Effect of restoration on early stages of salt marsh litter decomposition
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December 2014

Abstract

The restoration of impounded salt marshes is an opportunity to reverse widespread degradation of salt marsh ecosystems and recover their ecological and economic value. Leaf litter decomposition contributes to the health of these marshes through its influence on carbon and nutrient cycles, and environmental changes associated with restoration may alter decomposition processes. I measured the effects of salinity, tidal regime, and plant community on litter decomposition rate in a one-month field experiment and a two-week lab experiment. Litter from the native cordgrass, *Spartina alterniflora*, decomposed at a faster rate than the invasive reed, *Phragmites australis*. The difference in decay rate seems related to the lability of organic material rather than nitrogen content, given that *P. australis* contained more nitrogen than *S. alterniflora*. Low salinity treatments in both lab and field experiments decayed more rapidly than high salinity treatments, suggesting that salinity inhibited microbial activity on leaf litter. Manipulation of inundation regime did not affect decomposition. The reintroduction of tidal flow to an impounded salt marsh therefore could have mixed effects; a shift in plant community towards *S. alterniflora* could cause a decrease in carbon sequestration, while an increase in salinity might slow decomposition and increase sequestration.
Introduction

Salt marsh restoration is a major frontier for reversing the negative impacts of development on coastal areas. Salt marshes play critical ecological roles, providing habitats and supporting high productivity, and contribute ecosystem services including wastewater treatment and floodwater protection (Costanza et al. 1997, Boorman 1999). Many New England salt marshes are degraded due to partial or complete restriction of tidal flow by the construction of roads across wetlands. Impoundment prevents the inundation of high marsh areas and eventually causes brackish marsh waters to become fresh. These changes disturb native plant communities and often allow colonization by invasive species. Tidal flow has been reintroduced to some impounded salt marshes by widening or removing culverts that restrict tidal flow. The cascade of ecological responses at short and long time scales to the reintroduction of tidal flow remain under investigation.

In natural salt marshes, decomposition of organic matter contributes to a number of ecologically significant processes. At the global scale, decomposition rates in salt marshes are typically lower than organic matter inputs, and as a result they are important sinks in the global carbon cycle (Chmura et al. 2003). Decomposition also controls vertical accretion, which in the event of sea level rise will be crucial to maintaining the structure and productivity of marshes worldwide (Reed 1995, Morris et al. 2002). At the ecosystem scale, decomposition renders carbon and nutrients available to the marsh trophic system, supporting microbial, planktonic, and higher trophic structures (Mann 1988). Primary production in the middle and high marsh is largely retained within the
marsh as leaf litter (Bouchard and Lefeuvre 2000), so litter decay partially determines the amount of carbon that is either sequestered in the sediment, respired by the microbial loop, or made available to higher trophic levels.

The decay of leaf litter may be sensitive to ecological changes associated with restoration. The reintroduction of tidal flow supplies saltwater to previously fresh impounded marshes. In tidal freshwater marshes, saltwater intrusion has been shown to increase decomposition in sediments by fueling sulfate reduction and desorbing labile, low molecular weight organic material from mineral particles (Weston et al. 2006, Weston et al. 2011), and was also correlated to an increase in ecosystem respiration (Weston et al. 2014). However, in mesocosm experiments high salinity has been shown to inhibit microbial activity in the decay of leaf litter (Roache et al. 2006). The change in inundation regime associated with restoration of tidal flow may also influence litter decay, as different microbial communities and pathways may occur during inundation or exposure.

