COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./DUE DATE: **NSF 22-543** **03/23/2022**

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NSF PROPOSAL NUMBER

2224608

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OCE - LONG TERM ECOLOGICAL RESEARCH

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☐ AN ACCOMPLISHMENT-BASED RENEWAL

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NAME OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE: **MARINE BIOLOGICAL LABORATORY, THE**

ADDRESS OF AWARDEE ORGANIZATION, INCLUDING 9 DIGIT ZIP CODE:
7 M B L ST
WOODS HOLE,MA 02543-1015 US

AWARDEE ORGANIZATION CODE (IF KNOWN): **0021626000**

NAME OF PRIMARY PLACE OF PERF: **Marine Biological Laboratory**

ADDRESS OF PRIMARY PLACE OF PERF, INCLUDING 9 DIGIT ZIP CODE:
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WOODS HOLE,MA 02543-1015 US

IS AWARDEE ORGANIZATION (Check All That Apply) ☐ SMALL BUSINESS ☐ FOR-PROFIT ORGANIZATION ☐ MINORITY BUSINESS ☐ WOMAN-OWNED BUSINESS

☐ IF THIS IS A PRELIMINARY PROPOSAL THEN CHECK HERE

TITLE OF PROPOSED PROJECT: **LTER: Plum Island Ecosystems, the impact of changing landscapes and climate on interconnected coastal ecosystems**

SHOW LETTER OF INTENT ID IF APPLICABLE

REQUESTED AMOUNT: $ **7,649,999**

PROPOSED DURATION (1-60 MONTHS): **72** months

REQUESTED STARTING DATE: **10/01/2022**

SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE

THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW
☐ BEGINNING INVESTIGATOR
☐ DISCLOSURE OF LOBBYING ACTIVITIES
☐ PROPRIETARY & PRIVILEGED INFORMATION
☐ HISTORIC PLACES
☒ VERTEBRATE ANIMALS IACUC App. Date **04/29/2021**
PHS Animal Welfare Assurance Number **D16-00039**
☒ TYPE OF PROPOSAL **Research**
☐ HUMAN SUBJECTS Human Subjects Assurance Number ____
Exemption Subsection ____ or IRB App. Date ____
☐ FUNDING OF INT'L BRANCH CAMPUS OF U.S IHE
☐ FUNDING OF FOREIGN ORGANIZATION OR FOREIGN INDIVIDUAL
☐ INTERNATIONAL ACTIVITIES: COUNTRY/COUNTRIES INVOLVED
☒ COLLABORATIVE STATUS
A collaborative proposal from one organization (PAPPG II.D.3.a)

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Project Summary

Overview:
The Plum Island Ecosystems (PIE) LTER is a linked watershed-marsh-estuarine system connected to the Gulf of Maine where conditions are changing rapidly. Currently, rates of sea-level rise are accelerating, sea-surface temperatures in the Gulf of Maine are rising faster than most other places on earth, and precipitation is becoming more variable. We are also seeing substantial changes in the watershed, due to an increase in urbanization and from a large increase in wetland areas due to rapid growth in the beaver population. In PIE V we will examine the impact of changing landscapes and climate on these interconnected coastal ecosystems.

Intellectual Merit:
In PIE V we organized research around three questions that build on previous findings and integrate long-term studies with new observations, experiments, and model development. Activities within the three questions are coordinated across research groups and integrate across the entire watershed-marsh-estuary domain to facilitate a broader synthesis. Our first question is, "How are the sources and fates of organic matter and nutrients in the linked watershed/estuary system being altered by changing land use, sea-level rise (SLR), climate, and geomorphology?" We will examine how the linked coastal system influences estuarine production and water quality, and the role of coastal ecosystems in modulating carbon and nutrient fluxes to the nearshore ocean through a combination of expanded observations and modeling. Our second question, "How do food webs and energy flow respond to new geomorphic configurations, SLR, changing climate, and associated estuarine responses?", will be a new integrative effort to determine how the landscapes' ability to transfer energy to foodwebs changes with habitat. Given the importance of coastal ecosystems in supporting nearshore foodwebs, understanding how this link will change with climate, land use change and SLR is critical. Finally, we ask, "What internal feedbacks might accelerate, slow down, or even reverse the predicted changes in emergent marsh configuration and the fate of carbon, nutrients and energy?" We urgently need to understand more about internal feedbacks that might increase marsh resilience or compromise marsh survival as sea levels rise, the system warms, and watershed inputs continue to change. This will be a new direction for PIE. Finally, new collaborations will allow us to take advantage of a large-scale marsh restoration to test some of our hypotheses on feedback mechanisms within the system.

Broader Impacts:
PIE continues to advance our predictive understanding of the long-term response of coupled land-marsh-estuary-ocean ecosystems to changes in three key drivers: climate, sea level and human activities. Our research sheds light on the controls on marsh accretion and loss, blue carbon storage, the exchange of carbon and nutrients, and how food webs are changing with SLR, warming, and ecosystem responses. Given expected rates of SLR, salt marsh survival is in doubt. Agencies are eager for research and data that can help them better manage coastal systems and help in the design and evaluation of large-scale restoration efforts like those proposed for Plum Island marshes and beyond. PIE scientists participate in meeting with managers from local, regional and federal agencies and share data and information with them, and often participate in joint projects.

We will continue our K-12 Schoolyard program, "Salt Marsh Science", which provides ten schools with experiential learning opportunities for students and teachers. This program is in collaboration with Mass Audubon and serves approximately 1,000 students and 50 teachers per year in grades 5-12. Mass Audubon has added a new climate change focus that will make use of the vegetation transects teachers and students have been measuring for the past 25 years. We will also continue to work with the Gulf of Maine Institute (GOMI), an NGO that provides training to teachers and help them develop environmental community-based stewardship projects. Currently there are 5 schools participating, all within the watershed of the Merrimack River, including the Lowell Middlesex Academy where PIE has a teacher in the NSF RET program. PIE scientists are providing scientific support to GOMI for teachers to develop in-class programs which emphasizes watershed connectedness through shared data collection activities.

I. Site Introduction and History

The Plum Island Ecosystems (PIE) LTER site consists of a linked watershed-marsh-estuarine system connected to the Gulf of Maine (Fig. 1). The brackish and saline tidal wetlands of the PIE site form the major portion of the "Great Marsh", the largest contiguous acreage of intact marsh on the northeast coast of the United States. The marshes and the estuarine waters are influenced by three rivers: the Ipswich, the Parker and the Rowley, which collectively drain over 550 km^2 of upland. The coastal system is macrotidal –with a tidal range often exceeding 3 meters.

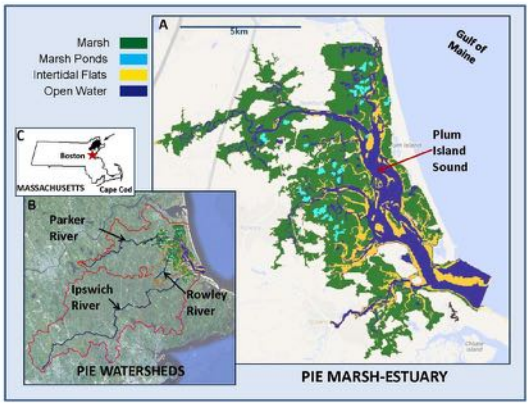


Fig. 1. The PIE marsh-estuary (A), the PIE domain consisting of the marsh- estuary and watersheds (B), the site location on the coast of Massachusetts (C).

PIE lies within the Acadian biogeographic province; a cold-water environment sharing many species with boreal and Arctic provinces of the western Atlantic Ocean. It is located 150 km north of Cape Cod, Massachusetts, which is the historical geographic boundary for many southern species. Therefore, PIE marshes historically lacked many of the invertebrate species which are believed to play key ecological roles in primary production in more southern marshes and estuaries (Pennings and Silliman 2005).

Conditions at PIE are now changing due to the rapidly warming waters of the Gulf of Maine (Pershing 2015). New studies now suggest that the five-decades-long warming trend has accelerated and that the movement of warmer water into the Gulf of Maine during summer has greatly strengthened, perhaps signaling a regime shift (Siedov et al. 2021). This has major implications for the fauna. We have already seen the expansion of several species of southern decapods into PIE marshes (detailed below) and suspect more species, both native to the south, and invasive, will make their way into the system.

In addition to temperature changes in the Gulf of Maine, we have seen other important changes in external climate drivers such as an increase in the rate of sea-level rise (SLR) and changes in the amount and timing of precipitation. Land-use change in the watershed is being driven by suburbanization and a large expansion in beaver ponds since the early 2000's. As detailed below, the changes in these drivers have led to changes in the geomorphology of the watershed, marsh and estuary. These geomorphic changes, combined with the direct impact of changing external drivers, are altering food webs, primary production, and the flows of energy, nutrients and carbon throughout the linked coastal ecosystem.

Historical legacies also play an important role. New England marshes were managed for centuries for the production of the high marsh vegetation, *Spartina patens* (salt marsh hay). Management included extensive ditching, channel alteration, control structures, and low elevations berms. Additional extensive ditching was done in the 1930's, for mosquito control. In the last few decades, perhaps exacerbated by accelerated SLR, many of these heavily managed areas have seen peat subsidence, waterlogging, and conversion of high marsh to low elevation marsh (Burdick et al. 2020). To counter this, and to preserve high marsh habitat for the endangered salt marsh sparrow, several small experimental restoration efforts are underway, and if successful, restoration activities may commence on over 2,000 acres.

History of Previous Research

The Plum Island Ecosystems LTER began in 1998 with the overall goal of developing a predictive understanding of the long-term response of coupled land-marsh-estuary-ocean ecosystems to changes in three key drivers: climate, sea level and human activities.

In PIE I we asked "*How will trophic structure and primary and secondary productivity in estuaries be affected by changes in organic matter, nutrient, and water fluxes caused by changing land cover, climate, and sea level?"*. During PIE I, we established long-term monitoring activities in the marsh, estuary and watersheds, most of which are still on-going. We carried out whole system isotope additions in the contrasting Parker and Rowley River estuaries to examine food web pathways. These studies revealed that hydrology played an enormous role in structuring the food web by altering water residence times. Pelagic phytoplankton dominate primary production in the high residence time, more turbid Parker estuary, whereas benthic production dominates in the shallow, more rapidly flowing Rowley estuary (Holmes et al. 2000, Tobias et al. 2003). These differences in primary producers cascade throughout the food web (Hughes et al. 2000). We also made substantial progress understanding how accelerated sea level rise impacts marsh processes and marsh grass production (Morris et al. 2002). We found marshes in PIE and South Carolina responded similarly to changes in relative SLR, despite the marshes being fundamentally different in structure (peat vs mineral). This link between relative SLR and marsh productivity led to the generally applicable Marsh Equilibrium Model (Morris et al. 2002) of marsh response to SLR, which has been widely applied and which is still being refined (Morris et al. 2021).

In PIE II we focused more heavily on the importance of the interactions of changing nutrient, water, and organic matter inputs within the "*hydrologic and geomorphic template*" (e.g., places where residence time was always short, and others where it was seasonally long). We quantified how hydrology controls N cycling both in the watershed (Wollheim et al 2008) and in the estuary through its impact on estuarine salinity (Giblin et al. 2010; Weston et al 2010). We found that hydrology structures both pelagic and sediment microbial communities (Crump et al. 2004; Bernhard 2007). We demonstrated that marsh creek drainage is an important source of carbon supporting estuarine water column respiration (Raymond and Hopkinson 2003; Vallino et al. 2005). Interactions between nutrient delivery, marsh grass production, and food web responses were examined in a large-scale, long term marsh creek-shed experiment (TIDE; Deegan et al. 2007). We documented substantial changes in the abundance and distribution of vegetation, ponds, and creeks throughout the system (Wilson et al 2014). Human altered river flows were impacting estuarine salinity regimes and the forms and quantities of nutrients delivered from the watershed. It became evident that we had to incorporate *geomorphic change* into our conceptual model.

PIE III directly addressed how geomorphic change shapes organic matter and nutrient transformations in linked watershed and coastal ecosystems. Our goal for PIE III was *"to understand how external drivers, ecosystem dynamics, and human activities interact to shape ecological processes in a mosaic of coastal landscapes and estuarine seascapes"*. Using this framework we (1) examined linkages and feedbacks between watersheds, marshes, estuaries, and the coastal ocean in more detail (e.g., Wollheim et al. 2013; Nelson et al. 2015; Leonardi et al. 2016); (2) identified the feedbacks between geomorphic change and ecosystem processes (e.g. Morris et al. 2013) and (3) dynamically incorporated the human dimension (Polsky et al. 2012; Runfola et al. 2013, Kaushal et al. 2014). We continued to examine the impact of *temporal change and variability* on ecological processes through long-term monitoring.

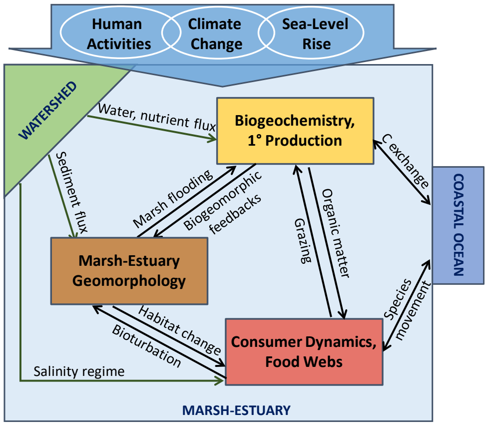


Fig. 2. The overall conceptual model for PIE IV showing the three external drivers, acting directly on the marsh and estuary and through changes in material fluxes from the watershed. The response of the marsh-estuarine system is modified by interactions within the system which either dampen or magnify the effect of external drivers.

II. Results of Prior

The focus of PIE IV is ***Dynamics of coastal ecosystems in a region of rapid climate change, sea-level rise, and human impacts***. During this cycle we built on topics that emerged as critical areas in PIE III, including sediment dynamics, species interactions, and the role of ocean warming on species changes. We continue to examine how external drivers alter the geomorphology, biogeochemistry, and population and community ecology of coastal ecosystems; however, we put more emphasis on the feedbacks *between* geomorphology, biogeochemistry, and communities (Fig. 2). We pursued questions on the role of humans as drivers through a cross-LTER Coastal SEES project.

A central question in PIE IV is: How will would SLR alter the geomorphic configuration of the marsh over the coming decades? PIE is currently dominated by high elevation marshes (Fig.3). Given expected rates of SLR over the next century, we predict that PIE will transition from a predominantly high-elevation marsh system dominated by *Spartina patens* to a lower elevation, more frequently flooded marsh dominated by *S. alterniflora*. (Note -the genus *Spartina* has been changed to *Sporobolus* but we use *Spartina* here to avoid confusion with previous work (Bortolus et al. 2019). Eventually, we expect to see an overall loss of marsh area and an increase in open water through erosion and as marshes are squeezed into smaller areas due to steep elevation gradients or human structures that limit upland migration. While driven by SLR, the trajectory of geomorphic change may be highly modified by internal feedbacks and, by human activities. Marsh "transition", erosion, and "squeeze" have important impacts on marsh productivity, community structure, and biogeochemistry. PIE IV focuses on understanding these impacts and how fast we might expect them to occur by addressing 3 questions.

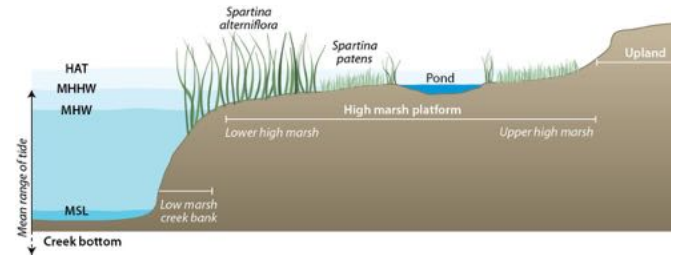


Fig. 3. Marsh elevation and vegetation types relative to tide height. (MSL-mean sea level, MHW-mean high water, MHHW-mean high high water, HAT highest astronomical tide). Note vegetation type changes with elevation.

Q1) How will the geomorphic configuration of the marsh and estuary be altered by changes in the watershed, sea-level rise, climate change, and feedbacks internal to the coastal system?

Much of our efforts on this question focused on determining sediment sources, documenting both short and long-term accretion rates, measuring lateral erosion and changes in marsh area, and determining if a change from high to low marsh was beginning to occur.

PIE LTER watersheds continue to see increased suburban development and population growth, while beaver pond abundance is simultaneously increasing (Fig.4). Despite extensive suburbanization, retention of sediments within the river network remains high, likely because of the shallow slope, abundant and expanding natural wetlands, and because much of the human development is skewed towards the upstream part of the watershed (Mineau et al. 2015, Huang et al. 2022). A synthesis of long-term data from the watershed indicates that sediment flux to the estuary varies 5-fold, primarily driven by precipitation and flow variability, not land use change. Hence, the current condition, where sediment fluxes from the watershed are very low and contribute little to maintenance of the estuarine marsh platform (Weston 2014, Hopkinson et al. 2018), will not be altered with land-use change. An additional important finding for management is that human dams in the watershed are not a large sink for sediments (Whitney et al. In Review) suggesting that planned dam removals will not result in major increases in sediment fluxes to the estuary.

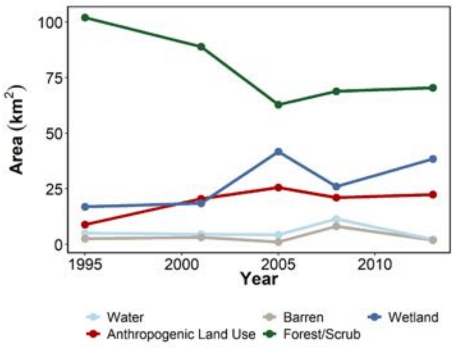
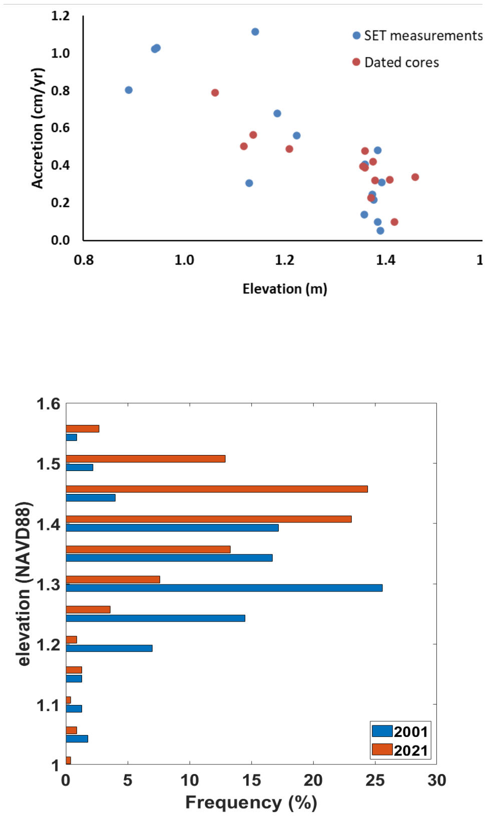


Fig. 4. Changes in land use from 1995 to 2013 based on classification of high resolution aerial imagery over the Parker River watershed (Whitney et al. 2021). Wetland area has increased since the early 2000's.

Low watershed sediment inputs make the marsh more vulnerable to accelerated SLR. We examined whether increases in SLR will lead to a conversion of the high elevation marsh to a lower elevation marsh by using a combination of long-term field measures, surveys, dated cores (Forbrich et al.

Fig. 5a. Accretion rates from SETs (Giblin et.al. 2022) or dated cores (Forbrich & Giblin 2018, Wilson et al 2014, Weston unpub.) vs. marsh elevation. 5b. Histogram of elevation (NAVD88) of vegetation transect points in 2001 and 2021 (Sundberg et al 2022a,b). Data binned in 5cm intervals.



2018), and modeling (Morris et al. 2020). Absolute elevation changes measured in 22 plots with very high temporal precision using Sediment Elevation Tables, (SETs, Cahoon et al. 2002) over 20 years show elevation is increasing faster at low elevations, primarily occupied by *S. alterniflora*, than in high marsh areas, which is consistent with accretion rates from dated cores (Fig. 5a). Rates of accumulation in the high marsh are at, or lower than, rates of SLR suggesting that if SLR continues to accelerate the high marsh zone will be lost. To determine trends in elevation and vegetation change across larger scales, in 2021 we re-visited 9 of 20 marsh transects measured in 2001. For most transect plots, we recorded elevation gains of over 10 cm during the 20 year time period (Fig. 5b), similar to our SET measurements. Notable exceptions occur along the creek bank and marsh edges, where several of the transect points recorded in 2001 were eroded away. A whole system sediment budget, coupled with data showing that the surface sediments are quite old (Hopkinson et al. 2018; Luk et al. 2021), indicates that a very important sediment source for marsh accretion is cannibalization from the lateral erosion of the marshes themselves (Hopkinson et al., 2018).

An additional sediment source is the periodic input of creek bottom sediments to the high marsh via ice rafting. In 2018 we saw a large sediment deposition event during a severe storm (FitzGerald et al. 2020, Wittyngham et al. 2021) leading to accumulation of several cm of sediment over significant areas of the marsh. Lesser storm rafting events occur more frequently. Of our 22 SETs, 17 showed at least one deposition event of 10 mm or more and 10 experienced multiple large deposition events over 20 years. These data support previous findings that ice rafting is important for marsh accretion in cold climates (Argow et al. 2011) and suggests that the frequency of winter climate extremes will influence marsh elevation gain.

Changes in marsh area will impact estuarine sediment transport, a feedback mechanism we explored using a high-resolution numerical model. Simulations indicate that increases in SLR augments the tidal prism, enhances ebb-dominated currents, and leads to a net loss of sediments for the entire system (Zhang et al. 2020). At the same time, lower tidal flats augment wave action and lateral marsh erosion (Carr et al 2018). A reduction in marsh extent reduces the ability of the coastal bay to retain sediments. We conclude that in the short-term salt marshes can continue accreting because SLR favors resuspension (Zhang et al 2020) and deposition on the platform. However, as marsh area is increasingly lost, the reduced trapping capacity jeopardizes the survival of the remaining marshes (Donatelli et al. 2020).

