Effects of Plant Communities and Seasonality on Wetland Greenhouse Gas Emissions
Statement of Work
Background:

Wetlands are important to carbon sequestration but are also large sources of greenhouse gases (GHG), accounting for ~30% of the methane (CH_4) emissions on Earth (1). Thus, wetlands are important in global carbon cycling and have the capacity to enhance global warming through the release of GHGs. Vegetation potentially mediates wetland carbon cycling by influencing GHG production rates, transport pathways, and ultimately, emissions to the atmosphere, depending on the traits of the present vegetation (2) and how that vegetation responds to changes in the environment (3). Wetlands support high levels of plant biodiversity and experience large environmental shifts seasonally, thus, the purpose of this study is to investigate the linkage between wetland plant biodiversity, seasonality, and GHG emissions. Understanding how different plant communities affect wetland carbon cycling will inform restoration and conservation efforts that may not only promote diversity but also reduce GHG emissions.

Plants directly and indirectly influence GHG dynamics with effects differing among functional types and species (1, 2). Plants exchange gas with the atmosphere (plant-mediated flux), provide carbon for GHG production via litter and the release of carbon substrates, and indirectly influence GHGs by changing the physical and chemical environment. Vegetation influences oxygen levels, pH, and litter quality, thereby influencing the microbial community, decomposition, and GHG production and consumption. Indeed, plant traits can regulate the total amount of anaerobic respiration and CH_4 production by influencing the availability of electron donors and electron acceptors in the sediment (2). Many of these traits are related to plant inputs of carbon and oxygen into the environment which are connected to plant productivity and photosynthesis (2). In fact, multiple studies report a correlation between rates of primary production and CH_4 emissions, though certain studies report a positive correlation (3) while others report a negative correlation (2). Similarly, physiological traits related to plant oxygen and carbon inputs into the sediment, such as root porosity and structure, may also be influenced by photosynthetic traits (2, 3, 4).

One functional trait that may drive CH_4 production is the photosynthetic pathway of the plant. C3 and C4 plants differ in the dark reactions of photosynthesis, leaf anatomy, the timing of photosynthesis (C4 plants are able to undergo photosynthesis when the stomata are closed), and the efficiency of carbon dioxide (CO_2) fixation (C4 plants are more efficient at fixing CO_2) and thus, the efficiency of photosynthesis (5). Moreover, C3 and C4 plants differ in root composition and the chemical composition of their root exudates (and thus, the lability of their root carbon exudates) with C4 root exudates typically having higher concentrations of organic acids and amino acids and C3 root exudates typically having higher concentrations of carbohydrates (4). Therefore, plant traits associated with productivity may have a distinct effect on GHGs. Indeed, plant photosynthetic pathways may be important in regulating wetland sediment respiration and carbon cycling (6).

Furthermore, plant community composition and biodiversity can have unique effects on GHG emissions due to the composition and co-occurrence of plant traits (7, 8). In fact, the traits and relative abundance of the present plant species and the effect of this diversity on dissolved organic matter quality may have stronger effects on GHG emissions than total biomass (9, 10). Wetland plant biodiversity and community composition likely also affect the seasonality of ecosystem functions due to species asynchrony and interspecific differences in response to common seasonal environmental change (e.g., hydrology, temperature) (11, 12, 13). Thus, as

plant productivity and potentially, physiology changes seasonally, GHG production and emissions may also change. For example, growth in certain woody and herbaceous species may be affected differently by the interaction of increased CO_2 levels in the atmosphere and changing water level, resulting in differences in CH_4 emissions, potentially due to differences in root morphology (3). Therefore, there may be an interaction between the combination of plant traits and seasonality that influences GHG production and emissions.

The objectives of my research are to understand the effects of plant functional types and diversity on wetland GHG emissions. I propose to investigate how wetland plant diversity (the co-occurrence of plant functional types and species) affects GHG dynamics across seasons, focusing on photosynthetic traits (C3 or C4 photosynthesis). I hypothesize that:

1. C4 monocultures will have lower GHG emissions than C3 monocultures due to the higher efficiency of photosynthesis and the higher degradability of C3 carbon exudates (4, 5).
2. Wetlands with more plant species and co-occurring C3 and C4 plants will have lower annual GHG emissions than less diverse wetlands due to dissolved organic matter diversity and higher rates of productivity due to niche partitioning (5, 7, 10).
3. Wetlands with greater plant diversity will have a weaker seasonal signal in GHG emissions as different species asynchronously photosynthesize, grow, and die-off, whereas low diversity wetlands will have more synchronous periods of photosynthesis, growth, and die-off, resulting in a greater seasonal variation in GHG emissions.

