatively consistent and used to correct for structural water loss of the clay samples (Hoogsteen et al., 2015). We subtracted the average LOI of samples without biofilm (from our control jars) from the LOI of all samples. The corrected LOI and the average bulk density of each layer of each jar were then used to calculate the total mass of organic matter in each layer of each jar. The mass of organic matter of the two layers of each jar were then summed to provide the total mass of organic matter per jar which was then used to determine the organic matter accumulation over time.

To measure total organic carbon, a subsample of sediment was treated with 6M HCl fumigation and analyzed for organic carbon using a COSTECH 1040 CHN Elemental Combustion Analyzer. An average of triplicate measurements of the concentration of organic carbon (mg/g) was converted to mass (g) of total organic carbon present in each layer of each jar. The mass of organic carbon in each of the two layers of each jar were summed to determine the mass of total organic carbon present in each jar with biofilm. This value was then used to determine the carbon accumulation over time.

V. Results

A. Chlorophyll-a

Sediment chlorophyll-a concentrations have been used to estimate biomass of

microphytobenthic communities (MacIntyre et al., 1996). Chlorophyll concentrations for this experiment represent only milligrams of chlorophyll-a, not pheophytin pigments, per one milliliter samples used for the analysis. For triplicate samples, the average chlorophyll concentration (mg/g) of those samples was used to determine the total chlorophyll content (mg) each layer of each jar. For all other samples, a single measurement of chlorophyll concentration was used to determine the total chlorophyll content of that layer because the percent deviation between the triplicate samples of each sedimentation rate ranged from <1% to 15% and any chlorophyll measurements for layers for jars without biofilm were negligible. The chlorophyll content (mg) of the two layers of each jar was added to give a total measure of chlorophyll in each jar. The total mass of chlorophyll was then converted to chlorophyll per area (mg/cm^2).

Figure 5 shows the chlorophyll content per area (mg/cm^2) for each jar of each sedimentation rate. There is a prevalent trend of increasing sediment chlorophyll content with increased sedimentation rate. The lowest sedimentation rate (equivalent vertical sedimentation rate of 12 mm/yr) contained approximately 26 mg/cm^2 while the average chlorophyll content for jars of Rate #5 (189 mm/yr) was over four times greater at approximately 113 mg/cm^2. It should be noted, however, that the weekly mass of sediment added for the highest rate is nearly 16 times greater than the weekly amount of sediment added for the lowest rate. The chlorophyll concentrations measured in this experiment are one to two orders of magnitude larger than many field measurements of surface chlorophyll concentrations therefore these values should be viewed as upper limits of biofilm production under ideal conditions (MacIntyre et al., 1996). *The increase in chlorophyll concentration per area with increased rates of sedimentation suggest that sedimentation promotes biofilm primary production.*

B. Organic Matter Loss on Ignition

Loss on ignition analysis has been used to estimate organic matter content in soils (Hoogsteen et al., 2015). The total mass loss on ignition for each jar was used to estimate the amount of organic matter that would be stored per meter squared per year in the sediments (under ideal conditions). Figure 6 shows the organic matter accumulation estimates for each jar of each sedimentation rate. As the sedimentation rate increases, more organic matter is accumulated in the sediments. Although the average organic accumulation of Rate #5 as less than that of Rate #4 (due to an extremely high loss on ignition of trial 2 of rate 4), the average organic accumulation of Rate #5 (456 $g/m^2/yr$), was over five times greater than the organic accumulation of Rate #1 (86 $g/m^2/yr$). While the organic matter accumulation between different sedimentation rates shows similar trends to the chlorophyll concentrations, loss on ignition analyses have been criticized to overestimate the organic content of sediments. Therefore, it is best to confirm results with a total organic carbon analysis (Veres, 2002).

