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Impacts of Shoreline Restoration and Source of Nutrient Enrichment on Macrophytes and Epiphytic Algal Communities

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Dedication

This master's thesis is dedicated to my parents, April and Tim Kleindl, who taught me to pursue my passions as well as Drs. Sarah Waters, Robert Verb, and Leslie Riley at Ohio Northern University who helped me make my passions a part of my everyday life. Your mentorship continues to shape me as a person, and I am forever grateful for your support and encouragement as I continue my pursuit of a scientific career.

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Abstract

Macrophytes and their epiphytic algal communities are integral for optimizing littoral ecosystem functioning in lakes. Epiphytic algae's placement on the plant's surface can reduce light and nutrient availability (i.e., nitrogen and phosphorus) for the host macrophyte. Macrophyte and epiphytic algal proximity complicates these primary producer group interactions and responses to bioavailable nutrients in the water column or porewater. For example, epiphytic algae may have a competitive advantage over surface water nutrients compared to macrophytes, which may have a competitive advantage over porewater nutrients via root systems.

Muskegon Lake's industrial history and designation as an Area of Concern prompted shoreline restoration, where macrophyte surveys were conducted pre- (2009-2010) and post- (2011-2012) restoration. For my thesis, I continued the macrophyte survey in 2018 to determine restoration impacts on the macrophyte community. An epiphytic algal survey also was included to evaluate interactions with their host macrophyte (*Vallisneria americana*) and to determine algal community structure variation across habitats. To further evaluate *V. americana*-epiphytic algal interactions, I examined both primary producer groups responses to source of nutrient enrichment (sediment porewater and/or surface water).

Fluctuations in hydrologic and meteorological conditions among all survey years, largely due to water levels, obscured restoration-induced macrophyte changes and slowed ecosystem improvement. By 2018, however, we had seen an increase in restored habitat quality compared to the reference habitat based on Coefficient of Conservatism values and macrophyte biomass

and density increases. My results also indicated a negative impact of epiphytic algal biomass and density on *V. americana* in Muskegon Lake and the mesocosm experiment. During the experiment, water column nutrient enrichment induced phytoplankton accumulation, reducing light and subsequent macrophyte and epiphytic algal biomass. Porewater nutrient enrichment helped alleviate the negative influence of phytoplankton biomass on macrophyte and epiphytic algal biomass when the water column was enriched.

These studies reinforced the importance of environmental variation and biological interactions in influencing macrophyte community structure. Managers can use this knowledge to choose restoration locations that will enhance macrophyte success: intermediate light and hydrologic exposure will help mitigate epiphytic algal growth, and shallow slope could help increase habitat resiliency to climactic scale environmental shifts.

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Abbreviations

Abd – Relative abundance

AOC – Area of Concern

B – Both treatment

BUI – Beneficial Use Impairment

C – Control treatment

C-Value – Coefficient of Conservatism

Chl-*a* – Chlorophyll-*a*

DO – Dissolved Oxygen

GrandT – Grand Trunk

HertL – Heritage Landing

Hrs – Hours

KH_2PO_4 – Potassium phosphate

Kmph – Kilometers per hour

L:D – Light and Dark Photoperiod

MI – Michigan

N – Nitrogen

N_2 - Dinitrogen

$NaNO_3$ – Sodium nitrate

NO_3^- - Nitrate

NO_2^- - Nitrite

NO_3^--N – Nitrogen in Nitrate

NMDS – Non-metric Multidimensional Scaling

NTU - Turbidity

NWRef – Northwest Reference

OM – Organic Matter

ORP – Oxidation Reduction Potential

P – Phosphorus

PAR – Photosynthetically Active Radiation

PCA – Principle Component Analysis

PoreNO3 – Porewater Nitrate

PoreSRP – Porewater Soluble Reactive Phosphorus

S – Sediment treatment

SC – Specific Conductance

SD – Standard Deviation

SedTKN – Sediment Total Kjeldahl Nitrogen

SedTP – Sediment Total Phosphorus

SRP – Soluble Reactive Phosphorus

T – Temperature

TDS – Total Dissolved Solids

TKN – Total Kjeldahl Nitrogen

TP – Total Phosphorus

WC – Water Column treatment

WChla – Phytoplankton Chlorophyll-*a*

WI – Wind Index

WL – Water Level

Chapter 1

Introduction

Coastal Wetland Loss – Muskegon Lake

Nearshore habitat and coastal wetlands provide numerous ecosystem services that are valuable to society (Steinman et al., 2017; Vadeboncoeur et al., 2011). These biologically productive environments stimulate economic growth through recreational and commercial fishing (Campbell et al., 2015), and can increase the value of lakeside property containing esthetically appealing natural shorelines (Leggett and Bockstael, 2000; Isely et al., 2018). Despite shoreline importance, human development has inflicted an array of disturbances on nearshore habitat, especially within the Laurentian Great Lakes region, including: dredging, shoreline hardening, pollution runoff, and external or internal nutrient loading (Schock et al., 2014; Steinman and Ogdahl, 2011; Whittier et al., 2002). The Great Lakes region has lost three fourths of its natural coastal habitat to industrial and urban development since European settlement, with the remaining river mouth and littoral ecosystems designated as poor habitat quality (Larson et al., 2013; Jude and Pappas, 1992). In response to habitat destruction and degradation, monitoring programs have been developed to evaluate the status of remaining wetlands (Grabas et al., 2012; Uzarski et al., 2017) and some locations have undergone restoration (Ogdahl and Steinman, 2015).

Muskegon Lake, located in central West Michigan, had suffered two centuries of environmental degradation due to industrialization and urbanization (Steinman et al., 2008). Approximately 65% of the entire lake's shoreline was hardened with materials such as rip-rap, seawall, slag, and slab wood (Steinman et al., 2008). These environmental impairments, among others, led to Muskegon Lake’s listing as a Great Lakes Area of Concern (AOC) in 1985 and the identification of Nine Beneficial Use Impairments (BUIs), including the loss of fish and wildlife habitat (Ogdahl and Steinman, 2015; Steinman et al., 2008). In an effort to increase habitat availability for fish and other wildlife, restoration at specific locations along the south shoreline occurred in 2010 and 2011 to remove unwanted fill material and replace the hardened shoreline with a natural, macrophyte-filled transition zone (Ogdahl and Steinman, 2015). Pre- and post-restoration habitat monitoring, with a focus on macrophyte communities, was used to evaluate the change in ecosystem quality over time (Ogdahl and Steinman, 2015).

Macrophytes as Keystone Contributors

Macrophytes exist within the shallow water littoral zone of a lake where ample light can reach the benthos (Chambers et al., 2008). Macrophyte roots embedded in the benthos restrict the resuspension of sediment particles, mitigate turbidity, and increase light attenuation (Bornette and Puijalon, 2011). Macrophyte shoots reduce water velocity within and adjacent to the plant bed, controlling particle resuspension as well as facilitating sedimentation (Madsen et al., 2001). Sedimentation results in greater organic matter and sediment particle accumulation in the macrophyte bed compared to unvegetated locations, stimulating macrophyte productivity (Madsen et al., 2001). Macrophytes also are critical to nutrient cycling in lakes; plants are considered nutrient sinks during the growing season and sources during senescence (Barko et al., 1991). The sequestering of nutrients by aquatic plants restricts nutrient availability to phytoplankton populations, helping control the formation of algal blooms at the water’s surface (Søndergaard and Moss, 1998).

Macrophytes, with their variety of morphologies, can increase habitat heterogeneity (Thomaz and Cunha, 2010). Waterfowl and other bird species rely on emergent macrophyte communities for essential breeding habitat (Bonter et al., 2009; Sierszen et al., 2012). Fish are dependent on macrophyte beds for juvenile development, predator protection, and food resources (Jude and Pappas, 1992). Macrophytes also provide shelter and substrate for macroinvertebrates; grazers specifically travel amongst the shoots and leaves to consume attached biofilms (Thomaz and Cunha, 2010). Epiphytic algae residing on the surfaces of macrophytes support the littoral food web by providing an energy base for higher trophic levels and contributing to carbon fixation (Allen, 1971; Dodds, 2003; Vadeboncoeur and Steinman, 2002). Epiphytic algae also are important sinks of nutrients, like nitrogen, and can increase organic nutrient concentrations in sediments (Young et al., 2005).

Macrophyte-Epiphytic Algal Matrix

Nutrients and light availability are two of the most important environmental variables influencing macrophytes and their epiphytic algal communities (Allen, 1971; Eminson and Moss, 1980). However, epiphytic algae form a barrier between the water column and macrophyte surface, essentially competing for light and nutrients with their host plant (Cattaneo et al., 1998; Strand and Weisner, 2001); epiphytic algae reduce macrophyte oxygen and carbon uptake, obstruct dissolved material transport including nitrogen (N) and phosphorus (P), and shade macrophyte surfaces (Nelson, 2017). Epiphytic algae may induce physical drag on macrophytes, increasing the risk of leaf loss during periods of high wind or wave action (Borowitzka and Lethbridge, 1989). In some extreme situations, epiphytic algae have brought about the loss of entire macrophyte communities in shallow lakes as well as seagrass beds

(Cambridge et al., 1986; Jones et al., 2002; Phillips et al., 1978). In response to excess algal growth, certain macrophyte species may release allelopathic chemicals, although this process may not reduce epiphytic algae (Erhard and Gross, 2006; Hilt et al., 2006). Macrophyte life history traits such as high leaf turnover could mitigate thick epiphytic algal layers (Eminson and Moss, 1980). Macrophyte bed density also could decrease epiphytic algal growth due to adjacent macrophytes shading the algal community (Öterler, 2017).

