2022

Providing New Insights On Sarracenia Purpurea’s Stoichiometry, Morphology, Obligate Invertebrates, And Plankton Communities In Response To N And P Deposition.

Lindsey Ann Pett

University of Vermont

Recommended Citation

Pett, Lindsey Ann, "Providing New Insights On Sarracenia Purpurea’s Stoichiometry, Morphology, Obligate Invertebrates, And Plankton Communities In Response To N And P Deposition." (2022). *Graduate College Dissertations and Theses*. 1576.
https://scholarworks.uvm.edu/graddis/1576

PROVIDING NEW INSIGHTS ON *SARRACENIA PURPUREA'S* STOICHIOMETRY, MORPHOLOGY, OBLIGATE INVERTEBRATES, AND PLANKTON COMMUNITIES IN RESPONSE TO N AND P DEPOSITION.

A Dissertation Presented

by

Lindsey A. Pett

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements
For the Degree of Doctor of Philosophy
Specializing in Biology

August, 2022

Defense Date: April 8, 2022
Dissertation Examination Committee:

Nicholas Gotelli, Ph.D., Advisor
Aimée Classen, Ph.D., Chairperson
Alison Brody, Ph.D.
Mindy Morales-Williams, Ph.D.
Cynthia J. Forehand, Ph.D., Dean of the Graduate College

ABSTRACT

For the past century, inputs of nitrogen and phosphorus to aquatic ecosystems have been chronically elevated by human activities. Nutrient loading occurs at multiple scales, from the discharge of wastewater into rivers to atmospheric inputs at the continental scale caused by the burning of fuels and use of fertilizers. Ecosystem components such as producer or consumer populations may respond stoichiometrically, either by actively regulating nutrient uptake or passively absorbing nutrients from their environment. Ecosystems may also respond to elevated nutrients through changes in species richness, species composition, abundance, or biomass, and these effects may cascade through food webs. Understanding these effects in natural systems is challenging because there are large-scale gradients in nutrient deposition across latitude, elevation, and growing season length. In this study, I quantified the responses of the microecosystem of the carnivorous pitcher plant *Sarracenia purpurea* to experimental manipulations of nutrients. At the small scale of a single bog in northern Vermont, I manipulated nitrogen and phosphorous inputs and nutrient ratios in a replicated ecological press experiment, and measured nutrient uptake, biomass, and abundance of various components of the *Sarracenia* microecosystem. I also measured the same components in a survey of 28 bogs from Florida to Maine. This transect encompassed geographic gradients of latitude, elevation, and growing season length, as well as gradients of annual atmospheric N and P deposition rates. I then compared the small-scale experimental effects of nutrient additions on ecosystem components to the large-scale correlations of annual N and P deposition rates with the same ecosystem components. I found strong concordance in both direction and magnitude of effects of nutrient deposition on pathways of nutrient transfer, but weaker effects on the trophic transfer of biomass and abundance. Pitcher fluid, which is analogous to lake and stream water in aquatic ecosystems, tracked experimental manipulations and depositional gradients in phosphorus, which is consistent with nutrient dynamics of surface waters in undisturbed watersheds. Experimental and natural nutrient additions also affected the growth and morphology of the leaves of *S. purpurea*. Carnivorous traits such as relative tube diameter responded readily to increasing N deposition at both small and large spatial scales, highlighting a new pathway for nutrient deposition to affect ecosystem processes by altering the habitat volume for the ecosystem. I also filled knowledge gaps of the model system *S. purpurea*. Algal, cyanobacteria, and rotifer community structure within *S. purpurea* has been greatly understudied within *S. purpurea's* native range. My research presents the first large-scale survey of algal inhabitants of *S. purpurea*, in which plants acted like larger ecosystems that support diverse phytoplankton assemblages of algae and cyanobacteria. Pitchers of *S. purpurea* were dominated by Chlorophyta in abundance whereas Cyanobacteria were dominant in biovolume. My final research aim was to determine if the inquiline *Metriocnemus knabi* aquatic larvae are capable of migration to new pitchers. With a manipulative greenhouse experiment, I provide the first evidence that aquatic larvae of *M. knabi* are capable of emigrating from unsuitable desiccated pitchers to suitable fluid-filled pitchers.

CITATIONS

Material from this dissertation has been accepted for publication in Northeastern Naturalist on February, 28, 2022 in the following form:

Pett, L.A., Linde, S., and Gotelli, N.J.. 2022. Midge larvae *Metriocnemus knabi* can emigrate to new pitchers within *Sarracenia purpurea* after pitcher drainage. Northeastern Naturalist.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Nicholas Gotelli, who has inspired my ecological journey since my undergraduate career. I have learned so much from Nick, whether it be on computation biology, ecological statistics, or the best field techniques to sample *Sarracenia*. I consider myself extremely privileged to have been mentored by one of my personal ecological heroes and cannot thank Nick enough for taking a chance on me five years ago.

I would also like to acknowledge my committee members. The chair of my committee Dr. Aimée Classen has continued to provide thought provoking feedback throughout my time at UVM and has greatly helped me determine the best way of presenting my work. Aimée has also provide valuable laboratory resources that without I would have not been able to complete my research. The graduate students in Aimée's lab were also a great resource as we were able to discuss and test different lab methodologies. I want to acknowledge and thank Dr. Alison Brody, who's work on plant insect interactions I have always admired. Alison's teaching philosophies and guidance have been instrumental in the way I teach and interact with students. Finally, I want to thank Dr. Mindy Morales. I have learned so much from Mindy's phycology course and without it I would not have been able to successfully complete my phytoplankton work. Mindy's teaching style of hands-on learning was extremely beneficial to me, and I greatly appreciate her patience as I probably asked every lab "is this even algae?". Mindy's iNaturalist observations are goals to any ecologist and I recommend everyone follow her for some great observations!

Additionally, I would like to thank our co-investigators on researching aquatic ecosystem stoichiometry. Dr. Angélica González and Mark Nessel have provided support and guidance throughout this research endeavor. I am so grateful that I was able to participate in this research with them both.

I want to acknowledge past and present Gotelli lab mates, Amanda Northrop, Alex Burnham, Lauren Ash, Emily Beasley, and George Ni. I am so grateful that I get to work with such wonderful and talented individuals. I consider these individuals not only lab mates but also close friends. I would also like to acknowledge Emily Mikucki and Lauren Ashlock for being such good friends that are always there to support me.

Some of my most fond memories at UVM are of mentoring undergrads. I want to thank all the undergrads that have helped both in the lab and field. These undergrads include Erin White, Rachel DuBrul, Jacquelyn Haytayan, Brendan Curran, Sophie Linde, and Lilly Carroll.

Finally, I would like to acknowledge my family, my mom who is the strongest most supportive person I know, my brother who instilled in me the love of nature, and my husband Sean who has supported me throughout my graduate career.

TABLE OF CONTENTS

INTRODUCTION

Macronutrients nitrogen (N) and phosphorus (P) continue to increase globally through atmospheric deposition as well as direct inputs, leading to changes in ecological stoichiometry, community structure, and overall ecosystem functioning (Fowler et al 2013; Penuelas et al., 2013; Elser et al., 2010; Meunier et al., 2016). Globally, inorganic N has doubled within the biosphere, this doubling can be contributed to anthropogenic effects such as burning of fossil fuels and the application of crop fertilizers. Within the biosphere in 2015 an estimated 165-259 Tg N yr^{-1} was produced, half of which can be attributed to anthropogenic effects (Fowler et al 2013; Carnicer et al., 2015). With the continuation of burning of fossil fuels and the human need for the expansion of food production, N input/deposition is predicted to increase to 1.9 kg N ha^{-1} $year^{-1}$ by the end of the 21st century (Lamarque et al., 2013). On a smaller scale human induced increases to atmospheric P from fertilizers, combustion, and mineral aerosols are lower than that of N, attributing to approximately 30% of atmospheric deposition (Wang et al., 2014). Historically, an increase of nutrients and nutrient imbalances coming into aquatic ecosystems has caused detrimental impacts to nutrient cycling, community structure, component dynamics, and may accelerate eutrophication (Elser et al., 2009; Carpenter et al., 1998; Elser et al 2007).

As aquatic ecosystems receive limiting nutrients of N or P, there may be an increase in growth of some primary producers such as aquatic macrophytes, algae, and blooms of cyanobacteria (Schindler 1977; Smith 1983). This increase in primary production can have cascading effects throughout the ecosystem, as it can diminish light penetration, deplete oxygen, and create dead zones. Overall, increases in nutrient concentrations and nutrient imbalances can result in biodiversity loss, shifts in community composition, decline or complete loss of habitat, change of component biomass, and a decline in environmental parameters such as turbidity and oxygen (Carpenter et al., 1998, Seehausen et al 1997; Smith 1998). In this work we refer to components as the discrete parts of the ecosystem, such as fluid, individual plankton and macroinvertebrate taxa, microbes, and plant tissue. Although there have been a wide range of studies conducted on the impact of nutrient inputs into aquatic systems, knowledge is still lacking on how each component within a system can respond and how components interact with one another under nutrient imbalances and inundations. Gradients in atmospheric nutrient deposition exist alongside other environmental gradients such as latitude, elevation, and growing season length which all can have similar impacts on ecosystem processes, confounding our understanding of the effects of nutrient deposition. Comparison between small scale direct nutrient input experiments, that eliminate confounding gradients, and large-scale gradients of nutrient deposition within the same type of aquatic ecosystem are rarely done but important because it will allow us to identify cause and effect.

N and P are essential to living organisms and play considerable roles in organisms and their environments. N is essential to life as it is a major structural component of DNA and RNA, present within muscles and tissues, a major component of proteins, nucleic acids, amino acids, chlorophyll, and serves as a nitrogenous base in ATP. Similarly, P is also a component of DNA, RNA, and ATP, it is present in phospholipid membranes, and is also prominent in bone, enamel, and present in insect exoskeletons. Besides singular nutrient content, ratios of these elements along with other macro-nutrients can give insight on aspects such as growth rate, nutrient limitation, life history adaptations, community structure, and behavior (Sterner and Elser 2002). For example, C:N ratios can be an indicator of food or resource quality, a low C:N ratio of plant tissue can mean a higher quality consumable to herbivores due to the increase in protein content (Haddad 2001). C:P or N:P ratios can give insight on growth rate, for example low C:P or N:P ratios mean a higher P content, P rich RNA promotes rapid rates of protein synthesis, which are more prominent in fast growing organisms (Elser et al 2000). Resource ratios are also used in predicting species diversity, aspects of coexistence, and dominance of a specific species (Tilman 1982, Grover and Grover 1997).

How an organism or ecosystem component responds elementally to input of nutrients can vary, organisms may show homeostatic regulation, in which their ratios will be regulated and remain the same or show plasticity in which their elemental content or ratios will vary, often tracking the resources coming into the system (Persson et al., 2010). Changes in nutrient for one component of a system can impact resources, processes, and functions for other components up the trophic ladder and for the entirety of the ecosystem. Turnover events can also occur due to changes in nutrient input/deposition of nutrients. Some organisms can reach elemental thresholds and are not able to efficiently utilize all available resources which can allow for the introduction of new organisms that can withstand the new resources and then potentially push out the original organism (Hecky 1982). Ecosystems may or may not experience stoichiometric flexibility, in which components making up the system show compensatory mechanisms (i.e., plasticity, regulation, compositional shifts) to maintain function. Historically, stoichiometric research has been limited to single groups commonly primary producers and neglect other components due to physical constraint. My work aims to expand on previous stoichiometric research by collecting data on stoichiometry, community structure, and environmental responses at an entire ecosystem level across a wide range of current and predicted concentrations and N:P ratios.

Capturing each component's response to nutrient input/deposition at a singular moment in time can be an impossible task on a large-scale aquatic system. *S. purpurea*, a carnivorous perennial herb makes an ideal model system, as each plant contains self-replicates in the form of tubular leaves that hold aquatic microcosms (Ellison et al., 2004). Although *S. purpureas* leaves are capable of both photosynthesizing and capturing prey, most of the nutrients used by the plant come from atmospheric deposition (Newell and Natase 1998; Kneitel and Miller 2002; Anderson and Midgley 2003; Juniper et al., 1989). The aquatic ecosystems contained within *S. purpurea's* leaves are complex and contain components like that of larger aquatic systems. Leaves are habitat to obligate invertebrates that aid in prey nutrient acquisition. Larval Diptera species that inhabit the leaves include a top predator *Fletcherimyia fletcheri* (Flesh fly), a shredder of prey *Metriocnemus kabi* (Pitcher plant midge), and an omnivorous filter feeder *Wyemoyia smithii* (Pitcher plant mosquito) that consumes shredded prey, bacteria, and protozoans (Addicott 1974; Butler et al., 2008; Forsyth Robertson 1975). Other obligate inhabitants are *Habrotrocha rosa* (Bdelloid rotifer), *Sarraceniopus gibsoni* (Histomatid mite), and facultative inhabitants of protozoans, bacteria, rotifers, phytoplankton and zooplankton

(Addicot 1974, Cochran-Stafira and von Ende 1998; Miller et al., 1994; Bledzki and Ellison 2003).

My research aim was to use a complete aquatic ecosystem to measure singular ecosystem component's response to varying concentrations and gradients of nutrient input or deposition. I used a press experiment on the micro aquatic ecosystem held within *S. purpurea's* leaves, into which predicted concentrations of N and P were inputted. These concentrations of N and P were derived from continental gradients across the east coast of the United States. We then linked results from the press experiment to a large scale continental survey of *S. purpurea* over varying gradients of N and P deposition at 28 sites along the east coast of the United States in the following year (Chapter I). From both the experiment and survey, stoichiometric, community structure, and biomass were measured to find concordant responses to nutrient inputs and deposition. In addition to my examination of aquatic ecosystem response to nutrient input and deposition, I attempted to clarify different conclusions in the literature on the morphological response of *S. purpurea* to nutrient inputs and deposition (Chapter II).

Although there is a wide range of work on this model system, there are some components of the system that are still understudied. I conducted the only large scale algal, cyanobacteria, and rotifer survey within *S. purpurea* (Chapter III). I also conducted an experiment on the obligate invertebrate *Metricocnemus knabi* larvae (Pitcher plant midge) to determine if these obligate aquatic larvae are capable of emigrating when faced with unsuitable habitat in the form of pitcher desiccation (Chapter IV). Both the plankton survey and observations of emigration contribute novel information to the current state of knowledge on this model system and the general ecology and natural history of these organisms.

By linking results from a small-scale press experiment, free of confounding environmental factors, to a large survey across the ecosystems range, we found strong concordance of nutrient transfers among ecosystem components from deposition to fluid, from fluid to plant tissue and microbes and through to its consumer, *W. smithii*. In this work, cyanobacteria are not included in the microbe component but are included in the plankton component. Microbes acted as a sink for nitrogen and phosphorus for fluid and detritus, altering both its nutrient content in as well as its overall biomass. *M. knabi,* although static in its nutrient content, increased in biomass in response to detrital biomass. We also confirmed *S. purpurea* morphology responds to nutrient gradients, reducing carnivorous traits in elevated nutrient environments. And we highlighted a new, yet obfuscating pathway for nutrient deposition to impact ecosystem function—the volume of the aquatic ecosystem itself can respond to gradients in nutrient deposition. My research on the plankton community of *S. purpurea* found that within *S. purprueas* native range the pitchers contained a diverse group of algae, cyanobacteria, and rotifers. My novel research on the obligate *M. knabi* larvae determined that the aquatic larvae is capable of successful emigration from a desiccated pitcher to a new viable fluid filled pitcher. By furthering our knowledge on *S. purpurea* it will fill gaps in both the natural history of the plants and its inhabitants but also how this aquatic microsystem functions.

LITERATURE CITED

Addicott J. F. (1974). Predation and Prey Community Structure: An Experimental Study of the Effect of Mosquito Larvae on the Protozoan Communities of Pitcher Plants. Ecology. **55**(3):475–492.

Anderson B. and Midgley J. J. (2003). Digestive mutualism, an alternate pathway in plant carnivory. Oikos. **102**(1):221–224.

Błedzki L. A. and Ellison A. M. (1998). Population growth and production of Habrotrocha rosa Donner (Rotifera: Bdelloidea) and its contribution to the nutrient supply of its host, the northern pitcher plant, Sarracenia purpurea L. (Sarraceniaceae). Hydrobiologia. **385**(1):193–200.

Butler J. L., Gotelli N. J., and Ellison A. M. (2008). Linking the Brown and Green: Nutrient Transformation and Fate in the Sarracenia Microecosystem. Ecology. **89**(4):898–904.

Carnicer J., Sardans J., Stefanescu C., Ubach A., Bartrons M., Asensio D., and Peñuelas J. 2015. Global biodiversity, stoichiometry and ecosystem function responses to human-induced C–N–P imbalances. Journal of Plant Physiology. **172**:82–91

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., and Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559–568.

Cochran-Stafira D. L. and Ende C. N. von. (1998). Integrating Bacteria into Food Webs: Studies with Sarracenia Purpurea Inquilines. Ecology. **79**(3):880–898.

Ellison A. M., Buckley H. L., Miller T. E., and Gotelli N. J. (2004). Morphological variation in Sarracenia purpurea (Sarraceniaceae): geographic, environmental, and taxonomic correlates. American Journal of Botany. **91**(11):1930–1935.

Elser, J. J., Peace, A. L., Kyle, M., Wojewodzic, M., McCrackin, M. L., Andersen, T., and Hessen, D. O. (2010). Atmospheric nitrogen deposition is associated with elevated phosphorus limitation of lake zooplankton. Ecology Letters, 13(10):1256-1261.

Elser J. J., Andersen T., Baron J.S., Bergstrom A.K., Jansson M., Kyle M., Nydick K. R., Steger L., and Hessen D. O. (2009). Shifts in Lake N:P Stoichiometry and Nutrient Limitation Driven by Atmospheric Nitrogen Deposition. Science. **326**(5954):835–837.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hullebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., and Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters. **10**:1135–1142.

Elser J. J., Sterner R.W., Gorokhova E., Fagan W.F., Markow T.A., Cotner J.B., Harrison J.F., Hobbie S.E., Odell G.M., and Weider L.W. (2000). Biological stoichiometry from genes to ecosystems. Ecology Letters. **3**(6):540–550.

Forsyth A. B. and Robertson R. J. (1975). K reproductive strategy and larval behavior of the pitcher plant sarcophagid fly, *Blaesoxipha fletcheri*. Canadian Journal of Zoology. **53**:174-179.

Fowler, D., Pyle, J. A., Raven, J. A., and Sutton, M. A. (2013). The global nitrogen cycle in the twenty-first century: introduction. Philosophical Transactions of the Royal Society B: Biological Sciences, **368**(1621):20130165.

Grover J. D., Grover J. P., and Hudziak J. (1997). Resource Competition. Springer Science & Business Media.

Haddad N. M., Tilman D., Haarstad J., Ritchie M., and Knops J. M. (2001). Contrasting effects of plant richness and composition on insect communities: a field experiment. The American Naturalist. **158**(1):17–35.

Hecky, R. E. (1982). East African lakes and their trophic efficiencies. Trophic Dynamics of Aquatic Ecosystems. American Association for the Advancement of Science Symposium.

Juniper B. E., Robins, R. J., and Joel D. M. (1989). The Carnivorous Plants. Academic. New York.

Kneitel J.M. and Miller T.E. (2002). Resource and Top-Predator Regulation in the Pitcher Plant (*Sarracenia purpurea*) Inquiline Community. Ecology. **83**(3):680–688.

Lamarque, J. F., Dentener, F., McConnell, J., Ro, C. U., Shaw, M., Vet, R., Bergmann, D., Cameron-Smith, P., Dalsorean, S., Doherty, R., Faluvegi, G., Ghan, S. J., Josse, B., Lee, Y. H., MacKenzie, I. A., Plummer, D., Shindell, D. T., Skeie, R. B., Stevenson, D. S. Strode, S., Zeng, G., Curran, M., Dahl-Jensen, D., Das, S., Fritzsche, D., and Nolan, M. (2013). Multi-model mean nitrogen and sulfur deposition from the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP): evaluation of historical and projected future changes. Atmospheric Chemistry an Physics, **13**(16):7997- 8018.

Meunier, C. L., Gundale, M. J., Sánchez, I. S., and Liess, A. (2016). Impact of nitrogen deposition on forest and lake food webs in nitrogen-limited environments. Global Change Biology, **22**(1):164-179.

Miller T., Cassill D., Johnson C., Kindell C., Lepis J., Mcinnes D., Bevis T., Mehlman D., and Richard B. (1994). Intraspecific and Interspecific Competition of Wyeomyia smithii (Coq.) (Culicidae) in Pitcher Plant Communities. The American Midland Naturalist. **131**(1):136–145.

Newell S. J. and Nastase A. J. (1998. Efficiency of insect capture by Sarracenia purpurea (Sarraceniaceae), the northern pitcher plant. American Journal of Botany. **85**(1):88–91.

Penuelas, J., Poulter, B., Sardans, J., Ciais, P., Van Der Velde, M., Bopp, L., Boucher O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., and Janssens, I. A. (2013). Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nature communications, **4**(1):1-10.

Persson J., Fink P., Goto A., Hood J.M., Jonas J., and Kato S. (2010). To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos. **119**(5):741–751.

Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes: natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. Science **195**:260–262.

Seehausen O., Alphen J. J. M. van, and Witte F. (1997). Cichlid Fish Diversity Threatened by Eutrophication That Curbs Sexual Selection. Science. **277**:1808–1811.

Smith V. H. (1983). Low Nitrogen to Phosphorus Ratios Favor Dominance by Blue-Green Algae in Lake Phytoplankton. Science. **221**:669–671.

Smith V. H. (1998). Cultural Eutrophication of Inland, Estuarine, and Coastal Waters. In: Pace ML, Groffman PM, editors. Successes, Limitations, and Frontiers in Ecosystem Science. New York, NY: Springer New York:7–49.

Sterner R. W. and Elser J. J. (2002). Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press.

Tilman D. (1982). Resource Competition and Community Structure. Princeton University Press. **Wang F, Li J, Wang X, Zhang W, Zou B, Neher DA, and Li, Z**. (2014). Nitrogen and phosphorus addition impact soil N_2 emission in a secondary tropical forest of South China. Scientific Reports. **4**:1-8.

CHAPTER I: AQUATIC ECOSYSTEM RESPONSE TO EXPERIMENTAL INPUT AND NATURAL GRADIENTS OF N AND P DEPOSITION USING THE MODEL SYSTEM *SARRACENIA PURPUREA*

ABSTRACT

Spatial gradients of nitrogen and phosphorous deposition and their ratio potentially control the ecosystem processes of nutrient uptake by producers and the transfer of those nutrients and biomass to their consumers. The rain-filled, cup-shaped leaves of the carnivorous pitcher plant *Sarracenia purpurea* support an aquatic ecosystem that is a model for studying these processes. I compared experimental manipulations of nutrients at the small-scale, free of the confounding factors of latitude, elevation, and growing season length, with large-scale variation in rates of atmospheric nutrient deposition. This comparison facilitates the identification of cause-and-effect relationships between atmospheric nutrient deposition and the structure and function of aquatic ecosystems. In a single ombrotrophic bog in northern Vermont, I measured the response of nutrient content, biomass, and abundance of ecosystem components to field manipulations of nitrogen and phosphorous inputs. At 28 bogs surveyed across gradients of latitude, elevation, and growing season length from Florida to Maine, I measured the response of nutrient content, biomass, and abundance of the same components and correlated them with atmospheric N and P deposition rates at each site. Across the algal taxa, consumer taxa, plant tissue, detritus, and pitcher fluid, both the direction and magnitude of the small-scale and large-scale standardized effects were highly concordant, with stronger effects of phosphorous input than nitrogen input regarding nutrient transfers. In contrast,

biomass responses of most ecosystem components to small-scale nutrient manipulations were weak and were largely uncoupled from biomass responses to large-scale variation in atmospheric deposition rates. In addition, taxa abundance responded largely to N:P ratios, often decoupled from absolute deposition rates of N or P. These results confirm that large-scale associations of atmospheric deposition with nutrient stoichiometry of primary producers and consumers reflect cause-and effect relationships, and that ecosystem stoichiometry may be more limited by the availability of phosphorous than nitrogen. In contrast, there is less evidence that biomass transfers among ecosystem components are related to atmospheric deposition and nutrient availability.

INTRODUCTION

Anthropogenic sources of nutrients play a significant role in altering the relative availability of macro-nutrients within the (Peñuelas et al. 2013). This alteration occurs at multiple spatial scales, from the discharge of wastewater into rivers, to industry creating continental sized gradients in atmospheric deposition. By their spatial nature, these gradients in nutrient deposition exist across other confounding gradients such as temperature (Paerl et al. 2019). Therefore, multi-scale studies are needed to confirm causation at large spatial scales with experimental methods at the small scale where other gradients do not exist (De Frenne et al. 2013).

The anthropogenic load of nitrogen in the biosphere was estimated to be 259 Tg N yr^{-1} in 2015, this value is similar in magnitude to the amount of fixed N in the biosphere produced by natural processes (Carnicer et al., 2015). As N has doubled in the atmosphere, P, which is extremely low in the atmosphere, has increased by roughly 30%

due to fertilizers, combustion, and mineral aerosols (Wang et al. 2014). These increases and imbalances of depositional nutrient inputs have led to the eutrophication of freshwater ecosystems in the Northern hemisphere (Wurtsbaugh et al. 2019). Nutrient enrichments and imbalances in aquatic ecosystems can have detrimental impacts, such as the deterioration of water quality, reduction of dissolved oxygen, production of turbidity, compositional shifts, loss of species, deterioration or complete loss of suitable habitat, and alteration of ecosystem functioning (Carpenter et al., 1998; Seehausen et al., 1997; Smith 1998; Karez et al. 2004; Rabalais 2002). With current and predicted increased inputs of N and P into aquatic ecosystems, it is necessary to continue to gather knowledge on how entire aquatic ecosystems and their components uptake and transfer N and P and how biomass and community structure respond.

Component stoichiometry, or their elemental N, P, and NP, can respond in two ways to nutrients entering an ecosystem, components can either show homeostatic regulation or plasticity in their elemental content or nutrient ratios. Homeostasis will occur when organisms or an ecosystem component are constrained in elemental content or a specific nutrient ratio and will not alter in response to changing of nutrients in the ecosystem or in its consumable resources (Persson et al., 2010). Opposite of homeostatic regulation, organisms or components may show plasticity in elemental content or nutrient ratios in response to nutrient input (Hillebrand et al., 2007; Bertram eta al.,2008; Sterner et al., 1998; Teurlincx et al., 2017).

The macro-nutrients of N and P are both limiting in freshwater systems. Generally, lake water responds readily to changes in atmospheric nutrient deposition. As lakes that receive high amounts of N deposition have elevated NO_3^- in surface waters and overall higher total nitrogen (TN) as well, total phosphorus (TP) in lake water is positively correlated to TP deposited (Elser et al. 2009; Camarero & Catalan 2012). Other freshwater systems such as rivers will have concentrations of dissolved inorganic N correlated to atmospheric inorganic nitrogen (Jaworski et al. 1997). These patterns in deposition are creating large imbalances in N:P ratios in freshwater aquatic ecosystems which can have cascading effects throughout an ecosystem leading to alterations of economically important components of freshwater ecosystems. N deposition can also increase the N:P ratio of plant stoichiometry in both aquatic macrophytes and terrestrial plants (Sardans and Peñuelas 2012). Lower trophic levels such as primary producers are often more readily affected by deposition by higher trophic levels. Insects often remain homeostatic in their nutrient ratios even with changes in nutrient deposition (Tao and Hunter 2012). Although some organisms are not directly affected by deposition, their resources may be. As resource stoichiometry changes, predators may change their feeding strategy to maintain a strict ratio. Although previous work highlights individual species or trophic levels respond to N or P deposition, there is minimal work on multiple or a complete ecosystem's stoichiometric response to atmospheric nutrient deposition.

