CONNECTICUT SEA GRANT PROJECT REPORT

Please complete this progress or final report form and return by the date indicated in the emailed progress report request from the Connecticut Sea Grant College Program. Fill in the requested information using your word processor (i.e., Microsoft Word), and e-mail the completed form to Syma Ebbin (syma.ebbin@uconn.edu), Research Coordinator, Connecticut Sea Grant College Program. Do NOT mail or fax hard copies. Please try to address the specific sections below. If applicable, you can attach files of electronic publications when you return the form. If you have questions, please call Syma Ebbin at (860) 405-9278.

Please fill out all of the following that apply to your specific research or development project. Pay particular attention to goals, accomplishments, benefits, impacts and publications, where applicable.

Name of Submitter: Beth Lawrence

Date of Report submission: April 30, 2020

Project #: R/CMB-42-CTNY Check one: [ ] Progress Report [X] Final report

Duration (dates) of entire project, including extensions: From [3/1/2017] to [2/28/2020].

Project Title or Topic: How will sea level rise-driven shifts in wetland vegetation alter ecosystem services?

Principal Investigator(s) and Affiliation(s):

1. Beth Lawrence/University of Connecticut/Dept. of Natural Resources & Environment, Center for Environmental Science & Engineering

2. Ashley Helton/University of Connecticut/ Dept. of Natural Resources & Environment, Center for Environmental Science & Engineering

3. Chris Elphick/University of Connecticut/ Dept. of Ecology & Evolutionary Biology, Center of Biological Risk

A. <u>COLLABORATORS AND PARTNERS</u>: *(List any additional organizations or partners involved in the project.)*

- Kimberly Williams, Smithtown High School
- Cadence Cambrial, North Haven High School
- Natural Resources Conservation Academy
- Roger Wolfe, CT DEEP

B. PROJECT GOALS AND OBJECTIVES:

Our overarching objectives are to quantify carbon (C) and nitrogen (N) cycling services in Long Island Sound (LIS) tidal marshes, project how those services will change under sea-level rise (SLR) scenarios, and develop educational materials to better communicate these changes and their implications to high school students. Specifically, the original objectives of the project were to:

1. Quantify carbon- and nitrogen-based services provided by dominant coastal marsh plant species.
2. Forecast how shifts in dominant marsh species will alter ecosystem service provision of LIS coastal wetlands.
3. Promote understanding of the complex interactions among climate change, SLR, coastal wetlands, and ecosystem services among diverse audiences in the LIS region.

C. <u>PROGRESS</u>: (*Summarize progress relative to project goals and objectives. Highlight outstanding accomplishments, outreach and education efforts; describe problems encountered and explain any delays.)*

We were given a one-year no cost extension in order to meet the project objectives. We finished collection of empirical data via a field survey and marsh organ experiment related to Objective 1, developed ecosystem service maps (Obj. 2), developed the climate change outreach module with regional high school teachers (Obj. 3), and have submitted two manuscripts (four additional manuscripts in prep) for publication in the peer reviewed literature (Obj. 3).

<u>Year 1 (3/1/2017-2/28/2018)</u>: We developed and received EPA approval for our QAPP in April 2017. Two MS-level graduate students began working on the project during summer 2017 and were integral to the site selection process. We received permission to sample from candidate sites, and during August 2017, we began our coastal wetland field campaign to investigate the role of tidal restoration and vegetation zonation on carbon and nitrogen-based ecosystem services. We sampled a total of 20 sites (10 restored, 10 unrestored) for a range of biological (% plant cover, above- and below-ground biomass, microbial community composition), soil physical and chemical parameters (pH, EC, SO_4^-, Cl^-, NO_3^-, NH_4^+, %OM, total C and N), and microbial process rates (denitrification, substrate induced respiration, carbon mineralization).

Given time and logistical constraints, we were unable to sample the total number of sites that we had intended to sample; we had proposed to sample 30 sites (10 unrestored sites, 10 tidally restored, 10 *Phragmites*-herbicide sites), but only sampled 20 (10 unrestored, 10 tidally restored). The *Phragmites* management sites were typically brackish marshes (more inland) where tidal flow restoration was not an option, and did not have all three plant species of interest (*Spartina alterniflora, Spartina patens*, and *Phragmites*). The on-the-ground reality of the marshes did not conform to our proposed experimental design. We considered sampling the *Phragmites* management sites differently, by comparing herbicide-managed areas with *Phragmites*-dominated areas and native-dominated areas within each site. However, this experimental design addresses a different question than the one we proposed; thus, given time constraints, we were unable to pursue it.

Year 2 (3/1/2018-2/28/2019): We conducted a marsh organ experiment at Barn Island WMA (Stonington, CT) during the 2018 growing season to test the interactive effects of sea-level rise and plant species composition. Manipulating the elevation of the marsh by installing PVC pipes of different heights allowed us to examine how different flooding frequencies altered plant biomass allocation patterns, as well as the suite of soil physical and chemical parameters measured during Year 1 of the project. Our findings indicate that carbon-based microbial processes were more greatly affected by plant treatment than by SLR treatments, highlighting the importance of plant-mediated ecosystem services.

We made significant progress on Objective 3 in Year 2. We presented our research findings to a wide variety of audiences during ten oral presentations in 2018 (see list below). MS students Aidan Barry and Sean Ooi served as "community partners" for the Natural Resources Conservation Academy's Conservation Ambassador Program, mentoring a high school student on a salt marsh ecology project. Additionally, we had a kickoff workshop with partner teachers in January 2019 to develop plans for the interactive climate change module; we identified learning objectives, outlined module components, created a time line and assigned tasks.

Year 3 (3/1/2019- 2/28/2020): Both graduate students associated with the project successfully defended their MS theses in June 2019. Additionally, two undergraduate research students associated with the project (Kayleigh Granville, Alaina Bisson) completed honors theses that are associated with project objectives. We are in the process of finalizing six manuscripts for publication in the peer-reviewed literature; we recently submitted two manuscripts (Barry et al., Donato et al. are appended) and we are actively working on four additional manuscripts that we intend to submit in the coming six months. We worked with regional high school teachers to finalize our interactive climate change module and began publicly disseminating the resource in January 2020 (see below).

D. PROJECT PUBLICATIONS, PRODUCTS, PRESENTATIONS AND PATENTS:
(Include published materials with complete references, as well as those which have been submitted but not yet published and those in press. Please attach electronic versions of any journal articles, reports, and abstracts not previously provided.)
[%]product is appended to this document; [**]undergraduate student, [*]graduate student

Journal Articles (List URLs):

- [%]Barry, A., Ooi, S., Elphick, C., Helton, A., Stevens, B. and B. Lawrence. Vegetation zonation drives salt marsh soil carbon mineralization and microbial communities. Submitted to Ecosystems on April 29, 2020.
- [%][**]Donato, M., [*]Johnson, O., Steven, B., Lawrence, BA. Nitrogen enrichment stimulates wetland plant responses whereas salt amendments alter sediment microbial communities and biogeochemical responses. Originally submitted to PLOS ONE January 31, 2020; revised version submitted April 23, 2020.

Conference Papers: NA

Proceedings or book chapters: NA

Web sites, Software, etc.:

- %Cambrial, C, Lawrence, B., Williams, K. 2020. Salt marsh-climate change teaching module: Impacts of climate change on Long Island Sound marshes: https://climate.uconn.edu/wp-content/uploads/sites/126/2020/01/Salt-marsh_Climate-change_module_final.pdf.

Technical Reports/Other Publications: NA

Other Products (including popular articles):

- "Connecticut's Marshes: Past, Present, and Uncertain Future." UConn Today article, available at: https://today.uconn.edu/2018/11/connecticuts-marshes-past-present-uncertain-future/
- "Scientists investigate effects of sea level rise on coastal wetlands." Naturally@UConn article (College of Agriculture, Health and Natural Resources), available at: https://naturally.uconn.edu/2017/07/04/scientists-investigate-effects-of-sea-level-rise-on-coastal-wetlands/

Publications planned / in progress:

- Ooi, S., Barry, A., Elphick, C., Lawrence, B., and A. Helton. *In prep.* Potential denitrification rates vary with dominant vegetation zones in southern New England coastal salt marshes. Target journal: Ecological Applications
- Granville, K, Ooi, S., Koenig, L., Lawrence, B., Elphick, C., Helton, A. *In prep.* Seasonal patterns of denitrification and N20 production in salt marshes. Target journal: Wetlands
- Barry, A., Ooi, S., Elphick, C., Helton, A., Stevens, B. and B. Lawrence. *In prep.* Plant-mediated carbon turnover overrides effects of sea level rise in a salt marsh field experiment. Target journal: Estuaries and Coasts
- Bisson, A., Barry, A., Meadows-McDonnell, Elphick, C., A. Helton, Lawrence, B. *In prep.* Impacts of salt marsh vegetation and sea level rise on soil carbon stability. Target journal: Plant and Soil.

Patents: *(List those awarded or pending as a result of this project.)*: NA

Presentations and Posters: *(Include name and date of the conference or meeting, whether it was a talk or poster, if it was invited, and who the presenter was.*):
NOTE: We had several planned presentations this spring at the Connecticut Conference on Natural Resources as well as the Society for Wetland Scientists meeting in Montreal, Quebec that were cancelled due to the COVID-19 pandemic. **indicates graduate student, **undergraduate student*

1. Lawrence, B. (presenter), Helton, A, Elphick, C., *Ooi, S., *Barry, A. How do vegetation shifts alter carbon and nitrogen based ecosystem services in southern New England salt

marshes? Coastal Estuarine Research Federation (invited talk); Advances in understanding sea level rise and coastal landscape change (Symposium). November 4, 2019. Mobile, AL.

2. *Barry, A. (presenter), *Ooi, S., Helton, A., Elphick, C, Steven, B., Lawrence, B. Plants drive carbon turnover under sea-level rise. May 30, 2019. Society for Wetlands Scientists Annual meeting (talk). May 30, 2019. Baltimore, Maryland.
3. *Ooi, S. (presenter), *Barry, A., **Granville, K., Lawrence, B., Elphick, C., Helton, A. Using vegetation zones to predict salt marsh denitrification. Society for Wetlands Scientists Annual meeting (talk). May 30, 2019. Baltimore, Maryland.
4. **Bisson, A. (presenter), Lawrence, B. Impacts of salt marsh vegetation and sea-level rise on soil carbon stability (poster). Society for Wetland Scientists Annual Meeting. May 30, 2019.
5. **Liu, F. (presenter), Helton, A., Elphick, C, Lawrence, B. How does sea level rise alter salt marsh plant biomass allocation and nitrogen content? UConn Frontiers in Undergraduate Research (poster). April 11, 2019, Storrs, CT
6. **Bisson, A. (presenter), Lawrence, B. Impacts of salt marsh vegetation and sea-level rise on soil carbon stability. UConn Frontiers in Undergraduate Research (poster). April 11, 2019, Storrs, CT.
7. **Granville, K. (presenter), *Ooi, S., Lawrence, B, Elphick, C., Helton, A. Seasonal patterns of denitrification in salt marshes. UConn Frontiers in Undergraduate Research (poster). April 11, 2019, Storrs, CT.
8. *Barry, A. (presenter), *Ooi, S., Helton, A., Elphick, C, Steven, B., Lawrence, B. Plants drive carbon turnover under sea-level rise. Connecticut Conference on Natural Resources (talk). March 2019. Storrs, Connecticut.
9. **Bisson, A. (presenter), Lawrence, B. Impacts of salt marsh vegetation and sea level rise on soil carbon stability. Connecticut Conference on Natural Resources (talk). March 2019. Storrs, Connecticut.
10. **Granville, K. (presenter), *Ooi, S., Lawrence, B, Elphick, C., Helton, A. Seasonal patterns of denitrification in salt marshes. Connecticut Conference on Natural Resources (talk). March 2019. Storrs, Connecticut.
11. *Ooi, S. (presenter), *Barry, A., **Granville, K., Lawrence, B., Elphick, C., Helton, A. Using vegetation zones to predict salt marsh denitrification. Connecticut Conference on Natural Resources (talk). March 2019. Storrs, Connecticut.
12. *Barry, A. (presenter), Ooi, S., Helton, A., Elphick, C, Steven, B., Lawrence, B. Plants drive carbon turnover under sea-level rise. Long Island Sound Study Research Conference (talk). March 2019. Port Jefferson, New York.
13. *Ooi, S. (presenter), *Barry, A., **Granville, K., Lawrence, B., Elphick, C., Helton, A. Using vegetation zones to predict salt marsh denitrification. Long Island Sound Study Research Conference (talk). March 2019. Port Jefferson, New York.
14. Lawrence, B. (presenter), Helton, A, Elphick, C. How will sea-level rise driven shifts in wetland vegetation alter carbon and nitrogen based ecosystem services? Long Island Sound Study, Science Technical Advisory Committee meeting (invited talk). November 16, 2018, Groton, CT.
15. Lawrence, B. (presenter). Marsh madness: invasive macrophytes and ecosystem service tradeoffs during wetland restoration. Carey Institute of Ecosystem Studies Fall Seminar Series (invited talk). November 2, 2018, Millbrook, NY

16. *Barry, A (presenter), *Ooi, S., Elphick, C., Helton, A. Steven, B., Lawrence, B. Salt marsh vegetation influence on carbon-based services and microbial communities. Connecticut Symbiosis Symposium (invited talk). October 2018. Connecticut Agricultural Experiment Station, New Haven, Connecticut
17. *Ooi, S, Barry A (co-presenters), Steven B, Elphick C, Helton A, Lawrence B. Effects of salt marsh tidal restoration on soil microbial process rates. Society of Ecological Restoration- New England Chapter Meeting (poster). October 2018. New Haven, CT
18. Lawrence, B. (presenter), Helton, A, Elphick, C. How will sea-level rise driven shifts in wetland vegetation alter carbon and nitrogen based ecosystem services? New York-Connecticut Sea Grant & Long Island Sound Study Principal Investigator Forum (invited talk). August 6, 2018, Groton, CT
19. *Barry, A. (presenter), *Ooi, S., Elphick, C., Helton, A. Steven, B., Lawrence, B. Salt marsh vegetation influence on carbon-based services and microbial communities. Society of Wetland Scientists Annual meeting (talk). June 2018. Denver, Colorado.
20. Lawrence, B (presenter). Towards a conceptual framework for understanding tradeoffs in biodiversity and carbon function in coastal wetlands. Society of Wetland Scientists Annual meeting (talk). June 2018. Denver, Colorado.
21. *Ooi, S. (presenter), *Barry, A., Lawrence, B., Elphick, C., Helton, A. Potential denitrification rates vary with salt marsh vegetation zones. Society of Wetland Scientists Annual meeting (talk). June 2018. Denver, Colorado.
22. *Barry, A (presenter), *Ooi, S., Elphick, C., Helton, A. Steven, B., Lawrence, B. Salt marsh vegetation influence on carbon-based services. New England Estuarine Research Society Spring 2018 Meeting (talk). April 27, 2018. Portsmouth, New Hampshire.
23. *Ooi, S. (presenter), *Barry, A., Lawrence, B., Elphick, C., Helton, A. Potential denitrification rates vary with salt marsh vegetation zones. New England Estuarine Research Society Spring 2018 Meeting (talk). April 27, 2018. Portsmouth, New Hampshire.
24. *Barry, A. (presenter), *Ooi, S., Elphick, C., Helton, A. Steven, B. Lawrence, B. Salt marsh vegetation influence on carbon-based services. Connecticut Conference on Natural Resources (talk). March 12, 2018. Storrs, Connecticut.
25. *Ooi, S. (presenter), *Barry, A., Lawrence, B., Elphick, C., Helton, A. Potential denitrification rates vary with salt marsh vegetation zones. Connecticut Conference on Natural Resources (talk). March 12 2018. Storrs, Connecticut.
26. **Donato, M., Lawrence, B. Effects of plant traits and water quality on carbon gas fluxes from freshwater wetlands. Connecticut Association of Wetland Scientists. March 8, 2018.
27. *Ooi, S. (presenter),*Barry, A., Helton, A., Elphick, C, and Lawrence, B. How does shifting wetland vegetation influence nutrient cycling in Connecticut coastal marshes? Joint Natural Resources and Environmental Engineering Graduate Student Symposium (poster). September 2017. University of Connecticut, Storrs, CT.
28. Lawrence, B. (presenter), Helton, A, and Elphick, C. How will sea-level driven shifts in wetland vegetation alter carbon and nitrogen based ecosystem services? Connecticut Institute for Resilience and Climate Adaptation Forum (invited poster). May 2017. University of Connecticut, Storrs, CT.

E. FUNDS LEVERAGED: *(If this Sea Grant funding facilitated the leveraging of additional funding for this or a related project, note the amount and source below.)*

- We received 25% match ($79,457) for this project from Connecticut Institute for Resilience and Climate Adaptation (CIRCA)
- UConn 2018 Summer Undergraduate Research Fellowships received by Kayleigh Granville and Alaina Bisson ($4000 each)
- B. Lawrence received Development Funds ($2,981) from Connecticut Sea Grant. "Translating climate science to high school audiences: developing a regionally relevant climate change module for southern New England." November 30, 2018- September 1, 2019.
- UConn Work-Study program. Undergraduate research assistant processing project-related samples (~8 hours/week x 14 weeks x 2 semesters= ~224 student technician hours x $10/hour = ~$2240). August 2018- May 2019.

F. STUDENTS: ***(****Document the number and type of students supported by this project.)*
*Note: **"Supported"** means supported by Sea Grant through financial or other means, such as Sea Grant federal, match, state and other leveraged funds. "**New"** students are those who **have not** worked on this project previously. "**Continuing"** students are those who **have** worked on this project previously. If a student volunteered time on this project, please use section G, below.*

Total number of **new*** K-12 students who worked with you: 1
Total number of **new** undergraduates who worked with you: 5
Total number of **new** Masters degree candidates who worked with you: 2
Total number of **new** Ph.D. candidates who worked with you: 0

Total number of **continuing**** K-12 students who worked with you: 0
Total number of **continuing** undergraduates who worked with you: 0
Total number of **continuing** Masters degree candidates who worked with you: 0
Total number of **continuing** Ph.D. candidates who worked with you: 0

Total number of volunteer hours: 80

*(Note: *__New__ students are those who have not worked on this project previously.*
***__Continuing__ students are those who have worked on this project previously.)*

In the case of graduate students, please list student names, degree pursued, and thesis or dissertation titles related to this project.

Student Name: Aidan Barry
Degree Sought: MS
Thesis or Dissertation Title: Salt Marsh Vegetation Influence on Carbon-based Services and Microbial Communities
Date of thesis completion: June 2019
Expected date of graduation:

Student Name: Sean Khan Ooi
Degree Sought: MS
Thesis or Dissertation Title: Potential denitrification rates vary with dominant vegetation zones in southern New England coastal salt marshes
Date of thesis completion: June 2019
Expected date of graduation:

G. VOLUNTEER HOURS:
An undergraduate student helped collect and process samples during 2017 (80 hours).

H. PICTORIAL: Please provide high resolution images/photos of personnel at work, in the field or laboratory, equipment being used, field sites, organism(s) of study. Attach images as separate files (do not embed). Include links to websites associated with the research project. Please include proper photo credits and a caption with date, location, names of people, and activity. These images are useful to document your project in future CTSG publications, websites and presentations.

- Lawrence Lab website: https://lawrencelabuconn.weebly.com/projects.html
- Attached photo ("Sean lab"): UConn MS student Sean Ooi quantifies salt marsh denitrification potential in the lab. Date: August, 2017. Photo credit: Beth Lawrence
- Attached photo ("Marsh organ"): Experimental marsh organ to test how flooding frequency and plants alter carbon and nitrogen-based ecosystem services. Date: May 2018. Photo credit: Beth Lawrence
- Attached photo ("Marsh org group"): UConn MS students Aidan Barry and Sean Ooi, with BS students Alaina Bisson and Kayleigh Granville at Barn Island NWR (Stonington, CT) in front of experimental marsh organ.

I. HONORS AND AWARDS: *(List any honors or awards received during the reporting period, for anyone working on the project. This can be for best paper or poster, university awards, etc.*) Specify:

- Beth Lawrence (PI) received the Early Career Teaching Excellence Award from UConn-American Association of University Professors; March 2020
- Sean Khan Ooi received the Graduate Student Excellence in Research and Creativity Award from the College of Agriculture, Health, and Natural Resources, University of Connecticut; March 2019
- Sean Ooi and Aidan Barry (MS students associated with project) received a best poster award at the Society for Ecological Restoration (New England Chapter); October 2018
- Alaina Bisson and Kayleigh Granville (undergraduate students associated with the project, mentored by Beth Lawrence and Ashley Helton, respectively) both received a Summer Undergraduate Research Fellowship ($4000 each) to pursue independent research related to project objectives; summer 2018

- Aidan Barry (MS student) was awarded a research grant ($1000) to support analysis of sediment microbial communities from the Society of Wetland Scientists- New England Chapter; May 2018
- Kayleigh Granville (undergraduate student) was accepted as a UConn "University Scholar," a prestigious undergraduate program at UConn that will allow her to pursue in-depth research related to project objectives; December 2017
- Ashley Helton (co-PI) received UConn's College of Agriculture Health and Natural Resources Kinsmen Teaching Award for excellence in undergraduate teaching and mentoring; April 2017
- Mary Donato and Kayleigh Granville (undergraduate students associated with the project, mentored by Beth Lawrence and Ashley Helton, respectively) received a Connecticut Association of Wetland Scientists Micheal Leflor Award ($1000 each) to pursue independent research related to project objectives; March 2017

FOR FINAL DEVELOPMENT AND RESEARCH GRANT REPORTS, PLEASE COMPLETE THIS SECTION:

J. PROJECT OUTCOMES AND IMPACTS

RELEVANCE OF PROJECT: *(Describe briefly the issue/problem / identified need(s) that led to this work.)*

Coastal marshes fringing the Long Island Sound are dynamic ecosystems positioned at the interface between land and sea, and provide an array of essential ecosystem services to society associated with improved water quality, carbon sequestration, and disturbance regulation. However, these valuable wetlands are increasingly altered by rising seas and invasive species, and have been altered by historical management efforts such as tidal manipulation. Direct effects of altered salinity and hydroperiods have been linked to changes in carbon and nitrogen cycling. Sea level rise and tidal restoration also alters plant species composition, which can affect carbon cycling and nitrogen removal rates. Our research fills a knowledge gap and will improve coastal management by explicitly quantifying the direct (elevated salinity and hydroperiod) and indirect (changes in plant species composition) effects of sea level rise and tidal restoration on carbon and nitrogen cycling.

RESPONSE: *(Describe briefly what key elements were undertaken to address the issue, problem or need, and who is/are the target audience(s) for the work.)*

We used field surveys, experimental manipulations, and modeling to quantify and forecast carbon and nitrogen ecosystem services that could be impacted by ecosystem management and sea level rise in Long Island Sound coastal marshes. We conducted a survey of 20 Connecticut salt marshes (10 tidally restored, 10 unrestricted references) in 2017 to quantify carbon mineralization, denitrification potential, microbial community composition, and a suite of plant and sediment characteristics. To disentangle the effects of vegetation, soils and hydrology on carbon and nitrogen cycling, we set up an manipulative "marsh organ" experiment in 2018 to test how the tidal hydrology of three sea level rise scenarios and plant composition altered carbon and nitrogen processes underlying key ecosystem services. We also used SLAMM models to

forecast vegetation composition under different sea level scenarios and scaled our empirical denitrification data to the Connecticut coast.

The target audiences for our work are land managers, fellow scientists, and students (high school, university undergraduate and graduate students). Our research questions centered on how sea level rise, invasive species, and tidal restoration alter the provision of carbon and nitrogen-based ecosystem services, which are focal issues with high relevance to coastal managers. Our work also strengthens our scientific understanding of the linkages between abiotic (hydroperiod, salinity) and biotic (plant and sediment microbial composition) factors, and the processes (carbon cycling, denitrification) underlying many salt marsh ecosystem services. Finally, our project aimed to improve understanding of the complex interactions among climate change, sea level rise, coastal wetlands, and ecosystem services among diverse audiences. We created curriculum for high school science teachers, mentored a high school student research project, mentored four undergraduate researchers, and two graduate students.

RESULTS: *(Summarize findings and significant achievements in terms of the research and any related education or outreach component; cite benefits, applications, and uses stemming from this project, including those expected in the future. Include qualitative and quantitative results.)*

Vegetation zonation is an important determinant of coastal wetland processes underpinning carbon and nitrogen-based ecosystem services. In our survey of 20 Connecticut salt marshes, we quantified carbon mineralization, potential denitrification, root-zone bacterial 16S rRNA genes, above and belowground biomass and a suite of sediment characteristics (soil pH and specific electrical conductivity, soil moisture, soil organic matter, and soil $SO4^{2-}$, Cl^-, NH_4^+ concentrations). While none of our parameters differed between unrestricted and tidally restored marshes, we observed strong differences among vegetation zones, with vegetation being a top predictor of microbial respiration and potential denitrification rates. Based on sea-level rise model projections, the replacement of *S. patens* by short-form *S. alterniflora* is expected to be widespread across the Connecticut coastline, decreasing statewide potential denitrification from the low-to-high marsh transitional zone by at least 121 kg N/ hr by 2085. Our results suggest that changes in vegetation zones can serve as landscape-scale predictors of the response of denitrification rates to rapid changes occurring in salt marshes.

To determine how sea-level rise (SLR) may impact carbon cycling and nitrogen removal rates among dominant salt marsh vegetation zones, we manipulated marsh elevation and vegetation composition using a marsh organ experiment. We quantified carbon fluxes (net ecosystem exchange, ecosystem respiration, soil carbon mineralization), and potential denitrification rates in response to three SLR-scenarios (present day, ~10-year SLR (+7.5cm), ~20-year SLR (+15cm)) and five vegetation treatments (*Spartina alterniflora*, *Spartina patens, Phragmites australis*, two unvegetated controls). Interestingly, most carbon flux metrics, denitrification rates, and soil parameters (electrical conductivity, soil moisture, SO_4^-, Cl^-, NH_4^+) were not responsive to our SLR treatments. In contrast, our vegetation treatments affected all carbon flux measurements; *S. alterniflora* and *S. patens* had greater CO_2 uptake and respiration rates compared to *P. australis*. Similar to our field survey, carbon mineralization assays indicated that soils associated with *Spartina* spp. emitted more CO_2 than *P. australis*, but potential denitrification did not vary among treatments. As marshes flood more frequently with projected

SLR, marsh vegetation composition is predicted to shift towards more flood-tolerant *Spartina* spp., which may lead to increased carbon turnover rates. While hydrologic conditions and tidal flow may influence the location of marsh vegetation, our findings suggest that plants, more so than incremental flooding, play a critical role in driving carbon cycling within a salt marsh.

Our data were quality assured by our Quality Assurance Officer, Dr. Lauren Koenig; please see appended letters detailing the results of her review of our data sets.

We currently have two project-related manuscripts in review (see appended Barry et al. and Donato et al.) and intend to submit an additional four manuscripts in the coming six months. Note that three of the six of these manuscripts will have undergraduates as lead authors, and the other three will have graduate students as lead authors. We have given at least 28 project related presentations during the project period to a diversity of audiences including management focused outlets and academic conferences. We have also created an interactive climate change teaching module for high school teachers, highlighting ecological responses of salt marshes to rising seas, socio-economic aspects of coastal management, and different approaches to studying climate change in coastal wetlands (see below for more details).

Consider the following as they apply to your research and any related outreach/education.

- What new tools, technologies, methods or information services were developed from this work? Have any been adopted / implemented for use and by whom?
  - We developed a climate change teaching module for high school science teachers that aligns with Next Generation Science Standards and Ocean Literacy Essential Principles. We worked with two regional educators (Candice Cambrial, Kimberly Williams) to develop a five-day interactive module (includes case studies, Mystery Scientist videos, etc.) that focuses on coastal wetlands of the Long Island Sound. The module engages students with regionally relevant examples of how global issues impact our local environment and how scientists study various aspects of climate change. It is publically available here: https://climate.uconn.edu/tools-assistance/teachers/
  - We released this to the public on January 29, 2020 and have disseminated it to our broad network of educators. We are unaware if any teachers have adopted it yet, but it is unlikely given the current Covid-19 pandemic.

- What are the environmental benefits of this work? Have policies been changed? How has conservation (of ecosystems, habitats or species) been improved?
  - We found no difference between tidally restored and unrestricted reference marshes in soil chemistry, plant biomass, soil carbon respiration, potential denitrification rates, or microbial communities, indicating that tidal restoration efforts over the past 40 years in Connecticut have not deviated from reference site levels. However, since tidal restoration and sea level rise change the composition and areal extent of salt marsh vegetation, scaling of empirical estimates to wetland extent would better reflect how tidal restoration alters carbon- and nitrogen-based processes at site and regional scales (Ooi et al. *in prep*). Our research suggests that vegetation could be utilized to do such scaling in southern New England coastal marshes, as we observed soil carbon mineralization and potential

denitrification rates across coastal Connecticut were strongly dependent on the dominant vegetation. Soils associated with *Phragmites australis* had lower rates of carbon mineralization and higher denitrification rates than *Spartina alterniflora* zones, suggesting potential environmental benefits associated with invasive *Phragmites.* While we are unaware of any policy changes based on our work, our findings clearly have management implications that could influence conservation practices, including invasive species management.

- What are the social payoffs of this work? Who has benefited from this work? Have attitudes / behaviors of target audience changed? Elaborate. Have policies been changed?
  - The most likely direct benefactors of this work are the students involved. The project fostered the professional development of five undergraduates and two graduate students. We designed our climate change teaching module to promote easy adoption and expect that it will be implemented by high school teachers throughout the region, which would greatly broaden the impact of our work.

- What are the economic implications / impacts of this work? (Where possible, please quantify.) Have new businesses been created /or existing businesses retained as a result of this research? Have new jobs been created or retained? Are new businesses or jobs anticipated?
  - Managers spend millions of dollars annually to control invasive *Phragmites australis* in the U.S. However, our work suggests that this species has some environmental benefits including enhanced carbon sequestration and nitrogen removal. Thus, while our work has not directly created new jobs or stimulated business activity, it contributes to the growing understanding that there are environmental trade-offs associated with controlling invasive *Phragmites*. For some wetlands, funds targeting control efforts should be reallocated to other conservation initiatives in light of the carbon accrual and nitrogen removal benefits associated with *Phragmites.*

K. Stakeholder Summary (This is an abstract of your research and findings written for a lay audience)

Coastal marshes provide an array of ecosystem services, including carbon sequestration and improved water quality via nitrogen removal, but the consequences of elevated sea level rise and ecosystem management on wetland vegetation and the provision of salt marsh services are unclear. We examined how sea level rise and tidal restoration alter carbon and nitrogen-based services in three dominant vegetation zones- *Spartina alterniflora* (low marsh), *Spartina patens* (high marsh), *Phragmites australis* (brackish marsh), using a 20-site field survey of coastal Connecticut, a manipulative experiment, and sea level rise models (i.e. Sea Levels Affecting Marsh Migration, SLAMM). While none of our parameters differed between unrestricted and tidally restored marshes, we observed strong differences among vegetation zones, with vegetation being a top predictor of microbial respiration and potential denitrification rates. Interestingly, invasive *Phragmites* zones had higher nitrogen removal rates and carbon sequestration indices than *Spartina alterniflora.* Likewise, when we manipulated species composition under three sea level rise scenarios (present day, 10 year, 20 year) using a marsh organ experiment, carbon responses differed among vegetation, but not sea level rise treatments.

Based on SLAMM projections, the replacement of *S. patens* by short-form *S. alterniflora* is expected to be widespread across the Connecticut coastline, decreasing statewide potential denitrification from the low-to-high marsh transitional zone by at least 121 kg N/ hr by 2085. Our results suggest that changes in vegetation zones can serve as landscape-scale predictors for rapid changes occurring in salt marshes. To convey the importance of salt marsh ecosystems and highlight the ecological consequences of sea level rise to diverse audiences, we developed a publically available, interactive climate change teaching module for high school teachers.

Dr. Beth Lawrence
Primary Investigator
Natural Resources and Environment
University of Connecticut

4 January 2018

Dear Dr. Lawrence,

The purpose of this memorandum is to summarize: 1) any deviations between the QA Project Plan (QAPP) and the field sampling and laboratory analyses conducted in 2017, 2) the results of QA/QC tests, and 3) whether the data meet the data quality objectives outlined in the QAPP. Field surveys and laboratory analyses associated with project objective one – to quantify how restoration and dominant coastal marsh plant species alter carbon and nitrogen-based ecosystem services – were conducted during summer and fall 2017. Field and laboratory methods generally followed protocols described in the QAPP. There was one substantial deviation between experimental procedures and the proposed project plan: 30 sites were initially proposed as part of the field survey, but due to logistical constraints, only 20 sites were sampled in 2017 (including 10 tidal flow restoration sites and 10 unrestored sites). In addition, an alternative protocol developed by the Lawrence Lab at the University of Connecticut was used to measure CO_2 mineralization in place of the SOP included in the QAPP (the new protocol is enclosed).

For each of the variables included in the salt marsh field survey, 10% of the 60 total samples (20 sites x 3 vegetation zones) were measured in triplicate in the field or the laboratory and treated as quality assurance samples to assess method precision and overall performance. The relative standard deviation of each set of triplicate samples was calculated as part of the QA/QC protocol, and was less than or equal to 20% for most measured variables, including CO_2 gas flux (C mineralization), sediment core pH, electrical conductivity, ash-free dry mass (AFDM), and most soil ions (SO_4^{2-}, Cl^-, and PO_4^{3-}). For lab replicates of NH_3, one out of six of the NH_3 QA replicate checks had a relative standard deviation greater than 20%. The relative standard deviation was greater than 20% for 3 out of 5 sets of QA replicate checks for denitrification potential (DEA), and greater than 20% for 4 out of 6 sets of QA replicate checks for NO_3^-. Relative plant cover was measured in duplicate in 18 plots spanning different sites and vegetation zones, and the calculated percent difference in plant cover was less than 25% in 16 of these QA plots.

From the QA/QC results described above, most variables collected and analyzed as part of project objective one meet or exceed the data quality objectives listed in the QAPP, and approximately 89% of plant cover QA samples met the data quality objectives. However, the majority of DEA and NO_3^- QA samples fell outside of the data quality targets, and I recommend that future tests should be conducted to determine why replicate DEA measurements from sediment cores are highly variable, whether modifications to the DEA protocol are warranted, and whether the extractable soil NO_3^- concentrations observed in the field survey (median 20 µg $N\ L^{-1}$) are below the limits of analytical quantification. Sample completeness was 100% for most variables measured, although a sample loss of 20% was experienced for sediment core bulk density due to sample handling error in the laboratory. Data preservation practices outlined in the QAPP were used for the 2017 field survey data. Raw data files indicate the personnel who

performed each task, all samples have a unique sample ID, and field sheets have been electronically copied and are stored in multiple locations.

Sincerely,

Lauren E. Koenig, Ph.D.
Quality Assurance Officer,
Postdoctoral Research Associate
University of Connecticut

Dr. Beth Lawrence
Primary Investigator
Department of Natural Resources and the Environment
University of Connecticut

26 February 2019

Dear Dr. Lawrence,

In this memorandum I have summarized: 1) any deviations from the QA Project Plan (QAPP) and the field sampling and laboratory analyses conducted during project year 2018, the results of QA/QC tests, and 3) whether the data meet the data quality objectives outlined in the QAPP. Field data collection and laboratory analyses associated with project objective two – to experimentally test how plant species, salinity, and hydroperiod alter carbon and nitrogen cycling – were conducted during summer and fall 2018. As outlined in the project QAPP, an in situ marsh organ experiment was implemented at the Barn Island Wildlife Management Area in summer 2018. The marsh organ experiment included three different locally-dominant species of salt marsh vegetation including *Spartina alterniflora*, *Spartina patens*, and *Phragmites australis*.

Field and laboratory methods generally followed protocols described in the QAPP, although the marsh organ sampling design ultimately deviated from the proposed project plan in three ways: Two salinity levels were initially proposed, but only one salinity environment was imposed during the field deployment. In addition, five elevations were proposed, but three elevations were simulated in the field (0, 8, and 15). Finally, two different types of un-vegetated experimental units were included in the marsh organ design (including soil plugs from high-elevation sites in the salt marsh often associated with the higher-elevation species, *S. patens* and *P. australis*, and from low-elevation sites in the marsh, often associated with *S. alterniflora*). These un-vegetated units were included in addition to the 3 focal vegetation species to act as control or reference samples. Five marsh organ platforms were deployed, and within each platform, three elevation treatments were considered for each of the five vegetation species, which includes the low and high-elevation un-vegetated controls (5 species x 3 elevations x 5 platform replicates = 75 samples for most variables, or 45 samples for variables related to plant biomass allocation).

For each of the variables included in the field and lab data collection, 10% of samples were measured in triplicate and treated as quality assurance samples to assess method precision and overall performance. The relative standard deviation of each set of triplicate samples was calculated as part of the QA/QC protocol, and was considered valid if equal to or less than 20%. The relative standard deviation was less than 20% for the majority of QA replicate sets for most reported variables (e.g. for pH, all 7 sets of QA replicate checks were below 20%; for biomass ash-free dry mass, 5 out of 7 sets of QA replicate checks were below 20%; for soil carbon mineralization, 4 out of 6 sets of QA replicate checks were below 20%). For lab replicates of substrate-induced respiration (SIR), the relative standard deviation exceeded 30% for 4 out of 6 sets of QA replicate checks, indicating poor agreement among repeated samples for this variable. Due to the design of the marsh organ experiment, above- and belowground biomass samples were assessed for each pipe within a platform and therefore could not be taken in triplicate.