Sources of Nitrogen and Phosphorus

N and P are the major growth-limiting nutrients for primary producers in lakes, including macrophytes and epiphytic algae (Elser et al., 2007). The inorganic and organic forms of these nutrients cycle through different locations within an aquatic ecosystem: the water column, sediment, and living organisms (Carpenter et al., 1998) (Figure 1.1). Both N and P externally enter the water column through surface runoff. Atmospheric deposition can also introduce N and particulate P into a water body (Brennan et al., 2016; Francis et al., 2007). Dissolved, biologically available N and P within the water column can be directly sequestered by macrophytes and epiphytic algae (Boström et al., 1988). Atmospheric nitrogen can be in the form of NH_x, NO_x, and N_2. N_2 must undergo nitrogen fixation before it is available for organism uptake (Elser et al., 2009; Francis et al., 2007).

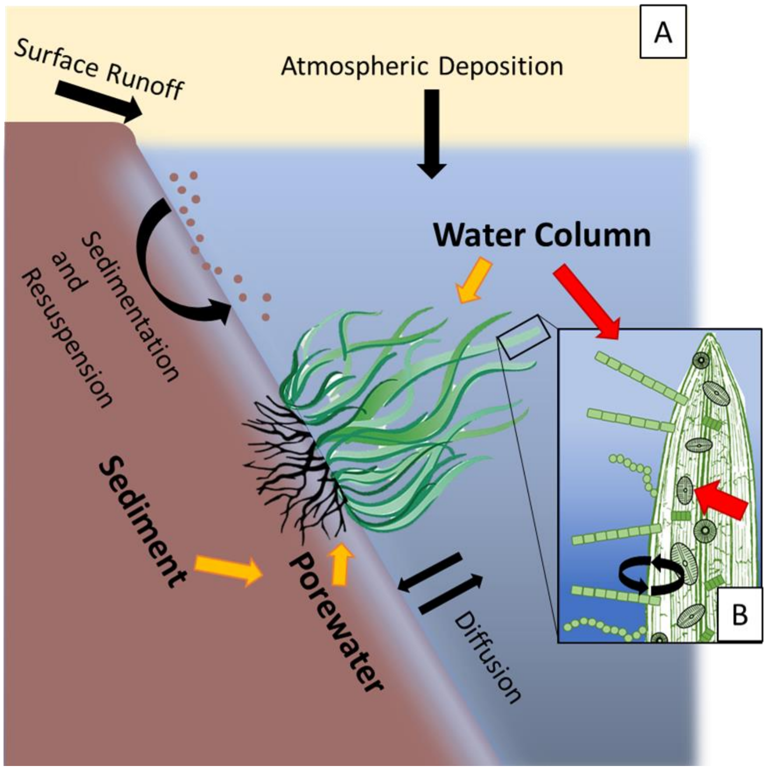

Figure 1.1: Representation of the sources of N and P available to primary producers in a lake littoral zone. (A) Black arrows indicate movement of nutrients into and within the aquatic ecosystem. Yellow arrows indicate which macrophyte structure would take up nutrients from each source. (B) Representation of the epiphytic algal community residing on the surface of a macrophyte leaf blade. Large red arrow represents direct epiphytic algal nutrient acquisition from the water column. Small red arrow indicates nutrient leaching from the macrophyte surface, available for epiphytic algal sequestration. Black arrows indicate internal nutrient cycling within the epiphytic algal biofilm.

Particulate N and P entering a lake from the surrounding landscape and watershed usually settle into the sediment nutrient pool (Saunders and Kalff, 2001; Søndergaard et al., 2001). This includes organic forms of N and P as well as sediment-bound P, which will become available to primary producers after mineralization and mobilization occurs, respectively (Boström et al., 1988; Saunders and Kalff, 2001; Søndergaard et al., 2001). Unbound N and P can reside in interstitial porewater found between benthic sediment particles. Exchanges across the sediment-water interface also occur and are influenced by: a nutrient diffusion gradient (Short, 1987), water turbulence resuspending N and P bound material, benthic organism uptake (Saunders and Kalff, 2001) and bioturbation (Nogaro and Steinman, 2014), and other transport mechanisms (Boström et al., 1988; Wetzel, 2001). The source, water column or sediment, containing the greatest biologically available nutrient concentrations will therefore stimulate the growth of primary producers in contact with that habitat.

Individual Responses to Nutrient Enrichment

Macrophytes sequester nutrients essential for maintaining productivity, growth, and reproduction through the water column and sediment (Figure 1.1) (Chambers et al., 1989; Madsen and Cedergreen, 2002). The importance of each location (hereafter referred to as nutrient source) containing bioavailable nutrients for macrophyte uptake may differ among environmental conditions (Barko and James, 1998). In general, nutrient limitation of N and P leads to an overall reduction in macrophyte growth (Madsen and Cedergreen, 2002). Increased water column nutrient availability can reduce root biomass since energy will be redirected away from root structures, decreasing submergent macrophyte biomass root:shoot ratios (O'Connell et al., 2015; Dülger et al., 2017). Macrophytes with roots mechanically removed can maintain productivity and experience high growth rates based solely on leaf nutrient uptake (Madsen and Cedergreen, 2002). Besides nutrient-rich environments stimulating macrophyte shoot growth, turbidity increases caused by phytoplankton accumulation may also invoke shoot growth to help leaves reach adequate light levels for growth (Phillips et al., 1978; Song et al., 2017). Root:shoot ratio increases have been documented in environments with low-fertile sediments since greater root system biomass and complexity increases the surface area available for nutrient uptake (Barko et al., 1988; Barko et al., 1991). However, in any habitat containing elevated water column-, interstitial water-, and/or sediment-nutrients, root:shoot ratios usually decline (Barko et al., 1991; Madsen and Cedergreen, 2002).

Although macrophyte responses to nutrient dynamics have been generalized, plants display species-specific responses based on their ideal nutrient optimum and sequestration methods (Grime et al., 1986; Mei and Zhang, 2015). Madsen et al. (2001) classified macrophytes into two simple growth forms: (1) meadow formers containing a basal meristem, where the majority of biomass is equally distributed along the vertical water column gradient and (2) canopy formers, which contain an apical meristem and their biomass is concentrated at the top of the plant canopy or at the water's surface. Meadow-formers like the genus *Vallisneria* are not as adept in nutrient-rich conditions compared to canopy-formers like *Myriophyllum* spp. that can compete much more successfully in nutrient-rich environments with turbid waters (Chambers and Kalff, 1987; Madsen et al., 2001). Additionally, the extent of macrophyte biomass change, for either growth form, will be contingent on the intensity and duration of nutrient increases as well as the influence of surrounding environmental pressures (Tang et al., 2019).

Unlike macrophytes, epiphytic algae do not directly interact with sediment nutrients; nutrients enter the epiphytic algal matrix through either the water column or from their host plant (Barko et al., 1991; Burkholder, 1996; Moeller et al., 1988; Sand-Jensen and Borum, 1991). Interstitial water exchange or lacustrine groundwater discharge may introduce higher concentrations of N and P into the water column, facilitating epiphyton growth (Périllon and Hilt, 2019; Vadeboncoeur and Steinman, 2002). Nutrients are also internally cycled within an established epiphytic algal biofilm, helping to sustain the current community and promote algal growth (Figure 1.1) (Mulholland et al., 1994; Sand-Jensen and Borum, 1991).

Regardless of location, nutrient increases in the absence of other stressors results in increased epiphytic algal productivity, growth, and biomass accumulation (Rosemond et al., 1993). Algae respond quickly to environmental changes as a result of rapid community turnover and can experience community composition shifts even with short-term nutrient spikes (Schneider et al., 2012). In general, nutrient-rich environments facilitate the growth of cell-stacking algal morphologies, which extend past the biofilm to directly exploit resources from the water column (Berthon et al., 2011; Burkholder, 1996). Higher surface area to volume ratio and advective nutrient accrual increases the nutrient uptake rates of cell-stacking algae about twofold (Burkholder et al., 1990; Dodds, 2003; Steinman et al., 1992). Elevated N and P concentrations may facilitate the replacement of algal species with lower nutrient optima with species with higher nutrient affinities (Berthon et al., 2011). Additionally, habitats with limited N availability would likely contain cyanobacterial taxa with N-fixing capabilities (Vitousek et al., 2002). Since algae community composition mirrors nutrient concentrations, as well as other physical and chemical environmental variables, algae are commonly used as indicators of trophic status or overall habitat quality (Potapova and Charles, 2003).

Purpose

The purpose of this study is to understand how environmental variables, restoration activities, and epiphytic algae play a role in macrophyte community success along Muskegon Lake's shoreline. Macrophyte and environmental surveys can help us better interpret restoration outcomes through the separation of restoration-induced macrophyte responses from responses due to the variation in physical habitat and fluctuating hydrologic conditions. Investigating the relationship between epiphytic algae and their host plant in Muskegon Lake provides insight to how biological interactions may vary among habitat conditions, may influence each primary producer group, and may affect restoration outcomes. Additionally, evaluating the role of nutrient source for epiphytic algae and their host macrophyte will help decipher the biological interactions within the macrophyte-epiphytic algal matrix in regard to resource competition. The use of epiphytic algae and macrophytes for evaluating habitat quality and environmental conditions will add to our understanding of primary producer biomonitoring.