In addition to stoichiometric responses, atmospheric deposition can also affect community assembly, nutrient input of N and P can impact species richness, abundance, and the overall structure of trophic levels (Oksanen et al. 1981, Abrams 1993, Tilman 1982, Dodson et al. 2000). Nitrogen addition can have negative effects on the diversity in an ecosystem, as plant species richness and abundance can decline with increasing nitrogen (Midolo et al., 2018). Nutrient addition in aquatic ecosystems often leads to compositional turnover of species, where at different nutrient concentrations certain species are favored over others (Dodds et al., 1989; Reed et al., 2016; Xu et al., 2010). Generally, P has a positive effect on aquatic invertebrate biomass because there are strong bottom-up pathways due to increased resource availability (Nessel et al. 2021). Acidification due to N deposition in aquatic ecosystems decreases invertebrate, vertebrate, algal diversity, and impacts community composition (Ormerod 1992). It is necessary for researchers to be aware of how nutrients are affecting community structure and how the structure is affecting overall ecosystem functioning, as diversity is needed for maintaining function (Tilman et al. 1997).

Sampling an entire aquatic ecosystem such as a lake or pond for response to nutrient input and deposition would be an arduous if not an impossible task. Using an aquatic ecosystem held within the phytotelmata, or water holding pitchers, of the model system *Sarracenia purpurea* (northern pitcher plant) allows for every individual and ecosystem component to be measured. *Sarracenia purpurea* inhabits nutrient deficient wetland ecosystems throughout the east coast of Canada and the United States (Ellison et al. 2004). *Sarracenia purpurea* has a poor root system and most of the nutrients that come into the system are from atmospheric deposition or invertebrate prey that enter the phytotelmata (Butler and Ellison 2007, Ellison and Gotelli 2002, Juniper et al. 1989, Chapin and Pastor 1995, Ellison and Gotelli 2001). Unlike, other carnivorous plant species, *S. purpurea* does not contain chitinases (Folkerts 1999, Gallie and Chang 1997). Prey that enters the system are initially shredded and broken down by obligate Diptera larvae *Fletcherimyia fletcheri* (flesh fly), *Metriocnemus knabi* (pitcher plant midge), and bacteria. Bacteria within *S. purpurea* are consumed by plankton species including an obligate bdelloid rotifer *Habrotrocha rosa*, an obligate histomatid mite *Sarraceniopus* *gibsoni*, protozoans, an obligate Diptera larvae *Wyeomyia smithii* (pitcher plant mosquito), of which will also consume plankton and protozoans (Addicott 1974; Butler et al., 2008; Forsyth Robertson 1975; Cochran-Stafira & von Ende 1998; Miller et al., 1994; Bledzki & Ellison 2003). These complex trophic interactions are like that of larger aquatic ecosystems, making it a suitable model system.

In this research we aimed to determine the effects of direct nutrient input of N, P, and N:P in a small-scale press experiment and then link those results to the effects of gradients of atmospheric nutrient deposition of N, P, and N:P across a large-scale continental survey. Concordance between responses at the small-scale, free of confounding environmental gradients, with responses at the large-scale provides strong evidence of causal pathways. The main objective of this study was to ascertain causal pathways of the effects of gradients of N, P, and N:P on aquatic ecosystems. It was hypothesized that ecosystem components within *S. purpurea* would vary in stoichiometric responses to input/deposition of nutrients. We predicted the components of pitcher fluid and plant tissue would be plastic in response to input/deposition, as larger aquatic systems like lakes and ponds will track wet depositional rates in their water chemistry. N and P are also common limiting nutrients within plants and with plants being capable of luxury consumption we predicted that plants would uptake and store excess nutrients coming into the system. We predicted that the microbial community and aquatic invertebrates will remain static in elemental content, as microbes can maintain optimal growth ratios through enzymatic adjustment or selective feeding. Aquatic invertebrates were also predicted to remain static in response to nutrient input/deposition due to assimilation, excretion, and selective feeding.

We were also interested in how community assemblage varies across gradients of nutrient input/deposition. The response of phytoplankton community structure to nutrient deposition often varies across other gradients such as temperature (Schulhof et al. 2019). In this work we'll explore these causal mechanisms. We hypothesized that structure of ecosystem components will change in response to nutrient input and atmospheric deposition of N and P. We predicted that abundance and biomass of obligate invertebrates and plankton will increase in response to their food which will become more available due to elevated N and P. Findings to these above-mentioned hypotheses will aid in filling current knowledge gaps of ecological stoichiometry and response of aquatic ecosystems to N and P inputs/deposition and provide evidence of direct causal pathways from nutrient deposition to ecosystem response.

MATERIALS & METHODS

Molly Bog Experimental Design

To measure the effect of nutrient input on aquatic ecosystem components, we experimentally manipulated nutrient input to the model system *S. purpurea* at Molly Bog, in Morristown, Vermont (44.500253°N, -72.639624°W) in the summer of 2018. We used a regression-based design with 5 concentrations of nitrogen 0.0875, 0.175, 0.35, 0.7, and 1.4 mg/l and 5 values of phosphorus 0.025, 0.05, 0.1, 0.2, 0.4 mg/l. Solutions of nitrogen and phosphorus were created using stock NH_4Cl and NaH_2PO_4, as these forms are readily available for the plant to uptake (Gray et al. 2006). In addition to the 5 x 5 regression-based design, 8 additional experimental N & P concentrations were included to replicate the most common ratios made from the regression concentration design (Figure 1). In total there were 9 treatment ratios among the 33 N & P concentrations. Ratios of the design included the N:P ratios of 3.5 and 7 replicated 6 times, 14 and 28 replicated 5 times, 1.75 replicated 4 times, 0.87 replicated 3 times, 0.44 replicated twice, and outlier ratios of 0.22 and 56 with no replicates. The concentrations of nitrogen and phosphorus were based on national atmospheric deposition data as well as predicted future deposition rates (National Atmospheric Deposition Program 2018, Galloway et al. 2008).

Molly Bog Experimental Protocol

A total of 38 mature non-flowering *S. purpurea* were chosen randomly at Molly Bog. All plants in the experiment were a minimum of 15 cm in basal diameter and contained a minimum of 5 living pitchers containing fluid. Experimental plants were separated from each other by a minimum of 0.5 meters. Of the 38 plants, 33 were randomly assigned concentrations of nitrogen and phosphorus and N:P ratios from the experimental design and 5 plants were randomly assigned as controls, receiving reversed osmosis (RO) water with minimal values of N and P (TN <0.02 mg/L, TP 0.005 mg/L). Once plants were chosen, fluid was removed from every pitcher. Pitchers of each plant were then rinsed with RO water and each given the assigned concentrations and ratios of nitrogen and phosphorus. This was a press experiment: for a total of 10 weeks starting on July 19, 2018, each week or after every rain event plants were topped off with the assigned solution of N and P.

Molly Bog Experiment Final Measurements

At the end of 10 weeks, ecosystem components of pitcher fluid, plant tissue, detritus, obligate invertebrates, and microbial community were collected for elemental analysis and plankton for species identification and biovolume calculation. Fluid from every pitcher in the plant was filtered through a 25 μ mesh filter to capture plankton and invertebrates, the volume of fluid was measured in a 100 ml graduated cylinder and then filtered through a 0.22 μ hydrophilic PTFE filter to capture the microbial community. The fluid was then homogenized into one sample for each plant. Samples were preserved with sulfuric acid based on EPA methodology and analyzed for ammonium, nitrate, phosphate, TN, and TP via ICP and CFA (Cary Institute of Ecosystem Studies, Millbrook, New York). Plant tissue of the youngest pitcher was collected from each plant and rinsed. Plant tissue was dried at 60°C for 72 hours and ground using a Thomas Wiley Mill (Thomas Scientific, Swedesboro, New Jersey) to a measurement of 1 mm. Plant tissue was analyzed for N content using combustion analysis using via LECO CHN628 (LECO Corporation, USA) and P by inductively coupled plasma atomic emission spectrophotometer (ICP-OES, Perkin Elmer Avio 200). Detritus from each pitcher was collected by scrapping the inside and bottom of each pitcher with a laboratory spatula. Detritus for each plant was dried at 60°C for 72 hours and homogenized. Detritus was analyzed for C, N, and P by the same means used for plant tissue. Obligate invertebrates were collected by picking them off the 25 μ mesh filter with flexible tweezers. The mesh filter was then inverted over a 50 ml conical tube and rinsed with RO to extract the plankton. The plankton samples were preserved in Lugols and measured using a PhycoTech Nannoplankton Counting Chamber. For each sample, 300 natural units were counted. Organisms were identified to lowest possible taxonomic identification at genus or species based on morphological attributes at 40x magnification using a Nikon 50i clinical grade microscope. Taxonomic identification was conducted using a variety of keys and resources (Komárek 2008; Komárek and Anagnostidis 2007; Wehr et al., 2015;

Hindák 2008; Patrick and Reimer 1966; Prescott et al., 1972; Smith 1950) and biovolume was calculated following Hillebrand et al. (1999) and Olenina (2006) methodologies. Obligate invertebrates were identified and counted. Once invertebrates were visually determined to have emptied their stomach contents (~24 hours), they were dried at 60°C for 72 hours. All invertebrates and prey were then individually weighed on a micro balance (ultra-micro balance, Sartorius, Göttingen, Germany, precision 0.1 μg) and then analyzed for N and P. The microbial community was collected by filtering pitcher fluid through a 0.22 μ hydrophilic polytetrafluorethylene membrane filter that was flash frozen in field and stored at -80°C until analysis. Filters were dried at 60°C until constant weight (~ 6 days) and then weighed on a microbalance and analyzed for N and P.

Large Scale Survey

To measure the effect of atmospheric nutrient deposition on aquatic ecosystem components, we conducted a survey of 28 sites along the Eastern coast of the United States from the Florida panhandle to Northern Maine from June 27th to August 20th, 2019 (Figure 2). At each site, we selected 15 random pitcher plants separated from each other by a minimum of 0.5 m. Plants were at least 15 cm in basal diameter, nonflowering, and contained a minimum of 5 pitchers. All measurements were identical to the Molly Bog Experiment. Only a subset of the samples (3 of the 15 plants) per site were the analyzed for N and P.

Statistical Analysis

To compare the direction and magnitude of the effect of nitrogen, phosphorus, and N:P on ecosystem components at the experimental scale to the large scale, we extracted standardized slope coefficients from paired Structural Equation Models (SEMs) fit by the R package *lavaan* (Rosseel, 2012). In this work, concordance was defined as directional agreement (+ and + or – and –) in slope coefficients between the experiment and survey. Discordance was defined as directional disagreement (+ and – or – and +) in slope coefficients between the experiment and survey. SEMs were chosen over simple linear models due to the functionality of covariance structures within SEMs that allow for slope coefficients to represent effects beyond other covarying independent variables. For example, the slope (or effect) of N deposition on the abundance of a component is the effect of N beyond the effect of NP or P. A total of 10 SEMs were fit using a maximum likelihood estimator and all response variables were scaled to 0 to 1 to meet the variance requirements of the SEM model fitting process. Of the 10 SEMs, 6 were for stoichiometry: 1 model for N in Molly Bog, 1 for N across the survey, 1 P Molly Bog, 1 P survey, 1 N:P Molly Bog, and 1 N:P survey. Of the remaining 4 SEMs, 2 were for biomass (biovolume in the case of algae): 1 for Molly Bog and 1 for across the survey. And finally, 2 were for abundance: 1 for Molly Bog and 1 for across the survey. Because microbes and detritus only have biomass data, their biomass values were included in the abundance SEMs to complete the food web. To account for the effect of fluid volume (ml) on biomass (mg) and abundance (count), these response variables were converted to densities by dividing by the total volume of fluid extracted from each plant except for the response variables pitcher biomass and pitcher count.

RESULTS

Stoichiometry

Phosphorus experimental inputs in Molly Bog (mb) and depositional phosphorus across the survey (s) had concordant positive effects on phosphorus concentrations in pitcher fluid (standardized coefficient for mb 0.31 and for s 0.13) and pitcher tissue (mb 0.32, s 0.43) (Table 1; Figure 3). Phosphorus content of detritus also had concordant positive effects on fluid concentrations (mb 0.06, s 0.33). Beyond the effects of phosphorus experimental input or deposition on tissue, fluid had positive effects on tissue content (mb 0.16, s 0.15). Microbial phosphorus content responded negatively to fluid phosphorus concentrations (mb -0.04, s -0.08).

Nitrogen experimental inputs and deposition over the survey did not have concordant effects on fluid (mb 0.15, s -0.07) or tissue (mb -0.16, s 0.26) nor between detritus and fluid (mb -0.03, s 0.03). Similar, to phosphorus, nitrogen concentrations in fluid had positive effects on nitrogen content of tissue (mb 0.16, s 0.20) and microbial nitrogen content responded negatively to fluid nitrogen concentrations (mb -0.23, s -0.01).

N:P of experimental inputs and deposition did not have concordant effects on fluid (mb 0.32, s -0.09) or tissue (mb -0.04, s 0.17) nor between detritus and fluid (mb -0.08, s 0.02). The N:P content of microbes responded negatively to the N:P of fluid (mb -0.18, s -0.06) and additionally to the N:P of detritus (mb -0.05, s -0.07). The N:P content of *W. smithii,* the bacteria consuming mosquito larvae, responded negatively to the N:P content of microbes (mb -0.23, s -0.09). The N, P, or N:P of *M. knabi*, the detritus consuming midge larvae, did not respond to the N, P, or N:P of detritus.

Biomass

Phosphorus experimental inputs in Molly Bog and depositional phosphorus across the survey had no concordant effects on pitcher biomass (mb -0.10, s 0.28) but had negative effects on the biovolume of Chlorophyta (green algae) (mb -0.18, s -0.78) (Table 2, Figure 4). Cyanobacteria, Bacillariophyta (diatoms), and plankton biovolume overall did not respond concordantly to inputs or deposition of P, while the biomass (mg/l) of microbes responded positively to phosphorus inputs and deposition (mb 0.13, s 0.48).

Microbial biomass (mb 0.14, s -0.20) and plant tissue biomass (mb 0.31, s -0.21) did not respond concordantly to nitrogen inputs or deposition nor did any plankton biovolume component or overall plankton biovolume. Microbial biomass (mb 0.71, s 0.06) and *M. knabi* biomass (mb 0.12, s 0.03) responded concordantly to the biomass of detritus (mg/l). *W. smithii* did not respond concordantly to any of the plankton biovolume components or overall plankton biovolume nor the biomass of microbes.

Abundance

Phosphorus experimental inputs in Molly Bog and depositional phosphorus across the survey had positive concordant effects on pitcher count (mb 0.14, s 0.35) but no concordant effects on components of plankton abundance (#/ml) or plankton abundance overall.

Nitrogen experimental inputs and deposition had concordant negative effects on the abundance (#/ml) of Cyanobacteria (mb -0.27, s -0.24), Chlorophyta (mb -0.03, s -0.18), plankton overall (mb -0.23, s -0.39), and pitcher count (mb -0.37, s -0.23) while

Bacillariophyta did not respond concordantly (mb -0.46, s 0.34). Beyond the concordant negative effects of nitrogen on pitcher count, overall plankton abundance, Chlorophyta abundance, and cyanobacteria, N:P deposition had a concordant positive effect on these components (Table 3, Figure 5).

The abundance *W. smithii* responded concordantly to Chlorophyta (mb 0.03, s 0.08) but not to overall plankton abundance nor Cyanobacteria or Bacillariophyta. The abundance *M. knabi* did not respond concordantly to the biomass of detritus (mb 0.28, s -0.01).

DISCUSSION

The goal of this study was to find casual effects of nutrient deposition on aquatic ecosystems by finding concordance between a small-scale experiment free of confounding environmental gradients to large-scale effects observed in a survey across the native range of the ecosystem.

Stoichiometry

The phosphorus concentration of pitcher fluid, the fluid being analogous to the water medium of larger aquatic ecosystems, responded positively to phosphorus concentrations in experimental inputs and atmospheric deposition. Atmospheric phosphorus deposition is largely confounded by terrestrial phosphorus inputs, but recent work has shown that high elevation lakes, largely free of watershed disturbances, may be more at risk of atmospheric phosphorus deposition than previously thought (Brahney et al. 2015). In addition, numerous Vermont oligotrophic lakes with largely untouched watershed are experiencing significant, positive trends in lake phosphorus concentrations, but little is known about potential causes (Merrell and Pierson, 2021)—perhaps atmospheric deposition of phosphorus is responsible. Monitoring of atmospheric phosphorus deposition is limited compared to nitrogen deposition, therefore, to better understand threats to oligotrophic lakes, more monitoring should be done. The phosphorus concentration of pitcher fluid also had a positive, concordant response to the phosphorus content of detritus, or the remains of prey and other organic materials captured by the pitchers. In stream watersheds with minimal disturbance, allochthonous input, or organic matter of natural origin, accounts for a major portion of phosphorus concentrations (Meyer and Likens 1979). The phosphorus concentration of pitcher tissue, analogous to macrophytes in aquatic ecosystems, had a concordant positive response to phosphorus inputs and deposition, and beyond just inputs and deposition, pitcher tissue responded positively to the phosphorus concentration of the pitcher fluid. Thus, pitcher tissue appears to be plastic in response to ambient phosphorus concentrations. Similar findings have been found for *Myriophyllum spicatum*, an important invasive species in North American lakes (Carpenter & Adams 1977). The phosphorus content of microbes had a concordant negative response to fluid phosphorus. The uptake of phosphorus by microbes may be faster than inputs/deposition into the system. Bacteria and lakes have been found to be extremely flexible in their phosphorus content (Godwin & Cotner, 2015). The microbes within the fluid of *S. purpurea* are acting similarly to phosphorus removing bacteria in wastewater treatment facilities (Kuba et al. 1997; Khoshmanesh et al. 2002). Our results demonstrated that bacteria were plastic sinks in their response to ambient phosphorus concentrations but not plastic in response to detritus phosphorus. The phosphorus content of *M. knabi* was static in response phosphorus content of detritus, its food source, and *W. smithii* was static in response to the phosphorus content of microbes, its food source. In experiments investigating diet treatments for insects as food and feed shows that the phosphorus content of insects was static across varying treatments (Oonincx & Finke et al. 2021).

The nitrogen concentration of pitcher fluid did not respond concordantly to nitrogen input or deposition nor detritus and therefore appears static. Nitrogen concentrations in lakes and rivers readily respond to atmospheric nitrogen deposition (Elser et al. 2009; Clarke, Mueller & Mast 2000; Jiang et al. 2022). The nitrogen content of plant tissue, though, did respond concordantly to the nitrogen concentration of pitcher fluid, similarly to the magnitude and direction of phosphorus mentioned above. Aquatic macrophytes are often plastic in their nitrogen content (Benoitn et al. 2007). Similarly, but less in magnitude compared to the phosphorus content of microbes, the nitrogen content of microbes responded negatively and fluid nitrogen concentration. Microbes are likely acting as a sink, removing nitrogen from the fluid. Similar to the phosphorus content of microbes and detritus, their primary consumers *W. smithii* and *M. knabi* did not respond concordantly and are generally static in their nitrogen content. Nearly all the variation in nitrogen content of insects could be accounted for by taxa identity, life stage, or gut contents (Fagan et al. 2002). One major concern we have with our work is the possibility of N escaping to the atmosphere through denitrification which is unaccounted for.

The N:P content of microbes had a concordant negative response to both the N:P concentration of pitcher fluid and the N:P content of detritus. Also, bacteria could be selectively feeding on detritus, altering their own N:P. The N:P content of *W. smithii* also concordant negative response to the N:P concentration of microbes, and therefore maybe selectively removing higher N:P microbes and leaving lower N:P microbes.

Biomass

The biomass of pitcher tissue had no concordant response to the phosphorus concentration of inputs and deposition, even though as mentioned above the phosphorus content of the tissue did respond to inputs and deposition. This is counterintuitive when considering phosphorus as a fertilizer for aquatic macrophytes (Carr 1998). The unique properties of *S. purpurea's* carnivorous trait may elucidate this phenomenon—see chapter II. In addition, biomass of pitchers may be responding to other environmental gradients not considered. The biovolume (cells/ul) of Chlorophyta (green algae) had a concordant negative response to phosphorus input and deposition. This too is counterintuitive as we would expect phosphorus to fertilize Chlorophyta, increasing their biovolume (Sagrario et al. 2005). The reduction in biovolume of Chlorophyta may be due to a competitive advantage of cyanobacteria at low N, but in this chapter, we did not investigate relative biovolume of plankton components. More analysis of our data should be done. In chapter II we will discuss potential reasons for this counterintuitive response such as the unique carnivorous traits that are responsive to phosphorus. Trophic interactions with *W. smithii* may also be confounding our understanding of phosphorus. Phosphorus had no concordant effects on other plankton components or plankton overall. The biomass of microbes had a concordant positive response to the phosphorus concentration of inputs and deposition. Biomass of microbes readily responds to ambient phosphorus concentrations in terrestrial ecosystems, but in freshwater literature much of the relationship studied between bacterial biomass and phosphorus is viewing bacteria as a facilitator of the release of phosphorus from lake sediments or phosphorus stimulating bacteria to breakdown leaf litter in streams (Liu et al. 2012). We find that across multiple, unknowable environmental gradients in the survey and in the controlled experimental design of Molly Bog, phosphorus consistently increased microbe biomass.

Nitrogen alone had no concordant effect on the biomass of pitcher tissue, plankton biovolume (cells/ul) or any of its components, nor on microbial biomass (mg/l). As mentioned above, this is counterintuitive because we generally think of nitrogen as a fertilizer. We find that nitrogen is not driving biomass or its transfer though the food web of this ecosystem and rather it is its ratio to phosphorus that does. But again, as mentioned above, N might be escaping to the atmosphere which we did not account for.

N:P concentrations of input and deposition had concordant positive effects on the biomass of pitcher tissue. A global review of nitrogen and phosphorus additions to terrestrial ecosystems found N:P explained more variation in the accumulation of above ground biomass than nitrogen alone (Peng et al. 2018). Conversely, N:P concentrations of input and deposition had concordant negative effects on the biovolume of Chlorophyta. At first glance this does make sense because just above we stated that Chlorophyta responded negatively to phosphorus—less phosphorus mathematically implies a larger N:P. But, considering the covariance structures in SEMs, the standardized coefficients reflect the effect of N:P if phosphorus is held constant. We can interpret this as follows: phosphorus may reduce Chlorophyta biovolume, but when the N:P is reduced beyond reductions in phosphorus, N:P still has deleterious effects on the biovolume of Chlorophyta independent of phosphorus. Additionally, warming temperatures shift the optimal N:P supply ratio upwards for algal communities potentially complicating our interpretation of results (Thrane, Hessn, & Anderson 2017). N:P concentrations of input and deposition had no concordant effects on microbes or any of the non-Chlorophyta algal taxa. Often top-down pressures override the effects of changes in ambient N:P, and warming can also flip the importance of top-down or bottom-up pressures on algal communities (Spilling et al. 2019; Shurin et al. 2012).

The biomass of microbes (mg/l) and the biomass *M. knabi* (mg/l) had concordant positive responses to their food source, detritus. In stream ecosystems bacterial biomass has a strong positive correlation with leaf litter biomass, a form of allochthonous input analogous to prey in pitchers, as well as detritivorous insect larvae and leaf litter in aquatic ecosystems (Hieber & Gessner 2002; Wissinger et al. 2018). The biomass of *W. smithii* (mg/l) did not respond concordantly to the biovolume of plankton or biomass of microbes.

Abundance

The number of pitchers per plant had a concordant positive response to phosphorus deposition and input. As mentioned above, phosphorus had no concordant effect on the biomass of the newest pitcher, therefore *S. purpurea* may be using phosphorus to produce more pitchers rather than pitcher mass of the youngest pitcher. Elevated phosphorus concentrations can stimulate dense shoot growth of certain aquatic macrophytes shifting the macrophyte from a single, large stem to multiple short stems (Dino, Thiebaut, & Muller 2007). Phosphorus input and deposition had no other concordant effect on plankton abundance (#/ml).

Nitrogen input and deposition had concordant negative effects and N:P had concordant positive effects on all the following components, pitcher count, over plankton abundance, Chlorophyta abundance, and Cyanobacteria abundance. Given N:P had no positive effect on biovolume of plankton, perhaps higher N:P is selecting for smaller bodied, more abundant taxa. Regarding cyanobacteria, higher N:P fertilizer in experimental ponds increased cyanobacteria abundance (Qin & Culver, 1996). We found a negative relationship with nitrogen and a positive relationship with N:P which is generally confusing because more N implies a larger N:P. Due to the covariance structures in SEMs holding N:P constant in the estimating of the standardized coefficient of N, we find that the effect of N:P can be truly independent of N. Overall, increasing N:P input and deposition can have positive effects on abundance of these plankton taxa while increasing nitrogen deleteriously effects abundance. Unlike the relationship between biomass of *W. smithii* and biomass of Chlorophyta, *W. smithii's* abundance had a concordant positive response to the abundance of Chlorophyta. Because the abundance of *W. smithii* did not respond to Cyanobacteria or Bacillariophyta or plankton overall, we find that *W. smithii* is selectively feeding on the potentially more palatable Chlorophyta (Ndebel-Murisa et al. 2010).

Conclusion

Our two-scale design of a press experiment in a single bog, free of confounding environmental gradients, coupled with a large-scale survey across the native range of the *S. purpurea* allowed us to identify pathways of N, P, and N:P through an aquatic ecosystem, confirm causal effects of N, P, and N:P on ecosystem processes of biomass transfer and ecosystem structure. We confirmed the plastic nutrient stoichiometry of some components and the static nutrient stoichiometry of others. Pitcher fluid phosphorus responded positively to phosphorus deposition across the Eastern United States as well as across experimentally manipulated pitchers in a single bog giving evidence for atmospheric deposition of phosphorus being significant source to high mountain lakes with historically undisturbed watersheds. Phosphorus content of pitcher tissue responded positively to both depositional phosphorus and fluid phosphorus concentration, inducing production of more pitcher shoots. Microbes acted as a nitrogen and phosphorus sink, depleting pitcher fluid of these nutrients and increasing its biomass with elevated phosphorus deposition and detrital accumulation. *M. knabi*, although increasing in biomass in response to increased detrital biomass, remained static in its stoichiometry. Although *W. smithii* did not respond in biomass or abundance to microbial biomass, a food source, its own N:P had an inverse response to the N:P content of microbes, highlighting a potential stoichiometric feeding strategy of the species. *W. smithii* also increased its abundance, selectively feeding on more palatable Chlorophyta. And finally, we identified a novel pattern: the effects of N:P on ecosystem structure can act independently of nitrogen and phosphorus, a phenomenon worth looking more into. Strong concordance between the small scale press experiment and the large scale continental survey were found, highlighting patterns of stoichiometry, biomass and community structure that exist beyond the confounding effects of latitude, elevation, and growing season length.

ACKNOWLEDGEMENTS

This research is based upon work funded by a grant to A.L.G. from the National Science Foundation (NSF) DEB-1754326. We thank the numerous entities that allowed for sampling of *S. purpurea*: Georgia Natural Heritage Program, Georgia Department of Natural Resources, YMCA Camp Greenville, The Nature Conservancy, Old Dominion University – Black Water Ecological Preserve, Phil Sheridan – Meadowview Biological Research Station, Prince George's Park, Pennsylvania Department of Conservation & Natural Resources, New Jersey Natural Lands Trust, Buffalo Audubon Society, Massachusetts Division of Fisheries & Wildlife, New Hampshire Audubon, Department of Natural & Cultural Resources NH, Paul Smiths College Visitor Interpretive Center, University of Maine, Department of Agriculture, Conservation & Forestry Bureau of Parks and Lands Maine.

TABLES & FIGURES

Table 1. Summary of Structural Equation Models for N, P, and N:P across Molly Bog and the Survey. est is the Estimated coefficient, se is the standard error, z is the z value, std.all is the standardized coefficient.

