EPA STAR FINAL REPORT

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Linking Impacts of Climate Change to Carbon and Phosphorus Dynamics Along a Salinity Gradient in Tidal Marshes

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TITLE: Linking Impacts of Climate Change to Carbon and Phosphorus Dynamics Along a Salinity Gradient in Tidal Marshes

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SUMMARY OF PROJECT DESCRIPTION, GOALS AND OBJECTIVES

Tidal freshwater marshes are often located in areas experiencing intense urbanization pressure, yet provide valuable services to coastal ecosystems. A climate change stressor that is unique to tidal freshwater marshes is the intrusion of salt water into previously freshwater zones. Marshes must accrete to keep pace with rising sea levels, and accretion rates depend on the balance between accumulation and decomposition of sediments. In tidal freshwater marshes, organic carbon (C) accumulation is a major mechanism of marsh accretion, and understanding how changes in salinity will alter pathways of microbial metabolism of marsh C is critical. Our overall ***objective*** was to understand how salt water intrusion affects the biogeochemical cycling of C, S, N and P, which in turn affects the balance between C accretion rates, and gaseous C losses from tidal freshwater marshes.

Over the past four years, we have undertaken an extensive effort to determine the impact of climate-change induced, salt-water intrusion on tidal freshwater marsh ecosystems in the Delaware Estuary. Our goal was to implement a novel, three-phase approach to determine changes in tidal marsh metabolism (e.g., CO_2 and CH_4 gas fluxes and SO_4^{2-} reduction), C and P sequestration (sediment deposition and burial), and changes in rates of organic matter decomposition at sites along a low-salinity transitional gradient in the Delaware Estuary. All three phases of the proposed project were implemented successfully with three complete field seasons of data for field components and one full year of data collection for the lab experiment. **Phase 1** involved finding suitable field sites in the spring and summer of 2005 & 2006 by making

appropriate biological (vegetation) and chemical (e.g., salinity) determinations. We spent several months on selecting sites in tributaries of the Delaware estuary with appropriate salinity levels and vegetation (given urbanization pressures, *Phragmites* invasions to large areas of the Delaware estuary made it difficult to find sites). We established 4 sites in the DE estuary that spanned a range in salinity. **Phase 2** consisted of two components: a laboratory manipulation experiment and field-based gas flux measurements. We have initiated a long-term laboratory experiments on cores collected from a site representing a freshwater end-member (i.e., Woodbury) of our salinity gradient (Results from the lab study are in press in the peer-reviewed journal, *Biogeochemistry*; see attached manuscript). To complement our lab study, we set up field plots in 2007 at three sites along our established salinity gradient, and measured net ecosystem production (NEE) over the field season. **Phase 3** was initiated in 2007, and involves a large-scale field manipulation (reciprocal transplanting of cores as a space for time substitution) to examine longer-term, ecosystem-level responses of marshes to elevated salinity. Since April 2007, we have measured Net Ecosystem Exchange (the balance in C production and consumption), monthly and in some cases bi-monthly, over the duration of the field season, for two seasons in both permanent and reciprocally transplanted plots). Phase 3 continued through the Fall of 2009.

Results of both the lab and field experiments from this funded research have provided us a stronger foundation to understand the response of Tidal Freshwater Marshes (TFMs) to climate change and salt water intrusion. The balance between marsh accretion and subsidence, and ultimately the ability of TFMs to outpace rising sea levels involves a complex interaction of the processes that drive plant production, microbial decomposition, sediment deposition and, ultimately, marsh accretion. The results of our work suggest that salt water intrusion will increase microbial decomposition and, together with declines in plant production, may put TFMs at risk of permanent inundation and create a positive feedback to the global C cycle.

SITE SELECTION

The Delaware River basin covers approximately 33,061 km^2 in DE PA, NJ and NY, and is one of the most populated and ecologically important areas of the mid-Atlantic region in the U.S. The Delaware River is the longest free-flowing [un-dammed] river east of the Mississippi, and extends 530 km from the confluence of its east and west branches in New York, and is a tidal estuary for 190 km before entering the Atlantic Ocean at the mouth of Delaware Bay. The Delaware River Basin is highly urbanized, especially the tidal portion. We have established four sites in the tidal portion of the Delaware River (Figure 1). Rancocas Creek is our freshwater end-member receiving no salt (negligible conductivity; Figure 2) for the duration of the growing season and beyond (pore water analysis of cores collected from Rancocas have verified the lack of salt water intrusion to a depth of 25 cm, Raccoon Creek is largely fresh with salinities ranging

from 0 to ~ 0.8 ppt (conductivity typically less than 2.0 mS cm^{-1} even during the driest months of the year; Figure 2), and Salem in Mannington Meadows receives salinity in the range of 1-5 ppt. Stow Creek is our salt water end-member with salinities ranging from 5 -12 ppt, and highest conductivity (Figure 2). We have set up boardwalks at all four sites, permanent square collars for gas fluxing (0.5 m x 0.5 m; Figure 3 left), and transplanted cores with collars that measure (30 cm in diameter; Figure 3 right). The transplanted cores were collected from Rancocas in 2007, and transplanted to each of the four sites (back transplanted to Rancocas, Raccoon, Salem, and Stow).

ACCOMPLISHMENTS

LAB EXPERIMENT-SUMMER 2007 & 2008

We completed a long-term, salinity-manipulation lab experiment that began in 2007. From both control and salinity-amended cores, we have approximately 14 months of CO_2 and CH_4 flux measurements, depth integrated concentrations of chloride, sulfate, dissolved inorganic carbon (DIC), ammonium, dissolved organic carbon (DOC), acetate, sediment organic C and methane, and depth specific rates of sulfate reduction, hydrogenotrophic methanogenesis, and acetoclastic methanogenesis. The bulk of these data are in press in the peer-reviewed journal, *Biogeochemistry.* Please see attached manuscript (Weston et al.) for figures and full interpretation. *Major Findings from the lab experiment include: (1) Salt water intrusion into tidal freshwater marshes (TFM) can significantly increase rates of microbial C mineralization. (2) The total amount of C mineralized as CO_2 and CH_4 from salt-water amended cores was ~ 37% greater than freshwater amended cores over the one year duration of the experiment. (3) Salinity intrusion increased rates of both sulfate reduction and, surprisingly, methanogenesis, resulting in increased CO_2 and CH_4 emission (as the product of these decomposition processes) from the TFM sediments undergoing salinity intrusion. (4) This increase in organic matter decomposition and carbon gas emission indicates that the vertical accretion potential of TFM experiencing salinity intrusion may be decreased, with negative implications for the ability of TFM to keep pace with rising sea levels and feedbacks to the global C cycle*.

FIELD EXPERIMENTS: PERMANENT PLOTS AND TRANSPLANTS

For both the permanent plots and transplanted cores we have three complete field seasons of biomass, photosynthetic efficiency, respiration rates, and methane fluxes (Figures 4-7). These data sets constitute the bulk of the project, and will likely be published as two, perhaps three manuscripts. Not surprisingly, transplants behaved differently than permanent plots early on (2007), especially in terms of biomass, but by the second field season, the native plants had grown into the transplanted cores, which initially contained the dominant vegetation at Rancocas (*Pontederia cordata*), and behaved more like the host site, biologically

and chemically (Figures 4-7). By the second year post-transplant, plant species reflected that of the host site and estimates were comparable to those in the permanent plots. Respiration rates, CH_4 Flux rates, photosynthetic efficiency, and aboveground biomass were all highest at Raccoon and Salem in the permanent field plots (Figure 4). During the last field season, patterns in the transplant plots were similar to field plots in that respiration rates and photosynthetic efficiency were highest at Raccoon and Salem, while above ground biomass was greatest at Salem and Stow. Interestingly, CH_4 fluxes were greatest at all sites in 2007, when first transplanted, but rates were typically low for all sites except Salem (Figure 5). Gross Primary Productivity (GPP) was greatest at Raccoon and Salem in the field plots while rates were similar for transplanted cores at all sites (Figure 6 & 7). Plants at Raccoon and Salem were more efficient photosynthetically (slope of relationship for GPP vs. PAR (photosynthetically active radiation) than the dominant vegetation at Raccoon and Stow in both field and transplant plots (Figures 4-7). The C:N ratio was highest at Raccoon, due to greater %C throughout the profile (Figures 11-14).

We also measured microbial rates of sulfate reduction and methanogenesis; rates of methane production through both the acetoclastic and hydrogenotrophic pathway also were measured (Figure 10). Rates of hydrogenotrophic methanogenesis are generally low, and acetoclastic methanogenesis is very low at Stow *in situ*, which is what we expected, but also supports the results we obtained in the lab experiment (see attached manuscript, Weston et al. 2010 in press in *Biogeochemistry*). We expected to find rates of acetoclastic methanogenesis highest at Rancocas, our freshwater end-member site, but rates were highest at Raccoon. Rates of acetoclastic methanogenesis may be lower at Rancocas because of site placement (plots were situated on bank instead of higher in the marsh) and type of soils at this site (lower organic C and higher mineral content; Figures 11-14). Acetoclastic methanogenesis is higher in transplants at Raccoon (at 3 and 6 months) and Stow (at 3 months) than in field cores, with no significant difference at Rancocas, which we expected (Figure 10); rates were slightly higher at Salem at 3 months.

CARBON, NITROGEN AND PHOSPHORUS

CARBON

Carbon (C) varied between 2 and 8 % in cores taken *in situ* with lowest values at Rancocas, highest values at Raccoon, both are freshwater sites. The lower % C found at Rancocas is due to the higher mineral content found there relative to the other sites, and is likely due to where the cores were taken (creek bank versus higher elevation in the marsh) (Figure 11). Total % C did varied with depth at both Rancocas and Raccoon, but varied little with depth for Stow and Salem (Figure 11). Interestingly, when comparing cores *in situ* collected to cores collected from transplant plots, the transplanted cores showed lower % C, which supports higher rates of C

metabolism (sulfate reduction, methanogenesis) measured in transplants, at least in the initial months following transplantation (Figure 10).

NITROGEN

Nitrogen ranged from 0.1 and 0.5 % with higher % N found in the more saline sites (Salem and Stow), and as we saw with % C, variation with depth was seen primarily in the freshwater sites, Rancocas and Raccoon (Figure 12). % N in transplant cores tended to be lower when compared to *in situ* depth profiles (Figure 12). C:N ratios ranged from 9 to 21, with higher ratios found in the freshwater sites than the more saline sites (Figure 13). Interestingly, at the more saline sites, the C:N ratio was higher in the transplanted cores than cores collected *in situ*, while in the freshwater site Raccoon, C:N ratios were higher in cores collected *in situ* than in transplanted cores.

PHOSPHORUS

There were significant differences in the soil P pools from the marshes along the salinity gradient. Total soil P, calculated as the sum of the P fractions from the sequential extraction, ranged from 600 to 4100 μg P gdw^{-1} across all sites, dates, and depths. Concentrations were highest at the freshwater end-member site, Rancocas (Figures 15-17). Concentrations at the three downstream sites were generally similar to each other but lower than at Rancocas. There were no repeatable changes in total P with depth or time across the four sites (data not shown).

The trends in inorganic P (sum of H_2O-P_i, Fe-P_i, Al-P_i, and Ca-P) paralleled those of total soil P, with concentrations highest at Rancocas and lower (but similar to each other) at Raccoon, Salem, and Stow. Inorganic P accounted for > 80 % of total P at Rancocas and decreased to ~60 % of total P at Stow (Figure). The concentrations of Fe-P_i and Al-P_i were highest at Rancocas, where Fe-P_i accounted for 60-70 % of total inorganic P (vs. only 20-50 % at the downstream sites, except for the April 2007 samples from Stow where Fe-P_i was <5 % of total P_i). The contribution of Al-Pi to total P_i was ~10-30 % at all sites. Ca-P had a similar contribution to total P_i, except at Rancocas where Ca-P accounted for 3-5 % of total P_i. Water-extractable inorganic P (H_2O-P_i) made the smallest contribution to soil inorganic P, accounting for 1-3% of total P_i at Rancocas and increasing steadily to Stow (9-24 % of total P_i).

In contrast with total P_i, soil organic P concentrations (P_o, sum of H_2O-P_o, Fe-Po, Al-P_o, HA-P, and Res-P) were generally similar at all four sites (Rancocas: 290-570 μg gdw^{-1}; Raccoon: 160-280 μg gdw^{-1}; Salem and Stow: 270-440 μg gdw^{-1}). Across the salinity gradient, organic P accounted for 11-19% of total soil P at Rancocas and increased to 28-50 % at Stow. Organic P associated with metals (Fe-P_o and Al-P_o fractions) decreased in concentration and significance to the total organic P pool when moving downstream from Rancocas to Stow. In contrast, the

concentrations and importance of the H_2O-P_o, HA-P, and Res-P organic P fractions increased from Rancocas to Stow.

TRANSPLANTS:

Total soil P: Cores transplanted to Raccoon, Salem, and Stow had lower total soil P concentrations in both July and October 2007 than did the donor marsh (Rancocas). At the three downstream sites, total soil P in the transplants was converging toward levels in the in situ soils at these sites. This appeared to be largely driven by decreases in inorganic P at all sites. There were also decreases in soil organic P in the Raccoon, Salem, and Stow transplants relative to the cores transplanted back into the donor marsh but the absolute magnitude of the decrease in organic P was less than the decreases in inorganic P (100-500 μg P gdw^{-1} vs. >1000-2000 μg P gdw^{-1})

ORGANIC P POOLS IN TRANSPLANTS:

H_2O-Po: concentrations rise throughout growing season in all transplanted cores … but not at any sites except Raccoon.

Fe-Po: With the exception of the October 2007 data point at Rancocas, which was (anomalously) high, all transplants showed the same temporal trends in Fe-Po concentrations, suggesting that Fe-Po concentrations are not significantly influenced by salinity/sulfate.

Al-Po: Concentrations of Al-Po in the transplants at Raccoon, Salem, and Stow were generally lower than in the transplants at Rancocas.

HA-P: Concentrations of HA-P generally showed the same temporal patterns in all transplanted cores, regardless of site. Interestingly, concentrations of HA-P in the transplanted cores at Raccoon, Salem, and Stow were generally lower than the cores transplanted at Rancocas, even though HA-P concentrations in the in situ samples from these downstream marshes were higher than those from the donor marsh (i.e., HA-P increased as salinity increased in the natural marshes but not in the transplants).

Res-P: Very few changes in Res-P in the transplants, regardless of site.

INORGANIC P POOLS IN TRANSPLANTS:

H_2O-Pi: Like H_2O-Po, concentrations of H_2O-Pi generally rose throughout the growing season in the transplants, regardless of site. H_2O-P may be more a function of soil type (see Sundareshwar and Morris 1999).

Fe-Pi: Relative to cores harvested and re-transplanted at Rancocas, Fe-Pi concentrations in the cores that were transplanted to Raccoon, Salem, and Stow were lower and closer to Fe-Pi concentrations in the natural marshes adjacent to the transplanted cores. This may reflect interactions between the Fe, S, and P cycles that also contribute to lower Fe-Pi concentrations in more saline sites.

Al-Pi: Similar trends as Fe-Pi. Some of this may be an ionic interaction since Al-Pi fraction includes some P sorbed to clays. Additionally, it may reflect Fe-S-P interactions, although the Al-P generally contains P associated with more-recalcitrant Fe minerals (vs. more-labile Fe minerals in the Fe-Pi pool).

Ca-P: At Raccoon and Salem, Ca-P in the transplants was higher than in the transplants at the donor marsh, Rancocas. However, there were no real differences in Ca-P between the donor marsh transplants and the transplants at Stow, the most saline site, even though Ca-P in the in situ cores was roughly 2x higher at Stow than at Rancocas.

MICROBIAL COMMUNITY COMPOSITION-LAB STUDY

The ability of marshes to keep pace with rising sea level depends upon accretion of C, and the accretion and decomposition of C is dependent on which microbes are dominant. This finding has important implications for microbial populations and what controls their abundance, population and community dynamics. To gain a mechanistic understanding of how and why the dominant microbial processes responded in the manner they did in the lab experiment described above (higher), we wanted to know how the community composition of sulfate reducing and methanogenic microbes responded to salinity intrusion. Weston incorporated a new component of the project (not initially proposed in the EPA grant) that links process-based biogeochemical rates with quantitative determinations of key functional genes for sulfate reducers and methanogens. Key populations of anaerobic microbes mediating the oxidation of organic matter were targeted using functional gene primers: Sulfate Reducers (dissimilatory sulfite reductase, *dsrAB*), Methanogens (methyl co-enzyme M reductase, *mcrA*), Denitrifiers (nitrite reductase, *nir*). Population sizes were determined using q-PCR techniques, and community composition was determined by selective cloning and sequencing. Weston extracted DNA from freshwater and saline marsh sediments, functional genes were PCR amplified using functional gene-specific primers, and *dsrAB* and *mcrA* products of appropriate size were obtained. Qualitatively, we found more *mcrA* functional gene products in freshwater sites and more *dsrAB* in saline sites. Preliminary data collected by Weston were promising enough to explore further. Over the summer of 2007 and 2008, Tanja Přsa, a senior thesis research student, with funding from the Biology Department added a molecular component to take advantage, and complement our biogeochemical process rate data to further understand the impact of salinity intrusion on C mineralization pathways in TFM. We examined the impacts of rising sea-level on the structure and metabolic activity of SRB in TFM sediments undergoing salinity intrusion in the field transplant experiment described above. In the spring of 2007, we transplanted 6 intact sediment cores (30 cm diameter, 25 cm deep) from a TFM to the same TFM (Rancocas) and to a down-estuary brackish marsh (Stow, salinity ~11 ppt). Sediment sub-cores were collected from transplants at both sites at the time of transplant (t=0) as well as 3 and 6 months post-transplant (mid-summer and early fall). Tanja Přsa has used this approach to

examine the community composition in the laboratory experiment, and on the transplanted sediment cores. We determined rates of dissimilatory sulfate reduction and examined the community composition of SRB by targeting the dissimilatory sulfite reductase alpha subunit (*dsr*A) functional gene. We constructed *dsr*A clone libraries for both control and salinity-impacted sediments at 0, 3, 6 months post-transplant and used phylogenetic analyses to determine changes in SRB community composition between the TFM and down-estuary brackish marsh over time. Rates of sulfate reduction rates were significantly higher at Stow than Rancocas (35.6 ± 17.9 and 2.5 ± 0.9; mean ± std dev, respectively; p=0.0141, ANOVA). Phylogenetic analyses of sulfate reducing bacteria show that the community composition of sulfate reducing bacteria at Stow was significantly different from Rancocas 3 and 6 months post-transplant ($p < 0.05$; ∫-LIBSHUFF). These results suggest that salinity intrusion into TFMs will result in increased sulfate reduction rates and changes in microbial SRB populations. These changes will alter C dynamics in TFMs, potentially altering accretion rates and putting TFMs in jeopardy as sea levels rise.

Ms. Přsa has presented this work at the Society of Wetland Scientists meeting in Washington D.C. in May 2008, where she won honorable mention for best student poster (please see attached poster presentation titled, Přsa SWS 2008). Ms. Přsa also presented her work at the Partnership for the Delaware River Estuary Science and Environmental Summit, held in Cape May, NJ in Janauary 2009; At this meeting, Tanja Přsa won an award for best student poster presentation, and was invited to submit an article to Estuary News, a publication of the Partnership for the Delaware River Estuary (see attached pdf of the newsletter or view the following link: http://www.delawareestuary.org/pdf/EstuaryNews/2010/WinterNews10.pdf). Weston and Prsa plan to publish the results of this study in a peer reviewed journal later this fall. I have also attached a copy of the newsletter at the end of this report (see page 11 of the newsletter or page 65 of the report).

We have several publications in various stages of publication. We expect, including the attached Biogeochemistry manuscript, a total of 5, peer-reviewed publications from the funded work.

PERSONNEL, INFRASTRUCTURE AND SCOPE OF WORK DURING THE COURSE OF THE PROJECT

At the end of August 2006, Vile moved from the Academy of Natural Sciences to a new position as Director of Grant Development and Research Assistant Professor in Biology at Villanova University. With this position, transfer of the EPA award followed Vile to Villanova. Nat Weston, a Postdoctoral Fellow funded by this EPA grant, transferred to Villanova with Vile from the Academy, and served as an unofficial co-PI for the entire duration of the project. In August 2008, Weston was hired as a tenure-track faculty member in the Department of Geography and The Environment at Villanova University. Dr. Scott Neubauer served as a co-PI

at the Baruch Marine Research Lab, The University of South Carolina. In April 2007, James Quinn, was hired as the senior research technician in Vile's lab, and remains so today. Additionally, several undergraduate students worked on various aspects of the project since May 2006 (Ashlie Smyth, Amanda Foskett, Tanja Přsa, Dan Russo, Pat Costello, Paul Weibel, Mariozza Santini, Michael Patson, and Justin Meschter). Tanja Přsa conducted senior thesis research under Vile & Weston to examine the effect of salinity intrusion on the microbial community structure of TFM sediments (see below). Her senior thesis research was funded through the Biology Department at Villanova, and took advantage of, and built upon, the one-year laboratory experiment funded through EPA that was recently completed in Vile's laboratory at Villanova University.

PUBLICATIONS/PRESENTATIONS (ASTERISK INDICATES STUDENT)

INVITED SEMINARS

Weston, N.B. March 2009. The impacts of climate change and sea level rise on tidal marshes in the Delaware River Estuary. Ursinus College, Collegeville, PA (Oral Presentation – *Invited*).

Weston, N.B. 2006. Ramifications of Rising Sea Levels and Salinity Intrusion into Tidal Freshwater Marshes: Shifting Microbial Communities and Pathways of Organic Matter Mineralization. Department of Biology, Villanova University, Villanova, PA (Oral Presentation – *Invited*).

CONFERENCE PRESENTATIONS

Weston, N.B., M.A. Vile, S.C. Neubauer and D.J. Velinsky. 2009. Sea-Level Rise and Salt-Water Intrusion Limit Vertical Accretion Potential in Tidal Freshwater Marshes of the Delaware River Estuary. Coastal and Estuarine Research Federation. Portalnd, OR (Oral Presentation).

Weston, N.B., M.A. Vile, S.C. Neubauer and D.J. Velinsky. June 2009. Climate change, sea level rise and salt-water intrusion in tidal freshwater marshes of the Delaware River Estuary. Society of Wetland Scientists. Madison, WI (Oral Presentation).

Weston, N.B. M.A. Vile, S. C. Neubauer and D. J. Velinsky. May 2009. Linking impacts of climate change to carbon and phosphorus dynamics along a salinity gradient in tidal marshes. Environmental Protection Agency Meeting, Seattle, WA (Oral Presentation).

Weston, N.B., M.A. Vile, S.C. Neubauer and D.J Velinksy. January 2009. The impact of climate change and sea level rise on tidal freshwater marshes of the Delaware River Estuary. Partnership for the Delaware River Estuary Science and Environmental Summit, Cape May, NJ (Oral Presentation).

*Prša, T., N.B. Weston and M.A. Vile. January 2009. Changes in metabolic activity and community composition of sulfate reducing bacteria in tidal freshwater marsh soils in

response to climate change and saltwater intrusion. Partnership for the Delaware River Estuary Science and Environmental Summit, Cape May, NJ (Poster Presentation – Best Student Poster Award).

*Prša, T., N.B. Weston and M.A. Vile. May 2008. Impact of rising sea levels and salinity intrusion on the metabolic activity and community composition of sulfate reducing bacteria in tidal freshwater marsh sediments. Society of Wetland Scientists, Washington, DC (Poster Presentation – Honorable Mention for best student poster).

Neubauer, S.C., C.B. Craft, M.A. Vile and N.B. Weston. May 2008. Tidal freshwater wetland responses to climate change. Society of Wetland Scientists, Washington, DC (Poster Presentation).

Vile MA, NB Weston, DJ Velinsky and S Neubauer. Assessing the Impact of Climate Change Induced Sea-Level Rise on Carbon Cycling Dynamics in Freshwater Tidal Marshes, 10th Annual Wetland Biogeochemistry Meeting, Annapolis, Maryland, April 1-4, 2007 (invited speaker for special session on sea-level rise on tidal marshes).

Weston, N.B., M.A. Vile, D.J. Velinsky, S.C. Neubauer and S.B. Joye. 2007. Shifting Pathways and Magnitude of Organic Matter Mineralization in Tidal Freshwater Marshes Following Sea-Level Rise. Estuarine Research Federation, Providence, RI (Oral Presentation).

Weston, N.B., M.A. Vile, D.J. Velinsky, S.B. Joye and S.C. Neubauer. 2007. Rising sea-levels and salinity intrusion into tidal freshwater marshes: Shifting microbial communities and pathways of organic matter mineralization. American Society of Limnology and Oceanography, Santa Fe, NM (Oral Presentation).

Giblin, A., N.B. Weston, J. Tucker, G. Banta, A. Bernhard and C. Hopkinson. 2007. Salinity Effects of Nitrogen Cycling in Estuaries. Estuarine Research Federation, Providence, RI (Oral Presentation).

Weston, N.B., M.A. Vile and S.B. Joye. 2006. Ramifications of Rising Sea Levels and Salinity Intrusion into Tidal Freshwater Marshes: Shifting Microbial Communities and Pathways of Organic Matter Mineralization. BIOGEOMON, Santa Cruz, CA (Oral Presentation).

PEER-REVIEWED PUBLICATIONS

*Weston, NB, MA Vile, DJ Velinsky, and SC Neubauer. 2010. Salt water intrusion and carbon cycling in a tidal freshwater marsh. *Biogeochemistry*, in press.

SIGNIFICANCE OF ACCOMPLISHMENTS

Tidal marshes are highly productive ecosystems that provide ecological services such as habitat for birds, fish and shellfish, storm-surge buffering and water quality mitigation. The loss of coastal marshes can have devastating ecosystem-level consequences, as previous hurricane events in the Gulf coast have demonstrated. Given that approximately 50% of the global

population lives within coastal regions, both the socioeconomic and environmental impacts of sea-level rise are far reaching. The response of TFM's to rising sea levels is a complex interaction of the processes that drive plant production, microbial decomposition, sediment deposition, and marsh accretion. A greater understanding of ecosystem-level responses of TFM to climate change is a major challenge that is of interest to scientists, land managers/planners, and increasingly, the general public. We have demonstrated how salinity-intrusion impacts C accumulation in freshwater tidal marshes.

STAKEHOLDERS AND/OR USERS OF RESULTS/DATA/PRODUCTS

We have disseminated our findings through peer-reviewed publications, conference presentations at national and international meetings, and invited seminars at various academic institutions in the greater Philadelphia area. Both Weston and Vile have worked on various outreach projects at the Academy of Natural Sciences in Philadelphia. Results of this research also have been disseminated to managers and stakeholders in the Delaware River and other coastal regions. Throughout this project, we have demonstrated a solid commitment to educating undergraduate students in the sciences through active involvement in the research. Additional undergraduate student involvement in this research was attained through Senior Thesis research at Villanova University, a RUI (Research at Undergraduate Institutions) institution. Additionally, this project supported an early-career scientist during his transition from a postdoctoral fellow into a tenure track position in the Department of Geography and the Environment at Villanova University.

Supplemental Keywords

Ecosystem, aquatic, habitat, environmental chemistry, biology, geology, ecology, hydrology, genetics, limnology climate models, northeast, Atlantic coast, midatlantic, ecosystem scaling, metabolism, marine, estuary

Table 1: Soil P concentrations for in situ cores collected from the Rancocas, Raccoon, Salem, and Stow marshes during 2007. Soil samples were sequentially extracted as described in the text. Values are averages and standard deviations across all dates and depths (n = 5 per marsh).