Scope

The first specific aim for this thesis focuses on the environmental and biological (i.e., macrophytes) metrics at three littoral habitats in Muskegon Lake before (2009-2010) and after (2011-2012, and 2018) restoration occurred, specifically two restored habitats along the south shoreline and one reference habitat along the north shoreline. Epiphytic algal communities on the macrophyte species *Vallisneria americana* and corresponding water quality variables also were examined at these habitats for one year (2018); results were interpreted in regard to macrophyte biological interactions and potential algal influence on restoration. To further highlight macrophyte and epiphytic algal interactions, the second specific aim was to evaluate *V. americana* and its attached epiphytic algal community responses to different sources of nutrient enrichment within a controlled experiment. Conclusions from the source of nutrient enrichment experiment can be applied to further research concerning macrophyte and epiphytic algal interactions but extrapolating results to other macrophyte taxa must be done with caution. Additionally, conditions simulated within an experimental setting are not fully representative of natural systems containing multiple stressors and should be interpreted with care.

Assumptions

1) I assumed macrophyte sampling and processing was unbiased and representative of the sampling locations.
2) I assumed epiphytic algae sampling from *Vallisneria americana* and subsequent processing was unbiased and representative of the sampling locations.
3) I assumed the epiphytic algae inoculum for the mesocosm experiment was representative of Muskegon Lake's epiphytic algal communities.
4) I assumed that commercially purchased *Vallisneria americana* macrophytes would be representative of Muskegon Lake's *V. americana*.
5) I assumed randomization of mesocosm treatments in the experiment buffered against biases in light and temperature.

Objectives

The objectives of my thesis were to: 1) determine if Muskegon Lake shoreline restoration improved habitat quality for macrophyte communities at the restored habitats compared to the reference habitat by examining macrophyte biological variables (e.g., richness, biomass, and density) and environmental characteristics (e.g., slope and water level) among the five survey years; 2) determine how epiphytic algae community structure on *V. americana* (i.e., density and chlorophyll-*a*) were influenced by environmental variables (e.g., light extinction coefficient, wind index, and water column nutrients) among the three transects; and 3) determine the influence of sediment vs. water column nutrient enrichment (N and P) on *V. americana* biomass and length and its epiphytic algal community structure (density, richness, diversity, and chlorophyll-*a*) in a mesocosm-based experiment with four treatment types: sediment porewater nutrient enrichment; water column nutrient enrichment; nutrient enrichment at both sources; and a control with no nutrient enrichment .

Significance

This thesis investigates how the macrophyte and epiphytic algal communities have responded to a restoration initiative on Muskegon Lake's shoreline. A successful restoration project, as determined in part by the macrophyte survey, will contribute to removing the loss of fish and wildlife habitat BUI and delisting Muskegon Lake as an AOC; monitoring techniques and habitat evaluation can then be applied to other restoration projects. Our results also can help managers choose restoration locations, assuming the land is available for restoration, which will maximize habitat rehabilitation. Surveying *V. americana* epiphytic algal communities for the first time in Muskegon Lake fills a gap in knowledge related to understanding the lake's littoral community structure. Examining the relationship between macrophytes and their attached algae in Muskegon Lake can help further explain differences in macrophyte community metrics among habitats. Epiphytic algal results can therefore be incorporated into potential adaptive management strategies, if needed, and will be beneficial for developing similar restoration activities at other locations.

The controlled experiment fills an important gap concerning macrophyte and epiphytic algae responses to nutrient enrichment. Often, only one source of nutrient enrichment (sediment porewater or water column) is studied in correlation with one primary producer group (macrophytes or epiphytic algae) For example, macrophyte responses to sediment porewater nutrients are studied separately from epiphytic algae responses to water column nutrient addition. This experiment examines macrophyte and epiphytic algal simultaneous responses to: sediment porewater nutrient enrichment; water column nutrient enrichment; enrichment in both sources. Results provide a more holistic understanding of how nutrients impact the macrophyte-epiphytic algae matrix, and the degree to which epiphytic algae inhibit macrophyte growth under the varying nutrient source circumstances. In conclusion, this thesis improves our understanding of the abiotic variables impacting Muskegon Lake macrophyte and epiphytic algal communities, and further untangles the complex interactions within the macrophyte-epiphytic algal matrix. This research contributes to the greater body of literature surrounding coastal wetland restoration, macrophyte biomonitoring, and the impacts of nutrient increase on primary producers.

Definitions

Atmospheric Deposition: Particulates and gases deposited from the atmosphere to the surfaces of terrestrial and aquatic ecosystems.

Autotroph: An organism that forms nutritional organic substances from inorganic substances like carbon dioxide, generally using energy from light or inorganic chemical reactions.

Beneficial Use Impairment: A change in the chemical, physical, or biological integrity of the Great Lakes system sufficient to cause significant environmental degradation.

Biomonitoring: Assessing the ecological condition of water bodies by examining their organismal communities.

Coastal wetland: A habitat inundated or saturated by surface or groundwater for a period of time, allowing that habitat to support vegetation adapted to saturated soil conditions. Coastal wetlands are located along the transition between land and a large body of water.

External Nutrient Loading: Input of nutrients from the surrounding terrestrial environment and watershed into a lake.

Epiphyte: A photosynthetic organism that grows on another photosynthetic organism but is not parasitic.

Great Lakes Area of Concern: A designated geographic area where significant impairment of beneficial uses has occurred as a result of human activities at the local level.

Inoculum: A small amount of substance containing algae from one location, which is used to start a community in a new location.

Internal Nutrient Loading: Nutrients are introduced into the surface water of lakes from the lake's sediment.

Littoral zone: A habitat in coastal environments that extends from the high-water mark to permanently submerged shoreline.

Macrophyte: An aquatic plant visible to the naked eye located in or near water.

Mesocosm: An experimental water enclosure designed to provide a limited body of water with close to natural conditions, and where environmental variables can be realistically manipulated.

Mineralization: The decomposition of chemical compounds in organic matter and nutrients within these compounds are released in a soluble inorganic form.

Mobilization: The release of inorganic nutrients, i.e., phosphorus, from sediment particles.

Nitrogen Fixation: The chemical process assimilating atmospheric nitrogen into organic compounds, carried out by certain microorganisms.

Primary producer: An organism that converts an abiotic source of energy (e.g. light) into energy stored in organic compounds.

Restoration: Renewing and recovering degraded, damaged, or destroyed habitats through human intervention and action.

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Chapter 2

Long-term Impacts of Shoreline Restoration on Macrophyte and Epiphytic Algae Communities

Abstract

Macrophytes and their epiphytic algal communities are critical components of the biologically productive littoral zones of lakes. Both communities respond to changes in light, nutrients, and physical habitat, and are increasingly used as bioindicators to detect anthropogenic alterations to aquatic ecosystems. With an industrial past, Muskegon Lake (MI) has undergone significant human disturbances, such as shoreline hardening and sediment contamination. Shoreline restoration during 2010 and 2011 presented an opportunity to use macrophytes as indicators of short-term (2009-2012) and longer-term (2018) post-restoration ecosystem status. Two restored habitats and one reference habitat were sampled during the five survey years along established transects perpendicular to the shoreline.

Significant variation in bathymetric slope (%) and hydrologic exposure (i.e., wind and wave index) among the three habitats influenced macrophyte richness, density, and biomass among all survey years. Despite substantial changes in water level, precipitation, and air temperature among survey years, restoration increased habitat quality at the two restored habitats compared to the reference habitat. A 1 m water level increase between 2012 and 2018 was associated with declines in macrophyte metrics, including a decrease in 2018 macrophyte richness at all transects. Greater epiphytic algal density and biomass, associated with high light and low wind/wave exposure, also may have impacted macrophyte biomass declines. Our findings suggest future restoration projects should consider restoration locations with habitat characteristics that optimize littoral rehabilitation (i.e., gentle slope and intermediate exposure) and can maintain macrophyte community metrics (i.e., richness, density, and biomass) through future climatic changes.

Keywords: macrophytes; epiphytic algae; shoreline restoration; Great Lakes; coastal wetlands; Muskegon Lake

Highlights

- Macrophyte metrics were evaluated to determine success of shoreline restoration
- Epiphytic algae were surveyed to determine biomass-related variation among habitats
- Macrophyte and algal dynamics were strongly influenced by physical habitat features
- Hydrologic changes also impacted macrophyte metrics
- Restoration potentially improved habitat quality but changing water levels conflated analysis

1. Introduction

Macrophyte communities provide multiple ecosystem services to littoral habitats in lakes including sediment stabilization, reduction of wave action, nutrient storage and cycling, and habitat refugia (Allen, 1971; Bornette and Puijalon, 2011; Thomaz and Cunha, 2010). Macrophytes are host to a diverse community residing both on the plants' surface and amongst their shoots and leaves; epiphytic algae are an ecologically significant group in this community because they cycle nutrients and form an energy base for aquatic food webs (Allen, 1971; Vadeboncoeur and Steinman, 2002).

Habitat characteristics associated with nutrient and light dynamics, such as sediment organic matter content (Squires and Lesack, 2003) and shoreline benthic gradient (Barko et al., 1991), often influence macrophyte presence and community structure (Eminson and Moss, 1980; Lacoul and Freedman, 2006). However, epiphytic algae's proximity to their host can reduce macrophyte resource availability by preventing dissolved materials, oxygen, carbon, and light from reaching the macrophyte (Allen, 1971; Eminson and Moss, 1980; Nelson, 2017; Sand-Jensen and Søndergaard, 1981).