C. Total Organic Carbon

Various equations attempt to translate the results of LOI to organic carbon content. A commonly accepted relationship, however, suggests that LOI values should be approximately twice the value of organic carbon (Veres, 2002). Total organic carbon analysis is used to determine the chemical components of the organic content of a sediment sample (Schumacher, 2002). Total organic carbon was measured for each layer of each jar with biofilm. The total organic carbon for each jar was used to calculate the potential organic carbon accumulation per square meter per year in the sediments (under ideal conditions). Figure 7 shows the organic carbon accumulation potential for each sedimentation rate from each trial. The error bars in the graph represent the standard deviation between the samples of that jar. Although there is greater variability in the total organic carbon analysis, the average organic carbon accumulation is higher

for higher rates of sedimentation. The average carbon accumulation of Rate #5 (211 g/m²/yr), is nearly seven times greater than the average carbon accumulation for Rate #1 (31 g/m²/yr). It can be noted that the highest and lowest values are also in a range that would be approximately half the values of organic matter accumulation calculated from LOI analysis, corroborating the methods cited in Veres (2002).

VI. Discussion

The increased concentration of organic matter with increased sedimentation suggests that biofilm undergoing rapid burial may allow for greater carbon accumulation. The consistent trend between the chlorophyll, LOI, and total organic carbon analyses confirm that the biofilm grown in this experiment can maintain itself and thrive under sedimentation rates nearly sixteen times the natural rate along the Gulf Coast (Cahoon et al., 2010). The ability these microbes to increase carbon accumulation under higher rates of sedimentation may be aided by their vertical migration (up to 5 mm) within sediments using extracellular polymeric secretions and their reproduction as they fill in the new available space of the new sediments above (Pinckney and Zingmark, 1993). If there are available nutrients and sufficient light penetration, biofilm growth can continue and allow for higher rates of carbon accumulation within the sediments.

In natural environments, organic carbon is consumed, decomposed, and released from sediments at varying rates (MacIntyre et al., 1996). Due to limitations of this experiment, we cannot isolate carbon accumulation from decomposition and we exclude bioturbation and predation. Not all of the organic carbon produced by biofilms would be stored within the sediments in a natural environment. We also cannot account for seasonal growth variability that would be occurring in a natural salt marsh. While our carbon accumulation rates are high, they represent upper limits of the ability of biofilms to produce and accumulate organic carbon within

sediments.

VII. Conclusion

The chlorophyll concentrations, LOI analysis and total organic carbon analysis all show a trend of increased biological production and organic concentration within the sediments with increased sedimentation rates. This is consistent with findings from Connor et al. (2001). This experiment demonstrated increased production with very high rates of weekly sedimentation and thus did not discover the proposed sedimentation maximum in which organic matter accumulation would begin to decrease within the limits of the sedimentation rates evaluated (up to 189 mm/yr). The results of this experiment are meant to represent the upper bounds of biofilm carbon accumulation potential under ideal salt marsh conditions over a short timescale.

VIII. References

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IX. Tables and Figures

Rate Number	Weekly Mass Added (g)	Sedimentation Rate ($g/cm^2/yr$)	Measured Vertical Accretion Rate (mm/yr)	Equivalent Vertical Sedimentation (mm/yr)
1	1.069	0.786	5.925	11.811
2	2.137	1.572	22.516	23.612
3	4.273	3.144	40.885	47.222
4	8.547	6.287	104.286	94.444
5	17.093	12.574	200.276	188.889

Table 1: Sedimentation rates used for weekly addition. Measured vertical accretion rate was calculated by dividing the difference in initial bed height and final bed height by time. Measured change in bed height over the experiment was also used to calculate the bulk density of the new sediment layers (0.6 g/cm^3) and then the equivalent vertical sedimentation rate achieved by each mass-based sedimentation rate.

Figure 1: Photograph of jar for sedimentation rate 5 after 11 weeks of sedimentation with biofilm.

