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Salinity Effects on *Phragmites* Decomposition Dynamics Among the Hudson River's Freshwater Tidal Wetlands

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Abstract Sea level rise due to climate change will expose Hudson River tidal marshes to chronic shifts in salinity, thus altering habitat conditions and biogeochemical processes. Increased salt intrusion may affect macroinvertebrate and microbial communities that are important in the decomposition of a dominant, invasive plant species *Phragmites australis*. We hypothesized that increased salinity intrusion will negatively affect macroinvertebrate and microbial litter breakdown processes. Field and laboratory experiments were conducted to test the effect of salinity on *Phragmites* decomposition. Leaf packets were incubated among tidal wetlands along a salinity gradient and used to compare microbial respiration, fungal biomass, and mass loss. In addition, salinity tolerance of a freshwater isopod (*Caecidotea sp.*) and heterotrophic microbes were examined using laboratory bioassays. Salinity negatively affected isopod survivorship and microbial activity in controlled laboratory experiments; however, salinity did not significantly account for in situ variation in *Phragmites* mass loss, microbial respiration, and fungal biomass among wetlands. Future studies need to include litter from additional wetland species and consider alternative controls on decomposition (e.g., variation in temperature or inorganic nutrients) in order to best forecast the long-term

impact of sea-level rise and salinity increases among tidal freshwater wetlands.

Keywords Tidal freshwater wetlands · Decomposition · Salinity · Respiration · *Phragmites* · Fungi

Introduction

Global sea-level rise is predicted to accelerate over the coming century, resulting in a 1–2 m increase by 2100 (Vermeer and Rahmstorf 2009). Environmental impacts include loss of coastal wetlands, increased coastal flooding, and salt intrusion of surface waters (Nicholls and Cazenave 2010). Coastal and tidal wetlands provide economically important ecosystem services (Costanza et al. 1998) such as flood control, sediment storage, and storm-surge protection (Mendelssohn et al. 1999; Quintino et al. 2009). Sea level rise threatens the long-term persistence and functioning of many coastal wetlands and these related ecosystem services (Titus 1988; Morris et al. 2002; Larsen et al. 2010; Nicholls and Cazenave 2010). Coastal freshwater, tidal wetlands are experiencing increased tidal amplitudes and increased probability of extreme storm surges, thus making them especially sensitive to increasing salinities (Quintino et al. 2009). Tidal wetlands are detritus-based systems that provide additional ecosystem services linked to nutrient uptake and retention, high rates of above- and below-ground net production, and secondary production/food supply (Findlay et al. 1990, 2002). In addition, wetland detritus supplies organic matter to adjacent rivers and coastal zones, and supports higher-trophic levels in a range of aquatic habitats (Jordan et al. 1989; Baldy et al. 1995; Mendelssohn et al. 1999; Komínková et al. 2000; Quintino et al. 2009).

Phragmites australis (common reed) has been present in eastern North American wetlands since pre-European times but has shown invasive attributes in the past few decades

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possibly due to a hybridization with the European strain (Saltonstall 2002). *Phragmites* stands are productive systems that enhance wetland biomass, modify community structure, and resource availability (Gessner 2001; Windham 2001; Findlay et al. 2002; Morris et al. 2002; Quintino et al. 2009). *Phragmites* is tolerant of brackish conditions and is expected to spread as the brackish zone of the Hudson River Estuary expands (Chambers et al. 2003). While there are several studies of *Phragmites* decomposition in general (Baldy et al. 1995; Komínková et al. 2000; Graça 2001; Findlay et al. 2002; Van Ryckegem et al. 2007) few studies have considered how increases in salinity may influence these decomposition processes and the fate of this large annual input of *Phragmites* litter (Reice and Herbst 1982; Hemminga et al. 1991; Mendelssohn et al. 1999; Piscart et al. 2005; Quintino et al. 2009). The extensive invasion of *Phragmites* along the eastern U.S. is likely to continue and will coincide with increases in salinity in many areas, thus it is important to assess the role of increasing salinity on factors controlling organic matter decomposition in tidal freshwater wetlands for future mitigation and wetland restoration projects (Mendelssohn et al. 1999).

