

## **Statement of Work**

### *Background*

Wetlands along coastlines are especially vulnerable to anthropogenic impacts (United Nations Environment Programme 2006) due to their positioning between highly concentrated human coastal development and rising seas associated with anthropogenic climate change. In addition, coastal wetlands provide a disproportionately large amount of valuable ecosystem services (MEA 2003) such as carbon sequestration and storage, water filtration, and coastal buffering, therefore, any loss or degradation of coastal wetlands will have particularly severe ramifications for human society and climate change resilience. Consequently, considerable interest is vested in conserving these ecosystems in the face of future change.

The capacity of tidal marshes to persist in the face of climate change is tightly linked to their ability to maintain their elevation relative to rising seas, which is strongly determined by rates of sediment and organic matter accumulation (Kirwin and Megonigal 2013). These rates also directly influence tidal marsh carbon sequestration and storage capacity. Many studies have projected changes in coastal marsh sediment accretion in response to a variety of sea level rise (SLR) scenarios (Ouyang *et al.* 2022), but we presently lack strong understanding of the potential impacts of SLR-associated increasing salinity and tidal inundation period on organic matter stabilization or concomitant marsh elevation changes.

Inputs of organic carbon into salt marsh soils primarily take the form of autochthonous root and shoot litter (Tanner *et al.* 2010, Saintilan *et al.* 2013), as salt marsh plants are highly productive (Chmura *et al.* 2003). Decomposition of plant litter in soil is therefore a primary factor determining the accrual of organic matter and elevation in salt marshes. Sea level rise alters soil redox conditions (through changes in inundation) and salinity and nutrient levels, thereby impacting microbial decomposer community functioning (Rath and Rousk 2015), associated organic matter decomposition, and potentially its stabilization (He *et al.* 2022), with important implications for coastal resiliency. In tidal marshes, salinity, nutrients, and inundation period tend to decrease along the elevation gradient from low (L) to mid (M) to high (H) marsh but are increased at all elevations by SLR due to increasing relative contributions of saltwater water to fresh (Sharpe and Baldwin 2012). Litter decomposition dynamics such as initial decomposition rates ( $k$ ) and stabilization ( $S$ ) can therefore be expected to be different and changing at all marsh elevations as SLR continues.

Beyond microbial community functioning, the quality of plant litter (carbon to nitrogen ratio, total nitrogen and phosphorous content, relative amount of carbon held in complex compounds such as lignocellulose, etc.) is a primary factor influencing litter decomposition (Cleveland *et al.* 2013). Plant litter quality varies based on plant organ (root vs. shoot) and can be expected to change as salinity and inundation dynamics alter the stresses exerted on plants during growth and nutrient assimilation (Hu and Schmidhalter 2005), therefore, an understanding of plant litter quality and organ-specific biomass accumulation changes will be important to understanding indirect changes to SLR of plant organic matter decomposition and stabilization.

To advance our understanding of the direct and indirect effects of changing salinity and inundation periods on plant litter decomposition, I thus propose to conduct both *in-situ* and *ex-situ* experiments evaluating salt marsh sediment stabilization dynamics by quantifying litter decomposition rates and litter quality of plant litter subjected to different tidal and salinity regimes. I will then interpret identified salinity and inundation direct effects on organic carbon storage and indirect effects on the ability of marsh elevation to keep pace with sea level rise and use this to predict potential SLR-induced changes in the valuable blue carbon storage capacity of tidal marshes.

### *Objectives and Hypotheses*

*Obj.1: Examine how manipulated salinity levels (0, 9, 18 ppt) alter litter quality of dominant salt marsh species.* H1: Litter quality will vary among salinity treatments. I predict that higher salinity will increase C:N ratios and decrease total N and P of both *Spartina alterniflora* and *Phragmites australis*, due to salinity-induced physiological stress that may limit uptake of nutrients.

*Obj.2: Evaluate how experimental salinity (0, 9, 18 ppt) and frequency of inundation (L, M, H) independently and interactively alter decomposition rates (k) and organic matter stabilization (S).* H2: Salinity and frequency of inundation will alter both *k* and *S*. I predict that elevated salinity and more frequent inundation will reduce microbial efficiency due to salt-induced stress and oxygen limitation. In this scenario, both *k* and *S* will decrease.

*Obj. 3: Test how elevation alters decomposition rates of S. alterniflora and P. australis in the field.* H3: Decomposition rates will vary with elevation. Higher salt marsh elevations are associated with shorter inundation periods and lower salinity; therefore, I anticipate decomposition rates to be greater for plant litter in these zones due to the lower oxidative and salinity stress placed on resident decomposer microbe communities.

### *Methods*

To address Obj. 1, I will leverage a recent hydroponic experiment examining manipulated salinity (0, 9, and 18 ppt) effects on carbon exudate rates of *S. alterniflora* and *P. australis*. Plants were grown in a controlled growth chamber environment in Hoagland's solution with altered salinity (0, 9, 18 ppt) while controlling for other variables (nutrients, pH, light, etc.). Following six weeks of plant growth from seedling, I harvested and dried above and belowground biomass. For this study, I will quantify relative biomass accumulation (root vs. shoot mass), grind and homogenize biomass in a ball mill, and quantify total carbon and nitrogen content of using a Costech 4010 elemental analyzer, and send remaining ground biomass samples for phosphorous measurement. I will use Analysis of Variance (ANOVA) tests of litter quality among salinity levels to assess the effects of increasing salinity on litter quality in common salt marsh grass species and then interpret my findings in the context of available literature regarding litter quality implications for soil organic matter decomposition and stabilization.