				(µg P g dry soil^{-1})				
Site		H_2O-P	Fe-P	Al-P	HA-P	Ca-P	Res-P	Total
Rancocas	Inorganic	61.9 ± 24.8	1749.8 ± 449.1	610.1 ± 226.1	--	96.0 ± 37.2	--	2514.8 ± 659.7
	Organic	12.4 ± 3.1	108.4 ± 55.7	170.3 ± 46.5	52.6 ± 12.4	--	77.4 ± 18.6	418.1 ± 117.7
	Total	71.2 ± 27.9	1855.1 ± 489.3	780.4 ± 263.2	52.6 ± 12.4	96.0 ± 37.2	77.4 ± 18.6	2932.9 ± 743.3
Raccoon	Inorganic	0.8 ± 0.5	6.0 ± 2.8	5.3 ± 1.3	--	5.6 ± 0.3	--	17.7 ± 3.8
	Organic	0.4 ± 0.2	1.0 ± 0.4	1.9 ± 1.1	1.6 ± 0.2	--	2.4 ± 0.2	7.3 ± 1.4
	Total	1.2 ± 0.7	7.0 ± 3.1	7.1 ± 2.2	1.6 ± 0.2	5.6 ± 0.3	2.4 ± 0.2	25.0 ± 5.1
Salem	Inorganic	4.0 ± 1.2	16.6 ± 9.1	8.0 ± 3.5	--	6.1 ± 0.3	--	34.7 ± 13.1
	Organic	0.9 ± 0.1	1.7 ± 0.6	2.9 ± 0.4	2.6 ± 0.6	--	3.1 ± 0.6	11.4 ± 1.8
	Total	4.9 ± 1.2	18.3 ± 9.6	10.9 ± 3.5	2.6 ± 0.6	6.1 ± 0.3	3.1 ± 0.6	46.1 ± 13.3
Stow	Inorganic	2.6 ± 0.3	5.9 ± 7.9	3.8 ± 0.2	--	5.3 ± 0.5	--	17.7 ± 8.0
	Organic	1.0 ± 0.1	1.6 ± 0.5	2.1 ± 0.4	2.9 ± 0.4	--	3.9 ± 1.0	11.4 ± 1.8
	Total	3.7 ± 0.3	7.4 ± 8.2	6.0 ± 0.3	2.9 ± 0.4	5.3 ± 0.5	3.9 ± 1.0	29.1 ± 8.9

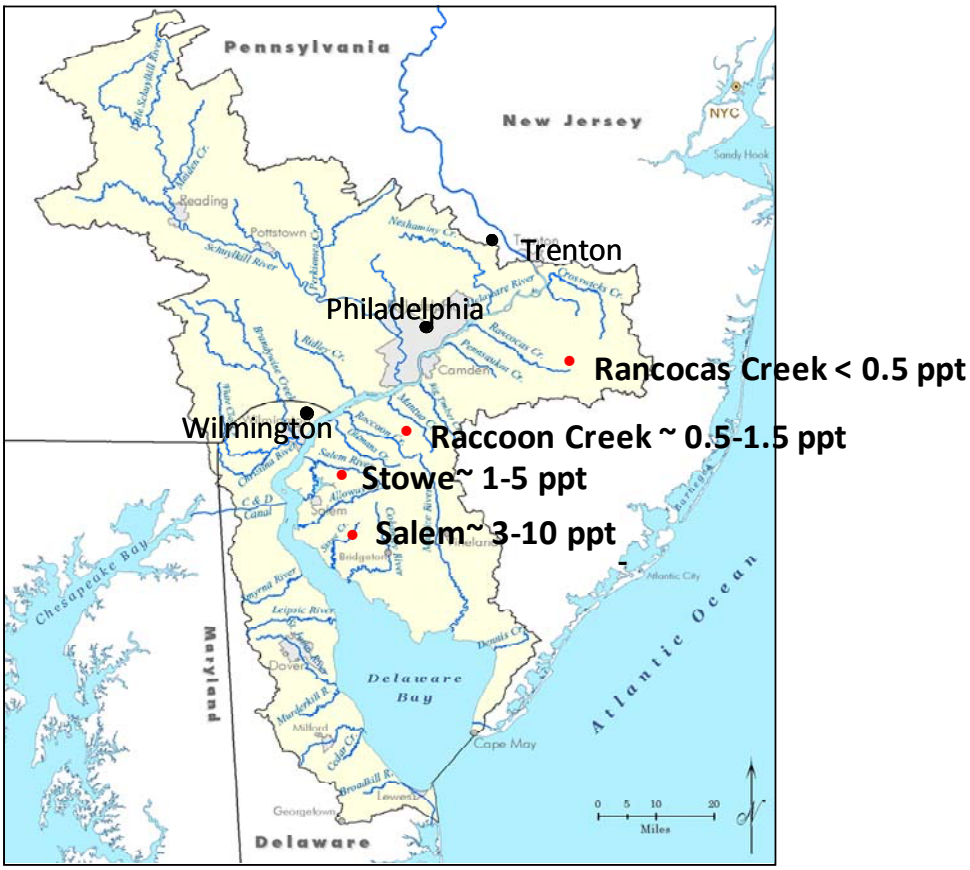


Figure 1. Field sites in along a salinity gradient in the DE Estuary.

Figure 3. Picture illustrating permanent square collars In the field (left) and transplanted cores (right).

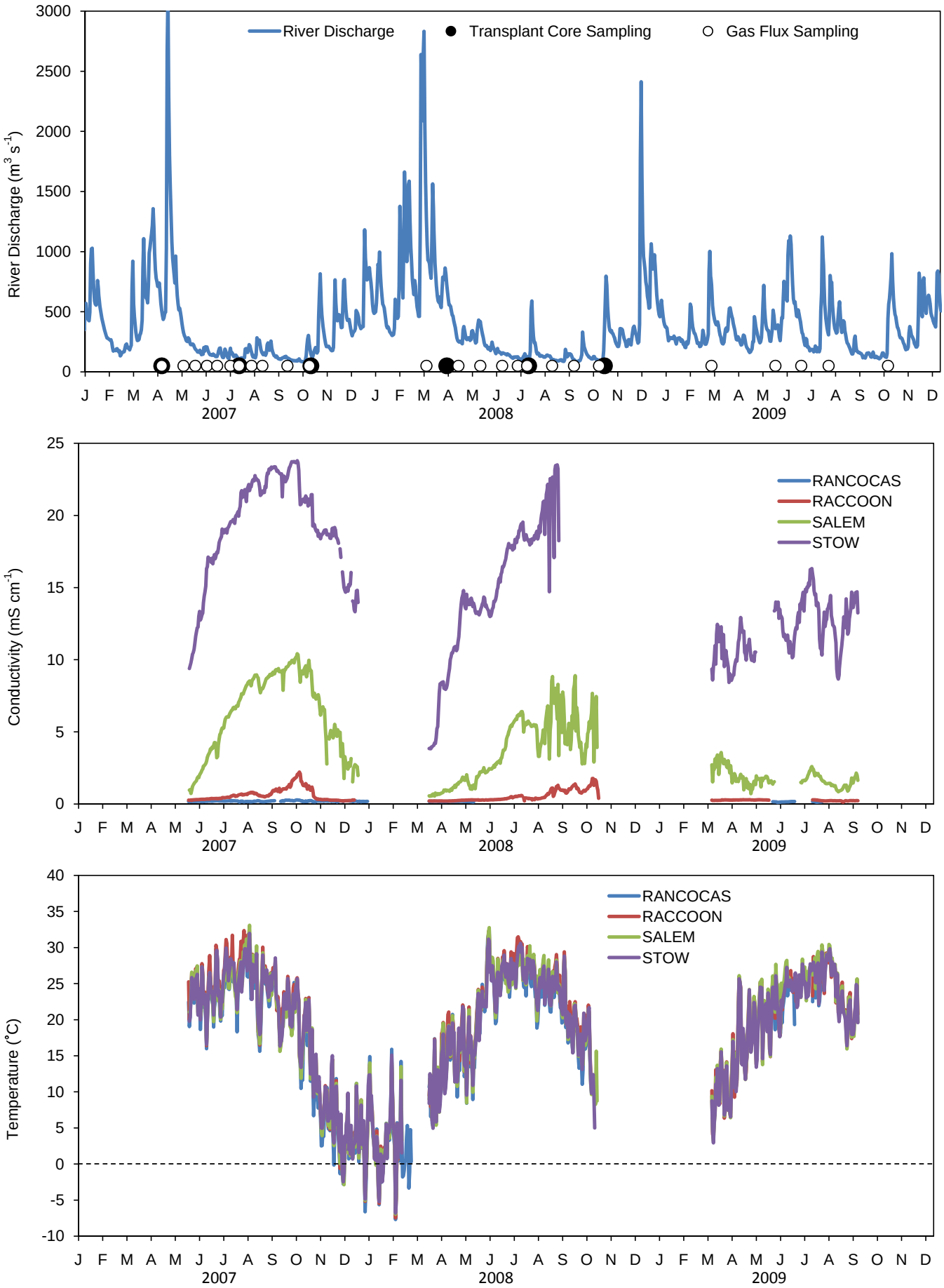


Figure 2. Delaware River mean daily discharge at Trenton, NJ (top, data from USGS site 01463500), mean daily conductivity (middle) and air temperature (bottom) at the four field sites in the Delaware River Estuary.

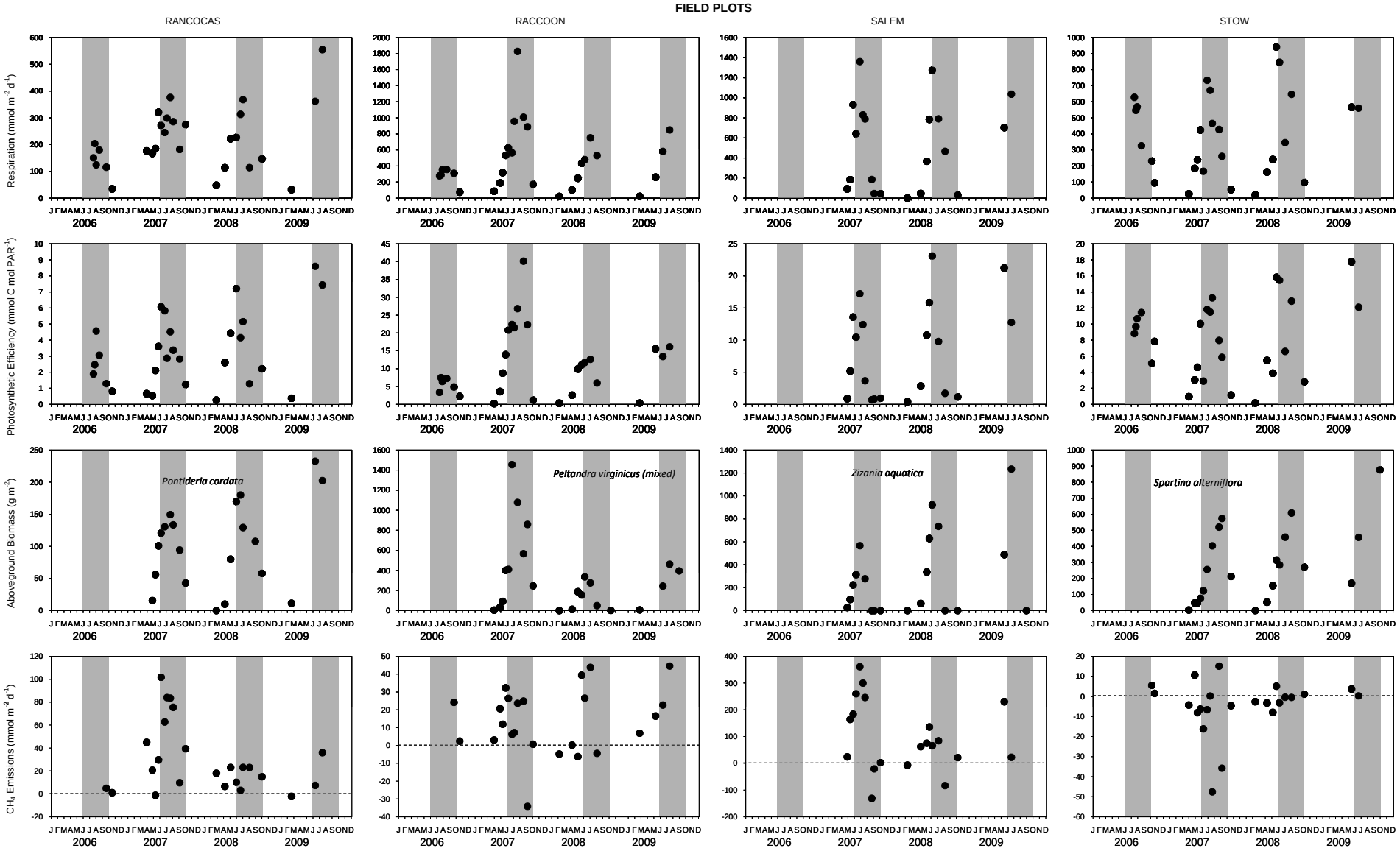


Figure 4. Average rates of ecosystem respiration and photosynthetic efficiency, aboveground biomass and methane emissions over 3-4 years in permanent field plots at fours sites in the Delaware River Estuary. The dominant plant species is indicated in the biomass plots, and the growing season (June - September) is indicated by shaded regions.

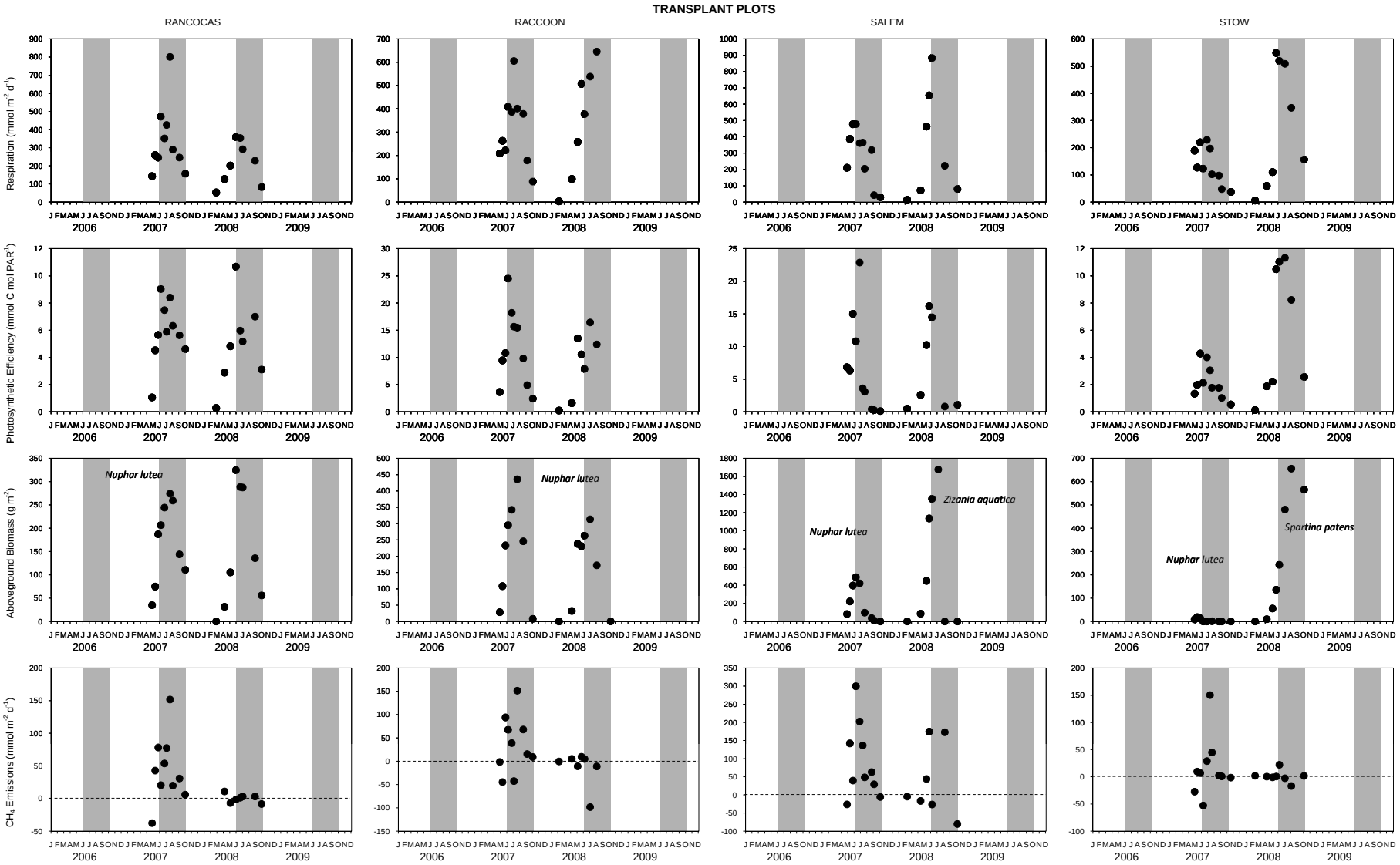


Figure 5. Average rates of ecosystem respiration and photosynthetic efficiency, aboveground biomass and methane emissions over 2 years in tidal freshwater marsh plots transplanted from the Rancocas site to four sites in the Delaware River Estuary. The dominant plant species is indicated in the biomass plots, and the growing season (June - September) is indicated by shaded regions.

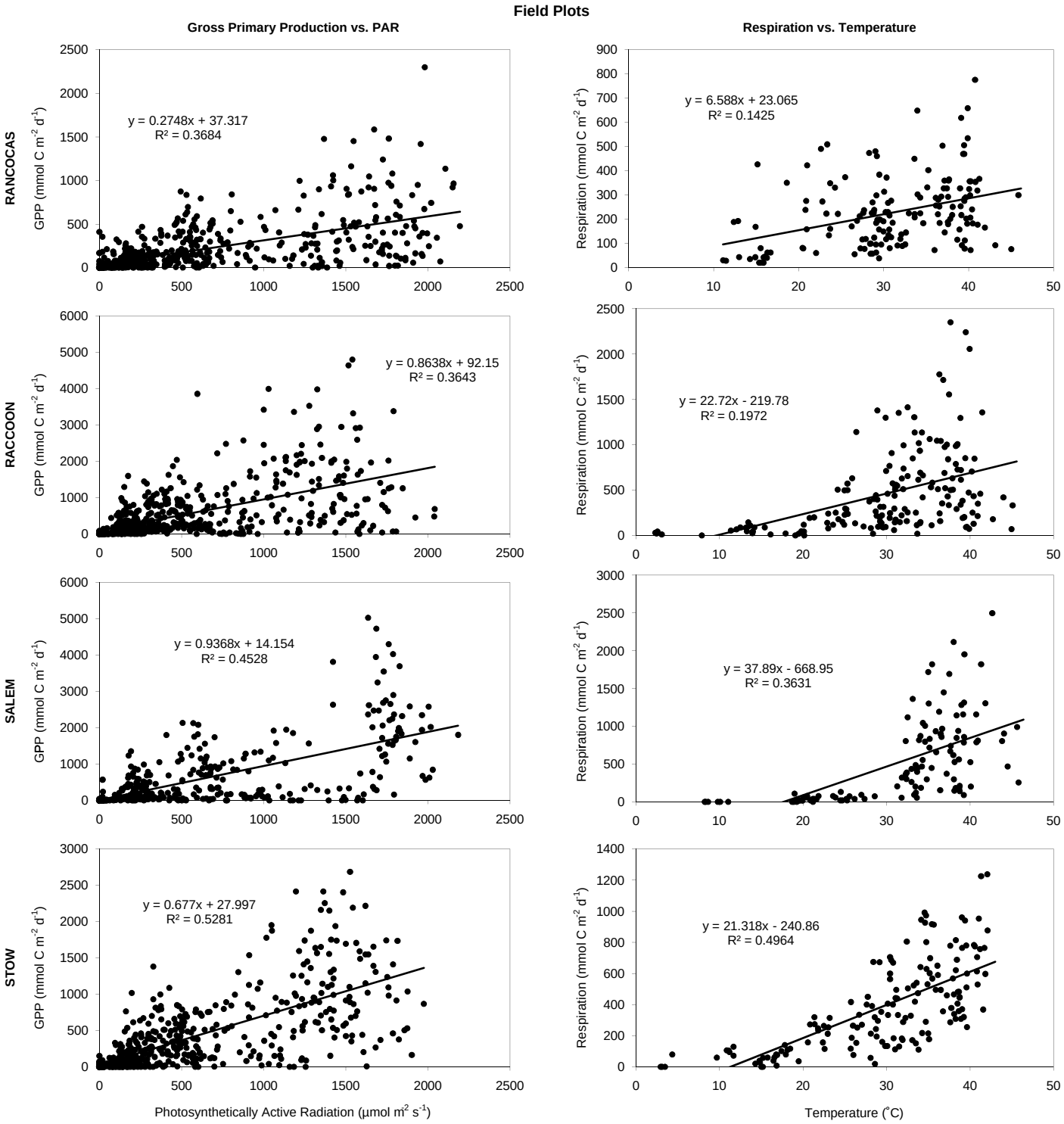


Figure 6. Relationship between gross primary production and photosynthetically active radiation (left) and between ecosystem respiration and temperature (right) in the permanent field plots at the four sites in the Delaware River Estuary over 4 years.

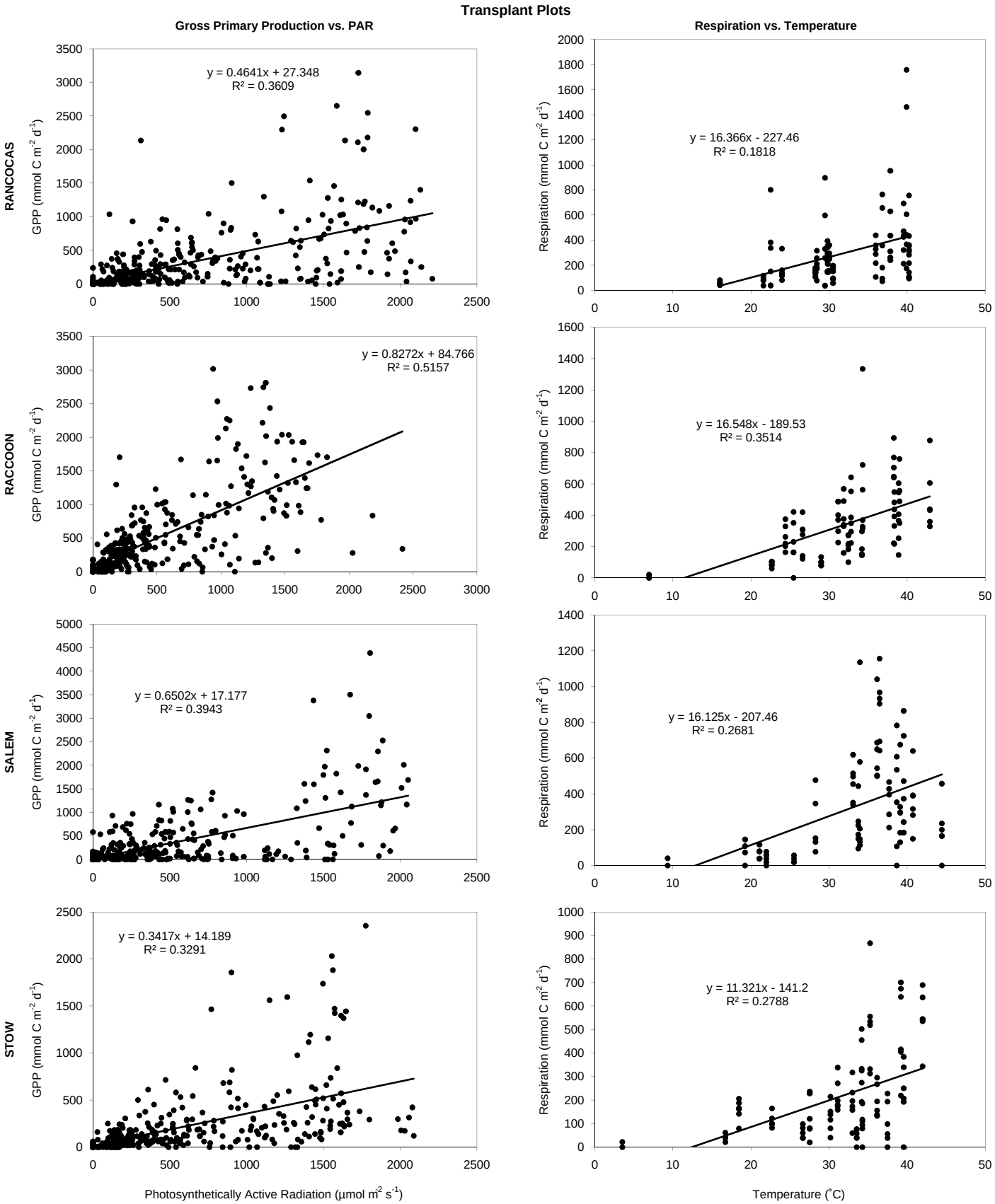


Figure 7. Relationship between gross primary production and photosynthetically active radiation (left) and between ecosystem respiration and temperature (right) in the transplanted plots at the four sites in the Delaware River Estuary over 4 years.

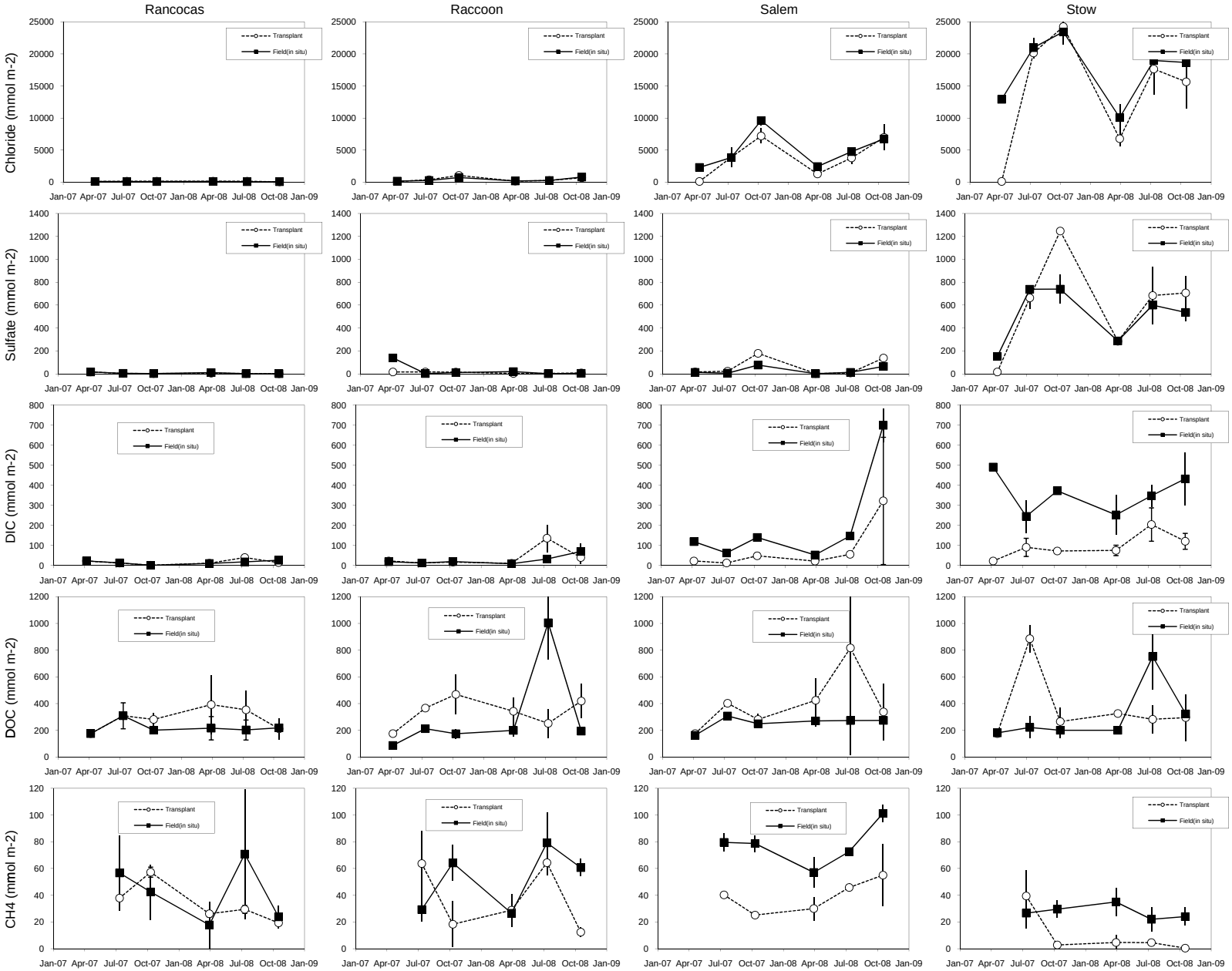


Figure 8. Soil porewater inventories (to a depth of 15 cm) of chloride, sulfate, dissolved inorganic carbon, dissolved organic carbon, and methane from four field sites in the Delaware River Estuary, and cores transplanted from the Rancocas tidal freshwater marsh site to the four field sites over a two-year period.