Given the lower accretion rates we measured in high-elevation marsh, we assessed whether we are seeing changes in the proportion of low and high marsh vegetation. The current elevations of the marsh platform at PIE are higher than the optimum elevation for *S. alterniflora* and lower than the

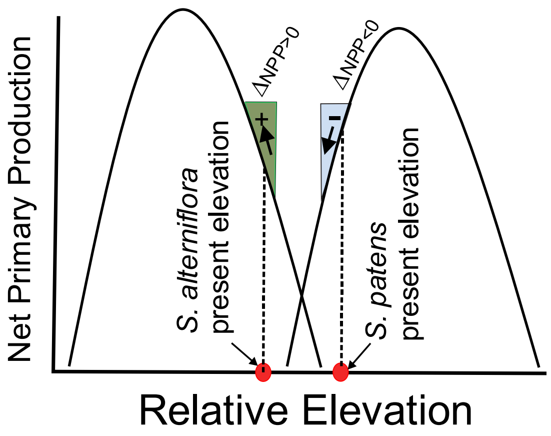
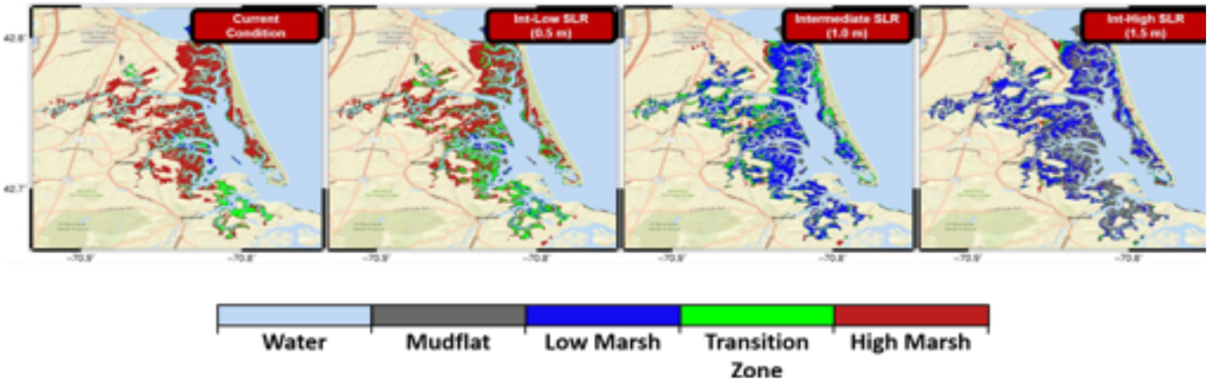


Fig. 6. Theoretical distribution of net primary production (NPP) of *S. alterniflora* and *S. patens* relative to mean sea level. As sea level rises relative to their present elevations, *S. alterniflora* is predicted to have greater NPP while NPP in *S. patens* will be reduced.

optimum for *S. patens* (Fig. 6). Consequently, we posit that as relative marsh elevation decreases, with sea level rising faster than marsh accretion, high marsh *S. patens* will be replaced by *S. alterniflora*. Evidence for this change so far is mixed. We revisited 8 long-term transects throughout the marsh in 2021 and found vegetation composition changed little since 2001(Sundberg et al. 2022a,b). Detailed annual transects across 6 higher elevation marsh areas (Sullivan et al. in prep) show some vegetation changes and our long-term monitoring site at Law's Point (an overall low elevation site) had a nearly 50% loss of the *S. patens* area. Overall, however, elevation and marsh composition show that in contrast to other wetlands (e.g. Gonneea et al. 2019) PIE marshes do not yet show signs of having passed a tipping point. At present, *most* high marsh monitoring sites are accreting enough material to keep up with the current rate of SLR. The current stability is very likely due to the elevation capital at PIE (e.g. Burns et al. 2021, Ganju et al. 2020), the relatively large tidal range (Burns et al. 2021, Gonneea et al. 2019), and the marsh cannibalization described above. However, field data that show *S. patens* is under stress (*see Q2 below*) and our modeling studies suggest a tipping point may be approaching (Fig. 7; Alizad et al. submitted)

We are using remote sensing techniques to quantify changes in creek area, ponds and marsh area, and changes in vegetation over the entire landscape. Marshes present special challenges, but we have made considerable progress developing novel, robust methods to evaluate change (Evenden and Pontius 2021; Khallaghi and Pontius 2021; Liu and Pontius 2021; Pontius 2019; Pontius et al. 2017a, 2017b).

Fig. 7. Current and predicted marsh vegetation changes using HYDRO-MEM, a combination of MEM with a hydrodynamic model including sediments, calibrated for PIE. With low rates of SLR high marsh is diminished and with the highest projections all high marsh is lost and marsh area is greatly reduced.



Q2) How will changing climate, watershed inputs, and marsh geomorphology interact to alter marsh and estuarine primary production, organic matter storage, and nutrient cycling?

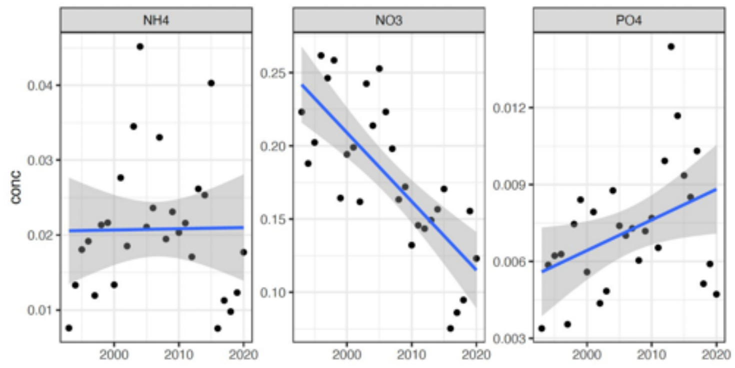


Fig. 8. Mean annual concentration of NH_4, NO_3 and PO_4 exported from the watersheds based on annual fluxes (composite method in Appling et al. 2016) and observed discharge. NO_3 has declined while PO_4 has increased since the mid-2000's, consistent with when beaver pond abundance increased. NH_4 is consistently low with high interannual variability.

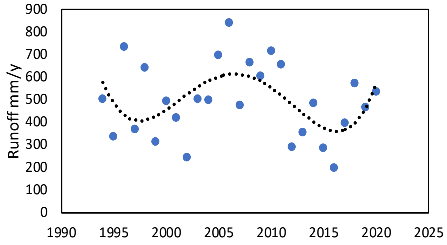


Fig. 9. Variability in annual runoff showing the cyclical nature of the discharge.

In the watershed, along with the increase in beaver ponds since the early 2000's, the rivers show a decline in the mean annual concentrations of nitrate, while phosphate is increasing (Fig. 8, updated from Morse et al. 2014). Annual fluxes of other constituents show no trends, instead, export is dominated by variability in annual discharge (Fig. 9) indicating that climate variability is currently more important than land use change. The decline of nitrate has occurred despite increased human activity in the watersheds, suggesting that expanding beaver ponds are altering the relative proportions of storage, emissions, and transport of inorganic nutrients to the estuary. Their impact on dissolved organic carbon (DOC) and nitrogen (DON) remains unclear. To better understand the processing of organic matter and nutrients in beaver ponds, and how these processes are altering consumption and loss, we have begun to quantify emissions of CH_4, CO_2, and N_2O in stream channels and fluvial wetlands.

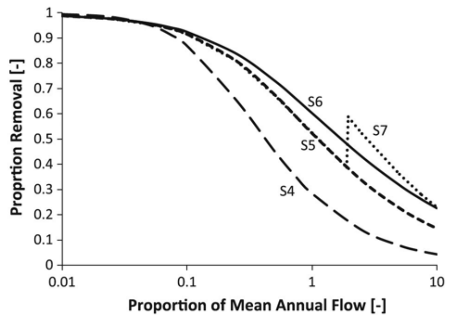


Fig. 10. Modeled relationship between the proportion of a constituent removed and flow conditions at various river network scale proportion for the Ipswich R. watershed. S4 assumes a channelized river network only, S5 adds lakes and reservoirs, S6 includes beaver ponds with density = 0.8 km^{-1} of river length and S7 assumes flood plains become active at 2x the mean annual flow.

While flowing streams are disproportionate sources of CH_4 and N_2O on a per area basis, the much greater extent of fluvial wetlands created by beaver dams results in total fluxes from wetlands being much higher (Robison et al. 2021, 2022, Bower 2020) and potentially increasing given the expansion of beaver ponds. The dynamics of storage, emission, and downstream carbon and nitrogen fluxes in response to beaver pond establishment and aging is an emphasis in PIE V.

To explore how changing climate and landscapes impact the flow of water and nutrients from watersheds, Wollheim et al. (2018) built a quantitative conceptual model of river network function, largely based upon PIE research. The model suggests that retention of sediment, carbon, and nutrients by river networks saturates with increasing flows, influenced by reaction rates and the presence of lentic waters such as beaver ponds (Fig.

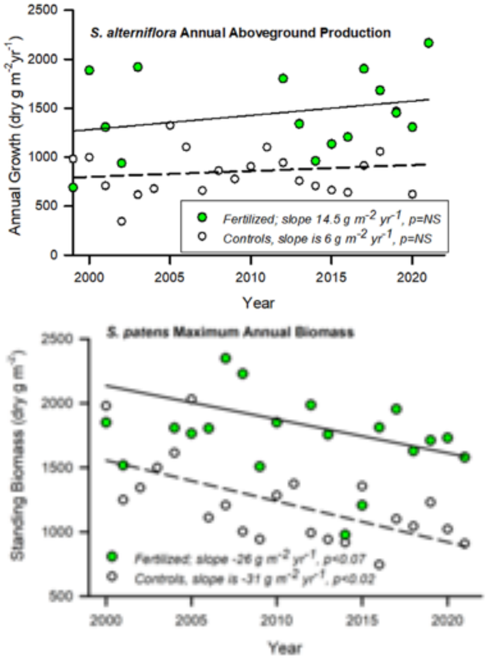


Fig. 11. Changes in annual net aboveground primary production of *S. alterniflora* and annual peak standing biomass of *S. patens* over time. As SLR increases *S. alterniflora* is becoming more productive while S. patens has declined. The common response of fertilized and unfertilized plots within sites show the trends are not driven by nutrient availability.

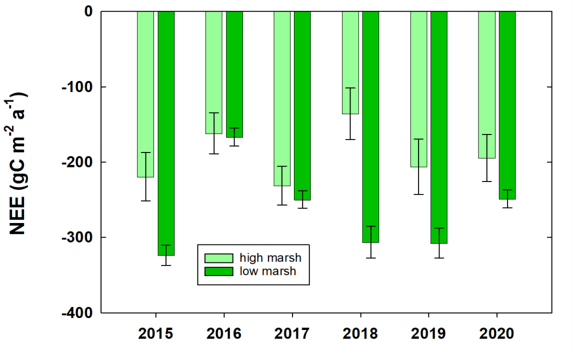


Fig. 12. Net Ecosystem Exchange (NEE) measured using eddy covariance towers located in the high and low marsh. NEE is typically greater (avg. 267±38 g m^{-2} y^{-1}) in the low marsh than in the high marsh (avg. 190±77 g m^{-2} y^{-1})

10;). Further, cumulative river network function tends to increase faster than watershed area (superlinear scaling) for processes with low to intermediate reaction rates (like denitrification or refractory carbon decomposition) because larger rivers in the network contribute disproportionately to whole network function, particularly at high flows (Wollheim et al. 2022). Model findings are consistent with the observation that NO_3^- is declining and support our hypothesis that beaver ponds have acted to counter increased loading due to land use change. Therefore, in order to predict changes in C and N fluxes from the watershed to the estuary, the shifting balance of anthropogenic reservoirs, towards beaver ponds which are primarily located in headwaters close to anthropogenic nutrient sources, must be considered.

In the marsh, efforts focused on determining how the predicted conversion from relatively high-elevation *S. patens* dominated marsh to a lower elevation *S. alterniflora* dominated marsh would alter ecosystem function. We use a 'space-for-time' approach (Picket 1989) in contrasting "creeksheds" dominated by areas of either low or high marsh. Given the current marsh elevation, the Marsh Equilibrium model (Morris et al. 2021) predicts that rising sea levels should increase the productivity of *S. alterniflora* and decrease *S. patens* productivity. These predicted changes in production are supported by field data, though we have not seen species replacement, (Fig. 11). We used eddy flux towers to measure differences in primary production and carbon storage in high vs. low marsh. Both marsh types are large carbon sinks, (Forbrich and Giblin 2015, Forbrich et al. 2018) but net CO_2 uptake (NEE) is typically greater in the low marsh than in the high marsh (Fig. 12). NEE at both sites greatly exceeds the long-term C burial rate measured using ^{210}Pb dating of soil cores (Forbrich et al. 2018). We made tidal exchanges measurement in creeks and expected to see large lateral exports of dissolved inorganic carbon (DIC) in outflowing tidal waters to account for the mismatch in burial vs NEE. Surprisingly, however, we did not observe DIC export but, rather, slight DIC uptake in both high- and low-elevation systems (Fig. 13). In contrast to DIC, our lateral exchange measurements did show a net export of organic carbon (DOC; Fig. 13) which is supported by our monthly porewater measurements showing higher DOC loss from low marsh areas. However, the calculated DOC loss is not large enough to account for the observed mismatch between NEE and burial, particularly in the low marsh. We participated in AmeriFlux's year of methane, and found the marsh was not a methane source, eliminating this as a possible reason for the discrepancy.

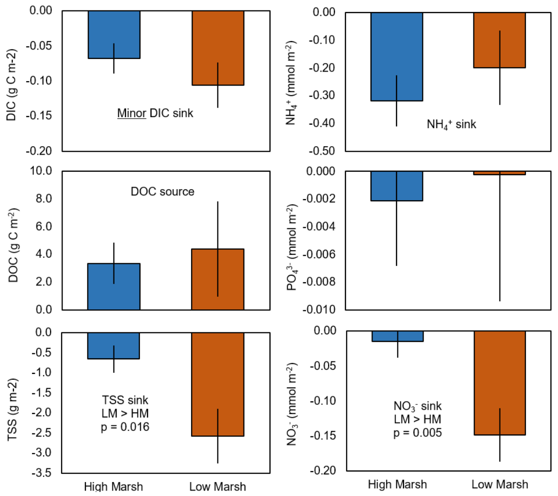


Fig. 13. Average (±SD) export (positive) or import (negative) of DIC, DOC, total suspended sediments (TSS), NH_4^+, NO_3^-, and phosphate PO_4^{-3} from high- and low-elevation marsh and tidal creeks

Net sediment fluxes also differ between creeks largely draining high-elevation marsh versus those largely draining low marsh. Low-elevation marshes retain greater amounts of suspended sediment than high-elevation marshes (Fig. 13), matching predictions that greater flooding duration in the low-elevation marshes results in greater sediment trapping (Morris et al. 2002). When annualized, sediment retention in the low-elevation marshes contributes significantly to marsh accretion (0.72 mm yr^{-1}) but has a very limited contribution in the high-elevation marsh (0.16 mm yr^{-1}).

Lateral exchange measurements of nutrients indicate that the tidal creeks and/or marshes are a sink for NH_4^+ and NO_3^-, and a marginal sink for PO_4^{3-}(Fig. 13). The low-elevation marsh systems demonstrated significantly greater NO_3^- uptake than high-elevation marshes, while there were no differences between marsh types for the other constituents measured. We also have observed lower rates of aquatic production in tidal creeks draining the low marsh than the high marsh. We conclude that greater connectivity between the marsh and creeks in the low-elevation marshes promotes nutrient uptake, and denitrification, in the marsh that lowers nutrient availability to the creeks reducing productivity (Fig. 13).

To better understand the seeming contradiction between studies that found nitrogen additions drive salt marsh loss (e.g. Deegan et al. 2012), and those that did not (e.g. Anisfeld and Hill 2012), we performed controlled experiments where we directly manipulated the quantity of nitrate available to microbes in salt marsh sediments. Added nitrate stimulated respiration by denitrification and dissimilatory nitrate reduction to ammonia (DNRA) and showed there was a pool of organic matter that was accessible for decomposition by microbes in the presence of nitrate that was not accessible when sulfate was the dominant electron acceptor (Bulseco et al. 2019). However, this “nitrate labile” pool was lower in sites with a history of long-term nitrate exposure (Bulseco-McKim 2018). Results from metagenomics highlighted that genes for central carbon metabolism, denitrification, and DNRA were all enhanced in the nitrate added treatment (Bulseco et al. 2020), and chemoautotrophy was suggested by enhanced genes for carbon fixation. We then used metagenomics to assemble genomes from this experiment. Again, the abundance of genes associated with carbon fixation and denitrification suggested that autotrophic denitrification may be more important in salt marsh sediments under high nitrate loading than previously recognized (Vineis 2022). Using these experiments and a literature meta-analysis of fertilization experiments, we discovered that the form of added nitrogen (ammonium vs. nitrate) had a major impact on the transformation and fate of added N (Bowen et al. 2020), with greater plant responses to ammonium and greater microbial responses to nitrate, helping explain the contradictory marsh fertilization studies.

Q3) How will key consumer dynamics and estuarine food webs be reshaped by changing environmental drivers, marsh-estuarine geomorphology and biogeochemistry?

We hypothesized that SLR will shift estuarine food webs toward dominance by marsh-marine food webs due to shifts in habitat configuration and productivity, with cascading consequences for trophic dynamics. Although we have not yet seen a loss of high marsh habitat in PIE marshes, an experiment revealed the importance of the high marsh to intermediate consumers. When altered creek geomorphology prevents mummichogs (a killifish *Fundulus heteroclitus)* from accessing high marsh prey, it results in decreased mummichog abundance (Nelson et al. 2019, Lesser et al. 2020a, 2021).

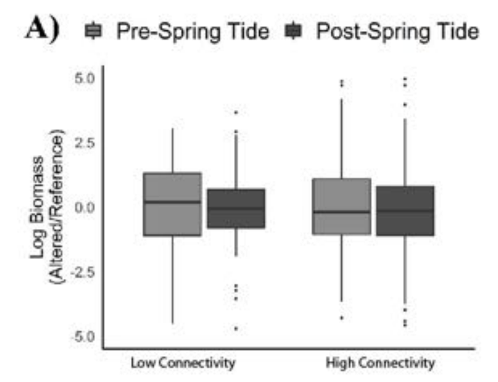


Fig. 14. Biomass of invertebrate prey showing no difference in creeks with high and low connectivity, and therefore access by fish.

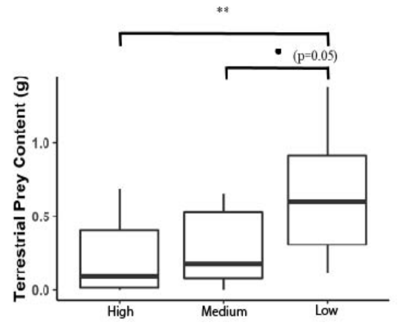


Fig. 15. Gut content of terrestrial prey in fish. Fish caught from creeks with low geomorphic disturbance fish have better access to terrestrial prey.

Reduced mummichog predation in the high marsh did not, however, lead to a reduction in invertebrate prey populations in those creeks (Lesser et al. 2020a; Fig 14). This indicates that while mummichog do not exhibit top-down control on high marsh communities, high marsh production is an important subsidy to creek food webs (Lesser et al. 2021; Fig 15). Therefore, predicted changes to less high marsh could result in a 50-66 % reduction in energy transfer from high marsh habitats to creek food webs disrupting the trophic relay function of these habitats (Lesser et al. 2021).

To understand the influence of long-term climatic shifts on resource use in PIE, we developed niche metrics based on a stable isotope mixing model to quantify long-term shifts in food web function in response to climatic variability (Lesser et al. 2020b). Using 10 years of environmental and food web data from the PIE long-term food web transects, we identified potential drivers of trophic niche metrics for multiple species over time and across space. We observed that while many factors correlated with species' niche metrics over time, these factors differed across space, due to contrasting environmental context, and between species, due to different modes of consumption and requirements for specific blends of production channels. Environmental factors also acted on niche components independently, highlighting the complex influence of context on food web variability (Fig. 16). In addition to SLR and changing habitats within the marsh, the ocean boundary of the Plum Island Ecosystem LTER, the Gulf of Maine, is warming faster than almost any other part of the world's oceans (Pershing et al. 2015). Because of this warming, southern species are now expanding their ranges into the Gulf of Maine and PIE. We have documented at least two range expansions into the PIE-LTER: the blue crab, *Callinectes sapidus*, and the mud fiddler crab, *Minuca* (formerly *Uca*) *pugnax* (Johnson 2014, Johnson 2015). Because we documented the range expansion of fiddler crabs as it occurred, we were able to examine how range expansions impact both the response of the species expanding their range as well as the impact on the ecosystem. Fiddler crabs are now found throughout Plum Island marshes, and since they were first discovered in 2014 their populations have, on average, tripled each year (Martínez-Soto & Johnson 2020) (Fig. 17), suggesting this climate migrant has successfully colonized PIE. We found that fiddler crabs at PIE are larger (Johnson et al. 2019), have escaped most of their parasites (Johnson et al. 2020a), and are better

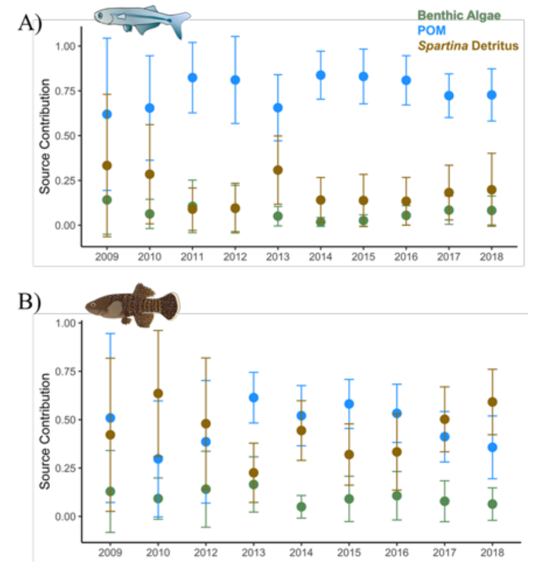


Fig. 16. Use of different resources over time by silversides (A) and the mummichog (B) determined using stable isotopes (X+/- SE).

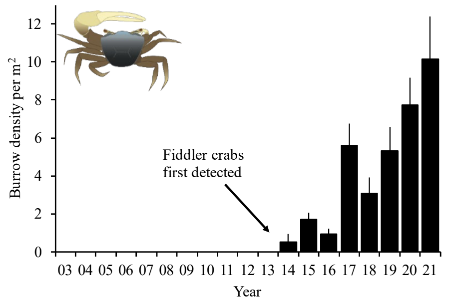
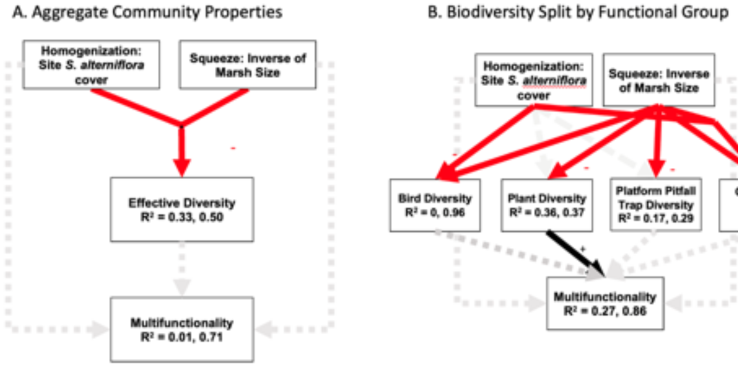


Fig. 17. Change in borrow density of fiddler crabs since they were first detected in 2014.

burrowers (Wong et al. 2021) compared to crabs south of Cape Cod (their historical range boundary). The fact that fiddler crabs in PIE are better burrowers than their historical range counterparts suggest local factors in PIE such as sediment strength may result in adaptation or phenotypic plasticity. We also documented ecosystem impacts, for example, fiddler crabs can reduce benthic microalgal biomass up to 70% (Johnson et al. 2020b). Crabs also have indirect negative effects on sediment strength but positive effects on *S. alterniflora* biomass and decomposition rates, suggesting increasing populations could have future impacts on marsh geomorphology, carbon cycling, and shift the abundances of multiple species connected indirectly through species interaction webs (Roy 2022).

Fig. 18. Contrasting SEMs of how marsh homogenization and squeeze combine to influence A) aggregate community properties, where effects combine to reduce net site effective diversity but have neither direct not indirect effects on multifunctionality, compared to B) functional group biodiversity, where not only do effects lower diversity of multiple functional groups of species, but by lowering plant diversity, multifunctionality is decreases as well (red arrows, negative interaction, black positive, dotted, no significant interaction).



We sampled marsh units of different sizes and elevations to understand how conversion to only low elevation marshes homogenization), and marsh loss (squeeze) will drive marsh community structure and ecosystem multifunctionality (Byrnes et al. 2014). Summarizing multiple ecosystem functions into a single metric has proven problematic, requiring development of metrics based on Hill numbers and Maximum Entropy approaches (Byrnes et al. Submitted). Data analyzed with Structural Equation Modeling revealed that marsh squeeze and homogenization work in concert to decrease species diversity, but depending on which functional groups change, will either maintain or decrease in multifunctionality (Fig. 18). Reducing marsh area lowers the abundance and diversity of both marine and terrestrial consumers with concomitant decreases in predation and herbivory rates. Smaller marshes have lower plant diversity and loss in plant diversity appears to be the primary driver of shifts in multifunctionality, supporting the concept that marshes are driven by bottom-up ecological processes.