I will address Obj. 2 by conducting a growth chamber experiment employing the Tea Bag Index (TBI) method (Keuskamp *et al.* 2013). I will estimate mass loss by incubating red and green tea

bags over a 90-day period in 2 L experimental units (plastic containers) filled with salt-marsh soil; I will test three salinity levels (0, 18, 36 ppt) and three tidal inundation levels (L, M, H) with five-fold replication using a method modified from MacTavish and Cohen (2014). Tidal inundation levels will be based on periods of inundation expected at each marsh elevation and derived from studies in natural systems. I will estimate  $k$  and  $S$  and use a two-way ANOVA to test for independent and interactive effects of salinity and frequency of inundation. These measurements will then be related to substrate-dependent organic matter loss and stabilization. Results will be analyzed in the context of SLR projections.

For Obj. 3, I will directly measure decomposition rates of *S. alterniflora* and *P. australis* litter using litter bags at an experimental restoration where sediment was added to the marsh surface in large mounds (“hummocks”) at Great Meadows Marsh in Stratford, CT. Litter bags will be filled with homogenized, field-derived plant material, placed along 3 different hummock elevations (L, M, and H representing different tidal inundation and salinity regimes), and sequentially removed and measured for mass loss over time. Decomposition rates will be compared among elevations and species using ANOVA, and I will investigate relationships among decomposition rates, soil salinity and frequency of inundation using regressions.

### **Experiment Status**

Seventy-two plants have already been grown and biomass collected and weighed for the salinity-manipulation hydroponic growth experiment (Obj. 1), but I still need to cut, homogenize, grind, roll, and process (or send for processing) root and shoot biomass for all samples prior to salinity- and species-specific litter quality analysis. Aside from securing a potential growth chamber space, no aspect of the litter bag TBI study (Obj. 2) has been started at this time. Finally, the field litter bag experiment (Obj. 3) has pre-established plots which have been under monitoring for multiple years, therefore, long-term salinity and water-level data is available, however, I still must gather litter, make litter bags, place them in plots, and conduct sequential removals and mass loss measurements.

### **Project Implications for Coastal Wetland Conservation**

Coastal wetlands are particularly vulnerable to impacts from sea level rise and associated saltwater intrusion but highly valued in part for their considerable blue carbon storage capacity. As coastal development continues and the coastal boundary migrates inland, salt marshes are being “squeezed” (Torio and Chmura 2013) or lost. Failure of salt marsh elevation gain to keep pace with SLR will facilitate further marsh loss and a concomitant loss of the systems’ valuable blue carbon storage capacity. In addition, direct effects of SLR-associated salinity and inundation changes on salt marsh organic carbon stability are unclear but may contribute to further losses in blue carbon storage services.

Understanding the factors contributing to salt marsh elevational and carbon storage stability is important to our ability to predict marsh stability, ecosystem service provisioning, and management needs in the future. The lack of studies and clear answers regarding the effects of salinity and inundation increases on organic matter stabilization in salt marshes currently places severe limitations on our ability to accurately, holistically predict future marsh carbon storage value. By conducting experiments examining litter quality and litter decomposition responses to

different SLR-related conditions, I aim to decrease this knowledge gap and generate data that can be applied to more accurate marsh loss and blue carbon storage service projections. Improved predictions can then inform more effective management to support the persistence of coastal wetlands and their services in a changing future.

**Budget**

<i>Objective #</i>	<i>Item</i>	<i>Cost</i>
1.	Consumables for C:N analysis (foil tins, Atropine, Eppendorf tubes, ethanol, gloves, KimWipes, etc.)	\$300
1.	Sample processing (\$6 per sample for C and N measurements, \$7.25 for Phosphorous measurements of 2 tissue types for 72 samples)	\$1908
2.	Experimental set up (plastic containers, tea bags), tidal simulation materials (buckets, Instant Ocean, 18 Tom Aquatics water pumps, 6 GE timer outlets, airline tubing, etc.) for 5 replicates of 9 conditions	\$2200
2.	Growth chamber rental (\$70 per month for 5 months)	\$300
3.	Field litter bag experiment supplies (fiberglass screening, staples, tags, dowels, clipboards, etc.)	\$200
3.	Mileage for travel to field site (\$0.63 per mile for 800 miles)	\$504
		\$5412

**Plans for Sharing research with Diverse Audiences**

As a researcher involved in the ongoing Great Meadows Marsh restoration currently underway in Stratford, CT, I partake in meetings with diverse stakeholder groups, including but not limited to, CT Deep, the Nature Conservancy, and Audubon Connecticut. My findings regarding salinity and tidal inundation impacts on salt marsh persistence and carbon storage capacity will be shared at these meetings and can inform adaptive management regarding thin layer placement depth and material significance. In addition, my participation in the planning and presentation of a salt marsh restoration practitioner workshop scheduled for the Fall of 2024 will also offer me the opportunity to share the findings of my proposed projects with community members and scientists who wish to increase their awareness of best practices and apply them in the field.

I further intend to share my work through peer-reviewed literature as well as at regional (New England Estuarine Research Society) and national (Ecological Society of America) conferences. Opportunities exist for my research to be shared with diverse audiences within the University of Connecticut, as well. My participation in UConn’s College of Agriculture, Health, and Natural Resources (CAHNR) annual graduate research forum in the Spring of 2025 will allow me to share my work with a broader audience while also considering the implications of other presented research for my study and potential future directions.

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