This study examined the effect of increasing salinity on *Phragmites* decomposition among tidal, riverine wetlands along a well-defined salinity gradient in the Hudson River. *Phragmites* litter packs were deployed among five tidal wetlands spanning a salinity range of ≤ 0.1 psu–15 psu (Yozzo et al. 2005) and analyzed for mass loss, (Findlay et al. 2002), microbial respiration (Findlay et al. 1990), and fungal biomass (Gessner 2001). The field experiment was complemented by controlled laboratory experiments that examined freshwater macroinvertebrate and microbial community salinity tolerance (Blasius and Merritt 2002; Baumann and Marschner 2013).

Methods

Site Description

The Hudson River Estuary extends 250 km (154 miles) from the Federal Lock and Dam at Troy, New York to The Battery, at the southern tip of Manhattan Island (Yozzo et al. 2005). Mean tidal amplitude ranges from 1.3 km (West Point) to 2.3 km (Troy). The estuary is tidal freshwater (< 0.1 psu) from Troy south to Poughkeepsie (River Kilometer “RKM” 121) (Yozzo et al. 2005). Depending on freshwater discharge, the salt front migrates between the Tappan Zee Bridge (RKM 48) and Newburgh (RKM 97) throughout the year (Limburg et al. 1986; Yozzo et al. 2005). Intertidal wetlands occur throughout the estuary and tidal freshwater wetlands are found from Albany south to Manitou Marsh (Yozzo et al. 2005). Oligohaline and mesohaline marshes occur along an increasing salinity gradient from Manitou Marsh south to New York City (Yozzo et al. 2005). This study was conducted at the

Piermont, Iona, Manitou, Con Hook, and Constitution Marshes from early June to late August 2012 (Fig. 1). Piermont Marsh is a tidal mesohaline marsh (5–15 psu) located along the west shoreline of the Hudson River about 26 km south of Iona Island (Yozzo and Osgood 2013). Piermont Marsh is covered predominately by *Phragmites* (65 % in 1991); native vegetation is restricted to the interior of the marsh (Yozzo and Osgood 2013). Iona Marsh is an oligohaline intertidal marsh and upland habitat, also predominately covered by *Phragmites* (70 % *Phragmites*; 30 % narrowleaf cattail, *Typha angustifolia*) as well as other minor vegetation: broadleaf emergent plants [e.g., pickerelweed (*Pontedaria cordata*) and arrow-arum (*Peltandra virginica*)] (Yozzo and Osgood 2013). Manitou Marsh is a freshwater to slightly brackish enclosed tidal marsh (0–7 psu) located on the eastern shore of the Hudson River (Limburg et al. 1986). Manitou Marsh is partially isolated from the Hudson River by a railroad and natural ledge and contains *Phragmites*, narrowleaf cattail, purple loosestrife (*Lythrum salicaria*) and spotted touch-me-not (*Impatiens capensis*). Con Hook Marsh is ~1 km distance north of Manitou Marsh and likely has similar salinity ranges. Constitution Marsh is a freshwater to slightly brackish, enclosed tidal marsh (0–5 psu) located on the eastern shore of the Hudson River (Limburg et al. 1986). Constitution Marsh is dominated by the narrowleaf cattail, but arrow-arum, pickerelweed, broadleaf arrowhead (*Sagittaria latifolia*), rice cutgrass (*Leersia oryzoides*), and wild rice (*Zizania sp.*) are also common, while *Phragmites* stands are minor and controlled.

We looked at historical records in order to guide our selection of salinities used in our laboratory experiment. Salinity data for Piermont, NY and Hastings, NY were analyzed using long-term USGS records (archived conductivity data provided by Gary Wall, USGS, Troy, NY). Salinity values from the Hastings and Piermont sites are based on shore-mounted sensors that sample the top 1–3 m (depending on tide stage). Daily averages were calculated from October 2005–November 2010 for Hastings (on the eastern shore opposite Piermont) and November 2010–September 2011 for Piermont due to differing lengths of records. During 13 % of the days sampled between 2005 and 2010, and 1.8 % of the days sampled between 2010 and 2011 the daily averages clearly reached 15 psu. During our field studies, salinity data were collected with YSI Sondes logging at 15 min intervals at depths of 1–2 m for all five sites, and averages were calculated during 9–14 days within the period 11 July–3 August. Additional salinity measurements were made from grab samples collected at the surface (< 1 m) during field work at each site from 11 July– 4 August.

