Temperature effects on the carbon content of marsh sediment measured through time using a CHN elemental analyzer and loss on ignition

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Abstract

Within the last decade, interest in conserving and restoring saltmarsh for their ability to rapidly sequester, and bury carbon has increased; however, the methodology for carbon analysis in marsh cores has not been standardized. Unless marsh sediment is dried or frozen after sampling, the carbon fraction is subject to microbial degradation through time. In this paper, we examine how saltmarsh-core storage temperature affects the carbon content of subsamples through time. We also compare CHN elemental analysis to loss on ignition (LOI) for measuring marsh carbon and organic matter, respectively. We used a marsh core from French Creek, NC, U.S.A, and extracted two 10-cm long sections from the upper and lower part of the core. These sections were mechanically homogenized and placed in 3 different temperature locations including: 1) a freezer at -20°C, 2) a refrigerator at 1.5°C, and 3) an indoor lab at 24°C. We subsampled each core section 9 times over 128 days for analysis of percent carbon (elemental analyzer) and percent organic matter (LOI). The upper part of the core had an initial value of 28.5 ± 2.2 % organic carbon and 57.1 ± 0.2 % organic matter, and the lower part had an initial value of 39.2 ± 5.9 % organic carbon and 77.6 ± 0.2 % organic matter. These results are in line with average carbon and organic matter content for saltmarshes in the area. We found that the carbon content of saltmarsh cores stored at temperatures below 24°C does not steadily decrease at a level below the resolution of our method (average standard deviation of 1.9%). Similarly, no decrease in organic matter below 0.45% (average standard deviation of the LOI analyses) was observed. CHN analysis introduces more uncertainty in measurements than LOI, which is likely due to incomplete sample homogenization, which is difficult to overcome. The LOI method uses an order of magnitude larger subsample than an elemental analyzer and when percent organic matter is converted to percent carbon using the conversion equation from Craft et al. (1991) it

allows for a more accurate and precise measurement when complete sample homogenization cannot be confirmed.

Introduction

Biotic systems in the ocean have been the principal drivers of carbon sequestration throughout Earth's history (Falkowski and Isozaki 2008), with highest carbon burial rates being measured in coastal ecosystems (Cebrian 2002). Vegetated coastal habitats (i.e. blue carbon habitats) cover less than 0.5% of seafloor bottom (Halpern et al. 2008) but are disproportionately valuable carbon sinks. In particular, mangroves (Bouillon et al. 2008; Nellemann et al. 2009; Donato et al. 2011), seagrasses (Cebrian and Duarte 1995) and saltmarshes (Cebrian 2002; Duarte et al. 2005) are strongly autotrophic environments that fix CO₂ in excess of what is respired. This excess material is buried in sediments at a rate accounting for roughly 46.9% of the ~250 Tg C buried throughout the entire ocean each year (Chmura et al., 2003; Duarte et al. 2005). Conserving and enhancing the ability of coastal environments to absorb and sequester carbon is a key component of strategies to combat anthropogenic climate change (Nellemann et al. 2009).

Saltmarshes have the highest rate of carbon burial of all blue-carbon habitats because sediment accumulation rates generally approximate the rate of relative sea-level rise (van de Plassche, O et al. 1998; DeLaune et al. 1978; Ouyang and Lee 2014). Their important role in carbon burial has led to many recent studies aimed at quantifying the carbon content of natural saltmarshes to improve assessment of the carbon reservoir (eg. Duarte et al. 2011), provide better estimates of carbon emissions from the degradation of saltmarsh, which is estimated at 25% of total global area from the 1800s (Bridgham et al., 2006; Duarte et al., 2008; McLeod et al., 2011; Pendleton et al., 2012, Theuerkauf et al., 2015), and determine the contribution that restoring and

conserving saltmarsh makes to increasing carbon storage (Burden et al. 2013). Although the focus on carbon sequestration in saltmarshes has intensified, systematic methods for carbon analysis have not been implemented. Critically assessing the accuracy of common marsh carbon-analysis methods should be a priority considering the great interest in including Blue Carbon in policy mechanisms for reducing greenhouse-gas emissions (Grimsditch et al., 2013; Luisetti et al., 2013; Ullman et al., 2013).