We advanced the maximum entropy production (MEP) based biogeochemistry model by including photoautotrophy and photoheterotrophy with our other reactions thereby realizing the full functional representation of microbial metabolisms; the model was extended to 1D space as well (Vallino & Huber 2018). To test the model's ability to capture microbial metabolism in complex environments, we used data collected from a meromictic pond (NSF award #1451356) that functions as a vertical estuary and exhibits all 5 modeled functional metabolisms. The MEP-based model accurately simulated the observed biogeochemical profiles and microbial functional groups. The model also demonstrated the importance of temporal strategies, such as circadian rhythms, and organization over space. These results support our main conjecture that living systems differ from abiotic processes in that the former increases entropy production over the latter by organizing over time and space, which we view a as a major contribution to understanding microbial biogeochemistry.

PIE Products

The PIE LTER has a total of 579 publications in its bibliography. Since 2016 we published 139 journal papers, 6 book chapters, and produced 7 Ph.D and 12 MS theses. We currently have 583 data sets in publicly available data repositories (*see data management plan*). Our data sets are frequently downloaded; between 2019-2021 we had more than 40,000 downloads of 515 separate data sets from EDI, and 1355 downloads from AmeriFlux for the period June 2019-February 2022.

PIE's Top 10 papers

Data sets associated with each paper are listed in the bibliography. Letters designate LTER core areas A) primary production, B) population dynamics and trophic structure, C) Organic matter accumulation D) Inorganic inputs and nutrient movement, E) Patterns and frequency of disturbance.

1. **Fagherazzi et al. 2020** *This invited review article focuses on what is currently known about physical processes and discusses quantitative models used to determine the future marsh evolution under accelerated sea level rise.* ***C, E***
2. **Nelson et al. 2019** *This paper illustrates the interaction between geomorphology, biogeochemistry and food webs.* ***B, E***
3. **Lesser et al. 2020** *This paper illustrates the importance of high marsh access to fish and the interactions between biogeochemistry and food webs*. **B,E**
4. **Johnson et al. 2020** *This paper illustrates the complex biotic responses to changing climate and illustrates how the enemy release hypothesis can apply to parasites for when organisms have planktonic larvae.* ***B***
5. **Wollheim et al. 2018** *This paper presents a modeling synthesis of dynamics of the entire river network across flow variability.* ***D, E***
6. **Hopkinson et al. 2018** *This paper synthesized data from the marsh, watershed and estuary to demonstrate marsh edge erosion is important for salt marsh vertical accretion in marshes with low sediment inputs from the watershed.* ***A, C, D, E***
7. **Forbrich et al. 2018** *This paper assessed how climatic variations affect marsh-atmosphere exchange of carbon dioxide in the short term and compared it to long-term burial rates based on radiometric dating. The data illustrated the importance of precipitation to net carbon exchanges in marsh systems and suggested substantial marsh carbon export.* ***A, C, E***
8. **Bulseco et al. 2019** *This paper used a controlled flow-through reactor experiment to test the role of nitrate as an electron acceptor, and its effect on organic matter decomposition and the associated microbial community in salt marsh sediments.* ***C, D***
9. **Bowen et al. 2020** *This paper synthesized many fertilization studies and shows that the form of nitrogen added impacts the influence it has on primary production and decomposition and helps resolve apparent contractions in the literature.* ***A, C, D***
10. **Pontius 2019** *This paper presents new quantitative methods and software to characterize error and temporal change for a categorical variable and applies the methods to analyze maps of land cover for our study region.* ***E***

Broader Impacts

PIE-LTER has a robust program in outreach and education; nearly every PI is involved in education or outreach at the local, regional, or national level. Highlights from our current grant are listed below.

● ***PIE-LTER Schoolyard Program*****:** In 2020 David Moon, Mass Audubon, took over our K-12 Schoolyard program after the death of Liz Duff. Our schoolyard program provides experiential learning opportunities for students and teachers in their own backyard. The program serves approximately 1,000 students and 50 teachers per year in grades 5-12 in 10 schools. Each year students present their findings at a conference attended by PIE scientists. In 2020 the Salt Marsh Science project continued with new staff and a limited number of schools due to COVID. Five schools continued data collection on six sites. In 2021 the project was restored to ten schools covering twelve sites, and an online symposium engaged students to present and hear about professionals' projects as have previous in-person symposia.

- ***Professional Development and Outreach**:* PIE scientists collaborated with former schoolyard director Liz Duff on a summer professional development course for teachers and by working with them to produce “Data Nuggets” and lesson plans for teachers. Currently we have created 9 data nugget lessons. Giblin ran a teacher professional development course in 2020 with the Wade Institute and Wollheim participated in the Museum Institute of Teaching Science course presenting a module on urban streams.
- ***Gulf of Maine Institute (GOMI)*****:** We have strong ties with the Gulf of Maine Institute, an NGO that provides training to teachers and helps them develop environmental community-based stewardship projects throughout the Gulf of Maine. Giblin is chair of the board and other members of the PIE team work with teachers and help edit the journal. With GOMI we are currently working with 5 Schools including Lowell Middlesex Academy (LMA). LMA is a charter school for students who previously dropped out of high school or are at risk of doing so and PIE has an RET at this school. GOMI is providing training for the teachers in community-based stewardship while PIE scientists are providing scientific support for teachers. All schools are within the watershed of the Merrimack River and we are emphasizing watershed connectedness through shared data collection activities.
- ***LTER-Children’s Book Series*****:** Titled "Save our Stream", (Polsky and Tucker, 2017) involves the story of two children who learn about the lawn-stream connection from a field scientist they encounter, and then take on the mission of educating their neighbors.
- ***Logan Science Journalism Course*****:** Each year 6-8 journalists participated in the 12-day hands-on Logan Science Journalism program on coastal eutrophication. This program offers fellowships to mid-career science journalists and immerses them in ecosystem and global change science. During this cycle both Deegan and Giblin have served as course directors.
- *Mentoring* ***for Grads and Undergrads*****:** During the summer, 10-14 undergraduate and graduate students work and live at our field house and many others come nearly daily from nearby colleges and universities. Students give presentations at the end of the summer on their work. This year we provided an opportunity for students to receive training in communication and outreach. Undergrads from Boston University and the MBL’s Semester in Environmental Sciences do part of their projects at PIE.
- ***Interactions with Policy Makers and Management Agencies*****:** PIE scientists serve on a large number of advisory boards of organizations interested in marsh sustainability, watershed issues and fisheries. Danielle Perry has taken over for Robert Buchsbaum as our local outreach representative and participates in many advisory groups including the Ipswich, Parker, Essex River Restoration coalition as a member of the Steering Committee. Wollheim is a member of the Ipswich Watershed Technical Advisory Committee and is co-chair of the Piscataqua Region Estuarine Partnership in NH, and member of the Portland Area Nutrient Group in ME. Fagherazzi advises managers of Parker River National Wildlife Refuge on the hydrology and geomorphology of salt marshes in Plum Island Sound and Giblin participates in meetings on salt marsh restoration and resilience with many local organizations and serves on the NEON STEAC. Tucker represents PIE as a member of The Great Marsh Coalition.
- ***Citizen Science:*** We continue to engage with volunteers in the watersheds to sample streams during the winter months, a relationship that has been ongoing for 15 years.
- ***Science and the Arts:*** In collaboration with the Parker River Fish and Wildlife service, PIE worked with two local artists to produce an exhibit “The Once and Future Saltmarsh” featuring three marsh inhabitants that have been affected by climate change: saltmarsh sparrow, fiddler crabs, and rainbow smelt. The exhibit was installed at the Parker River Wildlife Refuge in 2021.

Results of Supplemental Support

PIE received a supplement for an RET for a teacher from the Lowell Middlesex Academy (LMA) in 2019. The teacher, Melissa Chen, has been working with us for two years. The class focus was on the watershed concept and focused on water quality issues in Lowell. Due to COVID we were not able to do as much water sampling with the students as we had planned. However, we were able to support Ms. Chen and the students through zoom, and sent her class "Foldscopes", small microscopes the students build themselves, to explore their own backyard. By 2021 we were able to run several sampling trips with them and arranged for many of the students in the entire school to get an introduction to Plum Island marshes and learn about SLR with hands-on exercises. We continue to work with Ms. Chen and she is participating in the Emerald Web program through our association with GOMI.

PIE received an equipment supplement to replace obsolete equipment. We purchased 5 water quality sondes for continuous monitoring in the estuary, replaced data loggers on the weather station and eddy flux tower, refurbished sensors for the eddy flux tower and purchased a new fluorometer.

LTER and Other Cross Site Synthesis Activities

PIE PIs contribute to several LTER cross site comparisons and synthesis working groups. PIE investigator David Johnson is a member of the LTER CoRRE synthesis group (Community Responses to Resource Experiments). CoRRE has compiled 101 datasets, largely from LTER sites, of resource experiments on grasslands – including salt marshes – from across the globe to understand the impact of global-change drivers on community structure (Avolio et al. 2021; Komatsu et al. 2019, Langley et al. 2018). Jarrett Byrnes is a member of the NCEAS LTER-NCO Scaling-up productivity responses to changes in biodiversity working group (Hautier et al. 2020, O'Connor et al. 2021). Jim Morris participated in the cross-site study on the "Responses of Coastal Ecosystems to Climate Change: Insights from long-term ecological research (Reed et al. 2022). Johnson was on a study that investigated connectivity (Iwaniec et al. 2021) and Wollhiem participated in an LTER led study on the ecological impacts of COVID (Gaiser et al. in press). Fagherazzi and students participated in a comparison of the geometry of channel networks at PIE, VCR, GCE and marshes in the US and China (Liu et al. 2022) and collaborated with USGS on feedbacks between marsh extension and sediment dynamics (Donatelli et al. 2020). Deegan, Nelson and students participated in a national cross-site synthesis effort that used insights from LTER and other sites to examine saltmarsh and estuarine ecosystem function that resulted in 4 papers (Colombano et al. 2021, Waltham et al. 2021, Ziegler et al. 2021, Baker et al 2020).

III. Response to the Mid-Term Review

The mid-term review committee was very positive. They were supportive of the conceptual model and found that the research team had been very productive. The review team had suggestions for improvement in three areas (1) integration across projects, (2) internal communication, and (3) succession planning. Some of these we addressed immediately, and some are addressed in this renewal.

1. Improved Integration - The review team suggested that the site had matured to the point where we should strive for more integration and suggested increased modeling, as well as the development of material budgets across systems, as ways to improve integration. While they were supportive of the Hydro-MEM and MEP modeling activities they did not see the models making use of much long-term PIE specific data or providing cross group integration. We have now completed Hydro-MEM using PIE data (Alizad et al. submitted, Fig. 7) so the results of Hydro-MEM describing changes in vegetation cover will be used to set up the future scenarios for our biogeochemical model. We continue to use MEP to address important biogeochemical questions in PIE V but currently the model is too computationally expensive to integrate across the watershed- marsh-estuary domain in a dynamic way. Therefore, we have added two new integrative modeling projects in this round, one to better link element budgets with changes in geomorphology from the watershed to the ocean (*see QI*) and one to better link geomorphology and food webs (*see QII*). We are also focusing on some major gaps in the material budgets to work toward closing nutrient and carbon budgets in the future. The entire team will collaborate on a major restoration experiment which will serve as a test of our understanding of how changes in hydrology alter material and energy fluxes. Finally, as documented above, we have contributed to many cross-system syntheses projects in the last 3 years.

2. Communication - A second concern was about internal LTER communication, especially among non-PIs, and the lack of a central mechanism for assuring that everyone had been fully informed of safety procedures, Title IX issues, and other information. This was indeed a deficiency that we worked to address immediately. We now have a new registration process to assure everyone is aware of all policies and safety procedures and receives all necessary training. We adopted a Code of Conduct and established a DEI committee. The committee also recommended that we find a way to allow non-PIs to keep more informed of each other's work beyond the annual meeting, especially graduate students. We have established a graduate student listserv and increased graduate student communications through social media and will sponsor an annual graduate student symposium (*see management section*).

3. Succession Plans - The review team was concerned that we did not have enough time to recruit and hire a new Lead PI, or Co-lead PI to MBL in time for the next submission. This did indeed prove to be a challenge, particularly with COVID slowing down the process. However, we were able to successfully recruit a new faculty member, James McClelland, who is experienced in LTER oversight and has highly relevant scientific expertise. McClelland will be joining the MBL in summer 2022 and is prepared to take on increasing leadership responsibilities at PIE over the next six years. Giblin will continue as lead PI during the transition, with McClelland being appointed as Co-lead by year 4 and taking over as Lead PI for the next renewal. (*see site management*).

IV. New Research

In PIE V we organized research around three questions that build on previous findings and integrate long-term studies with new observations, experiments, and model development. Activities within the three questions are coordinated across our traditional research groups and integrate across the entire watershed-marsh-estuary domain to facilitate a broader synthesis. New collaborations will allow us to take advantage of a large-scale marsh restoration to test some of our hypotheses on feedback mechanisms within the system (Fig. 19).

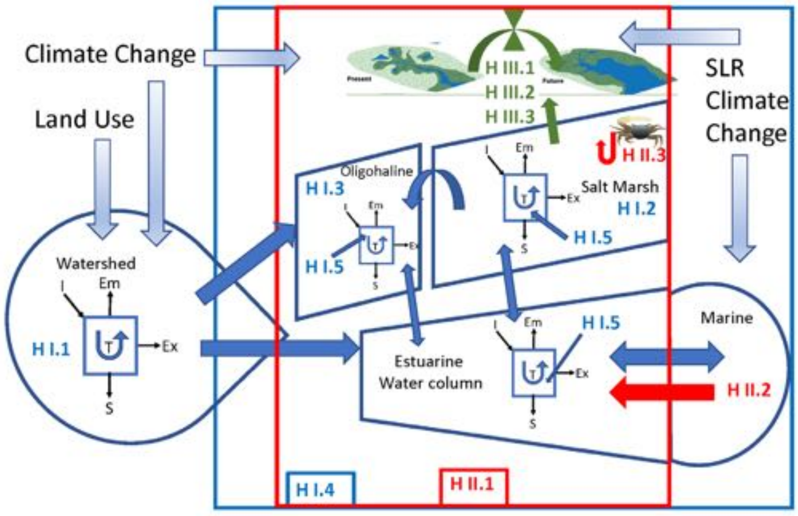


Fig. 19. The conceptual diagram of PIE V. In all questions we consider how the drivers of land-use change, climate change and sea level rise (SLR) are impacting the systems. For Q I (blue), in the watershed, estuarine water column, saline marsh and oligohaline marsh we will examine the inputs (I), storage (S), transformations (T), emissions (Em) and export (Ex) of carbon and nutrients. In Q II (red) we will examine how energy available to consumers changes across habitats and with climate change as new food webs are altered by species switches. For Q III (green) we will examine how internal feedbacks may accelerate or resist changes to the fate of the emergent marshes and alter the after of carbon, nutrients, and energy. (see text for details on the individual hypotheses).

The first question, ***QI. How are the sources and fates of organic matter and nutrients in the linked watershed/estuary system being altered by changing land use, SLR, climate, and geomorphology?*** examines the role of marshes in influencing estuarine production and water quality (Valiela and Cole 2002) and the role of coastal ecosystems in modulating carbon and nutrient fluxes to the nearshore ocean (Najjar et al. 2018). We will continue to examine material inputs (I), losses due to export (Ex) and gaseous emission (Em), transformations (T) and storage (S) in the watershed (H I.1) and saline marsh (H I.2). In this renewal, we will also expand work in the oligohaline marsh (H I.3). We will make continue and expand extensive measurements of dissolved and particulate constituents in the main estuaries to complement ongoing measurements of estuarine metabolism, and conduct focused lateral (tidal) exchange measurements at the whole estuary and sub-estuary scales to test linkages between watershed inputs, marsh-estuary connectivity, and the net system retention and/or export of carbon, sediments, and nutrients. These measurements will be used in a linked biogeochemical and hydrodynamic model to integrate nutrient, sediment, and carbon flows and to test our understanding of the processes governing the coupled biogeochemical cycles throughout the entire system (H I.4). We will focus more on DON and N_2 fixation as important but understudied parts of the N budget (H I.5). The second question, ***Q2*. *How will food webs and energy flow respond to new geomorphic configurations, SLR, and changing climate?*** will be a new integrative effort to determine how the landscapes' ability to produce energy changes with habitat. Given the importance of coastal ecosystems in supporting nearshore foodwebs, understanding how this link will change with climate, land use change and SLR is critical. By creating "E-scapes" we can examine how a changing geomorphic configuration alters food webs and niche space (H II.1). We will continue to follow changes in the food web structure triggered by climate change (H II.2) and the impacts of range expansions (H II.3) on food webs. Finally, our work showed that while we are seeing some shifts in the vegetated marsh, some areas seem to be resisting change, while others are changing rapidly. Thus, we are asking: ***Q.3 What internal feedbacks might accelerate, slow down, or even reverse the predicted changes in emergent marsh configuration and the fate of carbon, nutrients and energy?*** We urgently need to understand more about internal feedbacks that might increase marsh resilience or compromise marsh survival as sea levels rise, the system warms, and watershed inputs continue to change. These feedbacks include differences in the plant genetics and traits that impact plant growth, survival, and competition (H III.1); plant and microbial processes in the rhizosphere that help mediate carbon storage and nutrient fluxes (H III.2); and the arrival of new consumers, such as fiddler crabs, that may impact biogeochemistry and geomorphology (H III.3).

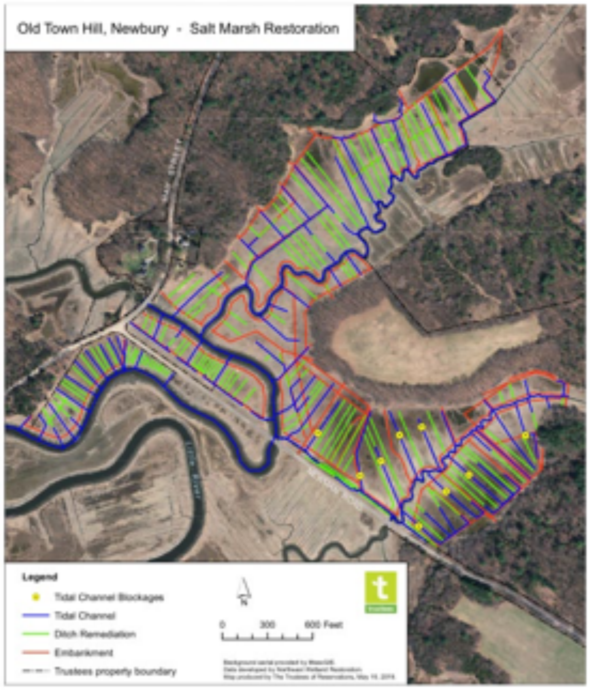


Fig. 20. A map showing modifications made to the salt marsh of Old Town Hill, Newbury which is owned and managed by the Trustees of Reservations. Map courtesy of Northeastern Wetland Restoration

An important alteration in the PIE system that may slow down or reverse vegetation changes caused by SLR is marsh restoration activities. Restoring marsh hydrology to more natural conditions can increase marsh resilience to SLR by boosting accretion rates and increasing carbon storage, although many questions remain (Poppe and Rybczyk 2021). Currently, Northeast Wetland Restoration is working with the Trustees of Reservations to restore natural hydrology to portions of the Great Marsh marsh by filling some ditches and opening others on approximately 100 acres in Old Town Hill (Fig. 20). UNH researchers Drs. Burdick and Moore carried out the baseline assessments in 2019 prior to restoration that was started in 2021. They will continue to measure water levels, plant cover, and sediment accretion annually with separate funding. We will take advantage of this restoration activity by making additional measurements as detailed below, to test hypotheses on biogeochemical and geomorphic feedbacks.

I. How are the sources and fates of organic matter and nutrients in the linked watershed/estuary system being altered by changing land use, SLR, climate and geomorphology?

H I.1 Increase in climate variability and beaver pond extent throughout the watersheds will lead to greater organic carbon and nitrogen exports relative to inorganic nitrogen exports, offsetting the effects of increasing suburbanization and dam removals.

Rationale: Freshwater ponds and wetlands in watersheds play an important role in controlling the transfer of nonpoint sources from anthropogenic land uses to coastal zones and can be important sources of greenhouse gases (Holgerson and Raymond 2016, Hansen et al. 2018, Schmadel et al. 2019). Suburban land use and population continue to grow in the PIE watersheds, while at the same time, beaver ponds are expanding (Whitney et al. 2021, Fig. W1), human reservoirs in the watersheds are declining (as seen in New England more generally; Gold et al. 2016), and climate variability is increasing (Huntington et al. 2009). This complex array of changing drivers has differential impacts on the sources and fates of carbon and nutrients in the watershed which affect the amount, form, and ratios that reach the estuary (Whitney et al. In Review, Bower 2020, Carey and Fulweiler 2013). Currently, annual export to the estuary of nitrate is declining, phosphate is increasing, while most other material exports, including organic nitrogen are without trends (Fig.8). At the same time, our monitored suburban headwater streams do not show these same trends, suggesting a changing transformation potential within the river network. Climate variability is an important driver of export variability, making subtle trends difficult to detect. Human reservoirs remove nitrate, but also produce DON and PON, so their impact on TN fluxes are minimal (Whitney et al. In Review).

We hypothesize that the strong decline in nitrate is due to the expansion of beaver ponds since the early 2000's (Fig. 4) when beaver populations exploded in response to changing trapping laws (MA DFW 2014). In contrast to human-built reservoirs, beaver ponds are abundant in headwater streams, representing a shift in water storage and transformations in the landscape. Beaver ponds are generally sinks for nitrate when inputs are high but may also be net sources of DOC and DON regardless of input levels, especially during wetter years. Thus, the ratio of organic to inorganic nutrients is increasing. Further, the transition to more beaver ponds suggests the watershed may be transitioning to a greater source of greenhouse gas. While river network scale modeling indicates beaver ponds may contribute to the declining nitrate trends (Wollheim et al. 2018), we have not studied beaver pond ecosystems in sufficient detail to test whether they are the mechanism for this decline, what the fate of this nitrogen is, the impact on greenhouse gas evasion, or whether these trends will continue as beaver ponds age.

Approach. We will focus on how carbon and nutrient sources, storage, gas emissions, and downstream fluxes in the watersheds are changing as a result of ongoing land use changes. The major new approach needed to address this focus and H I.1 is a greater emphasis on sampling beaver ponds of different types. We have begun measuring biogeochemical transformations in one beaver pond located in our reference catchment (Cart Cr.) that has low nutrient inputs (Whitney 2017). We will add monthly sampling in two additional beaver ponds located downstream of high nutrient inputs, to evaluate their net effect on inorganic and organic nutrients across seasons and flow conditions. We will target several storms at each beaver pond using Sigma autosamplers to understand how storm events affect beaver pond biogeochemical transformations. We will also sample these three beaver ponds for greenhouse gas emissions (dissolved and ebullitive CO_2, CH_4, N_2O) using techniques we use in stream channels (Robison et al. 2021, 2022). Finally, we will also conduct seasonal synoptic surveys of beaver pond chemistry (n=10-20 sites) distributed across a gradient of land use and pond ages (see below) and compare these relationships to those in non-beaver influenced streams, similar to the headwater catchment surveys we have done before (Wollheim et al. 2005, 2015a,b).

