Soil Composition and Plant Characteristics in Former Spartina Dieback Sites

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# Abstract

*Spartina* diebacks have been documented on salt marshes of the southeastern coast of the United States. Due the importance of *Spartina* to salt marsh structure and production, it is important that we understand the dynamics of *Spartina* growth and recovery from dieback so that we may better understand how anthropogenic changes affect the role of salt marsh communities in the global ecosystem. Sites classified by previous researchers as ‘dieback’ or ‘control’ sites in October 2011 were revisited in October 2015 to measure plant and soil characteristics. Former dieback sites had decreased plant cover and chlorophyll content. However, the increased organic soil carbon and water content suggests restored levels of sediment deposition. Some former dieback sites exhibited more growth than others, and these higher growth sites exhibited higher chlorophyll levels as well as more organic soil carbon. Altogether, the data suggest that even in a year of excellent growing conditions, the dieback sites were not fully recovered after four years, but even in these partially recovered states the carbon storage capacity of the soil begins to recover.

# Introduction

 Intertidal salt marshes in the southeastern United States are dominated by a salt-tolerant grass *Spartina alterniflora* Loisel (smooth cordgrass). The structure of the marsh is defined by *Spartina*, with the tall form *Spartina* on the creek bank, transitioning to intermediate form and short form *Spartina* in the slightly elevated levee marsh and high marsh, respectively (Teal 1958). Higher up on the marsh, *Spartina* is outcompeted by other, less salt-tolerant plants such as *Juncus roemerianus* Scheele (needlegrass rush). In addition to providing the physical structure of the marsh, *Spartina* is the most important primary producer in salt marshes and also causes sediment deposition (Teal 1962). Salt marshes are important “blue carbon” sinks, which are marine or coastal ecosystems capable of storing globally significant levels of carbon (Chmura et al. 2003). Unlike in a terrestrial carbon sink, carbon can be continually added to a blue carbon sink by continuous sediment deposition. This carbon can be built up over millennia, but is released on a decade time scale when salt marsh ecosystems are destroyed (Bu et al. 2015). They also act as a sink for heavy metals and excess nutrients and protect against coastal erosion (Williams, Bubb, and Lester 1994). Thus, it is important that we understand the dynamics of *Spartina* growth so that we may better understand how anthropogenic changes to the environment will affect these and other critical ecosystem services provided by salt marshes.

Over the last two decades, drastic *Spartina* diebacks have been documented on salt marshes of the southeastern coast of the United States (Alber et al. 2008). A dieback occurs when a group of neighboring plants rapidly dies off, usually occurring in multiple patches across an ecosystem. Many studies have documented the effects of top-down forces on diebacks, such as herbivory by the snail *Littoraria irrorata* and their control by *Uca*, a genus of fiddler crabs(Silliman et al. 2005; Gittman and Keller 2013) or infection by any one of a number of stem-borer species (Gaeta and Kornis 2011), as well as fungal infection, which can be mediated by *Littoraria* herbivory (Silliman and Newell 2003). Under normal conditions however, much of the *Spartina* biomass is consumed by detritivores and the distribution of *Spartina* is thought to be primarily controlled by bottom-up factors such as soil salinity, water content of soil, redox conditions, and nutrient availability (Mendelssohn and Seneca 1980). The large-scale diebacks seen on the southeastern coast of the United States have been attributed largely to unfavorable bottom-up factors, such as drought or sulfide toxicity stemming from waterlogging (Mendelssohn and McKee 1988; McKee, Mendelssohn, and Materne 2004; Alber et al. 2008).

There are few studies examining the recovery of *Spartina* after a dieback event. Some are artificial recoveries, either induced recovery by sediment-slurry addition (Tong et al. 2013) or done in a greenhouse (Brown and Pezeshki 2007). The sediment-slurry application, under some conditions, was able to restore the aboveground biomass to levels typically measured in salt marshes. However, in the same study, belowground biomass had not recovered after seven years. Dieback sites recover naturally as well, but these are difficult to study, since we do not know where, when, or how fast recovery occurs. The few studies documenting changes in plant and soil characteristics during dieback recoveries tend to occur spontaneously, such as when recovery occurs in the course of a dieback study (de Souza and Yoch 1997). However, some evidence suggests that soil at recovered dieback sites has similar levels of organic soil carbon, as measured by loss on ignition (LOI), to soil at vegetated control sites (Crawford and Stone 2014). Another study found that of a range of factors, including organic soil carbon, pore water salinity, and many others, only microbial nitrogen fixation was restored by recovery (de Souza and Yoch 1997).