As of February 2019, samples for %C and %N (plant biomass; soil) and soil ions have not yet been run in the laboratory, and data for field CO_2, CH_4, N_2O, and N_2 fluxes are still being processed, so I have not evaluated whether these data meet the proposed data quality objectives here. However, from the QA/QC results described above, most variables collected and analyzed as part of project objective two meet or exceed the data quality objectives listed in the QAPP. The one notable exception is SIR, for which all QA samples fell outside of the data quality targets; I recommend comparing the values reported from this field study with other ranges from the scientific literature to determine whether the SIR rates are below the limits of analytical quantification. For each of the variables included in the field and lab data collection, no sample loss was reported and the respective datasets are 100% complete (n = 75 or 45 samples, respectively). Data preservation practices outlined in the QAPP were used for the 2018 marsh organ experiment: field environmental data have been transferred to Excel files, raw data files indicate a unique sample ID, and all data are stored in multiple locations.

Please do not hesitate to contact me if you have any additional questions about the information presented here.

Sincerely,

Lauren E. Koenig, Ph.D.
Quality Assurance Officer
Postdoctoral Research Associate
University of Connecticut

ECOSYSTEMS Springer

Vegetation zonation drives salt marsh soil carbon mineralization and microbial communities

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Key Words:	carbon mineralization, microbial community, Phagmites, tidal restoration, salt marsh, Spartina, Connecticut

UCONN
UNIVERSITY OF CONNECTICUT

April 29, 2020

Dear Ecosystems Editorial and Reviewer Team,

We are submitting a research article titled "Vegetation zonation drives salt marsh soil carbon mineralization and microbial communities" for consideration in Ecosystems.

Ecosystems is the ideal outlet for our research as we investigate how tidal restoration and vegetation zonation alters salt marsh bacterial community structure and ecosystem processes. We quantified microbial respiration, root-zone bacterial 16S rRNA genes, above and belowground biomass, and a suite of sediment characteristics in 20 salt marshes across coastal Connecticut (USA). While none of our parameters differed between unrestricted and tidally restored marshes, we observed strong differences among vegetation zones, with vegetation being a top predictor of microbial respiration rates. We also observed distinct root-zone microbial communities associated with vegetation zones. Our findings suggest that dominant salt marsh vegetation zones are useful indicators of hydrologic conditions and could be used to estimate microbial respiration rates.

We confirm that this work has not been published previously or concurrently submitted for publication elsewhere. Sequences generated in this study are available in the NCBI sequence read archive under the accession number PRJNA555079.

Thank you for your time and consideration. We look forward to your review.

Sincerely,

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TITLE: Vegetation zonation drives salt marsh soil carbon mineralization and microbial communities

SHORTED TITLE (< 45 characters): Vegetation zonation drives carbon mineralization

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Author Contributions: AMH, CSE, and BAL conceived of and designed the study. AB, OK, AMH, SSB, and BAL performed the research. AB and BBS analyzed the data, and AB, SKO, AMH, BBS, CSE, and BAL wrote the paper.

ABSTRACT

Coastal marshes are important "blue carbon" reservoirs, but it is unclear how vegetation shifts associated with tidal restoration and sea-level rise alter microbial respiration rates and community composition. In 2017, we surveyed 20 Connecticut salt marshes (10 without tidal restrictions, 10 tidally restored) and sampled plants and soils from three vegetation zones dominated by *Spartina alterniflora* (short-form, < 30 cm tall), *S. patens,* and *Phragmites australis*. We quantified microbial respiration rates (SIR: substrate-induced respiration; carbon mineralization), root-zone bacterial 16S rRNA genes, above and belowground biomass and a suite of sediment characteristics (soil pH and specific electrical conductivity (EC), soil moisture, soil organic matter, and soil extracted $SO4^{2-}$, Cl^{-}, NH_4^{+} concentrations). While none of our parameters differed between unrestricted and tidally restored marshes, we observed strong differences among vegetation zones, with vegetation being a top predictor of microbial respiration rates. Electrical conductivity was a top predictor in our model set, with strong, positive correlations between EC and microbial respiration rates. Thus we observed elevated microbial respiration rates in more frequently inundated *S. alterniflora* zones than *P. australis* zones. We also observed distinct root-zone microbial communities associated with vegetation zones, with sulfate-reducing bacteria being more abundant in *Spartina* spp. zones. Our findings suggest that dominant salt marsh vegetation zones are useful indicators of hydrologic conditions and could be used to estimate microbial respiration rates; however, it is still unclear whether differences in microbial respiration and community composition among vegetation zones is driven by plant community, environmental conditions, or their interactions.

KEY WORDS: carbon mineralization, microbial community, *Phragmites*, restoration, saltmarsh, *Spartina*

MANUSCRIPT HIGHLIGHTS:

- Root biomass, soil chemistry, and microbial responses varied among vegetation zones
- Unrestricted and tidally restored salt marshes were similar in all measurements
- Vegetation zone was the best predictor of microbial carbon processing

Introduction

Coastal wetlands are among the most productive ecosystems on the planet and provide numerous services including storm mitigation (Shepard and others 2011), wildlife habitat (Brawley and others 1998), and provision of a major carbon sink (Barbier and others 2011; McLeod and others 2011). The high productivity of vascular plants in coastal marine ecosystems paired with the relatively slow rate of decomposition in flooded soils (Poffenbarger and others 2011) results in the accumulation of soil organic matter reservoirs. Coastal wetlands are modified by human-driven coastal development (Roman and others 1984) and threatened by sea-level rise (SLR) (McLeod and others 2011; Rodríguez and others 2017), both of which alter plant community composition and the biotic-abiotic feedbacks that underpin carbon cycling and sequestration; however, we lack a mechanistic understanding of how shifts in salt marsh plant community composition may alter microbial community composition and respiration rates.

Strong environmental gradients in salinity and flooding result in distinct zonation in salt marshes, with dominant plants exhibiting differential tolerance to these environmental drivers (Bertness 1991; Mitsch and Gosselink 2007). For example, *Spartina alterniflora* dominates low-lying, saturated soils, whereas *Phragmites australis* occupies higher, drier areas bordering marsh edges and brackish areas; *Spartina patens* typically dominates the high marsh between these two zones, in areas with intermediate flooding and salinity. Intensification of tidal flooding due to SLR, however, has induced landward migration of vegetation (Smith 2015; Raposa and others 2017), with low-marsh *S. alterniflora* migrating into areas historically dominated by high marsh species (Basso and others 2015; Field and others 2016). This is likely because *S. alterniflora* is more flood- and salt-tolerant and oxygenates its rhizosphere more readily than high marsh species (Bertness 1991). On the upper boundary of the marsh platform, dominance of invasive *P.*

australis (Basso and others 2015; Smith 2015) has resulted in high marsh communities, *S. patens* in particular, becoming constricted or squeezed (*sensu* Doody 2004).

Feedbacks between dominant plants and the soil can in turn alter carbon cycling and microbial composition, as wetland plants alter soil conditions by transporting atmospheric oxygen belowground via aerenchymous tissues, as well as exuding low molecular-weight carbon substrates into the rhizosphere (Sutton-Grier and Megonigal 2011; Mueller and others 2016). Invasive monotypes of *P. australis* are very productive and sequester more atmospheric carbon relative to native *Spartina* spp. in their aboveground biomass (Mozdzer and others 2013; Martin and Moseman-Valtierra 2015). However, higher rhizosphere oxidation by lower elevation *Spartina* spp. may stimulate greater microbial activity and diversity by introducing oxygen for aerobic respiration and replenish alternative electron acceptors in soils that would otherwise be enriched with anaerobic microorganisms (Emery and Fulweiler 2014). While carbon uptake in salt marshes is primarily dictated by the photosynthetic capacity of a few dominant graminoid species (e.g., *P. australis* and *Spartina* spp.), carbon mineralization (i.e., the microbial transformation of soil organic carbon to CO_2) is a dominant pathway for carbon emissions (Holmes and Mahall 1982; Howes and others 1985). Microorganisms are highly sensitive to alterations in their environment and species-specific rhizosphere environments can differ dramatically (Brune and others 2000; Rietl and others 2016), with interactions among vegetation, soil, and hydrology mediating carbon cycling (Gutknecht and others 2006; Moseman-Valtierra and others 2016). Bacterial assemblages in salt marsh soils may vary with plant characteristics such as root exudation despite little variation in abiotic environments (Rietl and others 2016). However, the extent to which coastal wetland vegetation and its influence on associated soil

microbial communities and carbon mineralization is largely unknown (Farrar and others 2003; Chaudhary and others 2018; Pietrangelo and others 2018).

Coastlines around the world are increasingly subject to development pressure (Sandi and others 2018), leading to modified tidal regimes that can alter the susceptibility of salt marshes to SLR (Rodríguez and others 2017). Coastal development promotes the construction of roads, dikes, and railroads that traverse the majority of coastal marshes today (Bertness and others 2002; Correll and others 2017). These physical barriers restrict the exchange of salt water and sediment between marine and coastal ecosystems, reducing flooding frequency, accelerating organic matter oxidation (Portnoy and Giblin 1997), and creating brackish conditions. Restricted marshes are often dominated by invasive *P. australis*, which has a competitive advantage in less saline environments and creates dense monotypic stands, reducing plant and wildlife diversity (Roman and others 1984; Chambers and others 1999). To promote salt marsh biodiversity and reduce subsidence associated with increased oxidation (Burdick and others 1997), restoration efforts in recent decades have focused on restoring tidal hydrology. Impoundment removal and tide-gate installation typically leads to increased flooding, salinity, and the return of *Spartina* dominance (Burdick and others 1997; Konisky and others 2006). However, the potential effects of tidal restoration and vegetation zonation on carbon-based services in salt marshes remains uncertain (Moreno-Mateos and others 2012).

In order to clarify how tidal restoration and dominant vegetation zones of southern New England salt marshes influence soil carbon cycling and microbial communities, we implemented a 20-site field survey in coastal Connecticut to evaluate how microbial respiration (carbon mineralization and substrate induced respiration) and microbial community composition in salt

marsh soils varied among dominant vegetation zones in tidally restored and tidally unrestricted salt marshes.

Methods

Study Sites- We sampled 20 polyhaline salt marshes along the north shore of the Long Island Sound in Connecticut, USA (Fig. 1). Sites were selected based on their restoration history and presence of target vegetation: *S. alterniflora* (short-form, < 30 cm tall), *S. patens*, and *P. australis*. We communicated with the Connecticut Department of Energy and Environmental Protection staff to identify 10 tidally unrestricted and 10 restored sites (R. Wolfe and H. Yamalis, *personal communication*). Salt marsh ditching was historically a pervasive strategy in New England (USA) to support marsh haying, grazing, and mosquito control (Miller and Egler 1950; Rozsa 1995); thus both our tidally unrestricted "reference" and tidally restored sites have a legacy of human disturbance. Our tidally restored sites were historically restricted, but had tidal flow restored via culvert replacement, fill removal, installation of self-regulating tide gates, or tide gate removal at various times from 1978 to 2012. We selected unrestricted sites (i.e., sites that did not experience tidal restrictions or subsequent restorations) based on their proximity to tidally restored sites to limit tidal and microclimate variation, but a paired design was not feasible (Fig. 1).

Field Sampling- At each of the 20 sites, we identified three candidate vegetation zones for sampling with relative cover of the target species > 50 % in an area > 35 m^2 and within 100 m of the two other vegetation zones. Within each vegetation zone, we established three 1-m^2 plots that were centered in the middle of the zone, perpendicular to the nearest tidal creek, and at least 5 m from each other or 1 m from the zone edge. We sampled all plots during the peak of the growing

season in mid-August 2017 and sampled within three hours of low tide to control for tidal influence. We visually estimated the percent cover to the nearest one percent of all species in each plot, and to ensure consistency across sampling teams conducted independent duplicate plots with all sampling members every nine plots. Aboveground biomass of a randomly selected 25 x 25 cm subplot was clipped at the soil surface and composited across the three plots in each zone. We collected three soil cores (5-cm diameter to 10-cm depth; 196-cm^3 volume) from each plot, which were composited by zone and used to estimate bulk density, belowground biomass, and microbial respiration rates. To characterize microbial communities, we used ethanol-sterilized spoons to subsample ~5 g of root-zone soil in the field. Samples collected for microbial analyses were from the initial soil cores and targeted soil rather than roots and are considered root-zone soils hereafter. Samples were transferred to sterile Whirl-Pak bags, placed on dry ice during transport to the University of Connecticut Storrs campus, and stored at -80°C until DNA was extracted.

Biomass & Sediment- To separate belowground biomass from the soil matrix, samples were washed over 2-mm sieves. All biomass (above and belowground) was dried at 65℃ for at least 72 hours prior to being weighed. A subsample of belowground biomass from each vegetation zone was separated into roots and rhizomes to estimate their relative abundance. Dried biomass was pulverized using a ball mill and analyzed for % C and % N content using a Costech ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Valencia, CA). Bulk density samples were dried at 105°C for at least 48 hours, weighed, pulverized, and similarly analyzed for % C and % N content. A subsample (~5 g) of 2-mm sieved, homogenized soil was dried at 105°C for 72 hours to quantify soil moisture fraction. We then estimated loss on ignition (LOI) on the subsample by combusting organic matter at 550°C for four hours. We calculated carbon density

by multiplying our bulk density data by % sediment C to determine carbon mass per unit soil volume.

Soil Wet Chemistry- We used 2-mm sieved, homogenized soils to quantify all soil chemistry parameters. Soil slurries (1:5 ratio of soil to deionized water) were used to determine soil specific electrical conductivity (EC) and pH on 10 g of soil. The slurries were well-mixed on a shaker table (160 rpms for 10 minutes) then allowed to settle for 15 minutes prior to taking measurements with an Orion Conductivity Cell and an Orion Star A215 pH Conductivity Meter Orion with Ross Ultra pH/ATC Triode at room temperature. We analyzed water extracts for chloride (Cl^-) and sulfate (SO_4^{2-}) by mixing 2.5 g of soil with 25 ml DI water. Samples were shaken at 200 rpms for 30 minutes and centrifuged at 2500 rpms for five minutes. The supernatant was filtered through Whatman GF/F filters, and then run on a Dionex Ion Chromatography System (ICS)-1100 (Thermo Fisher Scientific, Waltham, MA). We extracted ammonium (NH_4^+) and nitrate (NO_3^-) with 2 N KCl (1:10 ratio of soil to KCl), filtered using 110 mm Whatman paper (adapted from Keeney and Nelson 1982) and analyzed extracts on a SmartChem®200 discrete analyzer (Westcon Scientific Instruments, Brookfield, CT). We measured KCl-extractable NH_4^+ using the phenate method (APHA 1999) and KCl-extractable NO_3^- using cadmium reduction (APHA 1999) on a SmartChem® 200 discrete analyzer (Westco Scientific Instruments, Brookfield, CT). Only six samples of NO_3^- measurements were above detection limit (0.1 mg N L^{-1}) therefore we do not report NO_3^-, while 77 % of NH_4^+ samples were above detection limit (0.184 mg N L^{-1}) and are included in our analysis. Those below detection limit for NH_4^+ were set to 0.092 mg N L^{-1}, half the detection limit, for analysis.

Microbial Respiration Assays- We conducted assays using 2-mm sieved soils; thus carbon dioxide (CO_2) accumulation was associated with microbial activity of labile carbon substrates

rather than root respiration. We used substrate-induced respiration (SIR) (Anderson and Domsch 1978; West and Sparling 1986) as an index of soil microbial activity with excess carbon resources. Five grams of well-mixed sieved soil and 10 mL of yeast solution (20 mg yeast per g dry soil), were added to a 40 mL amber vial and sealed with a gas-tight septum and cap and were shaken horizontally throughout the duration of the experiment. Headspace CO_2 samples (1 mL) were injected into a LI840A CO_2/H_2O Gas Analyzer (LI-COR, Lincoln, NE) to quantify CO_2 concentrations at time zero, two, and four hours.

Aerobic carbon mineralization rates were measured as CO_2 and methane (CH_4) accumulation over a 24-hour period using a Picarro G2201-*i* gas analyzer and two, 16-port distribution manifolds (Johnson and others 2019). We added ~50 g of sieved soil to 196 mL glass canning jars, allowed them to come to room temperature, and connected them to the gas analyzer; headspace gas concentrations were measured approximately every two hours over a 24-hour period. We calculated gas flux rates, for both SIR and carbon mineralization, as the linear change in CO_2 or CH_4 concentration over time, corrected for temperature, atmospheric pressure, and volume, based on the ideal gas law. Methane accumulation rates were low and are not reported as over half of the samples were below detection limit (0.012 ppm CH_4 hr^{-1}).

Microbial Communities- We processed samples similar to Elmer and others (2017) in which a ~0.5 g subsample was aseptically transferred to a power bead tube (MO BIO Power Soil Kit, Carlsbad, CA) and DNA was extracted using the supplied protocols. DNA extractions were verified by gel electrophoresis and DNA was quantified with a NanoDrop spectrophotometer (NanoDrop Lite, Thermo Scientific, Waltham, MA).

The extractions were processed at the University of Connecticut's Microbial Analysis, Resources, and Services unit of the Center for Open Research Resources and Equipment for PCR

reactions and sequencing. Bacterial 16S rRNA genes were amplified using 30 ng of extracted DNA. The V4 region was amplified using primers 515F and 806R with Illumina adapters and dual indices (8 basepair golay on 3' (Caporaso and others 2012), and 8 basepair on the 5' (Kozich and others 2013). Samples were amplified in triplicate using GoTaq (Promega) with the addition of 10 µg BSA (New England BioLabs). The PCR reaction was incubated at 95˚C for 3.5 minutes, then 30 cycles of 30 s at 95.0°C, 30 s at 50.0°C and 90 s at 72.0°C, followed by final extension as 72.0°C for 10 minutes. PCR products were pooled for quantification and visualization using the QIAxcel DNA Fast Analysis (Qiagen). PCR products were normalized based on the concentration of DNA from 250-400 bp then pooled using the QIAgility liquid handling robot. The pooled PCR products were cleaned using the Mag-Bind RxnPure Plus (Omega Bio-tek) according to the manufacturer's protocol. The cleaned pool was sequenced on the MiSeq platform using v2 2 x 250 chemistry (Illumina, Inc).

Paired sequences were assembled into contigs using the make.contigs command with default parameters in the mothur software package, only retaining contigs of at least 253 bases. Each contig was further screened to remove any sequences with any ambiguous nucleotide calls or homopolymers of ≥ 7 bases. Potential chimeric sequences were removed from the dataset with the mothur utilization of vsearch. Sequences were clustered into operational taxonomic units (OTUs) with the OptiClust algorithim in mothur. For analyses of diversity and composition, an OTU definition of ≥ 97 % sequence identity was used. Taxonomic assignment of sequences was performed with the mothur utilization of classify.seqs against the SILVA 132 ribosomal database (Quast and others 2013).

Statistical Analysis- To compare how restoration and vegetation zones influenced our suite of response metrics, we compared 95 % confidence intervals (CIs) and considered non-overlapping

CIs to indicate differences among groups. To predict carbon mineralization and SIR rates, we used small sample size-corrected Akaike's information criterion (AICc) model selection to identify the most parsimonious linear mixed-effects model (*lme4* package), where site was coded as a random factor. Because of limited sample size, only models with up to four parameters (K) were included. We included vegetation zone and restoration status as parameters along with soil chemistry (EC, soil moisture fraction, and pH), plant biomass (aboveground, belowground), and interactions between soil chemistry and biomass in 38 candidate models. Other soil chemistry parameters (SO_4^{2-}, Cl^-, organic matter, soil moisture fraction) were not included in model selection as they were highly correlated (Pearson's correlation $r > 0.75$) with included parameters (Table 1). For all response variables, we tested for normality using Shapiro-Wilk tests, plotted fitted values against residuals, and log transformed responses to improve normality and homoscedasticity when necessary. We also tested for multicollinearity across our models by calculating variance inflation factors. All statistical analyses were run in R version 3.5.1 (R Core Team 2017).

OTU abundance data were uploaded to the phyloseq package for calculation of ordination plots and alpha diversity. Principal component analysis (PCA) was performed on data randomly rarefied to the sample size of the smallest sequence dataset (5645 sequences). Non-metric multidimensional scaling (NMDS) was performed when minimal variation was explained by PCA plots. Inter-sample distances were calculated with the Bray-Curtis metric and PERMANOVA statistics were calculated with the Adonis function in the vegan package. Differentially abundant OTUs were identified using the $\log_2$-fold ratio with the negative binomial generalized linear framework of the DESeq2 software package and post-hoc Wald test

(Love and others 2014). Sequences generated in this study are available in the NCBI sequence read archive under the accession number PRJNA555079.

Results

Primary explanatory variables- Several of our explanatory variables were highly correlated with one another (Table 1). For example, EC was strongly positively correlated with soil moisture fraction, soil organic matter, as well as with Cl^- and SO_4^{2-} concentrations; likewise, Cl^- and SO_4^{2-} concentrations were strongly positively correlated with one another, as well as with soil moisture fraction and organic matter (Table 1). Parameters included in our model selection all had low variance inflation factors; the highest value was belowground biomass which had a value of 4.8 and 4.3 for carbon mineralization and SIR models, respectively.

Effects of restoration and vegetation zones- The 10 tidally restored sites we sampled were restored from five to 40 years ago (R. Wolfe and H. Yamalis, *personal communication*), though we did not detect correlations between time since restoration and microbial respiration (carbon mineralization: $r = 0.01$; SIR: $r = 0.01$). None of our response variables differed between tidally restored and unrestricted marshes (Table 2). However, we observed strong differences among vegetation zones in sediment chemistry, plant biomass, and microbial respiration rates (Table 2). Sediment EC was highest in *S. alterniflora* zones near the tidal creeks and lowest in *P. australis* zones near the marsh edges, with Cl^- and SO_4^{2-} concentrations following a similar pattern; as expected, EC was strongly positively correlated with both Cl^- and SO_4^{2-} concentrations since the source of salinity is tidal flow. Even though EC was also correlated with soil moisture, soil moisture CI's largely overlapped among vegetation zones. Ammonium was greatest in unrestricted *S. patens* zones and lowest in unrestricted *S. alterniflora* zones.

Plant biomass and sediment quality varied among vegetation zones (Table 2). Aboveground biomass did not differ among vegetation zones, but tissue % C varied among zones, with *S. alterniflora* having the lowest % C. Belowground biomass was greater in *Spartina* spp. zones than *P. australis*, with *Spartina* spp. allocating a higher percentage of belowground biomass to roots (60 %) than *P. australis*, which had equal parts roots and rhizomes. Sediment C:N was lower in *P. australis* than *Spartina* spp. zones.

Carbon mineralization and SIR rates varied among vegetation zones with some minor differences between restored and unrestricted marshes. Carbon mineralization and SIR were positively correlated (Table 1; r = 0.73), and carbon mineralization rates were an order of magnitude lower than SIR results (Table 2). Average carbon mineralization was four times higher in *S. alterniflora* and restored *S. patens* marshes relative to *P. australis*-dominated marshes (Fig. 2). Similarly, average SIR was 1.25 greater in *S. alterniflora*-dominated and restored *S. patens* marshes than unrestricted *P. australis* marshes.

Model Selection- Our AICc model selection results suggest that vegetation zone alone was the most parsimonious predictor for carbon mineralization rates, as all other models had ΔAICc values greater than 2 (Table 3). This model explained 53 % of variation in carbon mineralization rates. For SIR, three candidate models had ΔAICc values less than two (Table 4), and explained between 39 and 41 % of the variation in SIR. The top models included EC and carbon density, but models with only vegetation and only EC had similar performance. Restoration status was not in any of the top models for carbon mineralization or SIR.

Microbial Communities- Rarified sequences were used to create a non-metric multidimensional scaling plot (Fig. 3). We did not observe differences in microbial communities between tidally restored and unrestricted sites ($p > 0.05$). The centroids of ellipses encompassing the three

vegetation zones differed (Adonis test p-value < 0.001), however, there was considerable overlap between microbial communities among vegetation zones (beta dispersion p value < 0.001), indicating that communities were not distinct. We did not observe differences in several diversity measurements among vegetation zones. The Shannon's diversity index mean (95 % CI) for *S. alterniflora*, *S. patens*, and *P. australis* were 4.96 (4.92 - 5.00), 4.95 (4.86 - 5.04), and 5.05 (4.97 - 5.13), respectively. The inverse Simpson index mean (95 % CI) for *S. alterniflora*, *S. patens*, and *P. australis* were 103.99 (95.87 - 112.11), 107.22 (94.40 - 120.52), and 123.79 (108.92 - 138.63), respectively.

Spartina spp. zones had sediment microbial communities that were more similar to each other than either was to *P. australis*-dominated zones (Fig. 4). Our data suggest that particular OTUs were more abundant in certain vegetation zones, with differentially abundant OTUs belonging largely to five phyla with a large proportion representing Proteobacteria and Bacteroidetes. Using Wald tests, we determined that there were 15 differentially abundant OTUs between *S. alterniflora* and *S. patens* zones; 88 between *S. patens* and *P. australis* zones, and 246 between *S. alterniflora* and *P. australis* zones (Appendix 1). Bacteroidetes and Proteobacteria phyla were often the differentiating phyla among vegetation zones.

Discussion

We conducted a 20-site field survey of Connecticut salt marshes to investigate the role of tidal restoration and vegetation zonation on microbial respiration rates and community structure. In contrast to our predictions, we did not observe differences between the tidally restored and unrestricted marshes we surveyed for microbial respiration, microbial community structure, or soil characteristics. We did find however that the suite of microbial and soil variables we

quantified were strongly associated with salt marsh vegetation zone, regardless of restoration status. This suggests that soil carbon cycling may be closely tied to salt marsh vegetation zones.

Differential vegetation zone responses- Carbon mineralization and SIR varied with vegetation zone, decreasing with distance from tidal creeks (i.e., *S. alterniflora* > *P. australis*), likely driven by a combination of environmental conditions and plant characteristics that are challenging to disentangle. We observed differences among salt marsh vegetation zones in belowground biomass, soil chemistry, microbial respiration rates, and microbial community structure, highlighting the interconnected nature of structure and function in salt marshes and the importance of tidal flooding in regulating vegetation zonation (Warren and others 2002). Vegetation type alone was one of the best supported models, perhaps because vegetation integrates several environmental variables that influence marsh processes. We observed higher belowground biomass and soil salinity (EC, Cl^-, SO_4^{2-}) in lower vegetation zones (*S. alterniflora* and *S. patens*) that are more frequently inundated than higher elevation *P. australis*-dominated zones. This aligns with ecological allocation principles, as low marsh plants allocate more resources to belowground biomass to increase structural support to limit erosion and increase surface area for root oxidation (Bertness 1991). Similarly, EC was a top predictor of SIR, possibly because of greater belowground biomass is associated with heightened salinity and depleted oxygen (Bertness 1991). Carbon density together with EC was also a top model for SIR; while carbon density was not correlated with SIR, perhaps it helped explain residual variation that was not accounted for by vegetation zone and EC.

Wetland plant inputs potentially play an important role in sediment microbial activity via priming (i.e., carbon exudates), as low molecular-weight carbon exudates are readily metabolized by microbial communities (Farrar and others 2003; Rietl and others 2016; Yarwood

2018). Salt marsh plants also alleviate thermodynamic constraints via rhizosphere oxygenation by aerenchymous tissue (e.g., Howes and Teal 1994), allowing aerobic microorganisms to rapidly metabolize carbon compounds (Sutton-Grier and Megonigal 2011; Chapman and others 2019). We focused our investigation on sediments within 10 cm of the surface, where the majority of belowground biomass resides in salt marshes (Santini and others 2019). While we observed less belowground biomass in *P. australis*-dominated surface soils, *P. australis* tends to root more deeply than native marsh species (Mozdzer and others 2016), thus the soils we sampled had less opportunity for plant-mediated interactions but may not be wholly representative of how *P. australis* affects microbial respiration. Litter quality also moderates feedbacks between plant community and salt marsh soil organic matter stabilization (Castellano and others 2015). Kim and others (2018) found that *P. australis* tissues had relatively high levels of phenol-rich, recalcitrant carbon compounds that were less readily used by microorganisms. We, however, did not see differences in biomass C:N ratios among our three target plant species, although sediments associated with *P. australis* did have lower C:N molar ratios (Table 1).

Plant inputs such as oxygen and carbon exudates are known to stimulate microbial activity (Rietl and others 2016), but it is less apparent how increased salinity influences carbon mineralization rates. Sulfate-rich marine water coupled with frequent inundation promotes sulfate-reduction and hydrogen sulfide production. Both hydrogen sulfide and Cl^- can inhibit plant nutrient uptake and ionic stress, which may alter interactions between vegetation and microbial activity (Luo and others 2019). We observed strong positive relationships between indices of salinity (EC, Cl^-, SO_4^{2-}) and our metrics of microbial respiration, and EC was a top predictor of SIR, but perhaps the breakdown of organic carbon is not explicitly driven by salinity

gradients but by another closely allied factor, such as availability of alternate electron acceptors or labile carbon as indicated by belowground biomass.

Laboratory assays vs. in-situ conditions- Utilizing systematic laboratory assays to estimate microbial respiration allowed us to compare soils collected from three vegetation zones across 20 salt marshes within a relatively small time frame (i.e, two weeks), but it is prudent to interpret these data with caution, as our laboratory assays were conducted under conditions quite distinct from *in-situ* environments. Under anoxic, reduced conditions prevalent in *S. alterniflora* zones in the field, labile carbon substrates likely accumulate; during our oxic laboratory incubations, these substrates could have metabolized quickly. Exposing anoxic soils to oxygen may increase carbon mineralization rates as anaerobes generally do not utilize complex organic carbon substances, but aerobes can rapidly recycle labile carbon (Kristensen and others 2008). While laboratory assays do not replicate field conditions, we observed differential carbon respiration rates across vegetation zones and our data suggest that *S. alterniflora* sediments have more labile carbon under oxic conditions than those from *P. australis*. Coupling anoxic and longer term incubations, as well as other methods that quantify labile and recalcitrant fractions of soil organic matter *in situ* (Keuscamp and other 2013) may provide further insight into the stability of carbon in salt marsh sediments. For example, we examined three-month long, *in-situ* decomposition rates of standardized substrate (i.e., Tea Bag Index; Keuscamp and others 2013) in a subset of the field sites present in the current study and in a marsh organ experiment and found consistently higher decomposition rates in *P. australis* vs. *Spartina* spp. (Bisson and others *in prep*). There are also potential modifications to the microbial community that could alter the functional traits of the sediment communities. For example, Proteobacteria are generally rapidly growing heterotrophs and are likely candidates to be stimulated by priming (Pascault and others 2013).

Effects of tidal restoration- Tidally restricted marshes are common along developed coastlines, resulting in a suite of consequences including surface elevation subsidence and reduced salinity levels that promote brackish vegetation such as invasive *P. australis* (Warren and others 2002). Reconnecting tidal hydrology by removing tide gates and widening culverts restores the exchange of salt water and sediments between restricted marshes and estuaries, allowing natural flooding regimes, salinity, and vegetation to rebound (Konisky and others 2006). Over the past several decades, the state of Connecticut has initiated >80 tidal restoration projects with over 730 hectares of tidal marsh restored, resulting in a 12 % increase in coastal marsh (Rozsa 2012). In brackish marshes in the region, Doroski and others (2019) observed higher potential denitrification rates and SIR rates with time since restoration, but they did not detect similar trends for carbon mineralization.

In surface sediments of salt marshes that were tidally restored between five to 40 years when we sampled in 2017, we observed no differences in microbial respiration or soil chemistry variables. Our findings suggest that surface soil microbial respiration responds relatively quickly to tidal restoration, as rates were analogous to reference unrestricted marshes. Surface sediments, such as those we sampled to 10-cm depth, may not be representative of management legacies, as fresh vegetation inputs (e.g., biomass, exudates, oxygen) in surface horizons and tidal inundation drive biogeochemical and microbial processes. Thus, perhaps it is not surprising that we observed similar surface soil chemistry and microbial respiration in tidally restored and unrestricted marshes within similar vegetation zones. We targeted surface sediments in this survey, anticipating them to be the most microbially-active (Craft and others 1999; Warren and others 2002), but this likely influenced our results. For example, our carbon density estimates were greater than those of Chmura and others (2003) and CEC (2015), who observed reduced

carbon density with depth (50cm and 20cm, respectively). We did not detect differences in soil chemistry responses (EC, pH, Cl^-, SO_4^{2-}, NH_4^+, organic matter, soil moisture fraction) between tidally restored and unrestricted marshes, likely because salt marsh vegetation zonation is highly constrained by the ability of the dominant species to withstand flooding and salinity stressors; thus vegetation can be considered a biological indicator of hydrologic and physio-chemical conditions (Smith and Warren 2012).

Microbial Communities- While vegetation has been identified as an important driver of microbial composition in terrestrial settings (Grayston and others 1998; Ladygina and Hedlund 2010), it is less well-studied in coastal wetlands primarily due to the numerous environmental factors (i.e., soil salinity, sulfide concentrations, redox potential) that can vary substantially over time and space (Rietl and others 2016). Our study helps bridge this gap as we systematically characterized microbial communities across dominant salt marsh vegetation zones in 20 marshes on the north shore of Long Island Sound, whereas previous studies typically examine only a few sites (Ravit and others 2003; Elmer and others 2017; Rietl and others 2016). We anticipated high variation across samples, because community structure is often highly site-specific (Ravit and others 2003; Bowen and others 2009; Simon and others 2017), but expected to observe general trends along environmental gradients. Although we did not observe strong differences in community composition by vegetation zones, certain bacteria and bacterial groups varied in abundance. For example, within *Spartina* spp.-dominated soils, we found higher proportions of sulfur-reducing delta-Proteobacteria, (Kearns and others 2016), suggesting that the physio-chemical environment may drive both vegetation zonation and microbial communities in coastal wetlands (Chrzanowski and Spurrier 1987). *Spartina* spp. zones were also associated with a higher abundance of the phylum Bacteroidetes, bacteria which are more abundant near living plant roots

(Elmer and others 2017; Gkarmiri and others 2017) and associated with the metabolism of recalcitrant carbon (Fierer and others 2007; Ai and others 2015). Given that we observed higher rates of carbon mineralization in *Spartina* spp.-dominated zones, the higher abundance of these two bacterial groups suggests that there may be feedbacks between vegetation, microbial community structure, and microbial process rates. While we observed higher abundance of bacteria that metabolize plant-derived carbon in vegetation zones with higher rates of microbial respiration, it is important to note that microbial abundance estimates from sequencing does not equate to microbial activity, as the data cannot differentiate between active and non-active sequences (Blazewicz and others 2017). For future studies, it would be valuable to quantify carbon cycling, microbial community structure, as well as examine extracellular enzymes associated with microbial metabolism and nutrient cycling. By isolating extracellular enzymes such as *beta*-glucosidase and phenol oxidase, we may gain a better understanding of which bacteria are actively metabolizing plant matter (Freeman and others 2001; Sinsabaugh 2010; Morrissey and others 2014).

In general, soils dominated by *Spartina* spp. shared more similar characteristics than with those of *P. australis* (Table 5), a pattern consistent with our observations of soil-root zone microbial communities. While there was considerable overlap among microbial communities (Fig. 4), the two *Spartina*-dominated zones had more similar bacterial structure than with *P. australis*-dominated soils. *Spartina alterniflora* and *S. patens* shared relatively similar microbial communities with only 15 OTUs differing in abundance between the two vegetation zones; whereas many more OTUs (246) differed in abundance between *S. alterniflora* and *P. australis* (Fig. 5). These OTUs were generally delta-Proteobacteria or Bacteroidetes, with more present in the *Spartina*-dominated zones. At least two of the OTUs that differed most in terms of relative

abundance, and were found in *P. australis*-dominated zones, were nitrogen fixing bacteria; Ooi and others (*in prep*) observed the greater denitrification potentials in these same *P. australis* zones, suggesting rapid utilization by the microbial community.

Management Implications- As coastal marshes are squeezed by both sea-level rise and coastal development (Doody 2004), coastal managers will increasingly need to make challenging decisions about tradeoffs between biodiversity, vegetation structure, and ecosystem function. Our findings suggest that vegetation may be a useful indicator of key carbon- and nitrogen-based processes in salt marshes as vegetation zones had distinct microbial respiration rates (current study) and denitrification potentials (Ooi and others *in prep*). Shifting coastal wetland vegetation may trigger a cascade of effects altering carbon cycling and storage capacity. As plant zones migrate inland or drown, there may be increased soil carbon respiration as more frequent flooding promotes species with dense belowground biomass (i.e., *S. alterniflora*), elevated salinity increases sulfate reduction, and senesced plant tissues increase the availability of labile carbon (Rooth and others 2003; Chambers and others 2011).