Shifts in plant community may be the most important change associated with restoration. In Cape Cod marshes and all across New England, natural marshes are often dominated by cordgrass, *Spartina* spp. This study considers *Spartina alterniflora* as the primary native vegetation. Disturbed marshes are frequently colonized by *Phragmites australis*, an invasive reed that grows aggressively in wetlands and can outcompete *Spartina* and other native species in low salinity environments. *Phragmites* grows very tall, up to 4 meters, and can produce twice as much biomass as *Spartina* (Windham 2001). *Phragmites* has been shown to decompose more slowly than *Spartina*, possibly due to its rigid woody stems containing recalcitrant carbon structures (Windham 2001).
However, *Phragmites* contains greater amounts of nitrogen than *Spartina*, and appears to therefore stimulate greater nitrogen mineralization during litter decay, accelerating the nitrogen cycle without changing the net ecosystem balance of nitrogen (Windham and Ehrenfeld 2003). Changes in plant community may therefore have mixed consequences for salt marsh restoration, and a multifactorial study of controls on litter decay can elucidate how restoration is likely to effect the drivers of decomposition.

For the purposes of the study I am considering the effects of salinity, inundation regime, and litter type on the early stages of litter decomposition. The first 2 to 3 days of litter decay is characterized by a rapid of loss organic material leaching off the litter, termed the leaching phase by Valiela et al. (1985). Though most leaching occurs in the span of a few days, it may continue several weeks. A second phase lasting up to a year, the decomposer phase, is characterized by microbial activity decomposing leaf material. The first month of decomposition therefore includes both the leaching phase and the early decomposer phase, and can be responsible for a significant portion of total litter decay.

**Methods**

*Site description*

The field site for this study is a salt marsh connected to Scornton Creek and located in the East Sandwich Game Farm in Cape Cod, MA (41° 43' 51.96" N, 70° 25' 38.04" W). The site is owned by Massachusetts Fish and Game and was used with permission. The site includes a portion of salt marsh that has not been affected by any dyking or construction, which I am referring to as the "natural" marsh (top right of Figure
1). There is a restored portion of marsh, which used to be impounded by a small bridge crossing a narrow portion of the marsh with a culvert too small to adequately allow tidal flow. In 2009 a much larger culvert was installed, and now the marsh is flooded completely with each high tide. I am using this portion of the marsh as the "restored" site. Above the restored site is an impounded marsh, which is disconnected from the restored marsh by a railroad. A small culvert allows a minimal amount of tidal water to reach the impounded marsh, and a small freshwater stream feeds the marsh from Nye Pond. This marsh is the "impounded" site.

Field experiment

I placed litter bags containing 5-10 g of leaf litter in the natural, restored, and impounded marshes. The litter bags were arranged along transects extending from the creek bank to the upland edge of the marsh, and litter bags at each point along the transects were placed in triplicate (Table 1). Within each marsh, litter from both plant types was placed in patches of both plants. Litter bags stayed in the marsh for 25 days in November and December. Temperatures infrequently dropped below freezing during this time, and mostly ranged from 5-10°C. The air-dry weight of litter was recorded before being placed in the marsh, and a sample of each litter type was oven-dried to obtain a conversion factor between air-dry and oven-dry weights. After litter bags were retrieved, they were rinsed to remove sediment and oven-dried. Mass loss calculated as the percent change in oven-dry weight.

After litter bags were retrieved from the field, the CO$_2$ respiration rate was measured on each bag from transect 3 (natural marsh, all litter types in both plant
patches) over the course of 200 seconds using a LI-6200 Portable Photosynthesis System (LI-COR).

**Lab experiment**

To simulate and control for some of the environmental factors I believe are influencing decomposition in the field, I incubated bins of water containing litter bags under treatments of salinity, inundation regime, and litter type (Table 2). Bins were incubated at 25°C for 2 weeks. I took water samples periodically throughout the incubation. Water samples in the tidal bins were taken at the end of a 12-hour inundation period. Samples were filtered through pre-ashed GF/F filters (Fisher# 0987464). For storage until analysis, samples for TDN were frozen, samples for spectrophotometer analysis were refrigerated for no more than 3 days, and samples for DOC were acidified using 50% phosphoric acid (0.1 mL acid per 25 mL sample) and refrigerated.

I measured DOC using an Aurora 1030W TOC Analyzer (OI Analytical). I measured nitrate content using a Lachat QuikChem 8500 Series 2 (Lachat Instruments). I measured TDN by digesting water samples with an alkaline persulfate oxidizing reagent and autoclave to convert all organic and inorganic nitrogen to nitrate, and then measured nitrate using the Lachat. I measure ammonium using a phenolhypochlorite method modified from Strickland and Parson (1972).