Beaver ponds differ in their size, landscape position, and age, all of which may also affect their hydrological and biogeochemical impacts (Burchstad et al. 2010). We will build on the coarse maps of wetland change at watershed scales (Whitney et al. 2021; Figure W1) to identify specific ponds and their ages using time series of aerial imagery. Critically, these images start in 1995 prior to the beaver pond expansion. We will continue our long-term monitoring at the current five stream locations: the three headwaters representing the dominant land uses (suburban, forest, wetland), and the two basin mouths (Parker and Ipswich) to continue tracking trends and variability both due to land use dynamics and climate (e.g., Fig. 9). For the first time we will deploy high frequency nitrate, conductivity, and fDOM sensors at these locations, in conjunction with similar deployments in the estuary (*See H I.4*), to quantify transformations along the watershed to estuarine continuum using approaches outlined in Wollheim et al. (2017) and Mulukutla et al. (2022). We will continue analyzing all major forms of inorganic and organic nutrients (NO_3^-, NH_4^+, PO_4^{3-}, TDN, TDP, DOC, PN, PC, PP) as well as Cl^-, and SO_4^{2-}. We will take samples of DOC and DON for additional analyses of DOM composition and lability (*See H I.4 and I.5*) to assess the biological impact of watershed DOM on estuarine nutrients and metabolism. We have only periodically sampled for silica but given its responsiveness to land use change (Fulweiler and Nixon 2005, Carey and Fulweiler 2013) and importance in coastal ecosystems (Humborg et al. 2000, Garnier et al. 2010), we will begin regularly analyzing dissolved and biogenic Si at all locations. Findings from the expanded beaver pond work will be used to add beaver pond dynamics to existing river network models of the PIE watersheds (Stewart et al. 2011, Wollheim et al. 2018, Huang et al. 2022) to test whether beaver ponds explain the decline in nitrate and changes in other biogeochemical fluxes.

H I.2 In the near-term, salt marsh primary production rates will increase as SLR leads to a transition from high marsh to more frequently flooded low marsh but production may become increasingly variable between years due to climate change.

Rationale: The productivity of coastal wetlands depends on numerous biological and abiotic factors (Mendelssohn and Morris 2000) that are likely to change in the future. One of the most important is the elevation of a site relative to mean sea level (Morris et al. 2002), because it determines flooding frequency and duration and thus influences soil salinity and sulfide levels. This interdependence of elevation, abiotic controls, and plant productivity leads to a site-specific optimum elevation for marsh vegetation (Morris et al. 2013). Low marshes at PIE (*S. alterniflora)* are situated above this optimum elevation, so we expect that with increasing sea levels, low marsh productivity will increase as the high marsh *S. patens* is replaced by more productive *S. alterniflora* (Morris et al. 2013; Fig. 6). Marsh carbon fluxes (NEE, GPP, R) at PIE show generally higher net CO_2 uptake in the low marsh than in the high marsh areas (see above) but are strongly influenced by meteorological drivers. For example, high marsh productivity is strongly influenced by rainfall - especially when tidal flushing occurs infrequently (Gross 1990, Forbrich et al. 2018). While the low marsh did not show an obvious response to rainfall, it responded more strongly to an extreme drought (in 2016; Fig. 12) than the high marsh, indicating different sensitivities to climate change stressors in the two marsh forms.

Approach: We will continue to measure biomass monthly during the growing season. We have two long-term sites, a low elevation site dominated by *S. alterniflora* and a higher *S. patens* site and we recently established a new *S. alterniflora* site that is higher in the tidal frame and where we expect to see the most rapid changes. We will continue to monitor marsh-atmosphere CO_2 exchange with two flux towers, one in the high marsh and one in the low marsh (Fig. 12) that are also part of the AmeriFlux network. We will develop a Bayesian framework to assess different timescales and lags in external and internal controls of net ecosystem exchange (Ogle et al. 2015) to better isolate climatic drivers. We will add sensors to continuously monitor redox potential and salinity at the flux tower sites to detect abiotic stressors in situ, which we expect will help explain more of the variability in CO_2 fluxes not currently accounted for by climatic drivers alone. *Restoration sites* - With other funding Burdick and Moore are monitoring biomass and vegetation changes at restoration sites as the hydrology is restored to more natural drainage. The

LTER will put in additional sensors to monitor redox and soil salinity at their sampling locations to help us understand marsh response to the restoration.

H I.3 The role of the upper oligohaline zone in buffering nutrient and carbon inputs to the lower estuary will decrease with SLR unless higher discharge can prevent increasing salinization.

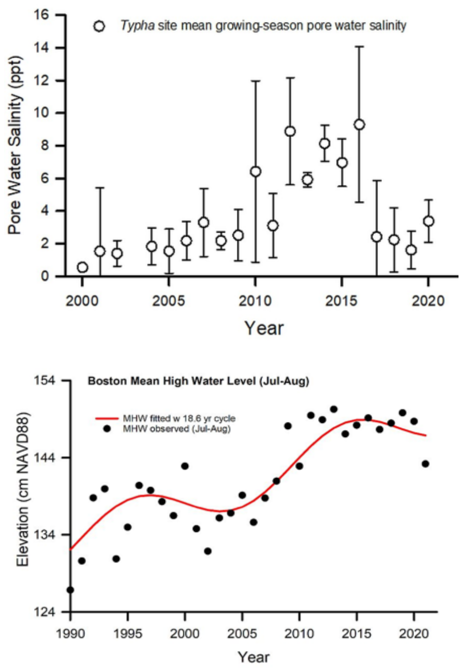


Fig. 21. Shows interannual variability of salinity in the *Typha* zone (top). Salinity responded to the rapid increase in mean high high water (MHHW; bottom) but began to decrease again due to an increase in runoff (Fig. 9). MHHW is altered both by SLR and by the 18.6 year cycle in tidal amplitude

Rationale: Oligohaline channels and marshes connect the watershed to the rest of the estuary and, despite their relatively small spatial extent, have a disproportionate influence on estuarine metabolism (Hopkinson et al. 1999), and nutrient cycling (Merrill and Cornwell 2002). The dominant plant in the oligohaline zone, *Typha angustifolia,* is highly productive, P limited (Morris and Sundberg 2003), and characterized by much lower levels of porewater nutrients than the saline portion of the estuary. In subtidal sediments, fluctuating estuarine salinity strongly controls sediment nutrient release by both physical processes and by decreasing N loss from denitrification (Weston et al. 2010, Giblin et al. 2010). We expect an even stronger influence on the vegetated marsh where salinity will impact primary production and nutrient cycling. While salinity changes in oligohaline marsh porewater are more muted than in the estuary, they do change seasonally and show pronounced variability between years. The long-term record shows porewater salinity increasing from 2008 to 2017 corresponding to a period of rising tidal amplitude, and low river discharge (Fig. 21, Fig. 9). Tidal amplitude is now decreasing, and a recent increase in precipitation and river discharge has reversed this salinity trend demonstrating the important influence of the watershed on this zone. However, as mean sea-level continues to increase we expect the oligohaline zone will experience more frequent high salinity intrusions. We have not measured rates of nitrate reduction in the oligohaline marsh of PIE, but based upon work in other low salinity marshes (Upreti et al. 2022), we expect denitrification currently dominates over DNRA in the oligohaline marsh. This contrasts with *Spartina* marshes where denitrification and DNRA are of equal importance (Koop-Jakobsen and Giblin 2010). If SLR leads to an increase in the salinity of the oligohaline marsh we expect that DNRA will be increasingly favored over denitrification leading to greater exports of N. A decrease in primary production may decrease C exports and increase P and Si export. Approach: We will continue our long-term monthly marsh porewater measurements of nutrients (and including Si), salinity and DOC at representative locations in the oligohaline marsh. In the adjacent water column we will measure oxygen, salinity, temperature, pH, fDOM, TSS, and chlorophyll fluorescence (*See H I.4*). We will measure rates of N$_2$ fixation in the oligohaline zone sediments over a seasonal cycle (*see H 1.5*) and conduct estuarine transects for dissolved constituents (*H I.4*) with emphasis on assessing changes in the fluxes of C, N, P and Si. We will measure denitrification and DNRA in the oligohaline zone over a seasonal cycle using established methods (Koop-Jakobsen and Giblin 2010). We will measure *T. angustifolia* biomass monthly during the growing season and equip the site with a new flux tower with a DOE funded study. This new monitoring site will record marsh-atmosphere CO$_2$ and CH$_4$ exchange as well as continuous soil biogeochemistry data (salinity, redox profiles, water levels, but see H3.2 for additional small-scale biogeochemical measurements). We will use this data to parametrize the response of productivity to salinity variation in the E3SM Land Model (ELM).

H I.4 As low marsh area increases, there will be an initial increase in connectivity between the marsh and the estuarine water column leading to greater inorganic nutrient uptake by the marsh, more organic matter export and changing nutrient ratios in the estuary.

Rationale: As detailed above and in H I.2, low-elevation *S. alterniflora* marshes appear to be more productive and are stronger sinks for nutrients from the water column than high-elevation marshes. Both systems appear to release small amounts of DOC and little or no DIC (Fig. 13). Given the large ratio of marsh to estuarine area in the Plum Island system, there should be little net N or P export to the coastal ocean, small losses of DOC, and low or negative DIC export. However, while we have solid measurements of nutrient and carbon exchange at the creekshed scale, we do not have a strong understanding of export from the entire Plum Island system. Our measurements may be strongly influenced by processes occurring in the very shallow creeks of our study sites. Our estimates of carbon export based on the difference between eddy covariance measurements of NEE and carbon accumulation in marsh soils are not matched by observations of carbon export during lateral exchange measurements (Fig. 13). Additionally, studies at the whole system scale suggested both DIC and DOC export from the Plum Island estuary as a whole (Raymond and Hopkinson 2003) and other studies have found that coastal systems are a large source of carbon to the coastal ocean (e.g. Wang et al. 2016).

In addition to C, N and P, we need a clearer understanding of silica (Si) cycling across the land-ocean continuum as it is important to our broader understanding of marine primary production, higher trophic level productivity, and global carbon cycling (Armburst 2009, Tréguer et al. 2021). However, the role of salt marshes in estuarine Si cycling is still an open question (Giblin et al. 2021). Salt marshes are considered hot spots of Si cycling because *Spartina sp.* sequester Si throughout their tissues. Observations in both fresh- and saltwater tidal wetlands have found them to be sinks of biogenic Si (BSi) and sources of dissolved Si (DSi) to adjacent waters (Struyf et al. 2006, Vieillard et al. 2011). Carey and Fulweiler (2014) found that a northeast salt marsh provided an order of magnitude more DSi than nearby rivers in spring, a time when estuarine waters are often depleted in DSi because of uptake and low watershed inputs, suggesting that marshes may be a critical source of DSi supporting spring diatom blooms. Approach: We will collect data on water column concentrations allowing us to create a detailed spatial and temporal picture of dissolved and particulate constituent concentrations throughout the estuarine system using both continuously recording sondes and by sampling along estuarine transects (Fig. 22). This will be combined with measurements of whole-

Fig. 22. A map showing the location of stations for water column sampling on transect surveys (white line), location of sondes in the estuary and watershed, and the location of stations for water column primary production, and benthic fluxes.

system aquatic metabolism along the same transects. Measurements of pelagic production and respiration, and benthic respiration and nutrient release will be made at selected stations. Because of the importance of DOC and DON in our system we will put additional emphasis on understanding their sources and fates (*see below and H I.5*). We currently measure marsh estuary exchange at the creekshed-scale using lateral/tidal exchange mass balances (Fig.13; Hopkinson & Weston in review). In PIE V we will transition to measuring tidal exchange at a cross-sectional transects at the mouth of Plum Island Sound and the estuarine mouths of the three main tributaries (Parker, Rowley, and Ipswich Rivers) expanding on previous studies (Zhao et al. 2010). Water quality models have been used to successfully develop comprehensive constituent budgets, estimate export to the ocean, and to separate out marsh contributions to estuarine carbon budgets (e.g. Clark et al. 2020). We will use the 3-D numerical hydrodynamic model Delft3D (Lesser et al. 2004) coupled with the estuarine biogeochemistry "water quality" model D-Water Quality to integrate information on watershed biogeochemical inputs, estuary-marsh interactions, water column transformations, and exchange of nutrients with the ocean (e.g. Fagherazzi et al 2013).

Water column constituents will be collected along transects from the ocean up all three estuaries three times per year (spring, summer, and fall), and include all dissolved and particulate forms of nutrients (now including DSi and BSi), DOC, DIC, particulate C and suspended sediments. During the transects we will also take measurements of dissolved oxygen, temperature, conductivity, fDOM, pH, chlorophyll, and turbidity with an YSI Exo sonde. These data will be supplemented with continuously collected data from 4 fixed stations, 3 in our three long-term stations along the Parker River and open Sound, and a new one in the upper Rowley River using these same sondes. These continuous measurements match similar measurements in the freshwater mouth of the Parker and Ipswich Rivers (*see HI.1*). The high frequency data will allow us to account for storm event scale responses (Mulukutla et al. 2022), an important consideration as storm frequency changes.

To calculate spatial patterns and whole-system aquatic metabolism (respiration and production) we will measure dissolved oxygen, salinity and temperature along a transect from the coastal ocean up to the head-of-tide in the Rowley, Parker, and Ipswich estuaries over five successive dawn and dusk periods. We will measure water-column production and respiration with light/dark bottle incubations at 9 stations three times per year (spring, summer, and fall). We currently conduct benthic flux measurements in the Parker, and we will expand them to include Rowley and Ipswich stations in years when these estuaries are the subject of intensive input and output budgets.

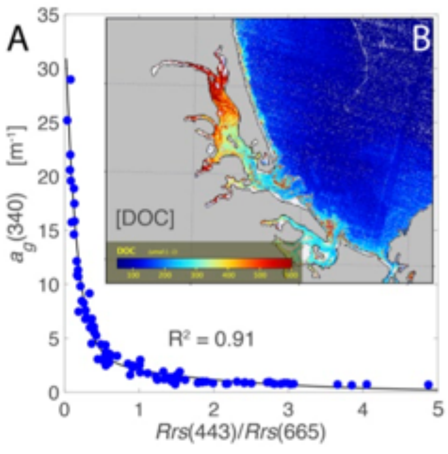


Fig. 23. (A) Relationship between the $a_g(340)$ (absorption coefficient of CDOM at 340 nm) and blue-red remote sensing reflectance ratio Rrs(443)/Rrs(665) for a range of samples collected in the estuary and the GoM. (B) Map of DOC concentrations applying the algorithm in panel (A) and the a_g (340)-DOC relationship (not shown) on an atmospherically corrected Landsat-8 Scene.

Tidal exchange will be measured by towing an acoustic doppler current profiler across the transect every 15 minutes from low-tide to the subsequent low-tide (~12 hours). Water samples will be obtained at several locations and depths across the transect on a similar timescale. By combining discharge information with measured concentrations, we will determine the net transport of carbon, nitrogen, phosphorus, silica, and sediment during flood and ebb, and by difference, the net tidal exchange of materials. These measurements will be conducted three times per year (spring, summer, fall) at the mouth of Plum Island Sound and one of the tributaries each year (Rowley, Parker, and Ipswich); the tributaries will be rotated such that we will focus on import/export of one each year.

We will leverage work on DOC by Fichot with complementary funded research (NASA and NSF) to inform and validate the modeling of carbon export and its transformations by microbes and photochemical processes. To supplement the transects described above, we will conduct monthly (April-to-November) transects along the salinity gradients of the estuaries, focusing on one estuary each year. These additional transects will have three primary goals. First, measurements of DOC and its optical properties (CDOM absorption coefficients and excitation-emission fluorescence matrices) will help better understand and constrain the relationship between DOC and its optical properties and its seasonal and spatial variability in PIE (Fichot and Benner, 2011) and help develop a robust optical proxy to optimize the accuracy of the DOC concentrations estimated from YSI fDOM sensors. Second, samples at selected locations along the transects will be collected for photochemical and microbial-incubation experiments. These controlled experiments will help determine quantitative measures of DOC reactivity (photomineralization, biomineralization, photo-biomineralization) and their variabilities in the estuarine system (Fichot and Benner, 2014, Zhu et al., 2020). These will be used directly to quantify DOC transformations from the marsh and rivers to the coastal ocean (Fichot and Miller, 2010, Fichot and Benner, 2014). Finally, measurements of water reflectance will also be collected to develop and validate optical algorithms applicable to existing and future high-spatial-resolution sensors (e.g., Landsat 8-9 and Sentinel 2A-B, GLIMR, EMIT). These algorithms will facilitate the mapping of DOC concentrations over the entire estuary with satellite imagery at 20 and 30-m spatial resolution (Fig. 23), to help understand how the spatial distribution of DOC in the estuary varies during different environmental conditions (e.g., river discharge, tidal stage), and will also provide data to validate the modeling results.

Modeling - We have previously used the hydrodynamic model Delft3D coupled to the wave model SWAN (Booij et al. 1999) to determine the high-resolution distribution of tidal currents and waves in Plum Island Sound. These coupled models allowed us to successfully study sediment dynamics within the PIE estuary (Zhang et al. 2019), the morphological evolution of the Sound driven by sea level rise (Zhang et al 2020), and to understand the complex feedbacks between hydrodynamics, bottom sediments, and microphytobenthos (Fagherazzi et al. 2014). Delft3D is the state of the art for sediment transport and hydrodynamics in coastal areas. The D-Water Quality module is used in conjunction with Delft3D to model biogeochemical processes, including carbon and nutrient cycling, and exchange of substances with the atmosphere (Deltares 2022). The biogeochemical modules and the hydrodynamic models are fully coupled thus allowing us to determine how changes in estuarine morphology affect biogeochemical cycles which is required to test this hypothesis. The model will be calibrated with field measurements from previous and proposed research spanning more than 20 years. Specifically, fluxes of nutrients from the watershed will be implemented at the river boundaries (e.g. Morse and Wollheim, 2014, Fig. 8), benthic fluxes will be calibrated with in situ data (Hopkinson et al. 1999, Giblin et al 2010, Weston et al. 2010, and ongoing), while nutrient sources and sinks within the marshes (e.g. Koop-Jakobsen and Giblin 2010; Hopkinson et al. 2018) will be included in the model. Model results will be tested with long-term biogeochemical surveys in the water column collected along the length of the estuary (e.g., Vallino et al 2005; Hopkinson et al. 2018) and the new data being collected as described above.

Once the model is fully calibrated and tested, we will quantify how future scenarios of sea-level rise will modify the exchange of nutrients and carbon between salt marshes, the open water sound, and the ocean. In a first set of scenarios, we will increase water levels thus transforming high marshes into low marshes. The model will quantify how rising sea level interacts with climate and watershed dynamics to affect estuarine nutrient uptake, primary productivity in the water column, exchanges within the estuary, and export to the ocean. In a second set of scenarios, we will progressively decrease salt marsh area to simulate degradation driven by climate change (see Donatelli et al. 2020). These simulations will quantify how marsh erosion affects nutrient recycling in the estuary.

Restoration Sites: A downscaled version of the hydrodynamic model with a finer mesh will be used to separately examine how water and sediment fluxes respond to the planned marsh restoration. Specifically, we will run simulations to determine how ditch remediation (i.e., closing man-made ditches), and microrunneling (i.e. the scouring of small channels to increase hydrological connectivity) are affecting sediment deposition on the marsh. To understand how restoration of marshes influences net tidal exchange, we will conduct in the restored creeksheds measurements of tidal carbon, nutrient, and sediment exchange 3 times a year in years 1,3 and 5.

H I.5 Changes in climate, sea level, and land use will increase the importance of net N fixation and DON mineralization as sources of reactive nitrogen to the marsh-estuary system

Rationale: While nitrogen cycling has been intensively studied in marsh-estuary systems, significant gaps in our understanding remain, especially concerning the production and mineralization of DON and the balance between N_2 fixation and gaseous losses due to denitrification (reviewed in Giblin et al. 2021). In this round we will focus on DON composition and mineralization in the water column and net nitrogen fixation in sediments.

Previous studies estimated that ~5% to 30% of river-supplied DOC is mineralized within estuaries (e.g. Uhlenhopp et al., 1995; Moran et al., 1999; Raymond and Bauer, 2000). While this makes a relatively minor contribution to estuarine carbon budgets, associated mineralization of river-supplied DON can be an important source of DIN (e.g. Knudsen-Leerbeck et al., 2017). Mineralization of ocean, marsh, and phytoplankton-derived DON also contribute to availability of DIN within estuaries, but their relative importance along estuarine gradients and how their contributions will change with climate, sea level, and land use are not yet known (Statham 2012).

Recent work by Hopkinson and Weston (in review) suggests that decomposition of river- and ocean-supplied DOC each account for about 10% of aquatic respiration within the Plum Island estuary, with a much larger but less well constrained contribution coming from decomposition of marsh-derived DOC. We anticipate that percentages of DON decomposed are larger (e.g., Petrone et al., 2009) for marsh and watershed DON inputs and that changes in climate, sea level, and land use will alter the relative contributions of DON as sources of DIN within the estuary. Work by Raymond and Bauer (2000) in the York River estuary demonstrated that proportions of DOC metabolized within the estuary varied from less than 5% to greater than 20% along the salinity gradient. These variations were, in part, attributed to changes in mixtures of DOC sources (i.e. river, marsh, ocean, and in-situ phytoplankton) and changing residence times. We anticipate that controls on DON mineralization will be qualitatively similar to those for DOC.

Net N fixation is the net balance between gaseous losses due to denitrification and gains due to N_2 fixation (in our system anammox is negligible; Koop-Jakobsen and Giblin 2009). Where both processes have been measured, denitrification often exceeds fixation (Merrill and Cornwell 2002; Valiela and Teal 1979, Foster and Fulweiler 2014), although there is growing evidence that the importance of N fixation in coastal sediments has been underestimated (Newell et al. 2016). In addition, studies have shown that the balance between N_2 gain and loss is influenced by external nitrate inputs so studies on N fixation in eutrophic systems may not reflect the balance in more oligotrophic systems (Li et al. 2021). A very rough N budget of the PIE marsh estuarine complex suggests that denitrification and burial exceed known inputs from the watershed and precipitation by nearly a factor of two. N fixation has not been measured but an N fixation rate on the order of 5 $g\ N\ m^{-2}\ yr^{-1}$ would be required to balance the budget. Given these large uncertainties, predicting how the system will respond in the future is difficult, but with declining DIN inputs from the watershed, the increasing terrestrial wetland area (Fig W1 and W2), and the expected eventual transition from a P limited oligohaline zone to an N limited salt marsh (H I.3), we hypothesize that N fixation will become increasingly important.

Approach – We will examine DON lability and associated release of DIN within the PIE system through analysis of spatial/temporal patterns in DON composition as well as incubation experiments. Water sampling for this DON work will be done in concert with the estuarine transect sampling for water quality (including DOC) described under hypothesis H I.4. Samples will be collected at all proposed stations for bulk DON analysis and at ~10 stations per trip for more detailed DON characterization. This work will leverage information about protein-like organic matter that can be gleaned from planned work on optical properties of DOM (e.g., Stedmon and Markager, 2005). In addition, we will measure amino acid composition using reverse phase high performance liquid chromatography (HPLC) and quantify contributions from other compound classes using ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Although these tools have not been used in past studies of the PIE system, we have successfully applied amino acid and FT-ICR MS analyses to study N sources and characterize organic matter in other systems (e.g. McClelland et al., 2003; Behnke et al., 2021). Amino acids, including free and combined forms, represent a major fraction of the characterizable DON compounds (e.g., Duan and Bianchi, 2007; Kutznetsova and Lee, 2002). As building blocks for labile peptides and proteins, total amino acid concentrations are correlated with organic matter quality. Amino acid composition also provides information about organic matter sources and degradation state (Dauwe and Middelburg, 1998; Liu et al., 2006; Liu et al., 2008). FT-ICR MS spectra consist of thousands of individual molecular peaks and intensities that will be evaluated in the mass range of 200–600 *m/z* (Koch and Dittmar, 2006). Molecular formulas will be assigned using PetroOrg software with mass errors of less than 200 ppb (excluding noise). While a comprehensive approach will be used to analyze the FT-ICR MS results, the subgroup of N-containing compounds will be given particular attention during this study. Samples for FT-ICR MS will be processed at MBL and run at the National High Magnetic Field Laboratory at Florida State University.

We have no data on N_2 fixation rates at PIE although genes associated with N fixation are present (Vineis 2022). We will begin with a broad survey of N_2 fixation in different habitats (low and high marsh, oligohaline zone, subtidal zones). While we are aware of the limitations of the traditional acetylene reduction assay (ARA; Fulweiler et al. 2015) we will use ARA calibrated with $^{30}N_2$ for the survey to determine spatial patterns of N fixation across the system. Measurements will also be made in the restoration area in years 2 and 5.