		P (Survey)					P (Molly Bog)				
Response	Predictor	est	se	z	pvalue	std.all	est	se	z	pvalue	std.all
Tissue	Fluid	0.14	0.07	2.01	0.04	0.15	0.16	0.15	1.11	0.27	0.16
Microbes	Fluid	-0.08	0.09	-0.85	0.39	-0.08	-0.04	0.16	-0.24	0.81	-0.04
Fluid	Deposition	0.92	0.52	1.76	0.08	0.13	0.20	0.08	2.62	0.01	0.31
Tissue	Deposition	2.76	0.52	5.28	0.00	0.43	0.21	0.11	1.87	0.06	0.32
Fluid	Detritus	0.26	0.12	2.26	0.02	0.33	0.07	0.16	0.46	0.65	0.06
Microbes	Detritus	0.07	0.07	0.97	0.33	0.08	-0.15	0.24	-0.64	0.52	-0.12
W. smithii	Microbes	0.08	0.13	0.62	0.54	0.09	-0.09	0.34	-0.25	0.80	-0.08
M. knabi	Detritus	-0.83	0.38	-2.17	0.03	-0.21	0.29	0.22	1.30	0.19	0.21

		N (Survey)					N (Molly Bog)				
Response	Predictor	est	se	z	pvalue	std.all	est	se	z	pvalue	std.all
Tissue	Fluid	0.26	0.08	3.17	0.00	0.20	0.15	0.13	1.19	0.23	0.16
Microbes	Fluid	0.00	0.11	-0.03	0.98	0.00	-0.28	0.20	-1.41	0.16	-0.23
Fluid	Deposition	-0.05	0.08	-0.60	0.55	-0.07	0.09	0.08	1.05	0.30	0.15
Tissue	Deposition	0.23	0.09	2.44	0.01	0.26	-0.09	0.08	-1.05	0.30	-0.16
Fluid	Detritus	0.00	0.01	0.25	0.80	0.03	0.00	0.01	-0.22	0.83	-0.03
Microbes	Detritus	0.01	0.01	0.98	0.33	0.12	0.00	0.01	-0.10	0.92	-0.01
W. smithii	Microbes	-0.23	0.13	-1.73	0.08	-0.22	0.25	0.17	1.47	0.14	0.26
M. knabi	Detritus	0.03	0.06	0.44	0.66	0.05	-0.03	0.01	-2.23	0.03	-0.34

		N:P (Survey)					N:P (Molly Bog)				
Response	Predictor	est	se	z	pvalue	std.all	est	se	z	pvalue	std.all
Tissue	Fluid	-0.11	0.05	-2.33	0.02	-0.06	0.13	0.06	2.13	0.03	0.11
Microbes	Fluid	-0.12	0.07	-1.84	0.07	-0.06	-0.20	0.08	-2.52	0.01	-0.18
Fluid	Deposition	0.15	0.10	1.50	0.13	0.17	-0.04	0.10	-0.37	0.71	-0.04
Tissue	Deposition	-0.05	0.07	-0.64	0.52	-0.09	0.25	0.20	1.23	0.22	0.32
Fluid	Detritus	0.02	0.08	0.20	0.84	0.02	-0.07	0.05	-1.55	0.12	-0.08
Microbes	Detritus	-0.12	0.13	-0.95	0.34	-0.07	-0.25	0.20	-1.25	0.21	-0.24
W. smithii	Microbes	-0.06	0.12	-0.53	0.60	-0.09	-0.05	0.13	-0.35	0.72	-0.05
M. knabi	Detritus	-0.05	0.10	-0.50	0.62	-0.04	0.63	0.21	2.96	0.00	0.36

Table 2. Summary of Structural Equation Models for N, P, and N:P and biomass (mg/ml) and biovolume (ug/ul) for plankton taxa across Molly Bog and the Survey. est is the Estimated coefficient, se is the standard error, z is the z value, std.all is the standardized coefficient.

		Survey					Molly Bog				
Response	Predictor	est	se	z	pvalue	std.all	est	se	z	pvalue	std.all
Pitcher mass	P	-0.15	0.35	-0.42	0.67	-0.15	-0.17	0.30	-0.55	0.58	-0.10
Pitcher mass	N	-0.17	0.22	-0.77	0.44	-0.21	0.23	0.20	1.17	0.24	0.31
Pitcher mass	N:P	0.24	0.25	0.93	0.35	0.26	0.12	0.30	0.39	0.70	0.10
Microbes	P	0.31	0.26	1.20	0.23	0.48	0.14	0.18	0.80	0.42	0.13
Microbes	N	-0.11	0.16	-0.65	0.51	-0.21	0.07	0.12	0.63	0.53	0.14
Microbes	N:P	0.15	0.19	0.79	0.43	0.25	-0.02	0.17	-0.14	0.89	-0.03
Plankton	P	-0.25	0.31	-0.82	0.41	-0.33	0.23	0.25	0.93	0.35	0.22
Plankton	N	0.13	0.19	0.69	0.49	0.22	-0.21	0.16	-1.31	0.19	-0.43
Plankton	N:P	-0.15	0.22	-0.66	0.51	-0.22	0.21	0.25	0.85	0.40	0.27
Bacillariophyta	P	-0.51	0.27	-1.90	0.06	-0.74	0.30	0.39	0.76	0.45	0.18
Bacillariophyta	N	0.18	0.17	1.10	0.27	0.34	-0.36	0.25	-1.44	0.15	-0.47
Bacillariophyta	N:P	-0.37	0.19	-1.91	0.06	-0.60	0.48	0.38	1.25	0.21	0.40
Chlorophyta	P	-0.64	0.32	-2.01	0.04	-0.78	-0.23	0.29	-0.79	0.43	-0.18
Chlorophyta	N	0.32	0.20	1.62	0.11	0.50	0.00	0.19	-0.02	0.99	-0.01
Chlorophyta	N:P	-0.54	0.23	-2.34	0.02	-0.74	-0.19	0.29	-0.67	0.51	-0.22
Cyanobacteria	P	-0.07	0.29	-0.25	0.81	-0.10	0.21	0.26	0.82	0.41	0.19
Cyanobacteria	N	0.09	0.18	0.51	0.61	0.16	-0.17	0.17	-1.04	0.30	-0.34
Cyanobacteria	N:P	-0.01	0.21	-0.05	0.96	-0.02	0.15	0.25	0.58	0.56	0.19
W. Smithii	Plankton	0.69	0.15	4.75	0.00	0.39	-29.66	0.09	-323.13	0.00	-0.75
W. Smithii	Microbes	-0.01	0.17	-0.04	0.96	0.00	0.70	0.11	6.16	0.00	0.02
W. Smithii	Bacillariophyta	-0.29	0.16	-1.79	0.07	-0.15	4.81	0.06	82.12	0.00	0.19
W. Smithii	Chlorophyta	-0.05	0.14	-0.35	0.73	-0.03	0.27	0.08	3.48	0.00	0.01
W. Smithii	Cyanobacteria	-0.81	0.15	-5.22	0.00	-0.43	26.50	0.09	294.37	0.00	0.68
Microbes	Detritus	0.03	0.06	0.58	0.56	0.06	0.64	0.11	5.78	0.00	0.71
M. knabi	Detritus	0.02	0.06	0.29	0.77	0.03	0.13	0.12	1.02	0.31	0.12

Table 3. Summary of Structural Equation Models for N, P, and N:P and abundance (#/ml) and pitcher counter per plant. Microbes and detritus are biomass values. across Molly Bog and the Survey. est is the Estimated coefficient, se is the standard error, z is the z value, std.all is the standardized coefficient.

		Survey					Molly Bog				
Response	Predictor	est	se	z	pvalue	std.all	est	se	z	pvalue	std.all
Pitcher count	P	0.13	0.22	0.58	0.56	0.20	0.24	0.37	0.64	0.52	0.14
Pitcher count	N	-0.09	0.15	-0.58	0.57	-0.15	-0.29	0.24	-1.21	0.23	-0.37
Pitcher count	N:P	0.04	0.18	0.23	0.82	0.06	0.36	0.37	0.98	0.33	0.30
Microbes	P	0.52	0.24	2.16	0.03	0.72	0.14	0.18	0.80	0.42	0.13
Microbes	N	-0.22	0.16	-1.32	0.19	-0.33	0.07	0.12	0.63	0.53	0.14
Microbes	N:P	0.25	0.19	1.29	0.20	0.35	-0.02	0.17	-0.14	0.89	-0.03
Plankton	P	0.47	0.31	1.51	0.13	0.52	-0.04	0.34	-0.13	0.90	-0.03
Plankton	N	-0.32	0.21	-1.50	0.13	-0.39	-0.16	0.22	-0.74	0.46	-0.23
Plankton	N:P	0.34	0.25	1.34	0.18	0.38	0.43	0.33	1.31	0.19	0.41
Bacillariophyta	P	-0.18	0.26	-0.68	0.50	-0.24	0.27	0.33	0.81	0.42	0.19
Bacillariophyta	N	0.09	0.18	0.48	0.63	0.13	-0.31	0.22	-1.42	0.15	-0.46
Bacillariophyta	N:P	-0.08	0.21	-0.36	0.72	-0.10	0.53	0.33	1.62	0.11	0.52
Chlorophyta	P	0.53	0.29	1.82	0.07	0.60	-0.03	0.27	-0.12	0.90	-0.03
Chlorophyta	N	-0.14	0.20	-0.71	0.48	-0.18	-0.02	0.17	-0.09	0.93	-0.03
Chlorophyta	N:P	0.21	0.23	0.91	0.36	0.24	0.10	0.26	0.40	0.69	0.13
Cyanobacteria	P	0.06	0.22	0.29	0.77	0.10	-0.12	0.30	-0.41	0.68	-0.09
Cyanobacteria	N	-0.14	0.15	-0.94	0.34	-0.24	-0.17	0.19	-0.88	0.38	-0.27
Cyanobacteria	N:P	0.10	0.18	0.59	0.55	0.17	0.40	0.29	1.36	0.17	0.42
W. Smithii	Plankton	-0.06	0.11	-0.59	0.56	-0.06	0.14	0.11	1.29	0.20	0.13
W. Smithii	Microbes	-0.21	0.13	-1.57	0.12	-0.17	0.19	0.18	1.06	0.29	0.13
W. Smithii	Bacillariophyta	0.07	0.13	0.56	0.57	0.06	-0.04	0.11	-0.33	0.74	-0.03
W. Smithii	Chlorophyta	0.07	0.11	0.65	0.52	0.07	0.04	0.14	0.30	0.76	0.03
W. Smithii	Cyanobacteria	-0.27	0.15	-1.81	0.07	-0.19	0.08	0.12	0.64	0.52	0.06
Microbes	Detritus	0.02	0.07	0.25	0.81	0.03	0.64	0.11	5.78	0.00	0.71
M. knabi	Detritus	0.00	0.05	0.03	0.98	0.00	0.29	0.12	2.40	0.02	0.28

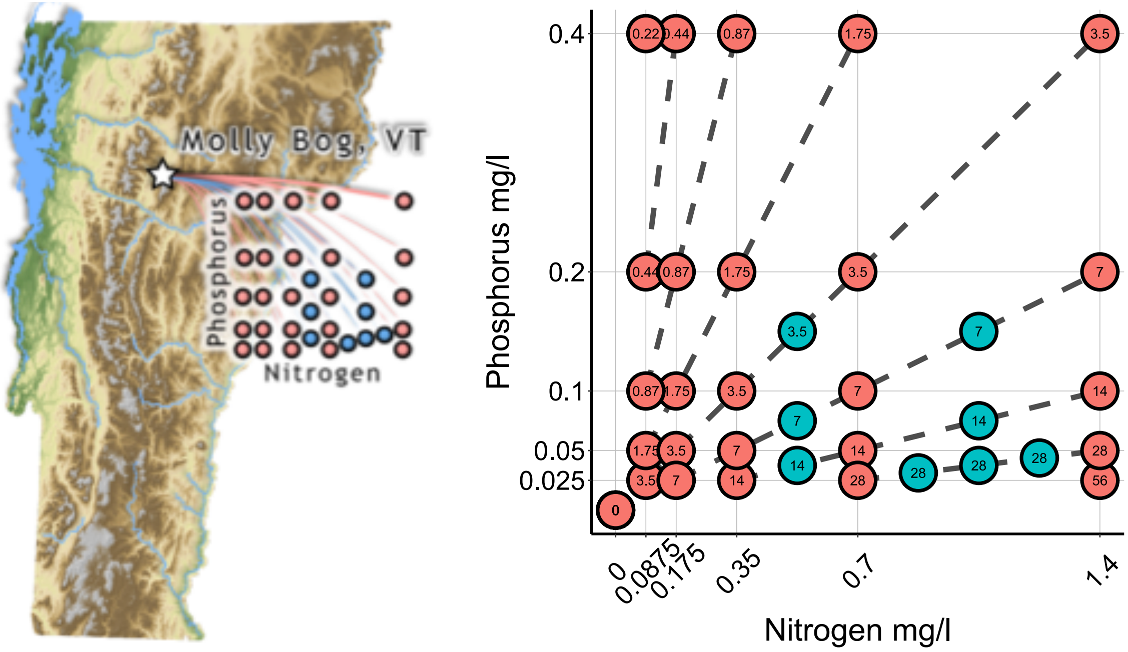

Figure 1. Experimental design of TN and TP concentrations (mg/l) for 33 experimental *Sarracenia purpurea* plants. Red circles represent a regression-based design, with blue circles representing 8 additional plants and concentrations of common ratios. Values within each circle represent the N:P ratios. Not shown are 5 points/control plants that received RO water. 2018 press experiment occurred at Molly Bog, Stowe, Vermont.

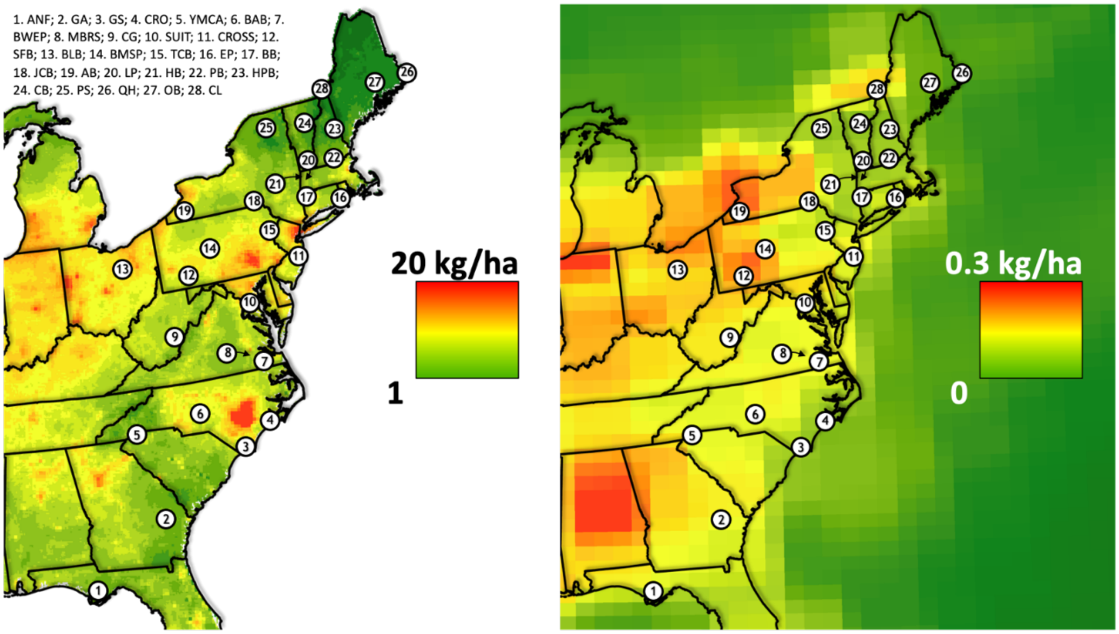

Figure 2. Map of N and P deposition in kg/ha of the Eastern United States (NADP 2019, Wang et al., 2017). White circles represent general site locations from the 2019 survey.

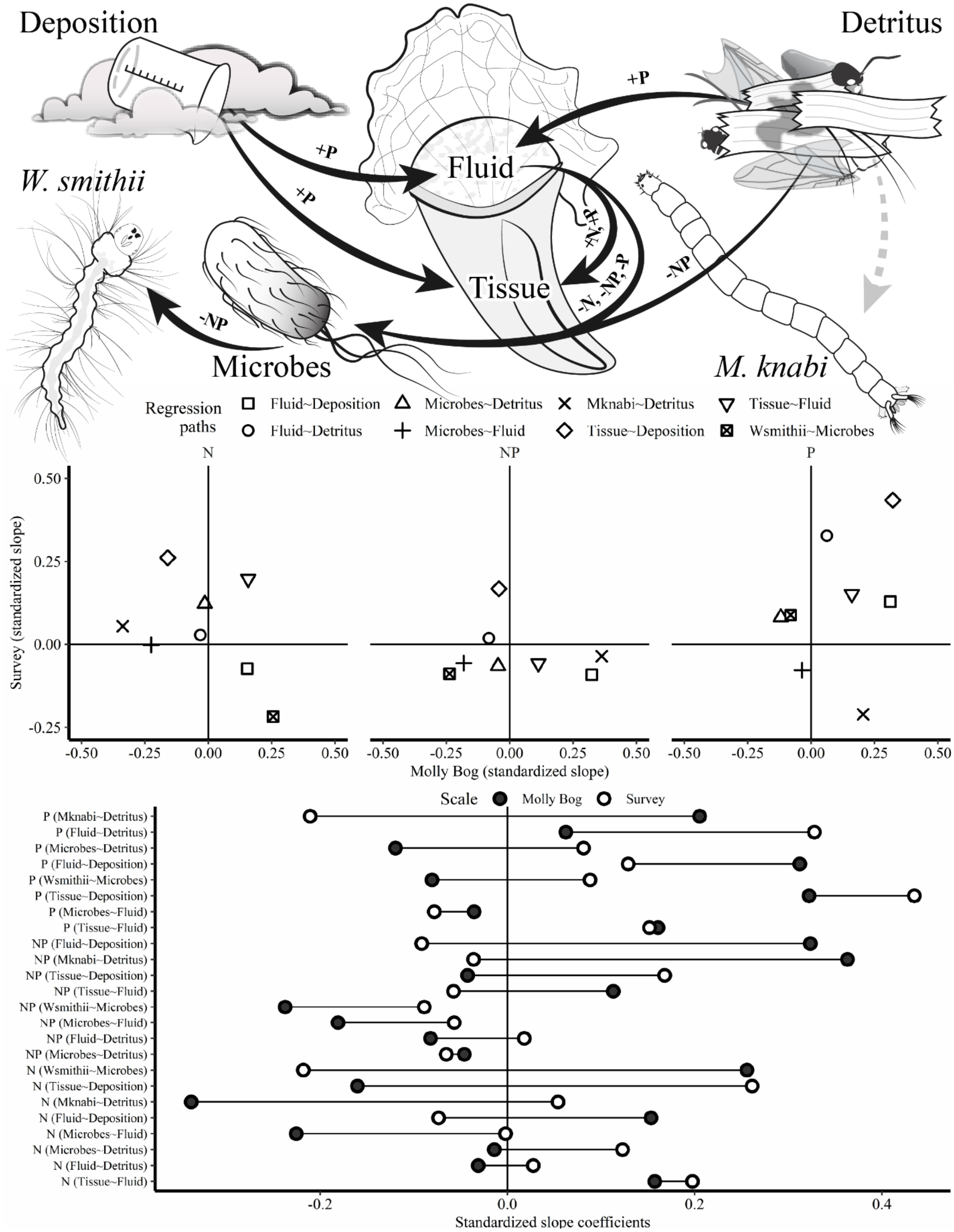

Figure 3. Top panel, regression paths of nutrient transfer among ecosystem components of the *S. purpurea* ecosystem. Dashed lines represent no concordance between Molly Bog and the Survey for N, P, or N:P in SEMs. Middle panel, concordance of standardized slope coefficients of SEMs with shapes representing unique regression paths. Bottom panel, concordance of standardized slope coefficients with connector lines overlapping the 0 vertical line representing disagreement between Molly Bog and the Survey.

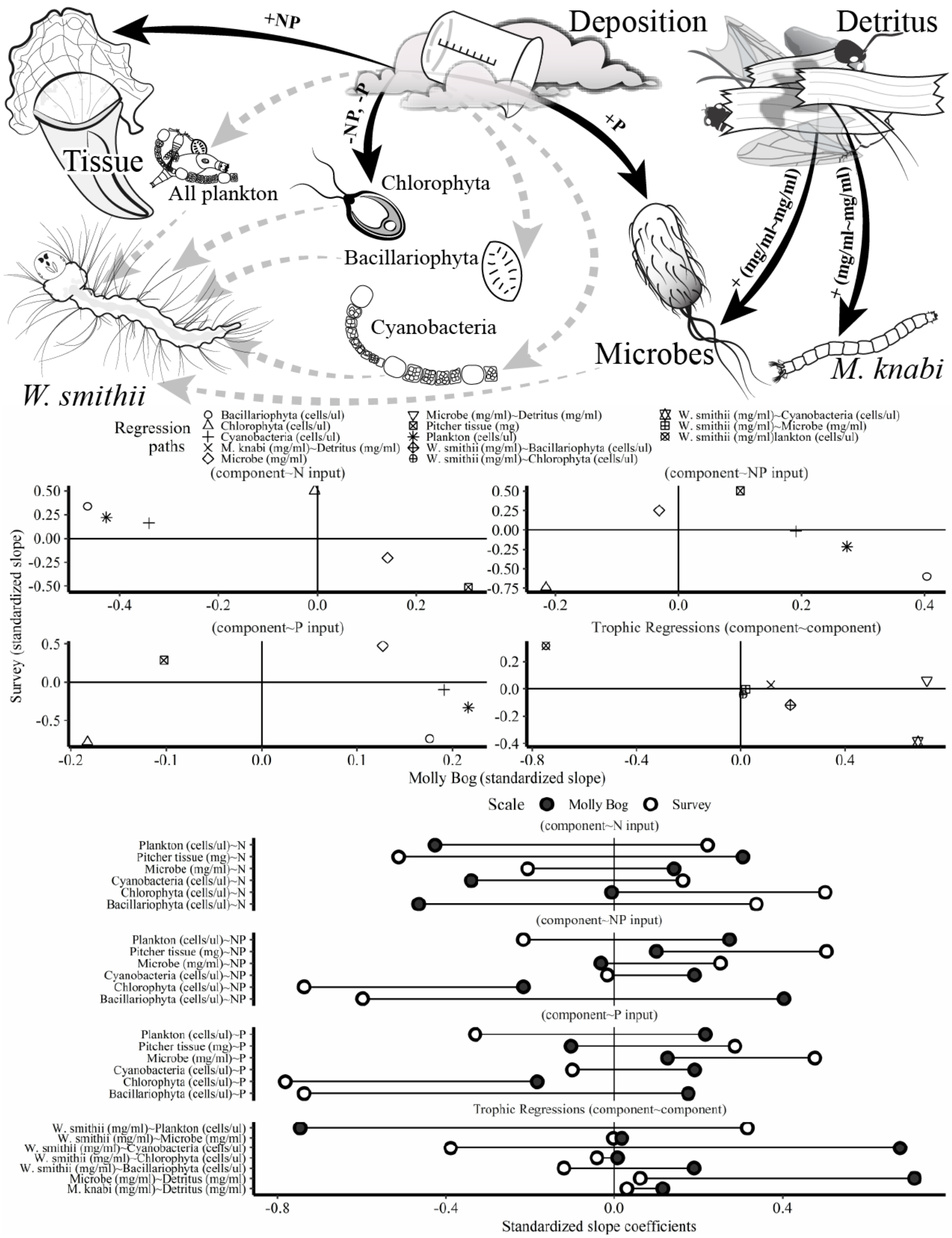

Figure 4. Top panel, regression paths of biomass transfer among ecosystem components of the *S. purpurea* ecosystem. Dashed lines represent no concordance between Molly Bog and the Survey for N, P, N:P, or biomass in SEMs. Middle panel, concordance of standardized slope coefficients of SEMs with shapes representing unique regression paths. Bottom panel, concordance of standardized slope coefficients with connector lines overlapping the 0 vertical line representing disagreement between Molly Bog and the Survey.

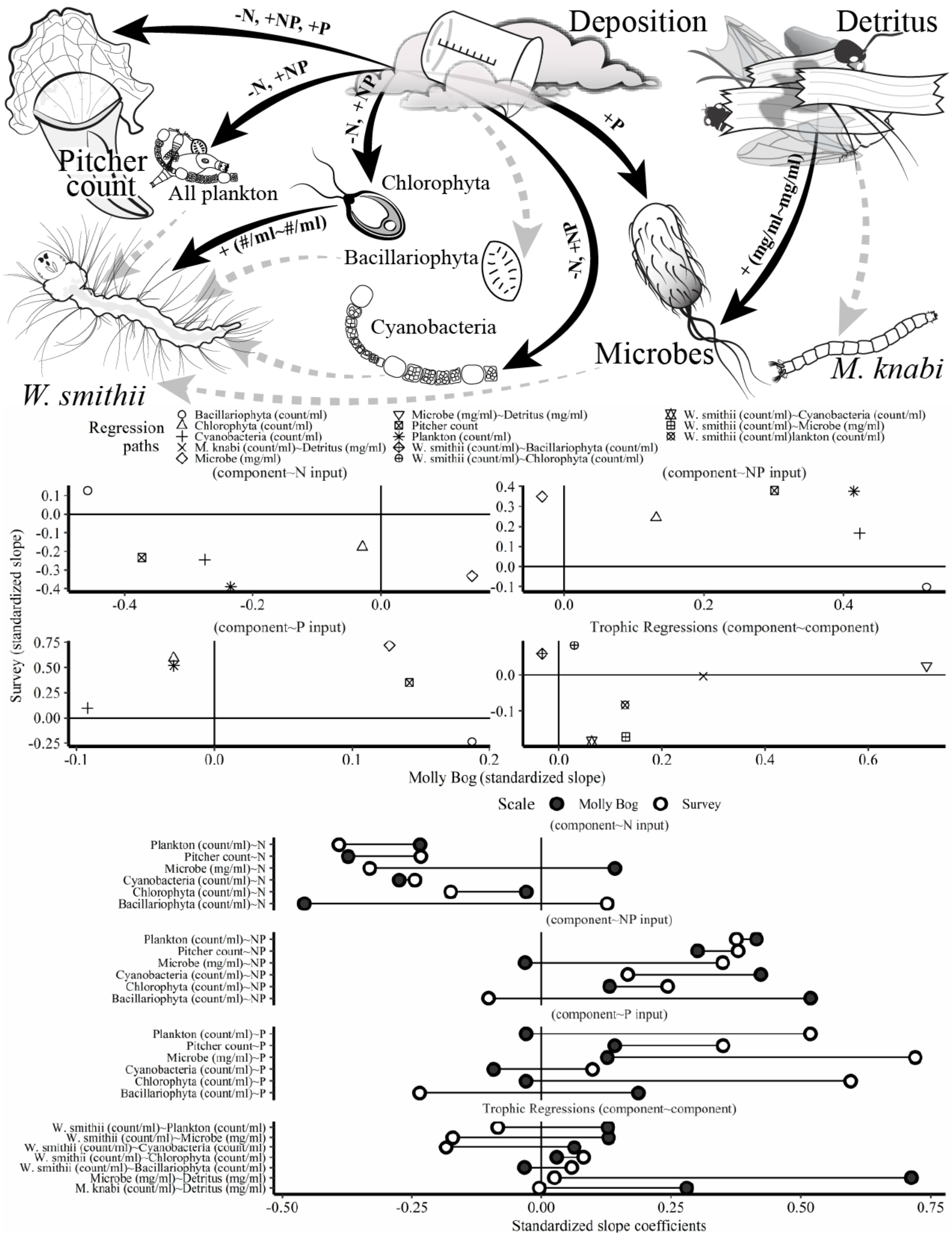

Figure 5. Top panel, regression paths of ecosystem structure of the *S. purpurea* ecosystem. Dashed lines represent no concordance between Molly Bog and the Survey for N, P, N:P, or abundance in SEMs. Middle panel, concordance of standardized slope coefficients of SEMs with shapes representing unique regression paths. Bottom panel, concordance of standardized slope coefficients with connector lines overlapping the 0 vertical line representing disagreement between Molly Bog and the Survey.