To assess microbial activity I measured BOD in water samples from the inundated treatments after 9 days of incubation. I agitated the samples to oxygenate them to a DO content of ~4 mg/L then sealed them in 60 mL BOD bottles with an oxygen electrode (Oxi 330i handheld meter and CellOx 325 sensor, WTW). Samples were allowed to sit
for 5 minutes while the oxygen reading stabilized and then oxygen consumption was recorded over a period of 5 minutes.

To estimate bioavailable dissolved organic carbon (BDOC) I took duplicate water samples from each bin for DOC analysis on the first sampling date, and only acidified one sample from each bin. The unacidified samples sat out at room temperature and were occasionally agitated to maintain oxygen supply and support microbial activity. After two weeks those samples were acidified and analyzed for DOC; the amount of DOC consumed in that time is an estimate of BDOC.

The molecular weight of the organic material in water samples was evaluated with an analysis of UV absorbance spectra following Helms et al. 2008. Absorbance was measured on a Shimadzu UV-1800 spectrophotometer on a spectrum from 230nm-1100nm. The spectral slope ratio ($S_R$) is the ratio between the slopes of log-transformed spectra on the intervals of 275-295nm and 350-400nm. Higher slope ratios are associated with low-molecular weight material (Helms et al. 2008).

**Results**

*Field experiment*

*Spartina* litter bags lost 10-20% of their initial dry mass, while *Phragmites* leaves and stems lost less than 5% (Figure 2). Some *Phragmites* litter bags gained a small amount of mass, likely attributable to sedimentation that was not fully rinsed off. Litter bags placed near the creek lost slightly less mass than bags near the upland boundary, though the trend was minor and inconsistent (Figure 3).
Litter bags showed consistent linear production of CO₂ during LI-COR measurements. Significantly higher respiration occurred on *Spartina* (mean 9.98 ppm kg⁻¹ s⁻¹) compared to *Phragmites* leaves (mean 4.37 ppm kg⁻¹ s⁻¹; p<0.01, n=12), and significantly lower respiration occurred on *Phragmites* stems (mean 1.71 ppm kg⁻¹ s⁻¹) than leaves (p<0.05, n=12; Figure 4).

**Lab experiment**

*Spartina* generally showed the greatest loss of mass during the lab incubation, ranging between 10% and 25%, followed by *Phragmites*, which ranged between 5% and 15%. *Phragmites* stems lost little mass, mostly 5% or lower (Figure 5). The low salinity treatment stimulated greater mass loss in *Spartina* (mean 24.1%) compared to the high salinity treatment (mean 10.6%), though the effect was less pronounced on the other litter types. No consistent trend in mass loss emerged between inundation regimes.

Elemental composition analysis showed high nitrogen content in *Phragmites* leaves. The leaves had lower C:N ratios than stems or *Spartina*, both in the initial litter samples and after the incubation period (Table 3). *Phragmites* stems had the highest C:N ratio in the initial samples, and after incubation the nitrogen content of some stems was below detection limit (<0.1%) so the C:N ratio may be inferred to have increased dramatically in those samples (denoted N/A). *Phragmites* leaves consistently decreased in N content after the incubation, as shown by their increase in C:N ratio, while *Spartina* showed only minor increase in C:N ratio.

Analysis of DOC concentration in water samples over the course of the incubation showed that salinity and litter type both influence the leaching of organic carbon. Low
salinity treatments stimulated higher release of DOC during the first 48 hours (the majority of the leaching phase) than the high salinity treatments among both *Spartina* and *Phragmites* leaves in both inundation treatments (Figure 6). However, there is not a clear effect on DOC leaching by inundation regime and no consistent difference between *Spartina* and *Phragmites* leaves.