The primary objective of many tidal restorations in mid-Atlantic and northeastern USA marshes has been to reduce the abundance of invasive *P. australis* (Chambers and others 1999). *Phragmites australis* reduces habitat quality for a range of species (Roman and others 1984), but the prioritization of ecosystem services such as carbon sequestration is becoming more common during ecological restoration (McLeod and others 2011; Nahlik and Fennessy 2016). Carbon mineralization rates suggest that invasive *P. australis* soils may be more effective at sequestering and storing carbon than native *Spartina* spp. Our findings reinforce those of others (Windham and Lathrop 1999; Moseman-Valtierra and others 2016) that, in terms of soil carbon flux, *P. australis* may maintain carbon more readily than either *Spartina* spp. Thus, a more attainable

goal may be to prioritize biodiversity and allocate financial resources to a subset of marshes most resistant to invasion and valuing carbon-based and resilience services (erosion, sediment accumulation) provided by marshes already dominated by *P. australis*.

Conclusions- We conducted a coastal marsh survey to investigate how tidal restoration and vegetation zonation affect microbial respiration and community structure in surface soils. We found no difference between tidally restored and unrestricted reference marshes in soil chemistry, plant biomass, soil carbon respiration rates, or microbial communities, indicating that tidal restoration efforts over the past 40 years in Connecticut have not deviated from reference site levels. However, since tidal restoration and SLR change the composition and areal extent of salt marsh vegetation, scaling of empirical estimates to wetland extent would better reflect how tidal restoration alters carbon- and nitrogen-based processes at site and regional scales (Ooi and others *in prep*). Our study suggests that vegetation could be utilized to do such scaling in southern New England coastal marshes, as we observed that rates of soil carbon mineralization and SIR across coastal Connecticut were strongly dependent on the dominant vegetation. Soils associated with *P. australis* had lower rates of carbon mineralization than *Spartina* spp. zones, suggesting potential for carbon sequestration. While we found several indices of soil salinity and plant biomass to be correlated with microbial respiration rates, flooding frequency, soil properties, and vegetation are inherently confounded during field surveys. Therefore, vegetation zones may be the most integrative index of numerous marsh processes that can be quantified both on the ground and via remote sensing.

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Table Legends

Table 1. Correlation matrix for primary explanatory variables (soil and biomass parameters) measured during a 2017 survey of 20 Connecticut salt marshes. Samples were collected from three vegetation zones at each site (n = 60). Pearson correlation coefficients (r) are above the greyed cells, and associated p-values are below. P-values < 0.05 are italicized and in bold. (SIR: Substrate-Induced Respiration; C Min: carbon mineralization; EC: electrical conductivity; AGB: aboveground biomass; BGB: belowground biomass).

Table 2. Mean (95% CIs) soil chemistry, carbon and nitrogen content, biomass, and microbial response parameters among 10 tidally restored and 10 unrestricted salt marshes in Connecticut from three target vegetation zones sampled in 2017. Responses with asterisks indicate non-overlap of 95% CIs among the six marsh types; superscripted letters indicate similarities among marsh types. (SIR: Substrate-Induced Respiration; C Min: carbon mineralization; EC: electrical conductivity; AGB: aboveground biomass; BGB: belowground biomass).

Table 3. Candidate mixed-effect models to explain variation in carbon mineralization rates across 20 Connecticut salt marshes. All models included intercept and random site effects. Number of model coefficients (K), Akaike's information criterion adjusted for small sample size (AIC_c), the difference in AICc between each candidate model and the top model (ΔAICc), and Akaike weights are reported.

Table 4. Candidate mixed-effect models to explain variation in substrate-induced respiration (SIR) rates across 20 Connecticut salt marshes. All models included intercept and random site effects. Number of model coefficients (K), Akaike's information criterion adjusted for small sample size (AIC_c), the difference in AICc between each candidate model and the top model (ΔAICc), and Akaike weights are reported.

Table 1.

	log SIR	log C Min	pH	EC	Soil Moisture Fraction	Organic Matter	AGB	BGB	Cl^-	SO_4^{2-}	NH_4^+	Sediment C:N	AGB C:N	BGB C:N	Carbon Density
log SIR		0.73	-0.18	0.63	0.54	0.33	-0.37	0.59	0.71	0.68	-0.45	0.69	0.47	0.10	0.01
log C Min	***<0.001***		0.01	0.49	0.28	0.01	-0.30	0.61	0.46	0.42	-0.41	0.67	0.45	0.20	0.28
pH	0.25	0.90		-0.26	-0.27	-0.30	0.02	-0.21	-0.32	-0.43	0.09	-0.10	-0.09	0.23	-0.11
EC	***<0.001***	*0.00*	0.09		0.80	0.61	-0.59	0.72	0.93	0.87	-0.34	0.53	0.30	0.22	0.09
Soil Moisture Fraction	***<0.001***	0.07	0.08	***<0.001***		0.86	-0.46	0.59	0.86	0.77	-0.16	0.39	0.16	0.06	0.12
Organic Matter	*0.03*	0.90	0.06	***<0.001***	***<0.001***		-0.34	0.37	0.65	0.56	0.00	0.08	-0.05	-0.05	0.05
AGB	*0.02*	0.05	0.90	***<0.001***	*0.00*	*0.03*		-0.42	-0.57	-0.48	0.01	-0.37	-0.10	-0.22	-0.23
BGB	***< 0.001***	***<0.001***	0.17	***<0.001***	***<0.001***	*0.02*	*0.01*		0.71	0.69	-0.38	0.75	0.38	0.25	0.29
Cl^-	***< 0.001***	*0.00*	*0.04*	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***		0.91	-0.31	0.54	0.27	0.14	0.10
SO_4^{2-}	***< 0.001***	*0.01*	*0.00*	***<0.001***	***<0.001***	***<0.001***	*0.00*	***<0.001***	***<0.001***		-0.26	0.59	0.33	0.15	0.13
NH_4^+	*0.00*	*0.01*	0.60	*0.03*	0.32	0.99	0.93	*0.01*	*0.04*	*0.10*		-0.42	-0.30	-0.02	0.10
Sediment C:N	***< 0.001***	***<0.001***	0.55	*0.00*	0.01	0.61	*0.02*	***<0.001***	***<0.001***	***<0.001***	*0.01*		0.64	0.29	0.16
AGB C:N	0.50	0.20	0.14	0.17	0.72	0.75	0.16	0.11	0.38	0.34	0.90	0.06		0.24	-0.03
BGB C:N	*0.00*	*0.00*	0.58	0.05	0.31	0.77	0.53	0.13	0.08	*0.03*	0.05	***<0.001***	0.10		0.02
Carbon Density	0.90	0.07	0.48	0.57	0.46	0.77	0.15	0.06	0.50	0.40	0.50	0.30	0.90	0.86	

Table 2.

	S. alterniflora		*S. patens*		*P. australis*	
	Unrestricted	**Restored**	**Unrestricted**	**Restored**	**Unrestricted**	**Restored**
Sediment properties						
pH	6.6 (6.1-7.2)^{a}	6.5 (6.0-6.9)^{a}	6.6 (6.4-6.9)^{a}	6.8 (6.6-7.1)^{a}	6.7 (6.3-7.0)^{a}	6.6 (6.3-7.0)^{a}
Organic Matter (%)	40.5 (31.0-49.9)^{a}	33.7 (23.1-44.2)^{a}	37.3 (24.8-49.8)^{a}	40.0 (26.3-53.6)^{a}	26.6 (10.6-42.7)^{a}	26.2 (11.4-40.9)^{a}
Soil Moisture Fraction	0.85 (0.78-0.93)^{a}	0.81 (0.71-0.91)^{a}	0.80 (0.73-0.87)^{a}	0.81 (0.72-0.91)^{a}	0.64 (0.51-0.78)^{a}	0.59 (0.39-0.79)^{a}
Bulk density (g cm^{-3})	0.79 (0.32-1.26)^{a}	0.72 (0.29-1.16)^{a}	0.74 (0.35-1.13)^{a}	0.79 (0.34-1.24)^{a}	1.22 (0.40-2.05)^{a}	1.50 (0.63-2.37)^{a}
Sediment chemistry						
*EC (mS cm^{-1})	8.2 (7.5-8.9)^{a}	7.7 (6.6-8.7)^{a}	7.0 (6.2-7.9)^{ac}	7.0 (6.1-7.8)^{ac}	5.1 (3.9-6.3)^{bc}	4.5 (3.0-6.0)^{b}
*Cl^{-} (mg g soil^{-1})	11.2 (10.2-12.2)^{a}	10.4 (8.4-12.5)^{a}	9.1 (8.0-10.2)^{a}	9.3 (7.9-10.6)^{a}	6.1 (4.2-7.9)^{b}	5.7 (3.4-8.0)^{b}
*SO$_4^{2-}$ (mg g soil^{-1})	1.6 (1.4-1.9)^{a}	1.5 (1.3-1.9)^{a}	1.4 (1.2-1.6)^{a}	1.5 (1.1-1.6)^{a}	0.8 (0.6-1.1)^{b}	0.8 (0.5-1.1)^{b}
*NH$_4^{+}$ (µg NH$_4^{+}$-N g soil^{-1})	3.9 (1.3-6.6)^{a}	6.2 (2.7-9.6)^{a}	14.1 (8.4-19.9)^{b}	6.4 (3.1-9.7)^{ab}	12.5 (7.7-17.3)^{b}	9.3 (5.5-13.2)^{ab}
Sediment %C	25.4 (20.8-30.0)^{a}	23.5 (18.5-28.4)^{a}	22.5 (16.0-28.9)^{a}	23.1 (16.7-29.5)^{a}	15.7 (8.6-22.8)^{a}	16.9 (7.7-26.0)^{a}
Sediment %N	1.21 (0.96-1.47)^{a}	1.13 (0.80-1.45)^{a}	1.03 (0.76-1.31)^{a}	1.14 (0.83-1.45)^{a}	0.97 (0.54-1.40)^{a}	0.99 (0.46-1.52)^{a}
*Sediment C:N	24.8 (23.1-26.5)^{a}	25.5 (22.4-28.5)^{a}	25.3 (22.8-27.8)^{a}	23.8 (21.8-25.9)^{a}	19.1 (17.4-20.9)^{b}	20.2 (18.7-21.6)^{b}
Carbon Density (g cm^{-3})	0.19 (0.10-0.28)^{a}	0.13 (0.11-0.15)^{a}	0.14 (0.10-.17)^{a}	0.13 (0.12-0.16)^{a}	0.12 (0.08-0.16)^{a}	0.10 (0.09-0.12)^{a}
Biomass						
AGB (kg m^{-2})	1.78 (1.00-2.56)^{a}	1.41 (1.22-1.59)^{a}	1.74 (1.44-2.04)^{a}	1.76 (1.42-2.11)^{a}	3.62 (1.31-5.93)^{a}	2.59 (1.87-3.32)^{a}
*AGB %C	40.1 (39.3-40.8)^{a}	40.9 (38.9-43.0)^{ab}	43.4 (42.6-44.2)^{b}	43.3 (42.3-44.3)^{b}	46.3 (41.3-51.3)^{b}	43.9 (42.6-45.1)^{b}
*AGB %N	1.01 (0.93-1.08)^{a}	1.03 (0.89-1.17)^{a}	0.77 (0.69-0.86)^{b}	0.83 (0.60-1.06)^{ab}	1.22 (1.05-1.40)^{a}	1.21 (0.93-1.49)^{a}
AGB C:N	50.1 (44.2-55.9)^{a}	57.0 (45.5-68.5)^{a}	52.7 (45.0-60.5)^{a}	57.3 (45.0-69.6)^{a}	49.1 (38.0-60.1)^{a}	53.0 (39.6-66.4)^{a}
*BGB (kg m^{-2})	13.9 (12.2-15.6)^{a}	12.6 (10.2-15.0)^{a}	10.3 (8.2-12.4)^{a}	10.5 (9.1-12.0)^{a}	4.9 (3.6-6.2)^{b}	5.5 (4.3-6.7)^{b}
BGB %C	44.1 (43.3-44.9)^{a}	44.6 (43.8-45.3)^{a}	45.0 (42.4-47.6)^{a}	45.4 (44.4-46.4)^{a}	40.8 (33.8-47.8)^{a}	38.2 (28.8-47.6)^{a}
BGB %N	0.90 (0.82-0.99)^{a}	0.84 (0.70-0.98)^{a}	0.83 (0.75-0.91)^{a}	0.94 (0.86-1.03)^{a}	0.97 (0.75-1.19)^{a}	0.90 (0.61-1.20)^{a}
BGB C:N	57.9 (52.1-63.6)^{a}	64.0 (54.1-73.8)^{a}	64.8 (56.6-73.0)^{a}	56.9 (51.3-62.4)^{a}	50.4 (44.8-55.9)^{a}	52.2 (39.7-64.7)^{a}
Microbial C process rates						
*log C Min (µmol g C^{-1} hr^{-1})	2.04 (1.59-2.49)^{a}	1.89 (1.66-2.12)^{a}	1.37 (0.81-1.92)^{ab}	1.69 (1.31-2.07)^{a}	0.55 (0.33-0.77)^{b}	0.68 (0.13-1.23)^{b}
*log SIR (µmol g C^{-1} hr^{-1})	4.62 (4.34-4.91)^{a}	4.32 (3.89-4.76)^{a}	4.09 (3.73-4.45)^{ab}	4.12 (3.79-4.45)^{a}	3.34 (2.91-3.77)^{b}	3.66 (3.27-4.04)^{ab}

Table 3.

Model	K	AICc	Δ AICc	Weight
Vegetation	3	91.63	0.00	0.54
Carbon Density + Belowground	4	93.98	2.35	0.17
Carbon Density + EC	4	95.03	3.40	0.10
Belowground	3	95.41	3.78	0.08
EC	3	97.01	5.38	0.04
pH + Belowground	4	97.90	6.27	0.02
pH + EC	4	98.38	6.75	0.02
EC + Belowground	4	99.46	7.82	0.01
Aboveground + Belowground	4	99.64	8.01	0.01
EC + Aboveground	4	101.38	9.75	0.00
Belowground + NH_4^+	4	101.87	10.24	0.00
Carbon Density + NH_4^+	4	102.84	11.21	0.00
Belowground + Belowground C:N	4	103.30	11.67	0.00
Carbon Density	3	103.37	11.74	0.00
Carbon Density + Belowground C:N	4	103.49	11.86	0.00
EC + NH_4^+	4	103.97	12.34	0.00
Carbon Density + Aboveground	4	105.03	13.40	0.00
EC + Belowground C:N	4	105.96	14.33	0.00
Belowground + Aboveground C:N	4	106.02	14.39	0.00
Carbon Density + pH	4	106.79	15.15	0.00
EC + Aboveground C:N	4	107.55	15.92	0.00
Aboveground	3	108.26	16.63	0.00
Aboveground + NH_4^+	4	109.01	17.38	0.00
Belowground C:N	3	109.12	17.49	0.00
NH_4^+	3	109.36	17.73	0.00
Aboveground + Belowground C:N	4	110.30	18.67	0.00
pH	3	111.54	19.91	0.00
Restoration	3	111.62	19.99	0.00
pH + Aboveground	4	111.87	20.24	0.00
Carbon Density + Aboveground C:N	4	112.14	20.51	0.00
pH + Belowground C:N	4	112.63	21.00	0.00
pH + NH_4^+	4	112.84	21.21	0.00
NH_4^++ Belowground C:N	4	113.57	21.94	0.00
Aboveground C:N	3	116.82	25.19	0.00
Aboveground + Aboveground C:N	4	117.96	26.33	0.00
NH_4^++ Aboveground C:N	4	117.97	26.34	0.00
Aboveground C:N + Belowground C:N	4	119.14	27.51	0.00
pH + Aboveground C:N	4	120.20	28.57	0.00

Table 4.

Model	K	AICc	Δ AICc	Weight
EC + Carbon Density	4	79.48	0.00	0.31
Vegetation	3	79.64	0.16	0.29
EC	3	80.38	0.89	0.20
Carbon Density + Belowground	4	82.38	2.90	0.07
pH + EC	4	84.58	5.09	0.02
EC + Belowground C:N	4	84.82	5.34	0.02
Belowground	3	84.94	5.45	0.02
EC + Aboveground	4	85.13	5.64	0.02
EC + NH_4^+	4	85.62	6.14	0.01
EC + Belowground	4	85.69	6.20	0.01
Aboveground + Belowground	4	88.71	9.23	0.00
pH + Belowground	4	88.92	9.43	0.00
Belowground + NH_4^+	4	90.55	11.06	0.00
Belowground + Belowground C:N	4	90.82	11.34	0.00
Carbon Density + Aboveground	4	91.19	11.70	0.00
EC + Aboveground C:N	4	91.38	11.89	0.00
Aboveground + NH_4^+	4	91.66	12.18	0.00
Carbon Density	3	92.54	13.06	0.00
Aboveground	3	92.59	13.11	0.00
Carbon Density + NH_4^+	4	92.66	13.17	0.00
Carbon Density + Belowground C:N	4	92.86	13.38	0.00
Aboveground + Belowground C:N	4	93.14	13.65	0.00
NH_4^+	3	93.85	14.37	0.00
Belowground C:N	3	93.89	14.40	0.00
Carbon Density + pH	4	95.06	15.58	0.00
pH + Aboveground	4	95.20	15.71	0.00
Belowground + Aboveground C:N	4	95.78	16.30	0.00
pH	3	96.29	16.80	0.00
pH + NH_4^+	4	96.93	17.45	0.00
pH + Belowground C:N	4	96.97	17.48	0.00
NH_4^++ Belowground C:N	4	97.53	18.04	0.00
Restoration	3	97.65	18.17	0.00
Carbon Density + Aboveground C:N	4	102.80	23.32	0.00
Aboveground + Aboveground C:N	4	103.31	23.83	0.00
Aboveground C:N	3	104.04	24.56	0.00
NH_4^++ Aboveground C:N	4	104.32	24.83	0.00
Aboveground C:N + Belowground C:N	4	104.71	25.23	0.00
pH + Aboveground C:N	4	106.06	26.58	0.00

Figure Legends.

Figure 1. Location of 20 tidal marshes along the Connecticut (CT) coast sampled during August, 2017; 10 tidally-restored (black circles) and 10 unrestricted (grey triangles) sites were sampled. Within each site, we collected samples from three dominant vegetation zones: *Spartina alterniflora*, *S. patens*, and *Phragmites australis*. Sites spanned the Connecticut coastline; our westernmost site was located in Westport, CT and our easternmost site was located in Stonington, CT. Salt marshes are less abundant along the urban western shoreline of CT, thus our sampling intensity was greater along the eastern shoreline.

Figure 2. Boxplots of laboratory assays of (A) carbon mineralization and (B) substrate-induced respiration (SIR) for soils collected from three dominant vegetation zones – *Spartina alterniflora*, *Spartina patens*, and *Phragmites australis* – in coastal marshes in Connecticut, USA (n = 20 sites). SIR had rates an order of magnitude higher than carbon mineralization due to the addition of yeast to all samples. Both responses demonstrate a similar pattern with greatest mineralization rates found in *S. alterniflora* zones and the lowest in *P. australis*.

Figure 3. Non-metric multidimensional scaling plot of ordinal distances between 60 sediment samples for microbial communities (20 sites x three vegetation zones) with a stress level of 0.19. Ellipses represent 95% confidence intervals around each vegetation zone's data based on a multivariate t-distribution. Ellipses demonstrate that the structure of microbial communities may be influenced by the conditions of each of the dominant vegetation zones, although there is clearly a lot of overlap.

Figure 4. Relative abundance of operational taxonomic units (OTUs) within the three vegetation zones. All data points represented in this figure correspond to a OTUs that were significantly more abundant in one of the vegetation zones than the others. Bacteria from the *S. alterniflora* zones had greater relative abundance than the other vegetation zones. Bacteria from Bacteroidetes and Proteobacteria phyla were often the differentiating phyla among vegetation zones.

Figure 1.

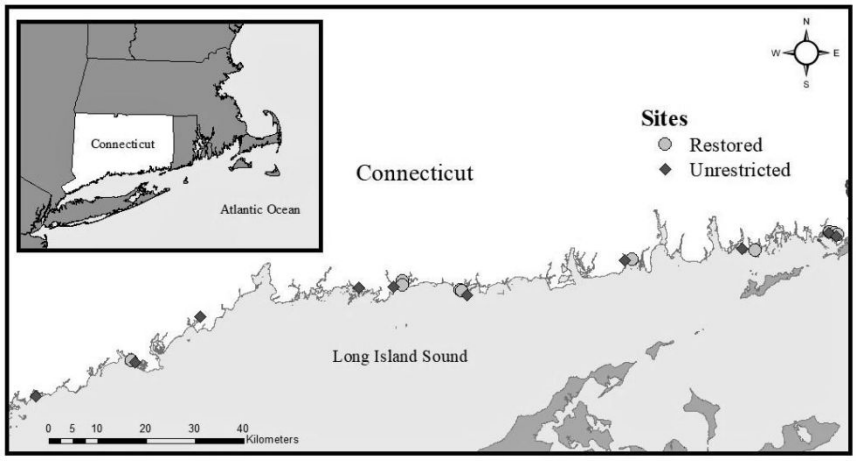


Figure 2.

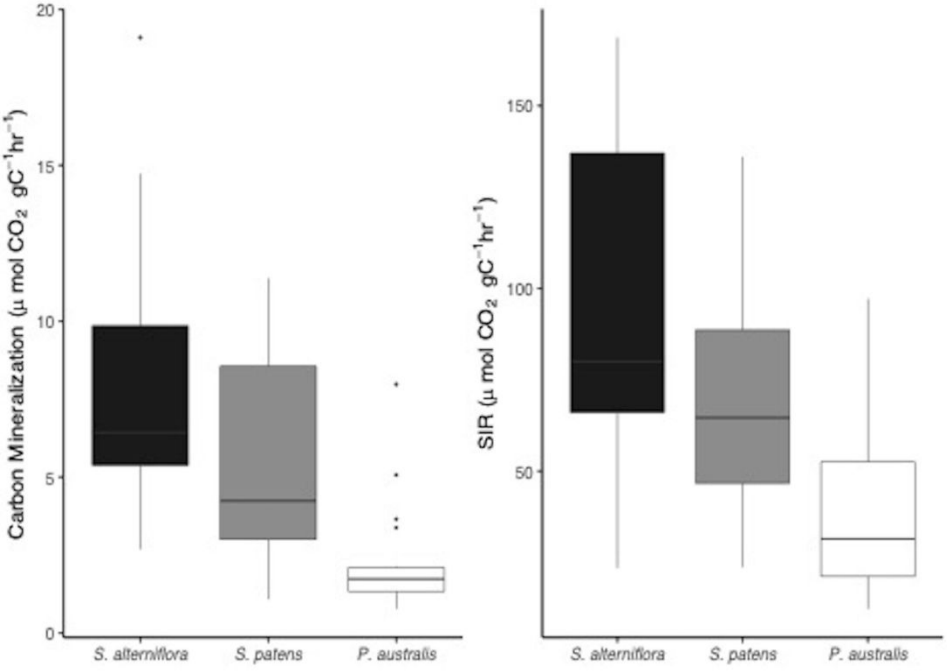


Figure 3.

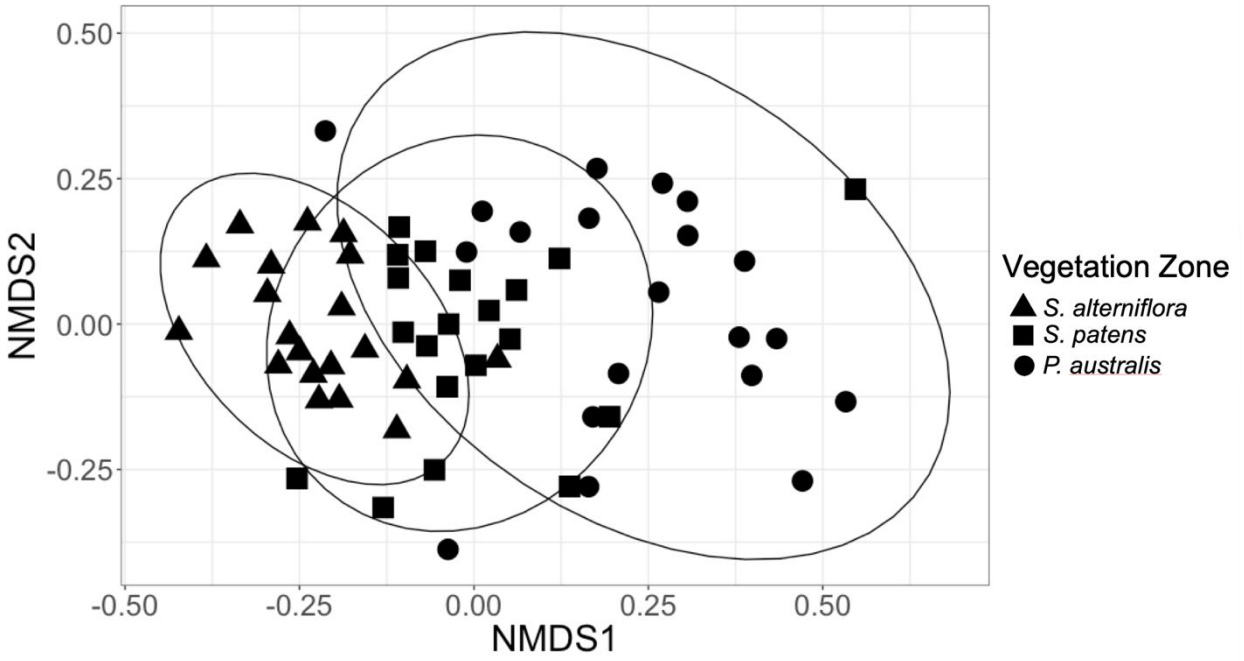
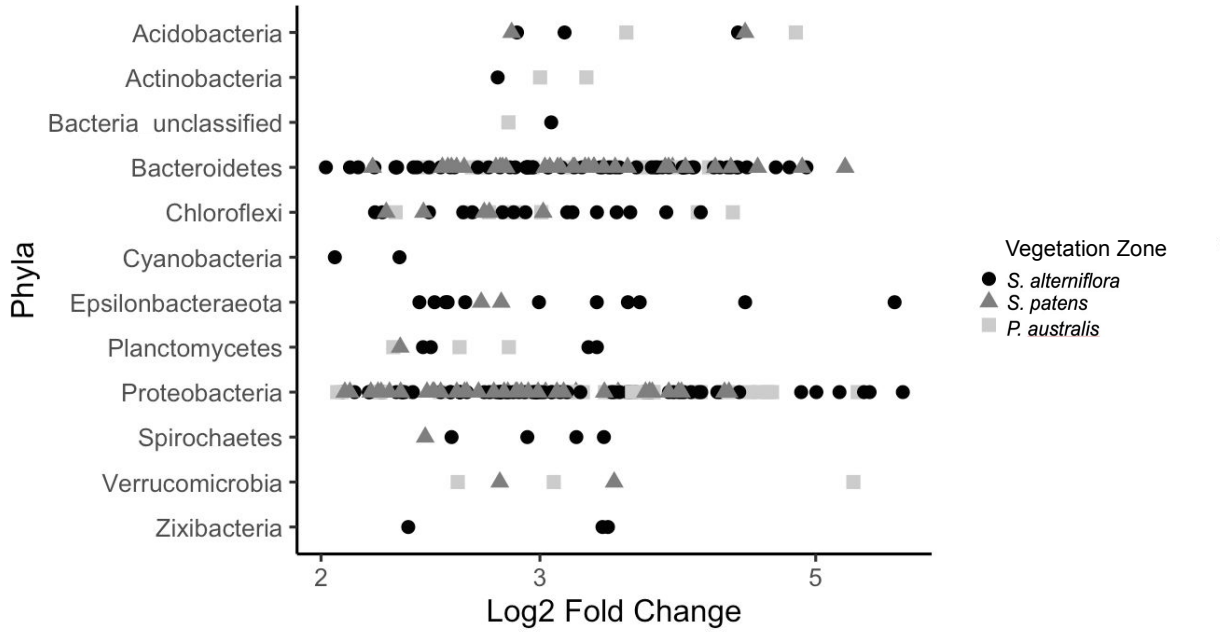


Figure 4.



APPENDICES/SUPPLEMENTAL MATERIAL

Appendix 1. List of differentially abundant operational taxonomic units with taxonomic information down to the genus level. Each OTU was found to be significantly elevated in abundance in one of the three vegetation zones.

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Vegetation Zone
Otu00002	Bacteria	Cyanobacteria	Oxyphotobacteria	Chloroplast	Chloroplast_fa	Chloroplast_ge	*S. alterniflora*
Otu00003	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. alterniflora*
Otu00005	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfosarcina	*S. alterniflora*
Otu00005	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfosarcina	*S. patens*
Otu00006	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Chromatiaceae_unclassified	*S. alterniflora*
Otu00006	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Chromatiaceae_unclassified	*S. alterniflora*
Otu00007	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00007	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. patens*
Otu00008	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Prolixibacteraceae_unclassified	*S. alterniflora*
Otu00008	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Prolixibacteraceae_unclassified	*S. patens*
Otu00009	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Sva0081_sediment_group	*S. alterniflora*
Otu00009	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Sva0081_sediment_group	*S. patens*
Otu00010	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidetes_VC2.1_Bac22	Bacteroidetes_VC2.1_Bac22_fa	Bacteroidetes_VC2.1_Bac22_ge	*S. alterniflora*
Otu00010	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidetes_VC2.1_Bac22	Bacteroidetes_VC2.1_Bac22_fa	Bacteroidetes_VC2.1_Bac22_ge	*S. patens*
Otu00011	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	uncultured	*S. alterniflora*
Otu00012	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00012	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. patens*
Otu00016	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Halieaceae	Halieaceae_unclassified	*S. alterniflora*
Otu00016	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Halieaceae	Halieaceae_unclassified	*S. patens*
Otu00017	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	Desulfatiglans	*S. alterniflora*
Otu00017	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	Desulfatiglans	*S. patens*

Otu00019	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Chromatiaceae_unclassified	*S. alterniflora*
Otu00021	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. alterniflora*
Otu00021	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. patens*
Otu00022	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*S. patens*
Otu00024	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	*S. alterniflora*
Otu00024	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	*S. patens*
Otu00025	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*S. patens*
Otu00027	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Prolixibacteraceae_ge	*S. alterniflora*
Otu00027	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Prolixibacteraceae_ge	*S. patens*
Otu00028	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfatitalea	*S. alterniflora*
Otu00028	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfatitalea	*S. patens*
Otu00030	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*S. alterniflora*
Otu00030	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*S. patens*
Otu00031	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium	*S. alterniflora*
Otu00031	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium	*S. patens*
Otu00032	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Sedimenticolaceae	Candidatus_Thiodiazotropha	*S. alterniflora*
Otu00032	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Sedimenticolaceae	Candidatus_Thiodiazotropha	*S. alterniflora*
Otu00033	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Sulfurovaceae	Sulfurovum	*S. alterniflora*
Otu00033	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Sulfurovaceae	Sulfurovum	*S. alterniflora*
Otu00033	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Sulfurovaceae	Sulfurovum	*S. patens*
Otu00034	Bacteria	Proteobacteria	Gammaproteobacteria	Ectothiorhodospirales	Ectothiorhodospiraceae	Thiogranum	*S. patens*
Otu00035	Bacteria	Proteobacteria	Gammaproteobacteria	B2M28	B2M28_fa	B2M28_ge	*S. patens*
Otu00039	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Oleiagrimonas	*S. alterniflora*
Otu00039	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Oleiagrimonas	*S. patens*
Otu00040	Bacteria	Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup_23	*S. alterniflora*
Otu00040	Bacteria	Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup_23	*S. patens*
Otu00044	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*

Otu00045	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	uncultured	*S. alterniflora*
Otu00045	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	uncultured	*S. patens*
Otu00047	Bacteria	Proteobacteria	Gammaproteobacteria	R7C24	R7C24_fa	R7C24_ge	*P. australis*
Otu00048	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Betaproteobacteriales_unclassified	Betaproteobacteriales_unclassified	*S. patens*
Otu00048	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Betaproteobacteriales_unclassified	Betaproteobacteriales_unclassified	*P. australis*
Otu00049	Bacteria	Proteobacteria	Gammaproteobacteria	Acidithiobacillales	Acidithiobacillaceae	9M32	*S. patens*
Otu00051	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	Desulfatiglans	*S. alterniflora*
Otu00051	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	Desulfatiglans	*S. patens*
Otu00053	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Fulvivirga	*S. alterniflora*
Otu00053	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Fulvivirga	*S. patens*
Otu00057	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*S. alterniflora*
Otu00058	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Robertkochia	*S. alterniflora*
Otu00058	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Robertkochia	*S. patens*
Otu00059	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00059	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00060	Bacteria	Cyanobacteria	Oxyphotobacteria	Chloroplast	Chloroplast_fa	Chloroplast_ge	*S. alterniflora*
Otu00061	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidetes_VC2.1_Bac22	Bacteroidetes_VC2.1_Bac22_fa	Bacteroidetes_VC2.1_Bac22_ge	*S. alterniflora*
Otu00061	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidetes_VC2.1_Bac22	Bacteroidetes_VC2.1_Bac22_fa	Bacteroidetes_VC2.1_Bac22_ge	*S. patens*
Otu00062	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*S. alterniflora*
Otu00064	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	SB-5	SB-5_ge	*S. alterniflora*
Otu00064	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	SB-5	SB-5_ge	*S. patens*
Otu00066	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*S. patens*
Otu00067	Bacteria	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Pedosphaeraceae_ge	*S. patens*
Otu00067	Bacteria	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Pedosphaeraceae_ge	*P. australis*
Otu00067	Bacteria	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Pedosphaeraceae_ge	*P. australis*
Otu00068	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriales_unclassified	Ignavibacteriales_unclassified	*S. patens*

Otu00069	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Sedimenticolaceae	uncultured	*S. alterniflora*
Otu00069	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Sedimenticolaceae	uncultured	*S. patens*
Otu00071	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacteraceae_unclassified	*S. alterniflora*
Otu00071	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacteraceae_unclassified	*S. patens*
Otu00073	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. alterniflora*
Otu00073	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. patens*
Otu00075	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. alterniflora*
Otu00075	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. patens*
Otu00078	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium	*S. alterniflora*
Otu00078	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium	*S. patens*
Otu00079	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriales_unclassified	Ignavibacteriales_unclassified	*S. patens*
Otu00080	Bacteria	Proteobacteria	Gammaproteobacteria	CCD24	CCD24_fa	CCD24_ge	*S. patens*
Otu00080	Bacteria	Proteobacteria	Gammaproteobacteria	CCD24	CCD24_fa	CCD24_ge	*P. australis*
Otu00081	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolineaceae_unclassified	*S. alterniflora*
Otu00081	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolineaceae_unclassified	*S. patens*
Otu00083	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	Desulfatiglans	*S. alterniflora*
Otu00084	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	*S. alterniflora*
Otu00084	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	*S. patens*
Otu00086	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. alterniflora*
Otu00086	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. patens*
Otu00092	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacteraceae_unclassified	*S. alterniflora*
Otu00092	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacteraceae_unclassified	*S. patens*
Otu00093	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	uncultured	*S. alterniflora*
Otu00093	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	uncultured	*S. patens*
Otu00094	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	*S. alterniflora*
Otu00100	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	MidBa8	MidBa8_ge	*S. alterniflora*
Otu00101	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	BIrii41_ge	*S. alterniflora*

Otu00101	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	BIrii41_ge	*S. patens*
Otu00103	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Arcobacteraceae	Arcobacter	*S. alterniflora*
Otu00103	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Arcobacteraceae	Arcobacter	*S. alterniflora*
Otu00105	Bacteria	Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	TPD-58	*S. alterniflora*
Otu00105	Bacteria	Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	TPD-58	*S. patens*
Otu00106	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	uncultured	*S. alterniflora*
Otu00106	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	uncultured	*S. patens*
Otu00108	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00108	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. patens*
Otu00110	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Prolixibacter	*S. alterniflora*
Otu00110	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Prolixibacter	*S. patens*
Otu00111	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*P. australis*
Otu00112	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Thiovulaceae	Sulfurimonas	*S. alterniflora*
Otu00112	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Thiovulaceae	Sulfurimonas	*S. patens*
Otu00113	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbaceae_unclassified	*S. alterniflora*
Otu00113	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbaceae_unclassified	*S. patens*
Otu00114	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloceanibacter	*S. alterniflora*
Otu00114	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloceanibacter	*S. patens*
Otu00115	Bacteria	Planctomycetes	Phycisphaerae	MSBL9	SG8-4	SG8-4_ge	*S. alterniflora*
Otu00115	Bacteria	Planctomycetes	Phycisphaerae	MSBL9	SG8-4	SG8-4_ge	*S. patens*
Otu00116	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	Desulfatiglans	*S. alterniflora*
Otu00118	Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	uncultured	*S. patens*
Otu00119	Bacteria	Proteobacteria	Gammaproteobacteria	Nitrosococcales	Nitrosococcaceae	Nitrosococcaceae_unclassified	*S. patens*
Otu00119	Bacteria	Proteobacteria	Gammaproteobacteria	Nitrosococcales	Nitrosococcaceae	Nitrosococcaceae_unclassified	*P. australis*
Otu00121	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	BIrii41_ge	*P. australis*
Otu00122	Bacteria	Chloroflexi	KD4-96	KD4-96_or	KD4-96_fa	KD4-96_ge	*S. alterniflora*