My aim was to better understand the process of *Spartina* recovery after a dieback by revisiting sites classified as dieback sites four year prior and evaluating their recovery. I also recorded the changes in several soil characteristics known to be affected by *Spartina* dieback.

# Methods

## Study sites

 Twenty-two sites were selected for study from (Crawford and Stone 2014). These sites were paired such that in 2011, one of the sites was a dieback (referred to as “2011 Dieback”) and its pair was a nearby putatively healthy plot (“2011 Control”). Sites were visited within 2 h of the low tide mark (before and after). I threw a 0.25-m2 quadrat three times to arbitrary locations within approximately 3 meters of the GPS coordinates for a particular site. Within each quadrat, I counted the number of live tillers and estimated the average height of the tillers. I also visually estimated the percent cover of live vegetation within the quadrat. SPAD meters measure the light absorbance of leaves in the red and near red spectrum and use these values to generate a SPAD reading that is proportional to chlorophyll content (Konica Minolta; Biber 2007). SPAD readings were taken from 8 leaves per quadrat (Konica Minolta Chlorophyll Meter SPAD-502Plus). Opposing sides of the quadrat were marked every 12.5 cm, and the closest leaf to each mark was selected for SPAD measurement.

## Soil Sampling and Processing

 Soil samples were collected from 18 of the 22 sites (9 of each group). Soil samples were taken immediately adjacent to each quadrat. Samples were taken in the hour leading up to low tide and within two hours after low tide. Soil was removed by cutting a circle approximately 20 cm in diameter using a standard spade shovel, then slicing at an angle approximately 15 cm deep to sever the section. The core was gently lifted out of the hole and slid into a plastic bag, which was sealed and transported back to the lab.

 In the lab, soil samples were cut into cuboids. The top of the sample was just below the start of solid soil and just above the root mass to avoid the top mud layer of variable depth. Samples were cut ~6 cm deep, and 4 to 7 cm on each side. Volumes of the samples were estimated using the lengths of the sides. The mass of the sample was recorded and the sample was dried for at least 4 d at 60 ˚C. After drying, the mass of the sample was recorded. Bulk density and water content were calculated. Samples were sieved to remove plant material, then pulverized to homogenize the sample.

## Estimating Organic Soil Carbon

 Crucibles were filled with the homogenized soil samples and the mass of each sample was recorded. The samples were subjected to 500 ˚C for 4 h. After cooling in the 60 ˚C drying oven, the mass of the post-burn sample was recorded. Loss on ignition (LOI) was calculated by dividing the loss of mass during the burn by the pre-burn mass of the sample. Percent organic carbon was calculated using the relationship established by Craft et al, 1991 ((0.40 ± 0.01)LOI + (0.0025 ± 0.0003)LOI2).

To calculate the change in organic carbon from 2011 to 2015, I calculated the average organic carbon content for each site in 2011, then compared each sample from 2015 to its corresponding 2011 site. Not all sites had data for organic soil carbon from 2011. The number of 2011 samples from each site ranged from 1 to 6, with an average of 2.6 samples per site.

## Data analysis

 All data analysis was conducted in R (version 3.2.2). Data were analyzed using a one-way ANOVA test with site as a blocking factor. Two former dieback sites were covered in *Salicornia depressa* Standl (Virginia glasswort), with very few *Spartina* tillers. These data were removed.

 For analysis of growth conditions at dieback sites, I grouped the dieback sites into two growth conditions based on average percent cover at each site. “Low Growth” sites were defined by average percent cover of <40%. “High Growth” sites were defined by average percent cover >40%. If normally distributed, means (±S.E.) were reported, but median values were reported if data were not normally distributed.

# Results

Summer 2015 exhibited good growing conditions for *Spartina* and very few diebacks were observed, especially relative to normal levels (S. Pennings, personal communication). All sites, including the 2011 Diebacks, exhibited some *Spartina* growth. However, the 2011 Control sites exhibit greater *Spartina* cover than the 2011 Dieback sites (2011 Control median: 60 ± 4%, 2011 Dieback median: 35 ± 4%; p = 0.007; Fig. 2a), as well as higher levels of chlorophyll, as determined by SPAD measurements, than 2011 Dieback sites (2011 Control mean: 42.6 ± 1.6, 2011 Dieback mean: 37.1 ± 1.8; p = 0.03; Fig. 2b). Thus, while the sites have begun to recover from the dieback state, they are not yet fully recovered to the levels seen in controls.