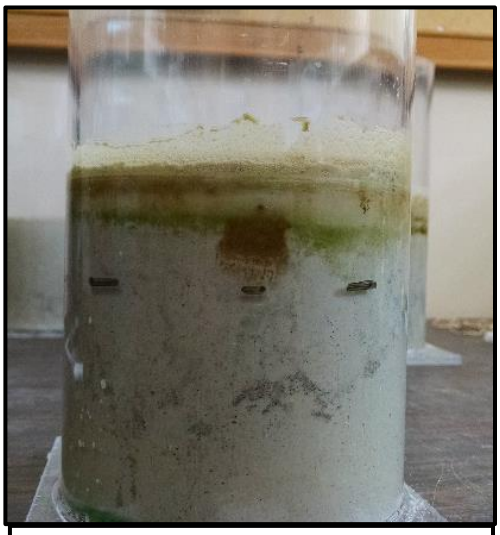

Figure 2: Photograph of jar for sedimentation rate 3 after 11 weeks of sedimentation with biofilm. Black dashes indicate the top layer (0-3 cm) to extracted for sampling and analysis.

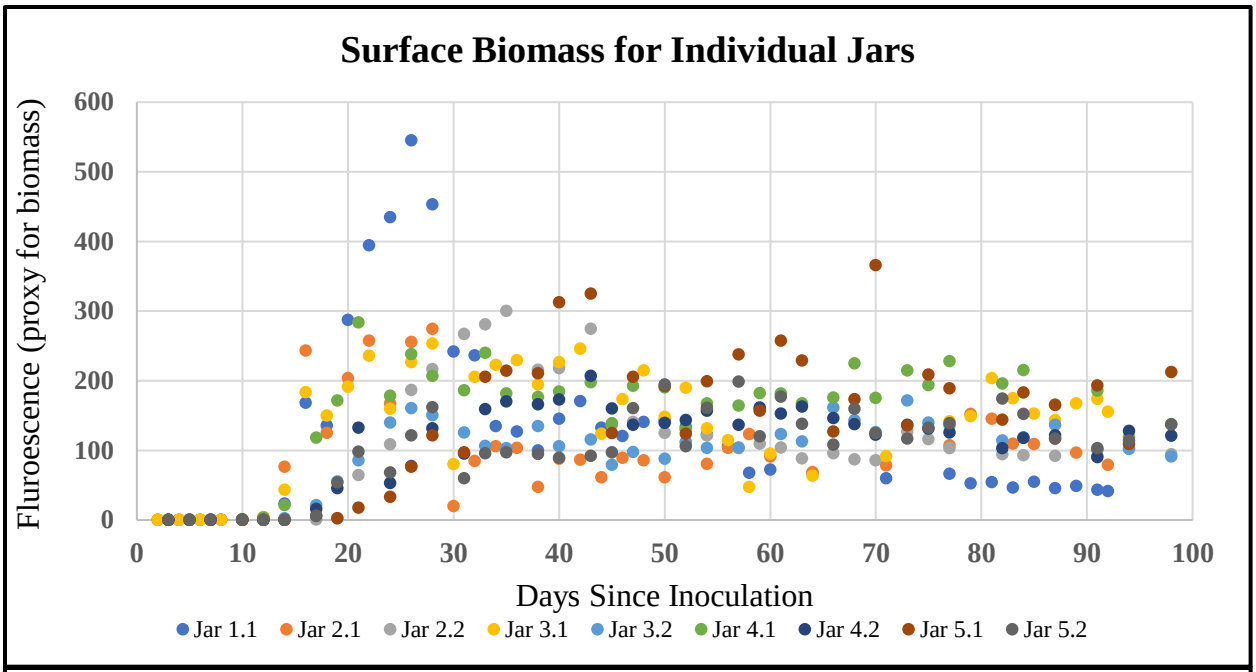

Figure 3: This graph displays the average pulse amplitude modulation (PAM) fluorescence records for individual jars during experimentation. Thirteen individual measurements were averaged for each jar for each day of monitoring. Each color represents an individual jar for the experiment.