Otu00122	Bacteria	Chloroflexi	KD4-96	KD4-96_or	KD4-96_fa	KD4-96_ge	*S. patens*
Otu00124	Bacteria	Proteobacteria	Gammaproteobacteria	Nitrosococcales	Nitrosococcaceae	Nitrosococcaceae_unclassified	*P. australis*
Otu00125	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00130	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Thiovulaceae	Sulfurimonas	*S. alterniflora*
Otu00132	Bacteria	Acidobacteria	Subgroup_6	Subgroup_6_or	Subgroup_6_fa	Subgroup_6_ge	*P. australis*
Otu00132	Bacteria	Acidobacteria	Subgroup_6	Subgroup_6_or	Subgroup_6_fa	Subgroup_6_ge	*P. australis*
Otu00133	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. alterniflora*
Otu00133	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. patens*
Otu00134	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Crocinitomicaceae	uncultured	*S. alterniflora*
Otu00134	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Crocinitomicaceae	uncultured	*S. patens*
Otu00135	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacteraceae_unclassified	*P. australis*
Otu00136	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Thiohalocapsa	*S. alterniflora*
Otu00138	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	uncultured	*S. alterniflora*
Otu00138	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	uncultured	*S. patens*
Otu00139	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	SB-5	SB-5_ge	*S. alterniflora*
Otu00143	Bacteria	Proteobacteria	Gammaproteobacteria	EPR3968-O8a-Bc78	EPR3968-O8a-Bc78_fa	EPR3968-O8a-Bc78_ge	*P. australis*
Otu00144	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00145	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	Cryomorphaceae_unclassified	*S. alterniflora*
Otu00145	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	Cryomorphaceae_unclassified	*S. patens*
Otu00148	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	uncultured	*S. alterniflora*
Otu00149	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. alterniflora*
Otu00151	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium	*S. alterniflora*
Otu00151	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium	*S. patens*
Otu00155	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	*S. alterniflora*
Otu00156	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaeta_2	*S. alterniflora*
Otu00157	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Rubripirellula	*S. alterniflora*
Otu00159	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. alterniflora*
Otu00163	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	uncultured	*S. patens*

Otu00163	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	uncultured	*P. australis*
Otu00164	Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	*P. australis*
Otu00165	Bacteria	Proteobacteria	Alphaproteobacteria	Kordiimonadales	uncultured	uncultured_ge	*S. alterniflora*
Otu00167	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaetaceae_unclassified	*S. alterniflora*
Otu00167	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaetaceae_unclassified	*S. patens*
Otu00168	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloceanibacter	*S. patens*
Otu00169	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00170	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Draconibacterium	*S. alterniflora*
Otu00170	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Draconibacterium	*S. patens*
Otu00171	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. alterniflora*
Otu00172	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	Lentimicrobiaceae_ge	*S. alterniflora*
Otu00172	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	Lentimicrobiaceae_ge	*S. patens*
Otu00173	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacteraceae_unclassified	*S. alterniflora*
Otu00175	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	SB-5	SB-5_ge	*S. alterniflora*
Otu00176	Bacteria	Chloroflexi	Anaerolineae	Ardenticatenales	uncultured	uncultured_ge	*S. alterniflora*
Otu00176	Bacteria	Chloroflexi	Anaerolineae	Ardenticatenales	uncultured	uncultured_ge	*S. patens*
Otu00177	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	Lentimicrobiaceae_ge	*S. alterniflora*
Otu00181	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Chromatiaceae_unclassified	*S. alterniflora*
Otu00181	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Chromatiaceae_unclassified	*S. alterniflora*
Otu00182	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobiaceae_unclassified	*S. alterniflora*
Otu00184	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	67-14_ge	*P. australis*
Otu00187	Bacteria	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	Thiomicrospiraceae_unclassified	*S. alterniflora*
Otu00190	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	Chryseolinea	*P. australis*
Otu00193	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	Sandaracinaceae_unclassified	*S. patens*
Otu00197	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	A4b_ge	*S. alterniflora*
Otu00199	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	67-14_ge	*S. alterniflora*
Otu00200	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	A4b_ge	*S. alterniflora*
Otu00200	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	A4b_ge	*S. patens*

Otu00201	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	*S. alterniflora*
Otu00203	Bacteria	Proteobacteria	Gammaproteobacteria	EPR3968-O8a-Bc78	EPR3968-O8a-Bc78_fa	EPR3968-O8a-Bc78_ge	*P. australis*
Otu00204	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Thiovulaceae	Sulfurimonas	*S. alterniflora*
Otu00206	Bacteria	Proteobacteria	Gammaproteobacteria	KI89A_clade	KI89A_clade_fa	KI89A_clade_ge	*S. alterniflora*
Otu00206	Bacteria	Proteobacteria	Gammaproteobacteria	KI89A_clade	KI89A_clade_fa	KI89A_clade_ge	*S. patens*
Otu00207	Bacteria	Proteobacteria	Gammaproteobacteria	Arenicellales	Arenicellaceae	Candidatus_Thiosymbion	*S. alterniflora*
Otu00207	Bacteria	Proteobacteria	Gammaproteobacteria	Arenicellales	Arenicellaceae	Candidatus_Thiosymbion	*S. patens*
Otu00209	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Prolixibacteraceae_unclassified	*S. alterniflora*
Otu00211	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriales_unclassified	Ignavibacteriales_unclassified	*S. alterniflora*
Otu00212	Bacteria	Chloroflexi	Anaerolineae	SBR1031	SBR1031_fa	SBR1031_ge	*S. alterniflora*
Otu00213	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	SB-5	SB-5_ge	*S. alterniflora*
Otu00214	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	Thiobacillus	*P. australis*
Otu00216	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	MND1	*P. australis*
Otu00216	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	MND1	*P. australis*
Otu00217	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfococcus	*S. alterniflora*
Otu00217	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfococcus	*S. patens*
Otu00220	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cytophagales_unclassified	Cytophagales_unclassified	*P. australis*
Otu00221	Bacteria	Zixibacteria	Zixibacteria_cl	Zixibacteria_or	Zixibacteria_fa	Zixibacteria_ge	*S. alterniflora*
Otu00222	Bacteria	Chloroflexi	KD4-96	KD4-96_or	KD4-96_fa	KD4-96_ge	*P. australis*
Otu00223	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. alterniflora*
Otu00224	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	*P. australis*
Otu00224	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	*P. australis*
Otu00226	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_unclassified	Rhizobiales_unclassified	*P. australis*
Otu00226	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_unclassified	Rhizobiales_unclassified	*P. australis*
Otu00227	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. alterniflora*
Otu00229	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*S. patens*
Otu00232	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	*S. alterniflora*

Otu00232	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	*S. patens*
Otu00233	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	uncultured	*P. australis*
Otu00236	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	uncultured	uncultured_ge	*P. australis*
Otu00238	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	uncultured	*S. alterniflora*
Otu00238	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	uncultured	*S. patens*
Otu00239	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	*P. australis*
Otu00239	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	*P. australis*
Otu00241	Bacteria	Proteobacteria	Gammaproteobacteria	KI89A_clade	KI89A_clade_fa	KI89A_clade_ge	*P. australis*
Otu00244	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	A4b_unclassified	*P. australis*
Otu00244	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	A4b_unclassified	*P. australis*
Otu00252	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Halieaceae	Halieaceae_unclassified	*S. alterniflora*
Otu00253	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	uncultured	*P. australis*
Otu00254	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	GOUTA6	*P. australis*
Otu00256	Bacteria	Proteobacteria	Gammaproteobacteria	Ectothiorhodospirales	Thioalkalispiraceae	Thioalkalispira	*S. alterniflora*
Otu00256	Bacteria	Proteobacteria	Gammaproteobacteria	Ectothiorhodospirales	Thioalkalispiraceae	Thioalkalispira	*S. patens*
Otu00258	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	SB-5	SB-5_ge	*S. alterniflora*
Otu00260	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Thiovulaceae	Sulfurimonas	*S. alterniflora*
Otu00261	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	uncultured	*P. australis*
Otu00263	Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiales_unclassified	Chromatiales_unclassified	*S. alterniflora*
Otu00265	Bacteria	Proteobacteria	Gammaproteobacteria	Tenderiales	Tenderiaceae	Candidatus_Tenderia	*S. alterniflora*
Otu00267	Bacteria	Actinobacteria	Acidimicrobiia	Actinomarinales	uncultured	uncultured_ge	*P. australis*
Otu00269	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosiaceae_unclassified	*S. alterniflora*
Otu00270	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Rhodopirellula	*P. australis*
Otu00272	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Nitrosomonadaceae_unclassified	*P. australis*
Otu00275	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Oricola	*S. alterniflora*
Otu00280	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	BIrii41_ge	*P. australis*
Otu00282	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	Saprospiraceae_unclassified	*S. alterniflora*

Otu00283	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	MidBa8	MidBa8_ge	*S. alterniflora*
Otu00284	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Thermomarinilinea	*S. alterniflora*
Otu00286	Bacteria	Chloroflexi	KD4-96	KD4-96_or	KD4-96_fa	KD4-96_ge	*S. alterniflora*
Otu00286	Bacteria	Chloroflexi	KD4-96	KD4-96_or	KD4-96_fa	KD4-96_ge	*S. patens*
Otu00289	Bacteria	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	*P. australis*
Otu00290	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	uncultured	*P. australis*
Otu00291	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriales_unclassified	Ignavibacteriales_unclassified	*P. australis*
Otu00292	Bacteria	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Pedosphaeraceae_ge	*S. patens*
Otu00293	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriales_unclassified	Ignavibacteriales_unclassified	*S. alterniflora*
Otu00293	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriales_unclassified	Ignavibacteriales_unclassified	*S. patens*
Otu00294	Bacteria	Zixibacteria	Zixibacteria_cl	Zixibacteria_or	Zixibacteria_fa	Zixibacteria_ge	*S. alterniflora*
Otu00296	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	Lentimicrobiaceae_ge	*S. alterniflora*
Otu00296	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	Lentimicrobiaceae_ge	*S. patens*
Otu00297	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Prolixibacteraceae_unclassified	*S. alterniflora*
Otu00303	Bacteria	Zixibacteria	Zixibacteria_cl	Zixibacteria_or	Zixibacteria_fa	Zixibacteria_ge	*S. alterniflora*
Otu00306	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00308	Bacteria	Bacteroidetes	Ignavibacteria	Kryptoniales	MSB-3C8	MSB-3C8_ge	*S. alterniflora*
Otu00309	Bacteria	Chloroflexi	Anaerolineae	SBR1031	SBR1031_fa	SBR1031_ge	*S. alterniflora*
Otu00310	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidetes_VC2.1_Bac22	Bacteroidetes_VC2.1_Bac22_fa	Bacteroidetes_VC2.1_Bac22_ge	*S. alterniflora*
Otu00312	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbaceae_unclassified	*S. alterniflora*
Otu00313	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes_BD2-2	Bacteroidetes_BD2-2_ge	*S. alterniflora*
Otu00315	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. alterniflora*
Otu00316	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Thiovulaceae	Sulfurimonas	*S. alterniflora*
Otu00318	Bacteria	Proteobacteria	Gammaproteobacteria	KI89A_clade	KI89A_clade_fa	KI89A_clade_ge	*P. australis*
Otu00323	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	WCHB1-32	*S. alterniflora*
Otu00323	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	WCHB1-32	*S. patens*
Otu00325	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobiaceae_unclassified	*P. australis*

Otu00329	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	BIrii41_ge	*P. australis*
Otu00332	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. alterniflora*
Otu00334	Bacteria	Proteobacteria	Zetaproteobacteria	Mariprofundales	Mariprofundaceae	Mariprofundus	*S. patens*
Otu00339	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. alterniflora*
Otu00341	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Thiovulaceae	Sulfurimonas	*S. alterniflora*
Otu00343	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	uncultured	*P. australis*
Otu00346	Bacteria	Chloroflexi	Anaerolineae	SBR1031	SBR1031_fa	SBR1031_ge	*S. alterniflora*
Otu00348	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	*S. alterniflora*
Otu00349	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00351	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Candidatus_Electrothrix	*S. alterniflora*
Otu00354	Bacteria	Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	Candidatus_Alysiosphaera	*S. alterniflora*
Otu00363	Bacteria	Planctomycetes	OM190	OM190_or	OM190_fa	OM190_ge	*S. alterniflora*
Otu00365	Bacteria	Proteobacteria	Gammaproteobacteria	EPR3968-O8a-Bc78	EPR3968-O8a-Bc78_fa	EPR3968-O8a-Bc78_ge	*P. australis*
Otu00366	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Arcobacteraceae	Arcobacter	*S. alterniflora*
Otu00369	Bacteria	Chloroflexi	Dehalococcoidia	S085	S085_fa	S085_ge	*P. australis*
Otu00370	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	MidBa8	MidBa8_ge	*S. alterniflora*
Otu00375	Bacteria	Chloroflexi	Anaerolineae	Ardenticatenales	uncultured	uncultured_ge	*S. alterniflora*
Otu00376	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	Melioribacteraceae	IheB3-7	*S. alterniflora*
Otu00378	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Phaselicystidaceae	Phaselicystis	*S. alterniflora*
Otu00378	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Phaselicystidaceae	Phaselicystis	*S. patens*
Otu00380	Bacteria	Chloroflexi	Gitt-GS-136	Gitt-GS-136_or	Gitt-GS-136_fa	Gitt-GS-136_ge	*P. australis*
Otu00381	Bacteria	Proteobacteria	Gammaproteobacteria	Ectothiorhodospirales	Thioalkalispiraceae	Thioalkalispiraceae_unclassified	*S. alterniflora*
Otu00385	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbaceae_unclassified	*S. alterniflora*
Otu00390	Bacteria	Proteobacteria	Deltaproteobacteria	RCP2-54	RCP2-54_fa	RCP2-54_ge	*P. australis*
Otu00390	Bacteria	Proteobacteria	Deltaproteobacteria	RCP2-54	RCP2-54_fa	RCP2-54_ge	*P. australis*
Otu00395	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	Saprospiraceae_unclassified	*S. alterniflora*
Otu00398	Bacteria	Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	Candidatus_Alysiosphaera	*S. alterniflora*

Otu00402	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodopirillaceae	Defluviicoccus	*S. alterniflora*
Otu00404	Bacteria	Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	Alterococcus	*P. australis*
Otu00417	Bacteria	Chloroflexi	Anaerolineae	SBR1031	SBR1031_fa	SBR1031_ge	*S. alterniflora*
Otu00418	Bacteria	Chloroflexi	Anaerolineae	Ardenticatenales	uncultured	uncultured_ge	*S. alterniflora*
Otu00421	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_unclassified	Rhizobiales_unclassified	*S. alterniflora*
Otu00422	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonadaceae_unclassified	*S. alterniflora*
Otu00425	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	A4b_ge	*P. australis*
Otu00436	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	P3OB-42	P3OB-42_ge	*S. alterniflora*
Otu00440	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Sediminispirochaeta	*S. alterniflora*
Otu00444	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaetaceae_unclassified	*S. alterniflora*
Otu00445	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacteraceae_unclassified	*S. alterniflora*
Otu00446	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	*P. australis*
Otu00457	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	*S. alterniflora*
Otu00459	Bacteria	Planctomycetes	Phycisphaerae	MSBL9	SG8-4	SG8-4_ge	*S. alterniflora*
Otu00473	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	Lentimicrobiaceae_ge	*S. alterniflora*
Otu00474	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Burkholderiaceae_unclassified	*P. australis*
Otu00477	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified	*S. alterniflora*
Otu00485	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	PHOS-HE36	PHOS-HE36_ge	*P. australis*
Otu00493	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	*S. alterniflora*
Otu00494	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	BIrii41_ge	*P. australis*
Otu00502	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	mle1-7	*P. australis*
Otu00508	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified	*S. alterniflora*
Otu00510	Bacteria	Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup_23	*S. alterniflora*
Otu00525	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	*S. alterniflora*
Otu00537	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacteraceae_unclassified	*S. alterniflora*
Otu00540	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	A4b_ge	*P. australis*
Otu00557	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	BIrii41_ge	*P. australis*

Otu00567	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	uncultured	*P. australis*
Otu00572	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pir4_lineage	*P. australis*
Otu00580	Bacteria	Planctomycetes	OM190	OM190_or	OM190_fa	OM190_ge	*P. australis*
Otu00596	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	A4b_ge	*P. australis*
Otu00605	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Xanthobacteraceae_unclassified	*P. australis*
Otu00614	Bacteria	Chloroflexi	OLB14	OLB14_or	OLB14_fa	OLB14_ge	*P. australis*

Tidally restored salt marsh at the Barn Island Wildlife Management Area (Stonington, Connecticut, USA)

564x423mm (72 x 72 DPI)

PLOS ONE

Nitrogen enrichment stimulates wetland plant responses whereas salt amendments alter sediment microbial communities and biogeochemical responses

--Manuscript Draft--

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Full Title:	Nitrogen enrichment stimulates wetland plant responses whereas salt amendments alter sediment microbial communities and biogeochemical responses
Short Title:	Experimental wetlands: N, road- and sea-salt effects on vegetation, biogeochemistry, and microbial responses
Corresponding Author:	Beth Lawrence University of Connecticut Storrs, CT UNITED STATES
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Abstract:	Freshwater wetlands of the temperate north are exposed to a range of pollutants that may alter their function, including nitrogen (N)-rich agricultural and urban runoff, seawater intrusion, and road salt contamination, though it is largely unknown how these drivers of change interact with the vegetation to affect wetland carbon (C) fluxes and microbial communities. We implemented a full factorial mesocosm (378.5 L tanks) experiment investigating C-related responses to three common wetland plants of eastern North America (Phragmites australis , Spartina pectinata , Typha latifolia), and four water quality treatments (fresh water control, N, road salt, sea salt). During the 2017 growing season, we quantified carbon dioxide (CO 2) and methane (CH 4) fluxes, above- and below-ground biomass, root porosity, light penetration, pore water chemistry (NH 4 + , NO 3 - , SO 4 - ², Cl - , DOC), soil C mineralization, as well as sediment microbial communities via 16S rRNA gene sequencing. Relative to freshwater controls, N enrichment stimulated plant biomass, which in turn increased CO 2 uptake and reduced light penetration, especially in Spartina stands. Root porosity was not affected by water quality, but was positively correlated with CH 4 emissions, suggesting that plants can be important conduits for CH 4 from anoxic sediment to the atmosphere. Sediment microbial composition was largely unaffected by N addition, whereas salt amendments induced structural shifts, reduced sediment community diversity, and reduced C mineralization rates, presumably due to osmotic stress. Methane emissions were suppressed by sea salt, but not road salt, providing evidence for the additional chemical control (SO 4 -2 availability) on this microbial-mediated process. Thus, N may have stimulated plant activity while salting treatments preferentially enriched specific microbial populations. Together our findings underpin the utility of combining plant and microbial responses, and highlight the need for more integrative studies to predict the consequences of a changing environment on freshwater wetlands.
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Question	**Response**
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April 23, 2020

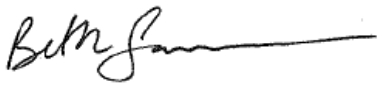
Dear Dr. Ana Lopes and PLOS ONE Reviewer Team,

Please consider the revised version of our research article (PONE-D-20-02931) titled "Nitrogen enrichment stimulates wetland plant responses whereas salt amendments alter sediment microbial communities and biogeochemical responses" for consideration for publication in PLOS ONE. We have addressed all editor and reviewer concerns, and detail how we have addressed them in our appended Response to Reviewers.

Also, Dr. Ana Lopes indicated that our manuscript was appropriate for the "Papers on the Microbial Ecology of Changing Environments" collection. We are interested in being considered for this collection, if that is still a possibility.

We greatly appreciate your time, thoughtful feedback, and consideration for publication. Please let me know if you have any questions about our manuscript.

Sincerely,



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Nitrogen enrichment stimulates wetland plant responses whereas salt amendments alter sediment microbial communities and biogeochemical responses

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Abstract

Freshwater wetlands of the temperate north are exposed to a range of pollutants that may alter their function, including nitrogen (N)-rich agricultural and urban runoff, seawater intrusion, and road salt contamination, though it is largely unknown how these drivers of change interact with the vegetation to affect wetland carbon (C) fluxes and microbial communities. We implemented a full factorial mesocosm (378.5 L tanks) experiment investigating C-related responses to three common wetland plants of eastern North America (*Phragmites australis*, *Spartina pectinata*, *Typha latifolia*), and four water quality treatments (fresh water control, N, road salt, sea salt). During the 2017 growing season, we quantified carbon dioxide (CO_2) and methane (CH_4) fluxes, above- and below-ground biomass, root porosity, light penetration, pore water chemistry (NH_4^+, NO_3^-, SO_4^{-2}, Cl^-, DOC), soil C mineralization, as well as sediment microbial communities via 16S rRNA gene sequencing. Relative to freshwater controls, N enrichment stimulated plant biomass, which in turn increased CO_2 uptake and reduced light penetration, especially in *Spartina* stands. Root porosity was not affected by water quality, but was positively correlated with CH_4 emissions, suggesting that plants can be important conduits for CH_4 from anoxic sediment to the atmosphere. Sediment microbial composition was largely unaffected by N addition, whereas salt amendments induced structural shifts, reduced sediment community diversity, and reduced C mineralization rates, presumably due to osmotic stress. Methane emissions were suppressed by sea salt, but not road salt, providing evidence for the additional chemical control (SO_4^{-2} availability) on this microbial-mediated process. Thus, N may have stimulated plant activity while salting treatments preferentially enriched specific microbial populations. Together our findings underpin the utility of combining plant and microbial responses, and highlight the need for more integrative studies to predict the consequences of a changing environment on freshwater wetlands.

Introduction

Wetlands play a disproportionate role in the global carbon (C) cycle; despite covering only 5-9% of the world's land surface [1], they store up to a third of terrestrial soil C [2,3] and contribute more than a third of global methane (CH_4) emissions, a potent greenhouse gas with 28-times the warming effect of CO_2 [4]. These highly productive ecosystems are increasingly dominated by monotypic graminoids [5] and have saturated soils that are key sites for anaerobic microbial processes. However, we currently have minimal understanding of how degraded water quality associated with anthropogenic activities affects the interactions among plant and microbial communities underlying wetland C processes.

Traits of dominant wetland macrophytes play an important role in wetland C cycling. Biomass production largely determines CO_2 assimilation rates and is often positively correlated with CH_4 emissions [6,7]. Plant allocation of resources belowground provides organic substrates to sediment microbial communities for anaerobic respiration [8,9], which can promote methanogenesis and increase CH_4 emissions [10]. However, the relationship between biomass and CH_4 emissions may not be so straightforward, as porous tissues of wetland plants (i.e., aerenchyma) link anoxic soil to the atmosphere; this could reduce net CH_4 emissions by promoting soil oxygenation via root-soil gas exchange [11,12], or increase net emissions by allowing CH_4 produced in underlying anoxic sediment to bypass oxidized surface sediments and waters [13,14]. Because root porosity varies among plant species and appears to be a plastic trait [15,16], we need to further elucidate its role in CH_4 emissions among common wetland plants subjected to impaired water quality.

Increasingly in the Anthropocene, wetland structure and function is determined by water quality because wetlands are "landscape sinks" [5] that accumulate materials and pollutants (e.g., nitrogen (N), salts) from watershed disturbances. Macrophytes such as species in the genera

Phragmites, *Spartina*, and *Typha* are well suited to invade and dominate wetlands [5, 17,18], thus changes in water quality associated with N enrichment or salt intrusion may give these plants a competitive advantage and indirectly affect C fluxes. For example, *Phragmites australis* is a salt-tolerant invader of brackish marshes and roadsides of eastern North America that tends to create large productive monocultures that have higher CH_4 emissions than native communities [19,20]. Similarly, N enrichment common in agricultural and urban landscapes promotes *Typha* dominance [21], whose invasion can increase soil CH_4 emissions [7]. Nitrogen enrichment promotes biomass production [22] with associated increases in CO_2 uptake, rhizosphere oxidation, C exudation, and microbial activity [23]. The consequent effects on CH_4 emissions are therefore mixed; in addition to the nuanced balance of oxygen and C inputs from increased biomass, the direct effect of increased N could favor other microbes over methanogens.

Elevated salinity associated with seawater intrusion and road deicing salts can induce osmotic stress, altering growth and composition of plant and microbial communities [24,25]. Further, saline conditions change the availability of terminal electron acceptors [26], and promote organic matter flocculation [27,28], which alter microbial respiration rates. Intrusion of sulfate-rich seawater into freshwater wetlands reduces soil CH_4 emissions, as sulfate reduction can be thermodynamically favored over methanogenesis [29,30]. Exponential usage of deicing salts, largely sodium chloride (NaCl) throughout the temperate north [31–33], has had severe ecological consequences [34,35]. Where water residence times are high, elevated concentrations of Na^+ can displace other cations (NH_4^+, Ca^+, K^+, Mg^+) through cation exchange [36], causing negative effects on biotic communities due to salt stress and altered nutrient availability [37,38]. However, the consequences of road salt pollution on wetland C emissions are less well understood, and may differ from those of seawater intrusion.

Our objective was to investigate how dominant wetland plants and common water quality impairments interact to alter components of freshwater wetland C cycling. We conducted a wetland mesocosm experiment during the 2016-2017 growing seasons to test how traits (i.e., biomass, root porosity) of three common wetland plants (*Phragmites australis, Spartina pectinata, Typha latifolia*, hereafter *Phragmites, Spartina, Typha*, respectively) and four water quality treatments (freshwater control, N, road salt, sea salt) interact to alter C gas fluxes (CO_2, CH_4, C mineralization) and sediment microbial communities.

Materials and methods

Experimental design

We implemented an outdoor mesocosm experiment at the University of Connecticut (Storrs, Connecticut, USA), consisting of 48 mesocosms which were 378.5 L plastic tanks (79 cm x 64 cm x 132 cm; Freeland Poly-Tuf Tank©; S1a Fig). In spring 2016, we planted monocultures of three wetland plant species (*Phragmites, Spartina, Typha*), and in 2017 we implemented four water quality treatments (freshwater control, N, road salt sea salt). We replicated each plant species-water quality treatment combination four-fold and randomly assigned treatments to the 48 mesocosms. We chose common wetland plants that occur throughout eastern North America and that vary in root porosity [16], biomass production, and salt tolerance; *Phragmites* and *Typha* tend to dominate fresh to brackish marshes, whereas *Spartina* is typically considered a freshwater grass, but occurs along the upland fringes of coastal marshes in eastern North America.

We filled the bottom of each mesocosm with 15 cm of sand, and then added 30 cm of commercially screened topsoil. In June 2016, we planted four, four-month old seedlings into

each mesocosm; we grew plants in a greenhouse using locally-collected, cold-stratified seed during spring 2016. Seedlings were allowed to establish during the 2016 growing season and were regularly watered to maintain saturated soils. In May 2017 we inoculated each mesocosm with 19 L of sediment collected from a nearby constructed freshwater wetland known to have methanogenic activity [39]. Water levels were maintained at 5 to 10 cm above the soil surface during the growing season (May-September) using ground water from a nearby well (pH: 7.12); water levels occasionally exceeded 10 cm after major rain events, but we ensured that water levels were consistent across tanks. Mesocosms were drained October-April when plants were dormant to prevent cracking of the plastic tubs during freezing conditions.

Water Quality Treatments

Water quality treatments (freshwater control, N, road salt, sea salt) were applied twice in 2017 (May, June). Powder forms of N and salt compounds were added to 1 L Nalgene bottles with 0.9 L of deionized (DI) water and shaken manually until fully dissolved. Once dissolved, solutions were poured evenly across assigned mesocosms; controls received 1 L of DI water. For the N treatment, we applied ammonium nitrate (NH_4NO_3) at a rate of 15 g N/year (two applications of 21.4 g of NH_4NO_3). We targeted a salinity of ~2 ppt for the two salt treatments, and during two application events added 300 g/year of dissolved salt (road salt: Diamond Crystal Winter Melt NaCl; sea salt: Instant Ocean® Sea Salt). Instant Ocean® is a commonly used saltwater aquarium additive with a similar chemical composition to seawater [40] and has been used to simulate seawater intrusion in other studies [41,42]. Treatment concentrations were selected based on previous experiments [43–45] as well as field measurements of salinity concentrations in Connecticut road-adjacent wetlands [38].

Response metrics & analysis

Carbon fluxes

We measured C fluxes during three sampling campaigns in 2017 (mid-July, August, and September; approximately one, two, and three months after the last dosing treatment). We used a Picarro G2201-*i* cavity ring-down spectrometer (Picarro Inc., Santa Clara, CA, USA) that measures CO_2 and CH_4 gas concentrations in real time (approximately every 3 s). A clear sampling chamber (base: 25 cm x 25 cm, height: 100 cm or 150 cm tall, depending on vegetation height) made of UV-resistant PVC film, and fitted with a vent tube, a sample port, and a fan to mix chamber air, was placed over a random quadrant of each mesocosm (S1b Fig). We connected the chamber to the Picarro gas analyzer via Swagelok® connections and Tygon® tubing, and deployed chambers for 10-minute incubations during daylight hours (10:00 to 16:00); an iButton temperature sensor (Maxim Integrated, San Jose, CA, USA) recorded in-chamber air temperature once every minute. Barometric pressure and ambient air temperature were also recorded, using a Kestrel 2500 Weather Meter (Nielsen-Kellerman, Boothwyn, PA, USA). Gas concentration measurements were corrected for the ideal gas law using temperature, pressure, and chamber volume. Flux rates were calculated based on linear changes in gas concentrations over time if R^2 values were > 0.85. For rates with R^2 values < 0.85, we visually inspected plots of concentration vs. time; rates that exhibited evidence or record of equipment malfunction (chamber tipping, etc.) or ebullition were removed from analysis (n = 5). If the linear regression of time vs. gas concentration did not differ from zero, we assigned the gas flux as zero.

Plant biomass & root porosity

We estimated aboveground biomass using species-specific allometric equations developed from ~50 oven-dried (65°C) stems of each species in 2016, relating stem height to dry

biomass; all equations were second order polynomials (*Typha*: $R^2 = 0.92$, 95% CI = ± 0.01 g; *Spartina*: $R^2 = 0.92$, 95% CI = ± 0.006 g; *Phragmites*: $R^2 = 0.94$, 95% CI = ± 0.004 g). All stem heights were measured in September 2017 to estimate aboveground biomass for each species-water quality treatment. Photosynthetically active radiation (PAR) measurements were taken at this time using a Decagon LP-80 Ceptometer (Decagon Devices, Pullman, WA, USA). Three measurements per mesocosm were averaged above the plant canopy and at the sediment surface to estimate the fraction of PAR (fPAR) transmitted through the canopy.

We installed in-growth root cores to measure 2017 root production in each tank (May-September 2017) [46]. Nylon mesh cylinders (5-cm diameter x 13-cm long) with a plastic base were packed with screened, root-free topsoil (same as that used to fill mesocosms) to a similar bulk density as the surrounding soil (~1.8 g dry soil/cm^3, average of 2016 soils). In-growth cores were installed into excavated holes of similar dimensions in May and were removed from the tanks by cutting around the outside of the core with a serrated knife and pulling it free of the tank sediment in September. Each core was emptied into a 2-mm sieve and soil was washed away with a garden hose to isolate the roots.

In the lab, we identified three root segments (~5 cm in length) per core that were elastic and light or white in color to estimate root porosity using methods similar to [15]. We blotted excess water from the outside of the roots with lab tissues then individually weighed each segment on a microbalance. To keep roots submerged under 500 mL of water in a 1 L side arm flask, we attached a paper clip to each root segment. The flask was attached to a vacuum pump for five minutes to replace all of the airspace in the root with water. The roots were removed from the water and weighed again. The difference of the two weights divided by the initial weight estimates the proportion of the root mass that was originally airspace. Roots sampled for

porosity were then returned to the bulk root sample from each mesocosm, dried ≧ 72 hours at 60°C, and weighed. Root porosity estimates for each mesocosm were averaged and then multiplied by belowground biomass to calculate total porosity. Belowground biomass estimates were calculated by scaling the mass of roots in the area of the ingrowth core to 1 m^2. Likewise, aboveground biomass estimates were scaled to units of g/m^2.

Water chemistry

We constructed wells to monitor and sample pore water chemistry. We cut 30-cm sections of PVC pipe (2.54-cm diameter), capped the bottom, sliced narrow slits to 7 cm, and wrapped 1-mm nylon screen around the slitted area to limit sediment intrusion. We pounded wells into the center of each tank to 15-cm depth. Conductivity, salinity, and porewater temperature measurements were taken during each gas sampling event using a YSI EcoSense® EC300A meter (YSI Incorporated, Yellow Springs, OH, USA). Pore water samples were taken from each mesocosm for analysis at the end of the growing season in September 2017. Pore water wells were purged and then water samples were collected with a nylon syringe and tubing and placed into acid-washed 50 mL centrifuge tubes. Samples were stored at 4°C until analysis. Water samples were centrifuged and filtered using 110-mm Whatman G/FF paper filters and analyzed for nitrate (NO_3^-) and ammonia (NH_4^+) on a SmartChem®200 discrete analyzer (Westco Scientific Instruments, Brookfield, CT, USA). Whatman G/FF-filtered samples were quantified for total organic carbon (TOC) on a Shimadzu Total Organic Carbon Analyzer using EPA Method 415.1, and sulfate (SO_4^{-2}) and chloride (Cl^-) on a Dionex Ion Chromatography System-1100 (Thermo Fisher Scientific, Waltham, MA, USA).

Carbon mineralization

Surface soil samples (5-cm diameter to 10-cm depth) were collected in September 2017 to estimate heterotrophic respiration rates. Soils were sieved through 2-mm brass screens; a 10-g subsample of sieved soil was dried at 105°C for 48 hours to calculate soil moisture content, and a 50-g subsample was placed in a 0.95-L canning jar. Jars were attached via a 15-port manifold sampling system to the Picarro G2201-*i* and their headspace CO_2 concentrations were sampled for six minutes approximately every four hours over a 24-hour period [47]; CO_2-free air soda lime blanks were used to flush the lines between samples. We converted gas accumulation rates measured as ppm/s to umol/s using the ideal gas law. Correcting for soil moisture content, we calculated C mineralization rates as the accumulation of gas over time per gram of dry soil.

Statistical Analysis

All statistical analyses were conducted using R Studio 1.1.419 using R 3.5.1. Data were log-transformed to improve normality of residuals and homogeneity of variances when necessary. We tested for fixed effects of plant species, water quality treatments, and their interaction on gas fluxes, biomass, total porosity, and water chemistry data using analyses of variance (ANOVA; *lme* and *aov* commands). Initial repeated measures ANOVA (*lme* command) indicated consistent treatment responses across our three sampling campaigns for both CO_2 ($F_{2,94}$ = 0.83, p = 0.439) and CH_4 fluxes ($F_{2,87}$ = 2.64, p = 0.077), thus we aggregated gas flux data into one data set for statistical analyses of gas flux response. We used only the September gas sampling campaign data to investigate relationships with the other variables as it aligned temporally with when we collected biomass and water chemistry data. We tested for correlations across the entire data set (i.e., no multivariate analysis separating by treatments due to limited sample size). Correlations between explanatory and response variables were analyzed using the *cor.test* command; we used Pearson's correlation coefficient (r) for parametric data and

Spearman rank correlation coefficients (r_s) for non-parametric data (i.e., when transformations did not improve normality). Means ± 1 SE are reported.

Sediment microbial characterization

Approximately 5 g soil samples were collected from the upper 10 cm of sediments using an ethanol-sterilized spoon. Samples were collected in the vicinity of the plants which contained a large amount of root material, however these were bulk soil samples from the root zone, not specifically rhizosphere soils. Soil samples were placed in sterile Whirl-pak bags, flash frozen on dry ice, and stored at -80 °C until further processing.

DNA was extracted from ~1 g of sediment using the DNeasy PowerSoil kit (Qiagen) using the manufacturer's protocols with the exception that bead mill beating was performed on a Retch MM301 Ball Mill (30 hz for 1 minute). The V4 region of bacterial 16S rRNA genes was amplified using primers 515F and 806R with Illumina adapters and dual indices (8 basepair golay on 3' [48], and 8 basepair on the 5' [49]). The amplification products were sequenced at UConn's MARS (Microbial Analysis, Resources, and Services) Illumina MiSeq platform.

Demultiplexed sequences were assembled into contigs and quality screened in the mothur software package (version 1.41.1.5; [50]). All sequences were selected to be at least 255 bp in length, contain no ambiguous bases, and no homopolymers of more than 8 bp. Chimeric sequences were identified with the mothur implementation of VSEARCH [51], and all potentially chimeric sequences were removed. Sequences were clustered into operational taxonomic units (OTUs) using a 100% sequence identity threshold, employing the OptiClust algorithm in mothur [52]. Taxonomic classification of sequences was performed with the Na¨ive Bayesian classifier [53] against the SILVA reference alignment (release 132) [54] in the mothur software package.