Macroinvertebrate and Microbial Tolerance to Salinity

A laboratory experiment was conducted to examine macroinvertebrate tolerance to salinity. A simple feeding experiment

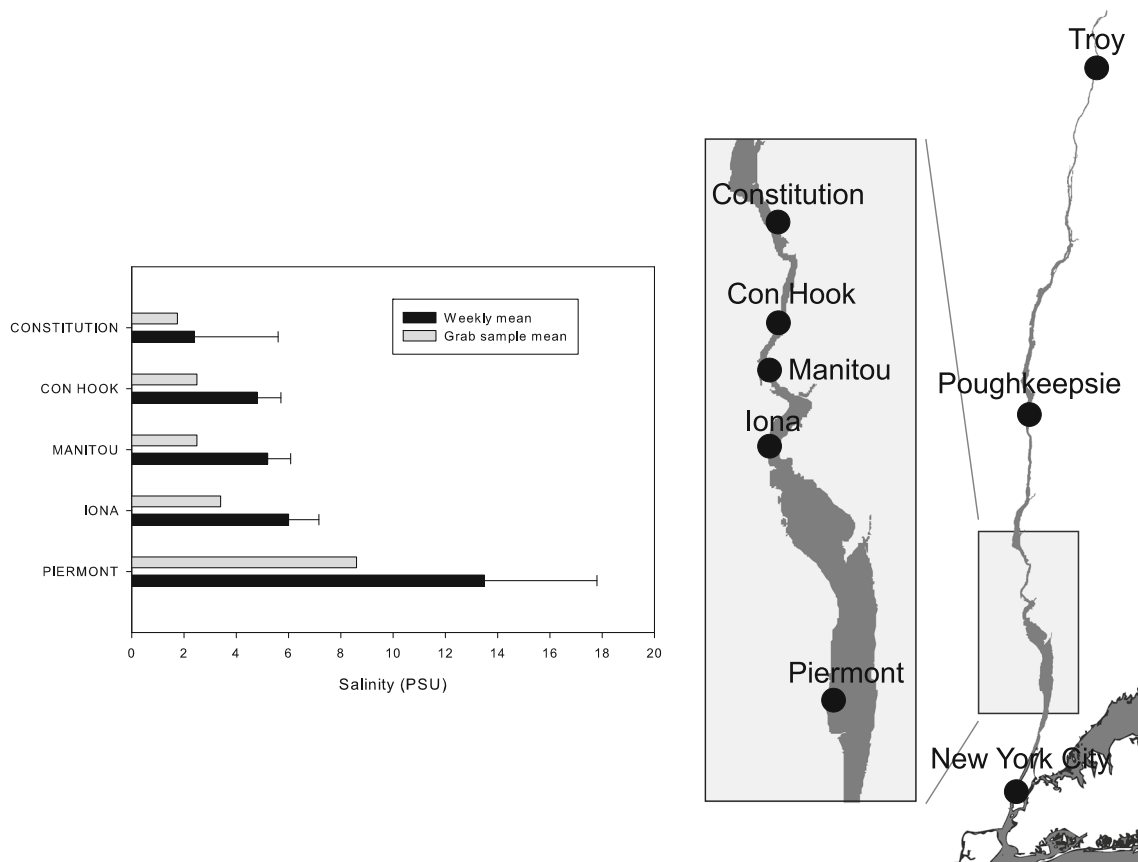


Fig. 1 Sampling locations along the Hudson River with respective mean salinity values from sondes measurements and grab samples collected mid July–August 2012. Error bars indicate the range. For grab samples,