Challenges associated with measuring the carbon content of saltmarsh sediment mainly arises from the wide range of particle size and percent carbon of the particles. For example, saltmarsh sediment is commonly composed of clay, sand, silt, vegetation, and fine-grained organic material such as fecal pellets and decomposed vegetation. Heterogeneity of saltmarsh sediment makes reproducibility of carbon-content measurements difficult. In addition, it is commonly thought that once a sediment sample is extracted from a marsh, its carbon content will decrease through time due to microbial degradation unless the sample is frozen immediately until it can be processed. The carbon content of a marsh sample is commonly measured using a CHN elemental analyzer (CHN) or loss on ignition (LOI), and it can be argued that CHN is superior because the analytical error for CHN (0.02 %OC) is an order of magnitude lower than analytical error for LOI (2.0 %OM, Heiri et al 2001). However, CHN uses a significantly smaller sample size (6-7 mg) than LOI (200-300 mg). The mass of sediment used for CHN may not be representative of the marsh, even with homogenization. With various marsh-sample storage methods, and with some studies using CHN and others using LOI, there may be variability in carbon measurements. In this paper, we explore how storage temperature affects carbon content in a marsh core through time. This includes analyzing the upper part of the core with labile and

reactive carbon and the lower part of the core, which has more recalcitrant carbon. Then we look at how propagated error in CHN compares to propagated error in LOI.

Methods

For this study we sampled the fringing saltmarsh at French Creek, which is located on the eastern shoreline of the New River Estuary, NC, about 20 km up the estuary from the tidal inlet. We collected a 4-inch diameter core from the *Juncus roemerianus*-dominated saltmarsh approximately 4 m from the marsh shoreline. The core sampled to 80 cm depth, and did not sample below the base of the marsh. Two 10-cm sections were sub-sampled from the core to represent the younger and older parts of the marsh. The younger section was collected from 5-15 cm (upper sample), and the older section from 65-75 cm in the core (lower sample). We pushed each section through a 2 mm sieve to remove macroflora, then put the sieved section in a blender and added enough DI water (less than 30 mL) to create a vortex and obtain a smooth homogenous texture. We separated each blended subsample into three resealable bags. A bag from the upper and lower parts of the core was placed in each of the following locations: the freezer at -20°C, the refrigerator at 1.5°C, and in the lab at 24°C. These temperatures are commonly used for core storage.

The bags were subsampled 9 times from day 0 to 128 on an exponential time scale. Each sampling day we took three separate subsamples from each of the six bags and oven-dried those at 105°C. On day 0, we only subsampled the ambient storage bags, because not enough time had elapsed for the sediment temperature to change in the climate-controlled environments. After drying, the subsamples were crushed to powder using a mortar and pestle and inorganic carbon was removed via HCL fumigation for 72 hours. A Costech Analytical Instruments Elemental

Combustion System 4010 was used for CHN analysis. We also used loss on ignition (LOI) to measure the percent organic matter (OM) in subsamples with an average mass of 261 ± 145 mg (± 1 standard deviation). Following the method of Heiri et al. (2001), we heated samples to 550° C for 4 hours in a muffle furnace to ensure all the organic matter combusted. Percent organic carbon measured using CHN and percent organic matter using LOI of the upper and lower subsamples was reported as a mean ± 1 standard deviation, based on the three subsamples taken each sampling day from the climate-controlled environments.