Prior to determining alpha-diversity via the nonparametric Shannon's diversity index (H'), data-sets were randomly subsampled to the size of the smallest dataset (omitting outliers with <1000 sequences), resulting in 5,720 sequences per dataset. Significant differences in OTU relative abundance were tested for with the ALDEx2 package. Prior to identifying significant differences, OTU count data were transformed using the centered log-ratio and normalized through Monte Carlo sampling with Bayesian sampling of 128 Dirichlet instances [55]. Both the Kruskal-Wallis and generalized linear model tests were performed and an OTU was considered to be significantly different in relative abundance if the p-value was $\leq$0.05 after adjusting for multiple testing with the Benjamini-Hochberg correction. The ternary plot of OTU relative abundance was generated with the ggtern extension package in R [56]. All raw sequence datasets are available in the NCBI Short Read Archive (SRA) under the BioProject ID PRJNA604015.

Results

Plant biomass and root porosity responses

Biomass production differed among vegetation above- and belowground (above: $F_{2,36}$ = 46.5, $p < 0.001$; below: $F_{2,36} = 6.8$, $p = 0.003$), as well as among water quality treatments aboveground (above: $F_{3,36} = 144.0$, $p < 0.001$), but we observed interactions between species and water quality treatment for aboveground biomass ($F_{6,36} = 6.5$, $p < 0.001$), principally because *Spartina* aboveground biomass responded strongly to N enrichment (Fig 1). fPAR transmission was strongly negatively correlated with aboveground biomass ($r = -0.80$, $p < 0.001$), and differed among species ($F_{2,42}$= 3.8, $p = 0.030$) and water quality treatments ($F_{3,42} = 24.3$, $p < 0.001$), with greater transmission through *Typha* (71.3% ± 1.9) than *Spartina* canopies (64.6% ± 3.5).

Nitrogen enrichment reduced fPAR (N: 52.9% ± 2.9) relative to the control and salt treatments (72.5% ± 1.1).

Fig 1. Biomass allocation by vegetation species-water quality treatment combinations. Mean (± SE) above- and belowground biomass by species-water quality combinations in 2017 in a full factorial mesocosm experiment where each treatment combination was replicated four-fold.

Root porosity did not differ across water quality treatments ($F_{2,36} = 1.85$, $p = 0.15$), but *Spartina* roots were more porous ($F_{2,36} = 7.6$, $p < 0.05$; Table 2) and had greater total root porosity ($F_{2,36} = 6.08$, $p < 0.05$; Table 1) than the other two species (Table 1).

Table 1. Root porosity differed among vegetation species.

Vegetation species	**Root porosity (%)**	**Total root porosity**
Phragmites	$25.5^{a} \pm 2.5$	$45.9^{a} \pm 8.8$
Spartina	$35.1^{b} \pm 3.1$	$137.1^{b} \pm 24.8$
Typha	$25.3^{a} \pm 2.5$	$46.6^{a} \pm 9.4$

Average (± 1 SE) 2017 root porosity (measured from 3 root subsamples per mesocosm) and total root porosity (% porosity x total belowground biomass) for each plant species (n = 16). Superscripts indicate significant differences between vegetation species after TukeyHSD post-hoc comparisons.

Carbon fluxes

We observed differences in CO_2 uptake among species ($F_{2,36}$ = 34.27 $p < 0.0001$) and water quality treatments ($F_{3,36}$ = 12.48, $p < 0.0001$), but did not observe an interactive effect of species and water quality treatment ($F_{6,36}$ = 1.12, p = 0.369). *Spartina* (36,239 ± 2744 µmol m^{-2} h^{-1}) and *Typha* (23,880 ± 1503 µmol m^{-2} h^{-1}) had greater CO_2 uptake than *Phragmites* (14,070 ± 1112 µmol m^{-2} h^{-1}; Fig 2a). Nitrogen addition (39,564 ± 3467 µmol m^{-2} h^{-1}) increased CO_2 uptake relative to freshwater controls (19,392 ± 1618 µmol m^{-2} h^{-1}) and sea salt treatments (19,751 ± 1990 µmol m^{-2} h^{-1}), but did not lead to different CO_2 uptake from our road salt treatment (20,211 ± 1467 µmol m^{-2} h^{-1}; Fig 2b).

Fig 2. CO_2 uptake differed among vegetation and water quality treatments. Boxplots of 2017 log-transformed CO_2 uptake rates (samples pooled across July, August, September sampling campaigns) by (a) vegetation and (b) water quality treatments. Note that measurements were estimates of net ecosystem exchange, integrating photosynthetic uptake, auto and heterotrophic respiration from transparent chambers. Differences between groups are indicated by non-overlap of letters, based on post-hoc Tukey contrasts.

Methane emissions differed strongly among vegetation species ($F_{3,36}$ = 40.83 , $p < 0.0001$; Fig 3a); *Spartina* (101.9 ± 12.4 µmol m^{-2} h^{-1}) had the highest CH_4 fluxes, followed by *Typha* (61.8 ± 10.7 µmol m^{-2} h^{-1}), and then *Phragmites* (21.0 ± 2.7 µmol m^{-2} h^{-1}). We also observed differences among water quality treatments ($F_{3,36}$ = 6.31 , p = 0.002; Fig 3b), with sea salt addition halving CH_4 emissions (34.7 ± 5.8 µmol m^{-2} h^{-1}) relative to the other water quality treatments (70.3 ± 7.7 µmol m^{-2} h^{-1}). Water quality treatment effects were consistent across

vegetation species, as we did not observe an interaction among these factors ($F_{6,36} = 1.10$, $p = 0.383$).

Fig 3. Boxplots of 2017 log-transformed CH_4 emissions (pooled data from July, August, and September) by (a) vegetation and (b) water quality treatments. Differences between groups are indicated by non-overlap of letters, based on post-hoc Tukey contrasts.

Pore water chemistry

Pore water chemistry was generally more responsive to water quality than vegetation treatments (Table 2), though SO_4^{-2} and DOC differed among species, with *Spartina* having lower concentrations (SO_4^{-2}: 1.03 ± 0.21 mg/L; DOC: 8.00 ± 1.25 mg/L) than *Typha* and *Phragmites* (SO_4^{-2}: 2.28 ± 0.53 mg/L; DOC: 10.14 ± 1.43 mg/L). Salt ions associated with experimental treatments differed as expected: Cl^- concentrations were much greater with road and sea salt addition (89.2 ± 4.6 mg/L) than control and N-enriched treatments (1.8 ± 0.5 mg/L), and sea salt treatment doubled SO_4^{-2} concentrations (3.2 ± 0.5 mg/L) compared to the other treatments (1.4 ± 0.9 mg/L). We did not observe treatment differences in NO_3^- nor NH_4^+ concentrations (Table 2); NO_3^- concentrations averaged 0.22 ± 0.05 mg/L among the 19 samples that were above our instrument's detection limit, whereas NH_4^+ concentrations averaged 1.00 ± 0.53 mg/L. Salt addition reduced porewater DOC concentrations, as we observed three times as much DOC in control and N-enriched mesocosms (14.9 ± 1.0 mg/L) than in road and sea salt tanks (5.0 ± 0.4 mg/L).

Table 2. **Pore water chemistry ANOVA results.**

	Vegetation			Water Quality		
Response	df	F	p	df	F	p
SO_4^{-2}	2, 41	6.3	**0.004**	3, 41	43.3	<**0.001**
Cl^-	2, 40	0.2	0.847	3, 40	94.7	<**0.001**
NO_3^-	2, 13	1.9	0.194	3, 13	0.9	0.434
NH_4^+	2, 36	0.1	0.903	3, 36	1.4	0.236
DOC	2, 38	6.2	**0.005**	3, 38	70.9	<**0.001**

Two-way ANOVA results that tested how pore water chemistry differed among vegetation and water quality treatments. Note that 29 NO_3^- samples were below instrument detection limit, resulting in low sample size.

Carbon mineralization

Soil C mineralization rates did not differ among vegetation ($F_{2,38} = 2.4$, $p = 0.11$) but were reduced with sea and road salt compared to freshwater controls and N enrichment ($F_{3,38} = 11.2$, $p < 0.001$) (Fig 4).

Fig 4. Carbon mineralization rates by vegetation and water quality treatments. Log-transformed sediment C mineralization rates estimated using 24-hour laboratory incubations did not differ among (a) vegetation, but differed among (b) water quality treatments. Differences between groups are indicated by non-overlap of letters, based on post-hoc Tukey contrasts.

Correlations with carbon fluxes

Aboveground biomass was positively correlated to CO_2 uptake ($r = 0.60$, $p < 0.0001$), but CH_4 emissions were not correlated with aboveground, belowground, nor total biomass. However, total root porosity was positively correlated with CH_4 emissions ($r = 0.38$, $p = 0.008$). Porewater chemistry associated with our salt treatments (SO_4^{-2}, Cl^-) influenced several C responses. We observed negative correlations between SO_4^{-2} concentration and CH_4 emissions ($r_s = -0.337$, $p = 0.024$), between Cl^- concentrations and C mineralization ($r_s = -0.577$, $p = < 0.0001$), and between DOC and C mineralization ($r_s = -0.769$, $p = < 0.0001$).

Bacterial community composition

Cluster Canonical Correlation Analysis (cluster-CCA) was used to investigate the relationship between bacterial 16S rRNA gene sequence datasets (Fig 5). Clustering by vegetation showed substantial overlap in community composition, although there was a significant difference in centroids between vegetation types (Fig 5a; $p = 0.005$). Clustering was more apparent when aggregated by water quality treatment, with the salt treatments separating distinctly from the control and N amendments (Fig 5b). However, there was no significant clustering that differentiated the controls and the N enrichment or between the two salting treatments (road or sea salt).

Fig 5. CCA-clustering of bacterial 16S rRNA gene datasets. (a) Data clustered by vegetation type. Significance of clustering was tested with the permutation anova CCA test and vegetation

was a significant factor for clustering (p = 0.005). (b) The same data clustered by water quality treatment, which was also a significant factor in dataset clustering (p = 0.001).

Bacterial diversity

Bacterial diversity was assessed by calculating the non-parametric Shannon's diversity index. When the datasets were clustered by vegetation, *Typha* showed the highest average diversity, with the lowest diversity amongst *Phragmites* (Fig 6a). Alternatively, water quality treatment showed a clear decrease in diversity associated with the salt treatments (Fig 6b).

Fig 6. **Bacterial diversity in sequence datasets**. (a) Diversity in datasets grouped by vegetation type. (b) Diversity in datasets grouped by water quality treatment. Significant differences between groups are indicated by non-overlap of letters, based on post-hoc Tukey contrasts.

Differentially abundant OTUs due to vegetation

The abundance of the numerically dominant OTUs were plotted as a ternary diagram to display their relative abundance among the three plant species (Fig 7a). Most OTUs belonged to five bacterial phyla, with the Proteobacteria being most common. The majority of the OTUs were present at similar relative abundances among the three vegetation types as evidenced by their clustering in the center of the ternary diagram (Fig 7a). Only two OTUs were identified as significantly different in relative abundance, and their abundances in each vegetation type is displayed in Fig 7b. Otu000028 was classified to the genus *Geobacter* (Phylum, Proteobacteria) and was enriched in the *Spartina* mesocosms. In contrast Otu000322, classified to the *Novosphingobium* (Phylum, Proteobacteria), was uniquely present in with *Typha*. Generally,

these data indicate that OTU relative abundance was sensitive to the different plant species, with only a very limited number of OTUs showing a shift in relative abundance in response to plant species.

Fig 7. **OTU relative abundance in association with vegetation.** Only the 1500 most abundant OTUs are displayed. (a) Ternary diagram displaying OTU abundance among the three plant species. The two OTUs identified as significantly different in relative abundance are indicated by the arrows. (b) Median counts per sample of each of the differentially abundant OTUs. A table showing the classification of the differentially abundant OTUs is shown in S1 Table.

Differentially abundant OTUs due to water quality treatment

OTU relative abundance in the controls was plotted against the treatments to test for shifts in abundance due to the different amendments. OTUs were present in similar relative abundance between control and N enrichment treatments, and no OTUs were identified as significantly different in relative abundance due to N (Fig 8a). In comparison, multiple OTUs were identified as differentially abundant due to the road and sea salt treatments. We further determined if the differentially abundant OTUs from the two salt treatments were unique or common to each condition (Fig 8b). A total of 86 OTUs were identified as significantly different of which 25 (29%) were common to both the road salt and sea salt treatments. In this regard, there appears to be a set of OTUs that share a similar response to salt, irrespective of the source. The differentially abundant OTUs identified as common to both salt treatments were predominantly within the phylum Proteobacteria (Fig 8c). Taken together, these data suggest that the differing plant responses to the N, road salt, and sea salt treatments were not matched by

similar responses in the sediment microbial community. Given that the soils for this survey were collected in the vicinity of the roots, but did not include the rhizosphere soils directly in contact with the roots, the influence of the plant on sediment communities did not appear to extend into the root zone soils. Instead, sediment microbial communities appeared to respond to changes in sediment properties, particularly those associated with salting, such as osmotic stress.

Fig 8. Differentially abundant OTUs due to treatment. (a) Each point represents a detected OTU and its counts in controls versus treatment. OTUs colored in red were identified as significantly different in abundance. (b) Differentially abundant OTUs unique and shared among the two salt treatments, (c) Taxonomic classification of differentially abundant OTUs in salt treatments.

Finally, we investigated those OTUs that were enriched in controls versus those that were enriched with salt and were shared between both the road salt and sea salt treatments (S1 Table). A diverse set of OTUs were identified, belonging to six different phyla and 17 families. All of the taxa were heterotrophic groups with a variety of different growth types and strategies. For instance, an OTU related to the genus *Sideroxydans*, an iron oxidizing group of bacteria [57], was enriched in the control samples (S1 Table). In contrast, three OTUs related to the genus *Geobacter* were enriched in the salted sediments (shared in both road salt and sea salt). Members of the *Geobacter* genus are thought to be the primary drivers of oxidizing organic matter coupled to the reduction of iron and manganese [58]. In this respect, these data point to a state change in the iron cycle in the mesocosms under the salt treatments, which points to a decreased availability of dissolved iron under elevated salt. The remaining OTUs largely belonged to general heterotrophic bacteria or were not able to be classified to taxonomic ranks deeper than

family, which limits the confidence that functional predictions can be made from these classifications.

Discussion

Wetlands play a major role in global C dynamics, but understanding how wetland plants, sediment microbial communities, and water quality interact is currently not well resolved. To help bridge this gap, we conducted a mesocosm experiment in which we manipulated plant species (globally dominant wetland genera- *Phragmites, Typha, Spartina*) and common water quality impairments (N-enrichment, salinization via road or sea salt) to investigate C and microbial responses. We found that plant species had strong effects on our response metrics, with largely similar patterns in response to water quality treatments across plant species. However, water quality treatments appeared to have distinct effects on plant vs. microbial responses; N enrichment increased biomass production and CO_2 uptake, whereas salinization reduced CH_4 emissions (with sea salt), reduced heterotrophic respiration, altered microbial composition, and decreased microbial diversity.

Biomass and C process responses

Rates and allocation of biomass production are the foundation of C cycling in wetlands. Not surprisingly, we observed that greater aboveground biomass promoted greater CO_2 uptake, and that N-enrichment amplified biomass production, particularly in *Spartina*, which had five times greater aboveground biomass production with N addition than controls. Anecdotally, we observed higher algae abundance in surface waters of *Typha* and *Phalaris* with N addition;

higher levels of PAR penetrating through sparser canopies may have stimulated algal production and resulted in similar increases in CO_2 uptake across all vegetation treatments with N addition. Interestingly, salt addition (300 $g/m^2/y$) did not reduce biomass production compared to freshwater controls at the relatively low, but environmentally relevant, salinity levels (2 ppt) we targeted. Dramatic biomass reductions for freshwater macrophytes were observed when salinity treatments exceeded 4 ppt in [45]. Likewise, [38] observed a wetland seed bank threshold of 2 ppt for species richness, diversity, and aboveground biomass, with reductions in plant responses in NaCl treatments > 2 ppt, suggesting that common freshwater wetland plants may be resilient to salinity levels ≤ 2 ppt.

However, biogeochemical processes appear to be more sensitive to salinization. We observed reduced CH_4 emissions with SO_4^{-2} rich sea salt addition; while we did not quantify how water quality treatments effected pH, under circumneutral pH, SO_4^{-2} is thermodynamically favored over the reduction of C compounds [59,60]. Likewise, we observed a negative correlation between SO_4^{-2} concentrations and CH_4 emissions across all treatments. Both salinity treatments decreased DOC concentrations, likely due to salt-induced flocculation which promotes particle aggregation [27,28], hence exclusion during filtration. We found no correlation between DOC and CH_4 emissions as found in other studies [61]. However, we observed decreased C mineralization rates (i.e., heterotrophic respiration) and decreased diversity of microbial communities in our salt treatments, potentially pointing to osmotic stress of certain microbial populations. Similar to [62], we did not observe an effect of N addition on C mineralization rates, indicating excess nutrients were assimilated by plants, algae or microbes in the water column, but not by soil microbes in soil.

In contrast to other studies [6,7], we did not observe positive correlations between biomass and CH_4 emissions, though total root porosity (% root porosity x root biomass) was positively correlated to CH_4 emissions. Still, our data suggest that porous plant tissue acted similarly to a straw, allowing methane produced in anoxic sediment to bypass surface oxic layers and travel into the atmosphere, as observed by others [13,63]. *Spartina* had greater total root porosity than the other two species, providing a large pathway for CH_4 to escape to the atmosphere. *Spartina* also had lower porewater SO_4^{-2} concentrations than other species; thus, elevated CH_4 emissions from *Spartina* would be expected, as these conditions may favor methanogenesis [59]. Why *Spartina* had lower porewater SO_4^{-2} concentrations is unclear, however, as rhizosperic oxygenation should decrease sulfate reduction, thereby maintaining large SO_4^{-2} pools. Elevated uptake of SO_4^{-2} by *Spartina* is plausible, as [64] observed differential species uptake.

Microbial community response

Plant species played a significant, if small role in sediment microbial community composition (Fig 5). The majority of the identified bacterial OTUs were present in all three mesocosms in similar proportions, with only two OTU's identified as significantly different in relative abundance between the three plant species (Fig 7). Thus, most bacteria appeared largely indifferent to plant species. The rhizosphere of wetland plants, the zone of soil directly in contact with the plant root has been shown to harbor elevated bacterial activity and altered communities in comparison to bulk soils [65,66]. However, in this study we did not specifically isolate rhizosphere soils. In this regard, the influence of the plant on sediment microbial communities may be mostly limited to sediments in direct contact with roots.

513 The salt treatments induced a reduction in the diversity of the sediment microbial
514 communities (Fig 6b). Furthermore, a substantial fraction of the bacterial OTUs that shifted in
515 relative abundance were common to both salt treatments, road salt and sea salt (Fig 8b). This
516 suggests that the elevated osmotic stress likely affected a similar group of bacteria. However, the
517 shifts in relative abundance due to the salt treatments were generally among the numerically rare
518 populations, whereas the most abundant OTUs were resilient to the treatments (Fig 8c). This
519 suggests that the dominant bacteria in the mesocosms were largely unaffected by the salt
520 treatment. The differentially abundant OTUs did point to an alteration in the iron cycle in the
521 sediments under the salt treatment. The enrichment of *Sideroxydans* in the control mesocosms in
522 comparison to an enrichment of *Geobacter* with elevated salt suggests a shift from iron oxidation
523 to iron reduction with the addition of salt. Further, similar to previous experimental findings
524 [62], we observed reduced mineralization of labile carbon from the salt treatments, which may
525 have been associated with reduced microbial diversity or shifts in community composition. Thus,
526 the osmotic or redox stress induced by the salt treatment did appear to shift biogeochemical
527 cycles in the sediments.
528 We hypothesized that we would observe a unique set of bacteria enriched in the sea salt
529 treatment. This is because the sulfates in sea-water are thought to support sulfate-reducing
530 communities which then outcompete methanogens. We observed a reduction of methane
531 emissions in the sea salt treatment, yet we did not observe an enrichment of sulfate reducers (S1
532 Table) which could indicate higher sensitivity to salinity than to redox conditions. Furthermore,
533 none of the differentially abundant OTUs in sea salt treatments were associated with
534 methanotrophic populations (S1 Table), bacteria capable of oxidizing methane [67]. As the
535 primers employed in this study were designed to amplify bacterial 16S rRNA genes, they were

not able to detect methanogenic archaea so we cannot directly address the effects of sea salt on methane producing populations. Thus, the sediment microbial data was not particularly predictive in the reduction of CH_4 observed under the sea salt treatment. However, our data only describe the composition of the sediment communities. It is possible that water quality treatments shifted the activity of particular microbial populations, such as sulfate-reducers, methanotrophs, or methanogens, without a concurrent alteration in their relative abundance. Future studies incorporating metrics of microbial activity may better address changes in the functions of the microbial community under differing water quality treatments.

Experimental design constraints

In the field, wetland vegetation, soils, and hydrology are often confounded, so a controlled mesocosm experiment allowed us to systematically test how vegetation and water quality treatments alter a range of biological and biogeochemical responses. However, relics of our experimental design should be considered when interpreting or comparing our results with other investigations. While invasive *Phragmites* is commonly known as an extremely productive and dominant species [68–70], the *Phragmites* we used in our study was a relatively short and sparse strain that sequestered less CO_2 and emitted less CH_4 than either *Spartina* or *Typha.* This is likely associated with the seed source we used; we collected seed from a population growing out of a groundwater seep at the base of a hill on UConn's campus, which may not be wholly representative of the species. We manipulated the hydroperiod of our mesocosms to promote reduced soils during the growing season; while draining the tanks during winter to prevent tanks from cracking may have altered microbial composition and redox conditions, consistent

manipulation of soils and hydrology allows us to draw inferences about responses to our vegetation and water quality treatments.

Conclusions

Wetlands are crucial landscape sinks, often occurring in low-lying areas that collect polluted or impaired runoff from surrounding watersheds, and are on the front lines of sea level rise, making them vulnerable to salt water intrusion. In turn, water quality can affect plant species composition and production rates, which are underlying drivers of wetland C cycling. Our results indicate that plant traits (biomass, root porosity) as well as species identity are important determinants of C gas flux. Particularly in areas vulnerable to invasive species and community shifts, presence or exclusion of key species has the potential to alter CO_2 uptake or CH_4 emission rates occurring within wetlands. Another important driver of C flux in freshwater wetlands is water quality. Different water quality impairments such as N, road salt, and sea salt affect C gas flux in different ways. Nitrogen enrichment's influence on biomass production and increased gas flux make it a prominent driver of change in wetlands exposed to agricultural runoff as well as wastewater. The reduction of CH_4 emissions due to salt-water intrusion of sea level rise exhibits the power of small water quality changes within the system. Although the relatively low concentrations of salt used in this experiment (2 ppt) did not significantly affect plant traits such as biomass production, they did alter the water and sediment chemistry enough to influence the sediment microbial communities therefore altering CH_4 emissions. Recent evidence suggests that engineered nanoparticles can exacerbate eutrophication in wetlands [71], highlighting the need to further examine the interactions among emerging contaminants, water quality, vegetation, and wetland carbon cycling.

The vegetation and water quality impairments used in this experiment are common throughout not only eastern North America, but also many locations worldwide. With the crucial role that wetlands play in the global C cycle, it is important to better understand the integration between plant performance and microbiology and how these factors influence C gas fluxes.

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Supporting information

S1 Fig. **Wetland mesocosm experimental setup.** (a) A mesocosm tank experiment was set up at the University of Connecticut in 2016-2017 to test how plant species and water quality treatments influenced carbon gas fluxes and sediment microbial communities. (b) Co-author O. Johnson monitors real time C fluxes using a transparent floating chamber connected to a Picarro g2201-*i* during the 2017 growing season.

S1 Table. **OTUs depleted or enriched in association with the salt treatments.**

Figure 1

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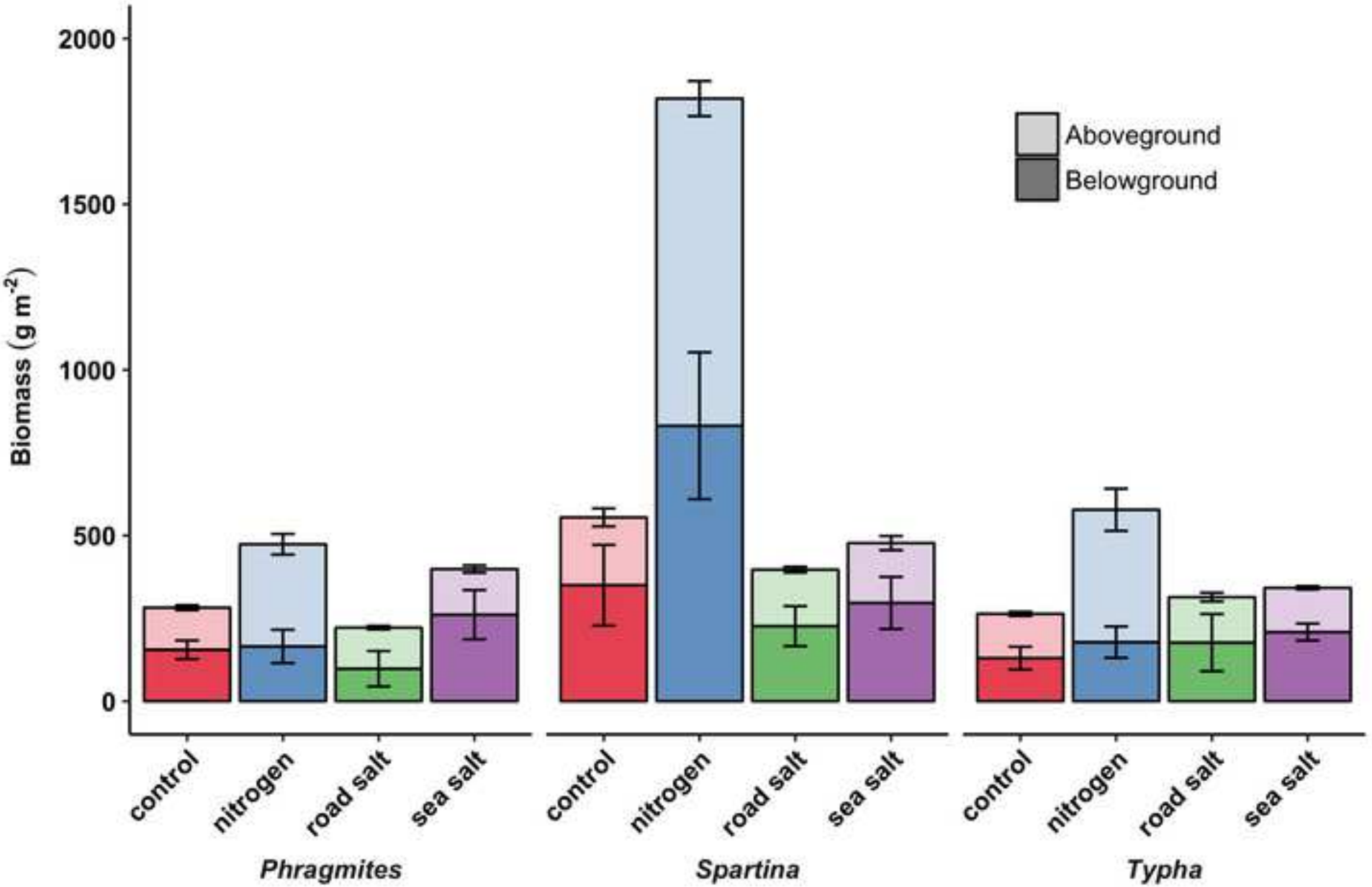


Figure 2

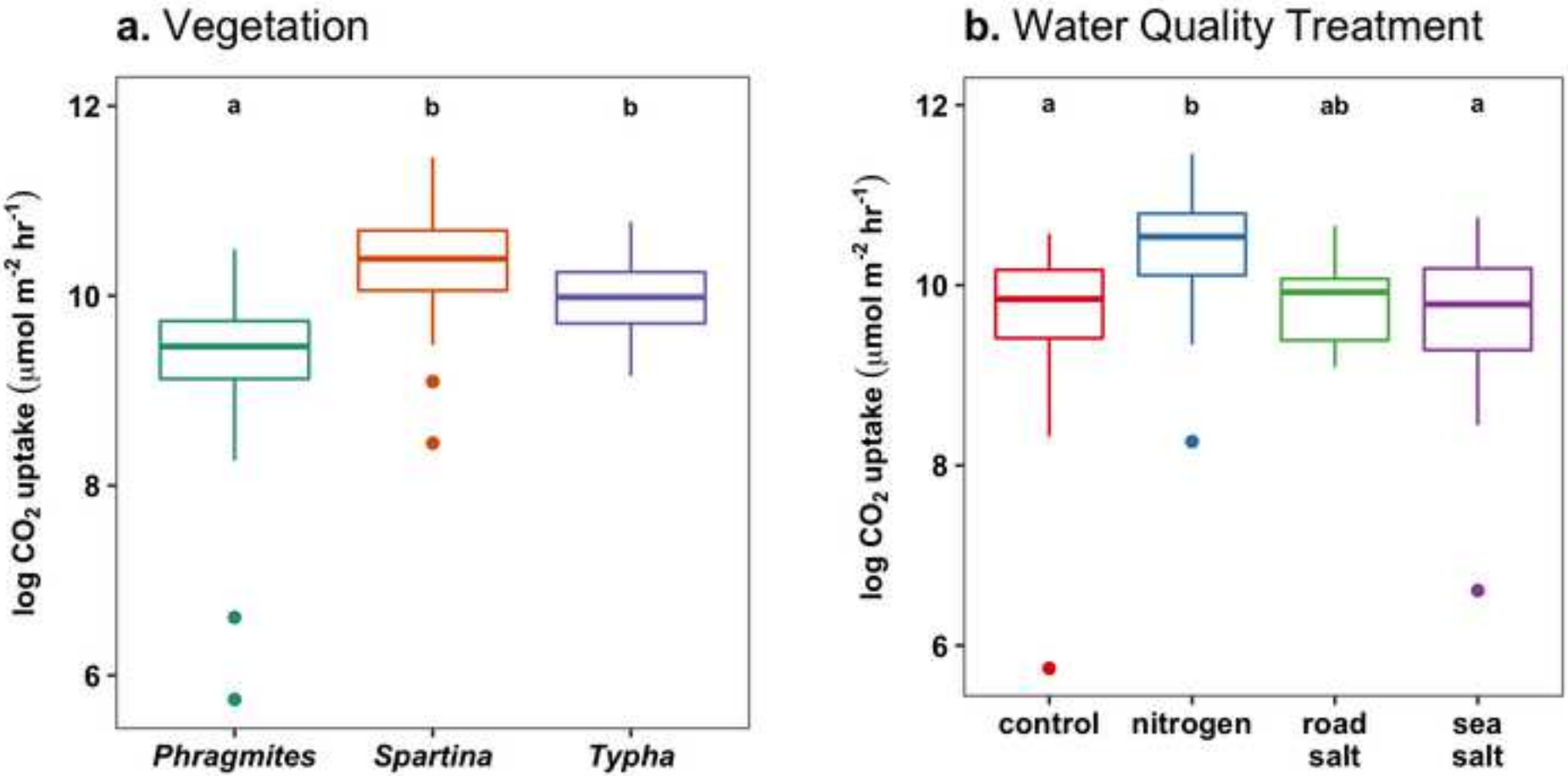
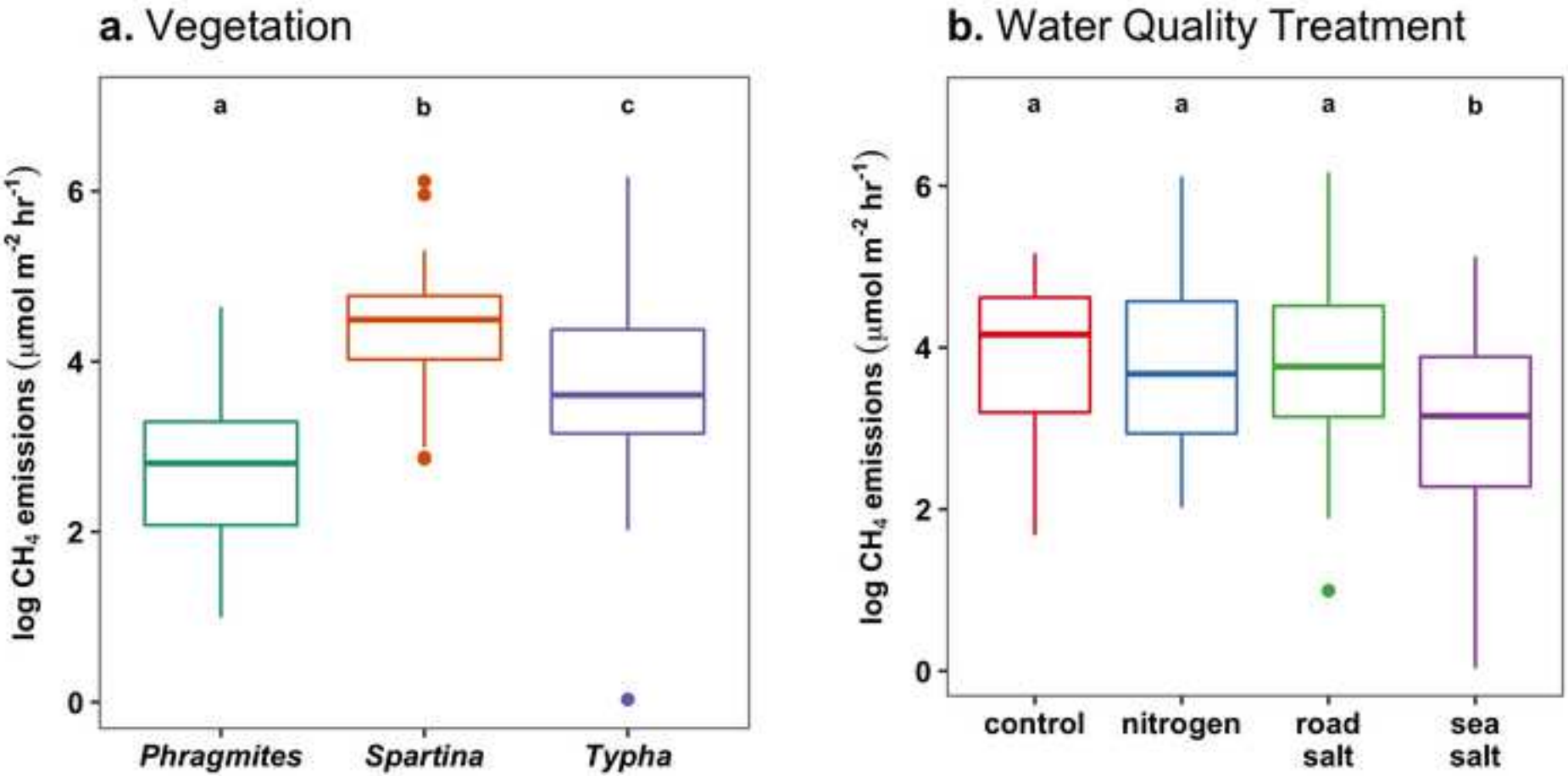


Figure 3



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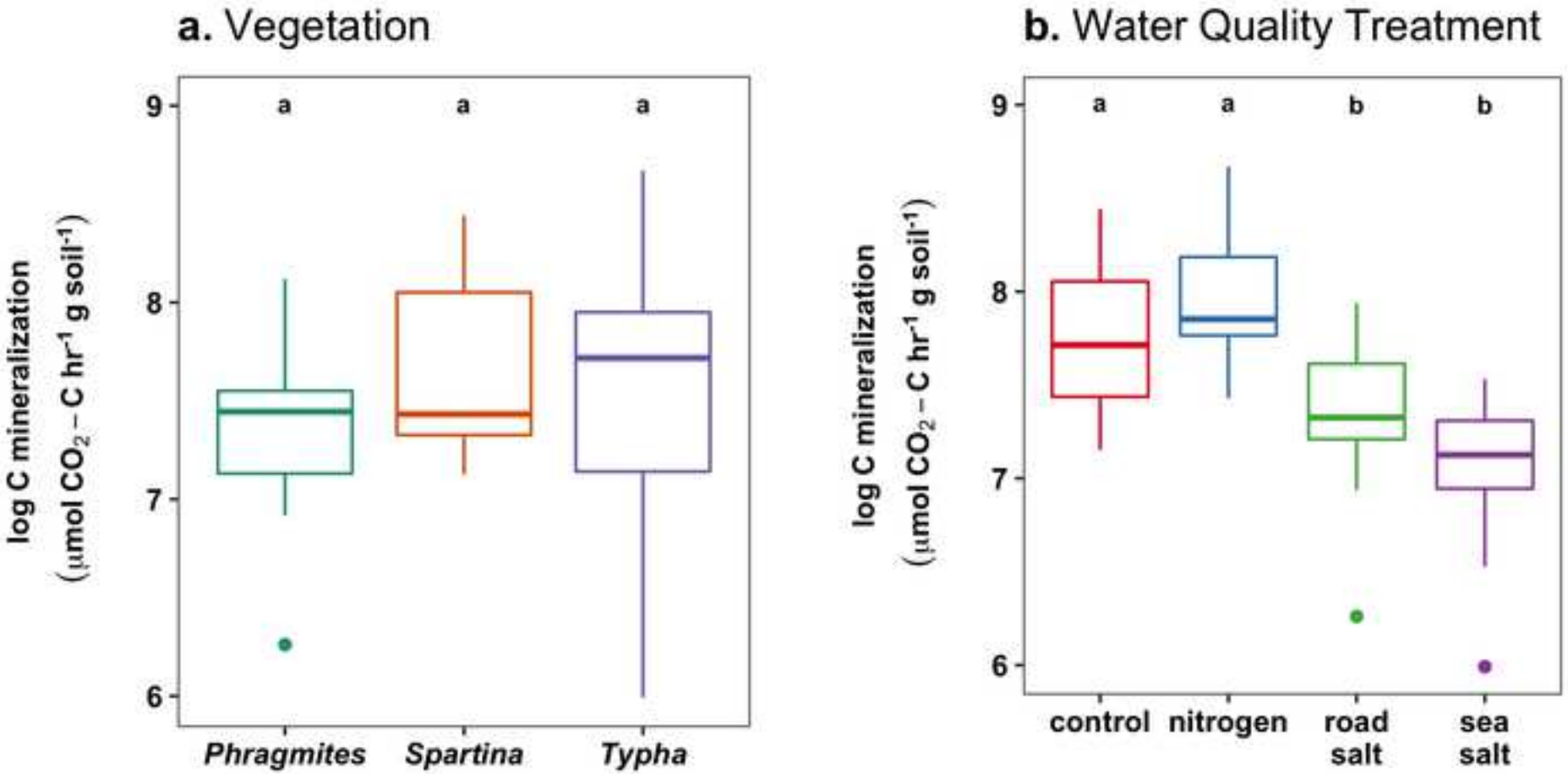