LITERATURE CITED

Aber, J. D., Goodale, C. L., Ollinger, S. V., Smith, M. L., Magill, A. H., Martin, M. E., Hallet, R. A., and Stoddard, J. L. (2003). Is nitrogen deposition altering the nitrogen status of northeastern forests? BioScience **53**(4):375-389.

Addicott J. F. (1974). Predation and Prey Community Structure: An Experimental Study of the Effect of Mosquito Larvae on the Protozoan Communities of Pitcher Plants. Ecology. **55**(3):475–492.

Carpenter, S. R., and Adams, M. S. (1977). The macrophyte tissue nutrient pool of a hardwater eutrophic lake: implications for macrophyte harvesting. Aquatic Botany, 3:239-255.

Brahney, J., Mahowald, N., Ward, D. S., Ballantyne, A. P., and Neff, J. C. (2015). Is atmospheric phosphorus pollution altering global alpine Lake stoichiometry? Global Biogeochemical Cycles **29**(9):1369-1383.

Butler, J. L. and Ellison, A. M. (2007). Nitrogen cycling dynamics in the carnivorous norther pitcher plant, *Sarracenia purpurea.* Functional Ecology **21**:835-843.

Carr, G. M. 1998. Macrophyte growth and sediment phosphorus and nitrogen in a Canadian prairie river. Freshwater Biology **39**(3):525-536.

Chapin, C.T., and J. Pastor. (1995). Nutrient limitation in the northern pitcher plant Sarracenia purpurea. Canadian Journal of Botany **73**:728-734.

Clark, G. M., Mueller, D. K., and Mast, M. A. (2000). Nutrient concentrations and yields in undeveloped stream basins in the United States. A Journal of the American Water Resources Association **36**(4):849-860.

Demars, B. O., and Edwards, A. C. (2007). Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. Freshwater Biology **52**(11):2073-2086.

De Frenne, P., Graae, B. J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., De Kort, H., De Schrijver, A., Diekmann, M., Eriksson, O., Gruwex, R., Hermy, M., Lenoir, J., Plue, J., Coomes, D. A., and Verheyen, K. (2013). Latitudinal gradients as natural laboratories to infer species' responses to temperature. Journal of Ecology **101**(3):784-795.

Di Nino, F., Thiébaut, G., and Muller, S. (2007). Phenology and phenotypic variation of genetically uniform populations of Elodea nuttallii (Planch.) H. St John at sites of different trophic states. Fundamental and applied limnology **168**(4):335.

Ellison, A. M., H. L. Buckley, T.E. Miller, and N.J. Gotelli. (2004). Morphological variation in Sarracenia purpurea (Sarraceniaceae): geographic, environmental, and taxonomic correlates. American Journal of Botany **91**:1930-1935.

Elser J. J., Andersen T., Baron J.S., Bergstrom A.K., Jansson M., Kyle M., Nydick K. R., Steger L., and Hessen D. O. (2009). Shifts in Lake N:P Stoichiometry and Nutrient Limitation Driven by Atmospheric Nitrogen Deposition. Science **326**(5954):835–837.

Ellison, A. M., and N. J. Gotelli. (2001). Evolutionary ecology of carnivorous plants. Trends in Ecology and Evolution **16**:623-629.

Ellison, A. M. and Gotelli, N.J. (2002). Nitrogen availability alters the expression of carnivory in the northern pitcher plant Sarracenia purpurea. Proceedings of the National Academy of Sciences of the United States of America **99**: 4409–4412.

Ellison A. M., Buckley H. L., Miller T. E., and Gotelli N. J. (2004). Morphological variation in Sarracenia purpurea (Sarraceniaceae): geographic, environmental, and taxonomic correlates. American Journal of Botany **91**(11):1930–1935.

Fagan, W. F., Siemann, E., Mitter, C., Denno, R. F., Huberty, A. F., Woods, H. A., and Elser, J. J. (2002). Nitrogen in insects: implications for trophic complexity and species diversification. The American Naturalist **160**(6): 784-802.

Folkerts, D. (1999). Pitcher plant wetlands of the southeastern United States, arthropod associates: 247-275. Invertebrates in freshwater wetlands of North America: ecology and management. John Wiley and Sons, New York, New York, USA.

Gallie, D. R., and S. C. Chang. (1997). Signal transduction in the carnivorous plant Sarracenia purpurea. Regulation of secre- tory hydrolase expression during development and in response to resources. Plant Physiology **115**:1461–1471.

Godwin, C. M., & Cotner, J. B. (2015). Aquatic heterotrophic bacteria have highly flexible phosphorus content and biomass stoichiometry. The ISME Journal, **9**(10): 2324-2327.

Gonzalez Sagrario, M. A., Jeppesen, E., Gomà, J., Søndergaard, M., Jensen, J. P., Lauridsen, T., and Landkildehus, F. (2005). Does high nitrogen loading prevent clear-water conditions in shallow lakes at moderately high phosphorus concentrations? Freshwater Biology **50**(1): 27-41.

Heard, S. B. (1994). Pitcher plant midges and mosquitoes: a processing chain commensalism. Ecology **75**:1647–1660.

Hieber, M., and Gessner, M. O. (2002). Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology **83**(4):1026-1038.

Jiang, X., Gao, G., Deng, J., Zhu, G., Tang, X., Shao, K., and Hu, Y. (2022). Nitrogen concentration response to the decline in atmospheric nitrogen deposition in a hypereutrophic lake. Environmental Pollution **300**:118952.

Juniper, B. E., R. J. Robinis, and D. M. Joel. (1989). The carnivorous plants. Academic Press, New York, New York, USA.

Karez, R., Engelbert, S., Kraufvelin, P., Pedersen, M. F., and Sommer, U. (2004). Biomass response and changes in composition of ephemeral macroalgal assemblages along an experimental gradient of nutrient enrichment. Aquatic Botany **78**(2):103-117.

Khoshmanesh, A., Hart, B. T., Duncan, A., and Beckett, R. (2002). Luxury uptake of phosphorus by sediment bacteria. Water Research **36**(3):774-778.

Kuba, T. M. C. M., Van Loosdrecht, M. C. M., Brandse, F. A., and Heijnen, J. J. (1997). Occurrence of denitrifying phosphorus removing bacteria in modified UCT-type wastewater treatment plants. Water Research **31**(4):777-786.

Matthaei, C. D., Piggott, J. J., & Townsend, C. R. (2010). Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction. Journal of Applied Ecology, **47**(3):639-649.

Merrell, K. and Pierson, O. (2021) Status of Vermont's Inland Lakes: Phosphorus Trends and Protection. Vermont Department of Environmental Conservation.

Ndebele-Murisa, M. R., Musil, C. F., & Raitt, L. (2010). A review of phytoplankton dynamics in tropical African lakes. South African Journal of Science **106**(1):13-18.

Oonincx, D. G. A. B., and Finke, M. D. (2021). Nutritional value of insects and ways to manipulate their composition. Journal of Insects as Food and Feed **7**(5):639-659.

Paerl, H. W., Havens, K. E., Hall, N. S., Otten, T. G., Zhu, M., Xu, H., Zhu, G., and Qin, B. (2019). Mitigating a global expansion of toxic cyanobacterial blooms: confounding effects and challenges posed by climate change. Marine and Freshwater Research **71**(5):579-592.

Peng, Y., Peng, Z., Zeng, X., and Houx, J. H. (2019). Effects of nitrogen-phosphorus imbalance on plant biomass production: a global perspective. Plant and Soil **436**(1):245-252.

Qin, J., and Culver, D. A. (1996). Effect of larval fish and nutrient enrichment on plankton dynamics in experimental ponds. Hydrobiologia **321**(2):109-118.

Schulhof, M. A., Shurin, J. B., Declerck, S. A., and Van de Waal, D. B. (2019). Phytoplankton growth and stoichiometric responses to warming, nutrient addition and grazing depend on lake productivity and cell size. Global change biology, **25**(8):2751-2762.

Yves Rosseel (2012). lavaan: An R Package for Structural Equation Modeling. Journal of Statistical Software, 48(2), 1-36. https://doi.org/10.18637/jss.v048.i02

Shurin, J. B., Clasen, J. L., Greig, H. S., Kratina, P., and Thompson, P. L. (2012). Warming shifts top-down and bottom-up control of pond food web structure and function. Philosophical Transactions of the Royal Society B: Biological Sciences **367**(1605):3008-3017.

Spilling, K., Camarena-Gómez, M. T., Lipsewers, T., Martinez-Varela, A., Díaz-Rosas, F., Eronen-Rasimus, E., Silva, N., con Dassow, P., and Montecino, V. (2019). Impacts of reduced inorganic N: P ratio on three distinct plankton communities in the Humboldt upwelling system. Marine Biology **166**(9):1-17.

Thrane, J. E., Hessen, D. O., and Andersen, T. (2017). Plasticity in algal stoichiometry: Experimental evidence of a temperature-induced shift in optimal supply N: P ratio. Limnology and Oceanography **62**(4):1346-1354.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann, E. (1997). The influence of functional diversity and composition on ecosystem processes. Science, **277**(5330):1300-1302.

Wissinger, S. A., Perchik, M. E., and Klemmer, A. J. (2018). Role of animal detritivores in the breakdown of emergent plant detritus in temporary ponds. Freshwater Science, **37**(4):826-835.

Wurtsbaugh, W. A., Paerl, H. W., and Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. Wiley Interdisciplinary Reviews: Water, **6**(5):e1373.

CHAPTER II: NITROGEN AND PHOSPHORUS DEPOSITION IMPACT *SARRACENIA PURPUREA* MORPHOLOGY AND HABITAT SIZE

ABSTRACT

Sarracenia purpurea, the northern pitcher plant, produces cup-shaped rain-filled leaves that support a complete aquatic ecosystem with a "brown" food web base of captured prey, microbes, phytoplankton, and invertebrate shredders, filter-feeders, and top carnivores. This system is often used as a tractable experimental model for larger aquatic ecosystems. However, the effects of inputs of nitrogen (N) and phosphorus (P), and the N:P ratio on the morphology of the carnivorous pitcher's trap are not well-studied. I utilized a two-scale study to test for concordant morphological responses to nutrients in a small-scale press experiment and a large-scale survey across gradients of atmospheric N, P, and N:P deposition. Concordance in these two studies is defined as the same directional response to variation in nutrients in the experiment and across the survey. We found a concordant negative response of relative tube diameter to nitrogen input in the press experiment as well as in the large-scale survey, confirming that nitrogen deposition reduces this trait of carnivory. However, there was no response of relative keel size to N additions, which contrasts with experiments conducted at this same site 20 years ago. Phosphorus acted independently of N:P by increasing carnivorous traits of relative tube diameter, opening width, and total length. These traits directly affect the water holding capacity and prey capture ability of the pitcher, both of which are likely to have important consequences for ecosystem-level processes.

INTRODUCTION

Sarracenia purpurea, the northern pitcher plant, and the ecosystem within its carnivorous phytotelmata is well studied by multiple investigators. But with multiple researchers studying such a small, unique system at different locations along environmental gradients, conflicting results and conclusions can arise.

Ellison et al. (2004) found pitchers with large keels, the largely photosynthetic portion, in sites with higher ammonium in pore water. And previously, Ellison and Gotelli (2002) found that high concentrations of N input or high N:P produced large non-carnivorous keels and small carnivorous tubes. These results were corroborated by field surveys across New England, correlating these morphologies with nitrogen in pore water. In the Adirondacks, Crumley (2015) found similar results where plant morphology elicited less carnivorous characteristics with increasing nitrogen deposition. Conversely, Wakefield et al. (2005) found that prey addition increased nitrogen concentrations in pitcher fluid, but pitcher morphology did not shift to larger keels or smaller tubes. Karbeg and Gale (2013) found similar results in that morphology of *S. purpurea* showed no relation to water chemistry, even at the highest nitrogen sites.

Here we used a small-scale press experiment of varying N, P, and N:P concentrations, free of confounding environmental factors, and a large-scale continental survey of gradients of N, P, and N:P to examine the causal effect of N, P, and N:P on *S. purpurea* morphology. In addition, we investigated causal relationships between these morphologies and water holding capacity and shed light on a new pathway for nutrients to impact ecosystem structure.

MATERIALS & METHODS

Molly Bog Experimental Design

To measure the effect of nutrient input on the morphology of *S. pururea*, we experimentally manipulated nutrient input to the model system *S. purpurea* at Molly Bog, in Morristown, Vermont (44.500253°N, -72.639624°W) in the summer of 2018. We used a regression-based design with 5 concentrations of nitrogen 0.0875, 0.175, 0.35, 0.7, and 1.4 mg/l and 5 values of phosphorus 0.025, 0.05, 0.1, 0.2, 0.4 mg/l (Figure 1, Chapter 1). Solutions of nitrogen and phosphorus were created using stock NH_4Cl and NaH_2PO_4, due to their past use in stoichiometric work and these forms are readily available for the plant to uptake (Gray et al. 2006). In addition to the 5 x 5 regression-based design, 8 additional experimental points were included to replicate the most common ratios made from the regression concentration design. In total there were 9 treatment ratios among the 33 treatment points or plants. Ratios of the design included the N:P ratios of 3.5 and 7 replicated 6 times, 14 and 28 replicated 5 times, 1.75 replicated 4 times, 0.87 replicated 3 times, 0.44 replicated twice, and outlier ratios of 0.22 and 56 with no replicates. The concentrations of nitrogen and phosphorus were based on national atmospheric deposition data as well as predicted future deposition rates (National Atmospheric Deposition Program 2018, Galloway et al. 2008).

Molly Bog Experimental Protocol

A total of 38 mature non-flowering *S. purpurea* were chosen randomly at Molly Bog. All plants in the experiment were a minimum of 15 cm in basal diameter and contained a minimum of 5 living pitchers. Experimental plants were separated from each other by a minimum of 0.5 meters. Of the 38 plants, 33 were randomly assigned concentrations of nitrogen and phosphorus and N:P ratios from the experimental design and 5 plants were randomly assigned as controls, receiving reversed osmosis (RO) water with minimal values of N and P (TN <0.02 mg/L, TP 0.005 mg/L) (Chapter 1, Figure 1) Once plants were chosen, fluid was removed from every pitcher. Pitchers of each plant were then rinsed with RO water and given the assigned concentrations and ratios for nitrogen and phosphorus maintained a press experiment: for a total of 10 weeks, each week or after every rain event pitchers would be topped off with the assigned solution of N and P.

Molly Bog Experiment Final Measurements

At the end of 10 weeks, morphological measurements were taken of each plant including basal diameter (BD) of the entire plant, opening pitcher width (OW), opening pitcher length (OL), total length (TL), keel width (KW), and total width (TW) of the youngest open pitcher, using flexible vinyl tape (±1 mm) and calipers (± 0.1 mm). The indices of relative keel size (KW/TW) and relative tube diameter (OW/TL) were also calculated. After morphological measurements, fluid was removed from every pitcher per plant and measured with a 100 ml graduated cylinder.

Large Scale Survey

To measure the effect of atmospheric nutrient deposition on pitcher morphology, we conducted a survey of 28 sites along the Eastern coast of the United States from the Florida panhandle to Northern Maine over the summer of 2019 (Figure 2, Chapter 1). At each site, we selected 15 random pitcher plants separated from each other by a minimum of 0.5 m. Plants were at least 15 cm in basal diameter, nonflowering, and contained a minimum of 5 pitchers. All morphological measurements were identical to the above-mentioned Molly Bog Experiment.

Statistical Analysis

To compare the direction and magnitude of the effect of nitrogen, phosphorus, and N:P pitcher morphology at the experimental scale to the large scale, we extracted standardized slope coefficients from paired Structural Equation Models (SEMs) fit by the R package *lavaan* (Rosseel, 2012). In this work, concordance was defined as directional agreement (+ and + or – and –) in slope coefficients between the experiment and survey. Discordance was defined as directional disagreement (+ and – or – and +) in slope coefficients between the experiment and survey. SEMs were chosen over simple linear models due to the functionality of covariance structures within SEMs that allow for slope coefficients to represent effects beyond other covarying independent variables. For example, the slope (or effect) of N deposition on a morphological trait is the effect of N beyond the effect of NP or P. A total of 2 SEMs were fit using a maximum likelihood estimator and all response variables were scaled to 0 to 1 to meet the variance requirements of the SEM model fitting process. Of the 2 SEMs, 1 was for Molly Bog and 1 was for the survey. In the morphology SEMs total volume of the plant was related to basal diameter (a plant wide measurement) and the volume of each pitcher was related to the morphological characteristics of the pitcher.

RESULTS

Phosphorus experimental inputs in Molly Bog (mb) and depositional phosphorus across the survey (s) had concordant positive effects on pitcher morphologies of total length (TL) (standardized coefficient for mb 0.42 and for s 0.44), opening length (OL) (mb 0.03, s 0.14), opening width (mb 0.40, s 0.53), and relative tube diameter (RelTD) (mb 0.04, s 0.20) (Table 1; Figure 1). Phosphorus did not have concordant effects on basal diameter (BD) (mb 0.50, s -0.60), total width (TW) (mb 0.36, s -0.01), keel width (KW) (mb 0.47, s -0.04), or relative keel size (RelKS) (mb -0.30, s -0.07).

Nitrogen experimental inputs in Molly Bog and depositional nitrogen across the survey had concordant negative effects on pitcher morphologies TL (mb -0.03, s -0.19), OW (mb -0.14, s -0.44), and RelTD (mb -0.22, s -0.35). Nitrogen did not have concordant effects on BD (mb -0.39, s 0.60), TW (mb -0.01, s 0.01), KW (mb -0.34, s 0.02), OL (mb 0.02, s -0.06), and RelKS (mb -0.46, s 0.01).

Beyond the effects of nitrogen and phosphorous alone, N:P of inputs and deposition affected concordantly TL (mb 0.42, s 0.37), TW (mb 0.28, s 0.12), OL (mb 0.14, s 0.36), and OW (mb 0.21, s 0.88). N:P of inputs and deposition did not have concordant effects on BD (mb 0.52, s -0.68), KW (mb 0.63, s -0.05), RelTD (mb -0.06, s 0.64), or RelKS (mb 0.59, s -0.20).

Total volume of pitcher fluid responded concordantly in Molly Bog and across the survey to BD (mb, 0.12, s 0.08) and individual pitcher volume responded positively to TL (mb 0.23, s 0.01), TW (mb 0.26, s 0.52) and negatively to KW (mb -0.17, s -0.40), and OL (mb -0.09, s -0.02). Pitcher volume did not respond to OW (mb 0.51, s -0.02), RelTD (mb -0.05, s 0.12), or RelKS (mb -0.08, s 0.10).

DISCUSSION

Here we aim to clarify knowledge on the response of *S. purpurea's* morphology to nitrogen, phosphorus, and N:P by finding concordant effects between a small-scale press experiment, free of confounding environmental factors, and a large-scale survey across gradients of nitrogen, phosphorus, and N:P.

Multiple pitcher morphologies responded concordantly to nitrogen, phosphorus, and N:P inputs and deposition, but not all. Total length, opening width, and relative tube diameter had concordant, negative responses to nitrogen deposition. Relative tube diameter, a measurement of a pitcher plants carnivory, was lower with elevated nitrogen deposition, corroborating Ellison and Gotelli (2002), but conversely relative keel size and keel width, traits of carnivory, did not respond to nitrogen concentrations (Wakefield et al., 2005). Relative tube diameter, opening width, opening length, and total length, all had concordant, positive responses to phosphorus. Previous studies have only found nitrogen to affect pitcher morphology, let alone increase carnivorous traits. Note that in chapter I we found pitcher tissue to readily uptake phosphorus. The hypothesized reason for a reduction in carnivorous traits with increased nitrogen deposition is clear: when nitrogen is readily available, the plant doesn't need to catch prey. An inverse relationship with phosphorus is more difficult to explain and should be investigated further.

Beyond just their individual responses to nitrogen and phosphorus, opening width, opening length, and total width had positive concordant responses to N:P. Given that N and P did act concordantly on TW, N:P may be acting truly independent of nitrogen and phosphorus. The positive response of opening length to N:P is more complicated due to phosphorus also having a positive response elicited from opening length. We would expect the opposite mathematically because increasing phosphorus should decrease N:P. But considering the covariance structure of SEMs, the standardized coefficients are based on holding covarying variables constant. Therefore, when phosphorus is held constant, increasing N:P increases opening length. The independence of N:P from nitrogen and phosphorus should be further investigated. More work should be done to find a biological explanation for this phenomenon.

Pitcher fluid volume had a positive concordant response to total length and total width and total plant fluid volume had a positive concordant response to basal diameter. Basal diameter generally means a larger plant, therefore more fluid makes sense. Total length equates to more space to hold water as well as total width. Keel width and opening length had concordant negative effects on pitcher fluid volume. Considering keel width is a general characteristic of non-carnivory, being functionally more photosynthetic, it makes sense that pitchers with large keels hold less water. Opening length is more complicated because we would assume a larger opening would be able to collect more water, increasing pitcher fluid volume. However, pitcher volume did not respond to increased relative keel size and increased relative tube diameter, two characteristics generally assumed to enhance carnivory. The carnivorous relevance of these ratios may not hold up linearly across the variety of keel widths and total widths and opening widths and total lengths. Here we point to carnivorous traits potentially confounding the effects of nitrogen, phosphorus, and N:P on ecosystem structure. We found causal links at the small scale and the large scale from nitrogen, phosphorus, and N:P to plant morphology which in turn affects total volume of pitcher fluid which defines the size of the ecosystem space. Nutrient deposition is acting on this aquatic ecosystem directly through the food web (Chapter I) and through alteration of the size of the ecosystem space itself, this chapter. To our knowledge, phytotelmata may be the only system where nutrient inputs can directly alter the size of its ecosystem.

Carnivorous morphological traits of *S. purpurea* readily responded to alterations of nutrient inputs at the scale of a press experiment in a single bog, free of confounding environmental gradients, and across a continental scale survey with gradients of nutrient deposition. Morphological characteristics also responded to N:P independently of nitrogen or phosphorus. Pitcher fluid volume responded to morphological traits that change across gradients of nutrient deposition highlighting a new, obfuscating pathway that may complicate the use of phytotelmata as model systems for larger aquatic ecosystems.

ACKNOWLEDGEMENTS

This research is based upon work funded by a grant to A.L.G. from the National Science Foundation (NSF) DEB-1754326. We thank to the numerous entities that allowed for sampling of *S. purpurea*: Georgia Natural Heritage Program, Georgia Department of Natural Resources, YMCA Camp Greenville, The Nature Conservancy, Old Dominion University – Black Water Ecological Preserve, Phil Sheridan – Meadowview Biological Research Station, Prince George's Park, Pennsylvania Department of Conservation & Natural Resources, New Jersey Natural Lands Trust, Buffalo Audubon Society, Massachusetts Division of Fisheries & Wildlife, New Hampshire Audubon, Department of Natural & Cultural Resources NH, Paul Smiths College Visitor Interpretive Center, University of Maine, Department of Agriculture, Conservation & Forestry Bureau of Parks and Lands Maine.

TABLES & FIGURES

Table 1. Summary of Structural Equation Models for N, P, and N:P and pitcher morphologies. across Molly Bog and the Survey. est is the Estimated coefficient, se is the standard error, z is the z value, std.all is the standardized coefficient.

		Survey					Molly Bog				
Response	Predictor	est	se	z	pvalue	std.all	est	se	z	pvalue	std.all
BD	Nitrogen	0.41	0.17	2.38	0.02	0.60	-0.26	0.14	-1.81	0.07	-0.39
BD	Phosphorus	-0.47	0.26	-1.80	0.07	-0.60	0.34	0.13	2.54	0.01	0.49
BD	N:P	-0.35	0.21	-1.67	0.09	-0.68	0.52	0.25	2.12	0.03	0.52
KW	Nitrogen	0.01	0.20	0.06	0.95	0.02	-0.26	0.16	-1.61	0.11	-0.34
KW	Phosphorus	-0.04	0.30	-0.12	0.91	-0.04	0.37	0.15	2.45	0.01	0.47
KW	N:P	-0.03	0.24	-0.13	0.90	-0.05	0.72	0.28	2.60	0.01	0.63
OL	Nitrogen	-0.04	0.18	-0.22	0.82	-0.06	0.01	0.18	0.07	0.95	0.02
OL	Phosphorus	0.11	0.28	0.41	0.68	0.14	0.02	0.17	0.14	0.89	0.03
OL	N:P	0.19	0.22	0.85	0.39	0.35	0.17	0.31	0.54	0.59	0.14
OW	Nitrogen	-0.32	0.19	-1.72	0.08	-0.44	-0.12	0.18	-0.65	0.52	-0.15
OW	Phosphorus	0.45	0.28	1.59	0.11	0.53	0.33	0.17	1.97	0.05	0.40
OW	N:P	0.49	0.23	2.15	0.03	0.88	0.26	0.31	0.83	0.40	0.21
TL	Nitrogen	-0.15	0.21	-0.75	0.45	-0.19	-0.01	0.05	-0.15	0.88	-0.03
TL	Phosphorus	0.40	0.31	1.28	0.20	0.44	0.11	0.05	2.14	0.03	0.42
TL	N:P	0.22	0.25	0.89	0.37	0.37	0.16	0.09	1.74	0.08	0.42
TW	Nitrogen	0.01	0.20	0.05	0.96	0.01	-0.01	0.18	-0.05	0.96	-0.01
TW	Phosphorus	-0.01	0.31	-0.02	0.98	-0.01	0.30	0.17	1.77	0.08	0.36
TW	N:P	0.07	0.25	0.28	0.78	0.12	0.35	0.32	1.09	0.28	0.28
RelKS	Nitrogen	0.01	0.23	0.02	0.98	0.01	-0.36	0.17	-2.12	0.03	-0.46
RelKS	Phosphorus	-0.07	0.34	-0.21	0.84	-0.07	0.23	0.16	1.48	0.14	0.29
RelKS	N:P	-0.13	0.28	-0.48	0.63	-0.20	0.69	0.29	2.37	0.02	0.59
RelTD	Nitrogen	-0.22	0.16	-1.39	0.16	-0.35	-0.17	0.17	-0.97	0.33	-0.22
RelTD	Phosphorus	0.15	0.24	0.62	0.54	0.20	0.03	0.16	0.21	0.83	0.04
RelTD	N:P	0.31	0.19	1.61	0.11	0.64	-0.06	0.30	-0.22	0.83	-0.06
Volume	RelKS	0.10	0.08	1.25	0.21	0.10	-0.06	0.10	-0.63	0.53	-0.08
Volume	OW	-0.02	0.09	-0.23	0.82	-0.02	0.39	0.10	4.06	0.00	0.51
Volume	KW	-0.45	0.09	-5.08	0.00	-0.40	-0.14	0.11	-1.30	0.19	-0.17
Volume	OL	-0.02	0.09	-0.25	0.80	-0.02	-0.07	0.10	-0.74	0.46	-0.09
Volume	TL	0.01	0.08	0.11	0.91	0.01	0.58	0.32	1.81	0.07	0.23
Volume	TW	0.56	0.09	6.52	0.00	0.52	0.19	0.09	2.04	0.04	0.26
Volume	RelTD	0.16	0.10	1.52	0.13	0.12	-0.04	0.10	-0.39	0.70	-0.05
Volume	BD	0.11	0.14	0.77	0.44	0.08	0.14	0.18	0.75	0.46	0.12

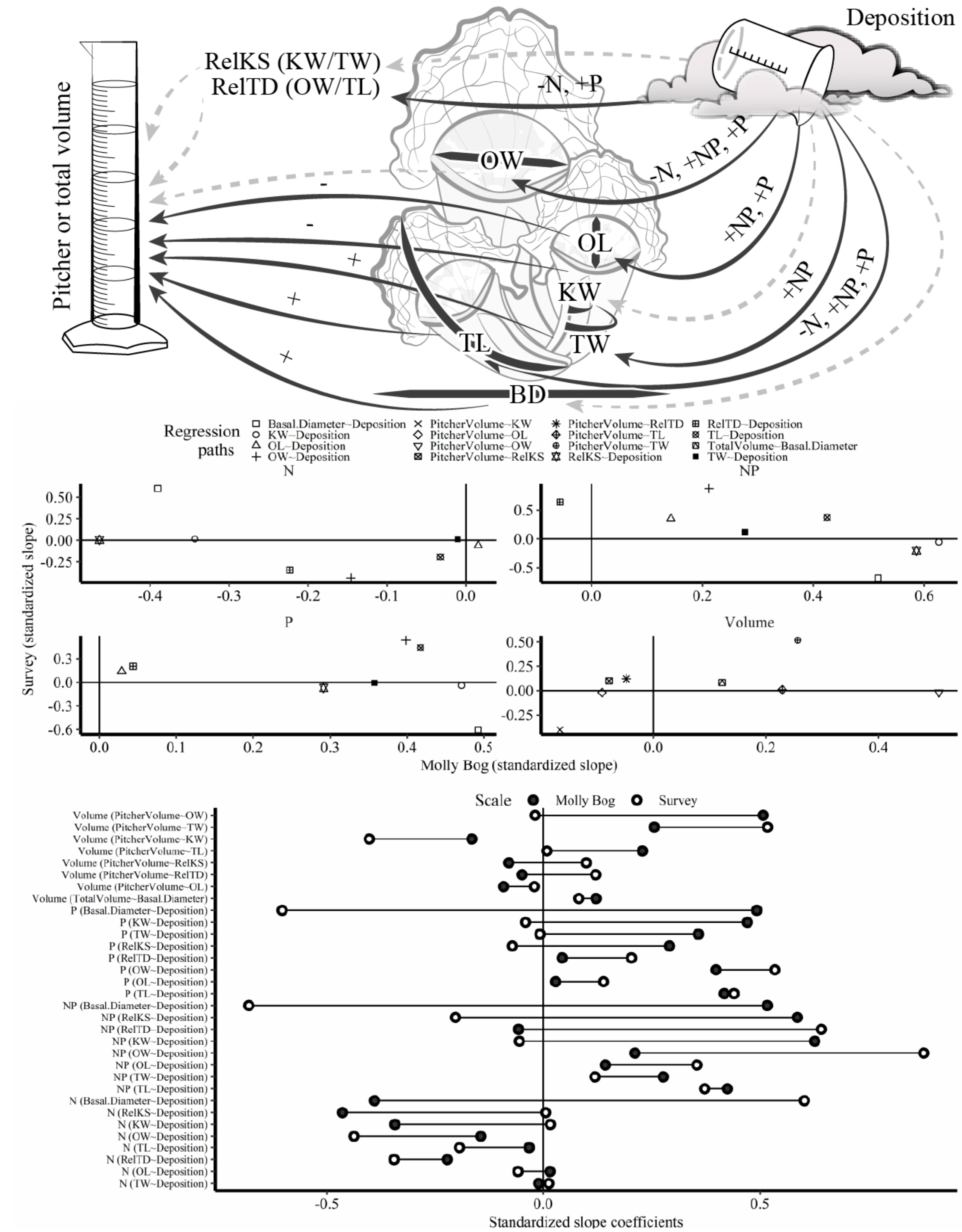

Figure 1. Top panel, regression paths of nutrient effects on *S. purpurea* morphologies and pitcher volume. Dashed lines represent no concordance between Molly Bog and the Survey for N, P, N:P, or volume in SEMs. Middle panel, concordance of standardized slope coefficients of SEMs with shapes representing unique regression paths. Bottom panel, concordance of standardized slope coefficients with connector lines overlapping the 0 vertical line representing disagreement between Molly Bog and the Survey.