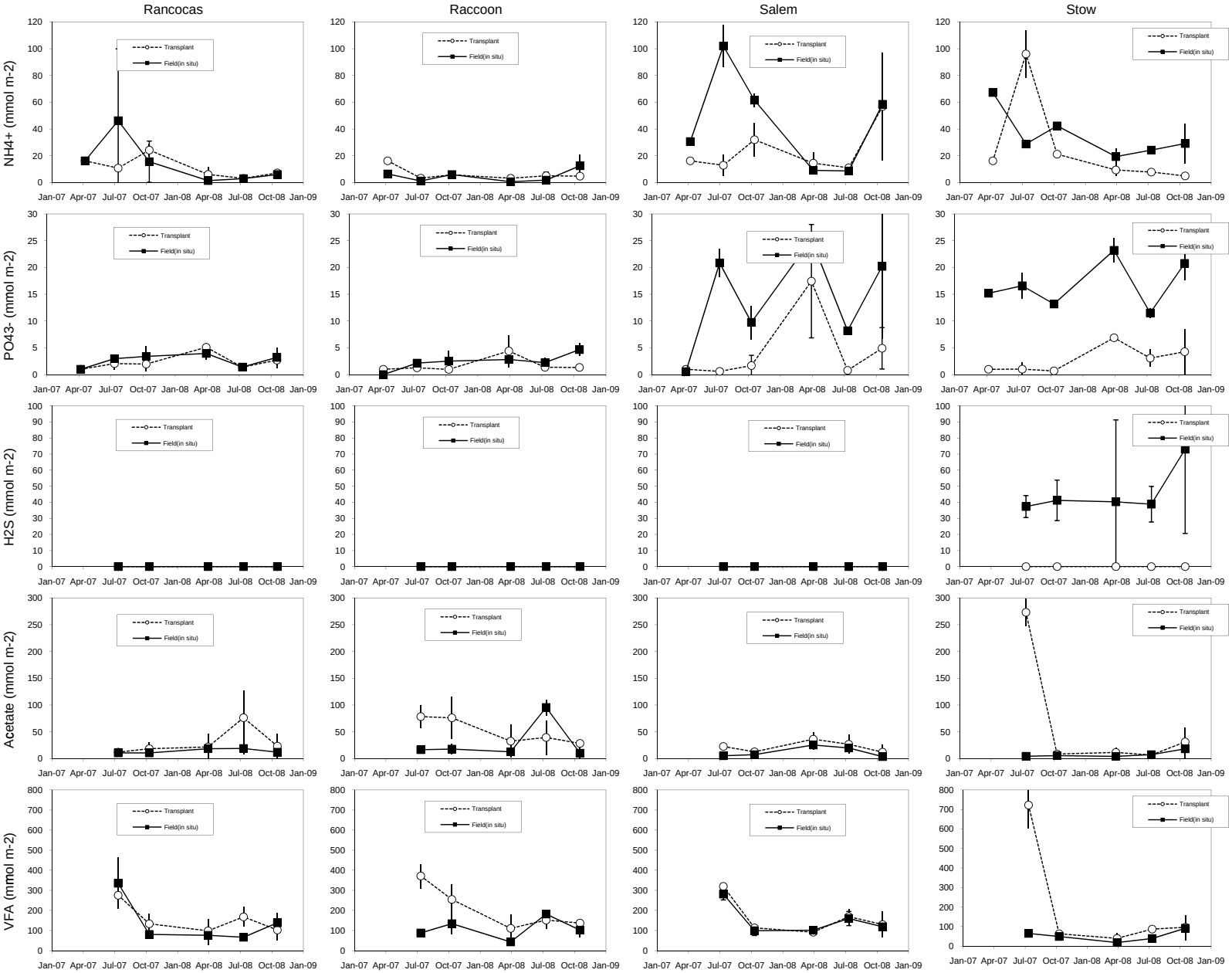


Figure 9. Soil porewater inventories (to a depth of 15 cm) of ammonium, phosphate, hydrogen sulfide, acetate, and total volatile fatty acids from four field sites in the Delaware River Estuary, and cores transplanted from the Rancocas tidal freshwater marsh site to the four field sites over a two-year period.

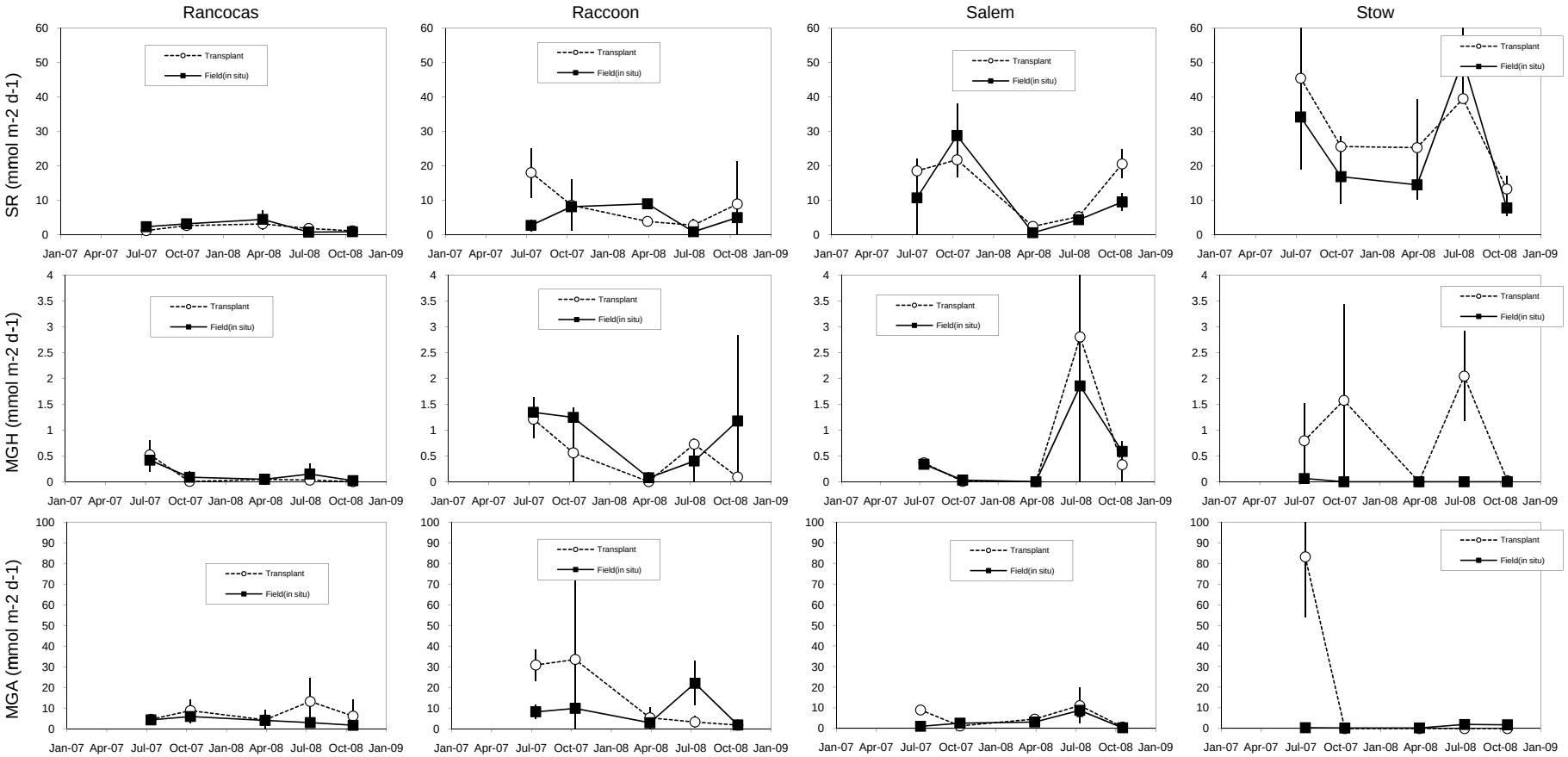


Figure 10. Rates of microbial sulfate reduction, hydrogenotrophic methanogenesis, and acetoclastic methanogenesis in soils (integrated to a depth of 15 cm) from four field sites in the Delaware River Estuary, and cores transplanted from the Rancocas tidal freshwater marsh site to the four field sites over a two-year period.

Figure 11.

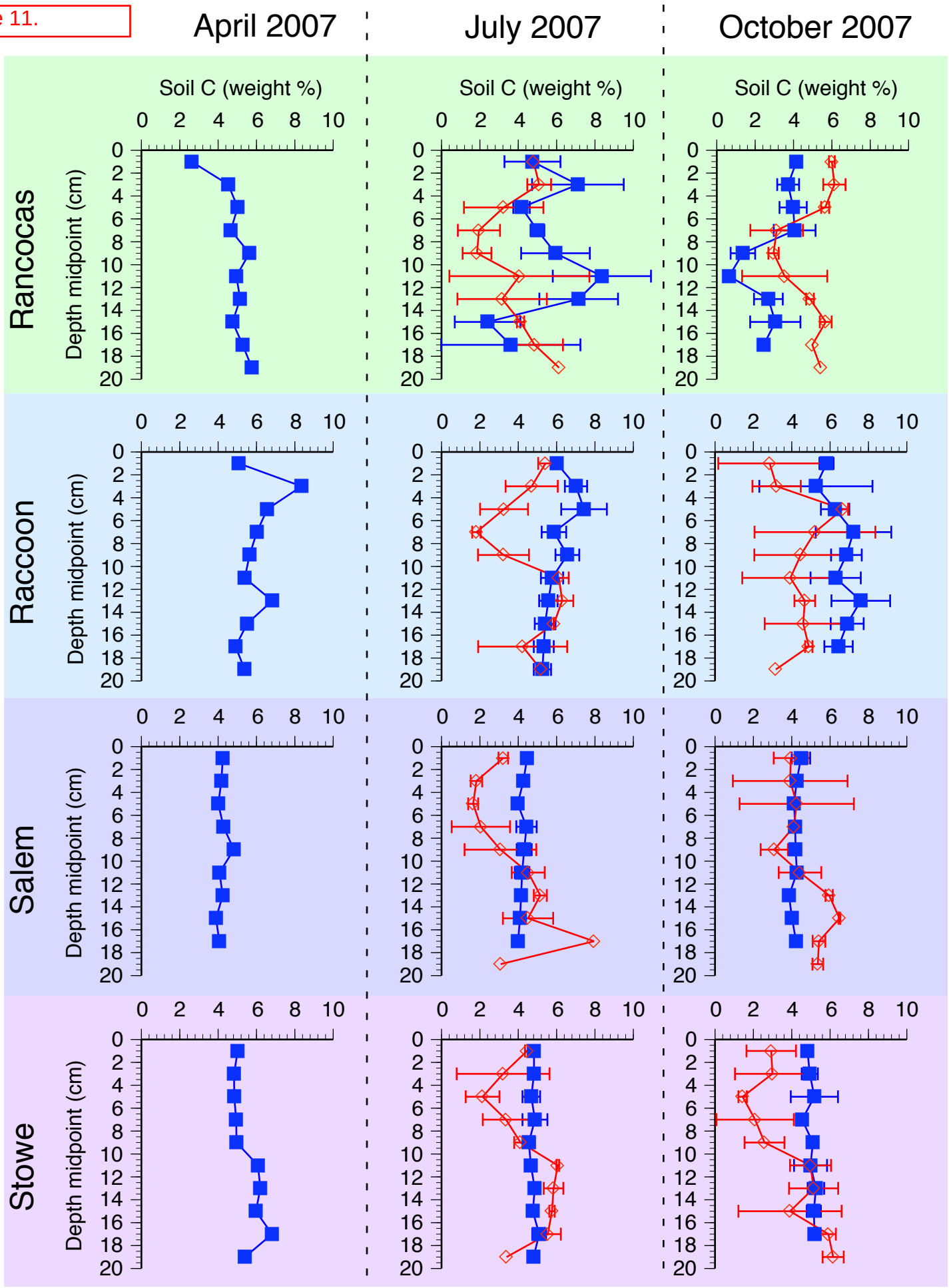

April 2007
July 2007
October 2007
Rancocas
Raccoon
Salem
Stowe
Soil C (weight %)
Depth midpoint (cm)
0 2 4 6 8 10
0 2 4 6 8 10 12 14 16 18 20

Figure 12.

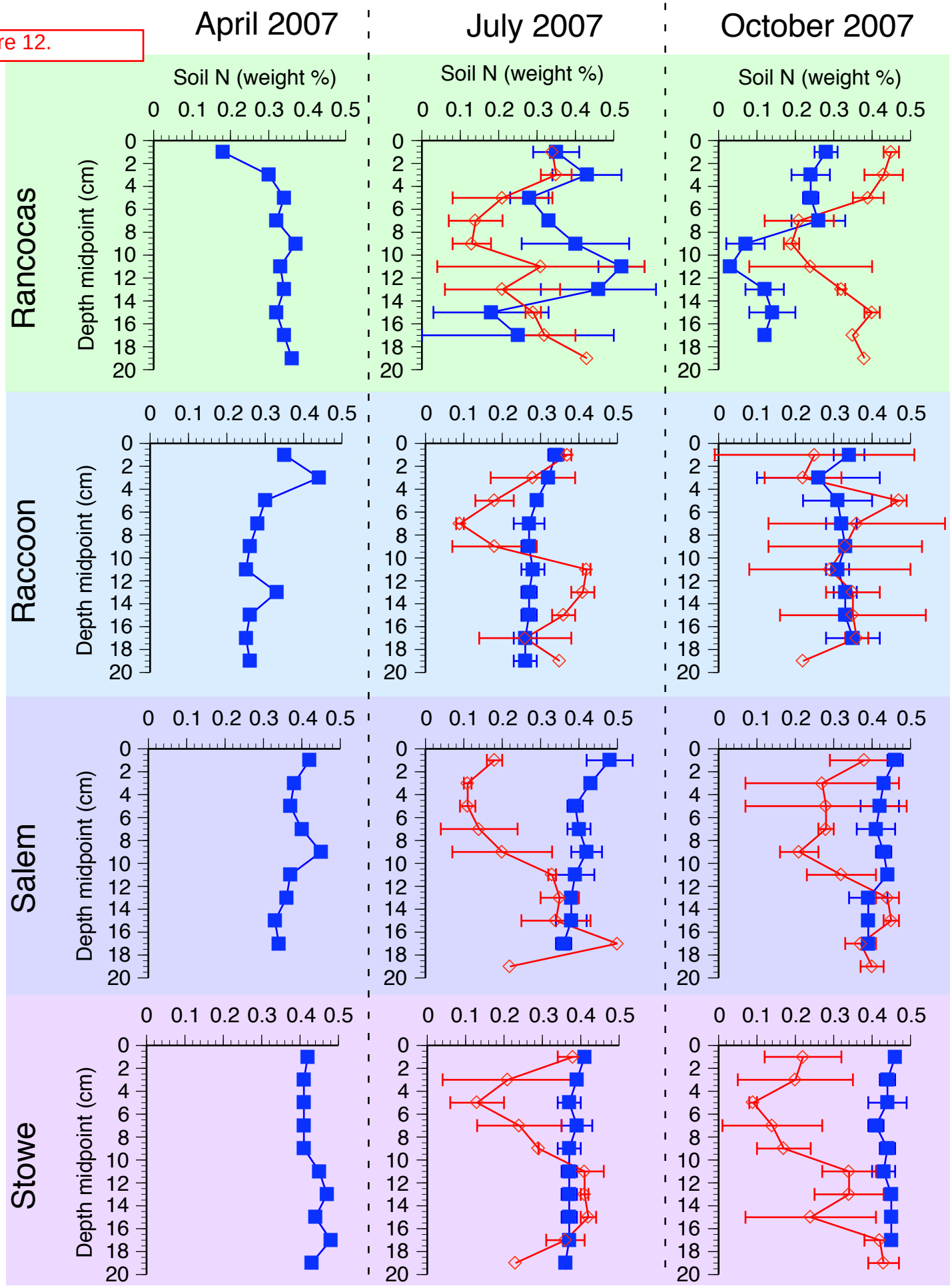

April 2007
July 2007
October 2007
Rancocas
Raccoon
Salem
Stowe
Soil N (weight %)
Depth midpoint (cm)
0 0.1 0.2 0.3 0.4 0.5
0 2 4 6 8 10 12 14 16 18 20

Figure 13.

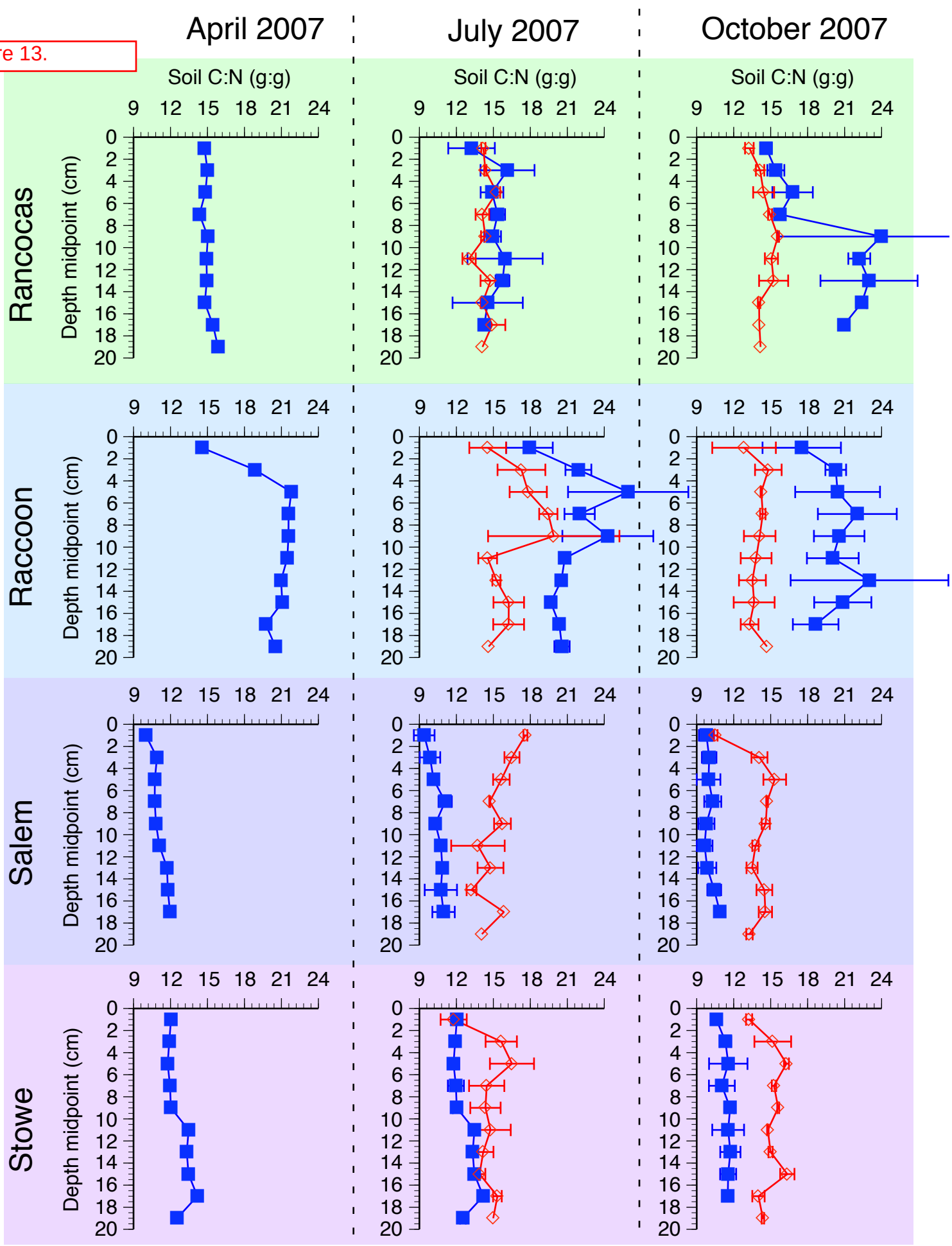
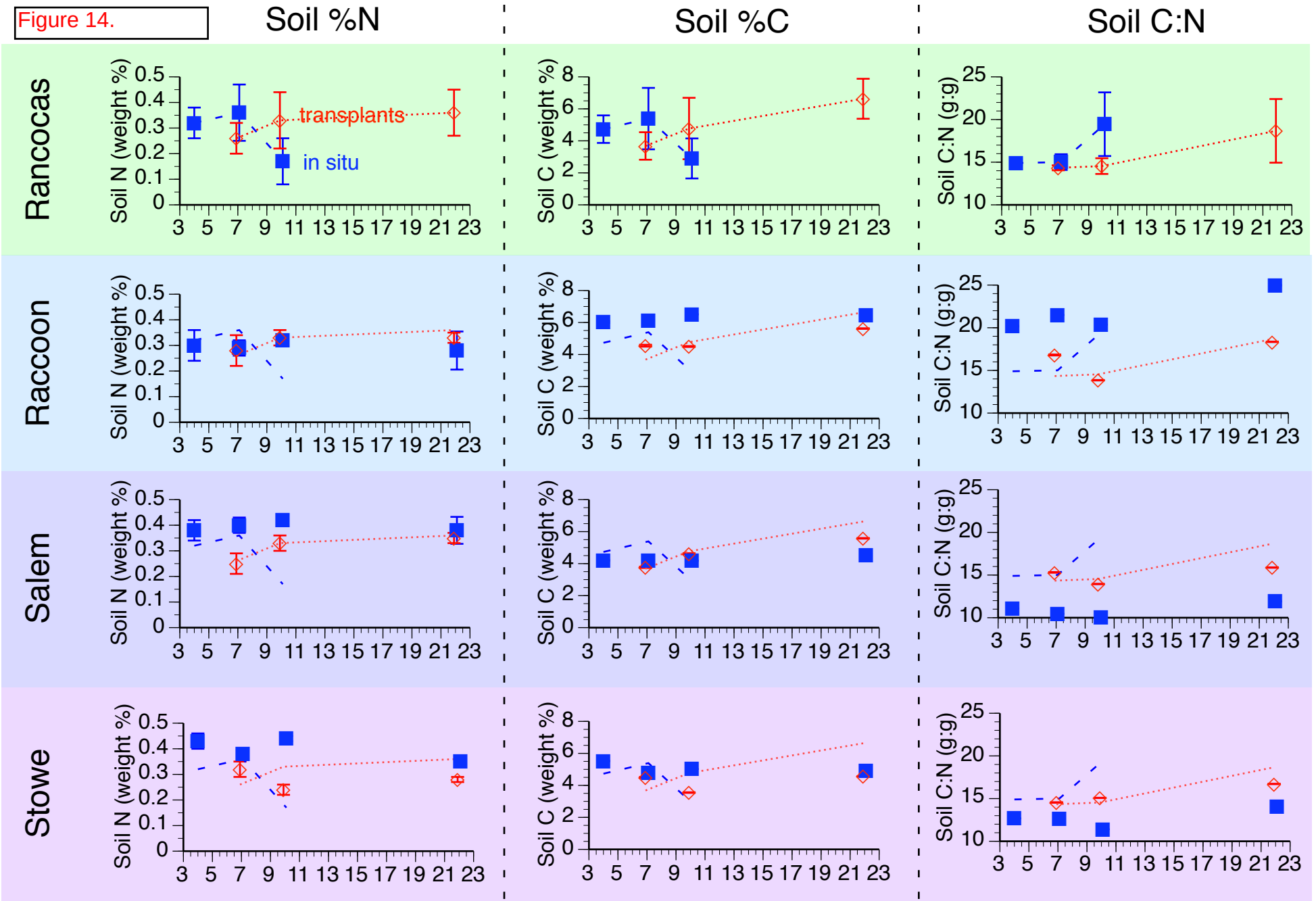

April 2007
July 2007
October 2007
Rancocas
Raccoon
Salem
Stowe
Soil C:N (g:g)
Depth midpoint (cm)
9 12 15 18 21 24
0 2 4 6 8 10 12 14 16 18 20

Figure 14.



Values represent the average %N, %N, or C:N ratio in the top 20 cm, averaged over two replicate soil cores (except for April in situ cores where n = 1 core). Error bars are ± 1 standard deviation and indicate variability of each parameter with depth. Dashed lines show the values of each parameter from Rancocas, the donor marsh for the transplant cores.

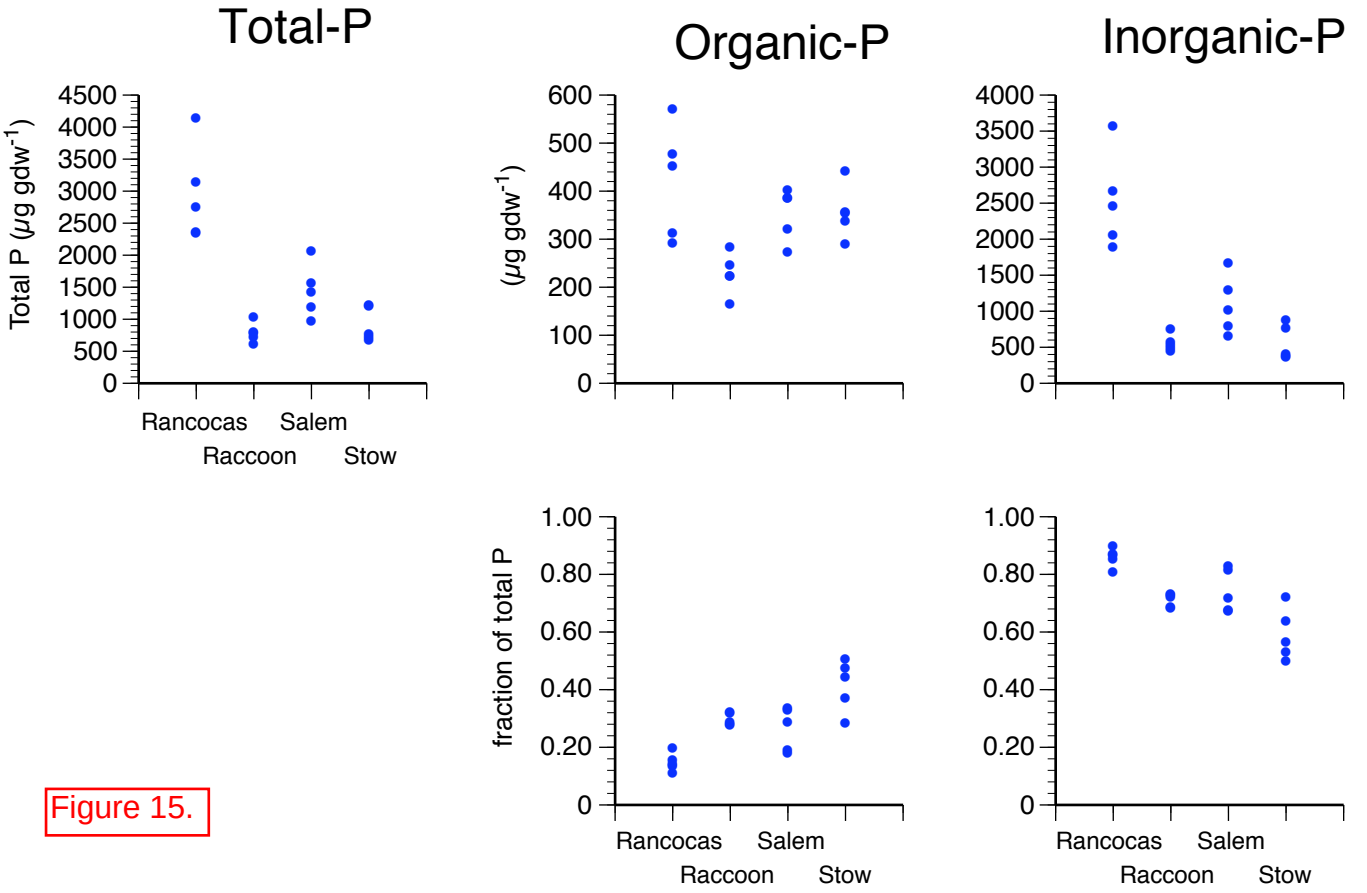

Total-P
Organic-P
Inorganic-P
Total P (μg gdw-1)
(μg gdw-1)
fraction of total P
Rancocas
Salem
Raccoon
Stow

Figure 15.