Figure 5

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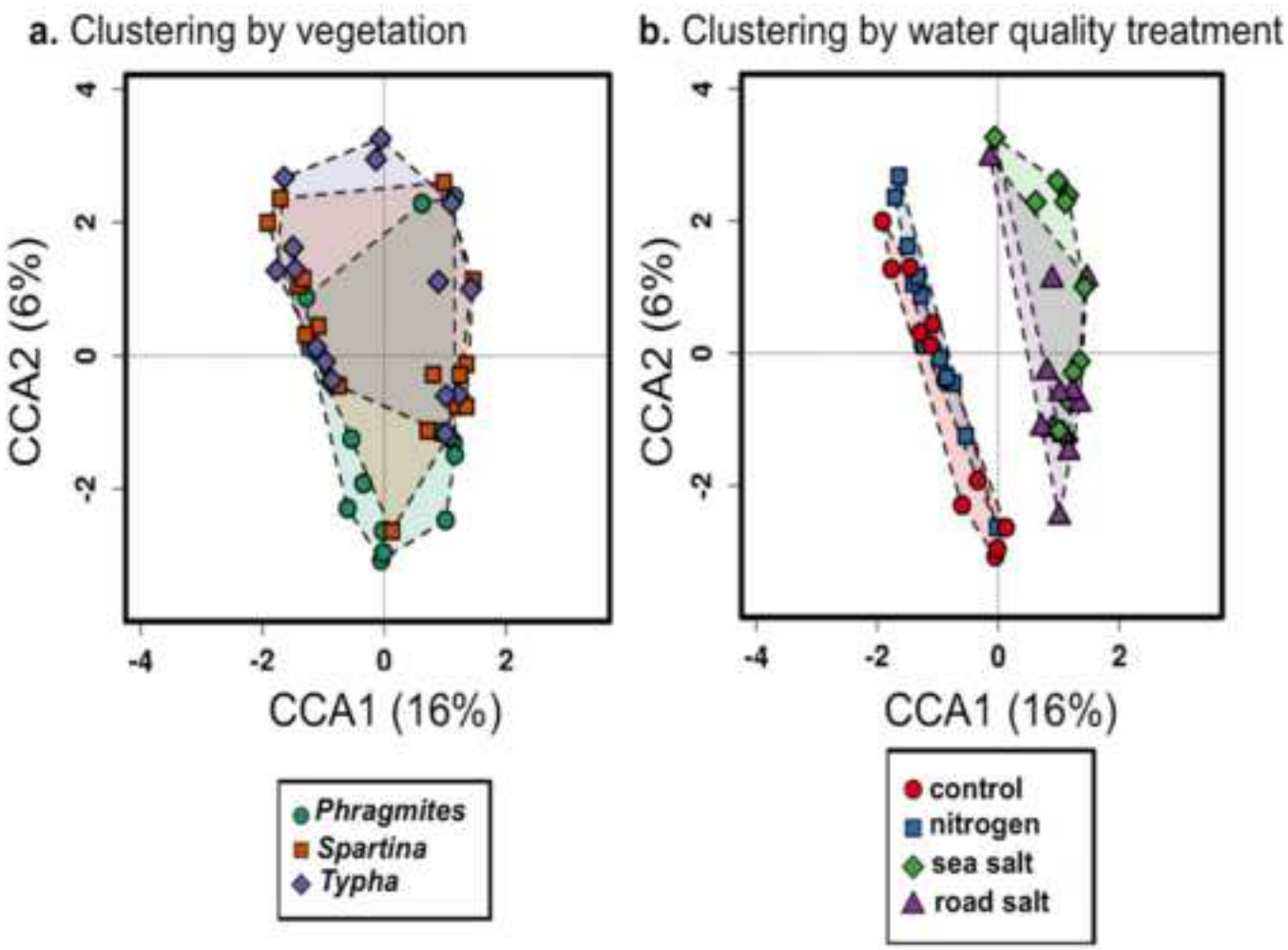


Figure 6

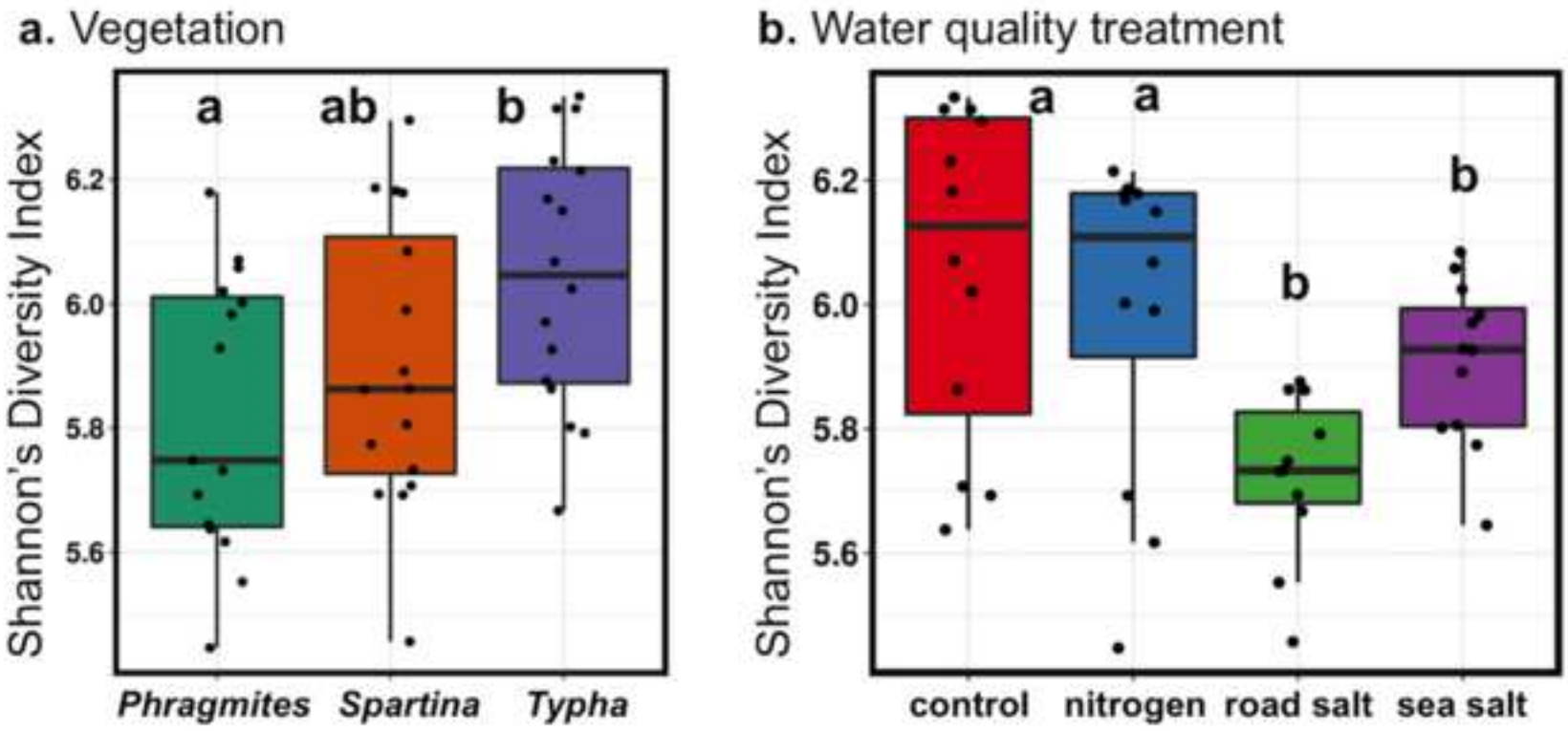


Figure 7

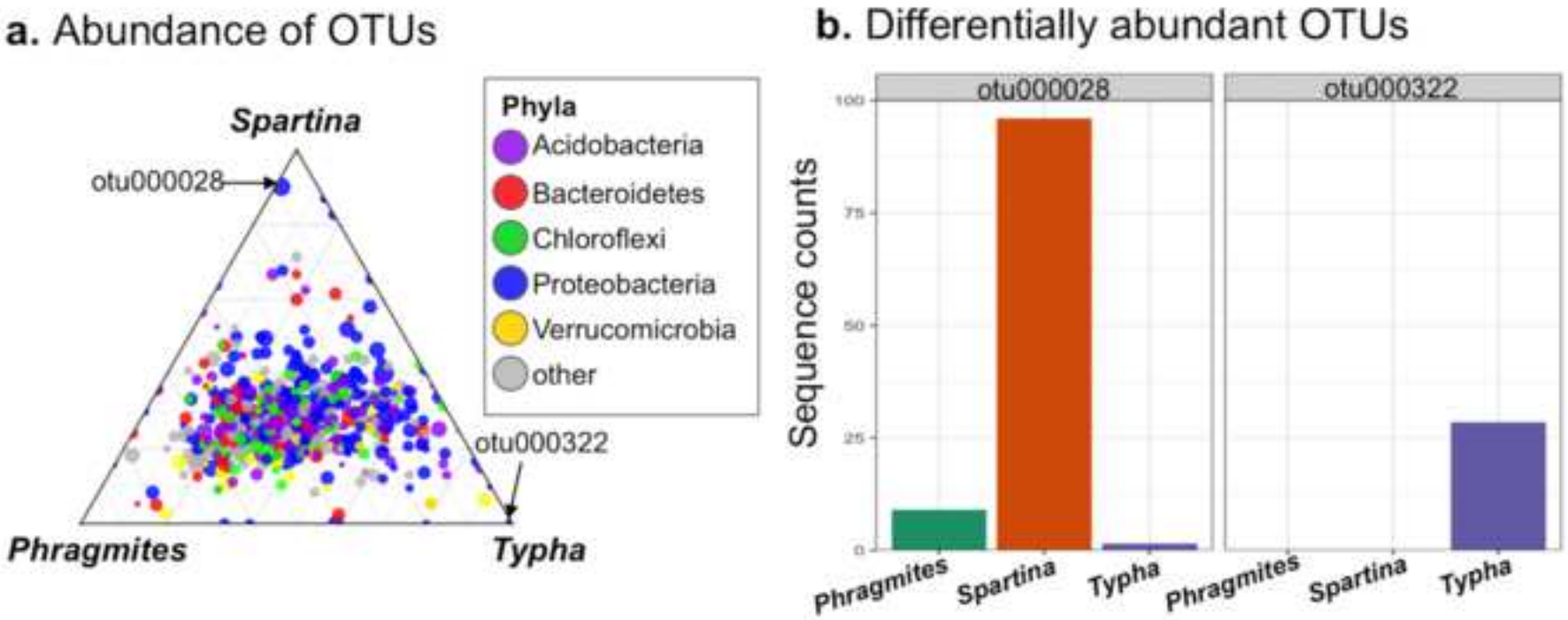
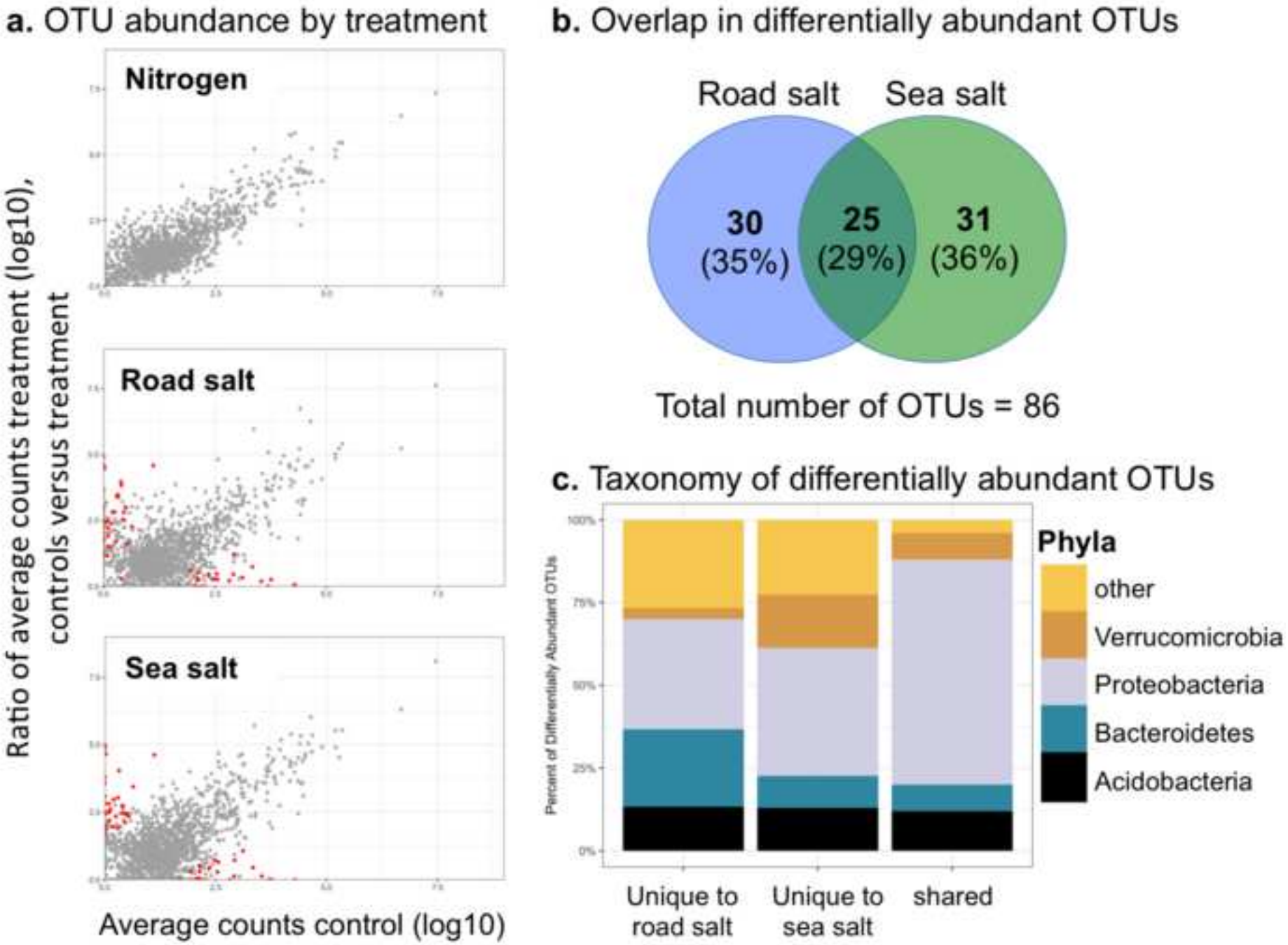


Figure 8



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Supporting Information
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Nitrogen enrichment stimulates wetland plant responses whereas salt amendments alter sediment microbial communities and biogeochemical responses

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Abstract

Freshwater wetlands of the temperate north are exposed to a range of pollutants that may alter their function, including nitrogen (N)-rich agricultural and urban runoff, seawater intrusion, and road salt contamination~~, and nitrogen-rich agricultural and urban runoff~~, though it is largely unknown how these drivers of change interact with the vegetation to affect wetland carbon (C) fluxes and microbial communities. We implemented a full factorial mesocosm (378.5 L tanks) experiment investigating C-related responses to three common wetland plants of eastern North America (*Phragmites australis*, *Spartina pectinata*, *Typha latifolia*), and four water quality treatments (fresh water control, N, road~~sea~~ salt, sea~~road~~ salt~~, nitrogen~~). During the 2017 growing season, we quantified carbon dioxide (CO_2) and methane (CH_4) fluxes, above- and below-ground biomass, root porosity, light penetration, pore water chemistry (NH_4^+, NO_3^-, SO_4^{-2}, Cl^-, DOC), soil C mineralization, as well as sediment microbial communities via 16S rRNA gene sequencing. Relative to freshwater controls, ~~nitrogen~~ N enrichment stimulated plant biomass, which in turn increased CO_2 uptake and reduced light penetration, especially in *Spartina* stands. Root porosity was not affected by water quality, but was positively correlated with CH_4 emissions, suggesting that plants can be important conduits for CH_4 from anoxic sediment to the atmosphere. Sediment microbial composition was largely unaffected by ~~nitrogen~~ N addition, whereas salt amendments induced structural shifts, reduced sediment community diversity, and reduced C mineralization rates, presumably due to osmotic stress. Methane emissions were suppressed by sea salt, but not road salt, providing evidence for the additional chemical control (SO_4^{-2} availability) on this microbial-mediated process. Thus, ~~nitrogen~~ N may have stimulated plant activity while salting treatments preferentially enriched specific microbial populations. Together our findings underpin the utility of combining plant and microbial responses, and

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47 highlight the need for more integrative studies to predict the consequences of a changing
48 environment on freshwater wetlands.
49

Introduction

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Wetlands play a disproportionate role in the global carbon (C) cycle; despite covering only 5-9% of the world's land surface [1], they store up to a third of terrestrial soil C [2,3] and contribute more than a third of global methane ~~(~~[CH_4]~~)~~ emissions, a potent greenhouse gas with 28-times the warming effect of CO_2 [4]. These highly productive ecosystems are increasingly dominated by monotypic graminoids [5] and have saturated soils that are key sites for anaerobic microbial processes. ~~-~~However, we currently have minimal understanding of how degraded water quality associated with anthropogenic activities affects the interactions among plant and microbial communities underlying wetland C processes.

Traits of dominant wetland macrophytes play an important role in wetland C cycling. Biomass production largely determines CO_2 assimilation rates and is often positively correlated with CH_4 emissions [6,7]. Plant allocation of resources belowground provides organic substrates to sediment microbial communities for anaerobic respiration [8,9], which can promote methanogenesis and increase CH_4 emissions [10]. However, the relationship between biomass and CH_4 emissions may not be so straightforward, as porous tissues of wetland plants (i.e., aerenchyma) link anoxic soil to the atmosphere; this could reduce net CH_4 emissions by promoting soil oxygenation via root-soil gas exchange [11,12], or increase net emissions by allowing CH_4 produced in underlying anoxic sediment to bypass oxidized surface sediments and waters [13,14]. Because root porosity varies among plant species and appears to be a plastic trait [15,16], we need to further elucidate its role in CH_4 emissions among common wetland plants subjected to impaired water quality.

Increasingly in the Anthropocene, wetland structure and function is determined by water quality because wetlands are "landscape sinks" [~~17~~5] that accumulate materials and pollutants (e.g., nitrogen (N), salts) from watershed disturbances. Macrophytes such as species in the

genera *Phragmites*, *Spartina*, and *Typha* are well suited to invade and dominate wetlands [5, 17~~7~~,18~~17–19~~], thus changes in water quality associated with ~~nitrogen~~ N enrichment or salt intrusion may give these plants a competitive advantage and indirectly affect C fluxes. For example, *Phragmites australis* is a salt-tolerant invader of brackish marshes and roadsides of eastern North America that tends to create large productive monocultures that have higher CH_4 emissions than native communities [19,20~~,21~~]. Similarly, ~~nitrogen~~ N enrichment common in agricultural and urban landscapes promotes *Typha* dominance [~~22~~21], whose invasion can increase soil CH_4 emissions [7]. Nitrogen enrichment promotes biomass production [~~23~~22] with associated increases in CO_2 uptake, rhizosphere oxidation, C exudation, and microbial activity [23~~424~~]. The consequent effects on CH_4 emissions are therefore mixed; in addition to the nuanced balance of oxygen and C inputs from increased biomass, the direct effect of increased ~~nitrogen~~ N could favor other microbes over methanogens.

Elevated salinity associated with seawater intrusion and road deicing salts can induce osmotic stress, altering growth and composition of plant and microbial communities [24,25~~,26~~]. Further, saline conditions change the availability of terminal electron acceptors [~~27~~26], and promote organic matter flocculation [27,28~~,29~~], which alter microbial respiration rates. Intrusion of sulfate-rich seawater into freshwater wetlands reduces soil CH_4 emissions, as sulfate reduction can be~~is~~ thermodynamically favored over methanogenesis [29,30~~,31~~]. Exponential usage of deicing salts, largely sodium chloride (NaCl) throughout the temperate north [31~~2~~–33~~432–34~~], has had severe ecological consequences [34,35~~,36~~]. Where water residence times are high, elevated concentrations of Na^+ can displace other cations (NH_4^+, Ca^+, K^+, Mg^+) through cation exchange [36~~737~~], causing negative effects on biotic communities due to salt stress and altered

nutrient availability [37~~8~~,38~~9~~38,39~~]~~]. However, the consequences of road salt pollution on wetland C emissions are less well understood, and may differ from those of seawater intrusion.

Our objective was to investigate how dominant wetland plants and common water quality impairments interact to alter components of freshwater wetland C cycling. We conducted a wetland mesocosm experiment during the 2016-2017 growing seasons to test how traits (i.e., biomass, root porosity) of three common wetland plants (*Phragmites australis*, *Spartina pectinata*, *Typha latifolia*, hereafter *Phragmites*, *Spartina*, *Typha*, respectively) and four water quality treatments (freshwater control, ~~nitrogen~~N, road salt, sea salt) interact to alter C gas fluxes (CO_2, CH_4, C mineralization) and sediment microbial communities.

Materials and m~~M~~ethods

Experimental design

We implemented an outdoor mesocosm experiment at the University of Connecticut (Storrs, Connecticut, USA), consisting of 48 mesocosms which were 378.5 L plastic tanks (79 cm x 64 cm x 132 cm; Freeland Poly-Tuf Tank©; S1a Fig). In spring 2016, we planted monocultures of three wetland plant species (*Phragmites*, *Spartina*, *Typha*), and in 2017 we implemented four water quality treatments (freshwater control, ~~nitrogen~~N ~~enrichment~~, ~~road salt, sea salt~~road salt sea salt). We replicated each plant species-water quality treatment combination four-fold and randomly assigned treatments to the 48 mesocosms. We chose common wetland plants that occur throughout eastern North America and that vary in root porosity [16], biomass production, and salt tolerance; *Phragmites* and *Typha* tend to dominate fresh to brackish marshes, whereas *Spartina* is typically considered a freshwater grass, but occurs along the

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upland fringes of coastal marshes ~~in the northeastern part of the United States~~in eastern North America.

We filled the bottom of each mesocosm with 15 cm of sand, and then added 30 cm of commercially screened topsoil. In June 2016, we planted four, four-month old seedlings into each mesocosm;~~P~~ we grew plants in a ~~were~~ greenhouse using ~~grown from~~ locally-collected, cold-stratified seed during spring 2016. ~~In June 2016, four, four-month old seedlings were planted into each mesocosm. We filled the bottom of each mesocosm with 15 cm of sand, and then added 30 cm of commercially screened topsoil.~~ Seedlings were allowed to establish during the 2016 growing season and were regularly watered to maintain saturated soils. In May 2017 we inoculated each mesocosm with 19 L of sediment collected from a nearby constructed freshwater wetland known to have methanogenic activity [~~40~~39]. Water levels were maintained at 5 to 10 cm above the soil surface during the growing season (May-September) using ground water from a nearby well (pH: 7.12); water levels ~~and~~ occasionally exceeded 10 cm after major rain events, but we ensured that water levels were consistent across tanks. Mesocosms were drained October-April when plants were dormant to prevent cracking of the plastic tubs during freezing conditions.

Water Quality Treatments

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Water quality treatments (freshwater control, ~~nitrogen~~N, road salt, sea salt) were applied twice in 2017 (May, June). Powder forms of ~~nitrogen~~N and salt compounds were added to 1 L Nalgene bottles with 0.9 L of deionized (DI) water and shaken manually until fully dissolved. Once dissolved, solutions were poured evenly across assigned mesocosms; controls received 1 L of DI water.~~.~~ For the ~~nitrogen~~N treatment, we applied ammonium nitrate (NH_4NO_3) at a rate of 15 g N/year (two applications of 21.4 g of NH_4NO_3). We targeted a salinity of ~2 ppt for the two

salt treatments, and during two application events added 300 g/year of dissolved salt (road salt: Diamond Crystal Winter Melt NaCl; sea salt: Instant Ocean® Sea Salt). Instant Ocean® is a commonly used saltwater aquarium additive with a similar chemical composition to seawater [40~~141~~] and has been used to simulate seawater intrusion in other studies [41,42~~,43~~]. Treatment concentrations were selected based on previous experiments [43~~4~~–45~~644–46~~] as well as field measurements of salinity concentrations in Connecticut road-adjacent wetlands [38~~939~~].

Response metrics & analysis

Carbon fluxes

We measured C fluxes during three sampling campaigns in 2017 (mid-July, August, and September; approximately one, two, and three months after the last dosing treatment). We used a Picarro G2201-*i* cavity ring-down spectrometer (Picarro Inc., Santa Clara, CA, USA) that measures CO_2 and CH_4 gas concentrations in real time (approximately every 3 s). A clear sampling chamber (base: 25 cm x 25 cm, height: 100 cm or 150 cm tall, depending on vegetation height) made of UV-resistant PVC film, and fitted with a vent tube, a sample port, and a fan to mix chamber air, was placed over a random quadrant of each mesocosm (S1b Fig). We connected the chamber to the Picarro gas analyzer via Swagelok® connections and Tygon® tubing, and deployed chambers for 10-minute incubations during daylight hours (10:00 to 16:00); an iButton temperature sensor (Maxim Integrated, San Jose, CA, USA)~~-~~ recorded in-chamber air temperature once every minute. ~~-~~Barometric pressure and ambient air temperature were also recorded, using a Kestrel 2500 Weather Meter (Nielsen-Kellerman, Boothwyn, PA, USA). Gas concentration measurements were corrected for the ideal gas law using temperature, pressure, and chamber volume. Flux rates were calculated based on linear changes in gas concentrations over time if R^2 values were > 0.85. ~~-~~For rates with R^2 values < 0.85, we visually

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inspected plots of concentration vs. time; rates that exhibited evidence or record of equipment malfunction (chamber tipping, etc.) or ebullition were removed from analysis (n = 5). If the linear regression of time vs. gas concentration did not differ from zero, we assigned the gas flux as zero.

Plant biomass & root porosity

We estimated aboveground biomass using species-specific allometric equations developed from ~50 oven-dried (65°C) stems of each species in 2016, relating stem height to dry biomass; ~~(A~~ all equations were second order polynomials ~~calculated in Excel, (with~~ *Typha*: R^2 = 0.92, 95% CI = ± 0.01 g; *Spartina*: R^2 = 0.92, 95% CI = ± 0.006 g; *Phragmites*: R^2 = 0.94, 95% CI = ± 0.004 g)~~)~~. All stem heights were measured in September 2017 to estimate aboveground biomass for each species-water quality treatment. Photosynthetically active radiation (PAR) measurements were taken at this time using a Decagon LP-80 Ceptometer (Decagon Devices, Pullman, WA, USA). Three measurements per mesocosm were averaged above the plant canopy and at the sediment surface to estimate the fraction of PAR (fPAR) transmitted through the canopy.

We installed in-growth root cores to measure 2017 root production in each tank (May-September 2017) [4~~7~~6]. Nylon mesh cylinders (5-cm diameter x 13-cm long) with a plastic base were packed with screened, root-free topsoil (same as that used to fill mesocosms) to a similar bulk density as the surrounding soil (~1.8 g dry soil/cm^3, average of 2016 soils). In-growth cores were installed into excavated holes of similar dimensions in May and were removed from the tanks by cutting around the outside of the core with a serrated knife and pulling it free of the tank sediment in September. Each core was emptied into a 2-mm sieve and soil was washed away with a garden hose to isolate the roots.

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In the lab, we identified three root segments (~5 cm in length) per core that were elastic and light or white in color to estimate root porosity using methods similar to [15]. We blotted excess water from the outside of the roots with lab tissues then individually weighed each segment on a microbalance. To keep roots submerged under 500 mL of water in a 1 L side arm flask, we attached a paper clip to each root segment. The flask was attached to a vacuum pump for five minutes to replace all of the airspace in the root with water. The roots were removed from the water and weighed again. The difference of the two weights divided by the initial weight estimates the proportion of the root mass that was originally airspace. Roots sampled for porosity were then returned to the bulk root sample from each mesocosm, dried ≧ 72 hours at 60°C, and weighed. Root porosity estimates for each mesocosm were averaged and then multiplied by belowground biomass to calculate total porosity. Belowground biomass estimates were calculated by scaling the mass of roots in the area of the ingrowth core to 1 m^2. Likewise, aboveground biomass estimates were scaled to units of g/m^2.

Water chemistry

We constructed wells to monitor and sample pore water chemistry. We cut 30-cm sections of PVC pipe (2.54-cm diameter), capped the bottom, sliced narrow slits to 7 cm, and wrapped 1-mm nylon screen around the slitted area to limit sediment intrusion. We pounded wells into the center of each tank to 15-cm depth. Conductivity, salinity, and porewater temperature measurements were taken during each gas sampling event using a YSI EcoSense® EC300A meter (YSI Incorporated, Yellow Springs, OH, USA). Pore water samples were taken from each mesocosm for analysis at the end of the growing season in September 2017. Pore water wells were purged and then water samples were collected with a nylon syringe and tubing and placed into acid-washed 50 mL centrifuge tubes. Samples were stored at 4°C until analysis.

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Water samples were centrifuged and filtered using 110-mm Whatman G/FF paper filters and analyzed for nitrate (NO_3^-) and ammonia (NH_4^+) on a SmartChem®200 discrete analyzer (Westco Scientific Instruments, Brookfield, CT, USA). Whatman G/FF-filtered samples were quantified for total organic carbon (TOC) on a Shimadzu Total Organic Carbon Analyzer using EPA Method 415.1, and sulfate (SO_4^{-2}) and chloride (Cl^-) on a Dionex Ion Chromatography System-1100 (Thermo Fisher Scientific, Waltham, MA, USA).

Carbon mineralization

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Surface soil samples (5-cm diameter to 10-cm depth) were collected in September 2017 to estimate heterotrophic respiration rates. ~~-~~Soils were sieved through 2-mm brass screens; a 10-g subsample of sieved soil was dried at 105°C for 48 hours to calculate soil moisture content, and a 50-g subsample was placed in a 0.95-L canning jar. Jars were attached via a 15-port manifold sampling system to ~~a~~the Picarro G2201-*i* and their headspace CO_2 concentrations were sampled for six minutes approximately every four hours over a 24-hour period [~~48~~47]; CO_2-free air soda lime blanks were used to flush the lines between samples. We converted gas accumulation rates measured as ppm/s to umol/s using the ideal gas law. ~~-~~Correcting for soil moisture content, we calculated C mineralization ~~flux~~ rates as the accumulation of gas over time per gram of dry soil.

Statistical Analysis

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All statistical analyses were conducted using R Studio 1.1.419 using R 3.5.1. Data were log-transformed to improve normality of residuals and homogeneity of variances when necessary. We tested for fixed effects of plant species, water quality treatments, and their interaction on gas fluxes, biomass, total porosity, and water chemistry data using analyses of variance (ANOVA; *lme* and *aov* commands). ~~Data were log-transformed to improve normality of residuals when necessary.~~ Initial repeated measures ANOVA (*lme* command) indicated

consistent treatment responses across our three sampling campaigns for both CO_2 ($F_{2,94}$ = 0.83, p = 0.439) and CH_4 fluxes ($F_{2,87}$ = 2.64, p = 0.077), thus we aggregated gas flux data into one data set for statistical analyses of gas flux response. We used only the September gas sampling campaign data to investigate relationships with the other variables as it aligned temporally with when we collected biomass and water chemistry data. We ~~analyzed~~tested for correlations across the entire data set (i.e., no multivariate analysis separating by treatments due to limited sample size). Correlations between explanatory and response variables were analyzed using the *cor.test* command; we used Pearson's correlation coefficient (r) for parametric data and Spearman rank correlation coefficients (r_s) for non-parametric data (i.e., when transformations did not improve normality). ~~Data were log-transformed to improve normality of residuals when necessary.~~ Means ± 1 SE are reported.

Sediment microbial characterization

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Approximately 5 g soil samples were collected from the upper 10 cm of sediments using an ethanol-sterilized spoon. Samples were collected in the vicinity of the plants which contained a large amount of root material, however these were bulk soil samples from the root zone, not specifically rhizosphere soils. Soil samples were placed in sterile Whirl-pak bags, flash frozen on dry ice, and stored at -80 °C until further processing.

DNA was extracted from ~1 g of sediment using the DNeasy PowerSoil kit (Qiagen) using the manufacturer's protocols with the exception that bead mill beating was performed on a Retch MM301 Ball Mill (30 hz for 1 minute). The V4 region of bacterial 16S rRNA genes was amplified using primers 515F and 806R with Illumina adapters and dual indices (8 basepair golay on 3' [48~~49~~], and 8 basepair on the 5' [49~~50~~]). The amplification products were

sequenced at UConn's MARS (Microbial Analysis, Resources, and Services) Illumina MiSeq platform.

Demultiplexed sequences were assembled into contigs and quality screened in the mothur software package (version 1.41.1.5; [5~~1~~0]). All sequences were selected to be at least 255 bp in length, contain no ambiguous bases, and no homopolymers of more than 8 bp. Chimeric sequences were identified with the mothur implementation of VSEARCH [51~~252~~], and all potentially chimeric sequences were removed. Sequences were clustered into operational taxonomic units (OTUs) using a 100% sequence identity threshold, employing the OptiClust algorithm in mothur [52~~353~~]. Taxonomic classification of sequences was performed with the Na¨ive Bayesian classifier [53~~454~~] against the SILVA reference alignment (release 132) [54~~555~~] in the mothur software package.

Prior to determining alpha-diversity via the nonparametric Shannon's diversity index (H'), data-sets were randomly subsampled to the size of the smallest dataset (omitting outliers with <1000 sequences), resulting in 5,720 sequences per dataset. Significant differences in OTU relative abundance were tested for with the ALDEx2 package. Prior to identifying significant differences, OTU count data were transformed using the centered log-ratio and normalized through Monte Carlo sampling with Bayesian sampling of 128 Dirichlet instances [55~~656~~]. Both the Kruskal-Wallis and generalized linear model tests were performed and an OTU was considered to be significantly different in relative abundance if the p-value was $\leq$0.05 after adjusting for multiple testing with the Benjamini-Hochberg correction. The ternary plot of OTU relative abundance was generated with the ggtern extension package in R [56~~757~~]. All raw sequence datasets are available in the NCBI Short Read Archive (SRA) under the BioProject ID PRJNA604015.

Results

Plant biomass and root porosity responses

Biomass production differed among vegetation above- and belowground (above: $F_{2,36}$ = 46.5, p < 0.001; below: $F_{2,36}$ = 6.8, p = 0.003~~; total: $F_{2,36}$ = 16.3, p < 0.001~~), as well as~~ and~~ among water quality treatments aboveground (above: $F_{3,36}$ = 144.0, p < 0.001~~; below: $F_{3,36}$ = 2.5, p = 0.078; total: $F_{3,36}$ = 16.8, p < 0.001~~), but we observed interactions between species and water quality treatment for aboveground biomass ($F_{6,36}$ = 6.5, p < 0.001), ~~and total biomass ($F_{6,36}$ = 2.2, p = 0.069),~~ principally because *Spartina* aboveground biomass responded strongly to N enrichment (Fig 1). fPAR transmission was strongly negatively correlated with aboveground biomass (r = -0.80, p < 0.001), and~~also~~ differed among species ($F_{2,42}$= 3.8, p = 0.030) and water quality treatments ($F_{3,42}$ = 24.3, p < 0.001), with greater transmission through *Typha* (71.3% ± 1.9) than *Spartina* canopies (64.6% ± 3.5). Nitrogen enrichment ~~increased biomass and~~ reduced fPAR (N~~ enrichment~~: 52.9% ± 2.9) relative to the ~~average of the~~ control and salt~~other water quality~~ treatments (72.5% ± 1.1).