LITERATURE CITED

Crumley, K. M. (2015). Effects of nitrogen deposition on nitrogen acquisition by Sarracenia purpurea in the Adirondack Mountains, New York, USA. The Journal of the Torrey Botanical Society, **143**(1):8-20.

Ellison, A. M., Buckley, H. L., Miller, T. E., and Gotelli, N. J. (2004). Morphological variation in Sarracenia purpurea (Sarraceniaceae): geographic, environmental, and taxonomic correlates. American Journal of Botany **91**(11):1930-1935.

Ellison, A. M., and Gotelli, N. J. (2002). Nitrogen availability alters the expression of carnivory in the northern pitcher plant, Sarracenia purpurea. Proceedings of the National Academy of Sciences **99**(7):4409-4412.

Karlberg, J. M., and Gale, M. R. (2013). Influence of surface water mineral nutrition on the plasticity of Sarracenia purpurea in Sphagnum fens, marl wetlands, and sand savannahs. Wetlands **33**(4):631-639.

Yves Rosseel (2012). lavaan: An R Package for Structural Equation Modeling. Journal of Statistical Software **48**(2):1-36.

Wakefield, A. E., Gotelli, N. J., Wittman, S. E., and Ellison, A. M. (2005). Prey addition alters nutrient stoichiometry of the carnivorous plant Sarracenia purpurea. Ecology **86**(7):1737-1743.

CHAPTER III: COMPREHENSIVE SURVEY OF ALGAL, CYANOBACTERIA, AND ROTIFER COMMUNITY STRUCTURE WITHIN NATIVE POPULATIONS OF *SARRACENIA PURPUREA*

ABSTRACT

At 28 sites along a latitudinal gradient from the Florida panhandle to northern Maine, I surveyed the abundance and diversity of the plankton assemblage (algal, cyanobacteria, and rotifer) associated with the rain-filled, cup-shaped leaves of the carnivorous pitcher plant *Sarracenia purpurea*. Chlorophyta was the numerically dominant taxa, although its number declined at higher latitudes, a pattern also documented in many freshwater lakes. Cyanobacteria were numerically dominant in leaves with high phosphorous concentrations, whereas Bacillariophyta were more common in leaves with low concentrations of ammonium and low pitcher fluid pH. Estimated species richness decreased at higher latitudes, Rotifera biovolume increased and Bacillariophyta biovolume decreased with increasing elevation. Collectively, the plankton diversity patterns we describe are similar to those seen in larger aquatic systems, which highlights the utility of *Sarracenia purpurea* as a model system for aquatic ecology.

INTRODUCTION

Phytotelmata are micro-aquatic ecosystems that are contained within a variety of terrestrial plants. These natural microcosms are found in bromeliads, tree-holes, heliconias, and pitcher plants such as *Sarracenia purpurea*, among others (Maguire 1971; Jalinsky et al 2014; Ramos and do Nascimento 2019). The diverse aquatic fauna inhabiting phytotelmata is composed of adult and larval aquatic insects, crustaceans, rotifers, zooplankton, protozoans, bacteria, and algae (Maguire 1971). Despite phytotelmata often being used as model systems to test general ecological theory, knowledge of their plankton species and the underlying environmental factors structuring them remains limited. Not until recently were plankton surveyed within *Sarracenia purpurea* L. (northern pitcher plant) (Dudley 1984; Gebühr et al. 2006)

Sarracenia purpurea is a long-lived carnivorous perennial with a native range distributed across the east coast of North America from the Florida Panhandle to Canada (Ellison et al. 2004). These plants inhabit nutrient poor ecosystems and rely on atmospheric deposition or invertebrate prey for nutrients (Butler and Ellison 2007, Ellison and Gotelli 2002, Juniper et al. 1989, Chapin and Pastor 1995, Ellison and Gotelli 2001). *S. purpurea* contain cup-shaped leaves that act as phytotelmata. Prey that enters the system are initially shredded by *Fletcherimyia fletcheri,* an obligate flesh fly larva and broken down by *Metriocnemus knabi,* an obligate midge larva, and bacteria (Addicott 1974, Heard 1994). Bacteria within *S. purpurea* are consumed by plankton, protozoans, and *Wyeomyia smithii,* an obligate mosquito larva. *W. smithii* also consumes plankton and protozoans, and besides *F. fletcheri* initially shredding prey, it will consume *W. smithii* and be cannibalistic in some instances.

Although much is known about *S. purpurea's* obligate Diptera inhabitants, little is known about the algal, cyanobacteria, and rotifer community that inhabit the phytotelmata, and the factors that can impact their community structure. To our knowledge there are only two studies of *S. purpurea's* plankton communities. Dudley (1984) observed 23 genera of algae in phytotelmata at one site in Pennsylvania and one site in New York. The diversity of algal species varied among sampling dates, bogs, and temperature of the phytotelmata fluid, but did not go into specific relationships as the main goal of the study was to determine if algae was present. Gebühr et al. (2006) observed 78 algal taxa and researched the development temporally of microalgae communities within *S. purpurea* at a site in Saxony, Germany, and a site in Blekinge, Sweden, where these plants are non-native and do not contain obligate Diptera larvae (Adlassing et al 2009, Walker 2015). *S. purpurea* phytotelmata not containing obligate invertebrates may affect plankton community structure as many of the obligate invertebrates are capable of consuming plankton such as *W. smithii,* an obligate filter feeder.

Research on plankton diversity in phytotelmata has been limited to bromeliads. Fluid parameters within bromeliad phytotelmata have been found to relate to algal community structure. In bromeliad phytotelmata many species of green algae are associated with high levels of DO (Ramos et al., 2018). pH may readily impact specific species but large taxonomic groups such as green algae have been found to prevail in both acidic and alkaline phytotelmata (Ramos et al., 2018). High nutrient concentrations in phytotelmata can also favor specific groups such as cyanobacteria, as nutrients such as ammonium is a main source of nitrogen for cyanobacteria species (Ramos and Moura 2019; Sigee 2005) Plant morphology can also impact algal community structures and biomass/biovolume. Marino et al. (2011) determined that habitat size is an important regulator of algal biomass, and the diameter of bromeliads is a driver of chlorophyll-a concentration.

In large aquatic ecosystems, plankton communities respond robustly to alterations of nutrient regimes (Dagenais-Bellefuielle and Morse, 2013; Vincon-Leite and Casenave, 2019). These relationships are not constant and will vary across other parameters at the same scale as the interaction of acidification and nitrogen or temperature and phosphorus (Eberlein et al. 2016; Thomas et al. 2017). Plankton communities also respond across large spatial scales like the negative response of plankton diversity to latitude and elevation across different aquatic ecosystems (Righetti et al. 2019).

My goal of this study was to comprehensively describe the phytoplankton communities across the native range of *S. purpurea*, compare our findings with two historical works, and investigate how these communities respond to plant scale abiotic fluid parameters within the phytotelmata, plant morphology, and site scale latitudinal, growing season, and elevational gradients. We hypothesized that fluid parameters of the phytotelmata fluid will impact algae community structure. We predicted a positive correlation between algal abundance and dissolved oxygen, as phototrophic algae produce oxygen. We also predicted that pH gradients will favor certain groups of algae over others, as certain species of green algae can increase in growth with low pH, while species of cyanobacteria can be suppressed with low pH (Graham et al. 1996; Yamamoto and Nakahara 2005). Plant morphology will also impact algal structure. As, Marino et al. (2011) found that habitat size of tank bromeliads significantly influences the biomass of algae. In comparison to Gebühr et al. (2006) we predicted to find fewer species than what was found in *S. purpureas* non-native areas. As noted above *S. purpurea* within North America contain organisms that can actively consume algae such as zooplankton and obligate Diptera larvae and mites. These findings give insight on what type of algal, cyanobacteria, and rotifers are present in native *S. purpurea* and how the community structure and biovolume respond to phytotelmata environmental parameters, habitat size, and gradients of latitude, elevation, and growing season length.

MATERIALS & METHODS

Sampling Sites

S. purpurea sampling took place from June to August 2019, along the Eastern seaboard of the United States, from the Florida Panhandle to Northern Maine (Figure 1). We sampled 28 natural populations of *S. purpurea*, sites ranged from floating bogs, seepage swamps, savannahs, kettle hole bogs, to wet meadows.

Field Sampling

Phytotelmata environmental parameters & plant morphology

At each site, we selected 15 random pitcher plants, separated among each other, by a minimum of 0.5 m. Plants were at least 15 cm in basal diameter, nonflowering, and contained a minimum of 5 pitchers. From each of these 15 plants, we sampled the pitchers/phytotelmata for physical-chemical and morphological measurements. Morphological measurements include entire plant basal diameter, fluid volume of each pitcher, pitcher opening width, pitcher opening length, keel width, total width, and total length. Pitcher fluid was measured for pH (±0.05), temperature, dissolved oxygen, electrical conductivity (± 2%), total dissolved solids (± 2%) using a Pancellent water quality probe and an AMTAST portable dissolved oxygen probe. We then collected the pitcher fluid of 5 pitchers from each of the 15 plants per site. Fluid was preserved using 0.1 ml of Sulfuric acid for every 50ml of fluid and stored at less than 6° C. Fluid was analyzed at the Cary Institute of Ecosystem Studies for ammonium, nitrate, total nitrogen, phosphate, and total phosphorus concentrations.

Phytoplankton collection & identification

For each of the five youngest phytotelmata/pitchers per plant, we removed the fluid via a plastic transfer pipette. The fluid collected was filtered through a 25 μm nylon mesh to capture the phytoplankton community, this was done before fluid preservation. The mesh was inverted and rinsed with RO water into a 50ml conical tube in which the plankton was preserved with Lugols Iodine solution.

In the laboratory, we measured plankton species richness, species composition, density, and biovolume, for 3 of the 15 plants collected for a total of 84 samples, using a PhycoTech Nannoplankton Counting Chamber. For each sample, 300 natural units were counted (Charles et al., 2002). The three plants per site chosen for plankton identification were those that had their fluid analyzed for chemical concentrations. Organisms were identified to lowest possible taxonomic identification genus or species based off morphological attributes at 40x magnification using a Nikon 50i clinical grade microscope. Taxonomic identification was conducted using a variety of keys and resources (Komárek 2008; Komárek and Anagnostidis 2007; Wehr et al., 2015; Hindák 2008; Patrick and Reimer 1966; Prescott et al., 1972; Smith 1950) and biovolume was calculated following Hillebrand et al. (1999) and Olenina (2006).

Data Analysis

For mixed effects models, all variables other than estimated species richness were scaled to 0 to 1. To model plankton community structure, we performed linear mixed effects models where estimated species richness, plankton density and biovolume, and relative abundance and biovolumes of plankton taxa were response variables (ACE, Chiu et al. 2011, R package *vegan* (Oksanen et al. 2020), and phytotelmata-scale predictors were treated as a fixed factor and latitude, elevation, site, and spring index (first leaf 2019, USA National Phenology Network) were treated as random factors in the models. Independent LMMs were performed for each community descriptor. LMMs were fit by restricted maximum likelihood (REML) using the package *lme4* and likelihood ratio tests were used to assess significance of random effects (Bates et al. 2015). The p-value of the fixed effect's coefficient in each final model was adjusted using Benjamini-Hochberg adjustment. A Poisson error distribution was used for the estimated species richness model and a Gaussian error distribution for other models. Reported R^2 is the variance explained by the fixed effect calculated using the R package *MuMIN* (Barton, 2020).

We used generalized linear models (GLMs) to analyze whether plankton community responses were affected by latitude, elevation, and spring index, with a Poisson error distribution for the estimated species richness model and a gaussian error distribution for all other models. Reported R^2 are pseudo R^2: 1 – (deviance / null deviance).

RESULTS

We identified 39 unique taxa representing individuals from Rotifera (rotifers), Bacillariophyta (diatoms), Chlorophyta, Charophyta, Cyanobacteria, and Ochrophyta (Table 2). Chlorophyta dominated plankton density and relative abundance followed by Cyanobacteria, Bacillariophyta, and varying low levels of Charophyta, Rotifera, Cryptophyta, and Ochrophyta (Figure 2) across a varying fluid parameters (Table 1). Of the relative abundances of plankton, Chlorophyta was the dominant plankton component for 73 plants, Cyanobacteria for 7, Bacillariophyta for 2, and Charophyta for 2. Conversely, Cyanobacteria replaced Chlorophyta as the dominant component when considering relative biovolume. Of the relative biovolumes of plankton, Cyanobacteria was the dominant plankton component for 59, Bacillariophyta for 22, and Rotifera for 3.

Plant scale

Of the plant-scale environmental parameters and morphological characteristics, ammonium, total nitrogen, phosphate, total phosphorus, basal diameter, and pH were significant predictors of plankton community structure while temperature, TDS, EC, DO, volume, volume per pitcher, and nitrate did not show any significant effects (Table 3, Figure 3). Plankton biovolume ($R^2=0.18$, $p<0.01$) responded positively to plant basal diameter. Plankton density responded positively to ammonium ($R^2=0.18$, $p<0.01$) and total nitrogen ($R^2=0.15$, $p<0.01$), but not to phosphate or total phosphorus. When looking at taxa responses, we found that Bacillariophyta relative abundance ($R^2=0.14$, $p<0.05$) responded negatively to pH, while its relative biovolume responded negatively to ammonium ($R^2=0.13$, $p<0.05$). Chlorophyta density ($R^2=0.08$, $p<0.05$) responded positively to total nitrogen. Cyanobacteria density also responded positively to total nitrogen ($R^2=0.22$, $p<0.01$), but more so than Chlorophyta. Cyanobacteria density and relative abundance responded positively to ammonium ($R^2=0.35, 0.28$, $p<0.01, 0.01$), phosphate ($R^2=0.23, 0.20$, $p<0.01, 0.01$), and total phosphorus ($R^2=0.18, 0.15$, $p<0.05, 0.05$), with a greater response to phosphate than to ammonium and a greater response to total nitrogen than to total phosphorus.

Site scale

The three continuous site scale characteristics of elevation, latitude, and spring index were significant predictors of site averaged community structure (Table 4, Figure 4). Bacillariophyta density ($R^2=0.14$, $p<0.05$), biovolume ($R^2=0.17$, $p<0.05$), and relative biovolume ($R^2=0.17$, $p<0.05$) responded negatively to elevation. Rotifera relative biovolume ($R^2=0.18$) responded positively to elevation. Charophyta relative biovolume ($R^2=0.16$, $p<0.05$) responded positively to latitude. Chlorophyta relative abundance responded negatively to latitude ($R^2=0.19$, $p<0.05$) and less so to spring index ($R^2=0.15$, $p<0.05$). Ochrophyta relative abundance responded positively to latitude. Estimated species richness responded negatively to spring index but less so to latitude.

DISCUSSION

Here, we provide the first survey of algae, cyanobacteria, and rotifers within the phytotelmata of *S. purpurea* over a large latitudinal gradient in the plants native range within the United States, compare our results with two previous studies, and then to investigate impacts of plant scale and site scale environmental conditions on plankton community structure. Although this study captured just a snapshot in time of each phytotelmatas community structure, our results were similar to those of Dudley (1984) and Gebühr (2006) as well as those from larger aquatic systems.

In comparison to Dudley (1984) we found 13 shared genera and 24 genera that Dudley did not find. We predicted that we would find more species than Dudley's work as our study contained more sites and more diverse environmental conditions. In comparison to Gebühr et al. (2006) we found 21 shared genera and 14 genera that Gebühr did not find. Gebühr found substantially more species than our study. We predicted to find less species than Gebühr, despite their fewer sites and plants, due to potential top-down trophic cascades (e.g., Pinito-Coelho et al. 2008). Gebühr's European communities did not have natural predators or grazers such as obligate Diptera larvae (*W. smithii, M. knabi)* and the obligate pitcher plant mite (*Sarraceniopus gibsoni*). Further work should examine trophic interactions between invertebrate predators and plankton prey in *S. purpurea* phytotelmata.

Plant scale

Chlorophyta dominated the relative abundance of plankton communities in pitcher plants. These results are like those reported for lakes where Chlorophyta also represent the dominant planktonic group (Grossman et al. 2016; Tian et al. 2013; Senerpont Domis, Mooji and Huisman, 2007; Morris, et al. 2006; De Stasio, Schrimpf and Cornwell, 2014, Yang et al. 2012). Despite an overall Chlorophyte dominance in pitcher plants, Cyanobacteria has been observed to dominate aquatic ecosystems, especially those largely eutrophicated (Paul et al. 2012; Lung'Ayia et al. 2000; Filstrup et al. 2016; Rao et al. 2021; Makarewicz et al. 1998; Smith, 1983, Trimbee and Prepas, 1987, Watson et al., 1997). In fact, Cyanobacteria replaced Chlorophyta as the dominant component overall when using relative biovolume as the ranking measure. Our mixed effects models show a significant, positive response of Cyanobacteria density and relative abundance to nitrogen and phosphorous concentrations. These results suggest that the nutrient status of pitcher plant fluid may drive the relative dominance of Cyanobacteria and Chlorophyta. Although more abundant, the Chlorophyta observed in pitcher plants were smaller, less clonal, than Cyanobacteria or Bacillariophyta. Relative biovolume and relative abundance of plankton are often not reported together, but when they are we find a similar loss of rank for Chlorophyta (e.g., Gong et al. 2020).

At the plant scale, Bacillariophyta relative abundance responded negatively to pH. The most abundant Bacillariophyta genera in our survey was *Eunotia*, a genus composed of many acidophilic diatom species which had optimal relative abundances of 30% at ~pH 4 (Table 2; Figure 5) (DeNicola 2000). Other, less abundant Bacillariophyta with optimal relative abundances near pH 4 in our study were *Synedra sp. Fragilaria sp., Pinnularia sp.* and *Melosira sp.* Although diatoms are often not associated with low pH, Pinnularia has been found to dominate abundance in low pH surficial water as well as in acidic streams (Luís et al 2009; Kwandrans 1993). In contrast to an increase in Bacillariophyta along lower pH, their relative biovolume responded negatively to ammonium concentrations. In fact, *Eunotia* had optimal relative biovolumes of 78% at < 4 mg/l ammonium. Considering ammonium is a weak acid we would expect the opposite relationship. Similar optimum curves were observed for *Synedra sp*. Previous studies have shown that elevated ammonium concentrations trigger development of sexual morphologies in some marine Bacillariophyta (Moore et al. 2017). Perhaps elevated ammonium concentrations can shift resources from vegetative growth to formation of auxospores in our freshwater *Eunotia* and *Synedra* genera. More work should be done to explore this relationship.

Cyanobacteria biovolume and overall plankton biovolume responded positively to plant basal diameter, or the overall size of the plant. Interestingly there were no relationships with pitcher plant fluid volume. Perhaps the volatile nature of water levels in these phytotelmata, due to climate and herbivory, disrupt these area/volume relationships, analogous to flow regime modification in lakes or perhaps the relationships are marginalized by more important factors such as nutrient concentrations (Zohary and Ostrovsky 2011; Soininen and Meier, 2014; Logue et al., 2012).

Overall, plankton density responded positively to ammonium and total nitrogen concentrations but not to phosphate or total phosphorus, suggesting that the plankton community inhabiting pitcher plants may be limited by nitrogen. In contrast to the general responses of the plankton community, cyanobacteria density and relative abundance responded positively to the concentration of ammonium, phosphate, TN, and TP. We expected a positive relationship between ammonium and cyanobacteria density/abundance as ammonium is the more easily assimilated form of inorganic N, although cyanobacteria are still capable of using nitrate and nitrite, supporting the relationship between TN and density/abundance (Ochoa-de-Alda et al., 1996). Similarly, Chlorophyta density increased with TN. The positive responses of Cyanobacteria and Chlorophyta to increases in N could be explained by the role that nitrogen availability plays on algal cell starvation, with low N supply preventing division and new growth (Zhang et al., 2019). Further, cyanobacteria density seems to be more sensitive to changes in TN compared to Chlorophyta, with similar responses found in other aquatic ecosystems (Donald et al. 2013). Our findings also showed that although Cyanobacteria increase along phosphorus concentrations as shown by other studies (Seale et al., 1987), they also respond more strongly to N than P, similar to those responses from lakes (Trimbee and Prepas 1987; Cremona et al. 2018; Jankowiak et al. 2019). In contrast, our results do not agree with findings reported in rivers where cyanobacteria responded more steeply to phosphate than to ammonium (Kim et al. 2017). Cyanobacteria in pitcher plant fluid may be more limited by nitrogen than cyanobacteria in streams.

Site scale

Across our geographical extent, Bacillariophyta density, biovolume, and relative biovolume responded negatively to elevation. Our environmental pH and ammonium displayed a significant positive correlation with elevation (Figure 6), therefore co-correlation producing the elevation relationship is likely. Studies investigating the influence elevational gradients on Bacillariophyta community structure have found either a lack of or a negative relationship (Heiikkinen, et al. 2021; Weckstrom et al. 2007; Gremmen et al. 2007). Although temperature tends to vary with elevation across gradients, in our study we did not find a significant effect of pitcher fluid temperature on plankton community structure but note these were one time temperature readings and might be poor representatives of fluid temperature and do not record diurnal swings which impact plankton communities (Beninca et al. 2011). In addition, atmospheric depositional gradients may also co-correlate with elevation and could provide an explanation for these relationships, but that is beyond the scope of this manuscript.

Similarly, to community-level responses, we did not find clear taxa-specific responses to environmental conditions. Although, Rotifera relative biovolume responded positively to elevation, it did not change with any plant scale variables or environmental conditions. Studies of Rotifera community structure find strong ‘bottom-up’ controls by diatom abundance (May et al. 2001). Therefore, the Rotifera and elevation relationship in our survey may not be the result of elevational gradients in diatom abundance because diatom abundance was inversely related to elevation. Rotifera exhibit strong altitude and seasonal polymorphisms. Cold temperatures associated at higher elevations delay the summertime reduction in the size of *Keratella cochlearis*, a species observed in our survey (Green 1981). Further work should explore polymorphisms in *S. purpurea's* obligate rotifer, *Habrotrocha rosa*.

The relative biovolume of Charophyta, and relative abundances of Chlorophyta and Ochrophyta responded to the latitudinal gradient in the survey. Charophyta and Ochrophyta, generally minor components of the plankton community, increased their relative biovolume and abundance with latitude. Conversely, Chlorophyta, the dominant group in pitcher plants, decreased its density at higher latitudes and responded negatively to spring index. In lakes, Chlorophyta dominance is often reduced at high latitudes and in winter months likely due to varying temperature optima and responses among plankton community components, for example Chlorophyta had a significant positive relationship with water temperature and Charophyta had none across lakes in the Alps (Pollingher, 1990; Salmaso et al., 2011).

Estimated species richness (ACE), responded negatively to latitude and more so to spring index, two co-correlated variables, and did not respond to any plant-scale variable. Negative latitudinal gradients in species richness occur across many taxonomic groups (Pianka, 1966; Schemske and Mittleback, 2017). For example, freshwater plankton richness follows a similar, negative pattern (Stomp et al., 2011). Gradients in temperature and growing season length are likely the mechanisms behind the negative relationship between latitude and plankton richness in the pitcher plants across this survey (Yvon-Durocher et al. 2015; Weyhenmery et al. 2013). A longer growing season (spring index) allows more time for the pitchers to acquire plankton stochastically through prey or weather events.

There has been no work investigating the pathways algae, cyanobacteria, and rotifers take to enter *S. purpurea's* phytotelmata. But we will briefly speculate. Genera of Chlorophyta (*Chlamydomonas, Chlorella*), Bacillariophyta (*Melosira*), and Cyanobacteria (*Chroococcus*) are commonly found in sphagnum moss or surface waters in bogs, and from personal observations entire populations of *S. purpurea* can become inundated with water (Schlichting and Sides 1969). Aquatic and terrestrial insects have also been found to successfully transport algae, rotifers, and cyanobacteria between habitats (Schlichting and Sides 1969, Revill et al., 1967). Insects could enter the plant as prey and then deposit algae or while ovipositing of eggs, leaving algal cells behind. We believe that introduction of algae, cyanobacteria, or rotifers most likely occurs through these two pathways of inundation of bog water and transportation via insects, atmospheric transport may also be plausible.