The percent of DOC consumed in water samples that sat out for two weeks is an estimate of biodegradable organic carbon (BDOC) and was similar between *Spartina* and *Phragmites* leaves (mean 33.0 and 35.8, respectively) but markedly lower in *Phragmites* stems (mean 17.3; Figure 7). BDOC was similar across salinity and inundation treatments.

Biological oxygen demand in water from inundated bins was higher among *Spartina* than either *Phragmites* leaves or stems (Figure 8). Low salinity treatments also supported higher BOD across all litter types.

During the initial 48 hours of incubation, *Phragmites* leaves leached the highest amount of nitrogen, which is consistent with their high nitrogen content. *Phragmites* leaves generally released over twice as much NH\(_4\) and DON than *Spartina*, and stems released very little nitrogen (Figures 9 and 10). No water samples contained significant levels of nitrate (>0.5 uM).

Analysis of UV absorbance spectra supplied information on composition of leached organic matter in water samples. The spectral slope ratio (S\(_R\)) is correlated to molecular weight, with higher S\(_R\) indicating lower molecular weight. After 48 hours in the inundated treatments, water samples from *Spartina* bins had higher S\(_R\) than *Phragmites* leaves or stems, indicating lower molecular weight leachate (Figure 11).
Discussion

Decomposition of Spartina versus Phragmites

Mass loss offers the most holistic indicator of decomposition, and *Spartina* consistently lost more mass than *Phragmites* in both lab and field experiments (Figure 2 and 5), which is consistent with the mass loss trends observed by Windham (2001) over the course of a year. The difference in mass loss between species does not appear to be related to nitrogen content; *Phragmites* leaves contain more nitrogen than *Spartina* (Table 3) and as a result supplied more nitrogen to overlying water during the leaching phase (Figure 9 and 10). Despite containing less nitrogen, *Spartina* supported higher microbial activity both directly on the litter, as measured by litter respiration rate (Figure 4), and in the water containing leached material, as measured by BOD (Figure 8).

Organic material containing high nitrogen content is generally expected to support high microbial activity (Valiela et al. 1984) and experimental addition of nitrogen to salt marsh ecosystems has stimulated decomposition processes in the high marsh and creek bank (Deegan et al. 2012). However, the microbial decomposer communities in this experiment did not respond to the greater nitrogen availability in *Phragmites* litter and leachate water.

Other factors such as the quality of the organic material must be responsible for differences in decomposition between species. Analysis of UV spectra shows that leached material from *Spartina* has higher $S_R$ than from *Phragmites*, indicating lower molecular weight material and supporting the notion that organic material from *Spartina* is more
labile than from *Phragmites* (Figure 11). However, BDOC estimates are similar between species (Figure 7), which confounds the UV analysis. More direct, mechanistic measures of lability and microbial activity are needed to clarify which characteristics of *Spartina* cause it to decompose more quickly than *Phragmites*.

**Effect of salinity**

Mass loss data from lab incubation suggest a strong salinity effect on decomposition rate. The trend is clearest in *Spartina* litter, where mass loss roughly doubled in high salinity treatments compared to low salinity treatments in both inundated and tidal bins. *Spartina* litter placed in the impounded marsh field site, where surface salinity water ranged from 1-3 ppt, also lost more mass (~20%) compared to the restored and natural sites (~15%) where surface salinity was variable, but ranged as high as 30 ppt. BOD measurements show a similar trend of reduced activity in high salinity water samples, suggesting that salinity either inhibited microbial activity or supported a microbial community that decomposed litter at a slower rate than those in the freshwater treatments. Any increase in anaerobic pathways such as sulfate reduction in the presence of salt water is therefore overshadowed by this inhibitory effect.

**Consequences of restoration**

Successful restoration is accompanied by an increase in salinity due to tidal flushing as well as a gradual shift in plant community from *Phragmites* towards native species such as *Spartina*. The leaching phase of litter decomposition and the early portion of the decomposer phase supply the marsh environment with a substantial portion of the
decomposable organic material in litter, and both phases seem affected by restoration. Replacement of *Phragmites* by *Spartina* during restoration may cause less organic material from litter to be sequestered in sediments, since *Spartina* decays proportionally more than *Phragmites* and produces less biomass annually (Windham 2001). However, the shift in plant community often takes many years and may never be complete. At the East Sandwich game farm marsh, for instance, *Phragmites* are present not only in the restored marsh but also in the natural marsh, demonstrating that restoration is not necessarily sufficient to return a marsh to a "natural" condition in which only native species thrive.