Modeling – To forecast how changes in salinity and organic matter availability alter microbial transformations of sediment N, we will extend our existing microbial metabolic network model (Algar & Vallino 2014) to include pathways for sulfate reduction, sulfide-induced DNRA (Murphy et al. 2020), canonical N fixation, and N fixation by sulfate reducing bacteria (Hou 2018) to augment the existing pathways of anammox, canonical denitrification, and DNRA. In our approach, metabolic pathway switching is determined by maximizing entropy production (i.e., free energy dissipation) over time and space (Vallino & Huber 2018), which will allow us to explore changes in microbial N processing as a function of organic matter and sulfate (i.e., salinity) inputs predicted from SLR.

Question II. How do food webs and energy flow respond to new geomorphic configurations, SLR, changing climate, and associated estuarine responses?

H II.1 As habitat network configurations shift across the landscape, consumers will respond by increasing their trophic niche size and exhibit greater variability in resource use.

Rationale: It is well known that the physical features of a land or seascape can exert control on the distribution and the structure of communities and food webs. This concept underpins the "Geomorphic-Trophic Hypothesis" developed by Hershey et al. (1999), which puts forth the idea that landscape features can limit the movement of species, consequently affecting the community structure and the overall ecosystem trophic structure. Consistent with this idea, previous work in PIE demonstrates that changing habitat configurations and geomorphology controls secondary production and energy flow by modulating foraging behavior. We demonstrated that nutrient enrichment led to geomorphic changes that decoupled connections between creek and marsh, leading to a tipping point in marsh food webs that altered energy flows and reduced the production of mummichogs (*Fundulus heteroclitus*) (Nelson et al. 2019, Fig. 15). This result is counter to traditional bottom-up food web theory that predicts the population should come into equilibrium with the new food supply (Power 1992, Endara & Coley 2011). Thus, despite continued stimulation of algal and invertebrate prey production by the nutrient addition, fish abundances declined to below pre-enrichment levels because they lost access to prey in the intermittently flooded marsh. These results demonstrate how geomorphic controls can override classic bottom-up control and emphasize the importance of changing habitat networks and landscape structure in controlling not only species distributions and abundance, but also energy flow through food webs.

As the abundance of open water, low marsh, high marsh, and their connectivity changes (Q1), we anticipate changes in habitat networks and landscape features will drive production changes that will alter food web structure and niche space. The energy available to a consumer is intimately linked to the kinds of habitats that a consumer experiences as it forages over a mosaic of habitat types (Turner 1989). The dominant energy pathways in a food web are determined by the types of primary productivity facilitated by a particular habitat (James et al. 2022; Harris et al. 2021) For example, when habitat such as salt marsh is formed by a dominant macrophyte, biotic material from that macrophyte can get incorporated into the detrital pool, making energy available to benthic consumers via that channel of primary production. Alternatively, some habitats primarily function as structure, but promote the growth of epilithic/epiphytic organisms and therefore make energy available primarily via those channels (Frankovich & Fourqurean 1997; Lesser et al. 2020). The collection of habitats within a system can vary in their ability to promote productivity via a particular channel (i.e., benthic microalgae, Pinckney and Zingmark 1993). Consumers adjust to this spatial variability in energy production by altering the degree to which they rely on a particular energy channel (Lesser et al. 2020) and/or changing foraging behavior across the system (i.e., niche space) (Micheli & Peterson 1999). Climate change will alter the types of habitats within PIE and the ability of particular habitat networks to support different production channels (Gilby et al. 2020).

As sea level rises and high marsh habitat is lost, so are the spatial food web subsidies provided by the monthly tidal connection of high marsh habitats to the aquatic food web. We hypothesize that as habitat networks shift across the landscape in PIE, consumers will respond by either adapting or move: i.e.. shifting the types and amounts of resources used where they are or move from regions of PIE that do not provide the resources they require. In either case, the change in the distribution of energy production across the landscape could generate novel niche space that can be exploited by climate migrants trying to establish themselves in PIE.

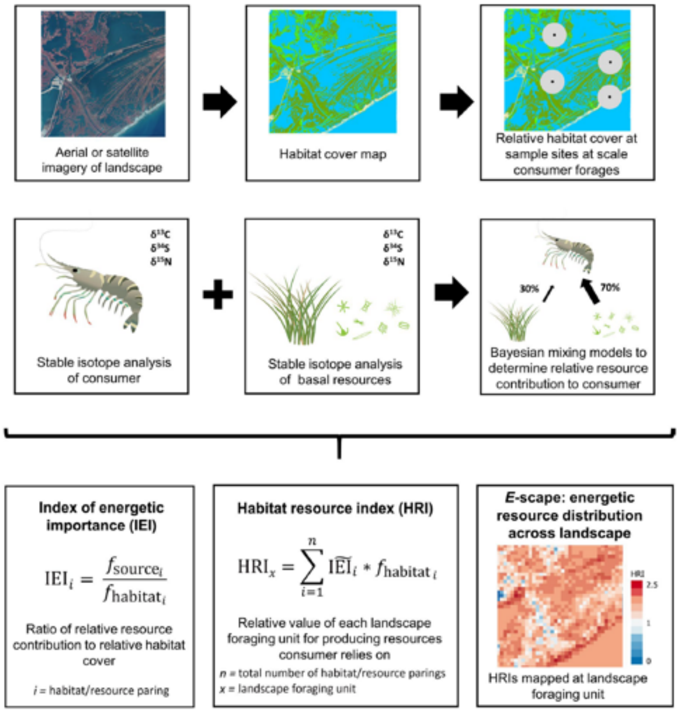


Fig. 24. Graphical abstract of E-scape construction. Habitat classifications are aggregated by the area of species movement. Species resource contributions are determined by Bayesian mixing models. The mixing model results, and habitat classifications are combined to generate an Energetic Landscape mosaic of resource availability.

Approach We conduct an annual census of fish and invertebrate consumers and basal resources for stable isotopes at three long-term monitoring sites along the estuary. We will continue to collect quantitative samples of nekton using lift traps across all habitats to monitor annual changes in nekton density, biomass, and diversity. We will also continue the long-term monitoring of benthic invertebrates in salt marshes with annual collections. We sample infauna (e.g., polychaetes) in two habitats (mudflats and low marsh) of six marshes. Epifauna (e.g., mussels, amphipods, gastropods) are sampled in the low and high marsh with quadrat sampling in the same six marshes. Fiddler crab densities are estimated based on burrow counts collected during quadrat surveys in the low marsh.

We will construct energetic landscape (E-scape) maps (e.g., Fig. 24) to test how changes in habitat configurations across the PIE landscape feedback to food web structure and the trophic niche of each of 7 major consumer species sampled as part of the long-term food web transects. The newly arrived climate migrant, fiddler crabs, have been added to the long-term sampling. These maps combine stable isotope analysis, Bayesian mixing models, and remote sensing to determine an Index of Energetic Importance for any given habitat as a foraging area of a species. The index is calculated by multiplying the contribution of a resource type (e.g., benthic algae) by the area of habitat that produces that resource (e.g., mudflat) scaled to the foraging area of the species being mapped (James et al. 2022; Fig. 24). The foraging areas will be modeled and validated using movement rages determined in previous studies (Kennedy et al. 2017, Lockfield et al. 2011).

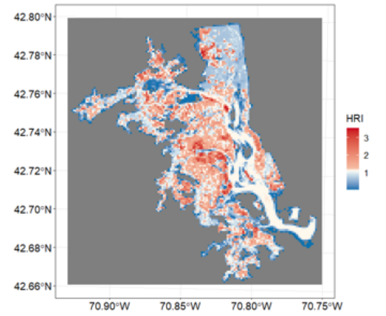


Fig. 25. An example of an E-scape showing the Habitat Resource Index (HRI) for mummichogs at PIE

We will use previous habitat classifications from PIE to synthesize and model ongoing food web responses to habitat change and create predictive models of how future change will further alter energy flow. We have already learned that incorporating these changes in habitat network and landscape geomorphology is critical to understanding how food webs will respond to changes in the distribution and availability of energy (Nelson et al. 2019, Lesser et al. 2021). The spatial mosaic of energy availability in the PIE estuarine system influences a consumer's trophic niche and provides a direct link between ecosystem processes that affect production and consumer resource use. To understand the feedbacks between changing habitat networks and landscapes we will combine remote sensing information and stable isotope ecology to produce consumer specific landscape maps of energetic resources for PIE (Fig. 25*)*. These GIS products can be used alone or in combination with existing models to test fundamental theories in several ecological disciplines (e.g., movement and landscape ecology) and improve habitat management and restoration practices.

Mapping: Advancement in remote sensing technology has allowed for finer spatial resolutions, which gives us the ability to identify more details concerning patches at individual time points (Ye et al. 2018) but makes characterizing temporal changes challenging (Pontius 2019). Our prior work focused on accounting for these issues so that we can now evaluate the configuration of landscape dynamics across various time intervals using new methods. We will use raw data from National Agriculture Imagery Program (NAIP) for 2008, 2010, 2012, 2016, 2018, and 2021 to classify the change in patches of land cover categories. The spectral data are sufficient to differentiate water from marsh and by including elevation, we can delineate the categories: water, tidal, low marsh, high marsh, and upland. We will then use the time series of maps to build *E-scapes* for each species through time (James et al. 2022). *How these energetic landscapes changed in the past can help to contextualize and reinterpret previously collected consumer biomass and abundance data.* We will analyze the connectivity of the water patches to track how far fish can swim through creeks to reach ponds, which gives insight into the changes in the extent of fish habitat. We will also characterize the

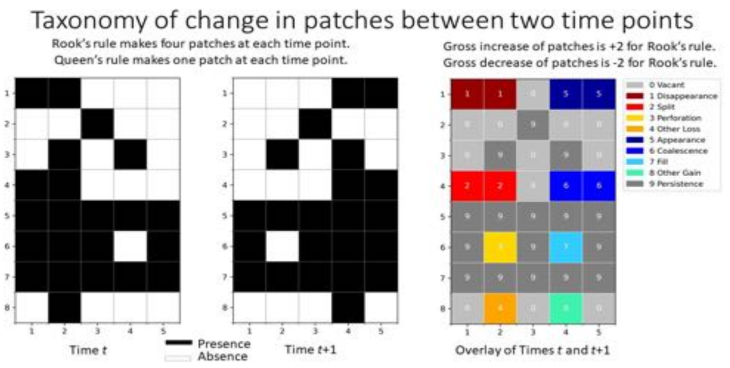


Fig. 26. Proposed taxonomy to analyze patches of change. Existing methods compute zero net change in patches from time *t* to *t*+1 but miss the gross change, while our proposed methods explain why the gross increase in patches is +2 and the gross decrease in patches is -2 for the Rook's rule.

temporal change in the configuration of patches. Existing methods (e.g., FRAGSATS) compute geographical pattern metrics concerning the number and shape of patches at a single time point. These methods can compute the net change of the number of patches between time points but cannot compute the gross change in the number of patches because the methods fail to track how individual patches change over time. We have begun to develop a taxonomy to track individual patches during a time series in a manner that allows a richer description of the landscape dynamics (Fig. 26). This approach recognizes that it is not simply the change in the number of each patch type that matters but also their spatial orientation. We will define the mathematical behavior of these new concepts and develop open-access computer programs that apply to any landscape.

Restoration Site: We will use the restoration sites as an experiment to determine if physical restoration also restores food web function. We will use E-scapes and niche hypervolume metrics of four of the dominant marsh species to determine if energetic resource restoration is followed by restoration of food web energy flow.

H II.2 Marine heatwaves and ocean warming will continue to alter food webs and biodiversity in the Gulf of Maine

Rationale: A striking feature of the Gulf of Maine (GoM) is that it is warming faster than almost any other part of the world's ocean (Pershing et al. 2015) and this warming trend seems to be accelerating (Siedov et al 2021). As a result, we are seeing more marine heatwaves (surface temperatures above the 90th percentile for more than five consecutive days). In addition, warm water from the Gulf Stream penetrates into the GoM more frequently than in the past. (Siedov et al. 2021). The GoM and PIE are north of an important zoogeographic barrier (Cape Cod), which is at the northern limit of many warm-water species. Therefore, it is critical we examine how marine heatwaves and ocean warming will influence the biodiversity and foodwebs of the PIE estuaries.

One prediction is that warming and heat waves will allow warm-water species to arrive from the south (i.e. climate migrants). We have seen this in PIE. After the 2012 marine heatwave in the Gulf of Maine we observed the blue crab, *Callinectes sapidus* (Johnson 2015) and the fiddler crab, *Minuca pugnax* (Johnson 2014) for the first time in the estuaries and salt marshes. Many marine climate migrants, especially invertebrates, arrive as larvae, which have pelagic stages that travel on the continental shelf and beyond. This allows for wide dispersal on ocean currents. As ocean temperatures rise, the thermally sensitive larvae can develop into juvenile and adult life stages. Another prediction is that cold-water species whose southern limit is the GoM may shift their range north. Thus, it is possible that some species may be lost from PIE as a result of warming. We hypothesize that marine heatwaves 1) open the door for southern species (e.g., species south of Cape Cod) to establish in the Gulf of Maine and continued ocean warming allows them to persist and 2) cold-water species at the southern edge of their distributions will disappear.

While we have detected two climate migrants in PIE so far, we may be overlooking others. This may be because their densities are too low or we are not adequately sampling for them. We also do not know if southern species are being introduced into PIE but are not able to be established for reasons other than temperature.

Approach – We will continue to look for new climate migrants through our annual biotic surveys (e.g., benthic invertebrate, fish, and plants). We will also use social media platforms such as iNaturalist to look for more visible species and compare to our own biodiversity list. Species we expect to expand into the GoM soon include, the sand fiddler crab, *Leptuca pugilator*, the purple marsh crab, *Sesarma reticulatum*, and the marsh periwinkle, *Littoraria irrorata*.

To anticipate new arrivals, and to determine which species may not be able to be established, we will take advantage of zooplankton samples collected by the National Oceanographic and Atmospheric Administration on the continental shelf as part of their Ecosystem Monitoring Surveys of the Gulf of Maine. These are the same surveys being used by the NES LTER. During these surveys, zooplankton are collected using bongo nets (60 cm diameter, 300 um mesh size) towed obliquely from 200 m in the late summer (August or September). We will use metabarcoding on a portion of the COI (mitochondrial cytochrome oxidase I) barcode region to identify species from these samples. Classification and identification of species based on COI sequences will use the North Atlantic regional MetaZooGene Atlas and Database (MZGdb), which includes 70,854 barcodes for zooplankton species reported to occur in the region. Selection of species for the MZGdb is based upon the NMFS COPEPOD database. We will target samples from the western Gulf of Maine in years before and after two recent marine heatwaves, 2012 and 2018. Because many marine species have pelagic larvae, and metabarcoding can discriminate among a wide array of taxa including fishes, polychaetes, bivalves, crustaceans (including crabs, copepods, amphipods), we can determine how biodiversity patterns are changing in the Gulf of Maine. To follow up we will also analyze zooplankton species in the GoM and within PIE in 2024 and 2027. If we find species in the zooplankton as larvae at PIE that have not been detected as adults, it indicates species that have arrived in the GoM but haven't yet established in PIE or so far haven't been detected.

H II.3 Fiddler crabs (M. pugnax) in the expanded range of PIE estuary will exhibit ecological release and be more fecund and store more energy than those in the historical range.
Rationale - As climate migrants expand their ranges, they can escape their enemies (i.e., parasites, predators) and competitors (Johnson et al. 2020a). This escape can, in turn, lead to changes in demographics, fitness, or niches compared to individuals in historical ranges. This is known as ecological release (MacArthur and Wilson 1967, Hermann et al. 2021). For instance, fiddler crabs in their expanded range (including PIE) had significantly fewer parasite species, on average, than crabs from their historical range (e.g., Virginia) indicating parasite escape (Johnson et al. 2020a). Fiddler crabs in PIE are also larger, suggesting ecological release (Johnson et al. 2019). However, over time, climate migrants may acquire more parasites in their new location or acquire novel parasites as PIE parasites switch hosts (time-since-establishment hypothesis). For instance, fiddler crabs in the expanded range had parasites novel to fiddler crabs (Johnson et al. 2020a). We will re-examine fiddler crabs for parasites to test for parasite escape and ecological release. If fiddler crabs continue to escape their parasites, they should invest less energy in defense and more into growth and reproduction (Phillips et al. 2010, Keogh et al. 2017). Thus, fiddler crabs in the expanded range should have greater fitness than crabs in the historical range. Specifically, fiddler crabs in the expanded range should be more fecund and have greater caloric density than crabs in the historical range.

Approach - We will retest for parasite escape by examining fiddler crabs for parasites in a comparative study between the expanded and historical range (N=5 marshes/range). It is likely that fiddler crabs in PIE will have gained new parasites since the last study (done in 2017; Johnson et al. 2020a), but we predict that fiddler crabs in the expanded range will still have escaped most historical parasites. To look at fitness, we will again use a comparative study design to compare crabs between the expanded and the historical range (N=10 crabs/marsh/range, N=5 marshes/range). For fecundity, we will estimate the number of eggs per female. We will determine caloric density of crabs with bomb calorimetry.

III. What internal feedbacks might accelerate, slow down, or even reverse the predicted changes in emergent marsh configuration and the fate of carbon, nutrients and energy?

H III.1 Genetic differentiation across the elevational gradient in Spartina alterniflora affects microbial community structure, carbon cycling and storage, and the response to changes in geomorphology.
Rationale: Plant genetic variation can have strong effects on ecosystem function and resilience to stressors (Hughes et al. 2008). In the low marsh, the dominant plant species *S. alterniflora* exhibits high levels of genetic and genotypic diversity at small spatial scales (Travis et al. 2002, Richards et al. 2004, Hughes & Lotterhos 2014, Hughes 2014), and this variation influences plant production, interactions with neighboring species including *Spartina patens*, and the response to stressors including sea level rise (Hughes 2014, Zerebecki et al. 2017, Noto and Hughes 2020, Zogg et al. 2021). At larger scales across the elevational gradient within marshes, there is adaptive divergence between two common growth forms

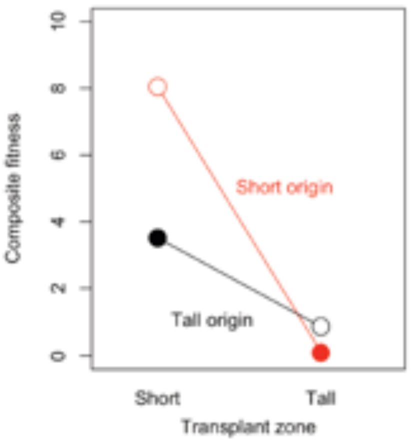


Fig 27. Composite expected fitness (survivorship and seed production) of short-origin transplants (red) and tall-origin transplants grown in either the short or tall transplant zone. Local treatments are open circles and foreign treatments are closed circles.

of *S. alterniflora (*Fig. 27*)*, tall form [~1-3 m stem height] at low elevations and short-form [<0.5 m] at high elevations, with key implications for how these plants will respond to expected changes in geomorphology (Fig. 27). Beyond the elevation range of the *S. alterniflora* distribution (i.e., tall vs short), we have little understanding of how intraspecific variation is distributed across the landscape in natural and restored marshes, or how this diversity impacts primary production, nitrogen demand, energy flow, carbon storage, and marsh persistence in response to SLR and subsequent geomorphological feedbacks.Microbes play a key role in mediating plant effects on C and N cycling and are known to vary based on plant species composition (Kearns et al. 2016). Our data also suggest strong genotype-specific effects on microbes under greenhouse conditions, but weaker effects in the field (Hanley et al. 2021). However, there are consistent differences in microbial community composition of both bacterial (Bowen et al. 2009) and fungal (Kearns et al. 2019) communities when comparing the distinct growth forms of *S. alterniflora*. Additionally, in a passively restored salt marsh complex, microbes shifted their community structure toward microbial communities present in reference marshes in advance of shifts in the plant community, indicating that microbes may be sentinel indicators of environmental change during restoration (Lynum et al. 2020). Understanding how plant intraspecific variation interacts with microbial community function will provide the link between plant-mediated marsh persistence, responses to restoration, and microbially-derived ecosystem services including carbon storage and nutrient removal.

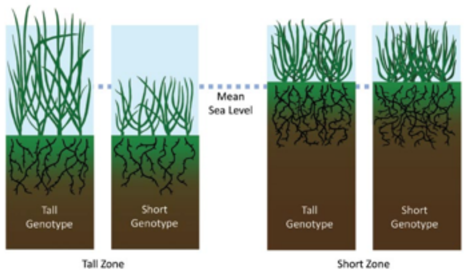


Fig. 28. Results of a transplant experiment. Tall-origin Spartina in the tall zone (left) is 60% taller and has 58% greater above-to-belowground biomass than short-origin *Spartina* illustrating the genetic constrain on short-origin plants. In contrast tall-origin and short-origin Spartina in the short zone (right) have similar morphology. *Adapted from Mudd and Fagherazzi (2016, fig 12.2) Image M. Freedman.*

Approach: We will examine relationships among *S. alterniflora* genetic variation, plant production, microbial community composition, and environmental variation at 8 locations across the elevational gradient along 20 permanent transects at natural and restored sites in years 2 and 5. At each location, we will measure plant biomass and collect *S. alterniflora* leaf tissue to quantify genetic variation using single nucleotide polymorphisms (SNPs), which have been used to document genetic divergence between tall and short growth forms (Zerebecki et al. 2021). We will also collect sediment samples (5 cm cores) from the rooting zone of the plants. Sediments cores will be homogenized across the depth and subsamples will be taken for microbial analysis. We will use standard methods for DNA sequencing (Kearns et al. 2016, Bulseco et al. 2019). Finally, we will measure elevation, porewater salinity, sediment redox potential, bulk density and carbon and nitrogen ratios in concert with our plant and microbial collections.

We will also conduct a field reciprocal transplant experiment at two elevations at one natural site and one restored site to test the relative importance of plant ecotype (tall vs short form), plant genetic diversity (number of genotypes), and plant species composition (*S. alterniflora* only vs. *S. alterniflora*-*S. patens* mix) on plant survival, biomass allocation, microbial community composition, and carbon storage. We will use tall-form and short-form genotypes of *Spartina alterniflora* reared from seed in a greenhouse, and field-collected *S. patens* that will be grown in common conditions in the greenhouse prior to the experiment to conduct a factorial manipulation of three treatments: (1) *S. alterniflora* ecotype (tall vs short); (2) *S. alterniflora* genotypic diversity (1 genotype vs 4 genotypes of a single ecotype vs 4 genotypes of both ecotypes (2 tall and 2 short)); (3) plant composition (*S. patens* present or absent), with 48 pots per elevation per site.

We will collect *S. alterniflora* seeds and *S. patens* transplants in the fall of year 2 and germinate/grow them using standard methods (e.g., Zerebecki et al. 2021). In spring of year 3, we will transplant plants to our field sites, which will be selected based on the results of our year 2 field survey. We will plant them in 33-cm diameter biodegradable pots filled with a known mass of commercial potting soil and planted flush with the surrounding sediment (Noto and Hughes 2020). We will sample the ^{13}C signature of that initial soil, and sample again at annual intervals over the course of the experiment, to measure the plant contribution to soil carbon using the natural abundance isotopic difference of the C4 plants. We will also collect leaf CHN samples and sediment cores for microbial analysis at the time of planting and annually thereafter. During each growing season, we will monitor plant stem density, height, and flowering stem density at monthly intervals. We will run the experiment until year 5. At the time of breakdown, we will take 3 cores per plot to measure plant biomass and biomass allocation. SNP and 16S rRNA gene amplicon analyses will follow standard methods.