$n=2$. Sonde values are means (ranges) of observations made at 15 min intervals for 9–14 days

was performed, giving the common freshwater Asellid (*Caecidotea sp.*) 1 cm leaf discs of *Phragmites*. Mass loss measurements of leaf discs were made 16 days later. *Caecidotea sp.* were collected at Constitution Marsh and were exposed to salt concentrations (0, 5, 10, 15, 20 psu), similar to conditions spanning the Hudson River Estuary (Blasius and Merritt 2002; Yozzo and Osgood 2013). Salt solutions were made using Instant Ocean® and freshwater from Constitution Marsh (0 psu at time of collection) and monitored using a YSI salinity meter. Isopods were held in aerated tanks until exposed to salt treatments. Each experimental unit was replicated 4× per treatment and contained four isopods, 20 ml of water varying in salinity, and some detritus. Isopods were exposed to salt treatments for 96 h with observations of behavior and mortality recorded every 24 h. A similar controlled, laboratory experiment was conducted to assess the effect of salinity on microbial respiration. *Phragmites* leaves were incubated at Constitution Marsh for 4 weeks to allow microbial colonization. Upon retrieval, 1 cm diameter leaf discs were placed into cups with water from Constitution Marsh (2.5 psu at time of collection) and salt amendments were added to reach 5, 10, 15, and 20 psu. Treatments lasted 96 h, with water changes every 24–48 h. After 96 h, ten leaf

discs were placed into 60 ml BOD bottles to measure respiration (change in dissolved oxygen, mg DO ml⁻¹ h⁻¹) over a 12–13 h interval.

In situ Mass Loss

To study the effect of a salinity gradient on mass loss, leaf litter packets containing 5 g dry weight of *Phragmites* leaves were deployed for approximately 4 and 8 weeks at the five marsh sites in summer 2012. Dead *Phragmites* litter from the previous growing season was taken from a stand at the Cary Institute of Ecosystem Studies to ensure uniformity of material and no prior exposure to salinity. Leaf packets were deployed 4–7 June. The first round of packets was collected 11–19 July, and the second round was collected 31 July–3 August. Leaf packets consisted of a 1 cm × 1 cm mesh size to allow macroinvertebrate colonization. Twenty-three leaf packets were deployed at each site and were held together by a nylon rope and PVC piping staked into the marsh sediment. Leaf packets were transported to the field individually in paper bags in order to collect mass loss during handling. Three leaf packets at each site were removed immediately to estimate mass loss during field transfer. Leaf packets

were placed in varying densities of *Phragmites* and narrowleaf cattail during low tide, where disturbance would be minimal. Attempts were made to place packets at the same elevation so they were inundated equally, but we do not have detailed water level data for each site. Leaf litter was dried at 70 °C for 24 h and combusted in a muffle furnace at 450 °C for 4 h for ash free dry mass (AFDM) determination. Mass loss during handling and field transfer, and mass removed for respiration discs and ergosterol segments were converted to an AFDM corresponding to the percent AFDM of original leaf matter from packets. These values were added back to the AFDM of litter from packets to obtain a final percent AFDM remaining after 4 and 8 weeks. The average mass loss due to handling and field transfer, and mass removed for respiration and fungal biomass (ergosterol) measurements were 2.7 % and 12.7 % respectively of the average mass of leaf litter from packets.

Microbial Respiration

To estimate rates of respiration after 4 and 8 weeks incubation, dissolved oxygen remaining in BOD bottles containing leaf litter associated microbes was measured. Prior to measurements, leaf litter was gently cleaned using Hudson River water from the corresponding site to minimize variation between laboratory and field conditions and prevent shock to microbial colonies. Leaf discs were cut using a core (1 cm diameter) immediately following retrieval of packets and placed in BOD bottles filled with corresponding Hudson River water at room temperature. BOD bottles were measured for initial DO concentration and a final DO concentration after a 12–13 h interval.