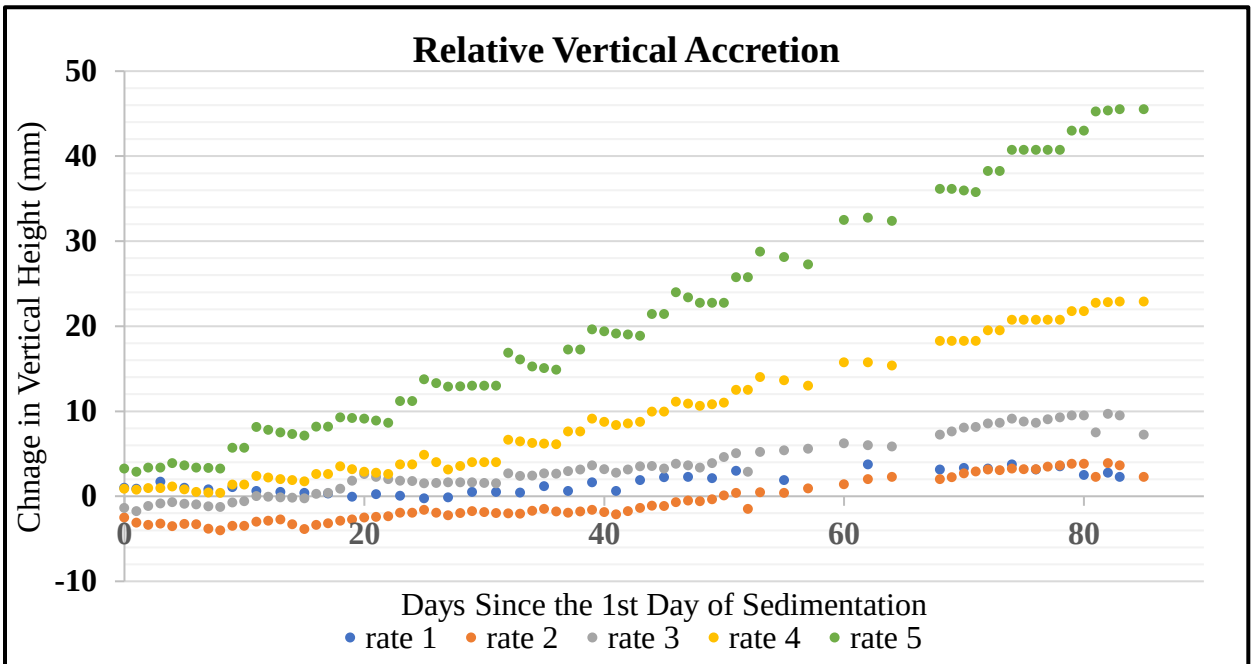

Figure 4: This figure shows the relative vertical accretion (mm) observed in each jar beginning with the first day of sedimentation and ending with the day of sampling. Each color represents that average change for a different sedimentation rate. The changes in the heights were normalized to the experimental consolidation curves.