Overall, the structure of plankton communities in *S. purpurea's* phytotelmata is similar to the structure of plankton communities in lakes. In our phytotelmata and in many lakes, Chlorophyta dominates, but can be replaced by Cyanobacteria under high concentrations of phosphorus. In acidic lakes, rivers, and *S. purpurea's* phytotelmata in this study, acidophilic diatoms of the genera *Eunotia* become more significant components of community structure. And, like plankton communities in lakes, the richness of *S. purpurea* phytotelmata responds negatively to latitude. From a qualitative comparison between our work and that done by Gebühr et al (2006) in Germany, we point to strong top-down controls of plankton community structure by obligate invertebrates only found in North America. From shifts in relative biovolumes, we identify potential polymorphisms in Rotifera induced by temperature gradients of elevation and from shifts in relative biovolumes, we highlighted a counterintuitive effect elevated ammonium concentrations on Bacillariophyta size potentially due to a trigger in their reproductive cycles. Experiments should be done to investigate the mechanisms behind these shifts. For example, *S. purpurea* in replicate could be grown in a greenhouse setting across different levels of temperature and ammonium while monitoring the growth of Rotifera and Bacillariophyta.

S. purpurea phytotelmata are small but contain complex plankton communities that respond to environmental pressures in ways comparable to large freshwater ecosystems. These algal, cyanobacteria, and rotifer communities should be accounted for more in the ecological studies and literature as they likely play an important role in ecosystem processing and functioning within the phytotelmata.

ACKNOWLEDGEMENTS

This research is based upon work funded by a grant to A.L.G. from the National Science Foundation (NSF) DEB-1754326. We thank to the numerous entities that allowed for sampling of *S. purpurea*: Georgia Natural Heritage Program, Georgia Department of Natural Resources, YMCA Camp Greenville, The Nature Conservancy, Old Dominion University – Black Water Ecological Preserve, Phil Sheridan – Meadowview Biological Research Station, Prince George's Park, Pennsylvania Department of Conservation & Natural Resources, New Jersey Natural Lands Trust, Buffalo Audubon Society, Massachusetts Division of Fisheries & Wildlife, New Hampshire Audubon, Department of Natural & Cultural Resources NH, Paul Smiths College Visitor Interpretive Center, University of Maine, Department of Agriculture, Conservation & Forestry Bureau of Parks and Lands Maine.

TABLES & FIGURES

Table 1. Mean, minimum, and maximum of plant scale characteristics of the 84 pitcher plants and site scale characteristics of the 28 sites in this study.

Plant scale characteristics	**Mean (min, max)**
Basal diameter (mm)	30.5 (15.2, 68.5)
Temperature (C)	31.24 (21.8, 42.8)
pH	5.452 (3, 8.22)
TDS	123.2 (11, 428)
EC	236.7 (22, 808)
DO (mg/l)	4.832 (1.69, 7.26)
Volume (ml)	75.61 (16.5, 178.5)
Volume per pitcher	15.12 (3.3, 35.7)
Ammonium (mg/l)	5.412 (0.3, 24.3)
Nitrate (mg/l)	0.108 (0.005, 0.74)
Phosphate (mg/l)	0.714 (0.013, 4.95)
Total nitrogen (mg/l)	10.85 (1.45, 70)
Total phosphorus (mg/l)	1.451 (0.005, 8.04)
Site scale characteristics	**Mean (min, max)**
Latitude	39.95 (30.09, 45.25)
Elevation (m)	307.3 (8, 1069)
Spring index	86.64 (15, 120.33)

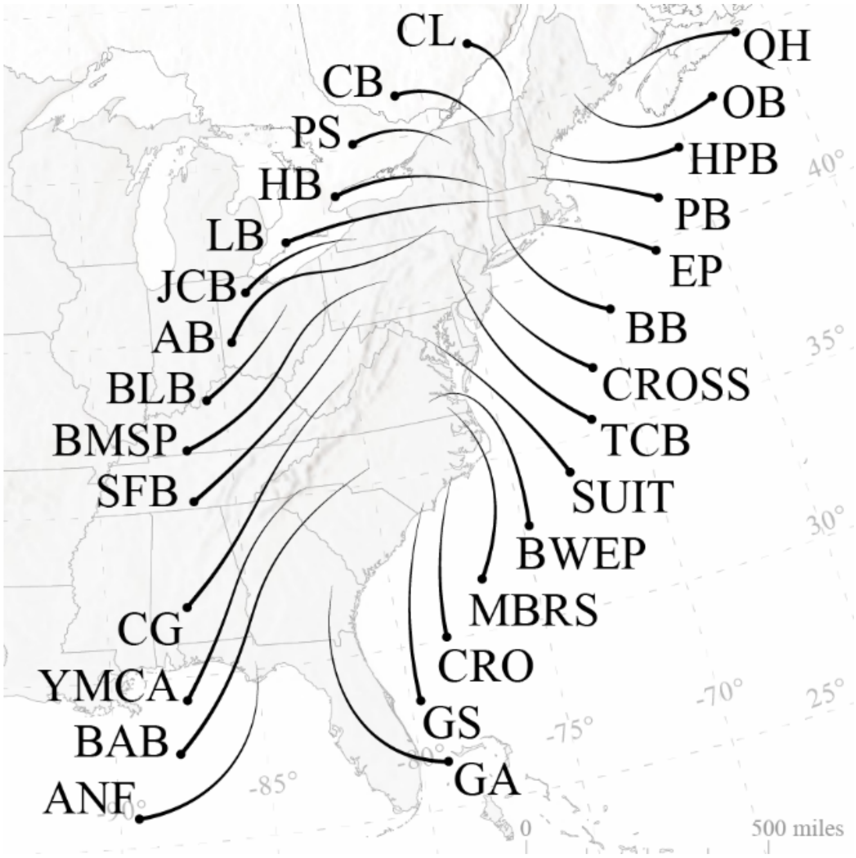

Figure 1. Location of the 28 sampling sites from 2019 across the east coast of the United States, where plankton samples were collected.

Table 2. The number of *S. purpurea* out of 3 possible plants per site with the presence of plankton taxa across the 28 sites.

Bacillariophyta	**CG**	**PB**	**QH**	**BWEP**	**MBRS**	**CROS**	**GA**	**AB**	**EP**	**JCB**	**SUIT**	**YMCA**	**BB**	**BAB**	**OB**	**TCB**	**ANF**	**BLB**	**CROSS**	**BS**	**HPB**	**LP**	**SFB**	**HB**	**BMSP**	**CB**	**CL**	**PS**
Eunotia sp.	1	1	2	3	3	3	3	3	3	3	2	2	1	3	3	3	3	1	3	1	2	0	3	0	0	0	0	1
Synedra sp.	1	1	1	1	2	1	1	1	0	3	0	2	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	3
Melosira sp.	2	0	1	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Eunotia arcus	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0	0	1	1	0	0	0
Pinnularia viridis	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0
Synedra ulna	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Aulacoseira sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Eunotia bidentula	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Fragilaria sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Asterionella formosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Navicula sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Stephanodiscus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Charophyta																												
Klebsormidium sp.	2	2	0	3	1	1	1	2	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	2	3	2
Penium polymorphum	1	2	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0
Cylindrocystis brebissonii	1	0	0	0	0	0	1	0	0	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Closterium sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cosmarium sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penium margaritaceum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Staurastrum sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorophyta																												
Chlorella homosphaera	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Chloromonas sp.	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3
Crucigenia sp.	1	3	3	3	3	3	3	2	2	2	3	3	0	3	2	1	3	3	1	2	2	1	0	1	0	0	1	0
Chlamydomonas sp.	1	0	0	2	1	1	2	1	2	0	1	2	0	0	0	2	3	0	0	2	0	0	0	0	0	0	0	0
Coenocystis sp.	1	0	0	1	0	2	0	3	1	0	0	0	2	0	0	0	0	1	0	0	0	2	1	0	0	0	0	0
Stigeoclonium sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0
Scenedesmus sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Choricystis minor	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptomonas sp.	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyanobacteria																												
Cyanogranis sp.	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Cyanodictyon reticulatum	3	3	2	1	3	3	2	3	3	3	3	3	3	3	1	3	3	3	3	3	0	3	3	3	3	0	0	0
Mersismopedia chondroidea	2	2	0	1	2	1	0	0	1	0	3	1	2	1	0	0	0	1	0	0	0	3	1	0	1	0	0	0
Chroococcus sp.	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0
Hapalosiphon sp.	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0
Oscillatoria sp.	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Ochrophyta																												
Synura sp.	3	3	2	1	1	1	1	0	3	3	1	0	0	2	1	2	0	0	0	0	3	3	0	3	1	0	1	0
Ochromonas sp.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotifera																												
Habotrocha rosa	1	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	0	2	0	1	0	0	0
Asplanchna sp.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Keratella cochlearis	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

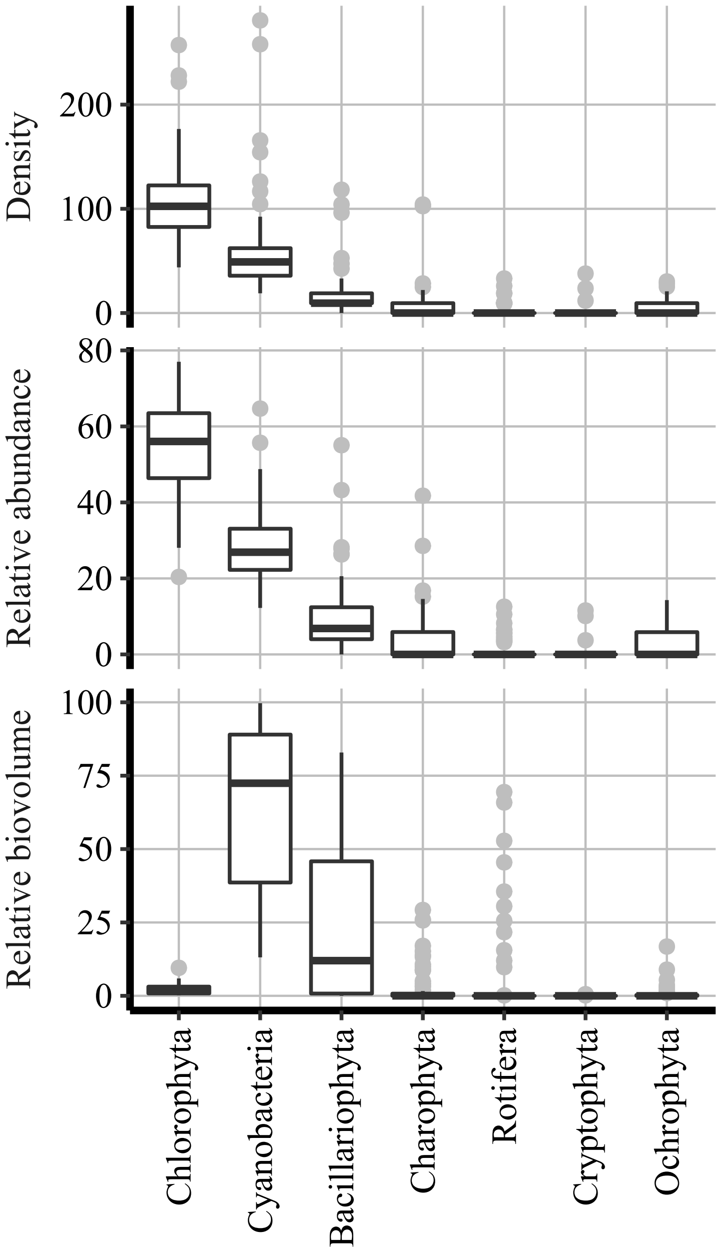

Figure 2. Distribution of plankton taxa group density (#/ml), relative abundance, and relative biovolume across the study area.

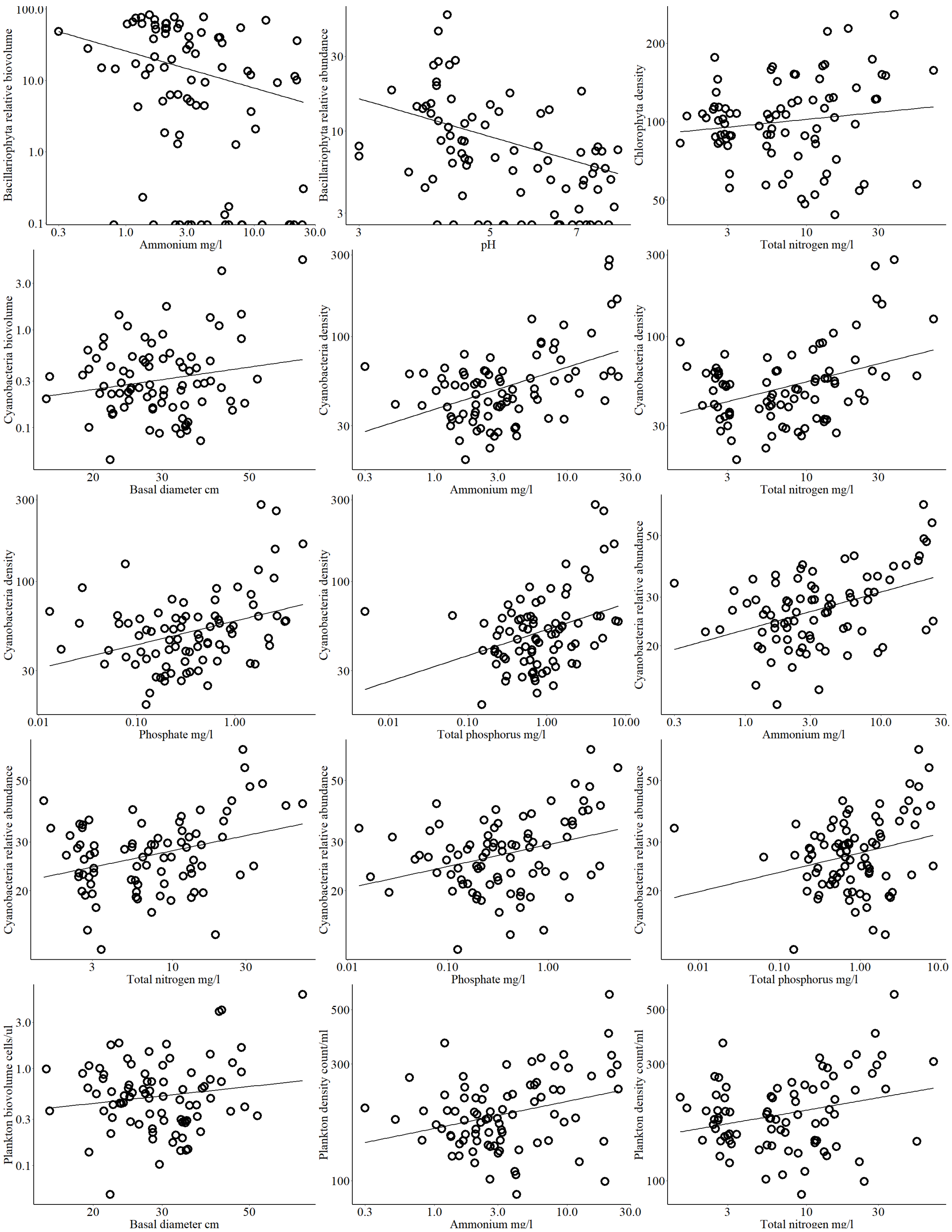

Figure 3. Significant relationships between plankton response variables and plant scale independent variables.

Table 3. Standardized slope coefficients of plant scale independent variables from mixed effects models. Only coefficients with p-values < 0.05 after Benjamini-Hochberg p-value adjustment were included. Fluid chemical parameters are in mg/l and basal diameter is in mm. Plankton densities are in # of organisms per ml and biovolumes are in ul. Relative refers to the plankton taxa group proportion to the whole plankton community.

	Plant scale independent variables (fixed effects)					
Plankton response variables	**Ammonium**	**Total nitrogen**	**Phosphate**	**Total phosphorus**	**Basal diameter**	**pH**
Bacillariophyta relative abundance						-0.2
Bacillariophyta relative biovolume	-0.48					
Chlorophyta density		0.32				
Cyanobacteria biovolume					0.37	
Cyanobacteria density	0.38	0.44	0.39	0.31		
Cyanobacteria relative abundance	0.37	0.32	0.43	0.3		
Plankton biovolume					0.38	
Plankton density	0.27	0.36				

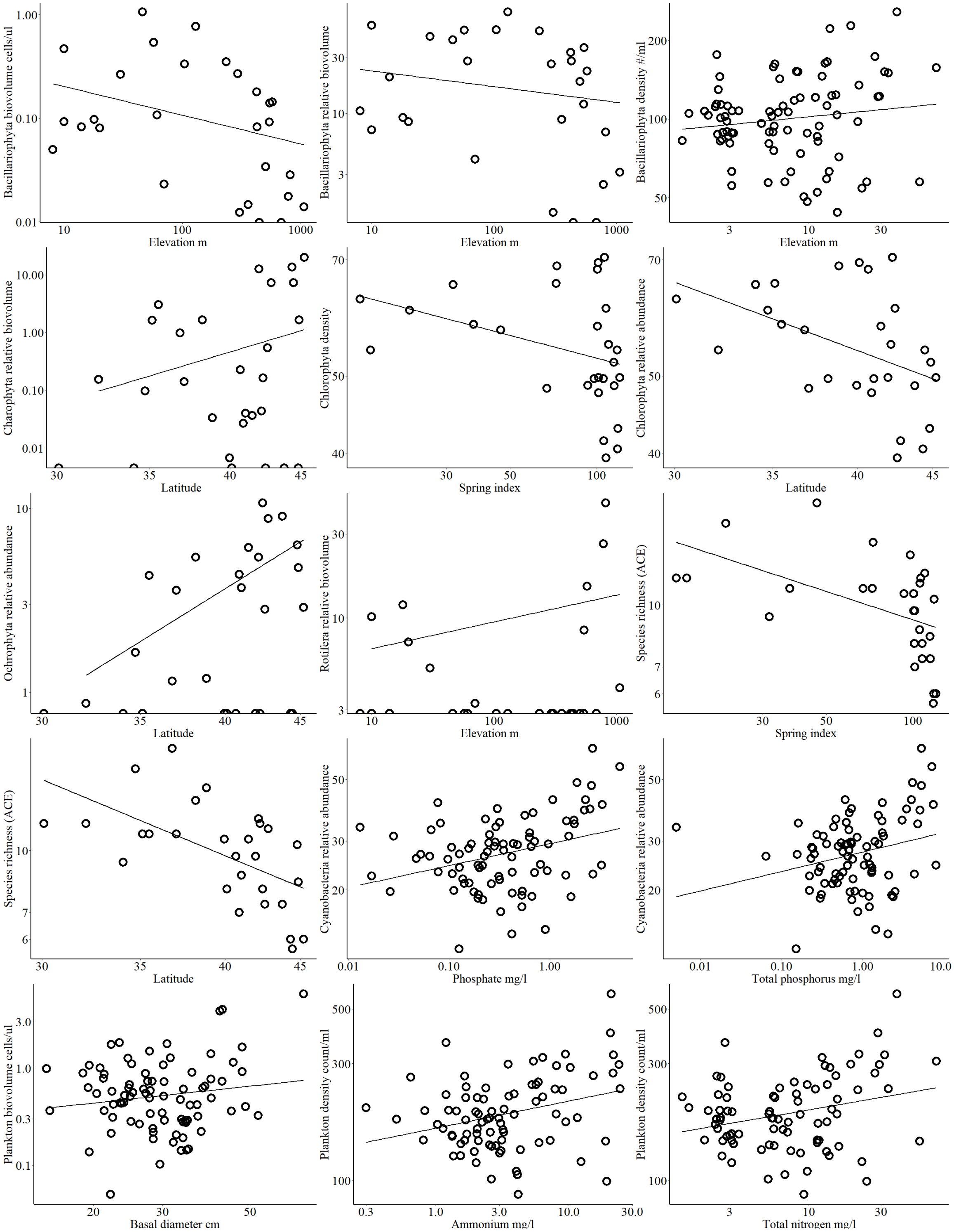

Figure 4. Significant relationships between plankton response variables and site scale independent variables.

Table 4. Standardized slope coefficients of site scale independent variables from generalized linear models. Only coefficients with p-values < 0.05 were included (* p<0.05, ** p<0.01). Plankton densities are in # of organisms per ml and biovolumes are in ul. Relative refers to the plankton taxa group proportion to the whole plankton community.

	Site scale independent variables		
Plankton response variables	**Elevation (m)**	**Spring index**	**Latitude**
Bacillariophyta biovolume	-0.35*		
Bacillariophyta relative biovolume	-0.35*		
Bacillariophyta density	-0.41*		
Charophyta relative biovolume			0.38*
Chlorophyta relative abundance		-0.35*	-0.47*
Ochrophyta relative abundance			0.42*
Rotifera relative biovolume	0.33*		
Estimate species richness (ACE)		-0.61**	-0.57**

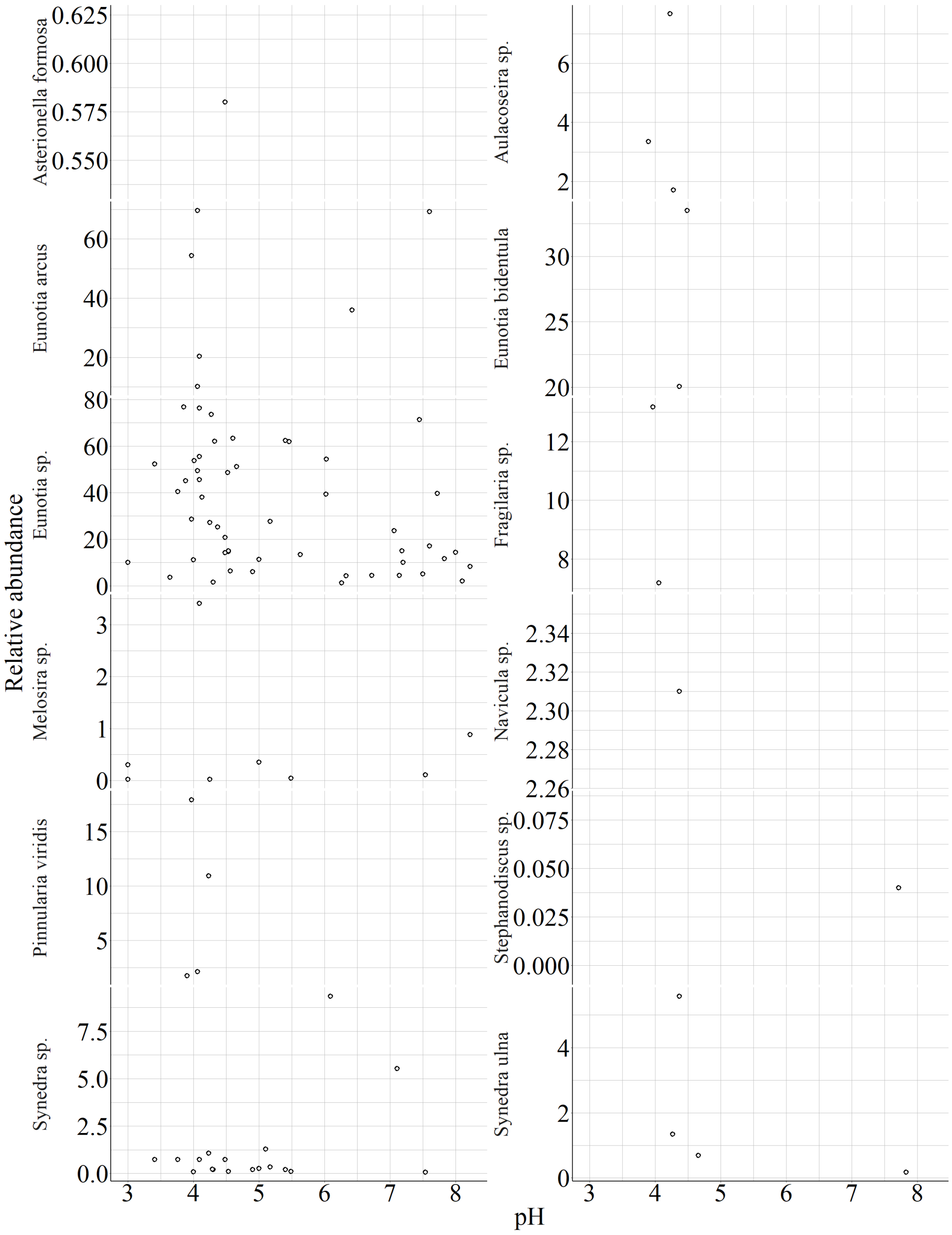

Figure 5. Optimal pitcher fluid pH by Bacillariophyta (diatom) taxa.

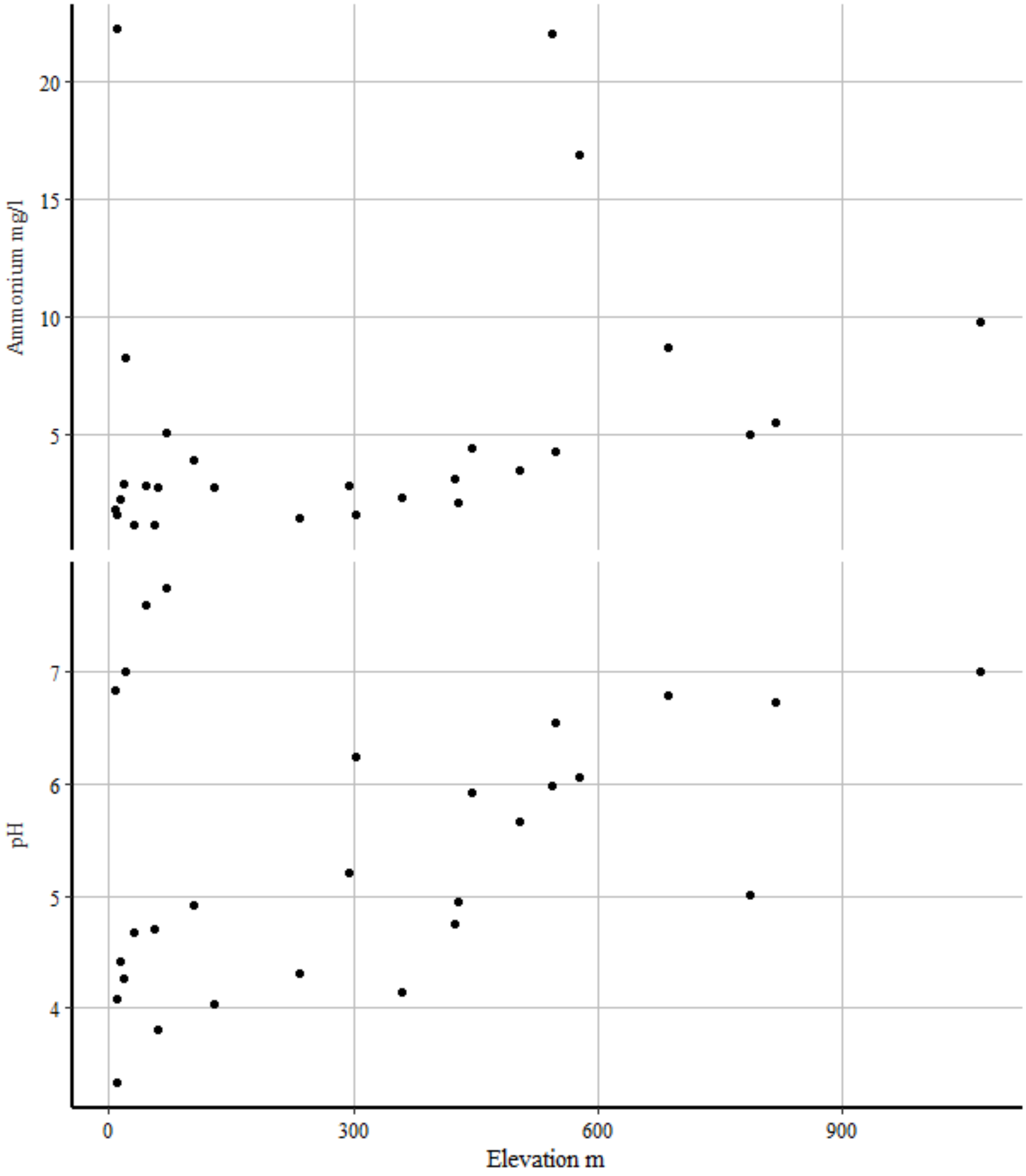

Figure 6. Significant co-correlation between elevation and pH and ammonium.