Fungal Biomass

To estimate fungal biomass, ergosterol (μg ergosterol/mg dry mass) content was measured (Gessner 2001; Gessner and Newell 2002). Upon retrieval, leaf material was cleaned with Hudson River water from corresponding sites and ten leaf segments (2 cm long) were made from random leaves. Leaf segments were stored in 20 ml methanol and placed in a freezer (−20 °C). For the first round of leaf packets, an additional ten leaf segments per leaf packet were made for AFDM determination. Samples were extracted for 2 h at 65 °C and then cooled in an ice bath. A 5 ml saponification solution containing 4 % KOH was used, followed by additional warming and cooling. A series of 10 ml and 5 ml pentane solutions were added and mixed. Pentane containing ergosterol was extracted and evaporated to dryness. Dissolved ergosterol residue was put in 1 ml methanol, sonicated and then filtered using acrodisks in 2 ml HPLC vials. Samples were stored in the freezer until HPLC analysis. The UV detector was set to 282 nm, while methanol as a mobile phase

was set to 1.0–1.5 ml/min. Ergosterol standards were included during the HPLC analysis. Retention time of ergosterol was dependent on flow rate, temperature, and column properties, but ranged from 4 to 5 min. A 20 μl injection of samples was used for analysis of peak area and amount of ergosterol.

Statistical Analysis

Statistica® software was used for statistical analysis. For laboratory components we used a simple one-way ANOVA. For field data which involved two separate collections we used a two-way ANOVA, treating both site and time as fixed effects. Model significance required $p\text{-value} \leq 0.05$. Data were log-transformed as needed to meet the assumption of normality (see figure legends). The least significant difference (LSD) post-hoc tests were used to delineate significance among treatments.

Results

Marsh Salinities Throughout our results, we report mean salinities in psu (e.g., Piermont: 13.5 psu, Constitution: 2.4 psu), but can be represented by their mean conductivity equivalents in units of dS/m (e.g., Piermont: 21.1 dS/m, Constitution: 3.7 dS/m). The mean salinities (psu) from field measurements for Piermont, Iona, Manitou, Con Hook, and Constitution Marshes are shown in Fig. 1. Spot measurements taken from 11 July–4 August for these marshes after 4 and 8 weeks were lower than the longer term means because the spot samples were collected at low tide (Fig. 1).

Laboratory Studies Results show *Caecidotea sp.* fed on leaf litter caused roughly a 2 %/day reduction in mass of leaf discs, indicating a significant consumption of litter. Cumulative mortality over the 96 h salinity exposure showed significant differences among treatments (Fig. 2, one-way ANOVA $p < 0.001$). No mortality occurred in 0 psu, indicating that mortality was due to salt treatments and isopods were provided suitable living conditions. Negative salinity effects on cumulative mortality were found with significant differences between treatments 10 psu or lower compared to 15 and 20 psu (Fig. 2). We found isopod behavior to exhibit lethargy and less responsiveness to prodding when exposed to salinity compared to the control. This behavior intensified in 10, 15, and 20 psu treatments with increased exposure. Microbial respiration rates ($\text{mg DO ml}^{-1} \text{ h}^{-1}$) significantly differed among treatments (Fig. 3, one-way ANOVA $p < 0.001$). Freshwater-colonized microbes had decreased respiration by 25–43 % at all salinities greater than 2.5 psu.

Field Studies In situ mass loss measures showed percent mass loss AFDM (g) after 4 and 8 weeks were significantly

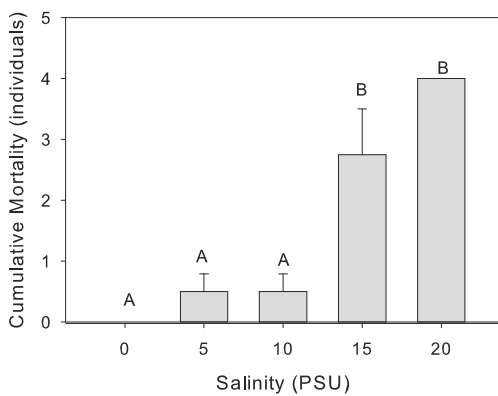


Fig. 2 Cumulative mortality of *Caecidotea sp.* after 96 h under different salinity treatments. Treatments differed significantly (one-way ANOVA $F_{4,15}=20.66$, $p<0.001$). Bars with different letters show differences among treatments and indicate the mean \pm SE (post-hoc, LSD, $p<0.05$)