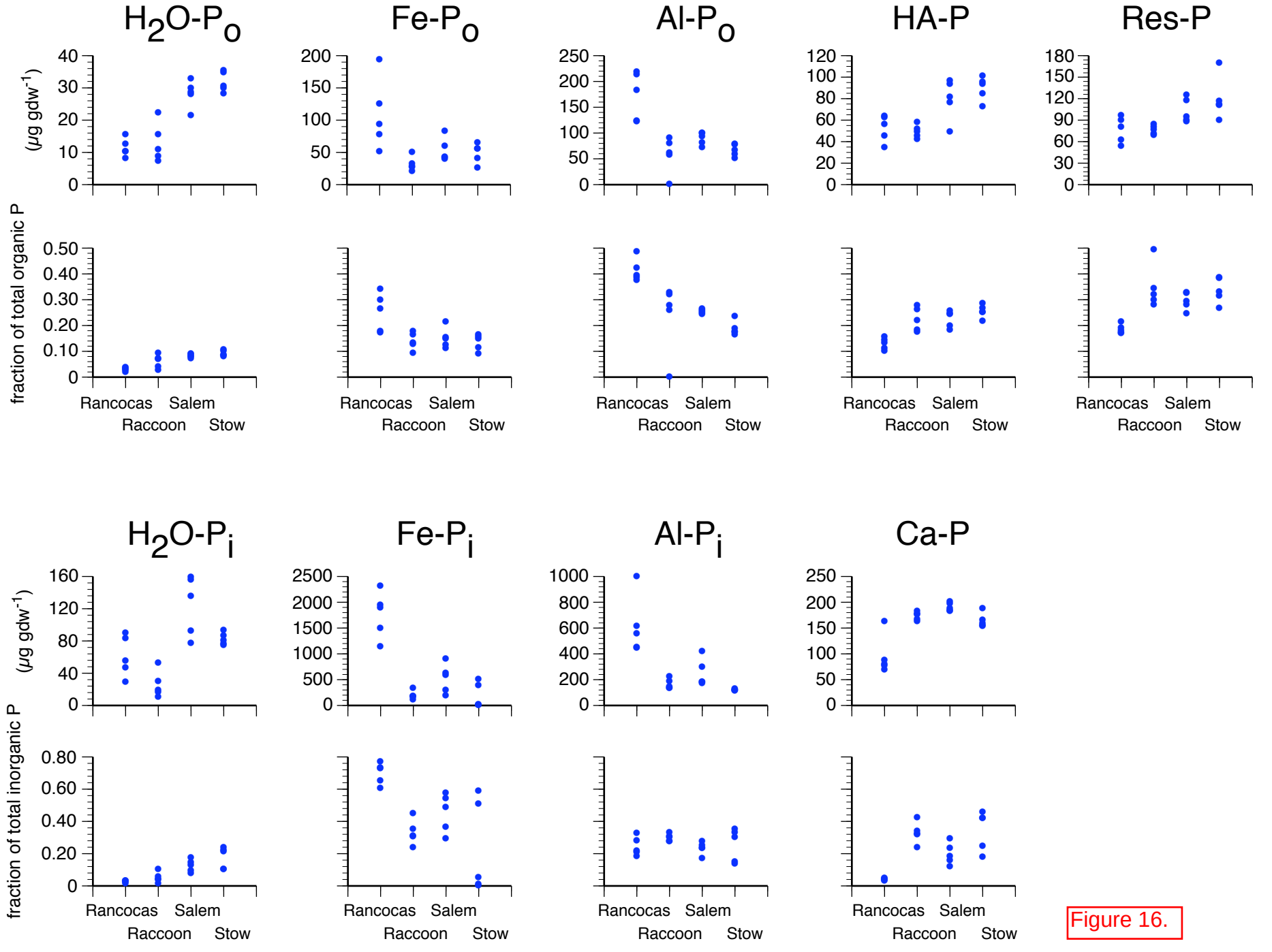

H2O-Po
Fe-Po
Al-Po
HA-P
Res-P
(μg gdw-1)
fraction of total organic P
H2O-Pi
Fe-Pi
Al-Pi
Ca-P
fraction of total inorganic P
Rancocas
Salem
Raccoon
Stow

Figure 16.

Figure 17.

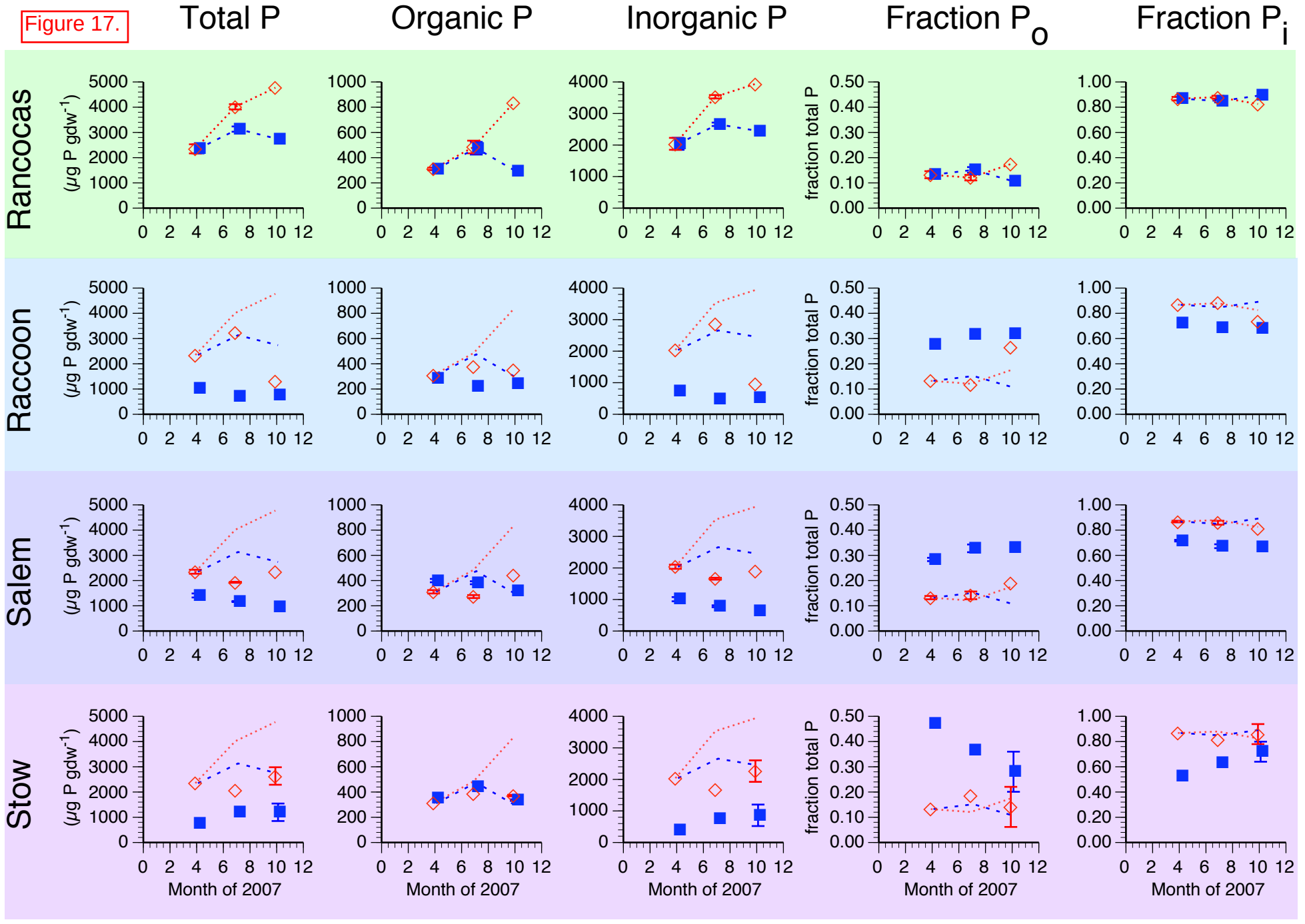

Total P
Organic P
Inorganic P
Fraction P_o
Fraction P_i
Rancocas
Raccoon
Salem
Stow
(µg P gdw^{-1})
fraction total P
Month of 2007

Figure 18.

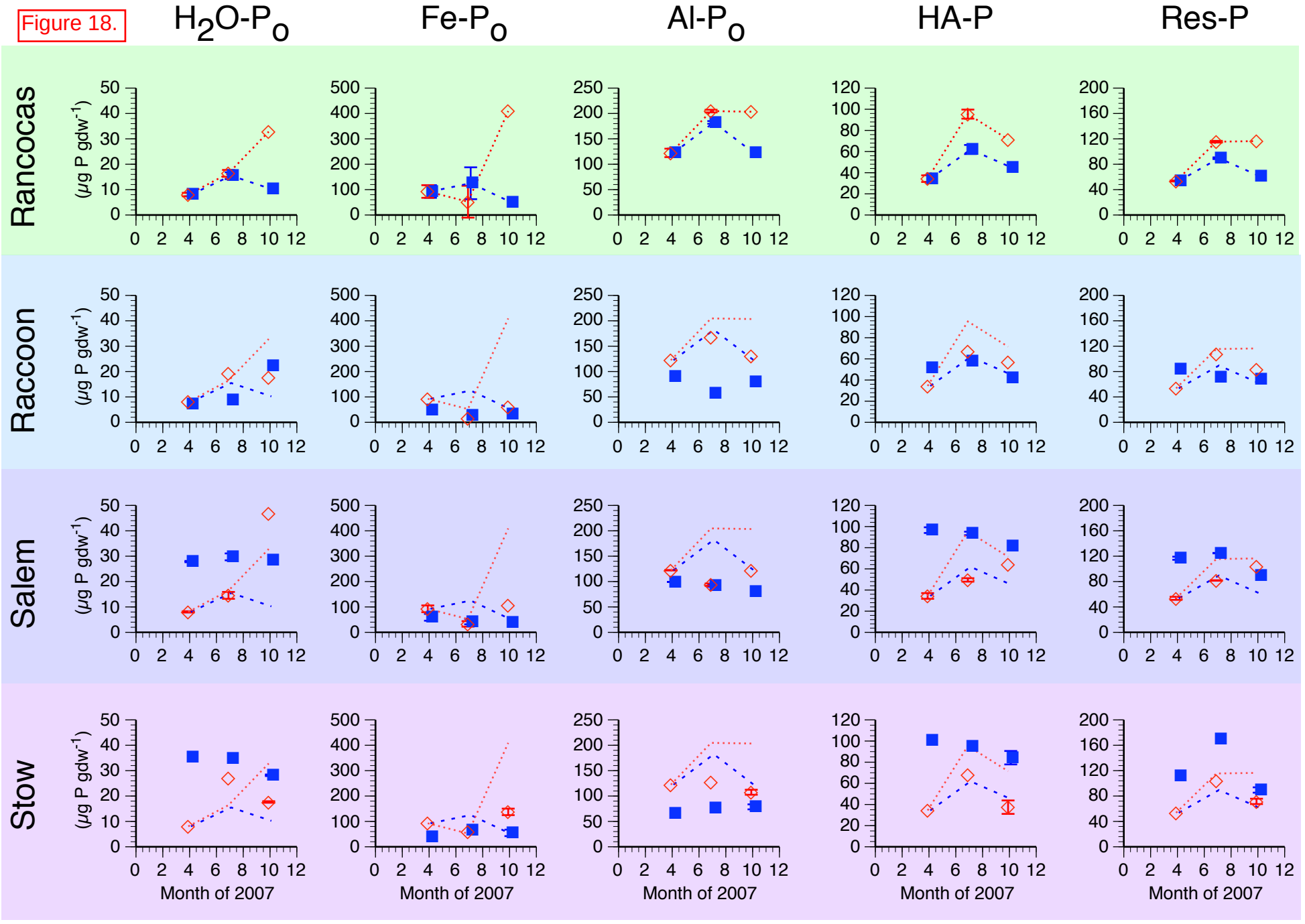

H2O-Po
Fe-Po
Al-Po
HA-P
Res-P
Rancocas
Raccoon
Salem
Stow
(µg P gdw-1)
Month of 2007

Figure 19.

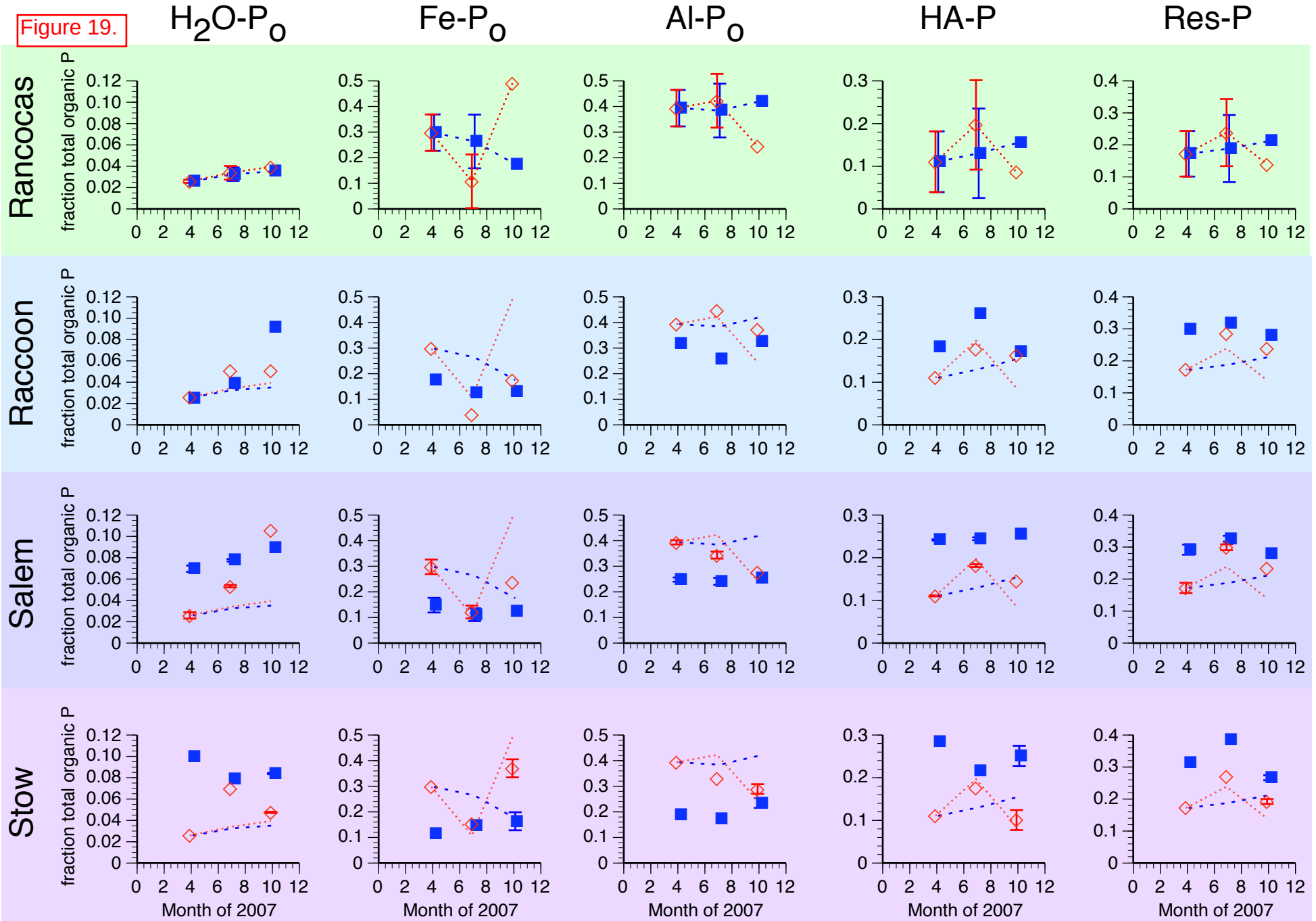

H2O-Po
Fe-Po
Al-Po
HA-P
Res-P
Rancocas
Raccoon
Salem
Stow
fraction total organic P
Month of 2007

Figure 20.

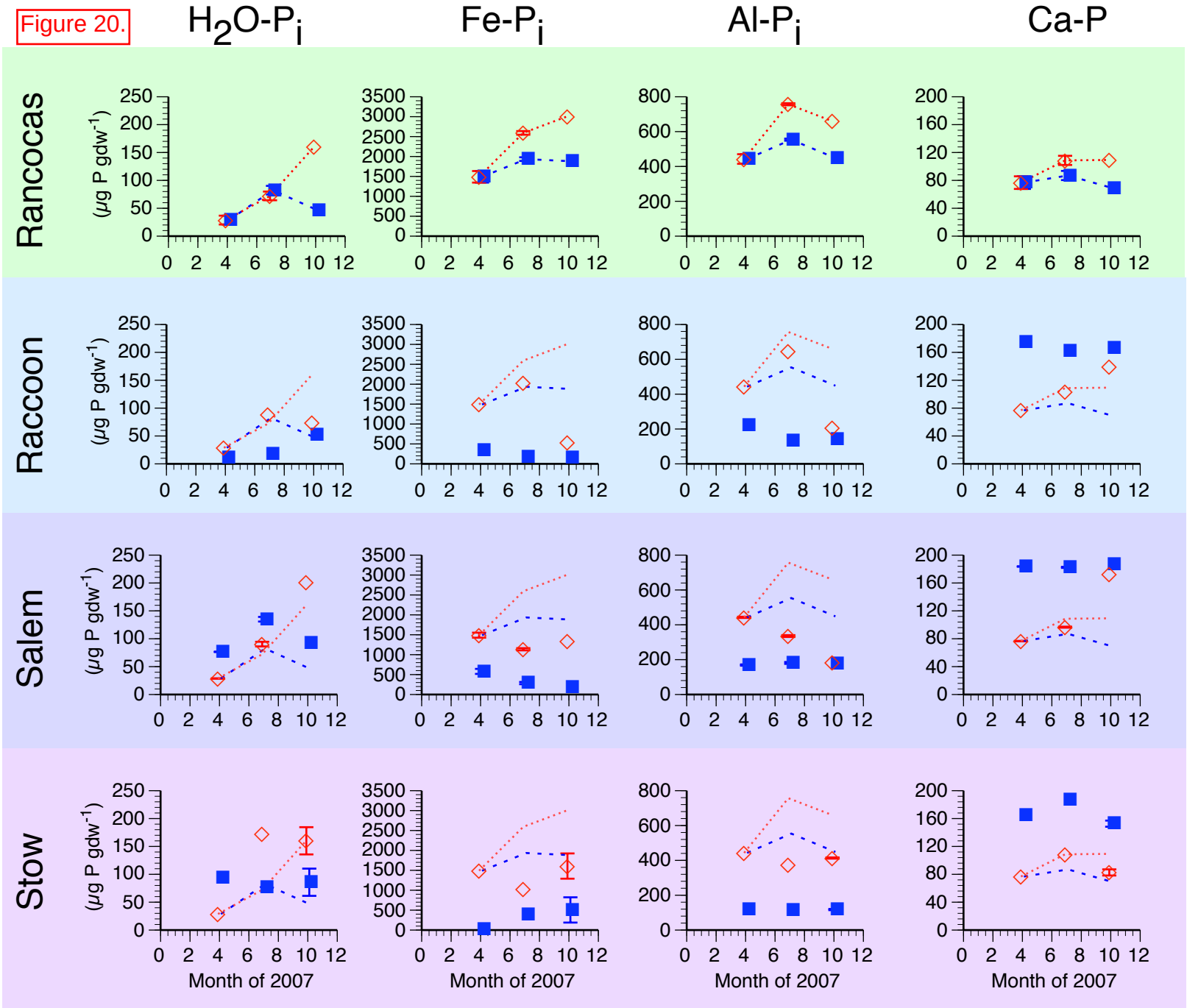
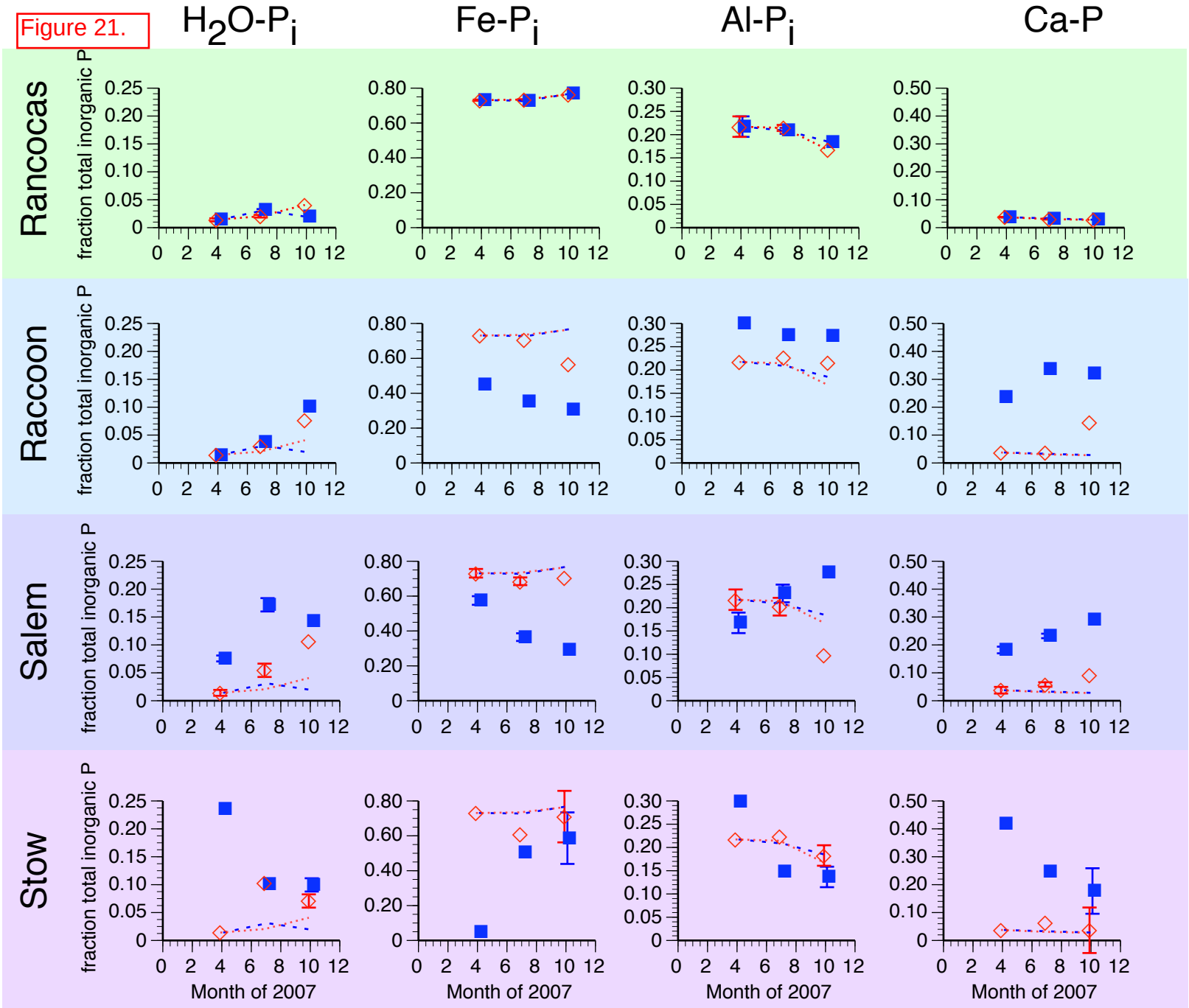

H2O-Pi
Fe-Pi
Al-Pi
Ca-P
Rancocas
Raccoon
Salem
Stow
(µg P gdw-1)
Month of 2007

Figure 21.


H2O-Pi
Fe-Pi
Al-Pi
Ca-P
Rancocas
Raccoon
Salem
Stow
fraction total inorganic P
Month of 2007

Biogeochemistry
DOI 10.1007/s10533-010-9427-4

Accelerated microbial organic matter mineralization following salt-water intrusion into tidal freshwater marsh soils

Nathaniel B. Weston · Melanie A. Vile · Scott C. Neubauer · David J. Velinsky

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Abstract The impact of salt-water intrusion on microbial organic carbon (C) mineralization in tidal freshwater marsh (TFM) soils was investigated in a year-long laboratory experiment in which intact soils were exposed to a simulated tidal cycle of freshwater or dilute salt-water. Gas fluxes [carbon dioxide (CO_2) and methane (CH_4)], rates of microbial processes (sulfate reduction and methanogenesis), and pore-water and solid phase biogeochemistry were measured throughout the experiment. Flux rates of CO_2 and, surprisingly, CH_4 increased significantly following salt-water intrusion, and remained elevated relative to freshwater cores for 6 and 5 months, respectively. Following salt-water intrusion, rates of sulfate reduction increased significantly and remained higher than rates in the freshwater controls throughout the experiment. Rates of acetoclastic methanogenesis were higher than rates of hydrogenotrophic methanogenesis, but the rates did not differ by salinity treatment. Soil organic C content decreased significantly in soils experiencing salt-water intrusion. Estimates of total organic C mineralized in freshwater and salt-water amended soils over the 1 year experiment using gas flux measurements (18.2 and 24.9 mol C m^{-2}, respectively) were similar to estimates obtained from microbial rates (37.8 and 56.2 mol C m^{-2}, respectively), and to losses in soil organic C content (0 and 44.1 mol C m^{-2}, respectively). These findings indicate that salt-water intrusion stimulates microbial decomposition, accelerates the loss of organic C from TFM soils, and may put TFMs at risk of permanent inundation.

Keywords Tidal freshwater marshes · Carbon · Organic matter mineralization · Sulfate reduction · Methanogenesis · Carbon dioxide · Methane · Delaware River

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Introduction

Tidal marshes have existed for at least the past 4000 years, when rates of sea level rise slowed enough to allow for their development (Redfield 1965). Sea level exerts an especially powerful influence on tidal marshes (Morris et al. 2002; Mudd et al. 2009). Tidal marshes are found at or near

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current mean local sea level, and maintain their elevation relative to rising sea levels through net accretion and vertical growth. Accretion in tidal marshes is largely driven by deposition of watershed-derived sediments and autochthonous organic matter produced by marsh macrophytes and the subsequent storage of these materials in marsh soils (Reed 1995; Morris et al. 2002). The rate of sea level rise has increased in the past century due to anthropogenic climate change, and future acceleration of sea level rise is predicted (Nakada and Inoue 2005; Church and White 2006). Increased rates of sea level rise may exceed the ability of some marshes to accrete vertically, resulting in permanent inundation and loss of marsh area (Reed 1995; Morris et al. 2002).