LITERATURE CITED

Addicott J.F. (1974) Predation and Prey Community Structure: An Experimental Study of the Effect of Mosquito Larvae on the Protozoan Communities of Pitcher Plants. *Ecology* **55**(3):475–492.

Adlassnig, W., Mayer, E., Peroutka, M., Pois, W., and Lichtscheidl, I. K. (2010). Two American Sarracenia species as neophyta in central Europe. *Phyton* **49**(2): 279–292.

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* **67**(1): 1–48.

Barton, K. (2020). MuMIn: Multi-Model Inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Butler J. L., Gotelli N. J., and Ellison A. M. (2008) Linking the Brown and Green: Nutrient Transformation and Fate in the Sarracenia Microecosystem. *Ecology* **89**(4):898–904.

Chapin, C. T. and Pastor, J. (1995) Nutrient limitations in the northern pitcher plant Sarracenia purpurea. Canadian Journal of *Botany* **73**(5): 728–734.

Chiu, C. H., Wang, Y. T., Walther, B. A. and Chao, A. (2014). Improved nonparametric lower bound of species richness via a modified Good-Turing frequency formula. Biometrics **70**: 671–682.

Cremona, F., Tuvikene, L., Haberman, J., Noges, P., and Noges, T. (2018). Factors controlling the three-decade long rise in cyanobacteria biomass in a eutrophic shallow lake. *Science of The Total Environment* **621**: 352-359.

DeNicola, D. M. (2000). A review of diatoms found in highly acidic environments. *Hydrobiologia*. **433**: 111-122.

Donald, D. B., Bogard, M. J., Finlay, K., Bunting, L., and Leavitt, P. R. (2013). Phytoplankton-specific response to enrichment of phosphorus-rich surface waters with ammonium, nitrate, and urea. *PloS one* **8**(1): e53277.

Dudley, J. L. (1984) Algal communities in the leaf liquid of the pitcher plant, Sarracenia purpurea L. *In Proceedings of the Pennsylvania Academy of Science*: 213–215. Pennsylvania Academy of Science.

Eberlein, T., Van de Waal, D. B., Brandenburg, K. M., John, U., Voss, M., Achterberg, E. P., and Rost, B. (2016). Interactive effects of ocean acidification and nitrogen limitation on two bloom-forming dinoflagellate species. *Marine ecology progress series 543*, 127-140.

Ellison, A. M. and Gotelli, N. J. (2001) Evolutionary ecology of carnivorous plants. *Trends in Ecology and Evolution* **16**: 623–629.

Ellison A. M. and Gotelli N. J. (2002) Nitrogen availability alters the expression of carnivory in the northern pitcher plant, Sarracenia purpurea. *PNAS* **99**(7): 4409–4412.

Ellison A. M., Buckley H. L., Miller T. E., and Gotelli N. J. (2004) Morphological variation in Sarracenia purpurea (Sarraceniaceae): geographic, environmental, and taxonomic correlates. *American Journal of Botany* **91**(11):1930–1935.

Gebühr, C., Pohlon, E., Schmidt, A. R., and Küsel, K. (2006). Development of microalgae communities in the phytotelmata of allochthonous populations of Sarracenia purpurea (Sarraceniaceae). *Plant Biology* **8**(6): 849–860.

Graham, J. M., Arancibia-Avila, P., and Graham, L. E. (1996). Effects of pH and selected metals on growth of the filamentous green alga Mougeotia under acidic conditions. *Limnology and Oceanography* **41**(2): 263-270.

Gremmen, N. J., Van De Vijver, B., Frenot, Y., and Lebouvier, M. (2007). Distribution of moss-inhabiting diatoms along an altitudinal gradient at sub-Antarctic Îles Kerguelen. Antarctic *Science* **19**(1): 17-24.

Heard, S. B. (1994) Pitcher-plant midges and mosquitoes: a processing chain commensalism. *Ecology* **75**(6): 1647-1660.

Heikkinen, J. M., Aalto, J., Rantamäki, O., Ruikkala, T., Soininen, J., and Pajunen, V. (2021). Observing diatom diversity and community composition along environmental gradients in subarctic mountain ponds. *Freshwater Biology*.

Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U., and Zohary, T. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of phycology* **35**(2): 403–424.

Hindák Frantisek Bratislava. (2008) Color atlas of cyanophytes VEDA, *Publishing House of the Slovak Academy of Sciences*.

Jankowiak, J., Hattenrath-Lehmann, T., Kramer, B. J., Ladds, M., and Gobler, C. J. (2019). Deciphering the effects of nitrogen, phosphorus, and temperature on cyanobacterial bloom intensification, diversity, and toxicity in western Lake Erie. *Limnology and Oceanography* **64**(3): 1347-1370.

Jalinsky, J.R., Wertenberger, R.A., Radocy, T.A., and Chaboo, C.S. (2014) Insects inhabiting two host plants, Heliconia stricta Huber (Heliconiaceae) and Calathea lutea Schult (Marantaceae), in southeastern Peru. *Journal of the Kansas Entomological Society* **87**(3): 299–311.

J**uniper B.E., Robins, R.J., and Joel, D.M.** (1989) The Carnivorous Plants. Academic. New York.

Kim, H., Jo, B. Y., and Kim, H. S. (2017). Effect of different concentrations and ratios of ammonium, nitrate, and phosphate on growth of the blue-green alga (cyanobacterium) Microcystis aeruginosa isolated from the Nakdong River, Korea. *Algae* **32**(4): 275-284.

Komárek, J. (2008) Cyanoprokaryota: Teil 1/Part 1: Chroococcales. *Spektrum Akademischer Verlag*

Komárek, J., and Anagnostidis, K. (2007). Süßwasserflora von Mitteleuropa, Bd. 19/2: Cyanoprokaryota: Bd. 2/Part 2: Oscillatoriales. *Elsveire GmbH*

Komárek, J. (2013) Süßwasserflora von Mitteleuropa, Bd. 19/3: Cyanoprokaryota. 3. Teil/3rd part: Heterocytous Genera. *Süßwasserflora von Mitteleuropa. Spektrum Academischer Verlag, Heidelberg.*

Krammer, K. and Lange-Bertalot H. (1991). Bacillariophyceae, 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. – In: Ettl H., Gerloff J., Heynig H. and Mollenhauer D. (eds.): Süßwasserflora von Mitteleuropa, Bd. 2(3): 1-576. Gustav Fisher Verlag, Stuttgart, Jena.

Logue, J. B., Langenheder, S., Andersson, A. F., Bertilsson, S., Drakare, S., Lanzén, A., and Lindström, E. S. (2012). Freshwater bacterioplankton richness in oligotrophic lakes depends on nutrient availability rather than on species–area relationships. *The ISME journal* **6**(6): 1127-1136.

Maguire Jr, B. (1971). Phytotelmata: biota and community structure determination in plant-held waters. *Annual review of ecology and systematics* **2**(1): 439–464.

May, L., Bailey-Watts, A. E., and Kirika, A. (2001). The relationship between Trichocerca pusilla (Jennings), Aulacoseira spp. and water temperature in Loch Leven, Scotland, UK. *In Rotifera IX* (29-34). Springer, Dordrecht.

Moore, E. R., Bullington, B. S., Weisberg, A. J., Jiang, Y., Chang, J., and Halsey, K. H. (2017). Morphological and transcriptomic evidence for ammonium induction of sexual reproduction in Thalassiosira pseudonana and other centric diatoms. *PLoS One* **12**(7): e0181098.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., and Wagner, H. (2013). Community ecology package. Package vegan. *R package version 2*(0).

Ochoa de Alda, J. A., Tapia, M. I., Franck, F., Llama, M. J., and Serra, J. L. (1996). Changes in nitrogen source modify distribution of excitation energy in the cyanobacterium Phormidium laminosum. *Physiologia Plantarum* **97**(1): 69–78.

Olenina, I. (2006). Biovolumes and size-classes of phytoplankton in the Baltic Sea.

Patrick, R., and Reimer, C. W. (1966) The diatoms of the United States. *Academy of Natural Sciences*.

Pianka, E. R. (1966). Latitudinal gradients in species diversity: a review of concepts. *The American Naturalist* **100**(910), 33-46.

Pinto-Coelho, R. M., Bezerra-Neto, J. F., Miranda, F., Mota, T. G., Resck, R., Santos, A. M., Maia-Barbosa, P. M., Mello, N., Marques, M. M., Campos, M. O., and Barbosa, F. A. R. (2008). The inverted trophic cascade in tropical plankton communities: impacts of exotic fish in the Middle Rio Doce Lake district, Minas Gerais, Brazil. *Brazilian Journal of Biology* **68**(4): 1025-1037.

Prescott, G. W., Croasdale, H., and Vinyard, W. C. (1972). *A synopsis of North American desmids*. University of Nebraska press.

Pollingher, U. (1990). Effects of latitude on phytoplankton composition and abundance in large lakes. *In Large Lakes* (pp. 368-402). Springer, Berlin, Heidelberg.

Ramos, G. J. P. and do Nascimento Moura, C. W. (2019) Algae and cyanobacteria in phytotelmata: diversity, ecological aspects, and conservation. *Biodiversity and Conservation* **28**(7):1667–1697.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Salmaso, N., Buzzi, F., Garibaldi, L., Morabito, G., and Simona, M. (2012). Effects of nutrient availability and temperature on phytoplankton development: a case study from large lakes south of the Alps. *Aquatic Sciences* **74**(3): 555-570.

Schemske, D. W., and Mittelbach, G. G. (2017). Latitudinal gradients in species diversity: reflections on Pianka's 1966 article and a look forward. *The American Naturalist* **189**(6): 599-603.

Schlichting Jr, H. E., and Sides, S. L. (1969). The passive transport of aquatic microorganisms by selected Hemiptera. *The Journal of Ecology* 759-764.

Seale, D. B., Boraas, M. E., and Warren, G. J. (1987). Effects of sodium and phosphate on growth of cyanobacteria. *Water Research* **21**(6): 625-631.

Smith, G. M. (1950) Fresh-water algae of the United States.

Soininen, J., and Meier, S. (2014). Phytoplankton richness is related to nutrient availability, not to pool size, in a subarctic rock pool system. *Hydrobiologia* **740**(1): 137-145.

Stomp, M., Huisman, J., Mittelbach, G. G., Litchman, E., and Klausmeier, C. A. (2011). Large-scale biodiversity patterns in freshwater phytoplankton. *Ecology* **92**(11): 2096-2107.

Trimbee, A. M., and Prepas, E. E. (1987). Evaluation of total phosphorus as a predictor of the relative biomass of blue-green algae with emphasis on Alberta lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **44**(7): 1337-1342.

USA National Phenology Network. 2019. Spring Indices, Current Year - First Leaf - Spring Index Date. 2020-01-01.

Walker. (2015) Risk assessment summary sheet Purple Pitcher plant (Sarracenia purpurea). GB non-native species secretariat.

Weckström, J., and Korhola, A. (2001). Patterns in the distribution, composition and diversity of diatom assemblages in relation to ecoclimatic factors in Arctic Lapland. *Journal of Biogeography* **28**(1): 31-45.

Wehr, J. D., Sheath, R. G., and Kociolek, J. P. (2015) *Freshwater algae of North America: ecology and classification*. Elsevier

Wetzel, R.G. (2001) Fundamental processes within natural and constructed wetland ecosystems: short-term versus long-term objectives. Water Science and Technology **44**(11-12): 1–8.

Weyhenmeyer, G. A., Peter, H., and Willén, E. V. A. (2013). Shifts in phytoplankton species richness and biomass along a latitudinal gradient–consequences for relationships between biodiversity and ecosystem functioning. *Freshwater Biology* **58**(3): 612-623.

Yamamoto, Y., and Nakahara, H. (2005). The formation and degradation of cyanobacterium Aphanizomenon flos-aquae blooms: the importance of pH, water temperature, and day length. *Limnology* 6(1): 1-6.

Yvon-Durocher, G., Allen, A. P., Cellamare, M., Dossena, M., Gaston, K. J., Leitao, M., Montoya, J. M., Reuman, D. C., Woodward, G., and Trimmer, M. (2015). Five years of experimental warming increases the biodiversity and productivity of phytoplankton. *PLoS biology* **13**(12): e1002324.

Zhang, Y., Wu, H., Yuan, C., Li, T., and Li, A. (2019). Growth, biochemical composition, and photosynthetic performance of Scenedesmus acuminatus during nitrogen starvation and resupply. *Journal of Applied Phycology* **31**(5): 2797-2809.

Zohary, T., and Ostrovsky, I. (2011). Ecological impacts of excessive water level fluctuations in stratified freshwater lakes. *Inland waters* **1**(1): 47-59.

CHAPTER IV: THE OBLIGATE INVERTEBRATE *METRIOCNEMUS KNABI* OF *SARRACENIA PURPUREA* IS CAPABLE OF EMIGRATING FROM A HISTORICALLY CLOSED MICROCOSM

ABSTRACT

The cup-shaped leaves of the northern pitcher plant *Sarracenia purpurea* fill with rainwater and contain an entire aquatic ecosystem, with captured prey forming the detrital base, microbes, and the aquatic life history stages of several associated invertebrate species. An unusual specialist herbivore of *S. purpurea,* the caterpillar of the pitcher-mining moth *Exyra fax*, drills a hole in the leaf by consuming plant tissue. The water in the leaf drains out, and the pitcher is no longer a suitable habitat for the obligate aquatic invertebrates. The obligate aquatic invertebrate larvae within *S. purpureas* pitchers have always been described as incapable of leaving the pitcher they were initially deposited in as eggs. The objective of this experiment was to determine if one of the obligate invertebrates, the pitcher plant midge *Metriocnemus knabi*, is capable of emigrating to new pitchers when faced with a dessicated pitcher after an attack by caterpillars of *E. fax*. In a greenhouse experiment, I placed midge larvae of varying abundances placed in experimental pitchers that were drained and desiccated as if they had been attacked by *E. fax* moth larvae. After desiccation, larval midges were allowed two weeks to emigrate. At the end of two weeks, experimental pitchers were dissected to determine if midges were present and if they survived desiccation. The remaining viable fluid filled pitchers were also dissected to determine if midge larvae were present. Of the initial abundance, 7.4% and 94.1% of *M. knabi* survived in place in desiccated and un-desiccated pitchers

respectively, 61.8% and 5.9% of *M. knabi* emigrated from desiccated and un-desiccated pitchers, and 38.2% and 0.00% of *M. knabi* respectively emigrated out of desiccated and un-desiccated pitcher and survived in a new pitcher. I present here the first evidence that the obligate invertebrate *M. knabi* is capable of emigration when faced with unsuitable habitat.

INTRODUCTION

Sarracenia purpurea L. a perennial carnivorous herb is unlike other carnivorous plants as it relies on obligate invertebrates to breakdown prey. One of these obligate invertebrates, midge larva *Metriocnemus knabi* (Coquillett), shreds and breaks down prey as they enter the system. *M. knabi* and other obligate inhabitants of *S. purpurea* rely on the pitchers to hold fluid, as desiccated pitchers are unsuitable habitat for these aquatic larvae.

A small number of herbivores are obligate consumers of carnivorous pitcher plants (Rymal and Folkerts 1982). Atwater et al. (2006) noted for *S. purpurea,* two noctuid moths, *Exyra fax* (Grote) and *Papaipema appassionata* (Harvey). The more common *E. fax* larvae consumes the interior plant tissue and in preparation for pupation chews a drainage whole at the base of the pitcher, causing fluid to drain from the pitchers (Rymal and Folkerts 1982, Fish 1976). *E. fax* in addition to desiccation can also close the pitcher mouth opening by creating a silken web or girdling the leaf, enclosing the pitcher opening, not allowing rainwater to accumulate creating unsuitable habitat for *S. purpureas* aquatic inhabitants (Atwater et al. 2006). Moth herbivore damage to *S. purpurea* is common across the eastern United States (personal observation).

There is little evidence *M. knabi* are capable of emigrating out of unsuitable pitchers to viable fluid filled pitchers while in its aquatic larval stage. To our knowledge only Paterson and Cameron (1982) make a singular note of the possibility after observing late instar stages of *M. knabi* in newly opened pitchers. In addition, Wiens (1972) observed that *M. knabi* larvae is incapable of swimming but can crawl, as they commonly observed *M. knabi* crawling up the interior of the pitcher wall.

Although it is known that *M. knabi* are capable of crawling on the interior pitcher wall, it is unknown if the *M. knabi* larvae can successfully emigrate out of its pitcher of origin to other surrounding pitchers. The objective of our experiment was to measure the success of *M. knabi* emigrating to viable fluid filled pitchers, when presented with mimicked *E. fax* moth herbivory causing desiccation of its origin pitcher. We hypothesized desiccated pitchers would induce emigration of *M. knabi* larvae. We also hypothesize that initial abundance of *M. knabi* larvae could impact success of emigration and survival in new pitchers. We predict that with increased abundance of *M. knabi* larvae in pitchers that emigration rate will increase due to competition for resources and habitat. This work will provide insight on the commonly used model system *S. purpurea* and whether this system is truly closed in terms of its larval aquatic invertebrates.

MATERIALS & METHODS

To measure the success of *M. knabi* emigrating out of desiccated pitchers of *S. purpurea* to viable fluid filled pitchers, we conducted a greenhouse experiment in the summer of 2021 using fifteen *S. purpurea* plants. All plants were cleaned and filled with purified reverse osmosis water and randomly placed outside in individual plastic pots. Pots were separated by ~13 cm within water holding trays inside insect tents. For each plant, we designated the largest pitcher as the experimental pitcher to be desiccated. The number of viable fluid filled pitchers per plant varied between 1 and 12.

M. knabi larvae were collected from Molly Bog, Morristown, Vermont. Five plants received high abundance (ten individuals) of *M. knabi* larvae in the experimental pitcher, five plants received medium abundance (five individuals) of *M. knabi* larvae, and five plants received low abundance (two individuals). Data from unpublished work on thirty-eight *S. purpurea* plants showed a median abundance of five *M. knabi* larvae, a minimum of one, and a maximum of fifty-three. After *M. knabi* larvae were placed in experimental pitchers we deposited aquarium fish flakes on day one as a food source. *M. knabi* larvae were then allowed to acclimate to pitchers for four days.

After four days, four of the five experimental pitchers of high, medium, and low abundance were desiccated, while one of each abundance was left fluid filled, serving as controls. To mimic desiccation caused by *E. fax*, we used a sterilized 18-gauge needle to punch a hole in the back of the leaf near the base of the plant. This location is described as zone 5 of the plant according to Lloyd (1954). Plants were then left for 2 weeks to desiccate and were exposed to ambient climatic conditions. After 2 weeks, experimental pitchers were clipped at the base of the plant and dissected. The number of midges present, and their status (living or dead) were determined. All other pitchers in the plant were then removed of fluid and dissected to determine presence of larvae and status of larvae.

Using the statistical software R (R Core Team 2019), we used two proportion χ^2-tests to test if there is a difference between 1) the proportion of initial abundance of larvae that survived in the experimental desiccated pitchers and the initial abundance of larvae that survived in the control pitchers, 2) the proportion of the initial abundance of larvae that emigrated from the experimental desiccated pitchers and the proportion of the initial abundance of larvae that emigrated from the control pitchers, 3) the proportion of initial abundance that survived in a new viable fluid filled pitcher emigrating from experimental desiccated pitchers and emigrating from control pitchers, and 4) pair-wise comparisons of the proportion of initial abundance surviving in new pitchers among the three levels of initial abundance.

RESULTS

After two weeks, 7.4% of the initial larvae survived in the experimental desiccated pitchers compared to 94.1% of those in the control pitchers, showing a significant difference between initial abundance of larvae surviving in experimental desiccated pitchers and control pitchers ($\chi 2=50.473$, $p<.001$). A significantly higher proportion of larvae (61.8%) emigrated from experimental desiccated pitchers compared to control pitchers (5.9%) ($\chi 2=14.829$, $p<.001$). After emigrating from experimental desiccated pitchers, 38.2% of larvae survived compared to 0.00% survival from control pitchers ($\chi 2=7.650$, $p<.01$) (Fig. 1).

The proportion of larvae surviving in new fluid filled pitchers was significantly greater coming from experimental pitchers with high (10) compared to medium (5) initial abundances ($\chi 2=12.574$, $p<.01$) and significantly greater in high (10) compared to low (2) abundance treatments ($\chi 2=7.747$, $p<.01$) (Fig. 2).

DISCUSSION

The objective of this experiment was to determine if *M. knabi* aquatic larvae are capable of emigrating from their pitcher of origin. Our results demonstrate that *M. knabi* larvae can emigrate out of desiccated pitchers and is capable of survival in new viable fluid filled pitchers. We also provide evidence *M. knabi* larvae may promote conspecific survival during emigration. However, 14 of the 85 original larvae were unaccounted for at the end of the experiment and should be considered when interpreting the proportional results.

The unaccounted-for larvae were most likely present in the sphagnum moss media that each plant was potted in, or a more unlikely scenario could be that midges were unidentifiable due to decomposition by protists and bacteria. The latter is less probable as numerous dead midges were found and not decomposed. *M. knabi* larvae could have also pupated, enclosed, and become an adult during the experiment if we initially gathered late-stage larvae. However, the experiment was surrounded by insect tents and no adult midges were found. Considering only one midge was unaccounted for in the control group, we deem it negligible. Future experiments should limit the *M. knabi* stage to only instars that would require more time than the experimental period to pupate and eclose.

We assume that no long-distance dispersal of *M. knabi* larvae occurred between plants. The larvae, which are at best poor swimmers, would have to leave experimental desiccated pitchers, traverse though the sphagnum, down the plastic pot, swim through the tray of water, and crawl back up to another plastic pot to reach a new plant (Wiens 1972). If long-distance dispersal was occurring in this environment, we would expect to see a net gain at least once in one of the plants, which we did not. We did find 3 live larvae within the tray water when it was filtered at the end of the experiment. Perhaps in a natural environment such as a sphagnum bog, long-distance dispersal is plausible.

Future work should compare the morphology of midge larvae that were able to emigrate in comparison to larvae that were not, as we believe larger posterior proleg hooks allow the midge to successfully crawl the pitcher walls. The ability of *M. knabi* larvae to promote conspecific survival during emigration should also be a continued investigation. It would be noteworthy to determine path of emigration for *M. knabi* larvae, as this could occur through the pitcher opening or through the drainage hole caused by *E. fax* moth larvae. *M. knabi* larvae are photosensitive so they may show preference through leaving via the drainage hole and not the pitcher opening. This work is the first proof that *M. knabi* is capable of successfully emigrating from an unsuitable desiccated pitcher to a new viable fluid filled pitcher and proves that this once deemed closed system in terms of its obligate invertebrates is no longer the case.

ACKNOWLEDGEMENTS

I would like to acknowledge Sophie Linde an undergraduate researcher on this experiment. We would like to thank the University of Vermont for funding and use of the Molly Bog Natural Area for larval midge collections.

TABLES & FIGURES

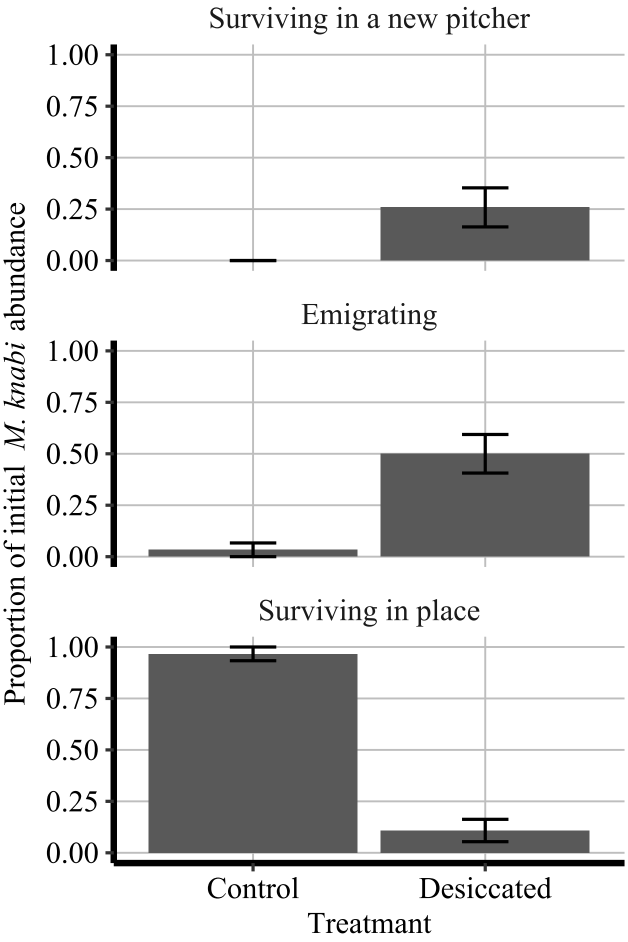

Figure 1. The difference of effect of control (fluid filled) and desiccated pitchers on the proportion of the initial *M. knabi* abundance surviving in place (bottom), emigrating (middle), and surviving in a new fluid filled pitcher (top). Error bars represent standard error.

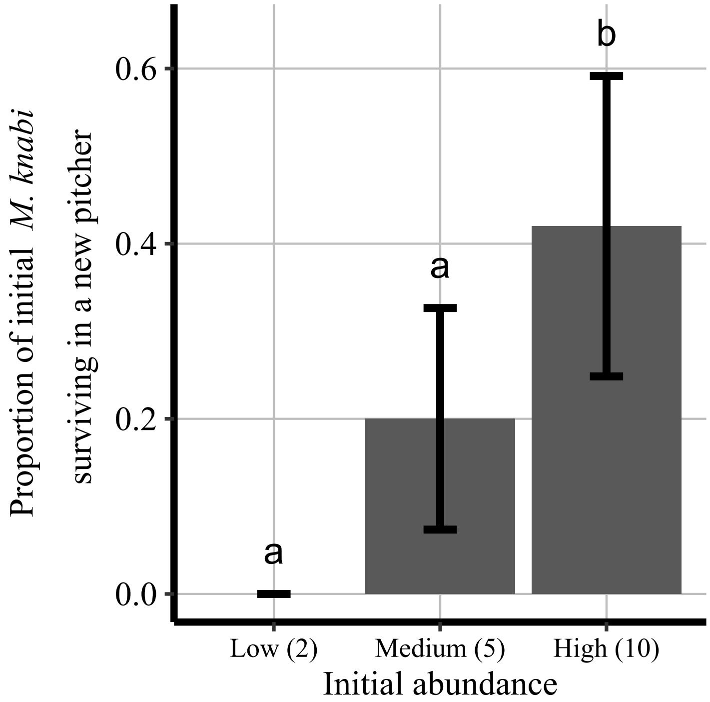

Figure 2. The proportion of initial *M. knabi* abundance surviving in a new pitcher among three different initial abundances, low (2 individuals), medium (5 individuals), and high (10 individuals). Repeat letters above bars show no significant difference ($p<.05$), error bars represent standard error.

LITERATURE CITED

Atwater, D. Z., Butler, J. L., and Ellison, A. M. (2006). Spatial distribution and impacts of moth herbivory on northern pitcher plants. Northeastern Naturalist. **13**(1): 43-56.