Results

Comparison of percent OC (CHN) and OM (LOI) through time of upper and lower subsamples

The marsh core subsamples showed a distinct color difference with the upper subsample being dark brown (7.5YR 3/2) and the lower subsample being very dark brown (7.5YR 2.5/2; Munsell Color System). The initial percent OC of the subsamples, via CHN, reflects those color differences with the upper section having a lower OC content ($28.5 \pm 2.2 \%$) than the lower section ($39.2 \pm 5.9 \%$). The percent OC for the subsamples stored in the freezer were generally consistent through time. There was no significant difference in percent OC between time zero and time 128 days for the upper subsample (P= 0.15) or the lower subsample (P=0.41) (Figure 1). The percent OC of the upper subsample is not significantly different between time zero and time 128 days for the ambient (P=0.13) treatment, but is significantly different for the refrigerated treatment (P= 0.05; Figure 2). The only significant negative relationship between % OC and time, was for the refrigerated treatment (r^2 =0.47; P=0.04). The percent OC of the lower subsample neither showed a significant relationship with time nor significant differences between the earliest and latest analysis times for any of the treatments. The percent OM of the upper and lower subsamples for all treatments showed no significant differences between time 0 and time 128 days (Figure 2).

LOI compared to CHN

Craft et al (1991) published a conversion equation for calculating percent OC based on percent OM from LOI using 250 marsh samples from 10 different *Spartina alterniflora* dominated marshes in North Carolina. With an r² value of 0.990 and the high number of samples and sample locations, this conversion is significant and widely used (e.g. Chmura et al 2003, Windham et al 2003). The %OM results from LOI plotted against %OC results from CHN (Figure 3), shows that the average of the points for the upper section falls below Craft's equation, while the average of the points for the lower section falls within the curve. The majority of the variation in both sections is along the vertical axis (%OC). The standard deviation for OC ranges from 0.16 to 8.64 %OC and the standard deviation for OM ranges from 0.04 to 2.01 %OM (Figure 4).

Interpretation and Discussion

The subsample stored in the freezer showed no significant change through time, as expected, because there should be no microbial activity to alter the OM or OC content at this temperature. The only treatment that showed a significant decrease in OC through time was the refrigerated upper section of the core. We disregard the significance of that result, which is supported by three observations: 1) the significance of the difference between time zero and time 128 days and the regression line was low, 2) the OM content of those same subsamples showed no significant decrease with time, and 3) the ambient subsample for the upper section of the core showed no significant decrease in both OC and OM with time. If the OC content of the refrigerated upper core section truly decreased over the 128 day period, then we would expect the ambient treatment to have shown a similar, if not more significant, trend because the microbial degradation of organic carbon would increase with increasing temperature, but that was not observed. We interpret the significant decrease in OC for the refrigerated upper section as being the product of a low sample size. All other results show that the percent of OC in each sampled marsh interval remains generally constant over 128 days (within measurement variance). As long as a core is stored within the -20°C to +24°C temperature range and subsampled for carbon within 4 months, the measured organic carbon content should represent the marsh when it was initially sampled.

In analyzing the reproducibility of organic carbon measurements in saltmarshes, we found that CHN analysis has a larger standard deviation in subsampling (1.99 %OC) as compared to LOI (0.45 %OM). We attribute the large standard deviation of the CHN results to the small sample mass analyzed with that method and incomplete homogenization of the sample. There may be hotspots or coldspots of carbon concentrations within individual core samples. We define hotspots and coldspots of carbon as being small subsamples that have a higher and lower concentration of OC than the mean OC content of the larger core interval, respectively. Saltmarshes are composed of a variety of different types of sediment including organic material and inorganic material of varying sizes, hardness, and texture making homogenization of a subsample difficult. Although we homogenized the marsh using the blender and a mortar and pestle, it is possible that small sediment components (<15mg of sediment) are OC hotspots and

coldspots. For example, after four days the CHN analysis of the upper core section ambient treatment returned two results with similar OC percentages (24.8 %OC and 25.2 %OC), and a third result that was about 10% higher (35.1 % OC), which must have subsampled an OC hotspot. When analyzing these same subsamples for LOI, they average 56.3 % OM with a standard deviation of 0.53. To explain the large difference in precision between CHN and LOI, it is likely that the marsh core interval was not homogenized to the small-scale required for precise CHN analysis, so a larger sample size was necessary to account for the heterogeneity of the subsample, but large subsamples cannot be run on an elemental analyzer. In this experiment, we used an average of 261 mg of sediment for LOI, and measurements proved to be more reproducible. If we used the same sample size for LOI as we used for CHN, it is likely that precision would be similar or lower than the CHN measurements. Even if there was complete sample homogenization, vegetation has a much lower specific gravity than many types of siliciclastic and carbonate grains, which makes extracting a representative sample from a container for CHN analysis challenging.