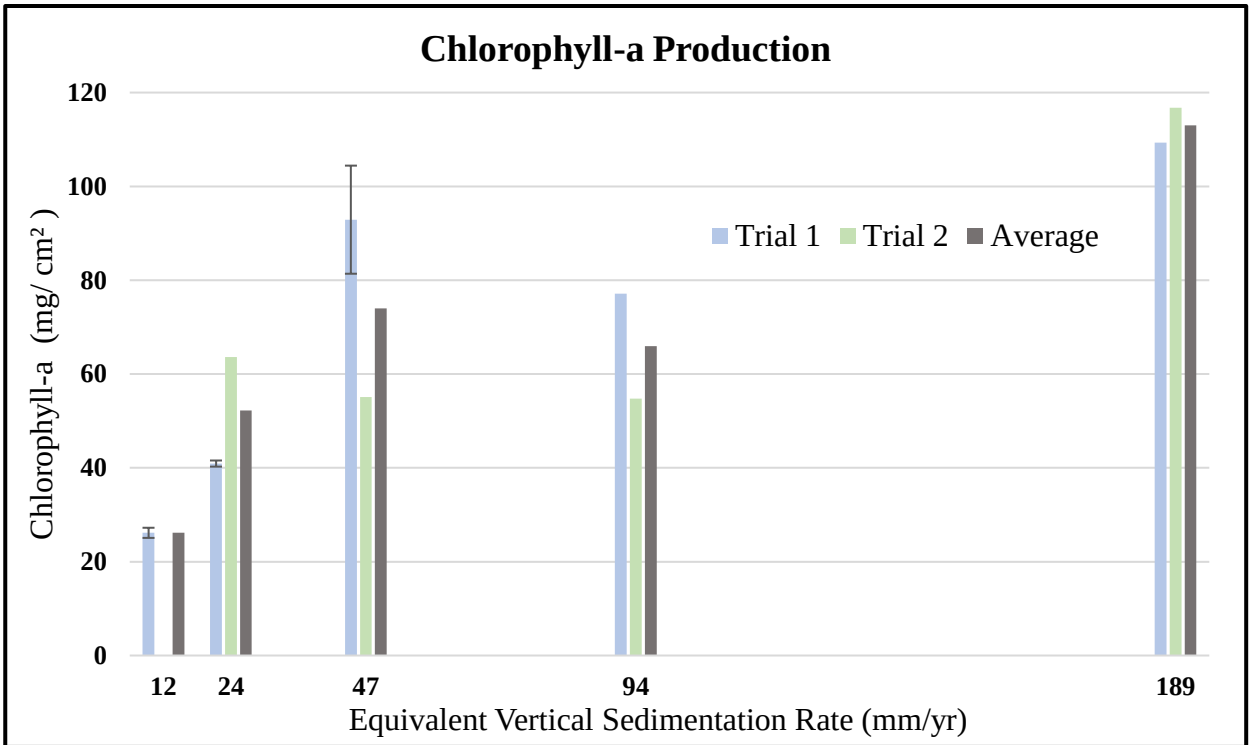

Figure 5: Chlorophyll-a (mg) per centimeter squared for each jar of vertical accretion rate. The blue and green bars represent replicate jars for each rate. The grey bars show the average of the two replicates. The error bars represent the standard deviation between triplicate measurements for those jars.