Fish, D. (1976). Insect-plant relationships of the insectivorous pitcher plant Sarracenia minor. Florida Entomologist: 199-203.

Lloyd, F. E. (1954). *The carnivorous plants*. Read Books Ltd.

Paterson, C. G. and Cameron, C. J. (1982). Seasonal dynamics and ecological strategies of the pitcher plant chironomid, Metriocnemus knabi Coq.(Diptera: Chironomidae), in southeast New Brunswick. Canadian Journal of Zoology. **60**:(12): 3075-3083.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rymal, D. E. and Folkerts, G. W. (1982). Insects associated with pitcher plants (Sarracenia: Sarraceniaceae), and their relationship to pitcher plant conservation: a review. Journal of the Alabama Academy of Science.

Wiens, A. P. (1972). Bionomics of the pitcher plant midge Metriocnemus knabi (Coquillett) (Diptera: Chironomidae).

COMPREHENSIVE BIBLIOGRAPHY

Aber, J. D., Goodale, C. L., Ollinger, S. V., Smith, M. L., Magill, A. H., Martin, M. E., Hallet, R. A., and Stoddard, J. L. (2003). Is nitrogen deposition altering the nitrogen status of northeastern forests? BioScience, **53**(4):375-389.

Addicott J. F. (1974). Predation and Prey Community Structure: An Experimental Study of the Effect of Mosquito Larvae on the Protozoan Communities of Pitcher Plants. Ecology. **55**(3):475–492.

Adlassnig, W., Mayer, E., Peroutka, M., Pois, W., and Lichtscheidl, I. K. (2010). Two American Sarracenia species as neophyta in central Europe. *Phyton* **49**(2): 279–292.

Anderson B. and Midgley J. J. (2003). Digestive mutualism, an alternate pathway in plant carnivory. Oikos. **102**(1):221–224.

Atwater, D. Z., Butler, J. L., and Ellison, A. M. (2006). Spatial distribution and impacts of moth herbivory on northern pitcher plants. Northeastern Naturalist. **13**(1): 43-56.

Barton, K. (2020). MuMIn: Multi-Model Inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* **67**(1): 1–48.

Błedzki L. A. and Ellison A. M. (1998). Population growth and production of Habrotrocha rosa Donner (Rotifera: Bdelloidea) and its contribution to the nutrient supply of its host, the northern pitcher plant, Sarracenia purpurea L. (Sarraceniaceae). Hydrobiologia. **385**(1):193–200.

Brahney, J., Mahowald, N., Ward, D. S., Ballantyne, A. P., and Neff, J. C. (2015). Is atmospheric phosphorus pollution altering global alpine Lake stoichiometry?. Global Biogeochemical Cycles, **29**(9):1369-1383.

Butler, J. L. and Ellison, A. M. (2007). Nitrogen cycling dynamics in the carnivorous norther pitcher plant, *Sarracenia purpurea* Functional Ecology **21**:835-843.

Butler J. L., Gotelli N. J., and Ellison A. M. (2008). Linking the Brown and Green: Nutrient Transformation and Fate in the Sarracenia Microecosystem. Ecology. **89**(4):898–904.

Carpenter, S. R., and Adams, M. S. (1977). The macrophyte tissue nutrient pool of a hardwater eutrophic lake: implications for macrophyte harvesting. Aquatic Botany, 3:239-255.

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., and Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559–568.

Carr, G. M. 1998. Macrophyte growth and sediment phosphorus and nitrogen in a Canadian prairie river. Freshwater Biology, **39**(3):525-536.

Chapin, C.T., and J. Pastor. (1995). Nutrient limitation in the northern pitcher plant Sarracenia purpurea. Canadian Journal of Botany **73**:728-734.

Chiu, C. H., Wang, Y. T., Walther, B. A. and Chao, A. (2014). Improved nonparametric lower bound of species richness via a modified Good-Turing frequency formula. Biometrics **70**: 671–682.

Clark, G. M., Mueller, D. K., and Mast, M. A. (2000). Nutrient concentrations and yields in undeveloped stream basins in the United States. A Journal of the American Water Resources Association, **36**(4):849-860.

Cochran-Stafira D. L. and Ende C. N. von. (1998). Integrating Bacteria into Food Webs: Studies with Sarracenia Purpurea Inquilines. Ecology. **79**(3):880–898.

Cremona, F., Tuvikene, L., Haberman, J., Noges, P., and Noges, T. (2018). Factors controlling the three-decade long rise in cyanobacteria biomass in a eutrophic shallow lake. *Science of The Total Environment* **621**: 352-359.

Demars, B. O., and Edwards, A. C. (2007). Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. Freshwater Biology, **52**(11):2073-2086.

De Frenne, P., Graae, B. J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., De Kort, H., De Schrijver, A., Diekmann, M., Eriksson, O., Gruwex, R., Hermy, M., Lenoir, J., Plue, J., Coomes, D. A., and Verheyen, K. (2013). Latitudinal gradients as natural laboratories to infer species' responses to temperature. Journal of Ecology, **101**(3):784-795.

DeNicola, D. M. (2000). A review of diatoms found in highly acidic environments. *Hydrobiologia*. **433**: 111-122.

Di Nino, F., Thiébaut, G., and Muller, S. (2007). Phenology and phenotypic variation of genetically uniform populations of Elodea nuttallii (Planch.) H. St John at sites of different trophic states. Fundamental and applied limnology, **168**(4):335.

Donald, D. B., Bogard, M. J., Finlay, K., Bunting, L., and Leavitt, P. R. (2013). Phytoplankton-specific response to enrichment of phosphorus-rich surface waters with ammonium, nitrate, and urea. *PloS one* **8**(1) e53277.

Dudley, J. L. (1984) Algal communities in the leaf liquid of the pitcher plant, Sarracenia purpurea L. *In Proceedings of the Pennsylvania Academy of Science*: 213–215. Pennsylvania Academy of Science.

Ellison, A. M., H. L. Buckley, T.E. Miller, and N.J. Gotelli. (2004). Morphological variation in Sarracenia purpurea (Sarraceniaceae): geographic, environmental, and taxonomic correlates. American Journal of Botany **91**:1930-1935.

Elser J. J., Andersen T., Baron J.S., Bergstrom A.K., Jansson M., Kyle M., Nydick K. R., Steger L., and Hessen D. O. (2009). Shifts in Lake N:P Stoichiometry and Nutrient Limitation Driven by Atmospheric Nitrogen Deposition. Science. **326**(5954):835–837.

Ellison, A.M., and N.J. Gotelli. (2001). Evolutionary ecology of carnivorous plants. Trends in Ecology and Evolution **16**:623-629.

Ellison, A. M. and Gotelli, N.J. (2002). Nitrogen availability alters the expression of carnivory in the northern pitcher plant Sarracenia purpurea. Proceedings of the National Academy of Sciences of the United States of America **99**: 4409–4412.

Ellison A. M., Buckley H. L., Miller T. E., and Gotelli N. J. (2004). Morphological variation in Sarracenia purpurea (Sarraceniaceae): geographic, environmental, and taxonomic correlates. American Journal of Botany. **91**(11):1930–1935.

Elser, J. J., Peace, A. L., Kyle, M., Wojewodzic, M., McCrackin, M. L., Andersen, T., and Hessen, D. O. (2010). Atmospheric nitrogen deposition is associated with elevated phosphorus limitation of lake zooplankton. Ecology Letters, 13(10):1256-1261.

Elser J. J., Andersen T., Baron J.S., Bergstrom A.K., Jansson M., Kyle M., Nydick K. R., Steger L., and Hessen D. O. (2009). Shifts in Lake N:P Stoichiometry and Nutrient Limitation Driven by Atmospheric Nitrogen Deposition. Science. **326**(5954):835–837.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hullebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., and Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters. **10**:1135–1142.

Elser J. J., Sterner R.W., Gorokhova E., Fagan W.F., Markow T.A., Cotner J.B., Harrison J.F., Hobbie S.E., Odell G.M., and Weider L.W. (2000). Biological stoichiometry from genes to ecosystems. Ecology Letters. **3**(6):540–550.

Fagan, W. F., Siemann, E., Mitter, C., Denno, R. F., Huberty, A. F., Woods, H. A., and Elser, J. J. (2002). Nitrogen in insects: implications for trophic complexity and species diversification. The American Naturalist, **160**(6), 784-802.

Fish, D. (1976). Insect-plant relationships of the insectivorous pitcher plant Sarracenia minor. Florida Entomologist: 199-203.

Folkerts, D. (1999). Pitcher plant wetlands of the southeastern United States, arthropod associates: 247-275. Invertebrates in freshwater wetlands of North America: ecology and management. John Wiley and Sons, New York, New York, USA.

Forsyth A. B. and Robertson R. J. (1975). K reproductive strategy and larval behavior of the pitcher plant sarcophagid fly, *Blaesoxipha fletcheri*. Canadian Journal of Zoology. **53**:174-179.

Fowler, D., Pyle, J. A., Raven, J. A., and Sutton, M. A. (2013). The global nitrogen cycle in the twenty-first century: introduction. Philosophical Transactions of the Royal Society B: Biological Sciences, **368**(1621):20130165.

Gallie, D.R., and S.C. Chang. (1997). Signal transduction in the carnivorous plant Sarracenia purpurea. Regulation of secre- tory hydrolase expression during development and in response to resources. Plant Physiology **115**:1461–1471.

Gebühr, C., Pohlon, E., Schmidt, A. R., and Küsel, K. (2006). Development of microalgae communities in the phytotelmata of allochthonous populations of Sarracenia purpurea (Sarraceniaceae). *Plant Biology* **8**(6): 849–860.

Godwin, C. M., & Cotner, J. B. (2015). Aquatic heterotrophic bacteria have highly flexible phosphorus content and biomass stoichiometry. The ISME Journal, **9**(10): 2324-2327.

Gonzalez Sagrario, M. A., Jeppesen, E., Gomà, J., Søndergaard, M., Jensen, J. P., Lauridsen, T., and Landkildehus, F. (2005). Does high nitrogen loading prevent clear-water conditions in shallow lakes at moderately high phosphorus concentrations? Freshwater Biology, **50**(1), 27-41.

Graham, J. M., Arancibia-Avila, P., and Graham, L. E. (1996). Effects of pH and selected metals on growth of the filamentous green alga Mougeotia under acidic conditions. *Limnology and Oceanography* **41**(2): 263-270.

Gremmen, N. J., Van De Vijver, B., Frenot, Y., and Lebouvier, M. (2007). Distribution of moss-inhabiting diatoms along an altitudinal gradient at sub-Antarctic Îles Kerguelen. Antarctic *Science* **19**(1): 17-24.

Grover J. D., Grover J. P., and Hudziak J. (1997). Resource Competition. Springer Science & Business Media.

Haddad N. M., Tilman D., Haarstad J., Ritchie M., and Knops J. M. (2001). Contrasting effects of plant richness and composition on insect communities: a field experiment. The American Naturalist. **158**(1):17–35.

Heard, S. B. (1994). Pitcher plant midges and mosquitoes: a processing chain commensalism. Ecology **75**:1647–1660.

Hecky, R. E. (1982). East African lakes and their trophic efficiencies. Trophic Dynamics of Aquatic Ecosystems. American Association for the Advancement of Science Symposium.

Heikkinen, J. M., Aalto, J., Rantamäki, O., Ruikkala, T., Soininen, J., and Pajunen, V. (2021). Observing diatom diversity and community composition along environmental gradients in subarctic mountain ponds. *Freshwater Biology*.

Hieber, M., and Gessner, M. O. (2002). Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology, **83**(4):1026-1038.

Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U., and Zohary, T. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of phycology* **35**(2): 403–424.

Hindák Frantisek Bratislava. (2008) Color atlas of cyanophytes VEDA, *Publishing House of the Slovak Academy of Sciences*.

Jankowiak, J., Hattenrath-Lehmann, T., Kramer, B. J., Ladds, M., and Gobler, C. J. (2019). Deciphering the effects of nitrogen, phosphorus, and temperature on cyanobacterial bloom intensification, diversity, and toxicity in western Lake Erie. *Limnology and Oceanography* **64**(3): 1347-1370.

Jalinsky, J.R., Wertenberger, R.A., Radocy, T.A., and Chaboo, C.S. (2014) Insects inhabiting two host plants, Heliconia stricta Huber (Heliconiaceae) and Calathea lutea Schult (Marantaceae), in southeastern Peru. *Journal of the Kansas Entomological Society* **87**(3): 299–311.

Jiang, X., Gao, G., Deng, J., Zhu, G., Tang, X., Shao, K., and Hu, Y. (2022). Nitrogen concentration response to the decline in atmospheric nitrogen deposition in a hypereutrophic lake. Environmental Pollution, **300**:118952.

Juniper, B.E., R.J. Robinis, and D.M. Joel. (1989). The carnivorous plants. Academic Press, New York, New York, USA.

Karez, R., Engelbert, S., Kraufvelin, P., Pedersen, M. F., and Sommer, U. (2004). Biomass response and changes in composition of ephemeral macroalgal assemblages along an experimental gradient of nutrient enrichment. Aquatic Botany, **78**(2):103-117.

Khoshmanesh, A., Hart, B. T., Duncan, A., and Beckett, R. (2002). Luxury uptake of phosphorus by sediment bacteria. Water Research, **36**(3):774-778.

Kim, H., Jo, B. Y., and Kim, H. S. (2017). Effect of different concentrations and ratios of ammonium, nitrate, and phosphate on growth of the blue-green alga (cyanobacterium) Microcystis aeruginosa isolated from the Nakdong River, Korea. *Algae* **32**(4): 275-284.

Kneitel J.M. and Miller T.E. (2002). Resource and Top-Predator Regulation in the Pitcher Plant (*Sarracenia purpurea*) Inquiline Community. Ecology. **83**(3):680–688.

Komárek, J. (2008) Cyanoprokaryota: Teil 1/Part 1: Chroococcales. *Spektrum Akademischer Verlag*

Komárek, J., and Anagnostidis, K. (2007). Süßwasserflora von Mitteleuropa, Bd. 19/2: Cyanoprokaryota: Bd. 2/Part 2: Oscillatoriales. *Elsveire GmbH*

Komárek, J. (2013) Süßwasserflora von Mitteleuropa, Bd. 19/3: Cyanoprokaryota. 3. Teil/3rd part: Heterocytous Genera. *Süßwasserflora von Mitteleuropa. Spektrum Academischer Verlag, Heidelberg.*

Krammer, K. and Lange-Bertalot H. (1991). Bacillariophyceae, 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. – In: Ettl H., Gerloff J., Heynig H. and Mollenhauer D. (eds.): Süßwasserflora von Mitteleuropa, Bd. 2(3): 1-576. Gustav Fisher Verlag, Stuttgart, Jena.

Kuba, T. M. C. M., Van Loosdrecht, M. C. M., Brandse, F. A., and Heijnen, J. J. (1997). Occurrence of denitrifying phosphorus removing bacteria in modified UCT-type wastewater treatment plants. Water Research, **31**(4):777-786.

Lamarque, J. F., Dentener, F., McConnell, J., Ro, C. U., Shaw, M., Vet, R., Bergmann, D., Cameron-Smith, P., Dalsorean, S., Doherty, R., Faluvegi, G., Ghan, S.J., Josse, B., Lee, Y.H., MacKenzie, I.A., Plummer, D., Shindell, D.T., Skeie, R.B., Stevenson, D.S. Strode, S., Zeng, G., Curran, M., Dahl-Jensen, D., Das, S., Fritzsche, D., and Nolan, M. (2013). Multi-model mean nitrogen and sulfur deposition from the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP): evaluation of historical and projected future changes. Atmospheric Chemistry and Physics, **13**(16):7997- 8018.

Logue, J. B., Langenheder, S., Andersson, A. F., Bertilsson, S., Drakare, S., Lanzén, A., and Lindström, E. S. (2012). Freshwater bacterioplankton richness in oligotrophic lakes depends on nutrient availability rather than on species–area relationships. *The ISME journal* **6**(6): 1127-1136.

Lloyd, F. E. (1954). *The carnivorous plants*. Read Books Ltd.

Maguire Jr, B. (1971). Phytotelmata: biota and community structure determination in plant-held waters. *Annual review of ecology and systematics* **2**(1): 439–464.

Matthaei, C. D., Piggott, J. J., & Townsend, C. R. (2010). Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction. Journal of Applied Ecology, **47**(3):639-649.

May, L., Bailey-Watts, A. E., and Kirika, A. (2001). The relationship between Trichocerca pusilla (Jennings), Aulacoseira spp. and water temperature in Loch Leven, Scotland, UK. *In Rotifera IX* (29-34). Springer, Dordrecht.

Meunier, C. L., Gundale, M. J., Sánchez, I. S., and Liess, A. (2016). Impact of nitrogen deposition on forest and lake food webs in nitrogen-limited environments. Global Change Biology, **22**(1):164-179.

Merrell, K. and Pierson, O. (2021) “Status of Vermont’s Inland Lakes: Phosphorus Trends and Protection.” Vermont Department of Environmental Conservation. Thursday, June 3, 2021. Online.

Miller T., Cassill D., Johnson C., Kindell C., Lepis J., Mcinnes D., Bevis T., Mehlman D., and Richard B. (1994). Intraspecific and Interspecific Competition of Wyeomyia smithii (Coq.) (Culicidae) in Pitcher Plant Communities. The American Midland Naturalist. **131**(1):136–145.

Moore, E. R., Bullington, B. S., Weisberg, A. J., Jiang, Y., Chang, J., and Halsey, K. H. (2017). Morphological and transcriptomic evidence for ammonium induction of sexual reproduction in Thalassiosira pseudonana and other centric diatoms. *PLoS One* **12**(7): e0181098.

Ndebele-Murisa, M. R., Musil, C. F., and Raitt, L. (2010). A review of phytoplankton dynamics in tropical African lakes. South African Journal of Science, **106**(1):13-18.

Newell S. J. and Nastase A. J. (1998. Efficiency of insect capture by Sarracenia purpurea (Sarraceniaceae), the northern pitcher plant. American Journal of Botany. **85**(1):88–91.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O’Hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., and Wagner, H. (2013). Community ecology package. Package vegan. *R package version 2*(0).

Ochoa de Alda, J. A., Tapia, M. I., Franck, F., Llama, M. J., and Serra, J. L. (1996). Changes in nitrogen source modify distribution of excitation energy in the cyanobacterium Phormidium laminosum. *Physiologia Plantarum* **97**(1): 69–78.

Olenina, I. (2006). Biovolumes and size-classes of phytoplankton in the Baltic Sea.

Oonincx, D. G. A. B., and Finke, M. D. (2021). Nutritional value of insects and ways to manipulate their composition. Journal of Insects as Food and Feed, **7**(5):639-659.

Paerl, H. W., Havens, K. E., Hall, N. S., Otten, T. G., Zhu, M., Xu, H., Zhu, G., and Qin, B. (2019). Mitigating a global expansion of toxic cyanobacterial blooms: confounding effects and challenges posed by climate change. Marine and Freshwater Research, **71**(5):579-592.

Paterson, C. G. and Cameron, C. J. (1982). Seasonal dynamics and ecological strategies of the pitcher plant chironomid, Metriocnemus knabi Coq.(Diptera: Chironomidae), in southeast New Brunswick. Canadian Journal of Zoology. **60**:(12): 3075-3083.

Patrick, R., and Reimer, C. W. (1966) The diatoms of the United States. *Academy of Natural Sciences*.

Peng, Y., Peng, Z., Zeng, X., and Houx, J. H. (2019). Effects of nitrogen-phosphorus imbalance on plant biomass production: a global perspective. Plant and Soil, **436**(1):245-252.

Penuelas, J., Poulter, B., Sardans, J., Ciais, P., Van Der Velde, M., Bopp, L., Boucher O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., and Janssens, I. A. (2013). Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nature communications, **4**(1):1-10.

Pianka, E. R. (1966). Latitudinal gradients in species diversity: a review of concepts. *The American Naturalist* **100**(910), 33-46.

Pinto-Coelho, R. M., Bezerra-Neto, J. F., Miranda, F., Mota, T. G., Resck, R., Santos, A. M., Maia-Barbosa, P. M., Mello, N., Marques, M. M., Campos, M. O., and Barbosa, F. A. R. (2008). The inverted trophic cascade in tropical plankton communities: impacts of exotic fish in the Middle Rio Doce Lake district, Minas Gerais, Brazil. *Brazilian Journal of Biology* **68**(4): 1025-1037.

Pollingher, U. (1990). Effects of latitude on phytoplankton composition and abundance in large lakes. *In Large Lakes* (pp. 368-402). Springer, Berlin, Heidelberg.

Persson J., Fink P., Goto A., Hood J.M., Jonas J., and Kato S. (2010). To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos. **119**(5):741–751.

Prescott, G. W., Croasdale, H., and Vinyard, W. C. (1972). *A synopsis of North American desmids*. University of Nebraska press.

Qin, J., and Culver, D. A. (1996). Effect of larval fish and nutrient enrichment on plankton dynamics in experimental ponds. Hydrobiologia, **321**(2):109-118.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for

Ramos, G. J. P. and do Nascimento Moura, C. W. (2019) Algae and cyanobacteria in phytotelmata: diversity, ecological aspects, and conservation. *Biodiversity and Conservation* **28**(7):1667–1697.

Rymal, D. E. and Folkerts, G. W. (1982). Insects associated with pitcher plants (Sarracenia: Sarraceniaceae), and their relationship to pitcher plant conservation: a review. Journal of the Alabama Academy of Science.

Salmaso, N., Buzzi, F., Garibaldi, L., Morabito, G., and Simona, M. (2012). Effects of nutrient availability and temperature on phytoplankton development: a case study from large lakes south of the Alps. *Aquatic Sciences* **74**(3): 555-570

Seale, D. B., Boraas, M. E., and Warren, G. J. (1987). Effects of sodium and phosphate on growth of cyanobacteria. *Water Research* **21**(6): 625-631..

Schemske, D. W., and Mittelbach, G. G. (2017). "Latitudinal gradients in species diversity": reflections on Pianka's 1966 article and a look forward. *The American Naturalist* **189**(6): 599-603.

Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes: natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. Science **195**:260–262.

Schlichting Jr, H. E., and Sides, S. L. (1969). The passive transport of aquatic microorganisms by selected Hemiptera. *The Journal of Ecology* 759-764.

Schulhof, M. A., Shurin, J. B., Declerck, S. A., and Van de Waal, D. B. (2019). Phytoplankton growth and stoichiometric responses to warming, nutrient addition and grazing depend on lake productivity and cell size. Global change biology, **25**(8):2751-2762.

Seehausen O., Alphen J.J.M. van, and Witte F. (1997). Cichlid Fish Diversity Threatened by Eutrophication That Curbs Sexual Selection. Science. **277**:1808–1811.

Shurin, J. B., Clasen, J. L., Greig, H. S., Kratina, P., and Thompson, P. L. (2012). Warming shifts top-down and bottom-up control of pond food web structure and function. Philosophical Transactions of the Royal Society B: Biological Sciences, **367**(1605):3008-3017.

Smith, G. M. (1950) Fresh-water algae of the United States.

Smith V. H. (1983). Low Nitrogen to Phosphorus Ratios Favor Dominance by Blue-Green Algae in Lake Phytoplankton. Science. **221**:669–671.

Smith V. H. (1998). Cultural Eutrophication of Inland, Estuarine, and Coastal Waters. In: Pace ML, Groffman PM, editors. Successes, Limitations, and Frontiers in Ecosystem Science. New York, NY: Springer New York:7–49.

Soininen, J., and Meier, S. (2014). Phytoplankton richness is related to nutrient availability, not to pool size, in a subarctic rock pool system. *Hydrobiologia* **740**(1): 137-145.

Spilling, K., Camarena-Gómez, M. T., Lipsewers, T., Martinez-Varela, A., Díaz-Rosas, F., Eronen-Rasimus, E., Silva, N., con Dassow, P., and Montecino, V. (2019). Impacts of reduced inorganic N: P ratio on three distinct plankton communities in the Humboldt upwelling system. Marine Biology, **166**(9):1-17.

Sterner R. W. and Elser J. J. (2002). Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press.

Stomp, M., Huisman, J., Mittelbach, G. G., Litchman, E., and Klausmeier, C. A. (2011). Large-scale biodiversity patterns in freshwater phytoplankton. *Ecology* **92**(11): 2096-2107.

Tilman D. (1982). Resource Competition and Community Structure. Princeton University Press.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann, E. (1997). The influence of functional diversity and composition on ecosystem processes. Science, **277**(5330):1300-1302.

Thrane, J. E., Hessen, D. O., & Andersen, T. (2017). Plasticity in algal stoichiometry: Experimental evidence of a temperature-induced shift in optimal supply N: P ratio. Limnology and Oceanography, **62**(4):1346-1354.

Trimbee, A. M., and Prepas, E. E. (1987). Evaluation of total phosphorus as a predictor of the relative biomass of blue-green algae with emphasis on Alberta lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **44**(7): 1337-1342.

USA National Phenology Network. 2019. Spring Indices, Current Year - First Leaf - Spring Index Date. 2020-01-01.

Wissinger, S. A., Perchik, M. E., and Klemmer, A. J. (2018). Role of animal detritivores in the breakdown of emergent plant detritus in temporary ponds. Freshwater Science, **37**(4):826-835.

Yves Rosseel (2012). lavaan: An R Package for Structural Equation Modeling. Journal of Statistical Software, 48(2), 1-36. https://doi.org/10.18637/jss.v048.i02

Wang F, Li J, Wang X, Zhang W, Zou B, Neher DA, and Li, Z. (2014). Nitrogen and phosphorus addition impact soil N_2 emission in a secondary tropical forest of South China. Scientific Reports. **4**:1-8.

Walker. (2015) Risk assessment summary sheet Purple Pitcher plant (Sarracenia purpurea). GB non-native species secretariat.

Weckström, J., and Korhola, A. (2001). Patterns in the distribution, composition and diversity of diatom assemblages in relation to ecoclimatic factors in Arctic Lapland. *Journal of Biogeography* **28**(1): 31-45.

Wehr, J. D., Sheath, R. G., and Kociolek, J. P. (2015) *Freshwater algae of North America: ecology and classification*. Elsevier

Wiens, A. P. (1972). Bionomics of the pitcher plant midge Metriocnemus knabi (Coquillett) (Diptera: Chironomidae).

Wetzel, R.G. (2001) Fundamental processes within natural and constructed wetland ecosystems: short-term versus long-term objectives. Water Science and Technology **44**(11-12): 1–8.

Weyhenmeyer, G. A., Peter, H., and Willén, E. V. A. (2013). Shifts in phytoplankton species richness and biomass along a latitudinal gradient–consequences for relationships between biodiversity and ecosystem functioning. *Freshwater Biology* **58**(3): 612-623.

Wurtsbaugh, W. A., Paerl, H. W., and Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. Wiley Interdisciplinary Reviews: Water, **6**(5):e1373.

Yamamoto, Y., and Nakahara, H. (2005). The formation and degradation of cyanobacterium Aphanizomenon flos-aquae blooms: the importance of pH, water temperature, and day length. *Limnology* 6(1): 1-6.

Yvon-Durocher, G., Allen, A. P., Cellamare, M., Dossena, M., Gaston, K. J., Leitao, M., Montoya, J. M., Reuman, D. C., Woodward, G., and Trimmer, M. (2015). Five years of experimental warming increases the biodiversity and productivity of phytoplankton. *PLoS biology* **13**(12): e1002324.

Zhang, Y., Wu, H., Yuan, C., Li, T., and Li, A. (2019). Growth, biochemical composition, and photosynthetic performance of Scenedesmus acuminatus during nitrogen starvation and resupply. *Journal of Applied Phycology* **31**(5): 2797-2809.

Zohary, T., and Ostrovsky, I. (2011). Ecological impacts of excessive water level fluctuations in stratified freshwater lakes. *Inland waters* **1**(1): 47-59.