Since macrophytes and their epiphytic algal communities reflect the quality of physical and chemical conditions in aquatic systems, both can be used as bioindicators of anthropogenic disturbances and restoration initiatives. For example, the European Water Framework Directive uses algae (Ballesteros et al., 2007) and macrophytes (Penning et al., 2008) to assess ecological health. Macrophytes also are used to measure coastal wetland status across the Laurentian Great Lakes region (Grabas et al., 2012; Uzarski et al., 2017). Assessments of ecosystem change are critical for the advancement of adaptive management strategies, for improving restoration techniques, and for increasing the value of restoration to investors and the public (Palmer et al., 2007). Although restoration evaluation has increased in recent years, the effectiveness of restoration activities remains unclear, potentially due to relatively short monitoring periods compared to the timescale required for ecological changes (Suding, 2011; Wortley et al., 2013).

Anthropogenic disturbances caused by human development, industry, and the transportation of goods have resulted in lake dredging, shoreline hardening, and pollution runoff (Whittier et al., 2002). Evidence of industrial degradation is visible throughout the Great Lakes region, which has lost three fourths of coastal habitats to development, with the remaining river mouth and littoral ecosystems designated as poor habitat quality (Larson et al., 2013). In Muskegon Lake, Michigan, historical industrialization negatively impacted 315 ha of natural shoreline, which was hardened with rip-rap, seawall, slag and slab wood, among other materials (Steinman et al., 2008), resulting in the loss of numerous ecosystem services. The disturbed littoral habitat made macrophytes an ideal focal point of restoration.

Macrophyte surveys occurred pre- (2009-2010) and post-shoreline restoration (2011-2012) in Muskegon Lake, from which Ogdahl and Steinman (2015) concluded that distinguishing restoration responses from environmental effects required a longer-term data set because of the physical disturbance associated with restoration efforts. Therefore, the first objective of this study was to determine, based on additional monitoring in 2018, if shoreline restoration had effectively improved habitat quality for macrophyte communities. Restored habitats were compared to a reference habitat with more natural shoreline, to separate the effects of environmental change from those associated with restoration activities. We hypothesized that shoreline restoration had increased macrophyte biomass, density, and richness apart from the influence of annual environmental variation.

Since macrophyte interactions with epiphytic algae can influence macrophyte growth characteristics, an epiphytic algae survey took place in 2018 at the same habitats as the macrophyte survey. The second objective of this study was to determine how epiphytic algal community structure was influenced by environmental variables (dissolved oxygen [DO], turbidity [NTU], pH, temperature [T], specific conductance [SC], redox potential [ORP], light extinction coefficient, wind index [WI], and water column nutrient concentrations) across the restored and reference habitats. We hypothesized that epiphytic algal density and chlorophyll-*a* (Chl-*a*) would vary among transects due to differences in environmental conditions, which in turn would influence macrophyte composition and abundance.

2. Methods

2.1 Habitat description

Muskegon Lake is a ~17 km^2 drowned river mouth lake located in central West Michigan, which receives inflow from the 7060 km^2 Muskegon River watershed, and connects directly into Lake Michigan (Figure 2.1). Environmental degradation began in the 1850's as the lumber industry developed along the lake's shoreline, with peak operation involving 47 active sawmills. Foundries and factories (i.e., metal finishing plants, a paper mill, and petrochemical storage facilities) concentrated along the south shoreline replaced the lumber industry in the early 20th century. These industries discarded slab wood, concrete, foundry slag, and other unwanted materials into the lake. Two centuries of urban development hardened 65% (32.64 km) of the entire shoreline with a disproportionately higher amount along the south shoreline (78%) compared to the north shoreline (45%), which has more residential and natural areas than the south (Steinman et al., 2008).

In response to habitat degredation and the listing of Muskegon Lake as a Great Lakes Area of Concern (AOC) in 1985, restoration took place in 2010 and 2011 to renaturalize habitats along the lake's south shoreline (Ogdahl and Steinman, 2015). Restoration included: (1) the removal of unwanted fill debris at (shoreline) or below (underwater) the ordinary high water mark, (2) shoreline vegetation planting, and (3) shoreline wetland restoration. After softening 4.17 km of the 49.99 km shoreline, 56.96% (28.47 km) remains hardened. During 2009-2012 and in 2018, two restored habitats (Heritage Landing and Grand Trunk) and one reference habitat (Northwest Reference) were surveyed (Figure 2.1) (Ogdahl and Steinman, 2015). In addition to the 2018 macrophyte survey, epiphytic algae communities on the macrophyte species *Vallisneria americana* were sampled at each of the three habitats.

2.2 Field protocols

A full description of the macrophyte survey methodology is included in Ogdahl and Steinman (2015). Briefly, macrophyte surveys took place in August during 2009-2012 and from July 16th-23rd in 2018. Sampling in 2018 occurred one month earlier than previous sampling events to accommodate the project's timeline. At each of the three survey locations (hereafter referred to as transects), a transect was established perpendicular to the shoreline and separated into standard distance categories for sampling. Transect lengths were determined by the farthest point of macrophyte growth defined as the last site of macrophyte presence before: (1) two consecutive sites with no presence, or (2) the absence of macrophytes at a site greater than 4.5 m deep, which is the maximum growth depth for macrophytes in Muskegon Lake (Ogdahl and Steinman, 2015). Restoration effects were expected to extend past the initial restoration location regardless of transect length, justifying the inclusion of the entire transect length during surveying. At each transect site, macrophyte taxa relative abundance (0-100%) was determined and overall plant cover was determined using a 0-4 ranking system: 0 = Bare; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; or 4 = 76–100%. Water depth also was measured at each transect site (Ogdahl and Steinman, 2015).

Macrophyte biomass and sediment organic matter (OM) were determined at one randomly chosen site within each selected category: 0-20 m from shore, 20-50 m, 50-100 m, 200-300 m, 300-400 m, 400-500 m, etc. (Ogdahl and Steinman, 2015). Randomly chosen sites were determined in 2009 and the same general locations were sampled for all survey years.

The same field personnel conducted surveys in 2009-2012 to exclude interpersonal variation; however, 2018 surveys were conducted by new personnel due to staff changes.

Epiphytic algae were not sampled in 2009-2012 but were collected as part of the 2018 macrophyte survey. Epiphytic algae were collected from the macrophyte *V. americana,* a subdominant and ubiquitous macrophyte in Muskegon Lake. *V. americana*'s long leaf blades with simple morphology made this taxon a better candidate for ensuring full removal of the epiphytic algal community instead of the dominant *Ceratophyllum demersum,* which contains morphologically complex and brittle leaflets. Along each transect, one site at a depth of 1 m was selected for sampling, where *V. americana* leaf blades almost reached the water's surface. Ten *V. americana* plants were randomly sampled from a 10 m-diameter area, approximately 5 m in any direction of the boat. The top 20 cm of each macrophyte was removed and placed on ice for transport to the lab for epiphytic algae analyses. Due to variation in bathymetric slope among the three transects, sampling sites were located at different distances from the shoreline to maintain the ~1 m water depth.

Physical and chemical variables also were measured at each epiphytic algae collection site. Water quality variables (DO, NTU, pH, T, SC, ORP) were measured with a YSI 6600 sonde. NTU was determined at all sites along each transect and averaged for the entire transect. A 1 L water sample was collected for analysis of water column soluble reactive phosphorus (SRP), total phosphorus (TP), total kjeldahl nitrogen (TKN), and nitrate (NO_3^-). Light intensity of photosynthetically active radiation (PAR) was measured using a Li-Cor quantum sensor. PAR measurements were used to quantify the light extinction coefficient.

2.3 Laboratory processing

Macrophyte, water, and sediment samples were refrigerated until processing. Macrophyte biomass was cleaned of sediment and *Dreissena* spp. mussels, and then dried at 85 °C for 96 hrs to determine plant dry weight. Dry weight also was determined for *V. americana* segments used for epiphytic algal removal. Sediments were then ashed at 550 °C for 4 hrs to determine OM concentrations (%), considered the difference between pre- and post-combustion sediment weights (Ogdahl and Steinman, 2015).

For the epiphytic algae survey, SRP, TKN, and NO_3^- subsamples were filtered through 0.45 µm acid washed filters. TP was digested with persulfate and stored at 4 °C. SRP, TP, and TKN were analyzed using a SEAL AQ2 discrete automated analyzer (APHA, 1998) and NO_3^-/NO_2^- was analyzed with ion chromatography on a Dionex ICS-2100 (APHA, 1998). For epiphytic algae removal, both sides of the *V. americana* blades were scrubbed with a toothbrush, and toothbrush bristles and leaf blades were rinsed with distilled water. Each macrophyte had its own respective toothbrush to limit epiphytic algae community contamination between samples. Photos of leaf blades were taken in order to determine macrophyte surface area for epiphytic algae colonization using ImageJ software (Schneider et al., 2012).

An aliquot of the toothbrush-removed epiphytic algae was used to determine Chl-*a* concentrations by filtering the sample through a GF/F filter (Whatman®) and freezing at −18 °C. Within 30 days of freezing, filters were ground and steeped in 90% buffered acetone for 24 hours in the dark. After centrifuging, Chl-*a* was analyzed using a Shimadzu UV-1601 spectrophotometer (Steinman et al., 2017). A 50 mL subsample of epiphytic algae was preserved with 1% Lugols solution and used for non-diatom algae identification in a Palmer-

Maloney nanoplankton counting chamber. Permanent slides for diatom identification were created by: boiling 10 mL of the 50 mL subsample in 30% hydrogen peroxide with potassium permanganate for 1 hour; performing a series of distilled water rinses to remove oxidation byproducts; evaporating the sample onto a coverslip; and mounting coverslips onto a microscope slide using heat and Naphrax©. All algae were identified to genus using a Nikon H550L Eclipse 80i light microscope.