Tidal marshes provide many critical ecosystem services, and the response of these ecosystems to climate change and sea level rise has received considerable attention from the scientific community (e.g., Morris et al. 2002; Mudd et al. 2009). Much of the attention has been on salt marshes, however, and relatively less is known about the impacts of climate change on tidal freshwater marshes (TFMs; see Neubauer and Craft 2009). TFMs are found in the tidally influenced freshwater portions of many estuaries, and approximately 20% of total tidal marsh area along the Atlantic and Gulf Coasts of the United States is TFM (Odum et al. 1984; Mitsch and Gosselink 1993). Both TFMs and salt marshes are highly productive ecosystems (Odum et al. 1984), serve as key habitats for many organisms (Mitsch and Gosselink 1993), and are efficient filters that can reduce the loading of nutrients from watersheds to coastal waters (Neubauer et al. 2005a; Gribsholt et al. 2005). Additionally, tidal marshes absorb storm surge and wave energy (Yang 1998), minimizing flooding and damage to adjacent upland areas during coastal storm events (Barbier et al. 2008). Although TFMs and salt marshes are functionally similar in many ways, differences in salinity and solute concentrations [especially sulfate (SO_4^{2-}) and hydrogen sulfide (H_2S)] lead to significant differences in microbial biogeochemical processes and dominant plant communities between these wetland types.

Climate change is predicted to alter future patterns and rates of precipitation, evaporation, and evapotranspiration (Smith et al. 2005; Milly et al. 2005). The combination of rising sea-levels and decreased river (freshwater) discharge will result in the upriver migration of the freshwater-saltwater mixing zone (i.e., salt-water intrusion) in some estuaries (Hamilton 1990; Knowles 2002), with potentially significant impacts on ecosystems in the tidal freshwater zone, including TFMs. Salinity-induced stress on freshwater plant communities is projected to decrease primary production and organic matter accumulation rates (Willis and Hester 2004; McKee and Mendelssohn 1989; Spalding and Hester 2007). In addition, rates and pathways of microbial organic matter mineralization can shift in response to changing salinities (Rysgaard et al. 1999; Canavan et al. 2006; Weston et al. 2006). Due to low SO_4^{2-} availability in freshwater (<0.1 mmol L^{-1}), methanogenesis (MG) is often a major pathway of anaerobic organic matter mineralization (Capone and Kiene 1988; Kelley et al. 1990), although microbial iron reduction and denitrification can also be important processes in freshwater wetlands (Roden and Wetzel 1996; Neubauer et al. 2005b; Gribsholt et al. 2005). Microbially-mediated SO_4^{2-} reduction (SR) replaces MG as a dominant anaerobic terminal C mineralization process in marine sediments and salt marsh soils (Jørgensen 1982; Capone and Kiene 1988) due to the greater availability of SO_4^{2-} in seawater ($\sim$28 mmol L^{-1}) and the higher energy yield of organic C degradation coupled to SR as compared to MG (Capone and Kiene 1988; Mishra et al. 2003). Therefore, salt-water intrusion into TFMs will likely alter pathways and rates of elemental cycling and drive shifts in overall ecosystem structure and functioning.

Previous studies have documented a positive relationship between salinity and decomposition in marsh soils (Craft 2007), and a shift from MG to SR following salt-water intrusion into tidal freshwater estuarine sediments (Weston et al. 2006). While these studies have suggested that salt-water intrusion may increase overall rates of organic matter decomposition, the impact of climate change on microbial C cycling in TFM soils remains unclear. Increased organic matter decomposition in response to salt-water intrusion has profound implications for the persistence of TFMs in coastal landscapes. In this study we incubated TFM cores in the laboratory under freshwater and dilute salt-water conditions and measured emissions of carbon dioxide (CO_2) and methane (CH_4), rates of SR and MG, and soil biogeochemistry throughout the 1-year experiment. We specifically excluded plants from the experimental design to

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minimize confounding factors, such as changes in C inputs as plants grow and senesce and the salinity-related deaths of freshwater plants, to focus on how salt-water intrusion impacts rates and pathways of microbial organic matter mineralization.

Methods

Study site and field sampling

The Delaware River is tidal as far north as Trenton, New Jersey, although saline water seldom reaches north of the Delaware–Pennsylvania border. Extensive TFMs are found along the main channel and in tributaries to the Delaware River between approximately Wilmington, Delaware and Trenton, New Jersey (Patrick et al. 1973; Field and Philipp 2000). We collected soils from the Woodbury Creek TFM (39° 51′ 33.05″ N, 75° 10′ 23.33″ W), approximately 2 km from the confluence of this small tributary and the Delaware River. This site is towards the lower end of the freshwater tidal portion of the Delaware River; just upriver of the highest reach of saline water in recent years. Vegetation at this site includes freshwater *Peltandra virginica* (arrow arum), *Pontederia cordata* (pickerelweed) and *Nuphar lutea* (yellow pond lily).

Experimental design

We collected 40 intact soil cores from the marsh platform at the Woodbury Creek study site at low tide in the early spring (17 April 2006), before plants emerged. Soils were collected in 10 cm (i.d.) polyvinylchloride tubes to a depth of approximately 25 cm, sealed at the bottom with gas- and water-tight end caps, and transported to the laboratory. Two cores were sectioned the following day for initial porewater biogeochemical measurements (see *Soil Biogeochemistry* below). Holes were drilled in the core barrel just above the soil surface. Subsequently, cores were randomly assigned to two separate tidal tanks which were housed in an environmental chamber at 20°C in the dark. The tidal tanks (100 L each) allowed the core surface to be exposed to air for a period of 6 h (low tide) followed by 6 h of inundation (high tide). Both tidal tanks were initially filled with artificial freshwater (AFW; Table 1), which was changed several times a week to maintain constant water chemistry. AFW chemistry was chosen to represent average ion and nutrient concentrations in the freshwater Delaware River.

Table 1 Composition of artificial freshwater (AFW) and artificial seawater (ASW) used in the salt-water intrusion experiment

Component	AFW	ASW
Cl^- (mmol L^{-1})	1.01	77.59
Mg^{2+} (mmol L^{-1})	0.10	3.58
Ca^{2+} (mmol L^{-1})	0.27	1.45
Na^+ (mmol L^{-1})	0.91	74.73
K^+ (mmol L^{-1})	0.18	1.52
SO_4^{2-} (mmol L^{-1})	0.04	3.98
HCO_3^- (μmol L^{-1})	668.8	668.8
NH_4^+ (μmol L^{-1})	28.1	28.1
PO_4^{3-} (μmol L^{-1})	11.0	11.0
NO_3^- (μmol L^{-1})	98.9	98.9
Salinity	0.06	4.95

After a 2 week pre-incubation period (days 14 to 0), the water in one tidal tank was replaced with dilute artificial seawater (ASW; Table 1). The ASW had a salinity of approximately 5 (about 14% of full strength seawater), which was attained through increasing major ion concentrations in proportion to seawater while maintaining nutrient and inorganic C concentrations as in the AFW (Table 1). Cores were exposed to simulated tidal flooding and drainage with AFW or ASW for 1 year (days 0–365). The water in both tanks was changed at least once weekly (more often during the first months of the experiments). We measured concentrations of dissolved inorganic C(DIC), chloride (Cl^-), SO_4^{2-}, ammonium (NH_4^+), nitrate + nitrite (NO_x), and phosphate (PO_4^{3-}) in the tidal tanks several times per week to ensure relatively constant chemistry (see *Soil Biogeochemistry* for analytical methods).

Gas flux rates

We measured rates of CO_2 and CH_4 gas emission from the soil cores 2 to 3 times per week during the initial 6 months of the experiment and once weekly in the last 6 months. Gas fluxes were measured during the low-tide portion of the tidal cycle when the

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soil surface was exposed. Cores were fitted with a gas-tight cap, providing approximately 1.2 L of headspace that was circulated with a small fan. An infra-red gas analyzer (PP Systems EGM-4) was connected to the cap in a flow-through configuration, and CO_2 concentration was measured in the headspace every 1 min for 10 min. When CO_2 measurements were complete, an initial headspace sample (3 mL) for CH_4 was obtained with a gas-tight syringe. Final CH_4 samples were obtained after approximately 1 h. CH_4 samples were analyzed immediately by flame ionization detection gas chromatography (Agilent 6890 N with Porapak Q column). Changes in CO_2 and CH_4 gas concentrations over time in the headspace were used to determine gas flux rates.

CO_2 gas flux rates were measured on all cores during each of 85 sampling dates for a total of 1453 CO_2 flux measurements. Due to logistical constraints, CH_4 flux was measured on a subset of 4 AFW and 4 ASW cores during each sampling (72 dates for a total of 618 CH_4 flux measurements). Equipment failure resulted in no CH_4 measurements from days 200 to 270.

To assess whether CO_2 and CH_4 flux rates differed between periods of core inundation and core exposure, we compared gaseous flux rates as described above with aqueous flux measurements. Aqueous flux rates were measured on duplicate cores from each treatment on 6 different dates (day 0, 5, 12, 27, 47 and 82; on day 0 only duplicate freshwater cores were incubated). Cores were capped without a gas headspace and incubated for approximately 8 h with continuous mixing of the overlying water. Water samples were obtained about every 2 h. For DIC measurements, 8 mL of headspace water was removed and placed into a glass vial, 50 μL of $HgCl_2$ was added to halt microbial activity, and the vial was capped without headspace. DIC concentrations were determined on a Shimadzu TOC-V_{CSH} instrument. For dissolved CH_4, 5 mL of sample was injected into a 12 mL headspace vial and preserved with 2 mL of 1 N HCl. Following equilibration, the concentration of CH_4 in the gas headspace of these vials was determined by gas chromatography. DIC and CH_4 flux rates under inundated conditions were then calculated from the changes in DIC and CH_4 concentrations in the flooded core headspace over time.

Rates of microbial sulfate reduction and methanogenesis

We sectioned soil cores periodically throughout the experiment to determine depth-specific rates of both microbial SR and MG and porewater and solid-phase biogeochemistry (see *Soil Biogeochemistry* below). Duplicate cores were sectioned after field collection (on day 14) and just prior to salt-water amendment (day 0). Duplicate cores were removed from the ASW tank and sectioned on days 5, 12, 27, 47, 82, 160 and 364, with sampling from the AFW tank occurring the following day. Due to the destructive nature of the sampling, the number of cores in each tidal tank decreased by two following each sampling timepoint.

Soil cores were sectioned in 2 cm depth increments to a depth of 20 cm in an O_2-free (N_2) atmosphere. Depth-specific rates of microbial SR, hydrogenotrophic MG (HMG) and acetoclastic MG (AMG) were determined on duplicate 2 cm^3 sub-samples from the 0–2, 2–4, 4–6, 8–10, 12–14 and 18–20 cm depths. Six intact sub-samples from each section were taken using 5 mL cut-off syringes that were immediately capped with silicon stoppers. Approximately 0.2 μCi of $^{35}SO_4^{2-}$, 1 μCi of $H^{14}CO_3^-$, and 0.2 μCi of $^{14}CH_3COOH$ were injected into separate sub-cores (2 each) and the samples were incubated at 20°C for 12-16 h. Sub-samples containing ^{35}S were then fixed in 10 mL of 20% zinc acetate and immediately frozen. Sub-samples containing ^{14}C were injected into a 12 mL headspace vial and immediately fixed with 2 mL of 6 N HCl to stop metabolic activity and convert DIC into CO_2. Activity of the total reduced sulfur (TRS) pool was quantified by liquid scintillation counting following cold distillation (Kallmeyer et al. 2004), and rates of SR were calculated as

$$SR = TR^{35}S \times \left(^{35}SO_4^{2-}\right)^{-1} \times \left[SO_4^{2-}\right] \times \varphi \times \alpha SR \times t^{-1} \quad (1)$$

where $^{35}SO_4^{2-}$ is the activity of the initial SO_4^{2-} added, $[SO_4^{2-}]$ is the concentration of SO_4^{2-} in the soil porewater, φ is the porosity of the soil (cm^3 water cm^{-3} soil), αSR is the isotope fractionation factor of SR (1.06; Jørgensen 1978) and t is incubation time.

The ^{14}C activities of CH_4 and CO_2 in MG samples were determined by gas chromatography. The gas headspace from acidified soil slurries was purged for

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10 min with helium and trapped onto a 5 cm length of Porapak Q column under liquid nitrogen. The trapped gases were then injected into a gas chromatograph (Agilent 6890 N) with a 1 m Porapak Q column for separation and quantification of CH_4 (by flame ionization detection) and CO_2 (by thermal conductivity detection), and quantification of $^{14}CH_4$ and $^{14}CO_2$ activities by gas counting (Raytest Raga Star). Purging and trapping efficiency was >99% for CH_4 and >95% for CO_2. The activity of samples was determined relative to the activity of $^{14}CO_2$ standards, after determining that the counting efficiency of $^{14}CH_4$ and $^{14}CO_2$ was equivalent. Rates of hydrogenotrophic HMG and AMG were quantified in a similar manner to SR rates (Eq. 1):

$$\mathrm{HMG} = {}^{14}\mathrm{CH_4} \times (\mathrm{DI14C})^{-1} \times [\mathrm{DIC}] \times \varphi \times \alpha\mathrm{HM} \times \mathrm{t}^{-1} \quad (2)$$

$$\mathrm{AMG} = {}^{14}\mathrm{CH_4} \times \left({}^{14}\mathrm{CH_3COOH}\right)^{-1} \times [\mathrm{CH_3COOH}] \times \varphi \times \alpha\mathrm{AM} \times \mathrm{t}^{-1} \quad (3)$$

where $^{14}CH_4$ is the activity of the measured CH_4, ($DI^{14}C$) and ($^{14}CH_3COOH$) are the activities of the DIC and acetate additions, respectively, [DIC] and [CH_3COOH] are the porewater concentrations of DIC and acetate, respectively, and αHM and αAM are the isotope fractionation factors for HMG and AMG, respectively (both 1.06, Orcutt et al. 2005).

Total organic C (CH_2O) mineralized through each anaerobic microbial pathway was estimated assuming the following stoichiometries:

$$\mathrm{SR}: 2\mathrm{CH_2O} + \mathrm{SO_4^{2-}} + 2\mathrm{H^+} \rightarrow 2\mathrm{CO_2} + \mathrm{H_2S} + 2\mathrm{H_2O} \quad (4)$$

$$\mathrm{AMG}: 2\mathrm{CH_2O} \rightarrow \mathrm{CH_4} + \mathrm{CO_2} \quad (5)$$

$$\mathrm{HMG}: \mathrm{CO_2} + 4\mathrm{H_2} \rightarrow \mathrm{CH_4} + 2\mathrm{H_2O} \quad (6)$$

The amount of SO_4^{2-} reduced (for SR; Eq. 1) or CH_4 produced (for MG; Eqs. 2 and 3) via each process was used to determine the total amount of organic C mineralized to CO_2 and CH_4. For SR and AMG, 2 mol C are mineralized per SO_4^{2-} reduced (Eq. 4) or CH_4 produced (Eq. 5), while there is no net C mineralization for HMG (Eq. 6). The rates of CO_2 and CH_4 production and total organic C (TC) mineralization are then:

$$\mathrm{CO_2} = 2 \cdot \mathrm{SR} + \mathrm{AMG} \quad (7)$$

$$\mathrm{CH_4} = \mathrm{AMG} + \mathrm{HMG} \quad (8)$$

$$\mathrm{TC} = 2 \cdot \mathrm{SR} + 2 \cdot \mathrm{AMG} \quad (9)$$

Soil biogeochemistry

Porewater and solid-phase biogeochemistry was determined on the same soil cores used for microbial SR and MG rates on each 2 cm soil section between the surface and 20 cm depth. Two cm^3 of soil was placed into an aluminum weigh dish for determination of bulk density, porosity, and elemental analysis after drying at 90°C. C and N content was determined on dried, ground soil using a Leco TruSpec CN analyzer. Carbonates did not contribute to the C content of these soils [unacidified = 0.997 (acidified) + 0.18; $R^2 = 0.88$; $n = 87$ samples from throughout the experiment and from both treatments] and the CN content reported here is for unacidified samples. For determination of porewater CH_4 concentrations, 2 cm^3 of soil was placed into duplicate 12 mL headspace vials which were immediately sealed. Four mL of 1 N HCl was injected into the vial, and the contents shaken vigorously to stop microbial activity and equilibrate the porewater gases with the vial headspace. CH_4 concentration was determined on the headspace of these vials by gas chromatography.

We placed 50 cm^3 of soil into centrifuge tubes under an N_2 atmosphere, centrifuged the soil at 4000 rpm for 15 min, and split aliquots of porewater into several vials for various analyses. One mL of unfiltered porewater was preserved with 50 µL of a saturated $HgCl_2$ solution for DIC analysis on a Shimadzu TOC-V_{CSH}. One mL of unfiltered porewater was pipetted into a 20% zinc acetate solution for later determination of reduced sulfide concentrations (Cline 1969). Four mL of 0.7 µm nominal filtered (GF/F) porewater was preserved with 50 µL of 6 N HNO_3, 2 mL of filtered sample was immediately frozen, and the remaining sample (1–5 mL) was filtered and refrigerated.

Porewater Cl^- and SO_4^{2-} (Dionex DX 500 ion chromatograph) and PO_4^{3-} (phosphomolybdate method; Murphy and Riley 1962) concentrations were determined on nitric acid acidified samples. Dissolved organic carbon (DOC) concentrations were determined by high-temperature combustion following sparging of acidified samples on a Shimadzu TOC-V_{CSH}. NH_4^+ (phenolhypochlorite method; Solorzano

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1969) and NO_x (flow injection autoanalyzer following cadmium reduction) concentrations were measured on un-acidified, refrigerated samples. Acetate was determined on frozen samples by high-pressure liquid chromatography (Agilent 1200 series) following sample derivitization (Albert and Martens 1997).

Data analysis

Porewater and solid phase biogeochemical variables and microbial rates were integrated over a 20 cm depth, with linear interpolations between data points when data were missing (e.g., rates were measured on only 6 of 10 depths). Porewater and solid phase measurements were converted to volumetric units (i.e., mmol cm^{-3}) using measured soil porosity and bulk density, respectively. Statistical analyses of the data were conducted using linear regressions and univariate analysis of variance (ANOVA) with least squares difference corrections of confidence intervals for main effects using SPSS (v16.0). Additional pairwise comparisons of means were made using T tests for independent samples.

Results

Gas flux

Gaseous CO_2 flux rates were significantly higher for cores undergoing salt-water intrusion (Fig. 1, $p < 0.001$, $F_{1,1452} = 95.38$). The CO_2 flux from the salt-water amended marsh soils increased above flux rates from freshwater controls rapidly (<1 week)

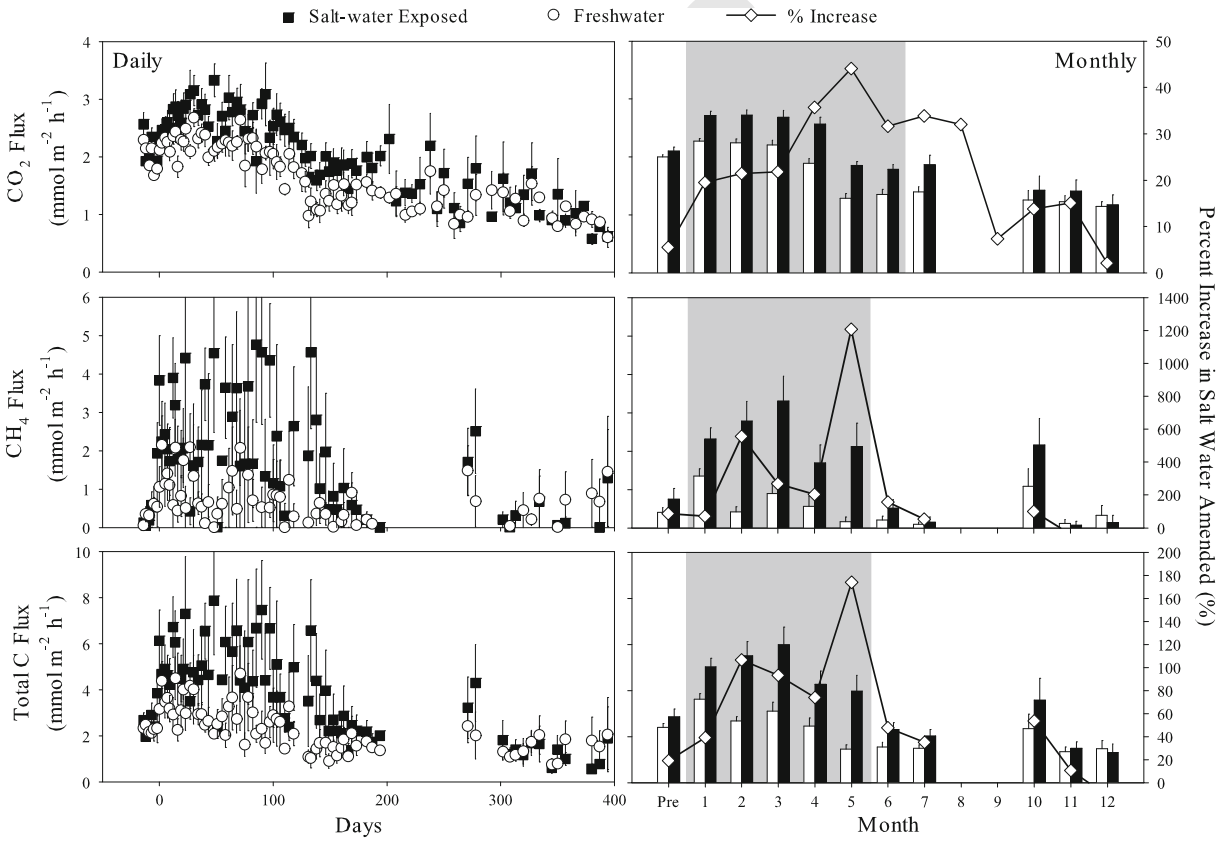

Fig. 1 Daily and monthly carbon dioxide (CO_2; *top*), methane (CH_4; *middle*) and total C (*bottom*) gas fluxes (mmol m^{-2} h^{-1}; mean ± SE) from freshwater soil cores and soil cores exposed to dilute salt-water. The percent increase in flux from salt-water amended soils versus freshwater controls for monthly averages are shown, and shading indicates months for which differences between treatments are significant ($p < 0.05$; months 1–6 for CO_2, 1–5 for CH_4 and 1–5 for total C). No CH_4 (and therefore total C) data are available from day 200 to day 270 due to equipment failure

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following salt-water intrusion and remained significantly higher for the first 6 months of the experiment (Fig. 1, $p < 0.05$). Maximum flux rates in both treatments were measured during months 1 through 3 averaging $\sim$2.7 mmol m^{-2} h^{-1} in the salt-water amended cores and $\sim$2.2 mmol m^{-2} h^{-1} in the freshwater cores (Fig. 1). The relative difference in CO_2 flux from salt-water amended soils increased to 20% in the first several months following salt-water intrusion, with a peak of 45% in the 5th month (Fig. 1). There was significant decline in CO_2 gas flux over time in both the freshwater (CO_2 flux $= -0.0037 \times$ day $+ 2.19$, $t = -18.02$, $p < 0.001$, $R^2 = 0.32$, $F_{1,696} = 324.61$) and salt-water amended soils (CO_2 flux $= -0.0040 \times$ day $+ 2.61$, $t = -12.61$, $p < 0.001$, $R^2 = 0.17$, $F_{1,756} = 159.08$).

CH_4 fluxes (Fig. 1) were significantly higher for cores undergoing salt-water intrusion ($p < 0.001$, $F_{1,617} = 44.04$) and this difference persisted for 5 months ($p < 0.05$, Fig. 1). CH_4 flux from salt-water amended cores peaked in month 3 with an average rate of about 3.3 mmol m^{-2} h^{-1} (Fig. 1). The flux of CH_4 from salt-water amended soils was 70% (in month 1) to 1200% (month 5) higher than flux rates from freshwater soils (Fig. 1). Note that CH_4 flux rates were not significantly different for 2 months (months 6 and 7) prior to data loss during months 8 and 9 (Fig. 1). As was observed for CO_2 flux, the flux of CH_4 declined significantly over time from the freshwater (CH_4 flux $= -0.0021 \times$ day $+ 0.84$, $t = -3.26$, $p = 0.001$, $R^2 = 0.03$, $F_{1,313} = 10.65$) and salt-water amended soils (CH_4 flux $= -0.0050 \times$ day $+ 2.15$, $t = -3.75$, $p < 0.001$, $R^2 = 0.04$, $F_{1,305} = 14.05$).

Overall, total gaseous C fluxes ($CO_2 + CH_4$) were significantly higher from salt-water amended marsh soils for 5 months following salt-water intrusion ($p < 0.001$, $F_1 = 52.46$), and C emissions ranged from 40% (in month 1) to 175% (in month 5) higher from the cores undergoing salt-water exposure than from freshwater cores (Fig. 1). Total C flux from salt-water impacted marsh soils peaked in month 3 at a rate of about 6 mmol C m^{-2} h^{-1} (Fig. 1).

During the first 90 days of the experiment (when inundated flux measurements were conducted) the average DIC flux rates when the soils were flooded were not significantly different than CO_2 gas fluxes when soils were exposed (Table 2; $p = 0.84$, $F_{1,865} = 0.66$). In contrast, there was a significant difference between exposed and inundated CH_4 fluxes (Table 2; $p = 0.02$; $F_{1,379} = 5.79$). The ratio of CH_4 emissions in inundated versus exposed cores ($R_{(Ind/Exp)}$) was 0.46 and 0.22 in freshwater and salt-water amended cores, respectively (Table 2).