different among sites (Fig. 4 top panel, two-way ANOVA $p<0.001$); however, there was no clear relationship between salinity and percent mass loss. Percent mass loss was highest at Iona Marsh ($62.95 \pm 1.46\%$) and lowest at Con Hook Marsh ($37.61 \pm 0.92\%$). Respiration also differed significantly among sites (Fig. 4 middle panel, two-way ANOVA $p<0.001$), but there was no clear relationship between salinity and in situ microbial respiration. Microbial respiration was highest at Piermont and Manitou with lowest values at Iona and Con Hook Marshes. Fungal biomass (reported as ergosterol) was significantly different among sites (Fig. 4 bottom panel, two-way ANOVA $p<0.001$), but there was no clear relationship between salinity and ergosterol content. Ergosterol content was highest at Con Hook Marsh and lowest at Iona Marsh. There was a significant effect of time on percent mass loss, respiration, and ergosterol content, indicating a difference in our results between 4 and 8 weeks (Fig. 4). We found a

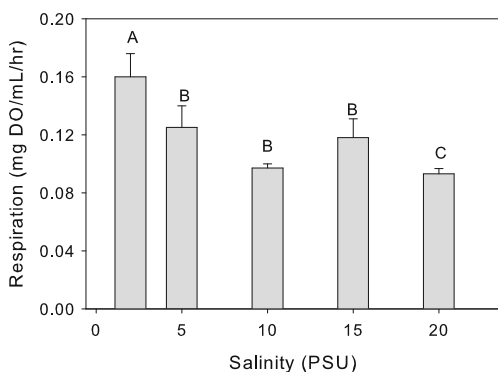


Fig. 3 Microbial respiration (change in dissolved oxygen, mg DO $\text{ml}^{-1} \text{h}^{-1}$) across salinity treatments (psu) after 96 h. Leaf matter had been incubated at the lowest salinity marsh (Constitution Marsh). Data were log-transformed to meet the assumption of normality. Treatments differed significantly (one-way ANOVA, $F_{4,19}=6.9$, $p=0.001$). Bars with different letters show differences among treatments and indicate the mean \pm SE (post-hoc, LSD, $p<0.05$). Note that the lowest salinity water from Constitution Marsh had 2.5 psu salinity