2.4 Data analyses

Macrophyte total taxa richness was calculated for each transect and survey year. Grass and tree species recorded along transects were excluded from richness values. Filamentous green algae and *Chara* spp. were included in macrophyte biomass calculations during the previous survey and therefore included in this study's richness values; filamentous green algae were treated as one taxon for richness calculations. Macrophyte density (g/m^2) and mean cover rank were determined for each transect and survey year (Ogdahl and Steinman, 2015). For this study, the State of Michigan's Coefficient of Conservatism (C-value) was applied to each collected taxon; a range from 0-10 represented the probability a species will occur within an undisturbed habitat. Taxa with a C-value=0 were either invasive or more likely to be found in highly degraded habitat while C-value=10 indicated taxa were more likely to be found in an ecologically healthy habitat, similar to pre-European settlement conditions (Bourdaghs et al., 2006). Mean C-values were determined for each transect per survey year and a mean C-value was determined for each restoration state: reference, pre-restoration, and post-restoration.

Hydrologic exposure, defined as bathymetric slope (%) and WI, was calculated for each transect and survey year. WI was calculated using a modified approach from Keddy (1982).

Fetch was measured at the origin and end of each transect along the four cardinal and four ordinal directions. The percentage of time (% frequency) wind speed exceeded 19.3 kmph was determined for each direction and summed. Transect origin and end WI were then averaged for each transect during all five survey years (Ogdahl and Steinman, 2015). Low WI values indicated protection from wind and wave action. Additional environmental variables were calculated for each survey year: total precipitation and mean air temperature during the growing season, and the change in Lake Michigan water level (WL) compared to the long-term mean (1917-2018) (CO-OPS, 2019; NCEI, 2019).

In addition to the macrophyte density, richness, and cover rank variables included in Ogdahl and Steinman's (2015) statistical analyses, macrophyte taxon-weighted relative abundance and total biomass were included in this study. Weighted relative abundance incorporated percent abundance and site cover rank into its value to increase the accuracy of an individual taxon's dominance within a transect. This way, a taxon with higher cover ranks contributed more towards the overall transect abundance than a taxon with lower cover ranks. To calculate weighted relative abundance, an individual taxon's relative abundance (0-100%) at a certain site was multiplied by its corresponding cover rank (0-4). The sum of all site-weighted relative abundances along a transect were then divided by the sum of all cover ranks in the transect to get a taxon's transect weighted relative abundance. Macrophyte total biomass (kg) per transect was calculated by multiplying transect total density by the total transect area.

Ogdahl and Steinman (2015) analyzed differences in physical variables (WI, slope, and OM) among transects using one-way ANOVAs. In this study, restoration state was added as a categorical variable to ANOVA analyses along with 2018 data; differences in physical parameters among transects and restoration states were tested using a type II two-way ANOVA with the Anova() function, a part of the *car* package in R (Fox and Weisberg, 2011). When significance was detected, a pairwise t-test with Bonferroni correction was used. Restoration states were defined as: (1) reference, which included 2009-2012 and 2018 data collected from Northwest Reference, (2) pre-restoration, which included 2009 and 2010 data collected from both restored transects, and (3) post-restoration, which included 2011, 2012, and 2018 data collected from both restored transects. Differences in the biological variables (total density, total biomass, total richness, and mean cover rank) among transects and restoration states also were tested using type II two-way ANOVAs and post-hoc pairwise t-tests. Normality was tested using Shapiro-Wilk, variance was tested using Levene's Test of equal variance, and all data were square-root transformed.

Principal component analyses (PCA) were performed to assess restoration-induced changes within the biological and environmental data, which incorporated prior survey years and the 2018 survey year. Biological data (total density, total biomass, total richness, and mean cover rank) and environmental data (OM, slope, total precipitation, air temperature [T], WI, transect length [L], and WL) were examined in separate PCAs among survey years (5 years: 2009-2012, 2018), transects (3 transects: Northwest Reference [NWRef], Heritage Landing [HertL], and Grand Trunk [GrandT]), and restoration states (3 states: pre-restoration, post-restoration, and reference). Relationships between biological and environmental data were evaluated using regression analysis, consistent with Ogdahl and Steinman (2015) analyses.

Epiphytic algal cell density (cells/mm^2) was determined using algae counts and the volume of the Palmer Maloney nanoplankton counting chamber. Differences among transects for Chl-*a* concentrations and cell density (cells/mm^2) were tested using Kruskal-Wallis and a post-hoc Wilcoxon test with Bonferroni correction. Normality was tested using Shapiro-Wilk, variance was tested using Levene's Test of equal variance, and data were square-root transformed. Relationships between biological metrics (Chl-*a* and density) and environmental data (DO, ORP, pH, T, NTU, PAR, light extinction, WI, SC, Depth, TP, SRP, NO_3^-, and TKN) were evaluated using regression analysis. All statistical analyses were performed using R version 3.5.2 (R Core Team, 2017).

3. Results

3.1.1 Macrophytes: environmental variables

In 2018, Northwest Reference's transect length (defined as the last site with macrophytes before two consecutive sites with plant absence or a site greater than 4.5 m deep) was the shortest compared to 2009-2012, while both restored transects remained the same length from 2012 to 2018, despite increased water depths (Table 2.1); indeed, compared to 2012, mean water depth increased between 0.44 m (Northwest Reference) and 0.69 m (Heritage Landing) (Table 2.1).

Slope was steepest for all transects in 2018 compared to survey years 2009-2012; regardless of year, Heritage Landing had a significantly steeper slope than Northwest Reference or Grand Trunk ($p_{\text{Transect}} < 0.001$, 2-way ANOVA, Table 2.1). In 2018, WI for Northwest Reference was lowest (i.e., low hydrologic exposure, 317.16) while Heritage Landing (44.62) and Grand Trunk (117.03) had intermediate WI compared to previous survey years (Table 2.1). Among all survey years, WI was significantly greatest at Northwest Reference and lowest at Heritage Landing ($p_{\text{Transect}} < 0.001$, 2-way ANOVA, Table 2.1), although WI values declined substantially at all three transects between 2012 and 2018. OM was greatest for Heritage Landing (9.37%) and Grand Trunk (25.47%) in 2018 and lowest for Northwest Reference (0.62%) in 2018 during the five survey years (Table 2.1). OM was significantly greatest at Grand Trunk and lowest at Northwest Reference for all survey years ($p_{\text{Transect}} = 0.002$, 2-way ANOVA). Differences among restoration state and interactions for slope, OM, and WI were not significant (2-way ANOVA, Table 2.1).

Mean T in 2018 (17.6 °C) fell within the previous range (16.0-19.1 °C, Table 2.A.1). 2018 precipitation accumulation (47.19 cm) was the second highest recorded over the five years (30.18-53.52 cm, Table 2.A.1). 2009 mean water level (-0.09 m) was below the long-term mean and continued decreasing through 2012 (-0.27 m, -0.30 m, and -0.43 m, Table 2.A.1). However, the mean water level in 2018 was much higher than the long-term mean (+0.47 m). Water level increase was consistent with deeper transect water depths in 2018.

For the environmental PCA, WL, slope, L, and WI had the most explanatory power with the first two PC axes explaining 65.7% of the dataset's variation (Figure 2.2A). Along the PC1 axis, transect clusters were clearly separated, with slope, L, and WI having strong effects (Figure 2.2B). Along the PC2 axis, environmental data strongly separated clusters by survey year (Figure 2.2C). Survey years 2009, 2011, and 2018 were associated with higher WL, greater precipitation, higher OM content, and lower T. Sampling years 2010 and 2012 were associated with high T and lower total precipitation, OM, and WL. The restoration state reference cluster separated from the pre- and post-restoration clusters (Figure 2.2D).

3.1.2 Macrophytes: biological factors and community composition

Northwest Reference's macrophyte richness was significantly greater than Heritage Landing's, with intermediate richness at Grand Trunk among survey years ($p_{\text{Transect}} = 0.04$, 2-way ANOVA, Figure 2.3A). Post-restoration, richness at all transects increased between 2011 and 2012, and then decreased in 2018 (Figure 2.3A). Macrophyte biomass also was significantly greatest at Northwest Reference, lowest at Heritage Landing, and intermediate at Grand Trunk ($p_{\text{Transect}} = 0.05$, 2-way ANOVA, Figure 2.3B). Both macrophyte biomass and density increased at all transects between 2011 and 2012. However, responses varied across transects in 2018 (although not significantly); both values declined at Heritage Landing but increased at Northwest Reference and Grand Trunk (Figures 2.3B and 2.3C). Macrophyte cover rank also increased in 2018 at Northwest Reference and Grand Trunk, whereas Heritage Landing's cover rank decreased (Table 2.1).

For the biological PCA, the first two PC axes explained 81.5% of the dataset's variation and macrophyte density and richness had the greatest explanatory power (Figure 2.4A). Transect cluster Northwest Reference was associated with greater macrophyte richness, cover, and biomass while the Heritage Landing cluster was associated with lower macrophyte values (Figure 2.4B). Survey year clusters were superimposed on one another with slight separation of the 2011 cluster, associated with low macrophyte density and biomass (Figure 2.4C). Restoration state clusters also did not separate from one another (Figure 2.4D).