Total CO_2 and CH_4 emissions over the 1-year experiment were calculated. As there was no significant difference between inundated and exposed CO_2/DIC flux (Table 2), the measured CO_2 gas fluxes (Fig. 1) were integrated over 1 year for 24 h per day. 14.2 mol CO_2 m^{-2} was emitted from freshwater soils compared with 17.3 mol CO_2 m^{-2} from soils exposed to salt-water. Because of the lower CH_4 emissions when soils were flooded (Table 2), the CH_4 gas flux measurements (Fig. 1) were assumed to represent CH_4 emissions for 12 h per day when soils were exposed. To determine CH_4 emissions for the remaining 12 h per day when soils were flooded, the CH_4 gas flux measurements (Fig. 1) were

Table 2 Average (±standard deviation; SD) carbon dioxide (CO_2) and methane (CH_4) flux rates (mmol m^{-2} h^{-1}) from freshwater and salt-water amended soil cores under exposed and inundated conditions, and the ratio of inundated to exposed flux ($R_{(Ind/Exp)}$) rates during the initial 90 days of the experiment

	CO_2						CH_4					
	Freshwater			Salt-amended			Freshwater			Salt-amended		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Exposed	2.17	0.54	396	2.54	0.91	449	0.86	1.37	182	2.12	2.68	177
Inundated	2.36	2.00	14	2.28	1.72	10	0.40	0.36	14	0.46	0.48	10
$R_{(Ind/Exp)}$	1.09			0.90			0.46			0.22		

The number of measurements (*n*) is shown. Note that the difference between exposed and inundated measurements is significantly different for CH_4 ($p = 0.02$; $F_{1,379} = 5.79$) but not for CO_2 ($p = 0.84$, $F_{1,865} = 0.66$)

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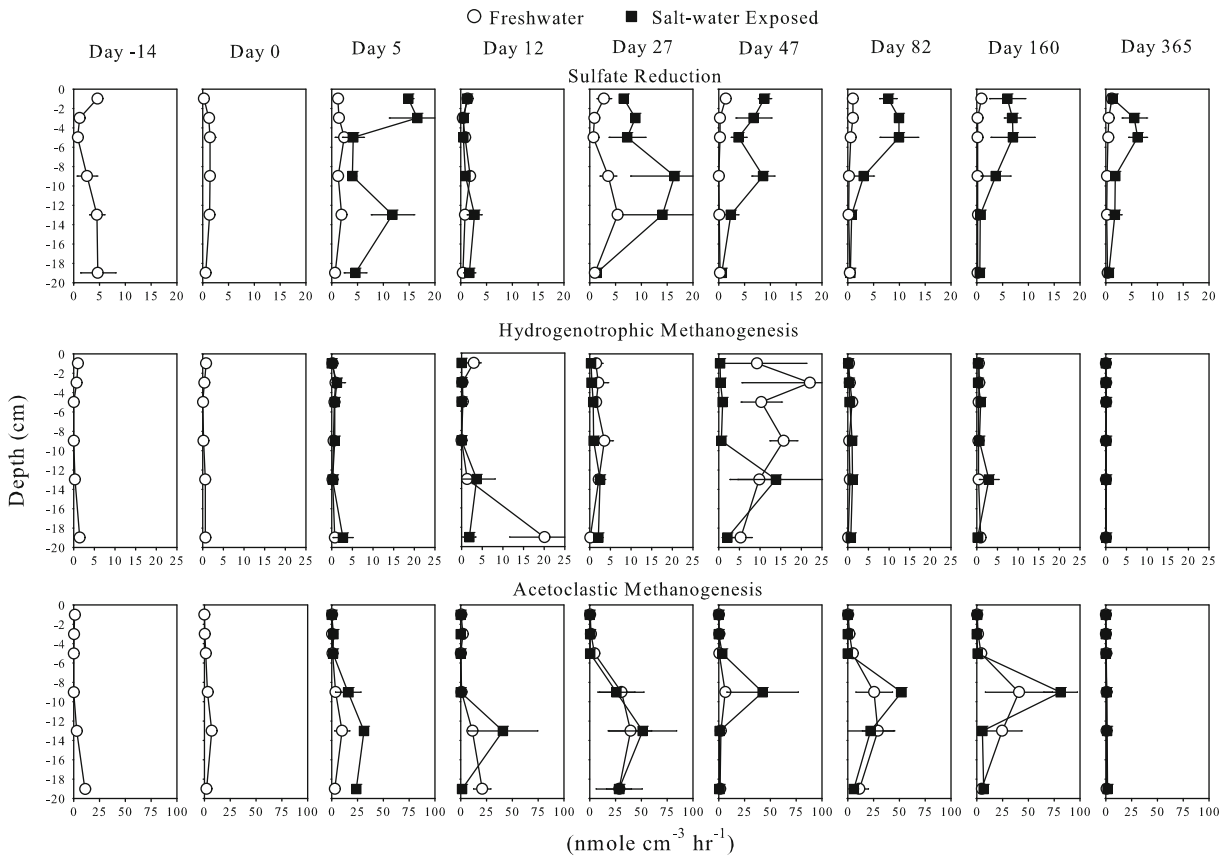

Fig. 2 Depth-specific rates of sulfate reduction (*top*; nmol SO_4^{2-} cm^{-3} h^{-1}), hydrogenotrophic methanogenesis (*middle*; nmol CH_4 cm^{-3} h^{-1}) and acetoclastic methanogenesis (*bottom*; nmol CH_4 cm^{-3} h^{-1}) in soils undergoing salt-water intrusion and in freshwater controls over time (average ± SE)

multiplied by the appropriate $R_{(Ind/Exp)}$ (Table 2). The total CH_4 flux over the 1 year experiment was calculated to be 3.9 and 7.5 mol m^{-2} from freshwater and salt-water amended cores, respectively. The total C gas flux over the 1 year experiment from freshwater cores was 18.2 mol m^{-2}, compared with 24.9 mol m^{-2} from salt-water amended soils.

Rates of microbial sulfate reduction and methanogenesis

Rates of SR ranged from 0 to approximately 16 nmol SO_4^{2-} cm^{-3} h^{-1}. SR was lower in the freshwater soils than in salt-water amended soils throughout the experiment. In the salt-water amended cores, SR rates increased at all depths on day 5, were not significantly different than freshwater rates on day 12, and were higher in the upper 10 cm of the soil column for the duration of the experiment (Fig. 2). SR became more confined to surface (0–5 cm) soils from 3 months until the termination of the experiment.

Depth-integrated rates of SR remained under 0.7 mmol SO_4^{2-} m^{-2} h^{-1} in freshwater soils and reached a maximum of 2.0 mmol SO_4^{2-} m^{-2} h^{-1} in salt-water impacted soils on day 27 after salt-water intrusion (Fig. 3). Salt-water amendment had a significant effect on SR rates ($p < 0.001$, $F_{1,29} = 25.40$). SR rates were significantly higher in salt-water impacted soils on all dates ($p < 0.05$, $t = 3.01$, $df = 2$) except for day 47 (Fig. 3). Total SR integrated over the 1 year experiment was 0.9 mol SO_4^{2-} m^{-2} in freshwater soils and 6.8 mol SO_4^{2-} m^{-2} in the salt-water amended soils.

Rates of HMG ranged from 0 to 22 nmol CH_4 cm^{-3} h^{-1}. HMG rates were variable and there was no clear pattern with depth (Fig. 2). Depth-integrated rates of HMG peaked in both freshwater and salt-water impacted soils on day 47, with the highest rates

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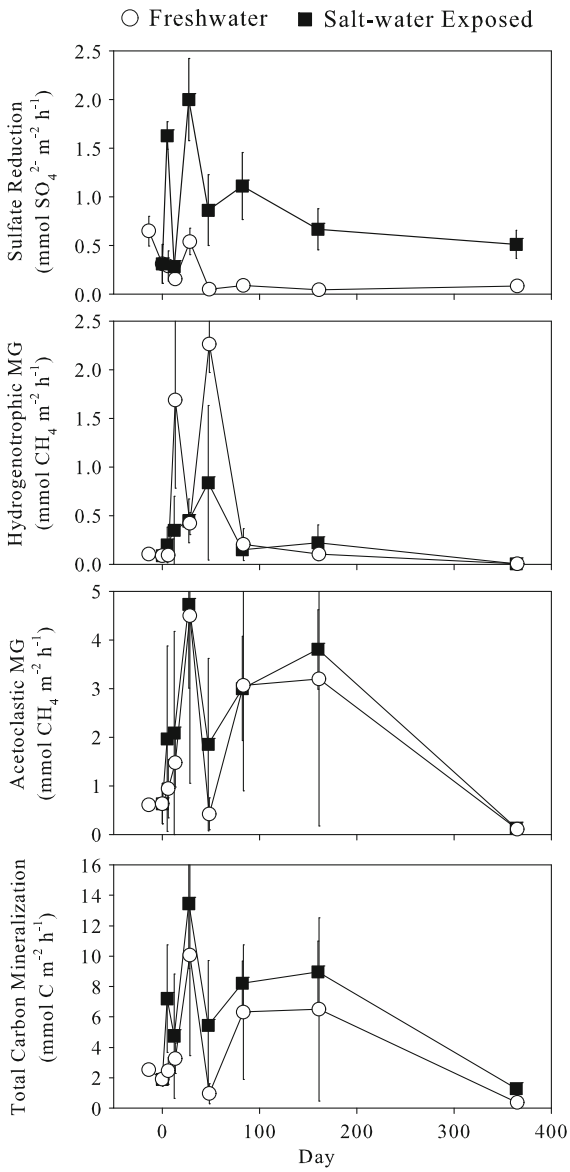

Fig. 3 Depth-integrated rates (mmol m^{-2} h^{-1}; average ± SE) of sulfate reduction, hydrogenotrophic and acetoclastic methanogenesis and total carbon mineralization (see text) over time in soil cores undergoing salinity intrusion and in freshwater controls

measured in freshwater soils (2.3 mmol CH_4 m^{-2} h^{-1}; Fig. 3). There were no significant differences in HMG rates between treatments ($p = 0.43$, $F_{1,29} = 0.63$), although note the high rates in freshwater soils on days 12 and 47 (Fig. 3). Rates of HMG integrated over 1 year were 2.8 mol CH_4 m^{-2} in the freshwater soils and 1.8 mol CH_4 m^{-2} in soils exposed to salt-water.

AMG rates of up to 80 nmol CH_4 cm^{-3} h^{-1} were measured. AMG was generally low in surface soils, and maximum rates were usually observed at deeper depths (>8 cm; Fig. 2). Depth integrated rates of AMG of over 4.0 mmol CH_4 m^{-2} h^{-1} were measured in both freshwater and salt-water impacted soils on day 27 (Fig. 3). There was no significant effect of salt-water amendment on AMG rates ($p = 0.25$, $F_{1,29} = 1.37$). Integrated over the 1 year experiment, rates of AMG were 18.1 mol CH_4 m^{-2} in freshwater and 21.4 mol CH_4 m^{-2} in salt-water amended soils.

Estimates of total C mineralized via anaerobic microbial pathways, calculated from measurements of SR and MG together with reaction stoichiometries in Eqs. 7–9, ranged from 0.4 mmol C m^{-2} h^{-1} (in freshwater soils at day 364) to 13.5 mmol C m^{-2} h^{-1} (in salt-water impacted soils on day 27; Fig. 3). Salt-water amendment significantly affected overall rates of TC ($p = 0.048$, $F_{1,29} = 4.26$), although differences were not significant between specific sampling dates ($p > 0.05$) except on day 364 ($p < 0.05$, $t = 3.27$, $df = 2$; Fig. 3). Rates of total C mineralization integrated over the 1 year experiment were 37.8 mol C m^{-2} (5% SR and 95% AMG) in freshwater soils and 56.2 C mol m^{-2} (24.0% SR and 76% AMG) in soils exposed to salt-water.

Soil biogeochemistry

Soil porosity (0.694 ml cm^{-3} ± 0.003, mean ± SE) and dry bulk density (0.500 g cm^{-3} ± 0.004) varied little with depth, time, or between salt-water and freshwater treatments (data not shown). Porewater Cl^- concentrations remained low in the freshwater soils throughout the experiment (Fig. 4). In the soils undergoing experimental salt-water intrusion, Cl^- concentrations in surface soils increased rapidly to reflect Cl^- concentrations in the ASW (Table 1), while concentrations at depth remained lower throughout most of the experiment. Total inventories of Cl^- in salt-water amended cores increased throughout the experiment (Fig. 4), reflecting the relatively slow diffusion-driven increase of Cl^- at depth. It took almost 3 months before Cl^- in the salt-water amended soils at depth (>16 cm) became significantly higher than Cl^- concentrations in the freshwater control soils, and a full year before inventories of Cl^- in amended cores were fully equilibrated with Cl^- concentrations in the overlying water (Fig. 4). There

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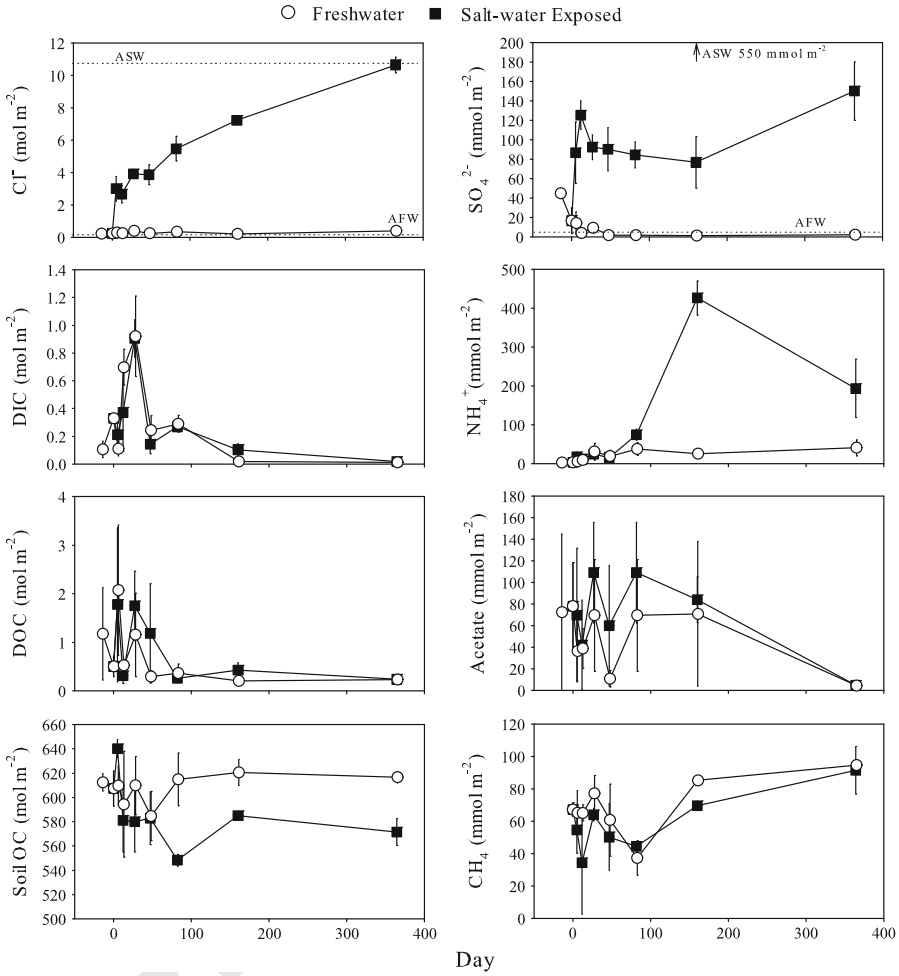

Fig. 4 Depth-integrated inventories (integrated to 20 cm; mean ± SE) of porewater chloride (Cl^-), sulfate (SO_4^{2-}), dissolved inorganic carbon (DIC), ammonium (NH_4^+), dissolved organic carbon (DOC), acetate and methane (CH_4), and inventories of soil organic C in freshwater and salt-water amended cores over time. Horizontal lines on select graphs denote theoretical inventories of cores fully equilibrated with overlying artificial freshwater (AFW) and/or seawater (ASW) used in the experiment (Table 1)

was an overall significant difference between Cl^- inventories in salt-water amended and freshwater soils ($p < 0.001$, $F_{1,29} = 88.70$), and Cl^- inventories were significantly greater in salt-water amended cores on all sampling dates post-amendment ($p < 0.05$, $t > 3.51$, $df = 2$).

Initial porewater SO_4^{2-} concentrations in cores collected from the TFM (day 14) indicated a subsurface SO_4^{2-} maximum of about 700 μmol L^{-1} at a depth of 7 cm (data not shown). This mid-depth peak in SO_4^{2-} concentrations in the freshwater cores decreased during the first several weeks of the experiment, such that by day 12 porewater SO_4^{2-} in freshwater cores did not exceed 100 μmol L^{-1} and this decrease is reflected in the SO_4^{2-} inventories (Fig. 4). SO_4^{2-} concentrations in salt-water amended cores increased rapidly on days 5 and 12, and then declined slightly through day 160 before increasing again at the end of the experiment (Fig. 4). SO_4^{2-} inventories remained far below equilibration with the overlying ASW (550 mmol m^{-2}) throughout the experiment (Fig. 4). SO_4^{2-} was limited to surface soils, and concentrations at depths below 10 cm remained low relative to overlying water concentrations (<500 μmol L^{-1}; see Table 1). There was a significant treatment effect on SO_4^{2-} inventories ($p < 0.001$, $F_{1,29} = 93.39$), and inventories of SO_4^{2-} were significantly greater in salt-water amended cores on days 12, 27, 82 and 364 ($p < 0.05$, $t > 3.99$, $df = 2$).

Porewater NH_4^+ concentrations were low initially (<150 μmol L^{-1}) and remained below 500 μmol L^{-1} in freshwater cores throughout the experiment (Fig. 4). Salt-water amendment impacted NH_4^+ inventories significantly ($p = 0.02$, $F_{1,29} = 6.01$), although NH_4^+ concentrations were not significantly

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different between freshwater and salt-water amended soils except on day 160 when NH_4^+ inventories in salt-water amended soils peaked at over 400 mmol m^{-2} (Fig. 4, $p < 0.05$, $t = 9.06$, $df = 2$). DIC concentrations were variable between replicate cores, and there were no significant differences between treatments for whole core inventories ($p = 0.90$, $F_{1,29} = 0.02$, Fig. 4). There was a consistent pattern over time for both treatments, in which inventories increased in both freshwater and salt-water amended soils until day 27 and then decreased. Porewater DIC inventories were quite low by the termination of the experiment (Fig. 4).

Porewater acetate concentrations were consistently higher at depth than in surface soils. Acetate concentrations in the top 4 cm remained below 400 µmol L^{-1}, while maximum concentrations exceeded 1 mmol L^{-1} at depths below 8 cm. Acetate concentrations were variable and there were no significant differences between treatments for whole core inventories ($p = 0.27$, $F_{1,29} = 1.27$), although acetate inventories were consistently larger in salt-water amended soils (Fig. 4). Inventories of porewater DOC were highly variable and there were no significant differences between salt-water amended and freshwater control soils (Fig. 4, $p = 0.57$, $F_{1,29} = 0.34$). DOC concentrations were consistently low by day 82, however, and remained low for the duration of the experiment (Fig. 4).

Whole core CH_4 inventories were not significantly different between freshwater and salt-water amended soils (Fig. 4, $p = 0.10$, $F_{1,29} = 2.96$), although inventories in both treatments increased over time ($p = 0.002$, $F_{1,29} = 12.18$). Soil inventories of PO_4^{3-} and NO_x were consistently low (<10 mmol m^{-2}), were not significantly different between treatments ($p > 0.05$) and did not exhibit patterns over time (data not shown). Porewater sulfide concentrations were below detection ($\sim$1 µmol L^{-1}) in all cores at all depths (data not shown).

Soil solid phase organic C ranged between 5.0 and 9.5% by weight, and total N ranged from 0.3 and 0.8% by weight. There was no significant change in N over time or between treatments ($p > 0.05$, data not shown). Inventories of organic C were significantly different between salt-water amended and freshwater soils ($p = 0.043$, $F_{1,29} = 4.50$), and organic C was significantly lower in salt-water amended soils on days 82, 160 and 364 compared to freshwater soils and to initial organic C values (Fig. 4, $p < 0.05$, $t < -2.98$, $df = 2$). The average soil inventory of organic C on these 3 sampling dates ($n = 6$ cores per treatment) was 568.2 (±7.7 SE) mol m^{-2} for salt-water amended cores versus 617.3 (± 7.5 SE) mol m^{-2} for freshwater cores and 612.2 (±8.3 SE) mol m^{-2} for the initial organic C inventory on day 14 (Fig. 4).

Discussion

Our research has documented that salt-water intrusion into TFM soils can significantly increase rates of microbial C mineralization (Fig. 5). We used three independent approaches to assess C mineralization, including (1) changes in soil organic C, (2) microbial sulfate reduction and methanogenesis rate measurements, and (3) C gas fluxes from soils following simulated salt-water intrusion and in freshwater controls. The lack of measurable decrease in the organic C in freshwater controls (Figs. 4, 5), coupled with the differences between rates CO_2 and CH_4 production by microbial mineralization and the flux of these gases from the soils (Fig. 5), suggests these three measures of soil C dynamics were prone to some uncertainties. That these three independent approaches agree on the relative impact of salt-water intrusion on microbial C cycling in TFM soils, however, clearly indicates that the mineralization of organic C accelerates following salt-water intrusion into tidal freshwater marshes.

The total amount of CO_2 and CH_4 released from salt-water amended cores (24.8 mol m^{-2}) was 36.9% greater than the total inorganic C flux from freshwater cores (18.2 mol m^{-2}) over the 1 year experiment. Similarly, the amount of organic matter mineralized via SR and MG within soils experiencing salt-water intrusion (56.2 mol m^{-2}) was greater than mineralization in freshwater soils (37.8 mol m^{-2}) by 49%. Finally, the higher rates of organic matter decomposition in salt-water amended soils were reflected in a loss of soil organic C (44.1 mol m^{-2}) from these soils (Fig. 5). These results reinforce earlier work about the effects of salinity on C turnover in a short-term (30 d) experiment with freshwater riverine sediments (Weston et al. 2006) and in a year-long root decomposition study along an estuarine salinity gradient (Craft 2007). However, unlike these earlier

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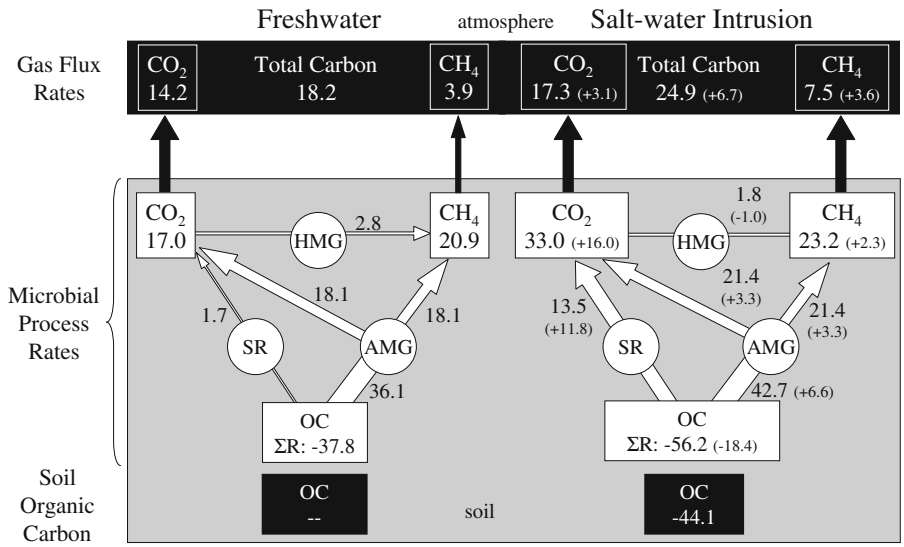

Fig. 5 Schematic of C cycling in freshwater tidal marsh soils (*left*) and soils undergoing salt-water intrusion (*right*) as calculated from measured gas fluxes (*top*, in black), microbial process rates (*middle*, in white) and soil organic C measurements (*bottom*, in black) from cores incubated for 1 year. All values are in units of mol C m^{-2}. Values for gas flux rates (Fig. 1) and microbial process rates (Fig. 3) are integrated over 1 year; organic C content (Fig. 4) is the difference between the initial organic C inventory (612.2 mol m^{-2}) and average soil organic C inventories from days 82, 160 and 364 in salt-water amended (568.2 mol m^{-2}) and freshwater soils (617.3 mol m^{-2}). The net production of carbon dioxide (CO_2) and methane (CH_4), the net consumption of organic carbon (OC) calculated as the sum of microbial process rates or the loss of soil organic carbon, and the rates of sulfate reduction (SR), acetoclastic methanogenesis (AMG) and hydrogenotrophic methanogenesis (HMG) are shown. Increases (+) or decreases (−) in salt-water amended soils relative to freshwater controls are shown in parentheses

studies, our research has shown that salt-water intrusion can accelerate rates of CH_4 emissions to the atmosphere, a finding that has implications not only for local rates of C preservation and marsh accretion, but also for regional-scale greenhouse gas budgets.

Experimental design considerations

The overall responses of TFMs to rising sea levels and salt-water intrusion will be determined by changes in microbial dynamics as well as plant processes. Our experimental design, in which soil cores were collected prior to spring plant emergence and incubated in the dark, intentionally precluded new C inputs to the soils via primary production so that we could focus our attention on understanding the effects of salt-water intrusion on microbially-mediated soil C mineralization. We acknowledge that wetland plants can influence C cycling by increasing soil C concentrations (Hines et al. 1989), accelerating rates and modifying pathways of anaerobic metabolism (Neubauer et al. 2005b), and "priming" the microbial utilization of recalcitrant soil C (Wolf et al. 2007). Further, rates of plant production and community composition can themselves be affected by salt-water intrusion (Spalding and Hester 2007). We suggest that the overall effects of excluding plants in our experimental design were to (1) lower total rates of organic C remineralization relative to a vegetated marsh and (2) cause organic matter limitation, leading to a progressive decline in CO_2 and CH_4 production and emission rates over the course of the experiment (Figs. 1, 3). Shifts in hydrology and drainage due to long-term incubation of soils in the laboratory, and the step-increase in salinity when simulating salt-water intrusion rather than pulses of saline water as would accompany salt-water intrusion in the field, also likely alter the overall rates of microbial processes in these soils. Regardless, we do not expect that the chosen experimental design would cause any difference in the relative patterns of soil C mineralization that were observed between freshwater and salt-exposed cores.

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Salt-water effects on anaerobic C mineralization rates

Salt-water inundation of TFM soils resulted in shifts in microbial pathways and increases in the overall rate of organic matter decomposition. Higher concentrations of SO_4^{2-} following salt-water intrusion fueled increased rates of SR (Figs. 2, 3) and likely contributed to higher CO_2 emissions from the cores (Fig. 1). Surprisingly, rates of MG did not decrease with salt-water intrusion (Figs. 2, 3) and CH_4 emissions from the salt-exposed cores increased by 70–1200% for 5 months relative to freshwater cores (Fig. 1). The overall gaseous C loss (Fig. 1) was significantly greater in TFM soils following salt-water intrusion relative to freshwater controls. Similarly, the inventory of organic C was significantly lower in salt-water amended soils 3, 6 and 12 months after exposure than in freshwater soils (Fig. 4), reflecting the increased mineralization of organic matter in these soils under higher salinity regimes. Weston et al. (2006) found a similar increase in organic matter decomposition in freshwater sediments following salt-water intrusion in a short-term experiment. In addition, Craft (2007) documented a negative relationship between both soil organic content and accumulation and the salinity of the overlying water in a survey of tidal freshwater and salt marshes, which he attributed to the availability of SO_4^{2-} and thus higher rates of SR in the more saline sites. Our results support these findings, and suggest that salt-water intrusion will stimulate decomposition in TFM soils.

SR and MG are terminal steps in the break down of organic matter, and are limited to relatively small organic compounds such as acetate (Weiss et al. 1991). These terminal metabolic processes therefore depend on the generation of low molecular weight DOC substrates by other processes. A microbial consortium converts particulate organic matter into low molecular weight DOC through hydrolysis and fermentation reactions (Arnosti et al. 1994; Fenchel and Findlay 1995). Greater inorganic C fluxes from TFM soils amended with salt-water (Fig. 1), which can be attributed to increased rates of SR and MG (Fig. 3), require either; (1) the utilization of a previously unused pool of low molecular weight DOC in the soils or (2) an increased supply of low molecular weight DOC via hydrolysis and fermentation.

Low molecular weight dissolved organic matter can adsorb onto mineral particles, and ion exchange plays an important role in the sorption of some compounds such as amino acids (Wang and Lee 1993; Liu and Lee 2007). Therefore, the intrusion of saline water with greater concentrations of dissolved ions into previously freshwater soils may desorb organic compounds from exchange sites making them available for terminal metabolism (e.g., Liu and Lee 2007) and may alter the availability of larger dissolved and particulate organic C, perhaps promoting hydrolytic and fermentative production of labile, low molecular weight, dissolved organic compounds. Similarly, NH_4^+ is a surface reactive ion that can also be desorbed upon addition of other cations (Rosenfeld 1979); evidence of NH_4^+ desorption in our study is reflected by the increase in porewater inventories of NH_4^+ in the salt-water amended soils from about 3 months until the termination of the experiment (Fig. 4). The increasing ionic strength of the porewater in the salt-water amended soils therefore clearly altered the soil sorption dynamics. There was no evidence of DOC or acetate desorption, however (Fig. 4), and other potential substrates for these terminal metabolic processes were not measured. Further investigation of the mechanisms influencing organic matter availability upon salt-water intrusion is required.