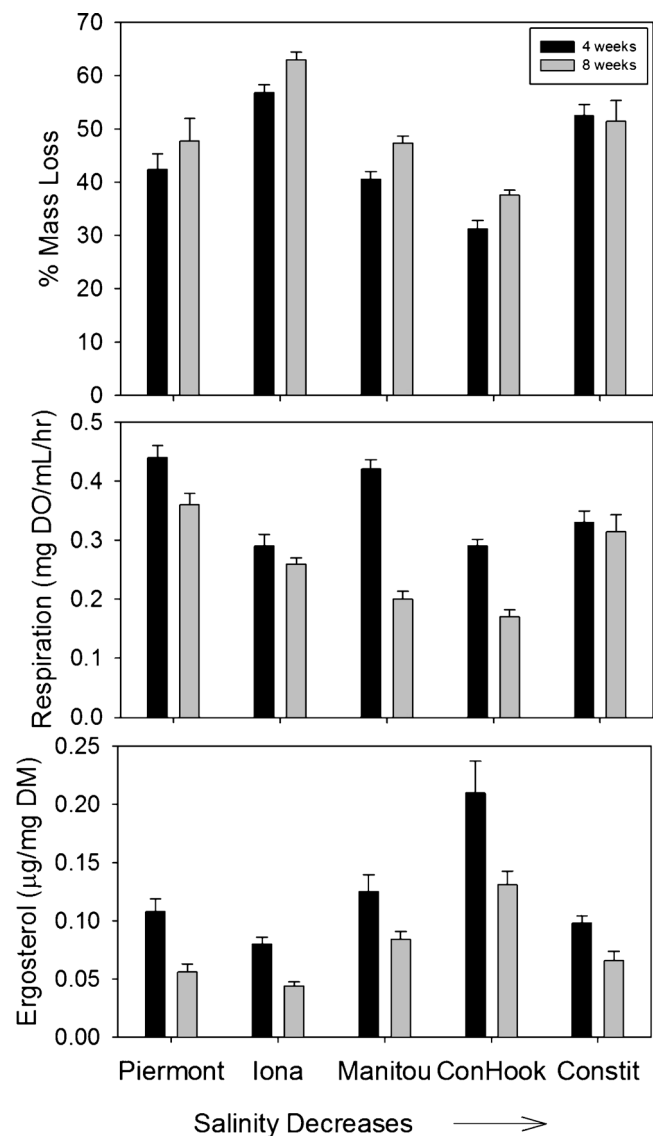


Fig. 4 (top panel) Percent Ash Free Dry Mass (AFDM) loss from leaf packets containing *Phragmites* after 4 and 8 weeks decomposition, across a salinity gradient from marsh sites farthest south (left) to farthest north (right) along the Hudson River Estuary. Both site and time differed significantly (two-way ANOVA, $F_{\text{site},4,90}=31.9$, $p<0.001$, $F_{\text{time},1,90}=9.7$, $p=0.002$), but site and time differences did not parallel the salinity gradient. (middle panel) Respiration from litter incubated at sites as described above. Both site and time differed significantly (two-way ANOVA, $F_{\text{site},4,90}=23.6$, $p<0.001$, $F_{\text{time},1,90}=62.4$, $p<0.001$, $F_{\text{interaction},4,90}=9.6$, $p<0.001$) but site and time differences did not parallel the salinity gradient. (bottom panel) Ergosterol content (μg ergosterol/mg dry mass) as an estimate of fungal biomass from litter incubated at sites described above. Ergosterol data were log-transformed. Site and time both differed significantly ($F_{\text{site},4,90}=26.2$, $p<0.001$, $F_{\text{time},1,90}=58.3$, $p<0.001$), but site and time differences did not parallel the salinity gradient

significant interaction between site and time for only respiration (two-way ANOVA $p<0.001$) indicating differences among sites were not consistent between the two times. What is relevant is that neither pattern for site or time parallels site salinity.

Discussion

Here we show that salinity differences relevant to brackish tidal wetlands affect some key processes of organic matter decomposition. Salinity changes as low as 10 psu negatively affected the freshwater isopod, *Caecidotea sp.*, and higher concentrations or longer exposure resulted in significant mortality. Macroinvertebrates are known to contribute to shredding and breakdown of leaf litter (Brinson et al. 1981; Webster and Benfield 1986; Graça 2001; Blasius and Merritt 2002; Collins et al. 2007) and we found that *Caecidotea sp.* consumed *Phragmites* litter in laboratory experiments. *Caecidotea sp.* is a common macroinvertebrate found in freshwater marshes along the Hudson River that shreds and decomposes *Phragmites* (Yozzo and Osgood 2013). Increasing salinity at sites near freshwater conditions would be expected to decrease decomposition of *Phragmites* by *Caecidotea sp.* Shifts in salinity might change the biodiversity of the reported 70 different macroinvertebrate taxa among Hudson River marshes, including common shredding macroinvertebrates, leading to further decreases in decomposition rates (Yozzo and Osgood 2013). Similar differences were seen of increasing mortality of *Gammarus* (Amphipoda) from exposure to high road salt concentrations in a laboratory study on the effect of road salts (NaCl) on macroinvertebrate communities in Lake Michigan (Blasius and Merritt 2002).