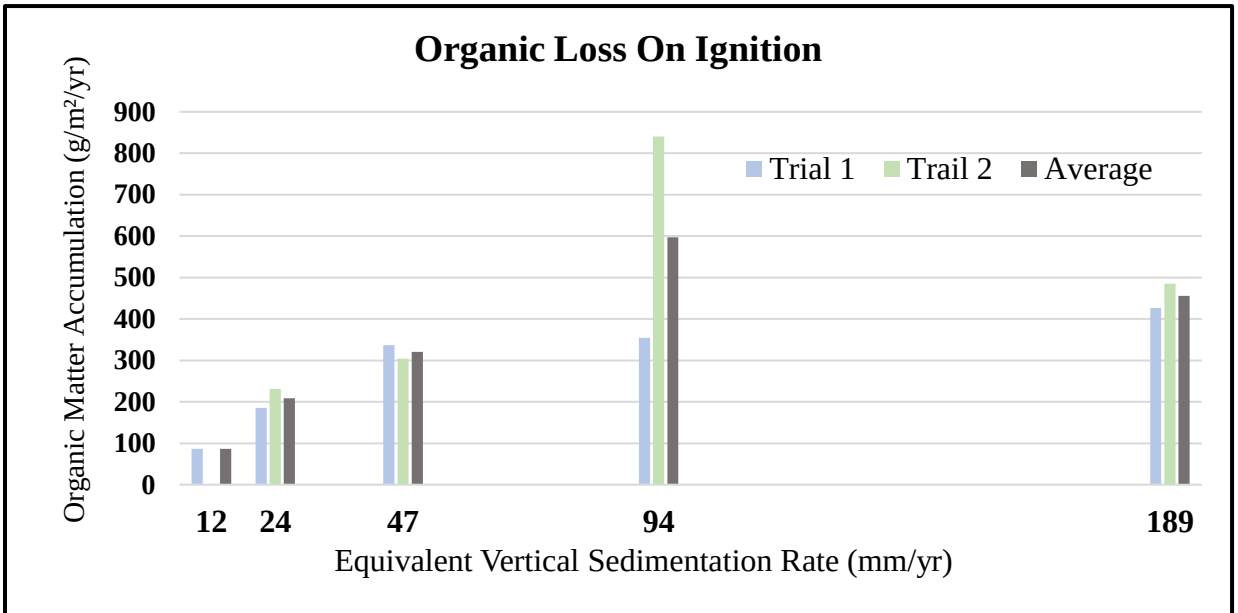

Figure 6: Organic matter accumulation (g/m²/yr) calculated from mass lost on ignition at 550°C for each jar of each vertical accretion rate. The blue and green bars represent replicates for each accretion rate. The grey bars represent the average organic accumulation of the replicate jars.

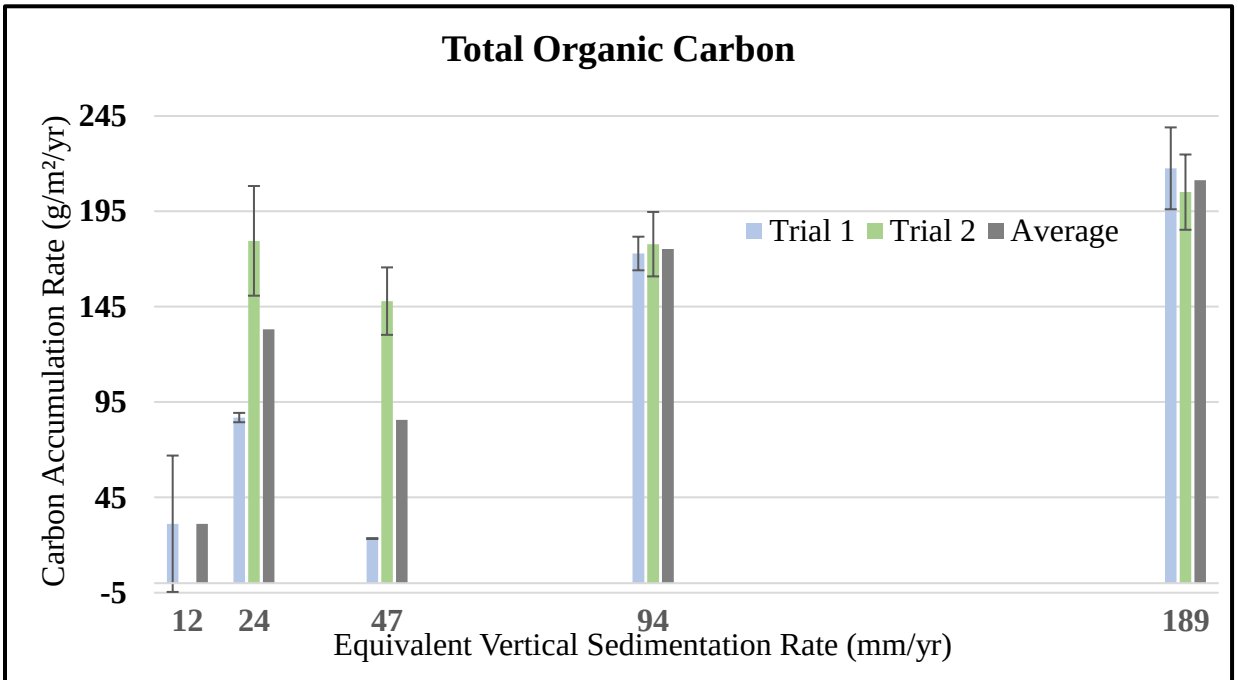

Figure 7: Organic carbon accumulation (g/m²/yr) calculated from total organic carbon concentration of each jar of each vertical accretion rate. The blue and green bars represent replicates for each accretion rate. The grey bars represent the average organic accumulation of the replicate jars. The error bars represent the standard deviation between triplicate measurements for each jar.