While a previous report has shown increased bulk density as well as decreased water and organic soil carbon content in dieback sites (Crawford and Stone 2014), none of these three measurements exhibited a difference between the 2011 Dieback and the 2011 Control sites, suggesting that the soil at dieback sites have recovered in terms of these three characteristics (bulk density: 2011 Control median = 0.360 ± 0.050 g/cm3, 2011 Dieback median = 0.535 ± 0.075 g/cm3, p = 0.16; water content: 2011 Control median = 0.690 ± 0.023%, 2011 Dieback median = 0.570 ± 0.034%, p = 0.09; organic carbon content: 2011 Control median = 11.3 ± 0.8%, 2011 Dieback median = 7.12 ± 1%; p = 0.138) (Fig. 2c-e). However, the one-way ANOVA test with blocking for site revealed that bulk density, water content, and organic soil carbon are all significantly influenced by site. This suggests that the sites themselves have large variations in these three factors regardless of 2011 status, and changes due to dieback status may be masked by site location. Since Crawford and Stone (2014) used the same sites, we used their data as site-specific baselines for these three characteristics and calculated the percentage change from 2011 to 2015. There was still no change in bulk density (p = 0.100) (Fig. 2f). The water content decreased in both control and dieback sites, but the decrease was significantly less in 2011 Dieback Sites (mean = -62.5 ± 2.0%) as compared to control sites (mean = -24.5 ± 7.5%) (p <0.001) (Fig. 2g). The increase in organic soil carbon in the 2011 Dieback sites (median = 87.7 ± 43.7%) is significantly greater than in the 2011 Control sites (median = 26.5 ± 21.0) (p = 0.015) (Fig. 2h), suggesting that the 2011 Dieback sites are accruing carbon more quickly than the 2011 Controls.

 The dieback sites had a heterogeneous appearance, and it was clear that they were in varying states of recovery. To establish various degrees of recovery, I used percent cover as a proxy for biomass and therefore as an indication of the recovery process (Schrift 2006). Four of the 2011 Dieback sites clustered at 50% *Spartina* cover, while five sites clustered around 30% (Fig. 3a). These two clusters were divided into two groups, High Growth (~50% cover) and Low Growth (~30% cover).

*Spartina* leaves in High Growth sites had more chlorophyll (SPAD reading mean = 43.1 ± 3.0) than the Low Growth sites (mean = 32.4 ± 1.5) (p < 0.001) (Fig. 3b). As with the 2011 site status, the growth status had no significant effect on net bulk density (High Growth mean = 0.545 ± 0.044 g/cm3, Low Growth mean = 0.615 ± 0.106 g/cm3, p = 0.180), water content (High Growth median = 0.580 ± 0.026%, Low Growth median = 0.570 ± 0.046%, p = 0.119), or organic soil carbon (High Growth median = 6.95 ± 1.10%, Low Growth median = 7.81 ± 1.45%, p = 0.176) (Fig. 3c-e). However, there were changes in the soil relative to the 2011 data. Bulk density measurements were decreased relative to their 2011 levels in both High and Low Growth sites, but the soil at the High Growth sites decreased more (mean: -44.2 ± 4.5%) than the Low Growth sites (mean: -13.9 ± 4.0%) (p = 0.003). While there was no significant difference in the percent change in water content (High Growth median: 2.9 ± 4.8%, Low Growth median: -55.2 ± 4.1%, p = 0.308), but this is due to a single large outlier in the Low Growth sites. Without it, the change in water content in the Low Growth sites (-57.7 ± 4.1%) is significantly lower than the High Growth sites (p = 0.013). In addition, the soil at High Growth sites have accrued more organic soil carbon since 2011 (mean = 362 ± 77%) than the soil at Low Growth sites (median = 65.6 ± 50.5%) (p = 0.028) (Fig. 3h).

# Discussion

 Our data suggest that even after four years that included a season of very favorable growth conditions, the dieback sites visited by Crawford and Stone (2015) had not fully recovered. Several studies have shown that *Spartina* has altered chlorophyll levels under various forms of stress, including heavy metal exposure and highly saline environments (Chai et al. 2013; Di Bella et al. 2014). Thus, the low chlorophyll levels in the 2011 Dieback sites, and particularly in the Low Growth sites, could represent greater degrees of stress in these recovering sites.