Fig 1. Biomass allocation by vegetation species-water quality treatment combinations. Mean (± SE) above- and belowground biomass by species-water quality combinations in 2017 in a full factorial mesocosm experiment where each treatment combination was replicated four-fold.

Root porosity did not differ across water quality treatments ($F_{2,36}$ = 1.85, p = 0.15), but *Spartina* roots were more porous ($F_{2,36}$ = 7.6, p < 0.05; Table 2) and had greater total root porosity ($F_{2,36}$ = 6.08, p < 0.05; Table 1) than the other two species (Table 1).

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Table 1. Root porosity differed among vegetation species. ~~Average (± 1 SE) 2017 root porosity (measured from 3 root subsamples per mesocosm) and total root porosity (% porosity x total belowground biomass) for each plant species (n = 16). Superscripts indicate significant differences between treatments after TukeyHSD post-hoc comparisons.~~

~~Species~~Vegetation species	R~~r~~oot ~~porosity~~ porosity (%)	Total root ~~porosity~~porosity
Phragmites	25.5[a] ± 2.5	45.9[a] ± 8.8
Spartina	35.1[b] ± 3.1	137.1[b] ± 24.8
Typha	25.3[a] ± 2.5	46.6[a] ± 9.4

Average (± 1 SE) 2017 root porosity (measured from 3 root subsamples per mesocosm) and total root porosity (% porosity x total belowground biomass) for each plant species (n = 16). Superscripts indicate significant differences between vegetation species after TukeyHSD post-hoc comparisons.

Carbon fluxes

We observed differences in CO_2 uptake among species ($F_{2,36}$ = 34.27 p < 0.0001) and ~~among~~ water quality treatments ($F_{3,36}$ = 12.48, p < 0.0001), but did not observe an ~~no~~ interactive effect of species and~~by~~ water quality treatment ~~interaction~~ ($F_{6,36}$ = 1.12, p = 0.369). *Spartina* (36,239~~.2~~ ± 2~~.~~744 µ~~µm~~mol m^{-2} h^{-1}) and *Typha* (23,880~~.9~~ ± 1~~.~~503 ~~mmol~~µmol m^{-2} h^{-1}) had greater CO_2 uptake than *Phragmites* (14,070~~.1~~ ± 1~~.~~112 ~~mmol~~µmol m^{-2} h^{-1}; Fig~~.~~ 2a). Nitrogen addition

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(39.5.64 ± 3.4675 ~~mmol~~µmol m^{-2} h^{-1}) increased CO_2 uptake relative to freshwater controls (19.392.4 ± 1.618 ~~mmol~~µmol m^{-2} h^{-1}) and sea salt treatments (19.751.8 ± 19902.0 ~~mmol~~µmol m^{-2} h^{-1}~~; Fig. 2b~~), but did not lead to different CO_2 uptake from~~than~~ our road salt treatment (~~mean~~20.211 ± 1467~~SE~~ ~~mmol~~µmol m^{-2} h^{-1}; Fig. 2b)~~.~~.

Fig 2. **CO_2 uptake differed among vegetation and water quality treatments.** Boxplots of 2017 log-transformed CO_2 uptake rates (samples pooled across July, August, September sampling campaigns) by (a) vegetation and (b) water quality treatments. Note that measurements were estimates of net ecosystem exchange, integrating photosynthetic uptake, auto and heterotrophic respiration from transparent chambers. -Differences between groups are indicated by non-overlap of letters, based on post-hoc Tukey contrasts.

Methane emissions differed strongly among vegetation species ($F_{3,36} = 40.83$, $p < 0.0001$; Fig 3a); *Spartina* (101.9 ± 12.4 µ~~u~~mol m^{-2} h^{-1}) had the highest CH_4 fluxes, followed by *Typha* (61.8 ± 10.7 µ~~u~~mol m^{-2} h^{-1}), and then *Phragmites* (21.0 ± 2.7 µ~~u~~mol m^{-2} h^{-1}). We also observed differences among water quality treatments ($F_{3,36} = 6.31$, $p = 0.002$; Fig 3b), with sea salt addition halving CH_4 emissions (34.7 ± 5.8 µ~~u~~mol m^{-2} h^{-1}) relative to the other water quality treatments (70.3 ± 7.7 µ~~u~~mol m^{-2} h^{-1}). Water quality treatment effects were consistent across vegetation species, as we did not observe an interaction among these factors ($F_{6,36} = 1.10$, $p = 0.383$).

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Fig 3. Boxplots of 2017 log-transformed CH_4 emissions (pooled data from July, August, and September) by (a) vegetation and (b) water quality treatments. Differences between groups are indicated by non-overlap of letters, based on post-hoc Tukey contrasts.

Pore water chemistry

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Pore water chemistry was generally more responsive to water quality than vegetation treatments (Table 2), though SO_4^{-2} and DOC differed among species, with *Spartina* having lower concentrations (SO_4^{-2}: 1.03 ± 0.21 mg/L; DOC: 8.00 ± 1.25 mg/L) than *Typha* and *Phragmites* (SO_4^{-2}: 2.28 ± 0.53 mg/L; DOC: 10.14 ± 1.43 mg/L). Salt ions associated with experimental treatments differed as expected: Cl^- concentrations were much greater with road and sea salt addition (89.2 ± 4.6 mg/L) than control and ~~nitrogen~~N-enriched treatments (1.8 ± 0.5 mg/L), and sea salt treatment doubled SO_4^{-2} concentrations (3.2 ± 0.5 mg/L) compared to the other treatments (1.4 ± 0.9 mg/L). We did not observe treatment differences in NO_3^- nor NH_4^+ concentrations (Table 2); NO_3^- concentrations averaged 0.22 ± 0.05 mg/L among the 19 samples that were above our instrument's detection limit, whereas NH_4^+ concentrations averaged 1.00 ± 0.53 mg/L. Salt addition reduced porewater DOC concentrations, as we observed three times as much DOC in control and ~~nitrogen~~N-enriched mesocosms (14.9 ± 1.0 mg/L) than in road and sea salt tanks (5.0 ± 0.4 mg/L).

Table 2. **Pore water chemistry ANOVA results.** ~~Two-way ANOVA results that tested how pore water chemistry differed among vegetation and water quality treatments. Note that 29 NO_3^- samples were below instrument detection limit, resulting in low sample size.~~

	Vegetation			Water Quality		
Response	df	F	p	df	F	p
SO_4^{-2}	2, 41	6.3	**0.004**	3, 41	43.3	<**0.001**
Cl^-	2, 40	0.2	0.847	3, 40	94.7	<**0.001**
NO_3^-	2, 13	1.9	0.194	3, 13	0.9	0.434
NH_4^+	2, 36	0.1	0.903	3, 36	1.4	0.236
DOC	2, 38	6.2	**0.005**	3, 38	70.9	<**0.001**

Two-way ANOVA results that tested how pore water chemistry differed among vegetation and water quality treatments. Note that 29 NO_3^- samples were below instrument detection limit, resulting in low sample size.

Carbon mineralization

Soil C mineralization rates did not differ among vegetation ~~species~~ ($F_{2,38}$ = 2.4, p = 0.11) but were reduced with sea and road salt compared to freshwater controls and ~~nitrogen~~N enrichment ($F_{3,38}$ = 11.2, p < 0.001) (Fig 4).

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Fig 4. Carbon mineralization rates by vegetation and water quality treatments. Log-transformed sediment C mineralization rates estimated using 24-hour laboratory incubations did not differ among (a) vegetation, but differed among (b) water quality treatments. Differences between groups are indicated by non-overlap of letters, based on post-hoc Tukey contrasts.

Correlations with carbon fluxes

Aboveground biomass was positively correlated to CO_2 uptake ($r = 0.60$, $p < 0.0001$), but CH_4 emissions were not correlated with aboveground, belowground, nor total biomass. However, total root porosity was positively correlated with CH_4 emissions ($r = 0.38$, $p = 0.008$). Porewater chemistry associated with our salt treatments (SO_4^{-2}, Cl^-) influenced several C responses. We observed negative correlations between SO_4^{-2} concentration and CH_4 emissions ($r_s = -0.337$, $p = 0.024$), between Cl^- concentrations and C mineralization ($r_s = -0.577$, $p = < 0.0001$), and between DOC and C mineralization ($r_s = -0.769$, $p = < 0.0001$).

Bacterial community composition

Cluster Canonical Correlation Analysis (cluster-CCA) was used to investigate the relationship between bacterial 16S rRNA gene sequence datasets (Fig 5). Clustering by ~~plant species~~vegetation showed substantial overlap in community composition, although there was a significant difference in centroids between vegetation types (Fig 5a~~A~~; $p = 0.005$). Clustering was more apparent when aggregated by water quality treatment, with the salt treatments separating distinctly from the control and ~~nitrogen~~N amendments (Fig 5b~~B~~). However, there was no

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significant clustering that differentiated the controls and the ~~nitrogen~~N ~~amendments~~ enrichment or between the two salting treatments (road or sea salt).

Fig 5. CCA-clustering of bacterial 16S rRNA gene datasets. (a) Data clustered by vegetation type. Significance of clustering was tested with the permutation anova CCA test and vegetation was a significant factor for clustering (p = 0.005). (b) The same data clustered by water quality treatment, which was also a significant factor in dataset clustering (p = 0.001).

Bacterial diversity

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Bacterial diversity was assessed by calculating the non-parametric Shannon's diversity index. -When the datasets were clustered by vegetation, *Typha* showed the highest average diversity, with the lowest diversity amongst *Phragmites* (Fig 6a). Alternatively, water quality treatment showed a clear decrease in diversity associated with the salt~~ing~~ treatments (Fig 6b).

Fig 6. Bacterial diversity in sequence datasets. (a) Diversity in datasets grouped by vegetation type. (b)~~.~~ Diversity in datasets grouped by water quality treatment. Significant differences between groups are indicated by non-overlap of letters, based on post-hoc Tukey contrasts.

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Differentially abundant OTUs due to vegetation

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The abundance of the numerically dominant OTUs were plotted as a ternary diagram to display their relative abundance among the three plant species (Fig 7a). Most OTUs belonged to five bacterial phyla, with the Proteobacteria being most common. The majority of the OTUs were present at similar relative abundances among the three vegetation types as evidenced by

their clustering in the center of the ternary diagram (Fig 7a). Only two OTUs were identified as significantly different in relative abundance, and their abundances in each vegetation type is displayed in Fig 7b. Otu000028 was classified to the genus *Geobacter* (Phylum, Proteobacteria) and was enriched in the *Spartina* mesocosms. In contrast Otu000322, classified to the *Novosphingobium* (Phylum, Proteobacteria), was uniquely present in with *Typha*. Generally, these data indicate that OTU relative abundance was sensitive to the different plant species, with only a very limited number of OTUs showing a shift in relative abundance in response to plant species.

Fig 7. OTU relative abundance in association with vegetation. Only the 1500 most abundant OTUs are displayed. (a) Ternary diagram displaying OTU abundance among the three plant species. The two OTUs identified as significantly different in relative abundance are indicated by the arrows. (b) Median counts per sample of each of the differentially abundant OTUs. A table showing the classification of the differentially abundant OTUs is shown in S1 Table.

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Differentially abundant OTUs due to water quality treatment

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OTU relative abundance in the controls was plotted against the treatments to test for shifts in abundance due to the different amendments. OTUs were present in similar relative abundance between control and ~~nitrogen~~N enrichment treatments, and no OTUs were identified as significantly different in relative abundance due to ~~nitrogen~~N (Fig 8a). In comparison, multiple OTUs were identified as differentially abundant due to the road and sea salt treatments. We further determined if the differentially abundant OTUs from the two salt~~ing~~ treatments were unique or common to each condition (Fig 8b). A total of 86 OTUs were identified as

significantly different of which 25 (29%) were common to both the road salt and sea salt treatments. In this regard, there appears to be a set of OTUs that share a similar response to salt, irrespective of the source. The differentially abundant OTUs identified as common to both salt~~ing~~ treatments were predominantly within the phylum Proteobacteria (Fig 8c). Taken together, these data suggest that the differ~~ren~~ing plant responses to the ~~nitrogen~~N, road~~sea~~ salt, and sea~~road~~ salt treatments were not matched by similar responses in the sediment microbial community. Given that the soils for this survey were collected in the vicinity of the roots, but did not include the rhizosphere soils directly in contact with the roots, the influence of the plant on sediment communities did not appear to extend into the root zone soils. Instead, sediment microbial communities appeared to respond to changes in sediment properties, particularly those associated with salting, such as osmotic stress.

~~.~~

Fig 8. Differentially abundant OTUs due to treatment. (a) Each point represents a detected OTU and its counts in controls versus treatment. OTUs colored in red were identified as significantly different in abundance. (b) Differentially abundant OTUs unique and shared among the two salt treatments, (c) Taxonomic classification of differentially abundant OTUs in salt treatments.

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Finally, we investigated those OTUs that were ~~depleted in the salt treatments (~~enriched in controls ~~)~~ versus those that were enriched with salt and were shared between both the road salt and sea salt treatments (S1 Table). A diverse set of OTUs were identified, belonging to six different phyla and 17 families. All of the taxa were heterotrophic groups with a variety of

different growth types and strategies. For instance, an OTU related to the genus *Sideroxydans*, an iron oxidizing group of bacteria [57] ~~(Muhling et al, 2016)~~, was enriched in the control samples (S1 Table ~~S1~~). In contrast, three OTUs related to the genus *Geobacter* were enriched in the salted sediments (shared in both road salt and sea salt). Members of the *Geobacter* genus are thought to be the primary drivers of oxidizing organic matter coupled to the reduction of iron and manganese [58] ~~(Lovley *et al.*, 2011)~~. In this respect, these data point to a state change in the iron cycle in the mesocosms under the salt treatments, which points to a decreased availability of dissolved iron under elevated salt. The remaining OTUs largely belonged to general heterotrophic bacteria or were not able to be classified to taxonomic ranks deeper than family, which limits the confidence that functional predictions can be made from these classifications. ~~there was no apparent pattern that differentiated those OTUs that were either enriched or depleted in the salt treatment.~~

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Discussion

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Wetlands play a major role in global C dynamics, but understanding how wetland plants, sediment microbial communities, and water quality interact is currently not well resolved. ~~-~~To help bridge this gap, we conducted a mesocosm experiment in which we manipulated plant species (globally dominant wetland genera- *Phragmites*, *Typha*, *Spartina*) and common water quality impairments (N-enrichment, salinization via road or sea salt) to investigate C and microbial responses. We found that plant species had strong effects on our response metrics, with largely similar ~~responses~~ patterns in response to water quality treatments across plant species. However, water quality treatments appeared to have distinct effects on plant vs. microbial responses; ~~nitrogen~~N enrichment increased biomass production and CO_2 uptake, whereas

salinization reduced ~~methane~~ CH_4 emissions (with sea salt), reduced heterotrophic respiration, altered microbial composition, and decreased microbial diversity.

Biomass and C process responses

Rates and allocation of biomass production are the foundation of C cycling in wetlands. Not surprisingly, we observed that greater aboveground biomass promoted greater CO_2 uptake, and that N-enrichment amplified biomass production, particularly in *Spartina*, which had five times greater aboveground biomass production with N addition than controls. ~~–~~ Anecdotally, we observed higher algae abundance in surface waters of *Typha* and *Phalaris* ~~*ragmites*~~ with N addition; higher levels of PAR penetrating through sparser canopies may have stimulated algal production and resulted in similar increases in CO_2 uptake across all vegetation treatments with N addition. Interestingly, salt addition (300 g/m^2/y) did not reduce biomass production compared to freshwater controls at the relatively low, but environmentally relevant, salinity ~~(2 ppt)~~ levels (2 ppt) ~~-~~we targeted. Dramatic biomass reductions for freshwater macrophytes were observed when salinity treatments exceeded 4 ppt in [4~~6~~5]. ~~-~~Likewise, [~~39~~38] observed a wetland seed bank threshold of 2 ppt ~~reduced~~ for species richness, diversity, and aboveground biomass, ~~seed bank plant responses~~with reductions in plant responses in NaCl treatments > 2 ppt ~~in NaCl treatments > 2 ppt~~, suggesting that common freshwater wetland plants may be~~are~~ resilient to salinity levels ~~<~~ ≤ 2 ppt.

However, biogeochemical processes appear to be more ~~responsive~~ sensitive to salinization. We observed reduced CH_4 emissions with SO_4^{-2} rich sea salt addition; while we did not quantify how water quality treatments effected pH, under circumneutral pH, ~~this is logical given that~~ SO_4^{-2} is thermodynamically favored over the reduction of C compounds

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[59,60].~~30,31]. [insert considerations from Gao et al. 2019 and Bethke et al. 2011 here].~~]

Likewise, we observed a negative correlation between SO_4^{-2} concentrations and CH_4 emissions across all treatments. Both salinity treatments decreased DOC concentrations, likely due to salt-induced flocculation which promotes particle aggregation [27,28~~,29~~], hence exclusion during filtration. ~~-~~We found no correlation between DOC and CH_4 emissions as found in other studies [61~~58~~]. However, we observed decreased C mineralization rates (i.e., heterotrophic respiration) and decreased diversity of microbial communities in our salt treatments,~~. C mineralization rates, which were negatively correlated to DOC concentrations. In conjunction, we found salt treatments decreased diversity of microbial communities,~~ potentially pointing to osmotic stress of certain microbial populations. Similar to [62], we did not observe an effect of N addition on C mineralization rates, indicating excess nutrients were assimilated by plants, algae or microbes in the water column, but not by soil microbes in soil.

In contrast to other studies [6,7], we did not observe positive correlations between biomass and CH_4 emissions, though total root poro~~s~~eity (% root porosity x root biomass) was positively correlated to CH_4 emissions. Still, o~~O~~ur data suggest that porous plant tissue acted similarly to a straw, allowing methane produced in anoxic sediment to bypass surface oxic layers and travel into the atmosphere, as observed by other ~~studies~~ -[13,63~~59~~]. *Spartina* had greater total root porosity than the other two species, ~~allowing~~ providing a large pathway for CH_4 to escape to the atmosphere. *Spartina* also had lower porewater SO_4^{-2} concentrations than other species; thus, elevated CH_4 emissions from *Spartina* would be expected, as these conditions may favor methanogenesis [59]. ~~-~~ Why *Spartina* had lower porewater SO_4^{-2} concentrations is unclear, however, as rhizosperic oxygenation should decrease sulfate reduction, thereby maintaining large

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SO_4^{-2} pools. Elevated uptake of SO_4^{-2} by *Spartina* is plausible, as [~~60~~64] observed differential species uptake.

~~While *Phragmites* is commonly known as an extremely productive and dominant species [61–63], the *Phragmites* we used in our study was a relatively short and sparse strain that sequestered less CO_2 and emitted less CH_4 than either *Spartina* or *Typha*. While this was surprising, it~~ This is ~~is likely associated with the seed source we used; we collected seed from a population growing out of a groundwater seep at the base of a hill on UConn's campus, which may not be wholly representative of the species.~~

Microbial community response

Plant species played a significant, if small role in sediment microbial community composition (Fig 5). The majority of the identified bacterial OTUs were present in all three mesocosms in similar proportions, with only two OTU's identified as significantly different in relative abundance between the three plant species (Fig 7). Thus, most bacteria appeared largely indifferent to plant species. The rhizosphere of wetland plants, the zone of soil directly in contact with the plant root has been shown to harbor elevated bacterial activity and altered communities in comparison to bulk soils [6~~4~~5,66~~5~~,~~65~~]. However, in this study we did not specifically isolate rhizosphere soils. In this regard, the influence of the plant on sediment microbial communities may be mostly limited to sediments in direct contact with roots.

The salt treatments induced a reduction in the diversity of the sediment microbial communities (Fig 6~~4~~b). Furthermore, a substantial fraction of the bacterial OTUs that shifted in relative abundance were common to both salt treatments, road salt and sea salt (Fig 8~~6~~b). This suggests that the elevated osmotic stress likely affected a similar group of bacteria. However, the

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555 shifts in relative abundance due to the salt treatments were generally among the numerically rare
556 populations, whereas the most abundant OTUs were resilient to the treatments (Fig 8c)~~6A)~~. This
557 suggests that the dominant bacteria in the mesocosms were largely unaffected by the salt
558 treatment. The differentially abundant OTUs did point to an alteration in the iron cycle in the
559 sediments under the salt treatment. The enrichment of *Sideroxydans* in the control mesocosms in
560 comparison to an enrichment of *Geobacter* with elevated salt suggests a shift from iron oxidation
561 to iron reduction with the addition of salt. Further, similar to previous experimental findings
562 [62], we observed reduced mineralization of labile carbon from the salt treatments, which may
563 have been associated with reduced microbial diversity or shifts in community composition. Thus,
564 the osmotic ~~and/~~or redox stress induced by the salt treatment did appear to shift biogeochemical
565 cycles in the sediments.

566 We hypothesized that we would observe a unique set of bacteria enriched in the sea salt
567 treatment. This is because the sulfates in sea-water are thought to support sulfate-reducing
568 communities which then outcompete methanogens. We observed a reduction of methane
569 emissions in the sea salt treatment, yet we did not observe an enrichment of sulfate reducers (S1
570 Table) which could indicate higher sensitivity to salinity than to redox conditions. ~~.~~Furthermore,
571 none of the differentially abundant OTUs in sea salt treatments were associated with
572 methanotrophic populations (S1 Table), bacteria capable of oxidizing methane [6~~6~~7]. As the
573 primers employed in this study were designed to amplify bacterial 16S rRNA genes, they were
574 not able to detect methanogenic archaea so we cannot directly address the effects of sea salt on
575 methane producing populations. Thus, the sediment microbial data was not particularly
576 predictive in the reduction of CH_4 observed under the sea salt treatment. However, our data only
577 describe the composition of the sediment communities. It is possible that water quality

treatments shifted the activity of particular microbial populations, such as sulfate-reducers, methanotrophs, or methanogens, without a concurrent alteration in their relative abundance. Future studies incorporating metrics of microbial activity may better address changes in the functions of the microbial community under differing water quality treatments.

Experimental design constraints

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In the field, wetland vegetation, soils, and hydrology are often confounded, so a controlled mesocosm experiment allowed us to systematically test how vegetation and water quality treatments alter a range of biological and biogeochemical responses. However, relics of our experimental design should be considered when interpreting or comparing our results with other investigations. While invasive *Phragmites* is commonly known as an extremely productive and dominant species [68–70], the *Phragmites* we used in our study was a relatively short and sparse strain that sequestered less CO_2 and emitted less CH_4 than either *Spartina* or *Typha*. This is likely associated with the seed source we used; we collected seed from a population growing out of a groundwater seep at the base of a hill on UConn's campus, which may not be wholly representative of the species. We manipulated the hydroperiod of our mesocosms to promote reduced soils during the growing season; while draining the tanks during winter to prevent tanks from cracking may have altered microbial composition and redox conditions, consistent manipulation of soils and hydrology allows us to draw inferences about responses to our vegetation and water quality treatments.

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Conclusions

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Wetlands are crucial landscape sinks, often occurring in low-lying areas that collect polluted or impaired runoff from surrounding watersheds, and are on the front lines of sea level rise, making them vulnerable to salt water intrusion. ~~-~~In turn, water quality can affect plant species composition and production rates, which are underlying drivers of wetland C cycling. Our results indicate that plant traits (biomass, root porosity) as well as species identity are important determinants of C gas flux. Particularly in areas vulnerable to invasive species and community shifts, presence or exclusion of key species has the potential to alter CO_2 uptake or CH_4 emission rates occurring within wetlands. Another ~~crucial~~ important driver of C flux in freshwater wetlands is water quality. Different water quality impairments such as N, road salt, and sea salt ~~,~~ ~~and nitrogen~~ affect C gas flux in different ways. Nitrogen enrichment's influence on biomass production and increased gas flux make it a prominent driver of change in wetlands exposed to agricultural runoff as well as wastewater. The reduction of CH_4 emissions due to salt-water intrusion of sea level rise exhibits the power of small water quality changes within the system. Although the relatively low concentrations of salt used in this experiment (2 ppt) did not significantly affect plant traits such as biomass production, they did alter the water and sediment chemistry enough to influence the sediment microbial communities therefore altering CH_4 emissions. Recent evidence suggests that engineered nanoparticles can exacerbate eutrophication in wetlands [71], highlighting the need to further examine the interactions among emerging contaminants, water quality, vegetation, and wetland carbon cycling.

The vegetation~~plant species~~ and water quality impairments used in this experiment are common throughout not only eastern North America~~New England~~, but also many locations worldwide. With the crucial role that wetlands play in the global C cycle, it is important to better

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understand the integration between plant performance and microbiology and how these factors influence C gas fluxes.

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Supporting information

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S1 Fig. Wetland mesocosm experimental setup. (a) A mesocosm tank experiment was set up at the University of Connecticut in 2016-2017 to test how plant species and water quality treatments influenced carbon gas fluxes and sediment microbial communities. (b) Co-author O. Johnson monitors real time C fluxes using a transparent floating chamber connected to a Picarro g2201-*i* during the 2017 growing season.

S1 Table. OTUs depleted or enriched in association with the salt treatments.

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~~Mühling, M., Poehlein, A., Stuhr, A., Voitel, M., Daniel, R., & Schlömann, M. (2016). Reconstruction of the metabolic potential of acidophilic Sideroxydans strains from the metagenome of an microaerophilic enrichment culture of acidophilic iron-oxidizing bacteria from a pilot plant for the treatment of acid mine drainage reveals metabolic versatility and adaptation to life at low pH. *Frontiers in microbiology*, *7*, 2082.~~

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PONE-D-20-02931 (Response to editor and reviewer comments detailed in blue text below)
Nitrogen enrichment stimulates wetland plant responses whereas salt amendments alter microbial communities and biogeochemical responses
PLOS ONE

Dear Dr. Lawrence,

Thank you for submitting your manuscript to PLOS ONE. After careful consideration, we feel that it has merit but does not fully meet PLOS ONE's publication criteria as it currently stands. Therefore, we invite you to submit a revised version of the manuscript that addresses the points raised during the review process.

In particular, criteria 3 and 4 (https://journals.plos.org/plosone/s/criteria-for-publication) have not been fully achieved. Experimental and statistical issues were raised by both reviewers, which need to be addressed by the authors as well as it is crucial to verify the values of tables/figures and text to avoid any misleading information.

Response: We thank the reviewers and editor for their thoughtful recommendations and have addressed all statistical issues raised and have also verified the values in all tables and figures. Please see our detailed responses below.

I also have few additional points:
- Have the authors considered the distinction between heterotrophs/organotrophs and heterotrophs/lithotrophs, when presenting the results in lines 416-419? These distinctions, in combination with data on salt tolerance could bring more light further into the discussion. These characteristics should then be included in table S1 and should also be used in the discussion of the results (lines 482-503).

Response: We have purposefully avoided too much discussion of potential functions of the differentially abundant OTUs for several reasons. First, we do not have cultures for the organisms so the identification is based on a single gene fragment, thus any identifications have to be taken with a grain of salt. To highlight this we have added the genus level classification to Table S1, which shows that many (12 of 13) OTUs could not be reliably classified. Second, changes in abundance are not always associated with a change in activity. So seeing an increase or decrease in an OTUs abundance does not mean any function assigned to that OTU would manifest as a net change in that activity in the community. Finally, many organisms show mixotrophic growth showing organo/lithotrophic characteristics depending on environmental conditions. So it is not always a simple matter to assign these functions.

That being said, we agree that there may be more information available than we presented and have added the following to the results (Lines 433-447): "For instance, an OTU related to the genus Sideroxydans, an iron oxidizing group of bacteria (Muhling et al, 2016), was enriched in the control samples (Table S1). In contrast, three OTUs related to the genus Geobacter were enriched in the salted sediments (shared in both road salt and sea salt). Members of the Geobacter genus are thought to be the primary drivers of oxidizing organic matter coupled to the reduction of iron and manganese (Lovley et al., 2011). In this respect, these data point to a state change in the iron cycle in the mesocosms under the salt treatments, which points to a decreased availability of dissolved iron under elevated salt. The remaining OTUs largely belonged to general heterotrophic bacteria or were not able to be classified to taxonomic ranks deeper than family, which limits the confidence that functional predictions can be made from these classifications."

and the discussion (Lines 513-527): "The differentially abundant OTUs did point to an alteration in the iron cycle in the sediments under the salt treatment. The enrichment of Sideroxydans in the control mesocosms in comparison to an enrichment of Geobacter with elevated salt suggests a shift from iron oxidation to iron reduction with the addition of salt. Further, similar to previous experimental findings [Craig and Zhu 2018], we observed reduced mineralization of labile carbon from the salt treatments, which may have been associated with reduced microbial diversity or shifts in community composition. Thus, the osmotic or redox stress induced by the salt treatment did appear to shift biogeochemical cycles in the sediments."

- The authors in the material and methods section clearly indicated that bacterial communities were not collected from the rhizosphere, but from bulk soil from the root zone. I believe the discussion in lines 473-481 would be improved if the authors also related the results with known effect of soil properties/soil parent material in the bacterial communities.

Response: We have added the following to the section (Lines: 420-426): "Taken together, these data suggest that the differing plant responses to the N, road salt, and sea salt treatments were not matched by similar responses in the sediment microbial community. Given that the soils for this survey were collected in the vicinity of the roots, but did not include the rhizosphere soils directly in contact with the roots, the influence of the plant on sediment communities did not appear to extend into the root zone soils. Instead, sediment microbial communities appeared to respond to changes in sediment properties, particularly those associated with salting, such as osmotic stress."

Some minor points:

Line 384-388, besides, the genus of the identified OTUs, also indicate in brackets the phylum;

Response: The suggested changes have been made.

Figure 8, in particular in Figure 8a, please change (Average counts treatment (log10)) by (ratio of average counts treatment (log10), controls versus treatment);

Response: The suggested changes have been made.

Line 456 replace "root porocity" by "root porosity".

Response: This change has been made.

Journal Requirements:

When submitting your revision, we need you to address these additional requirements.

1. Please ensure that your manuscript meets PLOS ONE's style requirements, including those for file naming. The PLOS ONE style templates can be found at

http://www.journals.plos.org/plosone/s/file?id=wjVg/PLOSOne_formatting_sample_main_body.pdf and http://www.journals.plos.org/plosone/s/file?id=ba62/PLOSOne_formatting_sample_title_authors_affiliations.pdf

Response: We now conform to PLOS ONE's style requirements.

2. We note that you have stated that you will provide repository information for your data at acceptance. Should your manuscript be accepted for publication, we will hold it until you provide the relevant accession numbers or DOIs necessary to access your data. If you wish to make changes to your Data Availability statement, please describe these changes in your cover letter and we will update your Data Availability statement to reflect the information you provide.

Response: All our raw sequence data are available in the NCBI Short Read Archive (SRA) under the BioProject ID PRJNA604015: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA604015. All other data will be available after acceptance in the Dryad database.

3. Please amend either the title on the online submission form (via Edit Submission) or the title in the manuscript so that they are identical.

Response: Titles are now identical.

4. Please include captions for your Supporting Information files at the end of your manuscript, and update any in-text citations to match accordingly. Please see our Supporting Information guidelines for more information: http://journals.plos.org/plosone/s/supporting-information

Response: Captions for Supporting Information files are now included at end of the manuscript according to guidelines provided.

5. Our internal editors have looked over your manuscript and determined that it is within the scope of our Call for Papers on the Microbial Ecology of Changing Environments. Additional information can be found on our announcement page: https://collections.plos.org/s/microbial-ecology. If you would like your manuscript to be considered for this collection, please let us know in your cover letter and we will ensure that your paper is treated as if you were responding to this call. If you would prefer to remove your manuscript from collection consideration, please specify this in the cover letter.

Response: Great- this sounds like a good opportunity and I will indicate our interest in being included in our cover letter if it is still feasible given our resubmission is beyond the April 10th deadline.

6. In your Methods section, please provide additional information regarding the permits you obtained for the work. Please ensure you have included the full name of the authority that approved the field site access and, if no permits were required, a brief statement explaining why.

Response: The experiment was conducted on property owned by the University of Connecticut, where I am a faculty member, thus, no permits were required.

Reviewers' comments:

Reviewer #1: Here Donato et al. analyzed the impacts of salt and nitrogen deposition on carbon cycling in wetland monocultures. They used 48, ~380 L tanks to make 4 replicates of 3 different species with 4 different water chemistry treatments. Overall the authors make a strong case for how salts impact both vegetation and microbial communities and thus the carbon cycling in wetlands. The study is original but some work needs could be done to contextualize the results within the broader literature to help expand the relevance of the mesocosm study the authors present (see papers below). This is particularly true when discussing the relevance of the OTU abundance on carbon cycling. Salt amendments lowered C mineralization, and altered OTU diversity, but the link between the two needs to be more carefully addressed within the discussion. Finally, the authors need to address the design components which could have impacted the conclusions.

Response: Please see our response to the editor above related to linking OTU abundance and function. We amended our discussion to address how OTU diversity may alter sulfur, iron, and carbon mineralization (Lines 433-447), and now reads "For instance, an OTU related to the genus *Sideroxydans*, an iron oxidizing group of bacteria [57], was enriched in the control samples (Table S1). In contrast, three OTUs related to the genus *Geobacter* were enriched in the salted sediments (shared in both road salt and sea salt). Members of the *Geobacter* genus are thought to be the primary drivers of oxidizing organic matter coupled to the reduction of iron and manganese [58]. In this respect, these data point to a state change in the iron cycle in the mesocosms under the salt treatments, which points to a decreased availability of dissolved iron under elevated salt. The remaining OTUs largely belonged to general heterotrophic bacteria or were not able to be classified to taxonomic ranks deeper than family, which limits the confidence that functional predictions can be made from these classifications."

It is unclear if the authors consider the impacts of draining the tanks on the reduction-oxidation potential of the micropores within the soil? Although the seasonal methane signal was not described in the text, the

relatively large p-value suggests that methane may not have been consistent across all sampling periods. Could exposure of the soil matrix to the atmosphere be part of the story? The authors should address this.

Response: As we describe, "initial repeated measures ANOVA (lme command) indicated consistent treatment responses across our three sampling campaigns for both CO2 ($F_{2,94} = 0.83$, $p = 0.439$) and CH4 fluxes ($F_{2,87} = 2.64$, $p = 0.077$), thus we aggregated gas flux data into one data set for statistical analyses of gas flux response." It is unclear what you mean by the "relatively large p-value"; did you intend to say relatively *low* p-value? We now consistently use an alpha level of 0.05 to infer statistical significance, so we stand by our decision to aggregate fluxes across sampling campaigns. However, it is certainly possible that there was an ecologically relevant temporal trend in CH4 fluxes; this could be related to how we manipulated hydrology, temperature, plant inputs, or their combination. We drained the tanks at the end of the growing season soon before the first freezing temperatures, and re-filled them as temperatures started to warm up; thus the tanks were filled with water when temperatures were prime for microbial activity and any oxygen dissolved in pore water was likely quickly consumed. We now address this and other possible issues associated with our experimental design in the discussion, in a section titled "Experimental design constraints" (Lines 546-559).

The Gibbs free energy that determines which terminal electron acceptor is the dominant metabolic pathway is pH dependent (See Bethke et al. 2011). If the soils and treatments varied in acidity, particularly around neutral conditions, metal reduction rates could be more relevant than the SO4 content in the context of limiting methanogenesis. If the pH wasn't measured, then the authors need convince the reader that the pH is similar across all treatments or they need to soften their language around 'favorablitily' of the kinetics that regulate microbial metabolisms.

Response: This is a great point and we soften our language about the kinetics of microbial metabolism as recommended, as others have found reduced pH with road salt addition (Craig and Zhu 2018, Kim and Koretsky 2013). Unfortunately, we did not measure soil pH during our study, but the water we used to maintain water levels was circum-neutral (pH of 7.18).

The statistics reported throughout the paper need some improvement. In one case the authors accept a null hypothesis with a p-value of 0.077 (line 222), but then reject it in separate analyses with a p-value of 0.078 (Ln 267) and 0.069 (Ln 269). Liner mixed effect models were poorly described but used to justify aggregating methane fluxes despite relatively low p-values. This may be acceptable, or may be problematic depending on the specific hierarchal model design.

Response: We apologize for the inconsistency. We re-wrote the results so that responses with $p > 0.05$ are not considered statistically significant. The sentence now reads (Lines: 270-274), "Biomass production differed among vegetation above- and belowground (above: $F_{2,36} = 46.5$, $p < 0.001$; below: $F_{2,36} = 6.8$, $p = 0.003$) and among water quality treatments aboveground (above: $F_{3,36} = 144.0$, $p < 0.001$), but we observed interactions between species and water quality treatment for aboveground biomass ($F_{6,36} = 6.5$, $p < 0.001$), principally because Spartina aboveground biomass responded strongly to N enrichment (Fig 1)."

The correlation analyses could also use some clarification, since it was unclear when Pearson correlation were used or when the Spearmen rank test was implemented. Clarity would help the reader understand the decisions around test selection, as it was initially assumed by this reviewer that the log transform was done to normalize the data. Why then use a Spearmen rank test in this instance, or if using Spearmen, why log transform the data?

Response: We changed the text to clarify why we used Pearson vs. Spearman's tests. The text now reads (Lines: 229-233): "We used Pearson's correlation coefficient (r) for parametric data and Spearman rank correlation coefficients (r_s) for non-parametric data (i.e., when transformations did not improve normality)." We clearly indicate in the results what test we used for each pair of variables by using the r or r_s notation.