H III.2 Fine-scale plant and microbial processes in the rhizosphere create biogeochemical diversity in the sediments that strongly influence organic matter storage, nutrient fluxes and accretion rates.
Rationale - Oxygen availability in sediments is a key variable that strongly impacts the cycling of carbon, nutrients, iron and sulfur. Oxygen availability is tightly linked to water level (e.g. Askaer et al. 2010), which controls diffusion into the sediment, and hence the tidal cycle can create regular and frequent changes of sediment aeration and anoxic conditions. However, wetland plants have adapted to frequent anaerobic conditions through the development of aerenchyma, which facilitate translocation of oxygen from air into the root system. Under some circumstances, this allows for the loss of oxygen across root surfaces, which generates small oxic root zones at depth in the otherwise anoxic sediment (Armstrong and Wright 1975; Koop-Jakobsen and Wenzhöfer, 2015; Koop-Jakobsen et al., 2018). This radial oxygen loss (ROL) is temporally and spatially dynamic and depends on environmental factors such as growth stage of the plant and photosynthetic rates. This creates steep gradients in redox conditions over short distances, creating microsites that act as hot spots of microbial reactions (*sensu* Kuzyakov and Blagodatskaya 2015) and that foster specific root associated microbial communities (Thomas et al. 2014). In addition, roots excrete labile organic substrates (Mucha et al., 2010), which in combination with the excreted oxygen, generate a potential for high microbial activity in the vicinity of the roots (Spivak and Reeve 2015). This results in the development of a patchy distribution of hot spots for microbial nutrient turnover and the degradation of organic carbon which has implications for carbon storage and greenhouse gas fluxes. As many of the key processes occur below the marsh surface and are closely linked to roots embedded in the sediment, they are particularly difficult to investigate under natural conditions. Investigations of marsh rhizospheres based on samples of bulk sediment and pore water miss this fine-scale spatial variation. Marsh plants vary greatly in belowground structures, ability to transport oxygen to roots, controls on ROL (Jovanovic et al. 2015; Li et al. 2021), and their root associated microbial community (Zhang et al 2021). A better understanding of the processes in the rhizosphere that mediate decomposition, nutrient cycling, and sediment redox status would greatly improve our ability to model these processes and forecast the impact of vegetation changes.

Approach – We will use a combination of in situ measurements to resolve spatiotemporal variation of redox conditions in the sediment in three major marsh plant species. In the *Typha* oligohaline zone (through separate DOE funding (*see H I.3*), we will deploy our new quantitative O_2-mapping field-robust planar optodes as well as "diffusive equilibrium in a thin film" (DET) samplers that provide 2D information on the distribution of Fe(II) and sulfide (Robertson et al. 2008) in multi-day campaigns. We will also make these measurements in the *S. alterniflora* and *S. patens* zones as part of this proposal. In situ as well as laboratory-based micro-profiling will elucidate the spatiotemporal dynamics of the pore water chemistry in the rhizosphere. In addition, we will explore using soil zymography to map microbial exo-enzymes (Razavi et al. 2019) to look for evidence of the spatial distribution of microbial activity. This work will be complemented by ongoing work by Bowen documenting the metagenomic and metatranscriptomic shifts in microbial communities as a function of depth in the marsh sediment (Vineis 2022) and as a result of changes in marsh elevation, work that is planned by a PhD student in the Bowen lab. These efforts will also be supported by Spivak's separately funded NSF project examining the mechanisms controlling soil organic matter preservation using experimental marsh organs in a minerogenic marsh (GCE) and an organic marsh (PIE). Spivak will use complementary laboratory and field experiments to test how 3 preservation mechanisms (oxygen availability, mineral protection, root exudate priming) operate under controlled and natural conditions, respectively.

Modeling - Through our DOE funded project, we will use the 2D measurements to inform mechanistic model development, particularly of biogeochemical processes modeled by PFLOTRAN, an open source, massively parallel subsurface flow and reactive transport model (e.g. Tang et al. 2016). We will particularly focus on the representation of roots and their impact on oxygen, iron, and sulfide concentrations in the soil. We will then couple this with the E3SM Land Model (ELM, see H1.3) to better study the impact of these small-scale processes on larger scale processes (productivity, decomposition, greenhouse gas release) and whether they make the marsh more or less vulnerable to SLR.

H III.3 The expansion of fiddler crabs into PIE will alter low marsh biogeochemistry but not impact low marsh sediment accretion.

<u>Rationale:</u> Thousands of climate migrants have been identified globally (Sorte et al. 2010, Poloczanska et al. 2013, Johnson 2015, Pecl et al. 2017), yet we know little about their ecosystem impacts in their expanded ranges. *Minuca pugnax* is a functionally novel species in the salt marshes of PIE. It is an ecosystem engineer that burrows into the marsh sediment up to 0.5 m deep. There are no other burrowing crabs in the salt marshes of PIE. As a result, there are novel ecological interactions that likely have strong influence on ecosystem functioning. Based upon previous studies we expect that fiddler crabs will reduce carbon storage in PIE marshes. Fiddler crabs in PIE are limited to the daily flooded, *S. alterniflora* low marsh. Their burrows can enhance soil redox potential and drainage (Michaels and Ziemann 2013). This, in turn, can enhance aboveground biomass (Gittman and Keller 2013, Williams and Johnson 2021) and reduce production of belowground biomass of *S. alterniflora* while simultaneously increasing decomposition of soil organic matter. For example, Thomas and Blum (2010) estimated fiddler crabs lead to a net loss of carbon of 3 g m^{-2} y^{-1} in a Virginia marsh and Grow (2022) found that they enhanced CO_2 and CH_4 efflux from sediments. As surface deposit feeders, *M. pugnax* can reduce the biomass of benthic microalgae by 70% (Johnson et al. 2020b; Fig. 29). Thus, fiddler crabs may reduce the ability of salt marshes in PIE to store carbon. Their per-capita impacts on carbon-storage in PIE may be larger than in their historical range because 1) crabs are larger, 2) crabs appear to dig more frequently, and 3) fiddler crabs are functionally novel ecosystem engineers in PIE. Although their populations are still 10x lower in PIE than

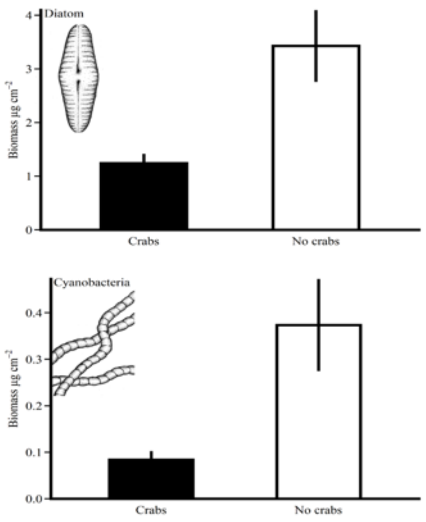


Fig. 29. Mean biomass of benthic diatoms (top) and cyanobacteria (bottom) in the presence (dark bars) or absence (open bars) of the fiddler crab.

in historical ranges (e.g., Rhode Island, Virginia) they are on average, tripling every year (Fig. DJ1). We have an outstanding opportunity to measure their impacts on carbon storage when their populations are low and examine their impact as their populations rise.

Fiddler crabs can affect geomorphology directly through burrowing, which can compromise soil strength and increase resuspension. They can influence it indirectly by increasing aboveground biomass (Bertness 1985, Williams and Johnson 2021). Due to the importance of sediment trapping by *S. alterniflora* and the contribution of organic matter via belowground production, this enhancement of aboveground biomass should enhance sediment deposition (Morris et al. 2002, Fagherazzi et al. 2013a, Morris et al. 2013), which would lead to greater vertical accretion, potentially enhancing the ability of low marsh to keep up with SLR. However, even when fiddler-crab abundances are high and aboveground biomass of *S. alterniflora* is stimulated within the historical range, we have not seen an increase in sedimentation (Williams and Johnson 2021). Thus, there may not be an indirect effect of fiddler crabs on sedimentation and any direct effect on erosion through burrowing is only present when the grass has been removed. We hypothesize that fiddler crabs will increase resuspension of sediments associated with their burrowing, but they will not have a strong impact on geomorphic processes such as sedimentation.

<u>Approach</u> To determine the effect of fiddler crabs on carbon storage at PIE we will focus on small scale process measurements in areas with different crab densities. Crab colonies occur at various densities (e.g., 16–130 m^2) and ten's of meters from each other (Martínez-Soto and Johnson 2020). We will measure the following in areas with and without crabs: soil strength, soil respiration, decomposition, belowground biomass, sediment bulk density, and organic matter content (loss-on-ignition). We will do this along a gradient of crab densities to generate a regression model to use for predictions. To measure the impact of fiddler crabs on geomorphology, we will also estimate burrow volume and annual sediment turnover associated with burrow digging and maintenance. To test for sedimentation, we will place sedimentation plates (LeMay 2007) in areas with and without fiddler crabs at various densities. We will also collect aboveground biomass at these sites.

Integration and Synthesis

Our research approach has been designed to help synthesize across boundaries (watershed, marsh, estuary, ocean) and disciplines (geomorphology, biogeochemistry and consumer dynamics - foodwebs) (Fig. 19). The linked hydrodynamic-biophysical model forces us to integrate nutrient, sediment, and carbon flows to test our understanding of the processes governing coupled biogeochemical cycles across boundaries and will move us towards improved predictive capacity of estuarine response to environmental change. The E-scapes model will link consumer dynamics to geomorphology and hot spots of primary production. Using these biogeochemical and consumer models we can explore scenarios to asses how changes in climate, sea-level, precipitation, and alterations to the geomorphology of the marsh itself alter system functioning. Our new research in the restoration sites will allow us to make observations in systems where the geomorphology and hydrology are rapidly changing to further test our synthesis. Throughout, our research is informed by cross-site work being done by the PIs in SC, and the VCR, GCE, FCE, and HFR LTER sites as well as other collaborations with other investigators.

Broader Impacts

● ***Benefits to Science and Society* -** PIE continues to advance our predictive understanding of the long-term response of coupled land-marsh-estuary-ocean ecosystems to changes in three key drivers: climate, sea level and human activities. Our research sheds light on the controls on marsh accretion and loss, blue carbon storage, the exchange of carbon and nutrients, and how food webs are changing with SLR, warming, and ecosystem responses. Given expected rates of SLR, salt marsh survival is in doubt. Local, and Federal agencies are eager for research and data that can help them better manage coastal systems and help in the design and evaluation of large-scale restoration efforts like those proposed for Plum Island marshes and beyond. We meet regularly with these agencies and discuss our research findings together.

● ***PIE-LTER Schoolyard Program*****:** David Moon, Mass Audubon, will continue to lead our K-12 Schoolyard program which provides ten schools with experiential learning opportunities for students and teachers. The program serves approximately 1,000 students and 50 teachers per year in grades 5-12. We hope to bring back the annual in-person conference (halted by COVID) where students present their findings at a conference attended by PIE scientists and local researchers. Historically the program had largely focused on examining the changes in salt marsh vegetation due to tidal restrictions and hydrologic restoration. Mass Audubon has added a new climate change focus that will make use of the vegetation transects teachers and students have been measuring for the past 25 years. In addition, work began on educational evaluation of project outcomes for students. Mass Audubon also plans to continue professional development activities begun with Liz Duff in collaboration with PIE scientists.
● ***Gulf of Maine Institute (GOMI)*****:** We will continue to work with the Gulf of Maine Institute (GOMI), an NGO that provides training to teachers and help them develop environmental community-based stewardship projects (*see results of prior*). Currently there are 5 schools participating, all within the watershed of the Merrimack River, including the Lowell Middlesex Academy where PIE has a teacher in the RET program. To facilitate hands on activities in a time when classes were disrupted by COVID, GOMI introduced the "Emerald Web"- a habitat sanctuary approach inspired by the work of Dr. Douglas Tallamy, (Tallamy 2019). Dr. Tallamy held a zoom with the teachers to outline the approach. Teachers and students have begun to design and plant habitat sanctuaries on school grounds where they will then collect data. PIE scientists are providing scientific support to GOMI for teachers to develop their in-class programs which emphasizes watershed connectedness through shared data collection activities.
● ***Logan Science Journalism Course*****:** We will continue to host 6-8 journalists per year through the 12-day hands-on Logan Science Journalism program. This immersive program offers fellowships to mid-career science journalists and focuses on the impact of climate and land use change in the coastal zone.
● ***Mentoring for Grads and Undergrads*****:** During the summer, 10-14 undergraduate and graduate students work and live at our field house and many others come nearly daily from nearby colleges and universities. Beginning last year, we made ongoing professional development available to undergrads during the summer through MBL, and all students give presentations at the end of the summer on their work. Undergrads from B.U. and the MBL's Semester in Environmental Sciences visit the site and do projects at Plum Island. We are now developing a better network for graduate students. Graduate students stay connected through a listserv and are made aware of both PIE and LTER network activities. We are also beginning an annual symposium for graduate students and will create more opportunities for interactions among graduate students from different institutions at our annual meeting.
● ***Interactions with Policy Makers and Management Agencies*****:** PIE scientists serve on a large number of advisory boards of organizations interested in marsh sustainability and watershed issues. We have continued all the relationships with groups described above (*see results of prior)* and we are now also participating in meetings of a cross agency group evaluating new potential restoration sites.

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The top ten most significant publications are shown in a box with the author in bold and data sets used in the paper are identified. Data sets are identified by their Data Package Identifiers. They are also identified in the data set table by shading by both package identifier and DOI.

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Facilities, Equipment & Other Resources

MARINE BIOLOGICAL LABORATORY

The Marine Biological Laboratory and The Ecosystems Center have well-equipped research laboratories. The Ecosystems Center occupies a 32,000-sq. ft. building that houses offices and several laboratories. Labs are all provided with ultrapure water via a recirculating reverse osmosis-deionization system. Instrumentation in the labs includes nutrient autoanalyzers; spectrophotometers; fluorometers; balances; pH meters; gas chromatographs with TCD, ECD and FID detectors for analysis of CO2, CH4, and nitrogen fixation; elemental analyzer for analysis of particulate C and N; TOC-TN analyzers; ion chromatograph; atomic absorption spectrophotometer; epifluorescence microscope; automatic titrators for alkalinity and Winkler O2. The Ecosystems Center maintains a stable isotope facility that runs samples for a reasonable cost. The lab utilizes both Dual- Inlet and Continuous-Flow Isotope Ratio Mass Spectrometer systems for the measurement of δ15N, δ13C, δ34S, δ18O and δ2H on various types of samples. Both natural abundance and isotopically enriched samples can be accommodated. Giblin's lab operates a membrane inlet mass spectrometer system (MIMS) used for denitrification and DNRA analyses, a DIC analyzer, a "NOx box" for NO3 analysis, and a well-type gamma counter for dating sediment cores.

The center also has a variety of incubators that allow for various controlled incubation experiments, including 4 environmental chambers, and an anaerobic chamber. Other center resources include a -80 freezer, 2 walk-in freezers and 2 walk-in refrigerators, an autoclave, and a high performance dish washer.

At the Ecosystems Center, computers and workstations obtained by individual projects are connected to the MBL-REGISTERED network for access to internal and external network resources. An additional MBL-GUEST network is available for visiting scientists, collaborators, and guests to access external network resources. A shared 1 TB drive maintained by MBL IT is available for Center and project files. This drive is backed up daily. The Center also provides access to ArcGIS, Matlab, and SigmaPlot software on the internal network to support analyses of field, laboratory, and simulation data, and the subsequent presentation of these analyses. In addition, backup software for project computers is available. Numerous printers are available, including a large format printer for poster preparation.

The Marine Biological Lab has laboratories with running sea water, several liquid scintillation counters, ultra- centrifuges, scanning electron microscopes, video-linked microscopes, digital microscopic analysis, and various balances, freezers, –80oC freezers to preserve DNA samples, and dissecting scopes.

The LTER has a variety of field equipment on site including three boats for inshore coastal use, several current meters, field instruments for monitoring water quality, a canopy analyzer, a Unispec for reflectance measurements, and the normal array of needed field gear such as plankton and zooplankton nets and tools.

The MBL owns the "Marshview Farmhouse" in Newbury which serves as a PIE field facility. An NSF Field Stations and Marine Lab grant in 2014 allowed us to create a 1,300 sq ft laboratory space as an addition to an existing barn, providing high quality space for on-site sample preparation and some analyses. The PIE LTER program maintains a number of field installations for monitoring on site. These include a weather station at Marshview, two eddy flux towers in the salt marsh for measuring the exchanges of CO2 and water between the marsh and the atmosphere, a network of water quality monitoring sondes in the estuary, pressure sensors for recording water levels in wells in the marsh and in the estuary, and a NOAA quality tide gauge near the southern entrance to Plum Island Sound. A third flux tower for measuring CO2, methane and H2O is being installed in the oligohaline zone this summer. We are cooperating with UNH for precise measurements continuous m of pH, oxygen, temperature in the Gulf of Maine using a SeapHOx. Our server is located in a nearby rental property and communicates with the sondes and the eddy flux towers via radio.

Leveraged Funding - Marshview is owned by the MBL. In addition to the lab there is a large house with a kitchen which sleeps 14. MBL maintains the property and pays for utilities and LTER investigators are not charged for use. Researchers reserve space at our annual March meeting. After LTER slots have been reserved, both facilities are available to other collaborating projects, and other researchers affiliated with federal and local partners as space allows. Outside investigators are charged a very small fee which is often waived for students.

The MBL Ecosystems Center has two vans and one pickup truck that are available for project use. Projects are charged for mileage only which covers the cost of gas and minimal maintenance.

Housing at Marshview is supplemented by the Rowley Field House, which is rented from a local land trust, the Essex County Greenbelt Association by the PIE LTER. This facility is reserved for shorter term stays and is also the site of our dock.

BOSTON UNIVERSITY

Fagherazzi - Boston University Computational Facility

The Delft3D coastal flooding model, ecogeomorphic evolution model, and agent-based model will be undertaken on the Boston University Shared Computing Cluster (SCC), located in the Holyoke, MA, LEED Platinum certified Massachusetts Green High Performance Computing Center (MGHPCC). Two pairs of 10Gigabit Ethernet network connections link the MGHPCC to the BU campus with extremely fast data transfer. The SCC is a heterogeneous Linux cluster composed of both shared and buy-in components (over 8,000 shared CPU cores, over 10,000 buy-in CPU cores, and 6 petabytes of storage— 90% buy-in—for research data) and provides a high-performance computing environment well-suited to the disciplines relevant to the current project: geographic information systems (GIS), statistics, data analysis, scientific/engineering simulation, and optimization. The MIDAS ABM (Bell 2016) is coded and simulated in MATLAB, which is installed on the SCC. The Delft3D-SWAN model is already installed on the SCC and it is run in parallel with more than 20 nodes.

Boston University Coastal Ecogeomorphology Laboratory

FACILITIES AND EQUIPMENT

The Coastal Geomorphology Lab is equipped with:

- Zodiac Inflatable Boat RIB Pro7 with 40hp Yamaha motor

- tripod frame
- one Sontek ADV for waves and currents measurements
- five OBS D&A Instrument Company for measurement of sediment concentrations
- four Nortek ADCP velocity profilers
- two TWR2050 Tide and Wave Recorders two steel tripods for tidal flats deployments
- one Partrac Cohesive Strength Meter for sediment critical shear stress measurements
- one Topcon Total Station
- Two Nortek Vector ADV
- one NexSens submersible datalogger with salinity and chlorophyll a probes
- one HOBO weather station
- one PA 500-6 Tritech altimeter for erosion
- one MiniWater6 velocity probe for velocity measurements
- one Hiperlite Topcon GPS rover and station
- one Waltz Diving PAM

Fichot - Boston University Aquatic Optics and Photo-biogeochemistry Laboratory
The Department of Earth and Environment and its affiliated centers at Boston University (BU) are well equipped to perform the proposed research. Field and laboratory equipment from the Aquatic Optics and Photo-biogeochemistry Laboratory will be available for this project and include the following major items:

1. A Biospherical® Compact-Optical Profiling System (C-OPS) profiler for radiometric measurement of UV-visible solar radiation during vertical profiles in water bodies (http://www.biospherical.com/index.php?option=com_content&view=article&id=66&Itemid=66). This instrument will facilitate measurements of surface remote-sensing reflectance and diffuse attenuation coefficients of downwelling irradiance at 19 wavelengths in the UV-Visible region. The wavelengths will closely match those of current ocean color sensors (e.g., MODIS, OLCI, OLI).
2. An ASD HandHeld 2 Pro VNIR Hand-held Spectroradiometer for the measurement of above-water hyperspectral remote-sensing reflectance (https://www.malvernpanalytical.com/en/products/product-range/asd-range/fieldspec-range/handheld-2-pro-vnir-hand-held-spectroradiometer/index.html)
3. A PerkinElmer UV Lambda 650 spectrophotometer outfitted with a 150-mm integrating sphere for the measurement of inherent optical properties of discrete samples (https://www.perkinelmer.com/labsolutions/resources/docs/BRO_Lambda950850650Americas.pdf).
4. A Shimadzu Total Organic Carbon analyzer TOC-L for the analysis of dissolved organic carbon in (http://www.shimadzu.com/an/toc/lab/toc-l.html).
5. A Costech ECS 4010 CHNSO Elemental Analyzer for the measurements of particulate organic carbon and nitrogen (http://www.costechanalytical.com/products/ecs4010.aspx).
6. A solar irradiation setup for performing photochemical experiments under very controlled illumination conditions, which includes an Atlas Suntest XLS+ solar simulator (http://atlas-mts.com/products/product-detail/pid/236/), a custom-made irradiation set-up, a circulating water bath, and an Optronics OL-756 UV-visible spectroradiometer (http://www.ghinstruments.com/products/spectroradiometers/ol-756-portable-uv-vis-spectroradiometer/) for NIST-traceable measurements of full-resolved irradiance in the 280-

700 nm range. The combination will facilitate accurate measurements of absorbed quanta by the dissolved organic matter of the samples.

7. The Hydrolight® software (http://www.sequoiasci.com/product/hydrolight/)
8. YSI Handheld ProDSS with GPS (30 m cable) and depth, salinity, temperature, dissolved oxygen, turbidity, and chlorophyll-a and phycocyanin fluorescence sensors.
9. YSI EXO-3 probe with depth, salinity, temperature, dissolved oxygen, turbidity, and DOM fluorescence probes.
10. Sampling devices: Horizontal Van Doorn bottle for sampling at discrete depths, Secchi disk.
11. Sc:an® Spectrolyzer V3 probe for in situ measurements of absorption and beam attenuation spectra in the UV-visible range. (https://www.s-can.at/en/product/spectrolyser-v3/).

Fulweiler – Coastal ecology and Biogeochemistry Laboratory
Ecological/Biogeochemical Equipment: membrane inlet mass spectrometer system (MIMS) with specially designed inlet system attached to a Balzers quadrupole MS; one in situ porewater sampling system/underwater mass spectrometer; a Shimadzu GC-2014 (with high sensitivity detectors (thermal conductivity: CO2, flame ionization detector: CH4, and electron capture: N2O); and a SEAL –Nutrient Autoanalyzer. Fulweiler also has a Unisense microprofiler which allows fine scale measurements of various dissolved gases, pH, etc. In addition, she has basic biogeochemical equipment including turner fluorometer, Hach dissolved oxygen, salinity, and pH probes, pH meters, redox meters, centrifuges, analytical balances, muffle furnaces, refrigerators, water baths, freezers, and ovens. Both water and soil/sediment samples can be analyzed in the laboratory. Nutrient analysis includes dissolved (NH4+, NO3- NO2-, DSi, DON, DOP) and particulate forms. Gas analyses include N2, Ar, O2, CH4, N2O, CO2, and 28,29,30N2. In addition, the Earth and Environment Department has a laboratory dedicated to the analysis of sediment characteristics and the Biology department has an autoclave.