The amount of both CO_2 and CH_4 produced by the measured microbial processes in marsh soils in both freshwater and salt-water amended soils exceeded the flux of these gases from the soils (Fig. 5). While processes other than SR and MG, such as iron reduction and denitrification (Roden and Wetzel 1996; Neubauer et al. 2005b; Gribsholt et al. 2005), may have contributed to the mineralization of organic matter, the measured rates of SR and MG were more than enough to support the measured inorganic C fluxes from these soils.) There was an increase in CH_4 production in soils following salt-water intrusion (Fig. 5; 2.3 mol CH_4 m^{-2}), which was lower than the increase in measured CH_4 flux (Fig. 5; 3.6 mol CH_4 m^{-2}). The difference between CH_4 production and flux (Δ = 17.0 and 15.7 mol CH_4 m^{-2} for freshwater and salt-water amended soils, respectively; Fig. 5) suggests a difference between production and flux from the soils and/or errors in the rate measurements. Porewater acetate concentrations measured in both freshwater and salt-water amended soils were

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relatively high (exceeding 1 mmol L^{-1} in some cases; data not shown), which may reflect increased acetate following centrifugation of soils (Shaw and McIntosh 1990; Hines et al. 1994). Artificially increased concentrations of porewater acetate would result in higher rates of AMG (Eq. 3), and lead to elevated estimates of CH_4 and CO_2 production (Fig. 5). CH_4 oxidation may also have played an important role in mitigating CH_4 emission from these TFM soils (Megonigal and Schlesinger 2002). The oxidation of CH_4 produces CO_2, but as with CH_4, the measured CO_2 gas fluxes could not account for the CO_2 produced via sulfate reduction and methanogenesis ($\Delta = 2.7$ and 15.7 mol CO_2 m^{-2} for freshwater and salt-water amended soils, respectively; Fig. 5). A total of 19.7 and 31.4 mol C m^{-2} was therefore apparently mineralized but not accounted for in gas fluxes from freshwater and salt-water soils, respectively (Fig. 5). The fate of this 'missing' carbon is unclear, though we suspect that estimates of CH_4 and CO_2 production were elevated due to artificially high porewater acetate concentrations. Some amount of the organic carbon substrate used during SR and MG would be assimilated by the microbes mediating these reactions, though growth yields do not typically exceed 10% and are often much lower (Widdel and Bak 1992; Maillacheruvu and Parkin 1996; Reeve et al. 1997; Habicht et al. 2005). Chemoautotrophic fixation and assimilation of CO_2 and CH_4 via methanotrophy, nitrification, reduced sulfur oxidation and other reactions may also reduce fluxes of these gasses from soils (e.g., Howarth 1984; Hadas et al. 2001). Fixation of carbon and an increase in microbial biomass and/or subsequent release of fixed C as DOC (DOC fluxes were not measured in this study, though soil inventories of DOC were substantial: Fig. 4) may account for some of this missing C. Alternatively, ebullition, which can be patchy both in space and time, could be responsible for some loss of CO_2 and CH_4 from soil that was not captured by the exposed or inundated core incubations. For example, ebullition accounted for ~50% of the total CH_4 flux (diffusion + ebullition) from subtidal freshwater river sediments (Chanton et al. 1989).

Pathways of anaerobic C mineralization

The energy yield of SR is greater than that of MG, and when SO_4^{2-} is available, sulfate reducers are expected to outcompete methanogens for organic matter substrates (Capone and Kiene 1988; Mishra et al. 2003). Increased rates of SR upon salt-water intrusion were therefore expected, and these findings support previous studies. For instance, Weston et al. (2006) found that the sulfate reducing microbial community in freshwater sediments of the Altamaha River, GA was able to adjust rapidly (<2 weeks) to higher SO_4^{2-} availability since sulfate reducers can multiply quickly upon the onset of positive growth conditions (e.g., Raskin et al. 1996). Although rates of SR increased in the current experiment, as hypothesized, the apparent stimulation (or at a minimum the lack of suppression) of methanogens was unexpected.

Depth-integrated rates of HMG in general were less than rates of AMG (Fig. 3). Although the differences were not significant, rates of HMG tended to be greater in freshwater soils than in salt-exposed, while rates of AMG tended to be higher in salt-water-impacted soils (Fig. 3). The two pathways of MG measured here are usually the major pathways of CH_4 production, but the utilization of other low molecular weight organic substrates, such as methanol and methyl amines, were not directly measured and could therefore account for a portion of the CH_4 generation (Oremland and Polcin 1982). Regardless of the specific substrate, however, results indicate that the increased CH_4 flux from TFM soils experiencing salt-water intrusion was due to the response of the methanogens utilizing organic matter substrates rather than hydrogen as the reductant.

MG was largely limited to deeper soils (>8 cm) while rates of SR were generally greater in surface soils (Fig. 2). SO_4^{2-} concentrations below about 10 cm remained relatively low in the salt-water amended cores, due to consumption of SO_4^{2-} via SR in surface soils and the slow diffusion of SO_4^{2-} at depth (see also Cl^- profiles; Fig. 4). The diffusion of Cl^- deeper into the soils relative to SO_4^{2-} may have desorbed organic matter and stimulated AMG at depth below the zone of active SR. However, rates of AMG were highest in the mid-depth soils, and there was substantial overlap in the zones of active SR and MG (Fig. 2). In the salt-water-impacted soils, there was actually a very weak but statistically significant positive relationship between AMG and SR [$AM = 0.91 \times SR$, $R^2 = 0.05$, $p = 0.04$]. Salt-water intrusion therefore stimulated both SR and MG

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(Fig. 5), and the apparent mechanism is enhanced availability of SO_4^{2-} (for SR) and organic matter (for both processes). While SR and MG often compete for substrates, contemporaneous SR and MG can occur when noncompetitive substrates are available (such as methanol and methylamines, which are not available to sulfate reducers; Oremland and Polcin 1982), when organic substrates are in abundance (e.g., Yoda et al. 1987) or due to fine scale heterogeneity in the distribution of electron acceptors and electron donors (Højberg et al. 1994). The increased CH_4 flux from soils experiencing salt-water intrusion was unexpected and conflicts with measurements along estuarine salinity gradients (e.g., Bartlett et al. 1987) and with prior experimental results using tidal freshwater river sediments (Weston et al. 2006). Further work is needed to determine the mechanism leading to enhanced CH_4 emissions following salt-water intrusion.

Implications for TFMs

Marsh accretion, which is necessary if marshes are to keep pace with rising sea levels, occurs through the accumulation of both organic matter and mineral sediments (Reed 1995; Morris et al. 2002). Across a diversity of TFMs, the accumulation of organic matter from both autochthonous and allochthonous sources contributes an average of 62% to vertical marsh growth (Neubauer 2008). Based on the loss of 44.1 mol soil C m^{-2} over 1 year due to salt-water intrusion (Fig. 5), we estimate that the increased rate of decomposition will lead to the loss of 5.8 mm of marsh elevation (assuming the % organic matter is twice the % organic C and a volumetric leverage of 5.5 cm^3 g^{-1} for organic matter in TFM soils; Neubauer 2008). For Delaware River TFMs, which have vertical accretion rates averaging 10 mm yr^{-1} (based on ^{137}Cs, ^{210}Pb, and pollen horizons; Orson et al. 1992; Church et al. 2006) and are exposed to a relative sea level rise rate of about 4 mm yr^{-1}, the loss of 5.8 mm of soil elevation is the difference between a site that is accreting considerably faster than sea level is rising and one that is growing at roughly the rate of today's sea level rise. While it is likely that the response of soil C mineralization to salt water intrusion will moderate after long-term exposure (e.g., Fig. 1), decreases in plant production also are likely and may hinder the vertical growth response of TFMs.

The tidal marsh plant community plays a key role in marsh accretion by supplying organic matter and by trapping allochthonous sediments and associated organic matter from tidal waters as water velocity slows due to friction within the plant canopy (Reed 1995; Pasternack and Brush 1998). In TFMs, salt-water intrusion associated with sea-level rise will adversely affect plant productivity (Willis and Hester 2004; Spalding and Hester 2007), and declines in plant production will limit the accretion potential of these marshes. Shifts in the dominant marsh macrophyte (from freshwater to salt-tolerant species) may play an important role in determining the fate of TFMs experiencing salt-water intrusion, and the rate of both sea-level rise and salinity increases relative to plant community shifts will likely determine the resilience of these ecosystems to climate change. Declines in plant productivity, coupled with increased organic matter decomposition rates as described here, create a scenario in which organic matter sequestration is severely limited in TFMs following salt-water intrusion. Future work involving experimental mesocosms, field transplants, or in situ manipulations that expose both TFM soils and plants to elevated salinities will be necessary since the overall response of TFMs to climate change and salt-water intrusion will be a complex interaction of the processes that drive plant production, microbial decomposition, sediment deposition and, ultimately, marsh accretion. Our work highlights that salt-water intrusion will increase microbial decomposition rates in TFM soils, can change the importance of metabolic pathways in unexpected ways (e.g., increases in CH_4 emissions), and may put TFMs at risk of permanent inundation as rates of sea level rise continue to accelerate.

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ESTUARY NEWS

NEWSLETTER OF THE PARTNERSHIP FOR THE DELAWARE ESTUARY: A NATIONAL ESTUARY PROGRAM

Partnerships Get Results

By Jennifer Adkins, Executive Director, Partnership for the Delaware Estuary

As you might guess from the name "Partnership for the Delaware Estuary," partnerships are a central part of everything we do. **This issue of *Estuary News* is dedicated to our partners and the great results they've produced.** From our state- and federal-agency partners, to the many nonprofit organizations we partner with across the region and the corporate and funding partners that provide critical resources for our work, none of the successes you'll read about in these pages would have been possible without these strong and effective partnerships.

One of our most successful partnerships has been the Delaware Bay Oyster Restoration Task Force, which has brought oyster populations in Delaware Bay back from the brink through shell planting. Last October, this partnership was recognized by the President of the United States with the Coastal America Partnership Award. In 2010 we will be working hard with partners on the Task Force to secure new resources to sustain the oyster-restoration project, which has run out of funding. Read more about this partnership effort on page 5.

continued on page 2

Credit: Haskin Shellfish Research Laboratory, Rutgers University

The Partnership for the Delaware Estuary and Rutgers University's Haskin Shellfish Research Laboratory team up to install a "living shoreline" along the Maurice River outside Bivalve, New Jersey, in 2008. The success of this and other installations is currently being monitored on a regular basis.

In this issue...

Partnerships continued from page 1

Over the last few years, our partnership with the Haskin Shellfish Research Laboratory (HSRL) of Rutgers University has expanded to more than just oyster restoration. The experts from the HSRL are critical partners in the Delaware Estuary Living Shoreline Initiative, a pilot project making use of their shellfish expertise and local capacity to help us stabilize eroding marshes using ribbed mussels. (See the story on page 6 for details.) They are also our partners for evaluating climate-change impacts on bivalve shellfish, one of three case studies in our pilot project for the Climate Ready Estuaries program. Through the same pilot program we are also working in partnership with the Philadelphia Water Department to assess the vulnerabilities of drinking-water systems, and we are working with The Academy of Natural Sciences in Philadelphia to assess the vulnerabilities of our tidal wetlands. (See the story on page 3 for details.)

In addition to working closely with the U.S. Environmental Protection Agency (EPA) to implement our National Estuary Program responsibilities, we collaborate with this federal agency on a number of specific projects. Over the last two years we've worked closely with the EPA to collect and analyze over 230 samples from the bay as part of our Delaware Estuary Benthic Inventory project, an effort to better understand the conditions and habitats on the bottom of Delaware Bay. Read more about this effort on page 4.

> *"...we are all indeed partners in the protection and enhancement of the Estuary."*

In this issue, you can also read about some exciting results from our new partnership with Cheyney University for freshwater-mussel restoration, on page 8. One of only a handful of historically black universities in our region, Cheyney is our partner for breeding freshwater mussels in the laboratory for eventual restoration to streams. Working together with Cheyney we are building the capacity needed to produce large numbers of baby mussels, while also creating new opportunities for students in a growing field of study and employment.

These are just a few examples of some partnerships that have really begun to pay off in the form of results for the PDE and the Delaware Estuary. But agencies and nonprofit organizations are not our only partners. Through our Corporate Environmental Stewardship Program we have formed partnerships with corporations to do projects that improve habitat and water quality on corporate and community lands. Last year, these ranged from tree plantings to a student symposium. These projects are highlighted on page 12 as examples of ways that corporations can make a difference on their own lands, and in their own communities.

Then of course there are our readers and supporters – some of our most important partners for keeping the Delaware Estuary clean and healthy. Wikipedia.org defines a partnership as an "entity in which partners...share with each other the profits or losses." By this measure, we are all indeed partners in the protection and enhancement of the Estuary. ■

MEETINGS CONTACT LIST

Meetings conducted by the Partnership for the Delaware Estuary's implementation and advisory committees occur on a regular basis and are open to the public. For meeting dates and times, please contact the individuals listed below:

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New Program to Help Assess Marsh Loss

A piece of marshland sits poised to erode away from the outskirts of Bivalve, New Jersey, where the Maurice River flows into Delaware Bay.

Tidal wetlands are a hallmark feature of the Delaware Estuary, forming a marshy fringe that extends from expansive salt marshes around the Delaware Bay to nationally rare freshwater tidal marshes along the urban corridor. Together, these wetlands provide perhaps the most critically important habitats in our watershed, important for flood protection, water quality, and habitat for fish and wildlife.

Despite their importance and signs that marshes may be declining in both extent and health, there has never been a coordinated and consistent assessment of their health over time. The Partnership for the Delaware Estuary (PDE) aims to change that with the Delaware Estuary Wetland Monitoring and Assessment Program (DEWMAP), which is now getting under way thanks to start-up funding from the U.S. Environmental Protection Agency. The DEWMAP will use information gathered from new fixed monitoring stations, remote sensing, and field assessments of wetland areas by PDE staff and other experts to determine and contrast conditions around the Estuary.

The PDE has also begun to work with the Barnegat Bay National Estuary Program in New Jersey to broaden the DEWMAP, and to link it to wetland monitoring initiatives being led by the state of Delaware and the Center for the Inland Bays of Rehoboth Beach, Delaware, for a more comprehensive mid-Atlantic look at tidal wetlands. So look for more on the DEWMAP in future issues of *Estuary News*, or get more information at www.DelawareEstuary.org/Science_Projects_Wetland_Assessment.asp.

Tool Available for Restoration Pros

The Delaware Estuary Watershed comprises a rich mosaic of "natural communities" across a diverse landscape. Natural communities are unique groups of plants and animals that reoccur within specific environmental settings. The plants and animals living in an area are like a unique fingerprint that also serves as a barometer for the environmental health of that area. When natural communities are impaired, the many species that depend upon them for habitat face a similar fate, and the benefits, or "ecological services" they provide are reduced or lost.

The Partnership for the Delaware Estuary has worked with NatureServe and The Nature Conservancy to prepare guides and maps of the natural communities across the Delaware Estuary Watershed. The *Guide to the Natural Communities of the Delaware Estuary* (*Guide*) describes 35 ecosystems and 185 plant-based community types known to occur here. This *Guide*, which is based on the National Vegetation Classification System (NVCS), was created to identify and help protect, preserve, and restore the unique array of species and habitat types that comprise the Delaware Estuary Watershed. It is a tool to help restoration managers design and carry out projects that use the right plants in the right places for healthier, more resilient habitats.

Since 2008, the Partnership for the Delaware Estuary has continued to use *The Guide to the Natural Communities of the Delaware Estuary* to restore local, streamside habitat at the University of Pennsylvania's New Bolton Center in Kennett Square, Pennsylvania.

For more information about the *Guide*, or to download NVCS documents, maps, and Geographic Information System layers, please visit www.DelawareEstuary.org/Science_Programs_NVCS_Downloadables.asp.

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Project Examines 'Communities' Under Water

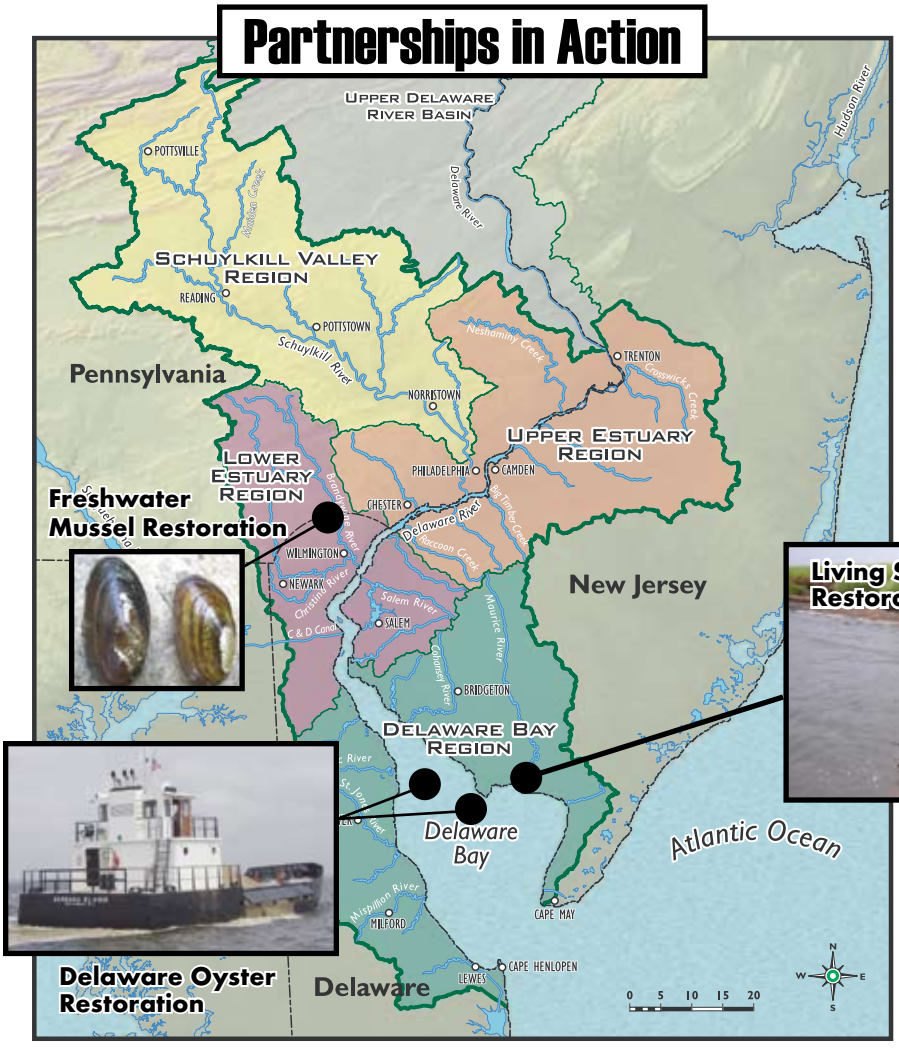
You may not see many animals in the Delaware Bay and River through their muddy waters, but the bottom is home to a plethora of animals and plants. The Delaware Estuary Benthic Inventory (DEBI) aims to identify and map these bottom-dwelling, or "benthic" communities, which play many crucial roles in the estuarine food web, in addition to cleaning the water, providing habitat and food for fish, and protecting shorelines against wave erosion.

In 2008, the Partnership for the Delaware Estuary (PDE) worked with partners to collect bottom samples from over 230 sites, from the mouth of Delaware Bay to as far north as Pennsylvania waters, in the most extensive bottom survey ever conducted here. Working with Dr. Doug Miller from the University of Delaware, the PDE continued this important effort in 2009 with a focus on hard-surface bottoms. Remotely operated vehicles and divers from the U.S. Environmental Protection Agency were used to film the bottom without disturbing it. Grab samplers and an oyster dredge were used to collect over 75 new samples from Lewes, Delaware, to Philadelphia. New discoveries in 2009 included a colony of freshwater mussels found in urban waters near Philadelphia, including a species previously thought to have been wiped out.

Credit: University of Delaware

Bill Hoffman of the U.S. Environmental Protection Agency hauls in a dredge full of sponges from the bottom of Delaware Bay on July 28. Since this discovery, researchers have learned these are *Cliona cellata*, a common sponge that, incidentally, is capable of boring holes into shells.

Partnerships in Action



Samples and data from 2008 and 2009 are being analyzed now, and will help us to create maps showing what animals live where. These will help resource managers identify critical fish habitats, protect sensitive areas when spills occur, target areas for restoration, and better assess environmental health in different areas of the Estuary.

To learn more, please log on to www.DelawareEstuary.org/Science_Projects_Baybottom.asp. ■

Bang for the Buck: Shell Planting in Delaware Bay

By Kathryn Ashton-Alcox, Field Researcher, Rutgers University, Haskin Shellfish Research Laboratory

U.S. Rep. Mike Castle, R-Del., congratulates members of the Delaware Bay Oyster Restoration Task Force during a bayside ceremony on October 4 where the group received a Coastal America Partnership Award, the only environmental award of its kind given by the White House.

How many taxpayer-funded programs can you think of that recycle a waste product, enhance a declining fisheries species, improve the environment, and provide a $40 return on every dollar spent? Not many, right? Well, the Delaware Bay Oyster Restoration Task Force's shell-planting program does all of those things.

Since 2005, the Task Force has been "planting," or strategically placing clam shell (a byproduct of clam processing) on the oyster beds of Delaware and New Jersey in order to enhance the oyster population on the beds. Oysters reproduce by releasing eggs and sperm into the water where fertilization occurs. The larvae then spend two to three weeks as plankton before they sink to the bottom in search of a clean, hard "substrate," or surface on which to cement themselves and continue shell growth, then never moving independently again. Since researchers knew there were larvae in the water, but few were showing up as "spat," or baby oysters, they identified the lack of clean substrate on the oyster beds as the likely reason for low oyster reproduction in Delaware Bay since 2000.

Broken clamshell provides an ideal substrate for these baby oysters when put down just before the larvae are likely to settle. Comparisons have shown that it is not the type of shell that matters to the oyster larvae. What matters is that the shell is clean, or not covered with fouling organisms or other growths, so timing is critical. Where the shell is put is also important. If the area has never supported natural oyster populations, or if it is too soft and muddy, it is likely that shell planting will not result in a successful oyster set. If shell is planted in an area where here are many predators, the spat will not survive either.

Following a successful pilot program conducted by the New Jersey Department of Environmental Protection in 2003, the Task Force formed to develop funding for large-scale shell planting to alleviate the continuing problem of low recruitment on oyster beds in the Delaware Bay. From 2005 to 2008, the Task Force obtained a total of $5 million from the Section 1135 Program of the U.S. Army Corps of Engineers to purchase and plant shell. This money was divided equally between New Jersey and Delaware and funded shell plants that covered 1,044 acres (423 hectares) over four years.

Each year, shell planting resulted in positive gains for the oyster population. Compared to natural shell on the beds (the native substrate), planted shell received up to seven times as many spat on average across all the sites. The contribution to oyster population enhancement provided by the shell plantings was very high compared to the modest proportion of acreage planted. For example, in 2008 only 0.8% of the New Jersey oyster acreage was planted, yet that small area yielded over 20% of the total spat on all the New Jersey beds.

Monitoring of the shell-planting sites shows that the clam shell continues to attract spat in subsequent years, albeit at the same rate as the native substrate. Because oyster shell disappears over time in the Delaware Bay, regular shell plantings are needed to prevent the loss of the oyster beds upon which so many other species depend. A self-imposed tax on the industry provides some funding for shell planting. However, additional funding is needed to plant enough shell to get oyster populations to a level where the system can be self-sustaining.

Projections of marketable bushels of oysters show that the number

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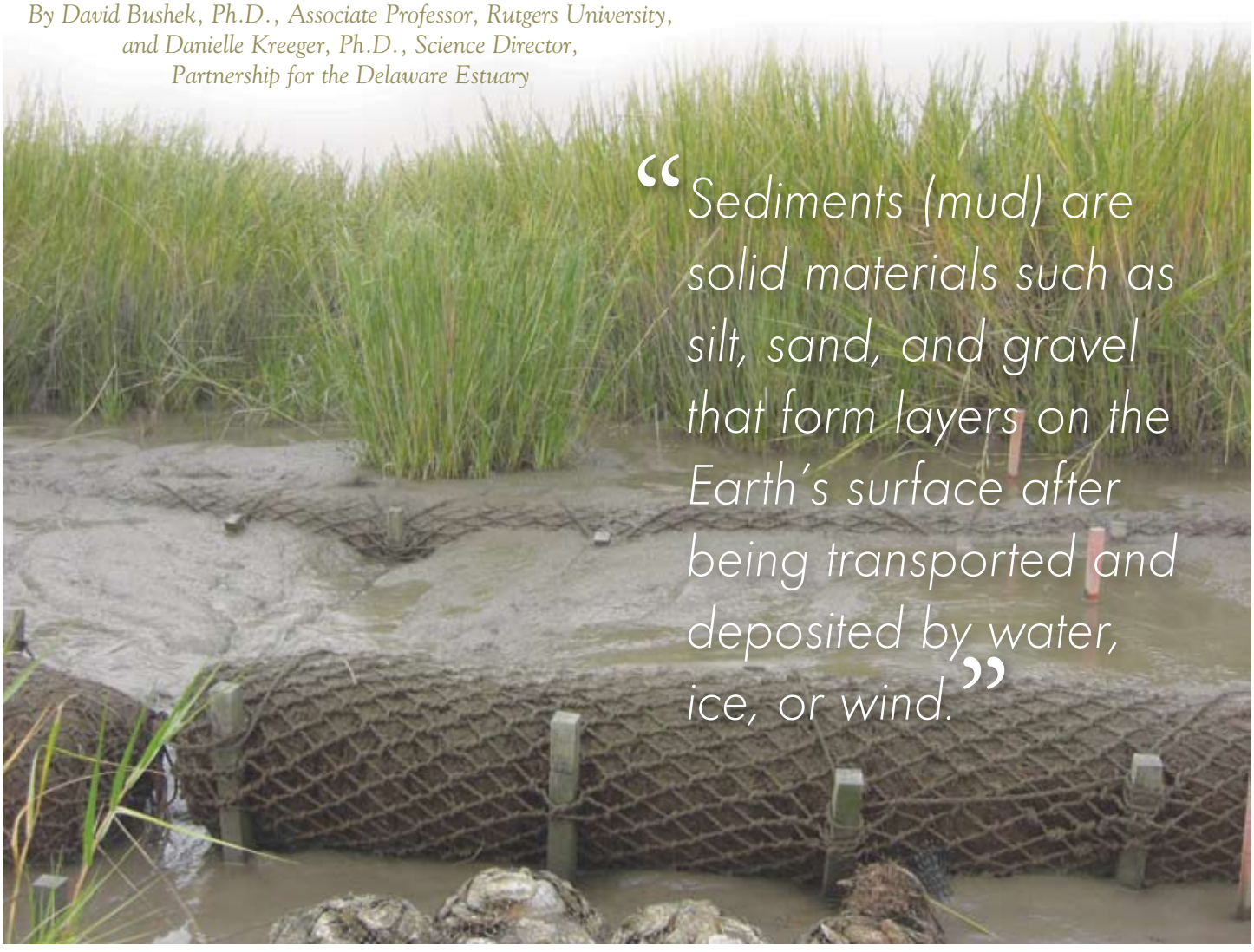
Scientists Flex Mussels to Protect Shorelines

By David Bushek, Ph.D., Associate Professor, Rutgers University, and Danielle Kreeger, Ph.D., Science Director, Partnership for the Delaware Estuary



Credit: Haskin Shellfish Research Laboratory, Rutgers University

Sediments (mud) are trapped by a barrier called a "biolog," which prevents them from eroding into the Maurice River near Bivalve, New Jersey.

With nowhere to move landward because of upland development, what's a salt marsh to do as sea level rises? Over millennia, salt marshes have migrated as sea level has risen and fallen. Salt marshes grow vertically by trapping sediments suspended in each flooding tide. But what happens if sediment availability declines or sea level rises faster than sediments can be trapped? Worse, what happens when a marsh is diked for long periods, depriving it of its daily dose of sediment from the rising and falling tides?

For nearly a century, dikes and other tidal restrictions around Delaware Bay have inadvertently slowed the natural build up of marshes by short-circuiting tidal sediment supply. Many dikes are no longer being maintained either by design or neglect. When a dike eventually fails, the former tidal wetland often finds itself too low to rebuild. Grasses, which previously thrived, struggle to maintain themselves and quickly drown. Excess nutrients, common in many marshes along tributaries, can cause grasses to invest less in belowground root production (peat), making the top-heavy plants more vulnerable to erosion. When salt marshes erode away, adjacent upland areas have no natural barrier against rising waters.