Methods: I will conduct a 2 year vegetation-manipulation experiment (7, 9), varying plant functional types (C3, C4, or both), and species diversity (low, high), at the Cornell Experimental Pond facility in Ithaca, New York using a randomized block design. Each of 3 artificial wetlands (30 m x 30 m) will be divided into 14 plots (1m x 1m with a 1m buffer zone containing the treatment community surrounding the plot), into which I will transplant only C3 species (e.g., *Carex* species, *Scirpus* species, *Typha Latifolia* (14, 15, 16, 17)), only C4 species (e.g., *Cyperus strigosus, Spartina pectinata, Panicum virgatum* (16, 18)), or a combination of C3 and C4 species at either low or high species diversity (2 vs. 4 species total) (Table 1). Each plot will contain the same number of individual plants and will be maintained by manually removing any non-experimental plants that may grow (9).

Table 1. Outline of the treatments of the proposed study. Species 1-4 represent C3 plant species, and species 5-8 represent C4 plant species. The semicolons separate different treatment plant communities that will be plotted for different combinations of functional type and diversity level to ensure that the observed results are due to diversity rather than a specific plant community. Each treatment will be performed in triplicate.

Functional Type	Monoculture	Low diversity	High diversity
C3 (*species 1 - 4)*	Species 1; Species 2	Species 1+2; Species 1+3	Species 1+2+3+4
C4 (*species 5 - 8)*	Species 5; Species 6	Species 5+6; Species 5+7	Species 5+6+7+8
C3 + C4	N/A	Species 1+5; Species 1+7	Species 1+2+5+6; Species 3+4+5+6

I will measure GHG emissions from each plot using a sampling chamber and a portable GHG analyzer (19), and I will collect porewater using vacuum syringes and porewater samplers to determine porewater GHG concentration via a headspace sampling technique (19), porewater dissolved oxygen percent saturation, and dissolved organic matter quantity (via loss on ignition methodology) and quality (fluorometrically) in the porewater (10). I will also measure root distribution using cores (20), the average height and width of each plant community, and the photosynthetic rate for one replicate of each treatment using a LI-6400 IRGA (LI-COR, Inc., Lincoln, NE) (2). I will sample each plot biweekly during the summer growing season and monthly the rest of the year. Following the 2-year experiment, I will harvest each plot, determine the total aboveground biomass by drying and weighing the plants, and determine the belowground ground biomass by removing the top 30 cm of sediment, rinsing the sediment, and drying and weighing the remaining roots. I will use mixed-effects models (blocked by wetland) to examine the primary effects of species richness and functional diversity on GHG emissions and the secondary influence on porewater concentrations, root distribution, and dissolved organic matter quality and quantity.

Impacts of Research on Coastal Wetlands

Wetlands are the largest natural source of CH_4 (21). Thus, understanding what factors drive GHG emissions from these ecosystems can strategically be used to conserve, restore, and potentially, build wetlands with lower GHG emissions. New York State is committed to carbon neutrality by 2050 and is currently inventorying statewide wetland GHG fluxes. My lab is leading this survey, and my research will contribute to these efforts to inform state carbon budgets and future coastal wetland conservation, restoration, and policy.

The original development of wetland conservation efforts and the continued input of government and non-government resources into wetland conservation are largely due to the ecosystem services that wetlands provide, including provisioning, regulating, cultural, and supporting services (22). Wetlands provide clear benefits for humans, such as carbon sequestration, flood mitigation, and water quality improvement, and wetlands are also crucial for many types of wildlife and maintaining biodiversity. By linking plant biodiversity to carbon cycling, my research will provide evidence that the services provided by wetlands are connected to one another and that in order to conserve the wetland functions that humans rely upon, we may also need to conserve the biodiversity of these systems. This is especially important given the bias associated with ecosystem service valuation and the emphasis placed on the impacts of ecosystems on society (23).

How Funds Will Be Used

Item	Description	Cost
Wetland plot establishment and chamber construction	Each plot will be cleared and a PVC sampling frame will be installed prior to introducing the plants. Two sampling chambers (1m x 1m x 1.5m) will be constructed with a PVC frame, UV resistant PVC film, and three fans each for air circulation.	$4,000

Porewater sampling supplies	15 porewater samplers and 15 vacuum syringes will need to be purchased. Porewater samplers will be installed 24 hours prior to sampling, thus, can be used in multiple plots over the experiment.	$1,000
Porewater: Sample collection and analysis	$3 per sample x 45 plots x 20 sampling days in two summers $3 per sample x 45 plots x 14 days in rest of year for two years	$4,590
	TOTAL	**$9,590**

I will use the majority of the **$5,000 from this grant** for establishing the experiment (wetland plot establishment, chamber construction, and the porewater sampling setup). Any remaining funds will go towards sample collection and analysis as described above. I will also recruit undergraduate students to assist in field work and will apply for departmental funding to support undergraduate assistants.

Dissemination of Results
I will present the results of this study at the American Geophysical Union 2024 Meeting in Washington, D.C. and the 2023 Cornell Ecology and Evolutionary Biology Graduate Student Symposium. The results of this study will also be written into a manuscript and submitted to the journal *Ecology* or *Ecosystems* for publication, and the data will be archived in a publicly-available data repository.