Fulweiler has dedicated space in the Earth and Environment Department at Boston University for her Marine Ecology and Molecular/Biogeochemistry laboratory. This space was renovated in 2016 and includes dedicated locations for biogeochemical and ecological laboratory work as well as environmental molecular work. Additionally, Fulweiler has separate laboratory space dedicated solely to the development and bench testing of instruments such as the in situ porewater sampling system/underwater mass spectrometer (POSSUMS). Fulweiler also has a wet lab which includes an environmental chamber with temperature and humidity control.

CLARK UNIVERSITY

Clark University has resources required for the subcontracted components of the proposed project. These resources are housed in the following parts of Clark University.

The George Perkins Marsh Institute (GPMI; www.clarku.edu/departments/marsh) conducts research funded by agencies such as the EPA, USDA, NSF, NASA, DOE, National Park Service, as well as state and local governmental agencies and private foundations. GPMI provides office space and support services, including a full-time Program Manager who is responsible for daily operation and budget management, as well as communications, graphic and web-design professionals. The Marsh Institute has provided the facilities, resources and expertise to implement numerous prior projects.

The Earth Observation and Remote Sensing (EORS) Lab provides computing and office space infrastructures for graduate students focused on earth system science, remote sensing, and geographic information science within the Graduate School of Geography (www.clarku.edu/departments/geography).

Clark Labs is a research center focused on the development of geographic information technologies (IDRISI and TERRSET GIS software), maintaining state-of-the-art computing and data facilities for software development and research applications.

The Kasperson Research Library offers one of the most extensive collections in North America on environmental risk and hazards, environment and development, and the human dimensions of global environmental change. The library's collection also includes holdings in international development, water resources, technology, energy policy, and sustainability.

MASSACHUSETTS AUDUBON SOCIETY

Mass Audubon has standard equipment for field sampling of plants, fish, birds, and other biota, such as binoculars, spotting scopes, rangefinders, tapes, quadrats, beach seines, nets, handheld GPS units, etc. Sieves and microscopes and are available for sorting and analyzing benthic samples. Massachusetts Audubon owns 240 acres of salt marsh and surrounding upland in the Plum Island Sound estuary that is the locus of some of our field studies. The organization also has two facilities in the region that could be used by the PIE LTER for meeting space. These are the Joppa Flats Nature Center in Newburyport and the Endicott Regional Center in Wenham. The former houses computer facilities for Mass Audubon's participation in the LTER.

NORTHEASTERN UNIVERSITY

Laboratory: Research will be conducted at the Northeastern University Marine Science Center (MSC), a full-service marine laboratory with computers, classrooms, laboratories, and a lab-wide flow-through seawater system, located 10 miles North of Northeastern University's main campus in Boston, MA, and approximately 20 miles south of Plum Island Sound, where half of the field research will take place.

Dedicated facilities in PI Bowen's and PI Hughes's labs include standard equipment for DNA/RNA extraction and amplification and gel electrophoresis, such as laminar flow hoods, a mixer mill, pipettes, heating blocks, vortexes, centrifuges, thermocyclers, a Bio-Rad CFX96 ™ Real-Time System, an Agilent AriaMx™ Real-Time PCR machine, a Qubit fluorometer for DNA/RNA quantification, an Agilent TapeStation™ for DNA/RNA quality checking a Blue Pippen for DNA fragment size selection, a Covaris Ultrasonicator for nucleic acid shearing, and basic laboratory equipment, such as freezers, refrigerators, drying ovens, incubators, pH meters, and balances. The Bowen lab also has a microprofiler for fine scale oxygen/redox profiles, a LiCor for measuring CO2 concentrations, a benchtop spectrophotometer, a fluorometer, and a model T200 chemiluminescence NOx analyzer for measurement of nutrient concentrations. We have sufficient -80°C freezer space to accommodate the samples generated from this work and we will archive all samples with the Ocean Genome Legacy to ensure there are sufficient backups in place. Metagenomic and amplicon sequencing will take place at the Tufts University Genomics core facility.

Co-PI Hughes has a dedicated 1400 ft2 greenhouse at the MSC with 28 mesocosms (53 cm tall * 84cm diameter; 204 L) and 16 1m*2m seawater tables with running seawater, UV-sterilized running seawater and freshwater irrigation which will be used for the hydroponic nutrient uptake experiments. Further, there is sufficient space that can be dedicated to rearing all the plants needed to transplant into the organs at both sites.

Computer: Computer resources and support are excellent. The MSC's IT infrastructure has been completely upgraded with hard-wired and wireless access throughout the Edwards Laboratory. To support the rapidly growing needs of its faculty for computational bioinformatics, the CSI/MSC recently purchased one High Performance Computing (HPC) server, a Dell R910, which is hosted by NU's Information Technology unit in the University's Data Center on the Boston campus. The server consists of a single node, which has 512 Gigabytes (GB) of RAM. The total memory capacity of this server is expandable to 2 Terabytes (TB) of RAM. The HPC server has four Intel® Xeon® E7-4870 (2.40 GHz, 30 M cache, 6.4 GT/s QPI, Turbo, HT, 10C) chips, which is equal to 40 physical cores of processing power and 80 virtual cores. We run the server with 200 GB of Hard Drive (HHD) space set up in a RAID 1 configuration (for the operating system + Programs) and 2 TB set up as working space for data analysis in RAID 5 configuration. All the HHD's are hot-swappable and run at 16k rpm, which facilitates fast access to data during analysis. The RAID 1 and 5 configurations ensure that all data is continuously backed up, in case of HHD failure. In addition, Northeastern is a founding member of the Massachusetts Green High Performance Computing Cluster, which provides massive computational support for all Northeastern faculty. The Bowen lab performs all metagenomics assemblies, MAG construction and comparative genomics using standard pipelines established on the Computing Cluster with data backup and storage both on the Cluster and locally.

Office: Office space is available at the MSC for Bowen, Hughes, and postdoctoral scholar Costa, and the graduate students and undergraduate researchers on the project.

Other: Our full-time Outreach Program staff at the MSC provides a diverse portfolio of programs and opportunities. These programs typically include NU undergraduate and graduate students as instructors who benefit from the opportunity to practice science communication with diverse audiences. In a typical year, over 7,500 adults and students visit the lab or participate in our Outreach programs. With support from the Americorps Massachusetts Promise Fellowship, the Outreach Program also coordinates the Beach Sisters initiative, which provides out-of-school-time STEM programming to 60 underserved girls from the City of Lynn annually and is poised for replication at other sites. The Outreach Program also operates COSA (Coastal Ocean Science Academy), an annual marine science academy that engages over 50 youth each year, many of whom hail from low-income communities and whose participation is made possible by alumni-supported scholarships. Thousands of other youths benefit each year from field- and/or classroom-based citizen science programs hosted by the Outreach Program. Northeastern is an active institutional member of the New England Ocean Science Education Collaborative, through which it has participated in the development and testing of approaches to connect informal science education centers with underserved audiences. Along with the Massachusetts Marine Educators, it also co-sponsors an annual High School Marine Science Symposium to bring academic and career awareness to area youth. We will build from this foundation to implement and assess the broader impacts proposed.

Lastly, Northeastern is widely recognized for its unique and excellent experience-based learning approach, and its Cooperative Education Program supports students seeking research opportunities. Each year, the University provides funds that allow two students to be employed on site through this “co-op” program at the Marine Science Center. In addition, the Marine Science Center funds multiple undergraduate summer research internships. We will engage students through these two programs to participate in the proposed research.

UNIVERSITY OF LOUISIANA AT LAFAYETTE

Biological Sampling and Stable Isotope Analysis

The Nelson Lab owns a 21’ aluminum hulled boat and drop sampler to conduct all of the quantitative sampling biological sampling. The LUMCON facility in Port Fourchon is equipped with a dock, wet lab, and dorm facilities to house the students. The Nelson Lab is located in Billeaud Hall on the main campus of the University of Louisiana at Lafayette, LA. The Nelson lab is equipped with all of the instrumentation needed to prepare tissue samples for stable isotope analysis and analyze the resulting data. Billeaud Hall contains a Grieve Hi-capacity convection drying oven for drying tissue and plant samples. The Nelson lab contains a Retsch-400 mixer mill for grinding tissue and plant samples, an Ultrahigh precision Ohaus balance for weighing samples, and all the accessory instruments needed to process samples for stable isotope analysis (mortar and pestle, spatula, etc.). The Nelson lab also contains all the computing equipment required to analyze the isotope data and build the stable isotope niches including a Dell M5800 with duel i7 Intel processors and 32 GB of RAM, installed with the R statistical program.

Drone Based Sampling and Analysis

The Nelson Lab owns a Quantum Systems Trinity F90+ vertical takeoff and landing fixed wing unmanned aerial system. The Trinity carries a MicaSense RedEdge MX and RedEdge MX Blue dual payload capable of taking 10 individual bands of light in the visible and non-visible spectrum. My lab has developed the full processing pipeline to analyze and categorize all of the drone imagery. I own a Dell Precision Tower 5810 desktop with 128 GB of RAM, an Intel Xeon CPU E5-1603 v3 @ 2.80GHz, and a NVIDIA Quadro M2000 GPU. I also own professional licenses for Pix4D Mapper (version 4.4.12) to create orthomosaics and 3D digital surface models (DSMs) using Structure from Motion (SfM) algorithms and eCognition Developer (v. 9.5, Trimble Germany GmbH, Munich, Germany) to create custom object based image analysis algorithms for habitat classification.

UNIVERSITY OF NEW HAMPSHIRE

General lab equipment available in support of this project includes muffle furnaces, drying ovens, 2 large incubators, filtration equipment (for TSS), autoclave, freeze dryer, hoods, sinks, walk-in freezer, walk-in refrigerator, acid-washing dishwasher, and ultrapure water (RO followed by Millipore Milli-Q with UV polishing) for reagent and standard preparation. Specific analytical instrumentation includes a Bay Instruments Membrane Inlet Mass Spectrometer (MIMS) for N2, Ar, and O2 gas measurements, Westco Smart Chem robotic automated colorimetric analyzer for nutrients, a Seal robotic automated colorimetric analyzer for nutrients, High Sensitivity Lachat FIA for low levels of phosphorus (2 cm path length), Shimadzu HPLC with fluorescence and UV/VIS detectors as well as a Horiba JY fluorometer, a Hewlett Packard HPLC optimized for amino acid analysis with pre-column derivatization, two Dionex ion chromatographs for simultaneous determination of cations and anions, a Shimadzu TOC VSH TOC/TON analyzer, a Perkin Elmer Model 2400 Series II CHN analyzer with microbalance, and a Shimadzu GC with headspace autosampler and three detectors (FID, ECD, and TCD) for analysis of CO2, CH4, and N2O in gaseous samples and the headspace above aqueous samples. Each of these instruments has an autosampler, and each has either its own chromatographic data handling station or is attached to an EZ Chrom data station.

Field equipment includes handheld conductivity meters, handheld dissolved oxygen meters, both Marsh McBirney and FlowTracker current meters for water velocity and discharge measurements, five Sigma autosamplers with level sensors that can be used for automated water (grab) sampling, as well as several HOBO stage, conductivity, dissolved oxygen loggers, fdom, and Satlantic SUNA nitrate analyzers that will be used with the proposed measurement plan.

The UNH Earth Systems Research Center's (ESRC) Science Computing Facility (SCF) has a wide range of computer servers, printers, plotters, archiving systems, software, data archives, and web based data distribution systems that are integrated using several internal networks and connected to the outside world through a high speed pipe. The overall SCF administration is provided by the Research Computing Center (RCC) located in the Institute for the Study of Earth, Oceans and Space (EOS). Scientific data processing and analysis support is distributed throughout workgroups within the center with additional centralized expertise provided by ESRC's Laboratory for Remote Sensing and Spatial Analysis. Within this proposal, we take advantage of this existing computer infrastructure, to meet our anticipated computational needs.

The main ESRC servers consist of high-end, multi-processor computing systems manufactured by Dell and SUN Microsystems. The Dell systems run Linux and are used for CPU intensive jobs, parallel modeling, and storage. They include several multi-node Beowulf clusters with over 25 Terabytes (TB) of RAID5 disk space, over twenty dual-CPU servers with a combined capacity in excess of 70 TB of RAID5 storage, and several other application and web servers. The Sun system is a Sunfire 280R that operates as both an application server and as the backup/archive server. Backups and archives are done using the Networker product from EMC. Most of the main servers share a gigabit (Gb) switch with the archive/backup system for high-speed communications. Nearline storage is done on a tape library unit. The tape library is a 120 slot Qualstar 46120 unit with 4 AIT-3 drives capable of 12.0 TB of native storage. All of this equipment is kept within a physically secured, humidity and temperature controlled machine room with UPS power. Final data and image products are produced from several ink-jet plotters and laser printers within the department. Additionally, several CD/DVD writers are used for data distribution.

ESRC also leverages the center's Laboratory for Remote Sensing and Spatial Analysis, a spatial information processing, analysis and distribution research laboratory. This laboratory provides geographic information system (GIS), Web Mapping, spatial data archiving, data distribution, remote sensing, image processing, cartography, large format printing and scanning support to several ESRC and EOS research projects. Staffed by professional geo-spatial information technicians, computer programmers, and graduate and undergraduate university students, the laboratory houses a multiple seat dual and quad core Linux, PC, and Mac OS computer cluster supplied with a variety of open source Remote Sensing, GIS, web mapping, image processing and cartography software and ESRI ArcGIS, Leica ERDAS Imagine, and IDL/ENVI, commercial site, block, and individually licensed GIS and Image processing software.

UNIVERSITY OF SOUTH CAROLINA

Facilities: The current proposed research will make use of the Baruch Institute's field laboratory at North Inlet consisting of a 24,000 ft2 research facility with a state-of-the-art analytical chemistry lab. The main research building contains 22 labs and 13 offices, library, computer laboratory, wet teaching laboratory, conference and teleconferencing facilities. Computer facilities at the lab are also excellent. Three modern dormitory buildings can accommodate 52 persons. Three cabins (7 persons each), all equipped with kitchens, provide living space for visiting students and researchers. A 2950 ft2 conference lodge is also available. The BMFL has a resident staff of 28, one of whom is a technician supported on various NSF projects (LTREB, PIE LTER, this proposal).

Other Resources: Morris manages a 1031 ft2 laboratory at the University of South Carolina, Columbia campus that is set up for analyzing samples typical for a field biology program. The lab has equipment for wet chemical analyses of water and soil (Lachat FIA autoanalyzer, Shimadzu spectrophotometer), plant ecophysiology (LiCOR 6200, Walz PAM fluorometer, GER 1500 Spectroradiometer, Turner Fluorometer), as well as drying cabinets, balances, muffle furnace, etc. Students have desk top computers in their offices equipped with Matlab, SAS, and ESRI ArcInfo.

VILLANOVA UNIVERSITY

Laboratory: Weston occupies a research laboratory within the Department of Geography and the Environment at Villanova University. Laboratory space totals 1400 square feet and includes analytical instrumentation to complete the proposed work. These facilities are well supplied with chemicals, reagents, standard and specialty glassware, pipettes, pH meters, balances, hotplates, ovens, centrifuges, and a deionized water system. In addition, the PI has access to an environmental chamber, a greenhouse, and a shared equipment room at Villanova University.

Office: Weston occupies a private office within the Department of Geography and the Environment at Villanova University with access to support services. Dell Latitude E6420 laptop for office and travel use, a Dell Dimension laptop for field use, and a Dell Optiplex 755 desktop for dedicated laboratory use.

Research Vessel: A boat (2007 G3 17’ aluminum boat) with outboard motor (40 hp Honda) are dedicated to field work associated with this proposal and other current support.

Equipment: The following equipment is housed within my laboratory, the shared equipment room at Villanova University, or within other faculty laboratories which I have access to:

- Canberra Low Energy Germanium Detector for gamma spectroscopy (7Be, 137Cs and 210Pb)
- Trimble 5800 Kinematic GPS System for high-accuracy mapping
- Agilent 6890N Gas Chromatograph with flame ionization and thermal conductivity detectors for CH4 and CO2 quantification in gas samples

- Leco TruSpec CN Analyzer for soil carbon and nitrogen analyses
- Shimadzu TOC-VCSH Total Carbon Analyzer for dissolved inorganic carbon and dissolved organic carbon and nitrogen quantification
- Agilent 6850 Gas Chromatograph with electron capture detector for nitrous oxide measurement
- Dionex DX-500 Ion Chromatograph for sulfate, chloride and other anion and cation analyses
- Hewlett Packard Liquid Scintillation Counter for measurement of radioactivity (14C and 35S) in liquid samples
- Agilent 1200 Series Liquid Chromatograph system for measurement of low molecular weight organic acids
- Glove Box for anaerobic sectioning and processing of soil cores
- Shimadzu Spectrophotometer for colorimetric analyses

VIRGINIA INSTITUTE OF MARINE SCIENCE

As part of William & Mary, the School of Marine Science at VIMS is among the three largest programs in the country with 55 faculty, a graduate student population between 90 and 100, and associated technical and support staff. In addition, there are 8-10 post-doctoral research associates training at the institute. The institute is located on a 42-acre campus in Gloucester Point, Virginia, at the mouth of the York River in Chesapeake Bay. It also has a satellite campus, the Eastern Shore Laboratory (ESL) on 5 acres at Wachapreague, VA, which serves as a field station with access to the estuarine and coastal ecosystems of Virginia’s Atlantic shore.

Laboratory and Field Equipment

Johnson has the necessary materials and equipment to complete the project including several stereo and compound microscopes (400x mag) with cameras, a high-precision balance (0.001 mg), fume hoods, drying ovens, -20°C and -80°C freezers, and adequate dry storage space. For field work, he has a shear vane, Russian peat corer, various push corers, and nets.
Office space: Johnson has a private office with computer processing power and cloud storage as well as several software packages (Excel and Word). VIMS provides cloud-storage via Box® of 100gb to all faculty. Office spaces for graduate students is available and assigned as needed.

VIMS Computer Resources: All faculty, research scientists, students and technicians are provided with computers that are networked and have access to a central database. The PIs collectively have more than 15 computers for analysis and documentation, with most connected to the internet via a Network Virginia DS-3 (45mbit/sec) connection. The VIMS (Gloucester Point) campus is connected to National Lambda Rail via a Gigabit ethernet connection through the W&M Main Campus. Both Apple Macintosh and IBM compatible systems are available.

OTHER RESOURCES – PERSONNEL

Dr. David Burdick, 0.01 months/year. Burdick is an ecosystem ecologist and an expert on salt marshes. He will help coordinate research efforts on salt marsh restoration site and continue his research on these sites on plant production and species changes.

Dr. Linda Deegan, 0.01 months/year. Deegan led the PIE consumer group until this current round. She led the large scale TIDE experiment, an experimental nutrient addition experiment, and is an expert on estuarine fish. She will continue to advise us on issues related to consumers.

Dr. Cedric Fichot, 0.2 months/year. Fichot is a biogeochemist with an expertise in marine optics, remote sensing, organic geochemistry, and photochemistry. He will help promote a better understanding of DOM dynamics in the estuary.

Dr. Inke Forbrich, 0.1 months/year. Forbrich is a landscape ecologist and an expert on measuring land-atmosphere exchange using eddy flux tower. She is the lead PI on the DOE funding project in the PIE oligohaline zone. She will be come a faculty member at the University of Toledo but will continue to coordinate all the eddy flux towers.

Dr. Robinson "Wally" Fulweiler, 0.5 months/year. Fulweiler is a biogeochemist with an expertise in Si and N cycling. She will also facilitate cross LTER comparison of Si cycling between Harvard Forest and PIE and work with Giblin on N_2 fixation.

Dr. David Johnson, 0.5 months/year. Johnson is a community ecologist who will continue the PIE long-term data collection on invertebrates and investigate the ecological impact of new species coming into PIE due to warming.

Dr. David Kimbro, 0.01 months/year. Kimbro is a population and community ecologist who works on salt marshes and oyster reefs. He will interact with the consumer group.

Dr. Gregg Moore, 0.01 months/year. Moore is a coastal ecologist focused on coastal wetland restoration ecology and managements. His work has a particular emphasis on anthropogenic impacts to wetlands and the management of invasive species. He will continue his work on the restoration sites and help coordinate new PIE efforts in these sites.

Dr. James Nelson, 0.1 months/year. Nelson will lead the consumer group. He uses a combination of field surveys, experimental approaches, stable isotope analysis and mathematical modeling to quantify the functional role of marine organisms in mediating energy flow, biogeochemical cycles and community structure in coastal ecosystems. He will continue the long-term data collection of nekton and carry out the E-Scapes modeling.

Dr. Amanda Spivak, 0.01 months/year. Spivak is an organic geochemist who is working to refine the role that estuaries and wetlands play in the global carbon cycle and predict their likelihood of recovery from disturbance. She will coordinate with the LTER and carry out a separately funded experiment on the role of plants in carbon storage.

Data Management Plan

As instructed in the solicitation, this information is being submitted as Other Supplementary Documentation.

DATA & INFORMATION MANAGEMENT PLAN

Introduction:

The goals of information management at PIE LTER are to: 1) curate and preserve high quality and well-documented data and metadata, 2) ensure that data and information are available to the community and to the public in a timely manner to facilitate scientific synthesis, education, and policy decisions, and 3) provide LTER and LTER-affiliated researchers education and training on best practices for collecting, curating, and publishing environmental data.

Data Availability

PIE data are handled in concurrence with the FAIR data principles assuring that the data are Findable, Accessible, Interoperable, and Reusable, and adhere to the LTER Network Data Access Policy assuring that the PIE data resources are made publicly available without due delay. PIE data is discoverable through the Data Observation Network for Earth (*DataONE)* and made accessible through the Environmental Data Initiative (EDI) Data Portal (https://portal.edirepository.org/) and the Biological and Chemical Oceanography Data Management Office (BCO-DMO) (https://www.bco-dmo.org/), which are both member nodes of DataOne. Eddy covariance data from PIE marshes are also available in the DOE AmeriFlux Network (https://ameriflux.lbl.gov). In addition, data are also available for direct download from the PIE-LTER web site. Microbial genomic sequence data are available through accession numbers in NIH GenBank (https://www.ncbi.nlm.nih.gov/genbank), which are also discoverable from PIE and EDI web sites. The integration of PIE data in these internationally renowned repositories are very valuable for data-curation in PIE, ensuring the long-term preservation in accordance with international data and metadata standards and facilitating discovery and access to PIE data resources for future re-use.

Information management in PIE begins with PIE's web site (https://pie-lter.ecosystems.mbl.edu/welcome-plum-island-ecosystems-lter), which provides descriptions of research-, education-, and outreach-activities, a data catalog, and a directory of personnel and publications associated with PIE. PIE's web site currently uses Drupal 7, a web site content management system. Several other LTER sites also have used Drupal, in a collaborative known as Drupal Ecological Information Management System (DEIMS). We will now be moving from this system as we upgrade to Drupal 9 (*see below*). PIE follows the guidelines in the LTER Network's IM Policy and NSF's Proposal and Awards Policies and Procedures Guide for Data Archiving.

To assure data quality and implementation of best practices for data management we have implemented the guidelines for full data life-cycle management outlined by DataOne (Plan, Collect, Assure, Describe, Preserve, Discover, Integrate and Analyze).

Data Life Cycle

Planning: PIE has a continuous data input from long term monitoring operations of i.e., water quality, climate, water level, population dynamics etc. with well-established data handling workflows. In addition, data inputs come from individual research projects at PIE, which content may vary from year to year. Descriptions of work from the individual research areas, which describe experiments, observational and geospatial studies, are used to identify the types and formats of data that will be produced and submitted to the PIE database from future research activities.

At our annual Spring PIE All Scientist Meeting (ASM), we give an overview of the data management activities and remind researchers of their data management duties. This provides opportunities for PIE researchers to discuss our information system and coordinate future data management needs for their research. Our data publication obligations are upheld by reminding PIE researchers several times a year via email, teleconference calls and during field sampling trips about their obligations to submit data to the PIE database.

Experience from PIE has shown that graduate students need more focus on data management, and as part of our data education activities, we plan to schedule an annual meeting between the Information

Manager (IM) and each graduate student and their advisor to help them properly curate their data-files and have them ready for submission. Short presentations and an opportunity for data management Q&A will also be provided at the annual fall graduate student symposium. Furthermore, this has the purpose of avoiding the huge rush to submit and publish data, once a paper has been accepted.