The increase in salinity from restored tidal flow, by contrast, is immediate. Increasing salinity can stimulate sulfate and iron reduction in marsh sediments while suppressing methanogenesis (Bartlett *et al.* 1987, Poffenbarger *et al.* 2011). But, salinity appears to reduce decomposition of litter on the marsh surface. Salt water intrusion could therefore slow the release of carbon and nutrients from litter to the marsh ecosystem and increase carbon sequestration. It is uncertain whether changes in salinity or plant community has the stronger influence on decomposition. An ecosystem-level approach will be needed to investigate the net impact of restoration on carbon and nutrient cycles.

**Acknowledgements**

Enormous gratitude goes to John Schade, Ken Foreman, Anne Giblin, and Rich McHorney for offering advice and resources on this project.
Figures

Figure 1. Satellite image of field site, showing natural marsh (top right, above culvert), restored marsh (center, between culvert and impoundment), and impounded marsh (bottom left). Image taken from Google Maps.

Table 1. Field experimental design. Transects extended from the creek bank to the upland marsh edge, with four points on each transect and triplicate litter bags at each point.

<table>
<thead>
<tr>
<th>Transect</th>
<th>Marsh</th>
<th>Litter treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transect 1</td>
<td>Natural</td>
<td>Spartina in Spartina patches, Phragmites in Phragmites patches</td>
</tr>
<tr>
<td>Transect 2</td>
<td>Natural</td>
<td>Spartina in Spartina patches, Phragmites in Phragmites patches</td>
</tr>
<tr>
<td>Transect 3</td>
<td>Natural</td>
<td>Spartina in both patches, Phragmites in both patches</td>
</tr>
<tr>
<td>Transect 4</td>
<td>Restored</td>
<td>Spartina in Spartina patches, Phragmites in Phragmites patches</td>
</tr>
<tr>
<td>Transect 5</td>
<td>Restored</td>
<td>Spartina in both patches, Phragmites in Phragmites patches</td>
</tr>
<tr>
<td>Transect 6</td>
<td>Restored</td>
<td>Spartina in both patches, Phragmites in both patches</td>
</tr>
<tr>
<td>Transect 7</td>
<td>Impounded</td>
<td>Both litter types in a Spartina patch</td>
</tr>
<tr>
<td>Transect 8</td>
<td>Impounded</td>
<td>Both litter types in a Phragmites patch</td>
</tr>
</tbody>
</table>
Table 2. Lab experimental design. Bins were incubated at 25°C for 2 weeks. Each bin contained three litter bags with 5-10 g plant material and 1.5 L of marsh water diluted with DI water according to salinity treatment. Tidal bins were inundated and dry on 12-hour cycles.

<table>
<thead>
<tr>
<th>Inundation regime</th>
<th>Salinity</th>
<th>Litter type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bin 1</td>
<td>Inundated</td>
<td>Salt (30 ppt)</td>
</tr>
<tr>
<td>Bin 2</td>
<td>Inundated</td>
<td>Salt (30 ppt)</td>
</tr>
<tr>
<td>Bin 3</td>
<td>Inundated</td>
<td>Salt (30 ppt)</td>
</tr>
<tr>
<td>Bin 4</td>
<td>Inundated</td>
<td>Fresh (1 ppt)</td>
</tr>
<tr>
<td>Bin 5</td>
<td>Inundated</td>
<td>Fresh (1 ppt)</td>
</tr>
<tr>
<td>Bin 6</td>
<td>Inundated</td>
<td>Fresh (1 ppt)</td>
</tr>
<tr>
<td>Bin 7</td>
<td>Tidal</td>
<td>Salt (30 ppt)</td>
</tr>
<tr>
<td>Bin 8</td>
<td>Tidal</td>
<td>Salt (30 ppt)</td>
</tr>
<tr>
<td>Bin 9</td>
<td>Tidal</td>
<td>Salt (30 ppt)</td>
</tr>
<tr>
<td>Bin 10</td>
<td>Tidal</td>
<td>Fresh (1 ppt)</td>
</tr>
<tr>
<td>Bin 11</td>
<td>Tidal</td>
<td>Fresh (1 ppt)</td>
</tr>
<tr>
<td>Bin 12</td>
<td>Tidal</td>
<td>Fresh (1 ppt)</td>
</tr>
</tbody>
</table>