References
1. Villa JA, Ju Y, Stephen T, et al (2020) Plant‑mediated methane transport in emergent and floating‑leaved species of a temperate freshwater mineral‑soil wetland. Limnol Oceanogr 65:1635–1650. https://doi.org/10.1002/lno.11467
2. Sutton-Grier AE, Megonigal JP (2011) Plant species traits regulate methane production in freshwater wetland soils. Soil Biology and Biochemistry 43:413–420. https://doi.org/10.1016/j.soilbio.2010.11.009
3. Vann CD, Megonigal JP (2003) Elevated CO2 and water depth regulation of methane emissions: comparison of a woody and non-woody wetland plant species
4. Sivaram AK, Logeshwaran P, Subashchandrabose SR, et al (2018) Comparison of plants with C3 and C4 carbon fixation pathways for remediation of polycyclic aromatic hydrocarbon contaminated soils. Sci Rep 8:2100. https://doi.org/10.1038/s41598-018-20317-0
5. Cui H (2021) Challenges and Approaches to Crop Improvement Through C3-to-C4 Engineering. Frontiers in Plant Science 12:.
6. Chen J, Wang Q, Li M, et al (2016) Does the different photosynthetic pathway of plants affect soil respiration in a subtropical wetland? Ecol Evol 6:8010–8017. https://doi.org/10.1002/ece3.2523
7. Schimel JP (1995) Plant transport and methane production as controls on methane flux from arctic wet meadow tundra. Biogeochemistry 28:183–200. https://doi.org/10.1007/BF02186458
8. Greenup AL, Bradford MA, McNamara NP, et al (2000) The role of Eriophorum vaginatum in CH4 flux from an ombrotrophic peatland. Plant and Soil 227:265–272. https://doi.org/10.1023/A:1026573727311

9. King J, Reeburgh W, Regli S (1998) Methane emission and transport Results of a vegetation removal by arctic sedges in Alaska: experiment . Journal of Geophysical Research 103:29083–29092

10. Ding H, Hu Q, Cai M, et al (2022) Effect of dissolved organic matter (DOM) on greenhouse gas emissions in rice varieties. Agriculture, Ecosystems & Environment 330:107870. https://doi.org/10.1016/j.agee.2022.107870

11. Dronova I, Taddeo S, Harris K (2022) Plant diversity reduces satellite-observed phenological variability in wetlands at a national scale. Sci Adv 8:eabl8214. https://doi.org/10.1126/sciadv.abl8214

12. Moor H, Rydin H, Hylander K, et al (2017) Towards a trait-based ecology of wetland vegetation. J Ecol 105:1623–1635. https://doi.org/10.1111/1365-2745.12734

13. Aben RCH, Velthuis M, Kazanjian G, et al (2022) Temperature response of aquatic greenhouse gas emissions differs between dominant plant types. Water Research 226:119251. https://doi.org/10.1016/j.watres.2022.119251

14. Bryson C, Carter R (2008) The significance of Cyperaceae as weeds. Monographs in Systematic Botany from the Missouri Botanical Garden 108:.

15. Bruhl J, Wilson K (2007) Towards a Comprehensive Survey of C3 and C4 Photosynthetic Pathways in Cyperaceae . Aliso: A Journal of Systematic and Floristic Botany 23:1.

16. Li M-R, Wedin DA, Tieszen LL (1999) C3 and C4 photosynthesis in Cyperus (Cyperaceae) in temperate eastern North America. Canadian Journal of Botany 77:209–218.

17. McNaughton SJ (1973) Comparative Photosynthesis of Quebec and California Ecotypes of Typha Latifolia. Ecology 54:1260–1270.

18. Sage RF, de Melo Peixoto M, Friesen P, Deen B (2015) C 4 bioenergy crops for cool climates, with special emphasis on perennial C 4 grasses. EXBOTJ 66:4195–4212. https://doi.org/10.1093/jxb/erv123

19. Bansal S, Johnson OF, Meier J, Zhu X (2020) Vegetation Affects Timing and Location of Wetland Methane Emissions. J Geophys Res Biogeosci 125:. https://doi.org/10.1029/2020JG005777

20. Li X, Minick KJ, Luff J, et al (2020) Effects of Microtopography on Absorptive and Transport Fine Root Biomass, Necromass, Production, Mortality and Decomposition in a Coastal Freshwater Forested Wetland, Southeastern USA. Ecosystems 23:1294–1308. https://doi.org/10.1007/s10021-019-00470-x

21. Carmichael MJ, Bernhardt ES, Bräuer SL, Smith WK (2014) The role of vegetation in methane flux to the atmosphere: should vegetation be included as a distinct category in the global methane budget? Biogeochemistry 119:1–24. https://doi.org/10.1007/s10533-014-9974-1

22. King SL, Laubhan MK, Tashjian P, et al (2021) Wetland Conservation: Challenges Related to Water Law and Farm Policy. Wetlands 41:54. https://doi.org/10.1007/s13157-021-01449-y

23. Costanza R, de Groot R, Sutton P, et al (2014) Changes in the global value of ecosystem services. Global Environmental Change 26:152–158. https://doi.org/10.1016/j.gloenvcha.2014.04.002