Macrophyte taxonomic composition was more similar between the restored transects than with the reference transect, and compared to 2009 to 2012 observations, Northwest Reference experienced a greater composition change in 2018 compared to Heritage Landing and Grand Trunk. *Typha* spp. (*augustifolia* x *glauca*, and *latifolia)*, *V. americana,* and *Phragmites australis* were abundant at Northwest Reference from 2009 to 2012, but were rare or absent in 2018, when they were replaced by *Wolffia* spp. and *Nymphaea* spp. (Figure 2.5). In contrast, *Ceratophyllum demersum* was the most abundant macrophyte at both restored transects in all years, with Heritage Landing containing a greater relative abundance of *Elodea* spp. and Grand Trunk containing a greater relative abundance of *V. americana*.

C-values for all three transects increased from 2012 to 2018, although increases were not statistically significant due to high variance (Table 2.A.2). Comparing the Northwest Reference 2018 C-value (4.22) to those of the restored transects, Grand Trunk's C-value (4.19) almost reached reference quality standards; however, Heritage Landing's C-value (3.56) was lower than reference standards (Table 2.A.2). For restoration states, reference (4.08) had a C-value greater than pre- (3.70) and post-restoration (3.71).

3.1.3 Macrophytes: biological and environmental interactions

The strongest correlations occurred between macrophyte density and richness with WI and slope. Macrophyte richness was positively correlated with WI and negatively correlated with slope (Table 2.2). Macrophyte density was positively associated with WI and negatively associated with precipitation and slope. When comparing environment variables together, WI had significantly negative correlations with slope and OM.

3.2 Epiphytic algae: environmental and biological variables

During the July 2018 sampling period, Heritage Landing had the greatest PAR at a 0.5 m depth and corresponding lowest light extinction (i.e., high-light availability) and NTU of the three transects (Table 2.A.3). WI was greatest at Northwest Reference (i.e., high wind and wave exposure), lowest at Heritage Landing, and intermediate at Grand Trunk. Grand Trunk had the greatest NO_3^- and TKN concentrations and the highest SC compared to the other two transects. Sampling depth also was greatest at Grand Trunk followed by Heritage Landing and then Northwest Reference; sampling depth was used as a surrogate for water level. For 2018, epiphytic algal Chl-*a* concentrations ($p < 0.001$, Kruskal Wallis, Figure 2.6A) and density ($p < 0.001$, Kruskal Wallis, Figure 2.6B) on *V. americana* were highest at Heritage Landing followed by Grand Trunk and Northwest Reference.

When comparing biological and environmental variables, Chl-*a* was not associated with N or P concentrations but did correlate with PAR, light extinction, and T (Table 2.3). Chl-*a* and DO were positively correlated at the time of sampling, which is expected given that algae evolve DO during photosynthesis. Epiphytic algal Chl-*a* was significantly positively correlated with SC and NTU and negatively correlated with WI. Epiphytic algal density was significantly negatively correlated with WI and light extinction and positively correlated with PAR (Table 2.3). Although not significant, water level was negatively correlated with density ($p = 0.81$, $R^2 = -0.002$) and positively correlated with Chl-*a* ($p = 0.20$, $R^2 = 0.06$).

4. Discussion

4.1 Macrophyte responses to restoration

The goal of continued transect monitoring was to determine the effectiveness of restoration in improving habitat quality, which involved the separation of macrophyte community responses to restoration from responses due to annual environmental variation. As noted in Ogdahl and Steinman (2015), macrophyte community spatial and temporal trends were dually associated with physical habitat (i.e., WI, transect length, and slope) and hydrologic characteristics (i.e., water level and precipitation), respectively, making it difficult to discern macrophyte responses to restoration. By 2018, however, we have seen a minimal net increase in post-restoration habitat quality at Heritage Landing and Grand Trunk compared to the reference transect. Declines in restored transect macrophyte biomass and density in 2011 may have been influenced by initial restoration disturbance, visible in the biological PCA. Habitat quality (C-value) increases from 2012 to 2018 at Heritage Landing and Grand Trunk compared to little change at Northwest Reference suggests that restoration has, in part, positively impacted restored transect macrophyte communities.

When examining restored transects separately, Heritage Landing and Grand Trunk's macrophyte responses were associated with the time since, and nature of, restoration. Restoration during April 2011 at Heritage Landing included the physical removal of underwater fill material along the sampling transect; this may account for low macrophyte density and biomass at Heritage Landing in 2011 and the subsequent habitat quality decrease in 2012. Less disruptive restoration at Grand Trunk in June 2010, adjacent to the sampling transect, may have influenced the less drastic macrophyte density and biomass declines in 2011 and subsequent increases in density, biomass, and habitat quality in 2012 and 2018.

Physical habitat similarities between Grand Trunk and Northwest Reference compared to Heritage Landing for all survey years may have facilitated Grand Trunk macrophyte community biological variables in reaching reference-quality standards. Gentle slopes and longer transect lengths at Grand Trunk and Northwest Reference likely increased habitat availability and heterogeneity (i.e., depth and light regimes) for different macrophyte morphologies, promoting increases in macrophyte richness, density, and biomass at those two transects (Duarte and Kalff, 1990). The Heritage Landing site was protected from wind and wave action in a shielded embayment while Grand Trunk and Northwest Reference were partially or not protected by an embayment, respectively, increasing their WI values. High WI and sediment OM at Grand Trunk and Northwest Reference may have increased habitat for less competitive macrophyte species (Wilson and Keddy, 1986) and prevented monodominance, also increasing macrophyte biomass and richness compared to Heritage Landing. This may explain why richness and biomass were positively correlated with WI in Muskegon Lake despite the usual negative correlation found in other lentic systems (Duarte and Kalff, 1986; Riis and Hawes, 2003).

Although environmental variables naturally shift, extreme precipitation events, temperature changes, and water level fluctuations are predicted to increase in frequency due to climate change (Havens and Steinman, 2013; Notaro et al., 2015). Water level increases can reduce light availability for macrophytes (Chow-Fraser et al., 1998) and precipitation can increase dissolved organic carbon loading, reducing light transmittance (Chen et al., 2016). Muskegon Lake macrophytes responded to increased water level or precipitation (2009, 2011, and 2018) with a decrease in macrophyte richness, especially evident during the 1 m water level rise from 2012 to 2018, when the emergent macrophytes *P. australis* and *Typha* spp. were absent at Grand Trunk and Northwest Reference compared to previous survey years. Emergent macrophyte physiological requirements are more easily surpassed by rising water levels than other morphologies (Zohary and Ostrovsky, 2011), inhibiting emergent plant growth and seed germination (Coops and Van Der Velde, 1995; Hudon et al., 2005) and decreasing overall habitat richness.

A decrease in Heritage Landing's macrophyte biomass, density, and cover rank in 2018, while these variables increased at Grand Trunk and Northwest Reference, may again be a product of physical habitat characteristics. Heritage Landing's steep slope, potentially reducing light availability, may have decreased habitat optima for macrophytes, prompting a negative response to rising water levels. Positive macrophyte responses at Grand Trunk and Northwest Reference in 2018 were likely influenced by other unmeasured environmental variables and in part by restoration at Grand Trunk. Future water level rises may plausibly decrease macrophyte biomass, density, and cover rank at Grand Trunk and Northwest Reference as the quality of macrophyte growing conditions declines.

4.2 Epiphytic algal dynamics among transects

Besides water level and precipitation potentially reducing light availability to macrophytes, increases in epiphytic algal density (µg/cm^2) can damage their macrophyte host by decreasing photosynthetic rate or increasing leaf loss; epiphytic algal density differences among transects in Muskegon Lake may have been sufficient to negatively impact macrophytes (Asaeda et al., 2004). Greater epiphytic algal biomass and density on *V. americana* at Heritage Landing, with the lowest overall macrophyte community density and biomass, supported epiphytic algae as an inhibitor to macrophyte growth. Northwest Reference and Grand Trunk had similar low epiphytic algal densities and biomass levels where macrophyte communities were more dense; it is possible that yearly variation in epiphytic algal communities at Grand Trunk more closely followed the reference transect compared to Heritage Landing; however, further monitoring would be required.

In terms of environmental influences on epiphytic algae, WI increase was potentially responsible for dislodging epiphytic algae with weak attachment features from their host macrophyte (Keddy, 1982; Strand and Weisner, 1996; Strand and Weisner, 2001). A subsequent increase in light attenuation after algal detachment may explain greater macrophyte density and richness where WI was high, in contrast to the usual view that physical disturbance causes leaf breakage or plant uprooting, harming the macrophyte. The dominance of *Cocconeis* at Northwest Reference, an adnate-attached diatom that can withstand disturbance driven environments (Berthon et al., 2011; Biggs et al., 1998), supported hydrologic exposure as a main driver of epiphytic algal community structure. The lowest *Cocconeis* relative abundance occurred at Heritage Landing, which was instead dominated by the colonial diatom *Fragliaria* and filamentous *Bulbochaete*, which favor low-disturbance regimes (Berthon et al., 2011). A higher light extinction coefficient at Northwest Reference (i.e., low light availability), lower PAR at a 0.5 m depth, and greater turbidity also could have further reduced epiphytic algal biomass and density (Hillebrand, 2005; Steinman et al., 1992; Sultana et al., 2004). Grazing invertebrate presence may have impacted epiphytic algal community structure (Jones et al., 2002); however, this biological interaction was beyond the scope of our study.