 Plant stress can have many causes. One of the most prominent causes of stress for plants, particularly for *Spartina*, is water content of the soil, either due to drought or to waterlogging (King et al. 1982; Mendelssohn and Seneca 1980). Previous evidence has shown that soil at dieback sites has decreased water retention, attributed to reduced rates of sedimentation (Crawford and Stone 2014). While the soil at both the former dieback and the control sites exhibit a decrease in the water content relative to 2011, this could be due to methodological differences. Thus, the relative changes will be discussed here. The 2011 Dieback sites exhibit a greater percent change in water content relative to 2011 levels than the 2011 Control sites, to the point that there is no significant difference between the two groups in the absolute water content. There are likely two mechanisms involved in this. The first is decreased evaporative loss due to increased plant cover. This is supported by the fact that, aside from a single large outlier in the Low Growth sites, the High Growth sites, with their increased *Spartina* cover, exhibit greater water retention in their soil relative to 2011 levels. Also, the recovery of the biomass will result in increased levels of sediment deposition, which has been previously suggested to lead to greater water retention (Stumpf 1983; Rawls et al. 2003).

The changes in organic soil carbon are also likely due to increased sedimentation (Stumpf 1983). While dieback sites have reduced organic soil carbon, the levels are no different between the recovering and control sites in this study. Under normal growth conditions, *Spartina* prevents erosion and promotes sediment deposition (Neumeier and Ciavola 2004; Stumpf 1983). Areas of dieback experience rapid erosion, carbon release, and decreased sediment deposition. Thus, the drastic percent change in organic soil carbon observed in recovering dieback sites is likely due to the deposition of new sediment rather than a change in the existing soil. The High Growth sites account for a greater portion of this recovery than the Low Growth sites, suggesting that the recovery of organic soil carbon is correlated to the aboveground biomass and its influence on sedimentation.

Belowground *Spartina* biomass decreases during a dieback, and prior evidence has suggested that it does not recover even after 7 years and near complete recovery of aboveground biomass (Tong et al. 2013). Indirect evidence in this study suggests otherwise. Bulk density is often considered to be solely the density of the soil at the study site. Here, the volume and the weight of soil samples were measured without removing the root mass, and the rhizomes appeared to constitute a significant portion of the sample. Thus, any differences recorded in the density may reflect changes in the belowground biomass rather than the actual soil composition. Crawford and Stone (2014), using the same techniques, found the dieback sites to have increased bulk density. Taken as an approximation of belowground biomass, this corroborates data from Tong et al (2013). However, the lack of a difference between the recovering sites and control sites and the greater decrease in bulk density in the High versus the Low Growth sites suggest that the recovery in belowground biomass mirrors the aboveground biomass recovery.

This study was limited by the limited time points for sampling. To more fully understand how sediment deposition is altered during dieback and recovery, long term studies that identify dieback sites in the early stages and follow them through the recovery process will be crucial. However, important insights can be garnered from looking at the sights on longer time scales. Together, the data presented here suggest that dieback sites do not recover at the same rates and often take more than 4 years to fully recover. Even before the sites are fully recovered, sedimentation and erosion control are restored, at least in part. The degree of restoration is correlated to the degree of recovery, and this recovery is reflected in the stress levels of the plant. Thus, while the recent dieback epidemic has dire implications for carbon release and shoreline erosion, recovery is possible on a relatively short timeline.

# Advice to Future Sapeloids

 Try to work together on a project. I had many ideas that I would have really liked to pursue while I was down there, but had to make sure I got at least enough data to test my original hypotheses. In many cases, working with a partner makes things go more than twice as fast. Also, if you’re on the marsh, it’s really nice to be around someone else. I’ll be willing to bet more people from my cohort will have something to say about that.

Try to set up your project so you can get all the data of the first part in the first couple of days. Then spend a good few hours analyzing that data and thinking about what it means before you go off to work on the next part. Even if you don’t necessarily change what you’re doing for the second part, it will give you a chance to evaluate how the project is progressing, and you might see trends that are more interesting than what you originally intended to study.

 The profs recommend not bringing back data for a reason. It’s a lot harder and slower to process samples back in Wisconsin. If you’re willing to put in the time, it could be worth it, but be warned it will take a lot of time.

 Try to think about the implications of what your data will mean if it doesn’t turn out the way you think it will. Just because you don’t get the results you expected, doesn’t mean you set up the design wrong or didn’t get enough data. It might just not work that way. So, when thinking about your project, think about what a “negative” result would look like, and what it would mean. It will keep you from getting frustrated, and from trying to over-interpret your results.

 You won’t have time. Period. There will always be more cool things to study. Pick the coolest, then, keep sleep to a minimum. Stay up late and chase ghost crabs, get up early and go hiking or canoeing. There’s a million cool things on that island, check out as many of them as possible. Picking a project that’s based on the tide cycle is a good way to ensure you have at least a few hours free. It also might entail getting up at 3 AM to catch the low tide.

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