Collection and Assurance – Each PI and associated research assistant/graduate student is responsible for their data collection, assurances of quality, data entry, validation and analysis, and data submission for their respective projects. We require that data and documentation have been reviewed by the data originators before they are submitted. The PIE IM takes responsibility for the data from the weather stations, water quality sondes, and the tide gauge. These sensor-based data are quality-controlled by flagging for outliers using an R-script based on methods described in Papale et al. (2006) in addition to manual checks.

With guidance from PIE IM, data are submitted to the PIE database using a standardized Excel metadata and data form (https://pie-lter.ecosystems.mbl.edu/sites/default/files/PIEMetadataBlank_0.xls). The form provides guidance on metadata entry, and standardization of units, measurement scale, and number type using “drop down”-lists. This ensures consistency and harmonization of data-entries from different sources.

Observational long term monitoring data, (ie., water quality, climate, water level, population dynamics) and long- and short-term experiments are also submitted to the PIE database using the above-mentioned Excel form. Geospatial data (boundary, census, elevation and bathymetry, land use, land cover, parcels and zoning) are typically submitted via ArcGIS formatted data raster or vector data packages. Microbial genomic sequence data that have been submitted to GenBank are made discoverable via a genomic reference file, (https://pie-lter.ecosystems.mbl.edu/content/microbial-genomic-reference-data), which describe bacterial genome citations, sequence accession numbers, study type, location, habitat and descriptive environmental conditions.

All files have a version number which is advanced anytime the data is changed. Notes are made on the changes in the metadata and the new file is transferred to the data repository. All data products are uniquely identified by a version number and DOI.

Description – The above-mentioned Excel metadata and data form is used as the initial step by researchers to describe and submit data to the PIE data repository. After submission the dataset is validated by the PIE IM using a script (Excel macro) to check metadata and data for completeness, consistency, and formats with the goal of providing high quality data and metadata suitable for generating Ecological Metadata Language (EML) metadata. The Excel macro facilitate metadata accuracy checks (standard units, package IDs, missing value codes, research locations), which are useful prior to submission to the EDI Data Portal, where extensive EML congruency checks are conducted. Upon a successful macro check, the metadata are entered, and data uploaded to the PIE web server where the dataset, data sources, and other content specific to PIE beyond what is needed in the meta data are (specific research area linked to the proposal, etc.) are updated or created. Once the data are in our web site, an EML metadata (xml) file is generated for uploading the dataset and corresponding data source (csv or zip file) to the EDI Data Portal national repository. After the uploaded dataset is accepted in EDI, a Digital Object Identifier (DOI) is assigned, and the dataset becomes publicly discoverable through DataOne.

Preservation – Preservation is assured by depositing the PIE data and metadata in national renowned data repositories: primarily EDI, but also other national databases such as AmeriFlux for atmospheric flux data and NCBI for microbial data. Locally, the PIE LTER web site is hosted on a shared (with ARC LTER) virtual Linus Server by the MBL. Backups of the MySQL database are made nightly. Weekly and monthly backups of the web site and the MySQL databases are stored for 1 month and 1 year respectively. The server is imaged and backed up by the MBL’s Veeam backups and disaster recovery platform each night.

The Marine Biological Lab (MBL) maintains an intranet providing online storage and cloud backup for PIs and RAs working on research projects using "Crash Plan". In addition, MBL has Office360 licenses for year-round researchers which include OneDrive for Business. Each researcher gets 5 TB of online storage with the ability to share folders and files with external researchers. Documents are shared using e-mail, Google Docs and OneDrive. As detailed in *Facilities and Equipment*, PIs at other institutions use their own University back-up systems for data that has not yet been submitted to the IM.

We currently use EndNote for managing bibliographies and publication citations. EndNote has the capability for exporting file formats for importing publications to our Drupal web site and BibTex formats for submission to the LTER Network Communications Office (NCO) and NSF.

Discovery – PIE adheres to the LTER Network Data Access Policy, https://lternet.edu/data-access-policy, with regard to data availability, metadata standards and data use agreements. Researchers funded by the PIE LTER are required to contribute data to the PIE LTER. It is recognized that investigators on PIE LTER have first opportunity for use of data in publications but there is also the expectation for timely submittal of data sets for incorporation into the PIE LTER data system and public data repositories. PIE strives to make data available within 1-2 years. The variability in time is dependent upon the time required for sample analysis and corresponding QA/QC of data and metadata. Data such as water quality, water level, discharge and meteorological observations from data-logging instruments and data that do not require a great deal of post-collection analysis are available within 3-6 months of collection. All other data is submitted within two years. Graduate students can request that their data be embargoed pending completion of their dissertations for a limited amount of time. Very large data sets, > 5GB, such as raw LiDAR are available upon request in addition to non-tabular scanned aerial imagery and maps.

PIE data are easily discoverable, accessible, and downloadable via our web site and the EDI Data Portal in accordance with the PIE data use agreement, https://pie-lter.ecosystems.mbl.edu/content/pie-lter-data-use-agreement and as outlined under the Creative Commons Attribution 4.0 International License, https://creativecommons.org/licenses/by/4.0. We request that datasets be cited, and the PIE LTER funding source and NSF be acknowledged in published papers. The PIE Data Catalog, https://pie-lter.ecosystems.mbl.edu/data, includes data set search capabilities using keywords, LTER Core areas and PIE Research Areas to provide easy access to datasets of interest.

Integration and Analysis - PIE researchers use a variety of software for data integration and analysis depending upon the data type and research goals. Excel spreadsheets are routinely used for chemical analyses, data entry and basic statistics. Larger data sets such as marsh sediment erosion table elevation heights are analyzed with SAS. Matlab is used routinely in eddy covariance analysis. ESRI ArcGIS and Clark Labs TerrSet IDRISI GIS Analysis and Image Processing software are used for geospatial data. To facilitate integration of PIE data in larger data collations, PIE data sets follow international standards for data and metadata and are available for downloads in a variety of recognized formats.

Analysis using Delft3D, SWAN and D-Water models will be undertaken on the Boston University Shared Computing Cluster (SCC), located in the Holyoke, MA, LEED Platinum certified Massachusetts Green High Performance Computing Center (MGHPCC). Two pairs of 10Gigabit Ethernet network connections link the MGHPCC to the BU campus with extremely fast data transfer. The SCC is a heterogeneous Linux cluster composed of both shared and buy-in components (over 8,000 shared CPU cores, over 10,000 buy-in CPU cores, and 6 petabytes of storage— 90% buy-in—for research data), and provides a high-performance computing environment well-suited to the disciplines relevant to the current project: geographic information systems (GIS), statistics, data analysis, scientific/engineering simulation, and optimization. The Delft3D-SWAN model is already installed on the SCC and it is run in parallel with more than 20 nodes on the shared cluster.

Information Management Team

Researchers work with the IM and the IM team to curate and facilitate future publish the data sets

The Information Manager (IM) has the responsibility for overseeing the overall integrity of the data and information system. Hap Garritt, a senior research assistant (SRA) with The Ecosystems Center, MBL, had been the information manager since 1998 and has just retired. We are in the process of interviewing for his replacement, and one should be in place by April. The new IM will be supported by Ketil Koop-Jakobsen, an Assistant Scientist at the MBL. Koop-Jakobsen will be an active scientific researcher on PIE V, but he also has a wealth of data management experience, which will be beneficial to the IM at PIE. From a preceding career in environmental data science, Koop-Jakobsen has experience from international projects on interoperability and data-exchange among environmental research infrastructures (project COOPEUS) and ocean observatories (project AtlanOS) for Bremen University, and data-projects on "Mesopelagic production" and "persistent identifiers in scientific data" for data publisher PANGAEA. The rest of the information management team consists of: Anne Giblin (Lead PI), and the coordinators of each research area (Nelson, Fagherazzi, Wollheim, Weston, Bowen), and Jane Tucker (SRA-Web). We also will continue to interact with Jim Laundre (ARC-LTER IM support) and research staff at the MBL with computer programming expertise. Management includes infrastructure support and guidance to process data for public availability on the PIE web site (https://pie-lter.ecosystems.mbl.edu/data) and public national data repositories.

The PIE IM will attend LTER Information Management meetings and video teleconferences to participate in network activities and provide updates about LTER Network data standards and new initiatives. The PIE Executive Committee is responsible for dealing with researchers who do not submit data in a timely manner to the database. The PIE Executive Committee reserves the right to withhold funding or restrict services and facilities to researchers who do not contribute their PIE-related data. We have explicitly included program coordinators in the data management team to assure the submissions of data to in a timely way.

Accomplishments 2016-2022

- PIE currently has 583 data sets in EDI and 33 additional data sets in repositories for microbial data which are referenced on our web site.
- We moved the PIE web site from Drupal 6 to 7 in December 2017. Drupal provides a much better content relationship system which is easily searchable across research areas. We are now in the process of upgrading to Drupal 9
- During PIE IV we have had a much higher percentage of successful dataset uploads to EDI, due to the successful implementation of new congruency metadata checks.
- Bibliography and Personnel updates were provided to the LTER NCO for use in the 40 year review. In addition, PIE routinely contributes to network databases including the Personnel, Bibliography and recently to the EDI ecocomDP community survey.
- PIE Eddy covariance data is now also uploaded to the Ameriflux network where it is frequently used. PIE data is downloaded from Ameriflux on average more than 60 times per month.
- Hap Garritt, our data manager participated in the LTER working group on how to accommodate non-tabular data in EDI for improved public access to these unique data resources. PIE has many very large data sets associated with LiDAR and many historical maps and aerial photos which are not easily made discoverable and accessible via standard data repositories. This group has produced a report with many recommendations (Gries et al. "Data Package Design for Special Cases Ver1. https://doi.org/10.6073/pasta/9d4c803578c3fbcb45fc23f13124d052).
- In 2019 we instituted a new site registration procedure in 2019. Each year everyone connected with the PIE LTER registers at Plum Island Ecosystems LTER Registration form

(https://form.jotform.com/93215313431143). This improved our internal communication and allows us to keep track of all users at our site. We also use the registration procedure to make sure everyone has received boat and safety training, read and agreed to our Principals of Community, and are fully aware of the MBL's Unlawful Harassment Policy. The registration site also collects all the information requested by the network for the NCO list of site personnel. The data can easily be downloaded to an excel file and users can easily re-register and just update the information without creating a new form. This also proved to be an invaluable way to keep everyone informed on MBL's Covid policies, and as required, register their vaccine status.

Plans for PIE V

Our most urgent task is to move our Drupal 7 web site to Drupal 9 as Drupal 7 is no longer being supported. This is being facilitated with MBL who has just migrated their site to Drupal 9. The ARC LTER is also upgrading to Drupal 9 and we will collaborate with them on the move. We will move to using the R script developed by the ARC-LTER (available from ARC-LTER GitHub) to parse the metadata from our Excel workbook template. This script creates the text template files required by EDI's EMLAssemplyline R script and then runs the EMLAssemplyline script to produce an EML file for uploading to the EDI portal. The PIE Drupal 9 web site will still provide information on the project, including documents, research sites, keywords, bibliography, and a dataset catalog. However, the data catalog will link directly to the public data repositories where the full metadata and data can be downloaded.

Additionally, the following tasks are planned to be conducted during PIE V:

- We will continue R script development for further QA/QC and analysis of long-term high frequency data sets such as climate, water quality, and water level and provide these tools to other team members, and have workshops for graduate students on using these tools.

- We will collaborate with EDI on the on-going effort to migrate PIE ClimDB/HydroDB data to Hydromet (harmonized database of meteorological and hydrologic data in CUAHSI ODM CSV).

- We will expand the description on project funding associated with various datasets.

- We will continue to make historical maps and aerial photos discoverable and accessible via data repositories using the best practices outlined in Gries et al. (above). We will also locate and store legacy data from former collaborating projects.

- We will create an EML data catalog of our "large data file"inventory (>100GB such as LiDAR) and provide accession number URLs to link to sequence data area in repository

- We will update the PIE clickable map of research sites and migrate it to Google Earth Pro for better functionality: https://www.google.com/maps/d/u/0/viewer?hl=en&oe=UTF8&vps=2&msa=0&ie=UTF8&mid=1025UGbPXWLCXwFv3F39ffolznSU&ll=42.74007414630872%2C-70.84255131687172&z=13

- We will update our web content on education and other topics. Updates are planned to coincide with migration of web site to Drupal 9 this spring.

PROJECT MANAGMENT

GOVERNANCE AND COORDINATION –Overall direction and management are provided by lead PI, Anne Giblin, and the executive committee. After the next mid-term review James McClelland and Giblin will serve as Co-lead PIs with the expectation that McClelland will lead PIE VI. McClelland has extensive experience studying watershed-estuary systems and has been the Co-lead for another LTER, so he has both the LTER experience, and the science background needed to lead the next renewal.

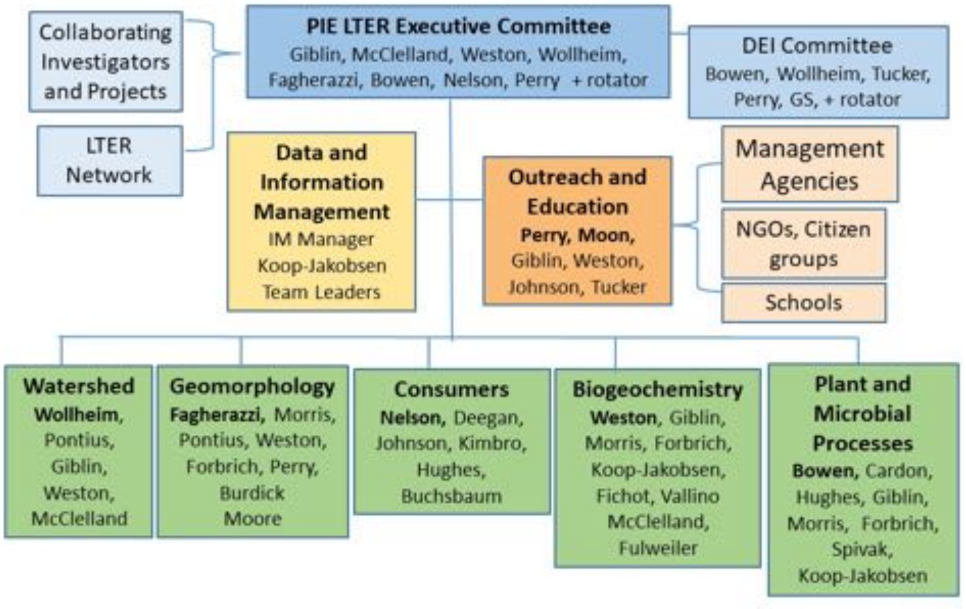
There have been several leadership changes for the renewal. James Nelson (ULL) will lead the consumer group and Sergio Fagherazzi (BU) will lead the geomorphology group. Both of these scientists are involved in other coastal LTERs (Nelson, FCE and Fagherazzi, VCR) which expands our ability to do cross site comparisons. The previous heads of these groups, Linda Deegan and James Morris, are still involved in PIE. Wil Wollheim (UNH) will continue to lead the watershed group and Nathanial Weston (Villanova) will continue as biogeochemistry lead. In recognition of the growing role that microbial process and plant genetics play in our questions we have a new group devoted to these areas led by Jen Bowen (NEU). There is a deliberate overlap between groups to facilitate communication, coordination, and synthesis. All of the group leads have been involved with the PIE LTER for more than 6 years. Danielle Perry (Mass Audubon) will take over for Robert Buchsbaum, who has retired, and Co-lead the outreach and education group with David Moon. Buchsbaum will still continue his annual bird census.

The executive committee will consist of Giblin, McClelland, the leadership of the major program areas, and a rotator (Fig.1) chosen by the Executive committee with a focus on individuals who are most likely to be future program coordinators.

The executive committee is consulted on financial matters, supplement requests, collects information for annual and final reports from other members of their program area, and facilitates transitions in leadership. The Executive Committee meets in person twice a year and by zoom every 1-2 months. Coordinators keep all other members of the LTER team informed about activities and findings in their program area. Each program area holds regular meetings that bring together PIs, students, post-docs and research assistants. Major research directions and strategic planning are discussed with all of the PIs and senior personnel involved in the LTER project and decisions are reached by consensus.

OTHER SENIOR PERSONEL – Joe Vallino will continue to advance MEP and advise on the modeling. David Johnson works on invertebrates and climate migrants. Ann Hughes will begin a new effort on plant genetics and plant traits. Inke Forbrich (MBL), runs the eddy covariance towers and works on C balances in marshes. She is the lead PI for the DOE project in the oligohaline zone. Amanda Spivak (UGA) is facilitating cross site comparisons between PIE and GCE

Fig. 1 PIE Management Structure, GS refers to Graduate Student Rep



with a separately funded NSF project. David Kimbo (NEU) adds additional depth to community ecology.

We have added six new scientists to the team. David Burdick and Gregg Moore (UNH) have been doing work in the Great Marsh for many years and are spearheading efforts to understand the efficacy of marsh restoration efforts in the region. Wally Fulweiler (BU) will work on Si cycling and N fixation. Si studies at PIE will complement her ongoing efforts on Si cycling at Harvard Forest. Zoe Cardon (MBL) is an ecosystems ecologist interested in rhizosphere processes, and is a PI on the DOE with Forbrich. Ketil Koop-Jakobsen (MBL) is a biogeochemist focusing on plant-soil interactions and also brings expertise in data management. Both Koop-Jakobsen and Cardon also bring expertise in new sensors to study belowground processes. Cedric Fichot (BU) will combine his expertise in marine optics, remote sensing, organic geochemistry and photochemistry to promote a better understanding of DOM dynamics in PIE and complement Jim McClelland's expertise in organic matter characterization, transport and cycling. McClelland will be leading the DON-oriented work for PIE V.

Three members of our team from PIE IV will not be participating in PIE V. Jarrett Byrnes (U. Mass-Boston) has decided to devote his energy to kelp research, Martha Mather (KSU) finished her work on striped bass at PIE and is now focusing on freshwater fish. Scott Hagen has sadly passed away.

PROJECT COMMUNICATION AND COORDINATION The site review team questioned how we kept track of the students, post-docs and casual researchers at our site. We realized that this was a real deficiency, as all of our "official" communications were through the PIs. Unless someone requested housing, MBL did not have a robust, centralized system to know who was at the site. We also had no way of making sure day visitors received all the needed information on Title IX policies, field safety training, emergency procedures, and who to go to for problems. To address this, we instituted a mandatory annual on-line registration system that allows us to track all PIE personnel and visitors to our facilities to make sure everyone has had a proper orientation to the site, safety training, and are aware of our Principles of Community. All records of training are logged. This enhanced communication method also proved invaluable allowing us to keep everyone informed on changing Covid protocols.

Each spring we hold a meeting of all scientists, post-docs, students and research assistants associated with the LTER. All personnel working on PIE LTER-related projects are invited to attend and present their findings. At our annual spring meeting we summarize the results of individual program areas, synthesize across disciplines, and plan the research program for the coming year. Graduate students present thesis ideas and participate in the planning for the upcoming field season. We also encourage representatives of governmental agencies and NGOs working in the area and teachers working with our education program to attend the annual meeting and give presentations on their work. This next round we are expanding opportunities for graduate student interactions and will hold a special graduate student symposium in the fall and connect graduate students through a list serve and social media.

EDUCATION and OUTREACH – PIE's education and outreach committee is led by Danielle Perry and David Moon and includes Anne Giblin, David Johnson, Jane Tucker and Nat Weston. David Moon directs our Schoolyard education program and is included in all discussions that involve K-12 education. Moon will work with PIE scientists on professional development courses for teachers. As mentioned in Broader Impacts, nearly all PIE scientists interact with local, state and federal management agencies. Giblin takes the lead in most interactions with Parker River NWR, and Giblin and all PIs work with Essex County Greenbelt on outreach activities. Before Covid, and again this fall, we jointly host community open house events at the Marshview field house. Wollheim is our primary contact with both the Ipswich and Parker River watershed associations and these groups have volunteers for our sampling programs. Vallino coordinates PIE modeling efforts with other regional modeling efforts.

Each year PIE scientists have the opportunity to request an REU from the two provided to the LTER. In addition, we normally have 3-4 additional undergraduates and several graduate students in residence. At the end of the summer we hold an all-day meeting where graduate and undergraduate students present their work to PIE scientists and local agency managers.

DIVERSITY EQUITY AND INCLUSION - PIE recently created a DEI committee, consisting of PIs, permanent staff, graduate students, and our education and outreach leaders. Jen Bowen serves as the initial chair. Bowen has extensive experience working on DEI related work, including serving on the steering committee for the National LTER DEI committee. She participated in the AdvanceGEO Train-the-Trainer program, among others, and is active in DEI efforts at her home institution. The rest of the committee consists of Wil Wollheim, head of the watershed group, Jane Tucker, Senior Research Assistant, Danielle Perry, Outreach Coordinator, and the PIE Graduate Student Representative to the LTER Network. Wollheim served on the UNH NR DEI Committee (2020-2021) and both Wollheim and Tucker participated in URGE (The NSF funded program Unlearning Racism in the Geosciences). We will offer an additional rotating slot to interested members of the PIE team. The goal of this committee is to promote actions across PIE that increases diversity, equity and inclusion at all levels within the LTER and to leverage the network of people and institutions associated with PIE in working toward expanding access to STEM throughout the PIE domain. To further that goal, we aim to build bridges with communities in our region that have historically been excluded from STEM fields. As a starting point, we are engaged in learning with an organization called the Indigenous New Hampshire Collaborative Collective (INHCC), an organization focused on framing the local history of our watersheds through an indigenous lens. INCC has been extremely interested in the removal of dams in coastal New England, which has been a major research effort in the PIE LTER and we have already had a considerable amount of dialogue with this group. Future efforts will continue to build foundations with community partners, including INHCC, to bring their perspectives into PIE efforts and provide pathways for building on shared interests.

We have made a concerted effort to increase diversity. At the K-12 level we have been able to increase the number of under-represented students participating in the program by adding the Collins Middle School (Salem, MA), and through our RET we reach students at Lowell Middlesex Academy, which serves a diverse population of at-risk students. Over the last two years we have also been more successful recruiting underserved students for our REU positions by actively engaging minority serving institutions. At all levels, the PIs at each of our institutions use list-servs set up by scientific societies and their own HR departments to assure a wide distribution of position announcements and leverage professional society recruiting efforts (e.g., AFS, ESA, ASLO, ASCE, SACNAS).

ADVISORY GROUPS – Last year we replaced our rotating with a standing advisory committee which will play an active role in PIE V. Committee members are Joel Kostka (Georgia Tech), Max Castorani (U. Virginia) and Shimon Anisfeld (Yale).

ENCOURAGING NON-LTER SCIENTIST PARTICIPATION AND COORDINATION - We broaden participation by writing grants with non-LTER investigators. We encourage non-LTER scientists and students to work at PIE and whenever possible assist them with site access, housing and computer facilities, and data and maps to help plan their research. Investigators coming for short periods of time are put in contact with the PI or research assistant / field technician most able to assist them. We actively help scientists from other LTER sites find study locations for comparative studies. Scientific and logistic coordination with non-LTER scientists is achieved through our annual meeting, and by interactions with PIE LTER investigators. Our program coordinators serve as the key points of contact for long-term projects.

MANAGEMENT OF FIELD FACILITIES -PIE LTER manages two field facilities, the Rowley House and the Marshview Farm. Marshview is owned by the MBL and sleeps 14. There is an MBL post-doc or research assistant in residence at Marshview who provides on-site supervision. The Rowley Field House, which is rented from a local land trust, the Essex County Greenbelt Association, sleeps 8. This facility is reserved for shorter term stays. Researchers reserve space at our annual March meeting. After LTER slots have been reserved, both facilities are available to other collaborating projects, and other researchers affiliated with federal and local partners as space allows.