4.3 Restoration impacts and future management implications

The benefits of shoreline recovery using macrophytes includes the repair of ecosystem services, the improvement of macroinvertebrate density and diversity, and the facilitation of fish community growth, thereby restoring littoral species interactions and food web structure (Brauns et al., 2011). Naturalized shoreline could also optimize lake esthetic appeal, influencing increases in lakeside property values (Leggett and Bockstael, 2000) and stimulating recreational usage through tourism and sport fishing (Campbell et al., 2015). Enhanced physical allure of restoration alone caused a projected $11.9 million housing increase within the Muskegon community (Isely et al., 2018). The improvement of repaired shoreline ecological integrity has also begun to stimulate economic growth for the local community and is calculated to generate six times the original cost of restoration ($10 million spent) (Isely et al., 2018).

Continued long-term monitoring has indicated that restored transects have improved in habitat quality; however, improvement was neither strong nor consistent, likely a result of substantial environmental changes among survey years. Based on our results, managers should anticipate variation in macrophyte response to restoration among habitats. In addition, monitoring timeframes should be extended if habitat recovery is delayed by changing environmental conditions. We recognize that restoration projects are subject to multiple pressures, with both funding and available locations being primary considerations. All things being equal, restoration projects should evaluate all potential habitats and choose locations with morphometric characteristics that optimize implemented improvements and can successfully adapt to climatic-scale variations (Lake, 2013). We suggest that habitats with gentle slopes, intermediate light availability (i.e., PAR and light extinction), and intermediate WI would be ideal for shoreline restoration, and would help mitigate epiphytic algal growth on their host macrophyte. Since shoreline projects often include multiple goals rarely centered around macrophytes, choosing habitats such as these will help maximize macrophyte community rejuvenation and effectively recover abiotic and biotic interactions within a littoral zone.

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Table 2.1: Transect length (m) (n = 15), mean (±SD) water depth (m) (n = 315) , bathymetric slope (%) (n = 15), WI (wind index) (n = 15), mean (±SD) OM (%) (n = 66), and mean (±SD) cover rank (n= 315) for the three Muskegon Lake transects over the five survey years. Type II two-way ANOVA results and post-hoc pairwise t-tests with Bonferroni correction are included for comparison of slope, WI, OM, and cover rank among transects. Asterisks on *P*-values for the two-way ANOVA results indicate significance $p < 0.05$ among transects for all survey years, for restoration state, or for their interaction. Superscripts on transect names indicate significant similarities or differences among transects.

		Pre-Restoration		Post-Restoration		
Variable	**Transect**	**2009**	**2010**	**2011**	**2012**	**2018**
Transect Length (m)	**NWRef**	650	800	750	650	600
	HertL	100	125	125	125	125
	GrandT	400	400	450	400	400
Water Depth (m)	**NWRef**	1.09 (1.08)	1.38 (1.46)	1.28 (1.34)	0.93 (1.17)	1.37 (0.96)
	HertL	2.05 (0.74)	2.14 (0.83)	2.27 (0.91)	1.93 (0.88)	2.62 (1.15)
	GrandT	0.82 (0.37)	0.81 (0.51)	1.06 (0.87)	0.59 (0.71)	1.27 (1.29)
Slope (%)	**NWRef** ^{a}	0.56	0.66	0.62	0.59	0.67
	HertL ^{b}	2.29	2.67	2.75	2.52	3.26
	GrandT ^{a}	0.44	0.67	0.89	0.81	1.04
P_{Transect} <0.001*	F=272.10	$P_{\text{Restoration}}$ =0.006*	F=11.55	$P_{\text{Interaction}}$ =0.31	F=1.15	*df*=1
WI	**NWRef** ^{a}	366.95	386.66	331.23	389.86	317.16
	HertL ^{b}	57.54	41.12	43.77	65.69	44.62
	GrandT ^{c}	136	112.39	104.19	154.91	117.03
P_{Transect} <0.001*	F=21.55	$P_{\text{Restoration}}$ =0.89	F=0.02	$P_{\text{Interaction}}$ =0.92	F=0.01	*df*=1
OM (%)	**NWRef** ^{a}	1.23 (1.58)	1.18 (1.60)	2.24 (5.85)	0.85 (0.37)	0.62 (0.32)

	HertL[b]	5.26 (5.87)	7.74 (4.79)	8.41 (5.21)	6.77 (4.21)	9.37 (2.93)
	GrandT[c]	22.03 (15.10)	25.38 (21.01)	15.93 (14.22)	25.39 (24.67)	25.47 (24.85)
P_{Transect} =0.002*	F=15.43	$P_{\text{Restoration}}$ =0.85	F=0.04	$P_{\text{Interaction}}$ =0.56	F=0.26	*df*=1
Cover Rank	**NWRef**	2.67 (1.82)	2.42 (1.79)	2.33 (1.28)	2.93 (1.43)	3.53 (1.03)
	HertL	3.58 (1.59)	3.23 (1.48)	2.77 (1.36)	3.00 (1.35)	2.53 (1.20)
	GrandT	2.77 (1.27)	3.64 (0.73)	3.57 (0.90)	3.27 (0.88)	3.63 (1.24)
P_{Transect} =0.21	F=1.77	$P_{\text{Restoration}}$ =0.62	F=0.62	$P_{\text{Interaction}}$ =0.46	F=0.57	*df*=1

Table 2.2: Linear regression results for significant correlations between variables for the macrophyte survey. Negative signs in front of R^2 values indicate a negative correlation between the two variables. Variable abbreviations are the same as in Table 2.1.

Regression	R^2	P	F	Df
Biological vs. Environmental:				
Richness vs. WI	0.55	0.002	16.01	13
Richness vs. Slope	-0.47	0.005	11.38	13
Density vs. WI	0.46	0.005	11.27	13
Density vs. Precipitation	-0.33	0.02	6.52	13
Density vs. Slope	-0.26	0.05	4.58	13
Biological vs. Biological:				
Biomass vs. Density	0.85	<0.001	75.45	13
Environmental vs. Environmental:				
Slope vs. WI	-0.64	<0.001	23.08	13
OM vs. WI	-0.26	0.05	4.49	13

Table 2.3: Linear regression results for significant correlations between environmental and biological variables for the *V. americana* epiphytic algae survey. Negative signs in front of R^2 values indicate a negative correlation between the two variables. Variable abbreviations are the same as in Table 2.1 along with chlorophyll-*a* (Chl-*a*), photosynthetically active radiation (PAR), dissolved oxygen (DO), total phosphorus (TP), and light extinction coefficient (Light Extinction).

Regression	R^2	P	F	Df
Chl-*a* vs. PAR	0.55	<0.001	34.12	28
Chl-*a* vs. Light Extinction	-0.65	<0.001	51.42	28
Chl-*a* vs. Temperature	0.31	0.001	12.8	28
Chl-*a* vs. DO	0.38	<0.001	16.95	28
Chl-*a* vs. Specific Conductance	0.36	<0.001	15.68	28
Chl-*a* vs. Turbidity	0.44	<0.001	22.3	28
Chl-*a* vs. WI	-0.69	<0.001	61.82	28
Density vs. PAR	0.43	<0.001	7.05	28
Density vs. Light Extinction	-0.28	0.002	11.12	28
Density vs. Temperature	0.35	<0.001	14.85	28
Density vs. DO	0.38	<0.001	16.92	28
Density vs. WI	-0.34	<0.001	14.73	28
Density vs. TP	0.16	0.03	5.44	28
Density vs. pH	0.16	0.03	5.41	28