Exposing microbes on *Phragmites* to acute salinity increases showed a strong negative effect on microbial respiration. Respiration decreased from salinity increases as subtle as 5 psu and was depressed by 43 % at higher salinities. Microbial assimilation of detritus is critical for softening of leaf tissue and contributes to the decomposition of leaf matter (Brinson et al. 1981; Webster and Benfield 1986; Gessner and Chauvet 1994; Baldy et al. 1995; Hieber and Gessner 2002). Acute increases in salinity would be expected to decrease decomposition of *Phragmites* by heterotrophic microbes adapted to freshwater conditions. Similar decreases in microbial respiration with increasing soil salinity were observed in a study of drying and rewetting (Baumann and Marschner 2013). Evidence from laboratory experiments also showed reduced microbial activity on a gradient of increasing salinity in a freshwater wetland near Gippsland Lakes, eastern Victoria, Australia (Roache et al. 2006). Variations among Hudson River marshes did not reveal clear differences in field respiration, fungal biomass, and mass loss from decomposition associated with differences in salinity exposure. There are at least two plausible reasons the in situ differences were not as clear as the controlled experiments: lower actual exposure and opportunity for acclimation in the field. The acute exposure regime used in experiments would mimic a rapid salinity incursion such as might occur during a storm surge. Such rapid increases are evident in the long-term salinity records at the Piermont and Hastings sites. Records from Piermont

show on 28 August 2011, Hurricane Irene caused a dramatic 9.5 psu increase in salinity (3.9 psu at low–13.4 psu at peak). The following day, salinity dropped and persisted at 0–1 psu. During the field exposures in summer 2012 the peak salinity at Piermont was 15.5 psu and persisted in the range of 14.7–15.5 psu for 7 days out of the 8 week incubation period. Therefore, the field exposures at even the most southerly site were not as high as in the lab experiment (maximum = 20 psu), possibly accounting for the lack of response. Aside from differences in actual exposure between lab and field conditions, the 8 weeks incubation at field sites might allow acclimation by both micro- and macroorganisms along with potential shifts in decomposer community composition. Such changes would buffer acute negative effects on decomposition processes, masking effects of salinity on processes occurring under field conditions. Recent studies on *Phragmites* decomposition along a full salinity gradient (34.6 psu at the mouth–0 psu at the head) of the Mira Channel, Ria de Averoio in Western Portugal, showed clearer relationships (Quintino et al. 2009). It was estimated that a 51 %, 71 %, 70 %, and 71 % mass loss occurred in 5 mm mesh sized litter bags containing 3 g *Phragmites* after 60 days in salinities (psu) of approximately 34.6, 16.2, 2.4, and 0.0, respectively. However, Quintino et al. 2009 found only small differences in rates of decomposition among salinity ranges less than 16 psu. This provides another plausible reason why in situ differences in decomposition were not seen across our field sites.

Studies examining relationships between salinity and microbial processes in other environments (e.g., terrestrial soils) may help inform our understanding of how increased salinity influences freshwater-tidal marsh processes. Decreasing micro-organism activity with increasing salinity was seen in a terrestrial ecosystem from shifts in species diversity (van Bruggen and Semenov 2000). Other studies in terrestrial ecosystems also indicated decreasing microbial biomass (Muhammad et al. 2006; Wichern et al. 2006), and activity (Rietz and Haynes 2003; Sardinha et al. 2003) with increasing salinity. Rising salinity along a gradient has been shown to reduce denitrification activity and the diversity of nitrogen cycling communities, suggesting nitrogen removal capacity will reduce as freshwater marshes become more saline (Larsen et al. 2010). Evidence from laboratory studies showed a decrease in leaf mass decay for three plant species along an increasing salinity gradient (Roache et al. 2006).

In years and decades to come, this pressing issue will become increasingly important as chronic changes in salinity will continue to alter and drive wetland processes. Consequences include adaptation, changes in productivity and community diversity, and diminishing of buffering and restorative services of wetlands. Future studies examining decomposition of *Phragmites* across a salinity regime are required to enhance the knowledge of wetland responses to salt intrusion from sea level rise. Increasing salinization in tidal wetlands is a current

issue for preventing the expansion of *Phragmites australis*, and for establishing restoration (Burdick and Konisky 2003). Climate change projections indicate continuous sea level rise; hence, this study provides a useful approach and initial benchmark for future studies examining projected salinity increases in tidal wetlands along the Hudson River and other freshwater-tidal rivers.

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