When comparing CHN to LOI on Craft's curve (1991), the samples from the upper core section fell below the curve. Craft's conversation was formulated from *Spartina alterniflora*-dominated saltmarshes, while the marsh we sampled is a *Juncus roemerianus*-dominated saltmarsh. The lower section of the saltmarsh core fell on the curve, but the origin of those sediments and the flora at the time of deposition is unknown. The discrepancy between our data from the upper core section and Craft's organic carbon conversion equation could indicate that the relationship between OM and OC is somewhat dependent on marsh-grass species.

Highly cited papers for reporting global marsh carbon include Chmura et al (2003) and Ouyang and Lee (2014). Both of these papers report OC content from various published and

unpublished resources that used mostly LOI converted to OC using Craft (1991), but some used the Walkley Black dichromate digestion method or elemental analysis to determine carbon content in the marshes studied. With LOI used as the main method to determine marsh carbon concentration, these papers most likely have reproducible results. Other papers including Theuerkauf et al. (2015), and Saintilan et al. (2013) that used CHN analysis to determine marsh carbon, may have reported numbers that do not accurately represent the organic carbon content. Theuerkauf et al. (2015) used a mortar and pestle for homogenization (similar to this study), so it is possible that the reported OC measurements include hotspots or coldspots. Other methods may homogenize saltmarsh samples to a fuller extent than the blender and mortar and pestle method used here. For example, Saintilan (2013) used a vibrator mill to create a powdered sediment prior to elemental analysis, which may homogenize the marsh sediment to a fuller extent. Complete sample homogenization is difficult to achieve, important for proper direct measurement of percent carbon and other homogenization methods should be tested to see if they provide more reproducible results via elemental analysis than a mortar and pestle.

Conclusions

- Marsh core sediments may be stored in temperatures ranging from -20°C to +24°C and subsampled for organic matter analysis for at least up to 128 days after obtaining the core from the field site, and the percent carbon (± 1.99) and percent organic matter (± 0.45) content will be representative of the original marsh.
- In lieu of full homogenization, loss on ignition is preferable to elemental analyzers because of the larger sample size used. Using a mortar and pestle or similar method, full homogenization of marsh sediment subsamples is difficult, so a larger subsample

is necessary to obtain reproducible and accurate results for determining the carbon content of the marsh.

3) Craft's equation (1991) is best used for *Spartina alterniflora*-dominated marshes, and may be slightly skewed when converting to OC from OM for marshes that have a different dominant macrophyte. However, if a sample is not completely homogenized, variance associated with CHN will likely be larger than any error introduced using LOI and Craft's conversion equation regardless of the dominant marsh-grass species.

Figure 1) These graphs are a comparison of (a) percent organic carbon, and (b) percent organic matter in the upper and lower sections for 128 days from CHN analysis using the samples kept in the freezer.



Figure 2) These graphs show the percent of organic carbon from CHN analysis (a,b) and percent organic matter from LOI (c,d) in each of the 3 storage locations for the upper (a,c) and lower (b,d) sections with time on a logarithmic scale.



Figure 3) Percent OC in the cores was measured with both CHN and LOI. We plotted CHN percent organic carbon versus LOI percent organic matter. The equation graphed is Craft's marsh carbon conversion equation. The points have the most variation vertically along the y-axis, which represents CHN %OC.



Figure 4) This shows the standard deviation in percent organic carbon versus the standard deviation for percent organic matter for all treatments. The average SD from CHN is 1.99 %OC and the average SD from LOI is 0.45 %OM. A one-to-one line is plotted for reference.



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