The Delaware Estuary Living Shorelines Initiative (DELSI) aims to slow the erosion of salt-marsh shorelines by taking advantage of a unique relationship between the dominant plant and animal: the salt-mash cordgrass *Spartina alterniflora* and the ribbed mus-

sel *Geukensia demissa*. Cordgrass and ribbed mussels have a *symbiotic*, or mutually beneficial relationship. Roots of the grass provide a habitat to which mussels attach thin, but very strong, byssal threads that hold them in place. Hundreds of threads help pull each mussel down into the mud, safely away from predators. In return, the mussels fertilize the mud with nutrients that are extracted from the plankton they eat as the tides pass. Grasses nourished by the extra nutrients grow denser along the edge which slows water currents, increasing the *sedimentation*, or trapping of suspended particles. The combined active and passive trapping of sediments builds up the marsh edge, forming a strong, natural, self-maintained levee.

By exploiting this mussel-plant relationship, scientists involved in the DELSI hope to protect salt-marsh shorelines around the Delaware Estuary. With support from the National Fish and Wildlife Foundation, New Jersey Sea Grant, New Jersey Department of Environmental Protection, Rutgers University, and the Partnership for the Delaware Estuary, we have been exploring methods to enhance mussel and plant densities at sites of marsh erosion using natural materials such as coconut fibers.

Fibers from the husks of coconuts, an industry byproduct, are spun into biodegradable twine called *coir* that is stitched into 20-foot-long *biologs*. These are installed in a semicircle mimicking the natural shoreline, to connect two points along an eroding marsh edge. Mussels placed into the coir logs readily attach with their strong byssal threads, and plugs of cordgrass salvaged from eroding areas can also be planted directly into the logs. The logs immediately trap sediments within and behind them, increasing the elevation of the marsh surface. As marsh plants and mussels colonize the elevated surface, resilience should increase.

Credit: Haskin Shellfish Research Laboratory, Rutgers University

Ribbed mussels are being examined as a tactic to help prevent salt marshes from eroding into Delaware Bay. By attaching to plant roots using "byssal" threads made of proteins, colonies of mussels may effectively armor the shoreline against waves whipped up by boats, currents, and wind.

Since the first DELSI installations in 2008, we've learned that logs fail in areas with lots of wave action, but that this appears to be a useful and cost-effective tactic at the back of coves, around marinas, and along shorelines where low-to-moderate wave action necessitates protection. We are still experimenting with methodologies and hope to soon establish a demonstration site at the Heislerville Fish and Wildlife Management Area along the Maurice River in Cumberland County, New Jersey. Beginning next year, we will begin to document the use of restored-versus-eroded areas by fish and wildlife.

For more information about the DELSI, please visit our website at www.DelawareEstuary.org/Science_Projects_Living_Shoreline.asp. ■

Bang for the Buck **continued from page 5**

of oysters produced from plantings each year can equal or exceed the total quota for the harvest of oysters, thanks in part to conservative harvest management by both states. This provides an opportunity to expand the industry while retaining a sustainable population. Economic estimates show high returns for each dollar invested in this program. The dockside return for each $1 spent averages $6.70. Using the usual economic multiplier (think "plateside" return) for fisheries products raises the "bang for the buck" number to an impressive $40 returned for every $1 spent! And the ecological return for this program is, of course, priceless. ■

Scientists on Verge of Restoring Native Mussel Species

By Danielle Kreeger, Ph.D., Science Director, Partnership for the Delaware Estuary, and Angela Padeletti, Science Specialist, Partnership for the Delaware Estuary

Freshwater mussels are the most imperiled of all animals and plants in North America, where most of our 300 native species are either extinct or are threatened with extinction. Of the 12-to-14 native species in the Delaware Estuary Watershed, only one is relatively common and most are listed as endangered or vulnerable by the states of Delaware, New Jersey and Pennsylvania. There are many reasons for the declines, including decreased water and habitat quality in our rivers and streams and declines in fish species that are needed for mussels to complete their life cycles. In the past few decades, conservation biologists have been leading the charge to help save our remaining species.

The Partnership for the Delaware Estuary (PDE) is working to raise awareness that populations of even common species of mussels appear to also be in decline. This has important ramifications because of the benefits, or "ecosystem services" that dense beds of mussels provide. Like their marine counterparts (oysters, clams, and marine mussels), each adult freshwater mussel filters up to 10 gallons of water a day. Natural beds of thousands of these animals collectively filter so much water that they improve water quality by removing nutrients and increasing light for bottom plants. Mussel beds also furnish habitat for fish and other organisms while stabilizing the bottom and helping to counteract the effects of polluted runoff.

As part of a watershed- and shellfish-based restora-

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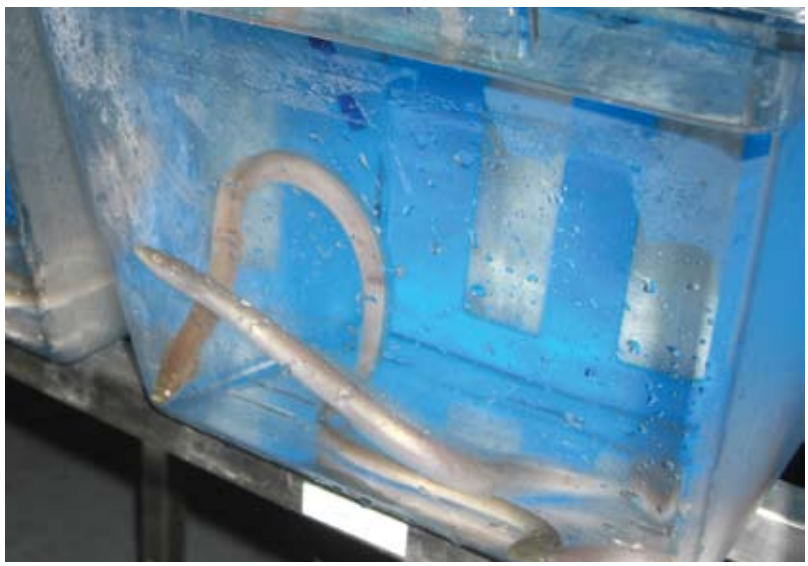
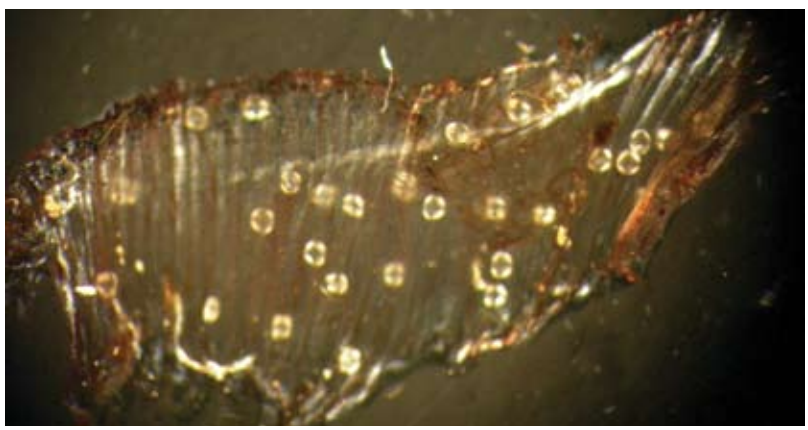
This freshwater mussel weighs just a few ounces, yet it can filter up to 10 gallons of water per day. Scientists at the Partnership for the Delaware Estuary are seeking to restore these shellfish to the Delaware River's tributaries in the hopes that, collectively, they will perform the same function as water-treatment plants.



American eels wait in aquariums inside a Cheyney University laboratory, where the Partnership for the Delaware Estuary is attempting to use them as hosts that will carry baby freshwater mussels up rivers so they can detach and grow.



Credit: Cheney University

Seen here under a microscope, baby mussels (tiny circles) hitch a ride aboard their host; in this case, a piece of gill from a fish. Later, when this fish swims up river, these babies will detach and find a new home on the bottom of a stream.

Researchers Seeking to Tame Oyster Disease in Delaware Bay

By Eileen Hofmann, Ph.D., Professor of Oceanography, Old Dominion University, Center for Coastal Physical Oceanography

Delaware Bay oyster (*Crassostrea virginica*) populations are influenced by two lethal parasites, *Perkinsus marinus* and *Haplosporidium nelsoni*, which cause Dermo and MSX (Multinucleated Sphere Unknown) diseases in oysters, respectively. The diseases do not affect humans, but they do affect oyster populations. Both diseases respond to environmental conditions, typically becoming more severe as temperature and salinity (salt level) rise.

Credit: Haskin Shellfish Research Laboratory, Rutgers University

Graduate student, Emily Scarpa (left), of Rutgers-Camden, and Jenny Paterno (right), a Stockton College intern, harvest eastern oysters from Delaware Bay using a dredge in June of 2009.

Delaware Bay oyster populations have battled MSX disease since 1957 and Dermo disease since 1990. Both diseases typically retreat to higher salinity in the lower part of the Bay following spring floods. But after a year of unusually low flows in the Delaware River from August 1984 to August 1985, MSX intensified in the upper Bay and killed 70 to 75% of the oysters. MSX disease prevalence fell dramatically after this drought and has never regained its preeminence in population control, suggesting that the oysters that repopulated the Bay after 1986 were dominated by MSX disease-resistant individuals. Yet MSX is still present because oysters with no history of MSX disease exposure quickly become heavily infected and die when exposed in the Bay. Such a system-wide population response has not been observed in other estuaries.

As part of the National Science Foundation Ecology of Infectious Diseases (EID) initiative, we have developed a program to understand how parasites and their hosts interact in dynamic estuarine systems like Delaware Bay, and how these interactions might be modified by climate change. We combined expertise in shellfish disease, genetics, and modeling in a collaborative effort to investigate: 1) the timeline of natural selection to establish disease resistance; 2) the role of disease refugia (disease-free areas within a habitat) in the adaptation of the genetic structure of a population; 3) the relationship between range contraction of a species and disease resistance in preventing local extinction of oysters; and 4) the effects of a warming climate on oyster lifespan, oyster reproduction, parasite transmission, and the consequences of shifts in the genetic structure of oysters.

Our EID group has undertaken field and laboratory studies focused on oyster genetics and disease dynamics designed to determine: 1) if suspected disease refuges harbor susceptible oyster populations and the mechanisms that create and maintain them; 2) if disease-resistant genes exist and disproportionally affect oyster diversity; and 3) if the number of parents that successfully produce offspring vary in space and time. The laboratory and field studies have identified genes related to

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Native Mussel Species

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tion strategy, the PDE is advocating for the restoration of native freshwater-mussel species and populations. To fully recover these important animals, we will need healthy riverside corridors, suitable water quantity and quality, and native fish hosts that pass freely up and down the rivers.

The good news is that once mussel communities begin to be reestablished, they will help do the work for us by improving water quality and enriching the habitat. For this reason, they are one of the few animals that are labeled "ecosystem engineers," because like oyster reefs, they build and maintain their own habitat that benefits other species.

In 2007, the PDE launched the Freshwater Mussel Recovery Program in collaboration with Cheyney University, The Academy of Natural Sciences, the U.S. Fish and Wildlife Service, and many others. New tactics were devised to decide which streams to target for mussel restoration based on their suitability for sustaining mussels. Hatchery techniques were developed using the latest science and focusing first on a common mussel species that has become impaired and patchy in distribution. Unlike oysters and other marine species, which have spawned and grown in the hatchery for over 100 years, only recently have scientists learned how to successfully produce baby freshwater mussels in hatcheries. Freshwater mussels have a complicated life history whereby a specific size and species of fish is needed as a host for the mussel's larval phase.

We are delighted to report that in 2009, we produced baby mussels and reared them through the crucial early-life stages at our Cheyney-based hatchery. This success was thanks in large part to scientists from The Academy of Natural Sciences and U.S. Geological Survey, who collected and supplied appropriate fish hosts, and funding from ConocoPhillips and the National Fish and Wildlife Foundation. We hope to repeat this success in 2010 and beyond, but with larger numbers so that we can rear juvenile mussels until they are ready for transplanting into selected streams, where no mussels have lived for quite some time. Eventually, we hope to expand this program to include other species to begin to rebuild the native-mussel population that once thrived across the Delaware Estuary's watershed.

For more information about this initiative, please visit www.DelawareEstuary.org/Science_Projects_Mussel_Restoration.asp. ■

Oyster Disease

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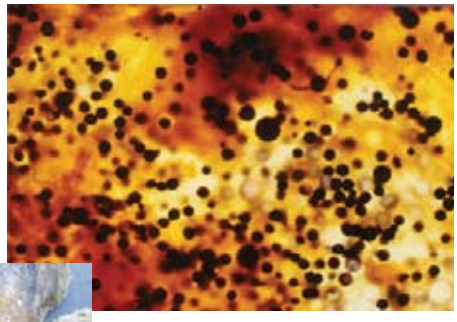
MSX and Dermo disease resistance, potential disease refugia and the mechanisms that allow them to exist, the differences among oysters from suspected refugia and high-disease areas, and the effect of space and time on the size of spawning populations.

We are integrating and extending the laboratory and field results using numerical models that include explicit genetic structure, disease processes, and post-settlement, oyster-population changes. These biological models are being coupled with a Delaware Bay circulation model to test scenarios of disease transmission, "larval," or baby-oyster transport, and current and future climate conditions on oyster diversity. Numerical particle-tracking experiments using the simulated circulation fields are providing potential transport pathways of oyster larvae and free-living disease pathogens, illustrating the importance of freshwater discharge rates and wind in determining these transport pathways, and highlighting the importance of oyster-larvae behavior in determining retention and final settling region.



Seen here under a microscope, oyster meat shows one sign of *Perkinsus marinus*, or Dermo disease: black spots. While proven to have no effect on humans, Dermo disease can be fatal to oysters.

Credit: Haskin Shellfish Research Laboratory, Rutgers University

Eastern oysters, or *Crassostrea virginica*

The findings noted above are promising. They clearly illustrate the need for multidisciplinary research to provide improved understanding of oyster disease dynamics in Delaware Bay. By extending field and laboratory findings with numerical modeling, our EID project provides an example of the type of research program necessary to allow for the development of a strategy; a strategy that will project the effects of a warming climate on Delaware Bay oyster populations in the coming decades. This knowledge will then inform management strategies to help protect the valuable resources of the Delaware Bay.

To learn more, please call Dr. Eileen Hoffman at (757) 683-5334. Hoffman is a member of the Delaware Bay EID Group, which includes scientists from Old Dominion University's Center for Coastal Physical Oceanography, Rutgers University's Haskin Shellfish Research Laboratory, Rutgers University's Institute of Marine and Coastal Sciences, and the University of Southern California's Department of Biological Sciences. ■

CLIMATE CHANGE UNDER THE MICROSCOPE

Credit: James Quinn of Villanova University

Villanova University student Tatjana Prša monitors soil samples that she transplanted from the Rancocas Creek in Burlington County to Stow Creek in Cumberland County during April of 2008. Prša won the Best Poster Award for this research at the Delaware Estuary Science and Environmental Summit in January of 2009.

How Will Sea-level Rise Impact Microbes in Delaware River Marsh Soils?

By Tatjana Prša, Graduate Student, Villanova University

Coastal wetlands are one of the most productive ecosystems on Earth. In addition to providing rich habitats for birds, fish, shellfish and other wildlife, wetlands also render important benefits, or "ecological services," to human populations by buffering storm surges during coastal storms, trapping pollutants, improving water quality and providing livelihoods for coastal residents.

These ecosystems, however, are the first to be impacted by sea-level rise, one of the major consequences of ongoing climate change. The marshes along the mid-Atlantic region, including the Delaware River and Bay, are already experiencing sea-level rise at an estimated rate of three-to-four millimeters per year. Along with the increased flooding of wetlands, sea-level rise also causes the movement of salt water further up the estuary into freshwater wetlands. The intrusion of salt water threatens freshwater drinking intakes for the City of Philadelphia, as well as other intakes for agriculture and drinking water on the Delaware River. Plants adapted to freshwater conditions may not be able to withstand higher salinity and they may eventually disappear and be replaced by other plants more tolerant of salt water.

Another impact of rising sea levels that is less obvious to the naked eye is the response of the communities of microbial organisms that live in marsh soils. These microbes are important for the overall functioning of marsh ecosystems. They are responsible for decomposition, a process in which organic matter (produced by the plants) is broken down into simpler organic molecules and gases. In marshes, the balance between rates of decomposition and accumulation of organic matter drive marsh stability: accumulation of organic matter must exceed decomposition so that

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An employee of Logan Generating Company in Logan Township, New Jersey, uses a skid loader on October 22 to transport native trees to a site on the power station's 31-acre property where they will be planted as part of an ongoing restoration project.

Corporations Help the PDE Redefine Habitat Restoration

By Laura Whalen, Restoration Specialist, Partnership for the Delaware Estuary

In habitat restoration, the question often arises: "What does restoration really mean?" The dictionary definition states that habitat restoration is "the return of a habitat to its original community structure, natural complement of species and natural functions." But this definition is changing as we learn what it really means to improve and restore habitat.

For many sites in the Delaware Estuary's watershed today, a more realistic goal for restoration efforts might be to maximize the structure, function, and benefits, or "ecosystem services" of the habitat as conditions in the Estuary change, due to climate, land-use, or other long-term changes. In other words, we need to restore for the future and not just try to return habitat to its original state, which may not be feasible or sustainable. In this way, members of the Partnership for the Delaware Estuary's (PDE) Corporate Environmental Stewardship Program (CESP) are restoring habitat in the Delaware Estuary's watershed smartly, with the PDE's help, and making their efforts count.

In 2009, the PDE worked with six CESP members to implement on-the-ground projects, including habitat enhancements on 14 acres.

Logan Generating Company

For nearly a decade, Logan Generating Company has been a member of the Partnership for the Delaware Estuary's CESP. Logan Generating has made commendable efforts to maintain and conserve its surrounding natural resources by taking an explicit interest in restoring its property. The project area at the Logan Generating Station in Logan Township, New Jersey, consists of approximately 31 acres of fields removed from agricultural production. On July 21, approximately 295 varying native and beneficial plants were planted there to start a series of new habitat-restoration projects that will add to the previously restored property. They also completed a second phase of tree planting

in October where about 200 large trees were planted in the buffer zone along the Delaware River.

Pepco Holdings, Inc

Pepco Holdings, Inc. is currently planning a rain-garden project at their site in Wilmington, Delaware. The rain garden will capture polluted runoff from one of the parking lots on Pepco's property, and the nutrients in the runoff will be absorbed by the native plants in the rain garden instead of leaching into the nearby Christina River. The rain garden site is in an urban location and will provide a good opportunity to educate people walking by about how to manage their own runoff. Pepco's rain garden may be among the first installed in the Delaware Estuary's watershed as part of a new "Rain Gardens for the Bay" campaign that the PDE is working on with the U.S. Environmental Protection Agency.

centocor research & development inc

In 2008, Centocor, Noramco, GBSC and McNeil (all Johnson & Johnson companies) began working with the PDE on a stream-restoration project at the University of Pennsylvania's New Bolton Center in Kennett Square, Pennsylvania. Phase one of the project involved planting native trees, shrubs, and grasses in a 10-foot-wide buffer along a stream that flows through a pasture where animals are allowed to roam. This was needed because nutrient-rich waste was causing bacteria and algae to grow in the tributary, reducing the amount of oxygen available for aquatic plants living downstream.

In July 2009, Centocor worked on the second phase of this project to plant additional trees and improve the buffer on the slope draining to the stream. Over 40 employees planted about 600 plants specific to this region. This buffer will eventually prevent horses and cows from walking into the stream. It will also filter nutrients from rainwater and snowmelt as they wash manure from the pasture into the waterway.

The New Bolton Center site is one of several demonstration plantings by the PDE that utilized *The Guide to the Natural Communities of the Delaware Estuary* (*Guide*) for the selection of plants. (Read an update about this *Guide* on page 3.)

Wheelabrator Gloucester Inc.
A Waste Management Company

Wheelabrator Gloucester is located on 153 acres on the shores of the Delaware River, and the property includes three primary habitat types: grasslands, upland forests and wetlands. As part of the Wildlife at Work program, the six-person Waste Management wildlife team actively manages 30 acres of the site for wildlife habitat enhancement and restoration. Wheelabrator is also continuing their environmental symposium program with a local middle school to educate the students on environmental issues. This year's project is planting a butterfly garden at the Gloucester County plant.

MANNINGTON.

In late October, the New Jersey Audubon Society (NJAS), Mannington Mills, the PDE, students from the Mannington Township School, and volunteers planted 1,150 trees and shrubs for wildlife-habitat improvement on Mannington's corporate property in Salem County. The planting was the second phase of a larger, ongoing habitat-improvement project that the NJAS is leading to increase the amount of quality wildlife habitat in Important Bird Areas in southern New Jersey.

"We think that one part of being a good neighbor is looking to improve the local ecosystems in our locations," said Dave Kitts, vice president - environment with Mannington Mills. "New Jersey Audubon has been working with numerous landowners in the area and we wanted to do our part to help keep local natural systems functioning properly and local wildlife healthy."

"This project is unique since it is a streamside restoration project and a habitat project that will provide both water quality and habitat benefits," said Beth Ciuzio, NJAS stewardship project director for southern New Jersey. Ciuzio is hoping to provide habitat for a group of birds that she says have been rapidly declining; birds that use abandoned farmland and shrubby areas. "What we've done is create scrub-shrub habitat, which is disappearing from the New Jersey landscape," she said. "The work done today will benefit bird species such as the blue-winged warbler, prairie warbler, field sparrow and brown thrasher."

ConocoPhillips

In addition to being a member of the CESP, ConocoPhillips has provided extra funding to support the Freshwater Mussel Recovery Program. This generous support has been instrumental to the success of the PDE's efforts to reproduce and restore native mussel populations in the Delaware Estuary, as detailed in the story on page 8.

Last September, the Freshwater Mussel Recovery Program was the focus of a CESP-member Eco-Excursion hosted by the PDE to showcase one example of a "Regional Restoration" project. CESP members toured the aquaculture labs at Cheyney University and learned about the PDE's efforts to promote the recovery of native freshwater mussels as part of a holistic initiative to restore shellfish populations and their benefits to the Delaware Estuary Watershed, from headwater streams to the coast.

After the tour, the group then went on a canoeing trip on the Brandywine River to see native mussels and other wildlife in their

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Corporations Help the PDE

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habitat for a fun-filled day on the Brandywine River in Pennsylvania.

Corporate environmental stewards combine assistance from the PDE with corporate funds and manpower to make tangible, environmental improvements in Delaware Estuary Watershed communities. For more information or to join the Corporate Environmental Stewardship Program, please contact Karen Johnson at (800) 445-4935, extension 101, or KJohnson@DelawareEstuary.org. ■

Members of the CESP are treated to a tour of the Partnership for the Delaware Estuary's Freshwater Mussel Recovery Program at Cheyney University on September 25, during the group's annual Eco-Excursion.

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Climate Change Under the Microscope

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marshes can grow vertically, keep pace with sea-level rise, and avoid permanent flooding.

In freshwater marshes, the microbes in charge of decomposition are predominantly methanogenic bacteria (as their name suggests, they convert organic matter largely into gaseous methane and carbon dioxide). In saltwater environments, the dominant microbial organisms are sulfate-reducing bacteria, which use sulfate (abundant in salt water) to decompose organic matter by creating carbon dioxide and gas hydrogen sulfide (this gas gives a distinct smell to saltwater marshes that resembles rotten eggs). So how does the community of microbes in freshwater marshes respond to saltwater intrusion, and what effect do changes in the microbial community have on rates of decomposition?

As part of the larger, long-term study conducted by Drs. Melanie Vile and Nathaniel Weston, where saltwater intrusion was simulated by transplanting marsh soils from a freshwater marsh (along Rancocas Creek) to a salt-marsh site (Stow Creek) in the Delaware River Estuary, I focused on the response of the sulfate-reducing bacteria. I measured rates of sulfate reduction and I used molecular techniques to target a specific gene (*dissimilatory sulfite reductase*; *dsr*) found only in sulfate-reducing bacteria to determine how the sulfate-reducing microbial community responded to saltwater intrusion.

My study suggested that within three months of saltwater intrusion, sulfate reduction rates increased significantly compared to the freshwater marsh soils. I also observed a shift in the community composition of sulfate-reducing bacteria toward a more diverse community in saltwater soils. The overall rate of decomposition increased as sulfate-reducing bacteria took advantage of the increased sulfate, suggesting that decay of organic matter in freshwater marsh soils and release of carbon dioxide will speed up following saltwater intrusion.

Coupled with other aspects of the study and ongoing field studies, these results paint a troubling picture for freshwater marshes that experience saltwater intrusion in the Delaware River Estuary. Increased decomposition in freshwater marshes may compromise their ability to keep pace with sea-level rise. This raises concerns about how these ecosystems will fare in the future, as the pace of sea-level rise is expected to increase. The response of these ecosystems to climate change will be complex, however, and there is much we do not yet understand about how microbial communities and plants will react to higher inundation and salinity.

This work was funded by the U.S. Environmental Protection Agency, and the Department of Biology at Villanova University. ■

ESTUARY EVENTS

Storm Drain Marking Program

March 12

Philadelphia, PA

The Partnership for the Delaware Estuary needs good Samaritans to protect Philadelphia's urban rivers by gluing signs onto storm drains that warn, "Yo! No Dumping! Drains to River." The deadline for registration is March 12, after which free training and materials will be provided prior to Earth Day. Volunteers do not need to live in Philadelphia to participate. To learn more, please call (800) 445-4935, extension 112. Registration forms and further information can be found online at DelawareEstuary.org.

Art Contest Deadline

March 5

Philadelphia, PA

Teachers, help your students show others how to "Protect Philadelphia's Hidden Streams" using a creative drawing. That or guide them in shooting a short video showcasing what pet waste is "doo-ing" to our water. Participants can win cool prizes and see their work used in an art exhibit or promotional campaign. Winners will also be recognized during a ceremony to be scheduled in close proximity to the 40th anniversary of Earth Day. Please visit DelawareEstuary.org for more insight.

Illustration credit: Nicholas Gambino of CCA Baldi Middle School in Philadelphia

Oyster Seminar

March 8 at 7 p.m.

Bridgeton, NJ

Discover how Delaware Bay's eastern oysters are faring at this installment of Rutgers University's "Jersey Roots, Global Reach" Seminar Series. Dr. Eric Powell from the Haskin Shellfish Research Laboratory will be visiting the Cousteau Center at Bridgeton to discuss how climate change, disease and other factors are affecting this signature species of the bay. Please call (856) 575-5580 for details, or visit http://marine.rutgers.edu for directions. And for more on oysters, see the article on page 5.

Featured on ecoDelaware.com

Great Green Expo

March 20, from 9 a.m. to 5 p.m.

Wilmington, DE

Green your lifestyle on this, the first Saturday of spring, as you browse amongst dozens of exhibitors inside The Chase Center on the Riverfront. Visitors will be treated to demonstrations, organic foods, and speakers such as Steve Thomas, star of Discovery's Planet Green television series *Renovation Nation* and the former host of *This Old House* on PBS. A portion of the proceeds from this event will be donated to the Partnership for the Delaware Estuary. Visit www.GreatGreenExpo.com for details.

Living-shoreline Seminar

April 5 at 7 p.m.

Bridgeton, NJ

See what scientists are doing to prevent pieces of South Jersey from washing away into Delaware Bay. This discussion on "living shorelines" will be given inside the Cousteau Center at Bridgeton by Dr. David Bushek of the Haskin Shellfish Research Laboratory. Log on to http://marine.rutgers.edu for directions, or call (856) 575-5580 for more insight into this and future installments of the "Jersey Roots, Global Reach" Seminar Series. Further explanation can also be found on page 7 of this newsletter. ■

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Partnership for the Delaware Estuary: a National Estuary Program

The Partnership for the Delaware Estuary, Inc., is a private, non-profit organization established in 1996. The Partnership leads collaborative and creative efforts to protect and enhance the Delaware Estuary and its tributaries for current and future generations. The Partnership is one of 28 National Estuary Programs. To find out how you can become one of our partners, call the Partnership at 1-800-445-4935 or visit our website at www.DelawareEstuary.org.

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