Furthermore, regression analysis may be more informative than Pearson in a number of analyses presented here, since it allows for multivariate analysis and post hoc analysis of residuals. For example, a properly constructed linear model could determine if the correlation between SO4 and methane is independent of the Spartina group, which can not be inferred from the Person correlation analysis presented in the text.

Response: If possible, we agree a multivariate analysis would have been more informative, however we did not have sufficient sample size to run multivariate analyses (i.e., we used gas flux data from a single sampling campaign, so total n = 48, divided by 4 water quality treatments = 12, divided by 3 plant species = 4 reps per group for multivariate analysis).

Recommended Papers:

Granberg, G., Sundh, I., Svensson, H., and Nilsson, M., Effects of temperature and nitrogen and sulfur deposition on methane emission from a boreal mire, Ecology, 82, 1982–1998, 2001.
Bethke, C. M., Sanford, R. A., Kirk, M. F., Jin, Q., and Flynn, T. M., The thermodynamic ladder in geomicrobiology, Am. J. Sci., 311, 183–210, 2011.
Herndon, E. M., Mann, B. F., Roy Chowdhury, T., Yang, Z., Wullschleger, S. D., Graham, D., Liang, L., and Gu, B., Pathways of anaerobic organic matter decomposition in tundra soils from Barrow, Alaska, J. Geophys. Res.-Biogeo., 120, 2345– 2359, 2015.
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Clark, M. G., Humphreys, E. R., Carey, S. K., Low methane emissions from a boreal wetland constructed on oil sand mine tailings, Biogeosciences, 17, 667-682, 2020.

Response: Thank you for the literature recommendations. We incorporated considerations from Gao et al. 2019 and Bethke et al. 2011 into the discussion.

Specific comments:

Line 84: Maybe some reclamation lit here? Response: We assume you are referring to mine reclamation, but that is unclear. Since our study focuses on the effects of common wetland pollutants of eastern North America (nitrogen, road and sea salt), we do not think it is necessary to integrate reclamation literature here.

Line 53: Are they highly productive, or do they just have low rates of respiration? The 5th assessment report WG1 is not a good source for suggesting that wetlands are becoming more monotypic graminoid dominant. Response: You are correct, this is not an appropriate citation. We now cite Zedler and Kercher 2004, which is the citation we were intending to use originally. We checked accuracy of all citations throughout manuscript.

Line 143: A schematic, of field picture to help visualize the experimental design would be helpful. Even if it's in the supplementary data.

Response: Thank you for the suggestion. We have added Supplemental Figure 1 which includes two photos: (a) our experimental mesocosm set up and (b) our gas sampling chamber configuration.

Line 161: What is the accuracy on the allometric equations?

Response: We added the R^2 and 95% Confidence Intervals for our allometric equations.

Line 273: Imprecise wording. I think it means it is relative to the control and the salt treatments? The control isn't really a "treatment".

Response: We clarified wording here. It now reads, "Nitrogen enrichment increased biomass and reduced fPAR (N-enrichment: 52.9% ± 2.9) relative to the average of the control and salt treatments (72.5% ± 1.1)."

Lines 290/91: This sentence is confusing. Do you simply mean there was a species and treatment effect but no interaction term? Simple wording clarification should be fine.
Response: We clarified wording here. It now reads, "We observed differences in CO2 uptake among species ($F_{2,36} = 34.27$ $p < 0.0001$) and among water quality treatments ($F_{3,36} = 12.48$, $p < 0.0001$), but did not observe an interactive effect of species and water quality treatment ($F_{6,36} = 1.12$, $p = 0.369$)."

Line 294/95: No mention the road salt treatment.
Response: We added road salt treatment result. It now reads, "Nitrogen addition (39,564 ± 3467 µmol m^{-2} h^{-1}) increased CO_2 uptake relative to freshwater controls (19,392 ± 1618 µmol m^{-2} h^{-1}) and sea salt treatments (19,751 ± 1990 µmol m^{-2} h^{-1}), but did not lead to different CO_2 uptake from our road salt treatment (20,211 ± 1467 µmol m^{-2} h^{-1}; Fig 2b)."

Line 304: There was an issue with the µ (/mu) symbol on the pdf.
Response: All gas flux rates are in units of µmol m^{-2} h^{-1}. Figures 2 and 3 are on a log scale. We checked the units to confirm they are accurate.

Line 441: ends with a citation, but is written as if it was a result from this study.
Response: Yes, we clarified that this line was referring to Ref #46. It now reads, "Dramatic biomass reductions for freshwater macrophytes were observed when salinity treatments exceeded 4 ppt in [46]."

Lines 429-430 is vague wording. Is it largely similar in response to water quality treatment or largely similar in the differences across species within each treatment?
Response: We clarified wording here. It now reads, "We found that plant species had strong effects on our response metrics, with largely similar patterns in response to water quality treatments across plant species."

Line 438-40: "environmentally relevant" and "we targeted" is redundant here. You already explained in the methods that you targeted environmentally relevant salinity profiles. Response: We felt it was appropriate to comment on the environmental relevance in the discussion as well.

Line 440-442: Did you mean "Other studies have found…"? Response: Yes, we clarified that this line was referring to Ref #46. It now reads, "Dramatic biomass reductions for freshwater macrophytes were observed when salinity treatments exceeded 4 ppt **in** [46]."

Line 442-443: Did Walker S. (2019) find only an effect at >2 ppt? Your wording leaves their results ambiguous. Did they find no effect at <2 or didn't include it in their study? Response: We clarified the text here so that it was clear that Walker et al. tested a range of salinities. It now reads, "Likewise, [39] observed a wetland seed bank threshold of 2 ppt for species richness, diversity, and aboveground biomass, with reductions in plant responses in NaCl treatments > 2 ppt, suggesting that common freshwater wetland plants may be resilient to salinity levels ≤ 2 ppt."

Line 447: "across all treatment" sounds like correlation analysis was performed within each treatment but was not reported. Response: We added clarification in the statistical analyses section that the correlation was for entire data set, not by treatment.

Lines 450-452: The correlation to DOC is a separate idea. Put it in a new sentence for clarity unless it was only negatively correlated in the salt treatments. Response: We made the suggested edit.

Line 453: Was the relationship between microbial community abundance and heterotrophic respiration examined, why wasn't it reported? I would assume some of these communities would have no impact on aerobic respiration. Response: We did not directly examine the relationship between microbial community and heterotrophic respiration as it is difficult to link community changes to flux rates given our methodology (see above response to editor). We see an overall decrease in diversity of the

community which could indicate lower functional diversity. However, the elevated respiration with nitrogen addition was not associated with a change in the microbial communities. We added these ideas to the discussion (Lines: 478-483).

Line 457: reference reads as if its on your own findings. Perhaps add "…as others [ref] have described"? The paragraph starting on Line 455 is difficult to follow. A lot of ideas that jump around and don't build on one another. Reorder the SO4-Spartina discussion so it builds to the conclusion, that methane is largest in these plots. The second sentence states that "Our data shows" but has two citations. You need to say what specifically in the other studies relate to what your data shows. Response: We clarified wording as suggested. It now reads, "Still, our data suggest that porous plant tissue acted similarly to a straw, allowing methane produced in anoxic sediment to bypass surface oxic layers and travel into the atmosphere, as observed by other studies [13,59]."

Lines 468/69: Why "this is surprising" is unclear, since expectations for production/emission by species was never discussed. Is the surprise simply referring to the productivity, the methane flux, or both? Response: We agree this was unclear, we removed "why this is surprising" and focused on the main idea, that "This is likely associated with the seed source we used…"

Line 483: Is this in reference to Fig 6b, not 4b? Response: Yes, we corrected figure reference.

Line 487: Again, I think this is Fig 8C. Fig 6A has nothing to do with rarity of populations. This sort of repeated mistake makes it hard to follow the discussion and has me wonder what else has been overlooked. Response: We corrected the figure reference and confirmed accurate figure numbers throughout the manuscript.

Line 492: I am not a microbiologists. For those who share this shortcoming, I wonder if you could add the primary metabolic pathway (i.e. sulphate reducers, methanogens, etc.) of the microbes to table S1 or perhaps list it when you first discuss the Phyla of the microbes? It would help readers follow the logic in the discussions on species abundances and reinforce the connection to the carbon cycle.

Response: Please see our response to the editor above. To reiterate briefly, it is difficult to assign a function to a microbe that we have only identified from a single gene fragment in a sequence-based assay. We have expanded the results and discussion to encompass some of the functional predictions we can make. Please see Lines 433-442, and 513-520.

Line 503: Did you look into composition/abundance and mineralization rates? I didn't see it in your manuscript but could be another link between microbes and the carbon cycle.
Response: See our response to previous comment and in our response to the editor.

Discussion: What are the implications of reduced fPAR on the carbon cycle with respect to your treatments? It was not included in the discussion, so is it relevant to the study?

Response: We included the potential importance of fPAR in the discussion (Lines 466-469), and the relevant next reads "Anecdotally, we observed higher algae abundance in surface waters of *Typha* and *Phalaris* with N addition; higher levels of PAR penetrating through sparser canopies may have stimulated algal production and resulted in similar increases in CO_2 uptake across all vegetation treatments with N addition."

Readability of Figure 8 is very low. Impossible to see axis labels. Increase resolution. Response: We revised Fig 8 to increase the readability as recommended.

Reviewer #2: Donato and collaborators are describing the carbon fluxes and microbial changes in wetland mesocosms exposed to nitrogen, road salt, or sea salt contaminations. They showed that carbon fluxes were mainly impaired by Nitrogen treatment through plant biomass changes, the salt pollution disturbed C mineralization, decrease in microbial diversity and shifts in the microbial community, and led to lower CH4 emissions.The authors have made a great effort in compiling a complex dataset in a very comprehensive study. The study is detailed, and the results well explained. The conclusions are interesting and will be of interest to the PLOS ONE readers. My main comments would be that the authors do not discuss the eutrophication of their system, neither the (likely high) reducing environment that the N and salt treatment likely generated. Though an additional discussion of that matter would be interesting, it should not prevent the publication of this paper that is already convincing as it is.

See my other comments below:
1. Was anything added to the control treatment (eg 1L of DIW?) Response: Yes, 1L of DI W was added to control treatments. We added this detail to the text.
2. Line 162: Please provide a reference or in SI the correlations used to calculate the plant biomass based on their height. Response: We added the R^2 and 95% Confidence Intervals for the equations.
3. It would be of interest to specify that the sampling campaigns were done 1months, 2 months, and 3 months after the last dosing treatment, during the summer (right?) Response: We clarified this so that the text now reads, "We measured C fluxes during three sampling campaigns in 2017 (mid-July, August, and September; approximately one, two, and three months after the last dosing treatment)."
4. Line 286: significances between treatments or between plant species? Line 280 = treatments do not impact root porosity) Response: Current Lines 284-286 are referring to our observation of no differences among water quality treatments, whereas Table 1 presents differences among plant species.
5. All plot, I would call the panels "treatments" rather than "Water quality", which is a trait itself. It is a bit confusing as it. Response: We manipulated vegetation and water quality, so consider them both "treatments." To avoid potential confusion, we changed the panel headers on Figures 2, 3, and 4 from "Water quality" to "Water quality treatments."
6. Line 303 and fig 3a: the number provided in the text vs the values plotted doesn't seem to match. Response: The values in Fig 3 are log-transformed data. We provided untransformed data in the text to allow easy comparison with other studies.
7. It has been shown in similar systems that redox variations can vary daily and along the year1, and be impacted by anthropogenic activities. These effects were likely mainly driven by the macrophyte photosynthesis, respiration, and life cycles. I am pointing this because based on my (rough) calculations, the authors have added approximately 5g of SO42- in their system (based on the characterization provided in ref 41 in the manuscript), which should represent a concentration in the 20L mesocosms of 250 mg/L. Despite the very high addition of SO42- added to their system the authors measured "only" 3.2 mg SO42- per L of water in their mesocosms. This likely indicates a reducing environment (which is not surprising since the sampling was done during the summer, as described in ref1).
My question is: could it be that some of the observed effects in the sea salt treatment (decrease of DOC, C mineralization) are due to a more reducing environment than the others? Did the authors measure dissolve CO2 and O2 concentrations in the mesocosms water? Response: We did not measure dissolved CO_2 or O_2 concentrations in mesocosm porewater. Since wetlands are characterized by reducing conditions, we intentionally manipulated the hydrology to promote anoxic, reducing conditions. We assume oxygen was consumed equally in all water-logged soils, leaving the explanations for observed effects to be related to terminal electron acceptor availability, and salt effects on organic matter and osmotic pressure. Sea salt treatments still had twice as much SO42- than any other treatment, indicating more SO42- available for sulfate reducers, and we infer the decreased DOC is due to increased flocculation of organic matter due to salt addition, and the decrease in C mineralization is due to osmotic stress on microbes.

8 Line 485: it could be osmotic stress and/or a more reducing environment.

Response: We have added redox stress to the section. Thank you for the suggestion.

9 Line 463: Spartina is also very responsive to the nitrogen treatment. Could it be an indicator of the eutrophication of the system, thus more reduction of the water, explaining the larger decrease in SO42- concentration? I am surprised that the authors do not discuss eutrophication here when implementing their systems with N at a very high rate (15 g N /year)

Response: It is possible that *Spartina* has higher nutrient use efficiency than the other species, as *Spartina* increased aboveground biomass 5x more than controls, compared to 3x increase of *Typha* and *Phragmites* relative to controls. Excess N and higher light penetration may have stimulated algal growth in surface waters, which we anecdotally observed in *Typha* and *Phragmites* tanks. Interestingly, we did not observe an effect of N addition on C mineralization rates, indicating excess nutrients were assimilated by plants, algae or microbes in water column, but not microbes in soil. We added these ideas to our discussion (Lines 463-469).

10 Line 491:Figure 5 also indicates that the 2salts treatments induce a shift toward the CCA1 factor, and the 2 populations are grouped. This could indicate that, at that period of the year (already under pretty high reducing conditions), the microorganisms are more sensitive to high salinity than to the redox conditions.
Response: Good point- we amended text to read "We observed a reduction of methane emissions in the sea salt treatment, yet we did not observe an enrichment of sulfate reducers (S1 Table) which could indicate higher sensitivity to salinity than to redox conditions"

11 I could be of interrest for the reader to add a picture of the mesocosms in supporting information
Response: We added a photo of both our experimental design and our gas sampling chamber set-up to the SI as recommended.

Cited reference
1 Andersen, et al. "Extreme diel dissolved oxygen and carbon cycles in shallow vegetated lakes." Proceedings of the Royal Society B: Biological Sciences 284.1862 (2017): 20171427.
2 Simonin, et al. "Engineered nanoparticles interact with nutrients to intensify eutrophication in a wetland ecosystem experiment." Ecological Applications 28.6 (2018): 1435-1449.

Response: Thank you for the suggested references. We integrated Simonin et al. 2018 into the discussion, and highlight the need to further investigate emerging contaminants in wetlands.

While revising your submission, please upload your figure files to the Preflight Analysis and Conversion Engine (PACE) digital diagnostic tool, https://pacev2.apexcovantage.com/. PACE helps ensure that figures meet PLOS requirements. To use PACE, you must first register as a user. Registration is free. Then, login and navigate to the UPLOAD tab, where you will find detailed instructions on how to use the tool. If you encounter any issues or have any questions when using PACE, please email us at figures@plos.org. Please note that Supporting Information files do not need this step.

Response: All our figures (including revised versions) meet PLOS requirements, as determined by uploading to PACE.

Impacts of Climate Change on Long Island Sound Salt Marshes

Developed by: [1]Candice Cambrial, [2]Beth Lawrence, [3]Kimberly Williams

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Focus

The natural and anthropogenic impacts of climate change on salt marshes.

Salt marshes are critical habitats at the interface of land and sea that fringe the Long Island Sound. Photo: B. Lawrence

Focus Question

How are scientists in our region studying the various impacts of climate change on salt marsh habitat?

Audience

9th/10th grade Biology or General Science students as well as upper level science elective courses such as Environmental Science or Marine Science, as appropriate.

Learning Objectives

Students will obtain an overview of a variety of different techniques for climate change research.

Students will describe carbon- and nitrogen-based services associated with dominant coastal marsh plant species.

Students will identify that shifts in dominant marsh species will alter ecosystem service provision of Long Island Sound coastal wetlands.

Students will gain an understanding of the complex interactions among climate change, sea level rise, coastal wetlands, and ecosystem services among diverse audiences in the Long Island Sound region.

Materials

- Computer or individual ‘smart’ device
- EdPuzzle account
- Case Study handout & PowerPoint
- Drowned sparrow nest & viable sparrow nest printed (recommend images printed on opposite sides and laminated if possible)
- LCD/Projector with audio

- Interactive PowerPoint guided notes worksheet
- Mystery Scientist guided notes worksheet
- CER student worksheet

Audio/Visual Equipment
Computers/Internet access
LCD for PowerPoint presentation (audio required)

Teaching Time
Five teaching periods/days estimating a 45 min class duration. Teachers with block scheduling will be able to complete the unit in three class meetings.

Seating Arrangement
Students will work in small groups of 4 - 5, in pairs and individually over the course of the unit.

Key Words
Anthropogenic
Biodiversity
Biogeochemistry
Carbon and Carbon Sequestration
Ecosystem Services
Greenhouse Effect
Greenhouse Gas
Invasive Species
Nitrogen and Denitrification
Photon
Vegetation
Salt Marshes
Wetlands

Background Information/Teacher Preparation

- Teachers should be familiar with the basics of climate change and what causes it. The climate change video used in the EdPuzzle is a good primer for teachers as well as students.

- Additional background for the “Polar Bear of the Salt Marsh” is included with the case study.

- Explanation and examples are provided for the Claim, Evidence and Reasoning technique with the unit materials.

Learning Procedure

Summary:

- Day 1 should be used to pre-teach or refresh students about the basics of climate change they will need to understand to meet the learning objectives of this module by completing The Greenhouse Effect PHET.
- Day 2 should be used to conduct the Polar Bear of the Salt Marsh case study parts 1, 2 and 3.
- Day 3 should be used to generate student interest and discussion (phenomenon) with the drowned sparrow warm up activity followed by the lead researcher's interactive Powerpoint.
- Day 4 should be used to conduct the Mystery Scientist Activity.
- Day 5 should be used to complete the unit assessment CER based on the Mystery Scientist Activity.

Procedure:

Note- all handouts associated with module materials are provided after page 7, but can also be downloaded via provided links. Teacher materials (including teaching notes and answer keys) can be accessed via the links in the table below.

Time line (*45 min periods)	Content Covered	Materials
Pre-work	Basic review of climate change. EdPuzzle - free for students and teachers.	Review video of climate change: • https://youtu.be/XFmovUAWQ640423 867UQ EdPuzzle: https://edpuzzle.com/media/5d5d7378ef145440951f9ea4
Day 1	Essential information to pre-teach, or refresh students about the basics of climate change they will need to understand to meet the learning objectives of this module. Climate Change: what is it & causes of. Potential activity/discussions: -The Greenhouse Effect PHET (for classrooms with computer access) - See PHET site for additional optional support materials (ie- worksheets & diagrams) -Review results of EdPuzzle questions with students	Interactive Simulations: https://phet.colorado.edu/en/simulation/greenhouse

Day 2	Case Study Parts 1 -> 3 : "The Polar Bear of the Salt Marsh?" from National Center for Case Study Teaching in Science	• Case Study Link: http://sciencecases.lib.buffalo.edu/collection/detail.html?case_id=1011&id=1011
		• Teaching notes and answer key posted here:
		  • saltmarsh_sparrow_teachingnotes.pdf
		  • saltmarsh_sparrow_answer key.pdf

Day 3	Drowned Sparrow Do-Now/Opener/Warm-Up:
	Pass out photos of non-drowned (viable) sparrow nest and drowned (non-viable) sparrow nest on opposite sides of a laminated sheet to each lab group for discussion.
	
	Interactive Powerpoint by researcher (~25 min) with directed notes: Overview of saltmarshes and scientist's research
	
	Optional extension: NY Times Article
		• Suggested phenomena: image of baby sparrows in nest and nest drowning.
		  • Regular Nest: https://images.app.goo.gl/giN5s4zVkwyzTBfD6
		  • Drowned Nest:https://www.audubon.org/news/the-saltmarsh-sparrow-creeping-dangerously-close-extinction
		• Interactive Powerpoint: https://kaltura.uconn.edu/media/HBL-Rec01_bal15101_20190815-151824/1_0e1n3m2j
		• Interactive directed notes:https://drive.google.com/file/d/1ffQhjOXXoOyqQracOUKV15PwzK6ozA8Q/view?usp=sharing
		• Answer key: https://docs.google.com/document/d/1g61ZwtfJshZxRBDWfGprTiOOI4ZOmpPHELsmxErypCA/edit?usp=sharing
		Extension Article: https://www.nytimes.com/2018/09/17/science/saltmarsh-sparrow-extinction.html

Day 4	Mystery Scientist Activity
	Objectives: "Meet the Scientists"
	• Watch assigned videos in groups of 4-6 (note: there are 5 total Mystery Scientist videos, labeled A-E)
	• Suggested ideas for sharing results:
	  • Jigsaw results with students-each group sharing out to class
	  • Have students fill in results on a large scale table
	  • Matching activity/game-match laminated photos with appropriate scientist
		• Mystery Scientist Guided Notes: https://docs.google.com/document/d/1_pu9TZpXp-H0bOys4MMgkoZGbnSU6fy0UXjURNFO9AU/edit?usp=sharing
		• What do Mystery Scientists Do? Videos:https://www.youtube.com/channel/UCeh-g0Hcgu-_z9C-MSq_yeQ/videos

	Closure/HW: 'Ask the Scientist' • Now that you have learned about what your Mystery Scientist does, if you could talk to them right now, what would you ask or suggest to them about their research? What about their experiments made you wonder or wish you knew more? What more do you want to know about their research?	
Day 5	Do Now/ Warm Up: Brain storm student question/responses for 'Ask the Scientist'. Optional: teacher email a curated selection to the scientist(s). Video Reveal of Mystery Scientist. (Suggested whole class activity) CER - start in class, finish as HW assignment. Assessed for grading. Introduce focus question. "Is the scientist helping us learn more about climate change?" Pick a scientist from the collection. CER - Students will make a claim using evidence provided to address the question. Evidence taken directly from mystery scientist guided notes. *(Crafting your Reasoning document should be downloaded for best viewing. *google doc instructions)* Options: • Group or individual assessment activity. • In class or homework assignment. IEP students - provide resources with highlighted preselected evidence/data for them to choose from. Three sample CERs have been provided for classrooms unfamiliar with the Claim Evidence Reasoning technique.	• Mystery Scientist Identifier videos: https://www.youtube.com/channel/UCqsxQpiOWsXiiJ8anoE6tUw?view_as=subscriber • CER outline (student copy): https://drive.google.com/file/d/1KtkD5J4K5ed1Gb6rLYVnuG9uAEbqc99B/view?usp=sharing • CER outline with sentence starters: https://drive.google.com/file/d/1sFo8wNjXBoUUCmGxcIbMvSlUdcuCr_Hj/view?usp=sharing • Crafting your Reasoning: https://drive.google.com/file/d/1Ye2DcM_mlTpJshuro21yiA4fPpXUozbZ/view?usp=sharing • Sample CER: https://drive.google.com/file/d/1exkapvzwWhpJt9pqAkEQdwq4aL854G4-/view?usp=sharing • Grading Rubric: https://drive.google.com/file/d/1yJeKR3-gWYgGYYPb3SCuOWQf2QpOnobR/view?usp=sharing

The "Me" Connection

- Explain how human development of coastal land has impacted the salt marsh habitat.
- Describe how anthropogenic actions have caused sea level rise.

Connection to Other Subjects

History/Geography, Economics

Evaluation

EdPuzzle answers, Case Study answers, Interactive PowerPoint worksheet, Mystery Scientist guided notes and CER worksheet.

Extensions

Day 3: Read and discuss 'Saltmarsh Sparrows Fight to Keep Their Heads Above Water' article published by the NY Times.
Day 4: Utilize the student generated responses to the 'Ask the Scientist' activity to email a select number of questions to the researchers who participated in the Mystery Scientist activity videos.
Day 5: Complete the Polar Bear of the Salt Marsh case study (Part 4 & 5)

Resources/Helpful Links:

Review video of climate change:
https://youtu.be/XFmovUAWQ640 423 867UQ

Ocean Literacy Link:
http://oceanliteracy.wp2.coexploration.org/ocean-literacy-framework/

Instructional Resource News Platform: Newsela:
https://newsela.com/

LIS Salt marsh response to SLR graphic:
http://2pywec11qb6ms796h1llfxn1.wpengine.netdna-cdn.com/wp-content/uploads/2015/08/SLAMMdid-you-know-fact-sheet2-V05.pdf

How LIS was formed (animation):
https://www.youtube.com/watch?v=eeeIgDs4SdY

Sea Level Rise by State:
https://sealevelrise.org/states/

Greenhouse Gas simulator:
https://phet.colorado.edu/en/simulation/greenhouse

PHET - Greenhouse Effect
https://phet.colorado.edu/en/simulation/legacy/greenhouse

MIT's greenhouse gas simulator:
https://www.climateinteractive.org/tools/mits-greenhouse-gas-simulator/

How sun's energy gets to earth's surface:
https://science360.gov/obj/tkn-video/4ee36f26-71e6-41cd-bdcf-662c4dca6e9b/earths-heat-balance-suns-energy

Greenhouse Gas Activities:
https://authoring.concord.org/sequences/388

Scientific Inquiry, Literacy and Numeracy

- Scientific inquiry is a thoughtful and coordinated attempt to search out, describe, explain and predict natural phenomena.
- Scientific inquiry progresses through a continuous process of questioning, data collection, analysis and interpretation.
- Scientific inquiry requires the sharing of findings and ideas for critical review by colleagues and other scientists.
- Scientific literacy includes speaking, listening, presenting, interpreting, reading and writing about science.
- Scientific literacy also includes the ability to search for and assess the relevance and credibility of scientific information found in various print and electronic media.
- Scientific numeracy includes the ability to use mathematical operations and procedures to calculate, analyze and present scientific data and ideas.

Next Generation Science Standards

HS-ESS3-1. Construct an explanation based on evidence for how the availability of natural resources, occurrence of natural hazards, and changes in climate have influenced human activity.

Ocean Literacy Essential Principles and Fundamental Concepts

Essential Principle 6: The ocean and humans are inextricably interconnected
Fundamental concept e: Humans affect the ocean in a variety of ways. Laws, regulations and resource management affect what is taken out and put into the ocean. Human development and activity leads to pollution (point source, non-point source, and noise pollution) and physical modifications (changes to beaches, shores and rivers). In addition, humans have removed most of the large vertebrates from the ocean.

NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

The Polar Bear of the Salt Marsh?

by
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Christopher R. Field, University of Maryland, Annapolis, MD

Part I – What's Going On?

Katie was horrified. A sudden feeling of unease overtook her. Looking at the drowned nestlings floating in a tangle of saltmarsh grass made her sick to her stomach. This was the fifth drowned saltmarsh sparrow nest she had discovered this breeding season. Katie had been exploring the wetland adjacent to her house in coastal Connecticut since her dad had given her a set of binoculars for her eighth birthday ten years ago. A competent naturalist, she knew that saltmarsh sparrows were ground-nesting birds, endemic to the tidal marshes of the eastern United States and were decreasing in population size throughout southern New England. She noted another drowned nest in her field notebook and asked herself, *What could be going on here?*

Question

1. What factors could lead to drowned nests in a tidal salt marsh?

Part II – Rising Sea Levels

Since it was low tide, Katie decided to tromp through the marsh to the Barn Island Wildlife Management Area headquarters to see if she could talk with somebody who might have more information. Different salt marsh plants can tolerate different amounts of flooding and salt concentrations. This variation in physical stress tolerance leads to vegetation zones or bands, each dominated by different grass-like plants. Katie traversed the band of vegetation closest to the ocean where cordgrass *(Spartina alterniflora)* exclusively dominates the daily flooded low-marsh elevations. In southern New England salt marshes, marsh hay *(Spartina patens)* dominates the intermediately flooded band, and black rush *(Juncus gerardii)* occupies the higher, drier, and less salty marsh elevations. Marsh hay and black rush are excluded from the low marsh by low soil oxygen levels and high salt concentrations. Cordgrass has the ability to oxygenate its root zone and has physiological adaptations to deal with high salinity, allowing it to tolerate the frequently flooded and salty low-marsh zone.

After a hot slog through the marsh, Katie was relieved to arrive at the Barn Island headquarters and see Chris Smith, a natural resource manager for the Connecticut Department of Energy and Environmental Protection (DEEP). Katie blurted out, "Chris, I found another drowned nest of saltmarsh sparrows this afternoon. That's the fifth one this season! Have you heard reports from other people like this?"

Chris laughed, "Hi Katie, nice to see you too." In a more serious tone, he added, "Actually, I've had several birders report nest drownings this breeding season, and it seems like more and more are documented each year." Chris was thoughtful for a moment and then pulled out a recent issue of a preeminent scientific journal and said, "Check out this article. Maybe there's something in here."

"Wow, I didn't know global mean sea-level has risen 14–22cm in the last century. That's crazy!" exclaimed Katie as she skimmed the article. "Actually, their models suggest that about 70% of sea-level rise since 1970 is attributable to human activities, especially greenhouse gas emissions."

Chris responds, "So sea levels are rising, but I'm unclear how…"

As Katie continued reading the article she responded, "The two biggest contributors to sea-level rise are thermal expansion of the oceans—as water warms, it takes up more volume—and glacier mass loss. Basically, the earth is warming up due to our use of fossil fuels and causing water to expand and ice to melt." Katie continued, "But what's going on in Connecticut? Is that what's drowning all these saltmarsh sparrow nests?"

Questions

2. What kind of information, either biotic or abiotic, could Katie and Chris use to determine whether sea-level rise is occurring in salt marshes in Connecticut?

3. Sea-level rise of 14–22 cm over 100 years may not seem like much (1.4–2.2mm per year), but consider how the slope of the land determines how much will be inundated. Will steeply or gently sloped areas be more impacted? Try sketching the two situations.

4. Make a diagram showing the three dominant vegetation zones of the salt marsh, indicating relative elevation and distance to the ocean. Based on salt and flooding tolerance thresholds of the dominant plant species, predict how plants will shift in response to sea-level rise; show this on your diagram.

Part III – Vegetation

"There's a researcher at the University of Connecticut that monitored vegetation in 55, 1-hectare plots in 12 different salt marsh complexes along the Connecticut coastline in 2003 and 2013," Chris said pensively. "I wonder whether we could determine if sea-level is rising here by comparing the change in occurrence of the different plant species."

Katie jumped at the suggestion and exclaimed, "Let's do it!"

Question

5. Do the data in Figure 1 provide support for rising sea levels in coastal Connecticut? Why or why not? What other information would support this hypothesis?

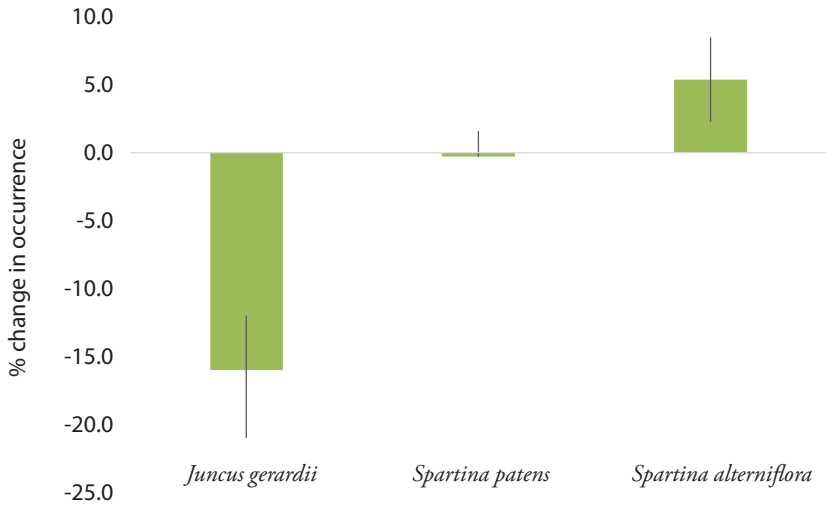

Figure 1. Mean percentage change in occurrence for the dominant plant species in 55, 1-ha plots in Connecticut salt marshes surveyed in 2003 and 2013 (data adapted from Field *et al.,* 2016).

Part IV – The Future

While Katie was data crunching, Chris looked into the literature and found that sea-level rise in southern New England is predicted to be much higher than the global average (Yin *et al.,* 2009; Boon, 2012; Sallenger e*t al.,* 2012). Observed sea-level trends at tide stations in southern New England range from 2.44 to 2.87 mm/year over the past 50 years (NOAA; www.tidesandcurrents.noaa.gov) and from 1980 to 2009 increases in the rate of sea-level rise have been 3–4 times the global average (Sallenger *et al.,* 2012). Even with no future carbon emissions, coastal areas face over 0.5 m of sea-level rise over the next century, with more than 1 m possible (Schaeffer *et al.,* 2012).

"Yikes!" exclaimed Katie. "Well, couldn't saltmarsh plants move in response to increased flooding? Can't we just expect marshes to migrate landward?"

Chris responded, "Maybe. Let's look at some satellite images of coastal Connecticut and think about it."

Question

6. Brainstorm three potential challenges to marsh migration.

References

Boon, J.D. 2012. Evidence of sea level acceleration at US and Canadian tide stations, Atlantic Coast, North America. *Journal of Coastal Research* 28(6): 1437–45.

Field, C.R., C. Gjerdrum, and C.S. Elphick. 2016. Forest resistance to sea-level rise prevents landward migration of tidal marsh. *Biological Conservation* 201: 363–9.

Sallenger, Jr, A.H., K.S. Doran, and P.A. Howd. 2012. Hotspot of accelerated sea-level rise on the Atlantic coast of North America. *Nature Climate Change* 2(12): 884.

Schaeffer, M., W. Hare, S. Rahmstorf, and M. Vermeer. 2012. Long-term sea-level rise implied by 1.5 C and 2 C warming levels. *Nature Climate Change* 2(12): 867.

Yin, J., M.E. Schlesinger, and R.J. Stouffer. 2009. Model projections of rapid sea-level rise on the northeast coast of the United States. *Nature Geoscience* 2: 262–6.

Part V – How to Respond?

Imagine that you own a $1.5 million house in Old Saybrook in the marsh migration zone. What would you do in the face of sea-level rise?

You will be assigned one of the following five sea-level response strategies to research for the next class meeting. Spend about thirty minutes researching your assigned strategy and develop a list of pros and cons and bring it with you to class next time.

- Beach nourishment
- Sea wall construction
- Conservation easement
- Sell property
- Put house on stilts (adaptation)

You will share your list with others so make sure that you are prepared!

Name:_____ Date:_____ Class:_____

Interactive Directed Notes on the Salt Marsh Scientist Talk

Link to interactive Powerpoint: https://kaltura.uconn.edu/media/HBL-Rec01_bal15101_20190815-151824/1_0e1n3m2j

THINK - PAIR - SHARE
Why are coastal marshes important?
...did you miss anything important? Use the space below!

COMPARE and CONTRAST	
Compare and contrast Carbon and Nitrogen-based ecosystem services provided by salt marshes.	
COMPARE (What is similar?)	CONTRAST (What is different?)

BRAINSTORM	
The narrator reviewed some of the reasons wetlands have been lost. Brainstorm TWO ways they can be restored.	
1.	2.

NOTES
What is the BIG question? (What is the research question?)

THINK LIKE A SCIENTIST
The researchers sampled three plots in each zone and 20 different sites. Why did the researchers sample so many sites?

FIVE SENTENCE ESSAY
What should we do with the invasive grass the researchers analyzed? Support your response with evidence from the presentation!

Name:_____ Date:_____ Class:_____

Mystery Scientist Guided Notes

Directions: Watch the 'mystery scientist' video you have been assigned to answer the questions below. You answers do NOT need to be in complete sentences, bullet points are fine. Your task is to make notes on this information to help you with a future challenge! HINT: put the captions on the video to help your team.

What part of sea level rise or climate change does this scientist study?

What parts of the ecosystem is this scientist focused on? Ex: sediment, water chemistry, grasses, fish, birds, etc.

WHY is this scientist focused on this in particular (why is it important)?

Mystery Scientist Guided Notes

How do they do their research? Ex: observational studies, experiments, etc.

What type of equipment they use? Ex:quadrat frames, mist nets, satellite imagery, etc.

How is their research currently being used (or could be used)?

Claim-Evidence-Reasoning (C-E-R)

Student Graphic Organizer

Question: Is the scientist helping us learn more about climate change?
****Use your Mystery Scientist Guided Notes!**

C (Claim) Write a statement that responds to the question.	
E (Evidence) Provide information from your the video to support your claim. Your evidence should be appropriate (relevant) and sufficient (enough to convince someone that your claim is correct). Bullet points or sentences.	
R (Reasoning) Use scientific principles and knowledge that you have about the topic to explain why your evidence (data) supports your claim. In other words, explain how the information you chose from the video helps or doesn't help people learn more about climate change.	