Figure 2. Loss of mass in litter bags along transects in three connected marsh sites. Values represent mean ± SE (n=24) of bags along all transects in each marsh.
Figure 3. Mass loss of litter bags along transects that had all litter types at each point. Point 1 is at the edge of the high marsh nearest the creek bank and point 4 is near the upland boundary of the marsh. Error bars are SE, n=3.

Figure 4. Respiration rates of litter from transect 3 in the natural marsh site. Values represent mean ± SE (n=12) of carbon flux rate.
Figure 5. Mass loss in litter bags during the two-week lab incubation. Values are mean ± SE of triplicate litter bags in each bin. Where error bars are absent, only one data point was available due to some bags in salinity treatments gaining mass due to salt accumulation.

Table 3. C:N composition of litter before and after two-week incubation. For initial samples, n=5, and for samples after incubation, n=2. Parentheses contain SE.

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Inundated, high salinity</th>
<th>Inundated, low salinity</th>
<th>Tidal, high salinity</th>
<th>Tidal, low salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spartina</td>
<td>106.9 (1.1)</td>
<td>116.6 (11.4)</td>
<td>102.3 (1.3)</td>
<td>97.9 (27.4)</td>
<td>110.8 (11.8)</td>
</tr>
<tr>
<td>Phragmites leaf</td>
<td>41.6 (0.6)</td>
<td>73.5 (9.9)</td>
<td>54.6 (5.7)</td>
<td>50.7 (1.3)</td>
<td>54.4 (3.0)</td>
</tr>
<tr>
<td>Phragmites stem</td>
<td>147.1 (4.2)</td>
<td>N/A</td>
<td>152.4 (N/A)</td>
<td>N/A</td>
<td>112.4 (3.4)</td>
</tr>
</tbody>
</table>
Figure 6. Dissolved organic carbon after 48 hours of lab incubation. Values for tidal bins are the sum of DOC measured at the end of the first two 12-hour inundation periods.

Figure 7. Consumption of DOC in water samples containing leached organic material that were allowed to sit at room temperature, loosely capped and periodically agitated to prevent anoxia. No notable trends occurred between salinity and inundation treatments. Error bars show SE, n=6.
Figure 8. Biological oxygen demand in unfiltered water samples from inundated treatments over the course of 5 minutes, after agitating to oxygenate water. Error bars show SE, n=2.

Figure 9. Ammonium leached into bin water after 48 hours of lab incubation. Values for tidal treatments represent the sum of NH₄ measurements from the first 2 inundation periods. Ammonium concentrations declined in all bins after four days.
Figure 10. Dissolved organic nitrogen leached into bin water after 48 hours of lab incubation. Values for tidal treatments represent the sum of DON measurements from the first 2 inundation periods. DON concentrations declined in all bins after four days.

Figure 11. Spectral slope ratio ($S_R$) of UV absorbance spectra of water samples from inundated treatments after 48 hours of lab incubation. Calculated as the ratio between slopes of log-transformed spectra on intervals from 275-295nm and 350-400nm, following Helms et al. 2008.
References
Windham, L. 2001. Comparison of biomass production and decomposition between Phragmites australis (common reed) and Spartina patens (salt hay grass) in brackish tidal marshes of New Jersey, USA. Wetlands 21:179-188.
cycling processes within a brackish tidal marsh. Ecological Applications 13:883-897.