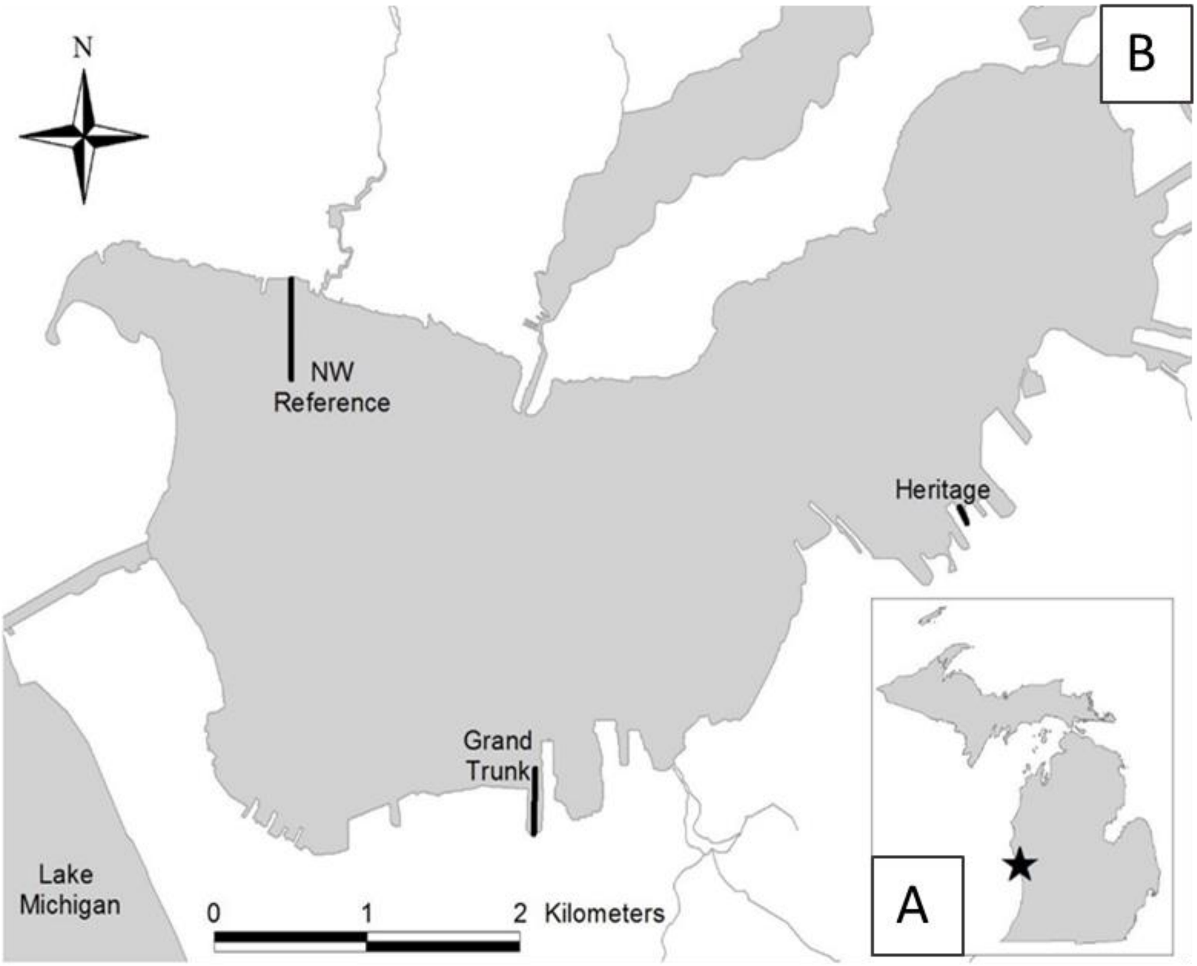

Figure 2.1: (A) Map of the state of Michigan with the location of Muskegon Lake indicated by a black star. (B) Muskegon Lake with the three macrophyte survey transects indicated by black lines showing the length of each transect. Restored transects (Grand Trunk and Heritage Landing) are perpendicular to the southern shoreline and Northwest Reference is perpendicular to the northern shoreline.

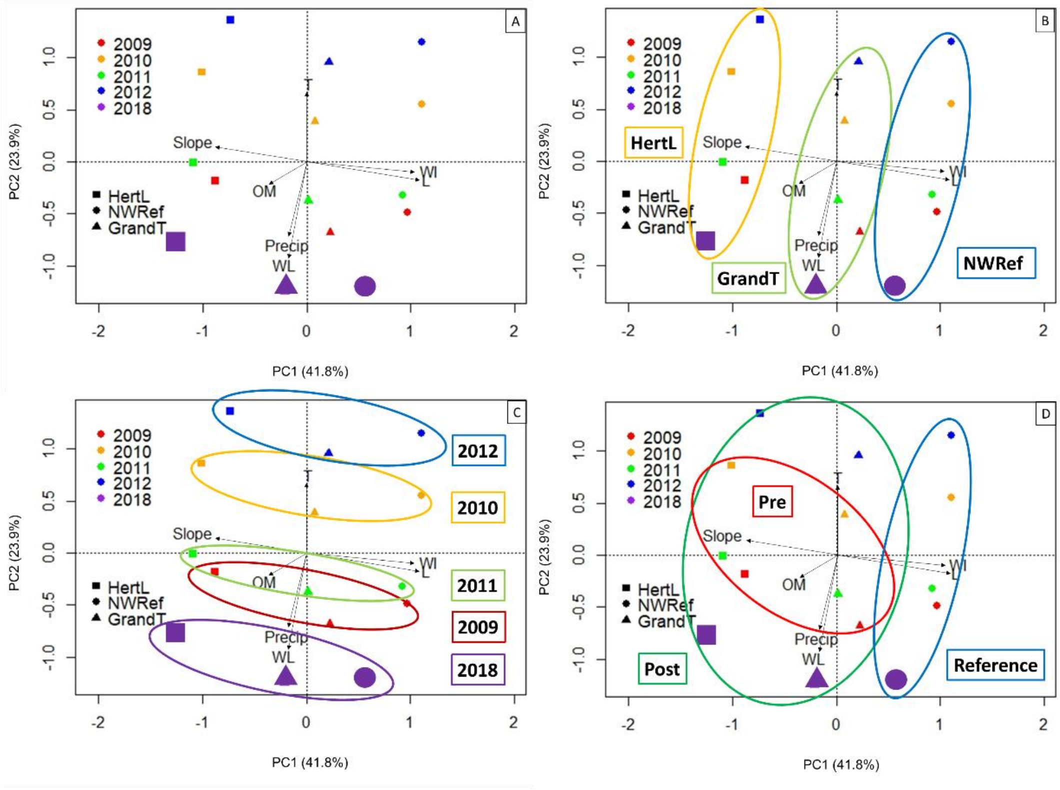

Figure 2.2: (A) A PCA biplot of environmental data (sediment organic matter [OM], annual total precipitation [Precip], air temperature [T], slope, wind index [WI], transect length [L], and water level relative to the long-term mean [WL]) where 41.8% and 23.9% of the data are explained by PC1 and PC2, respectively. Symbol shapes represent the different transects (NWRef, HertL, and GrandT) and colors represent the sampling years pre- (2009 and 2010) and post-restoration (2011, 2012, and 2018). Each symbol represents one transect per survey year. Vector length is positively correlated with each variable's explanatory power in the dataset. (B) Environmental data clustered by transect (blue = NWRef, yellow = HertL, and green = GrandT). (C) Environmental data clustered by survey years (red = 2009, orange = 2010, green = 2011, blue = 2012, and purple = 2018). (D) Environmental data clustered by restoration state (blue = reference transect, red = pre-restoration, and green = post-restoration). 2018 symbols (purple) intentionally increased in size to differentiate from other years.

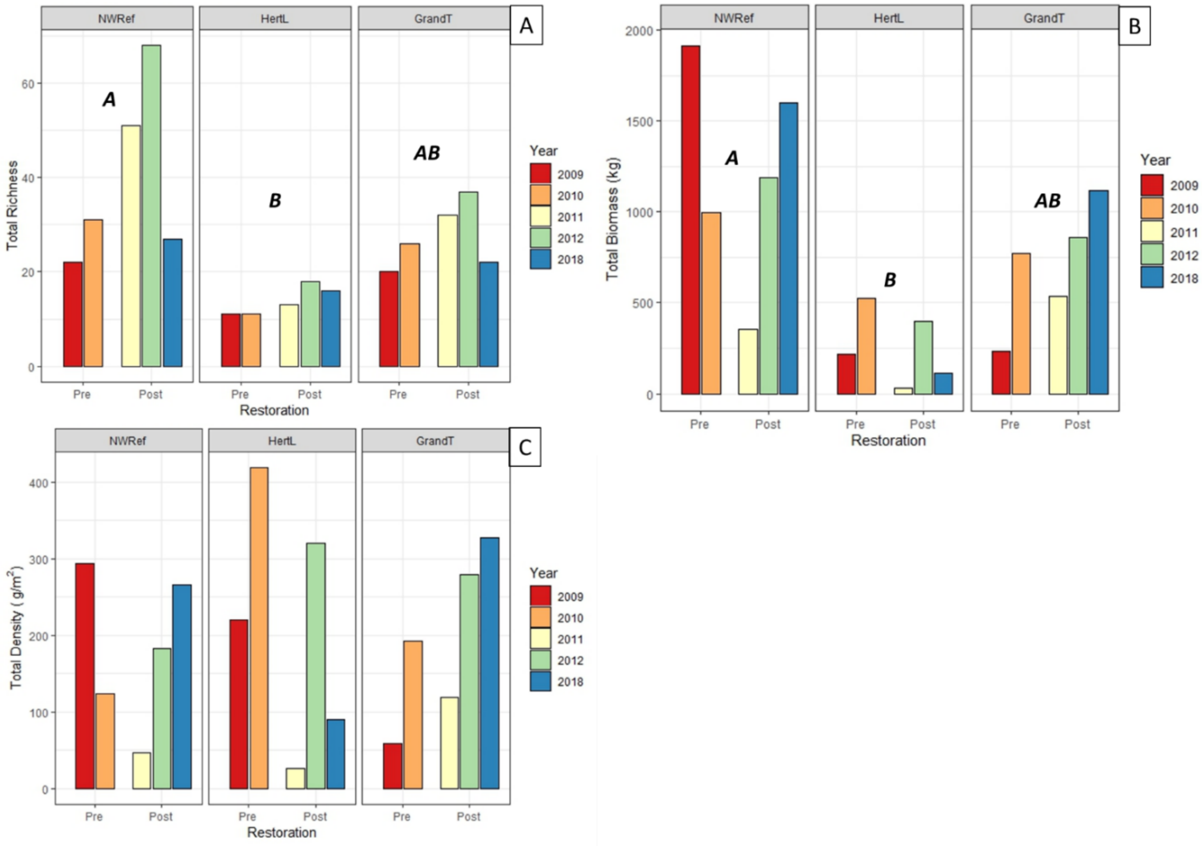

Figure 2.3: Macrophyte biological variables at each transect pre- (2009 and 2010) and post-restoration (2011, 2012, and 2018). Each bar represents the sum of a biological variable at all sampled sites along each transect per survey year. Letters above groupings represent statistically significant differences among transects (Two-way ANOVA). (A) Macrophyte total richness at each transect for all five survey years (n = 315). (B) Macrophyte total biomass (kg) at each transect for all five survey years (n = 66). (C) Macrophyte total density (g/m^2) at each transect for all five survey years (n = 66).

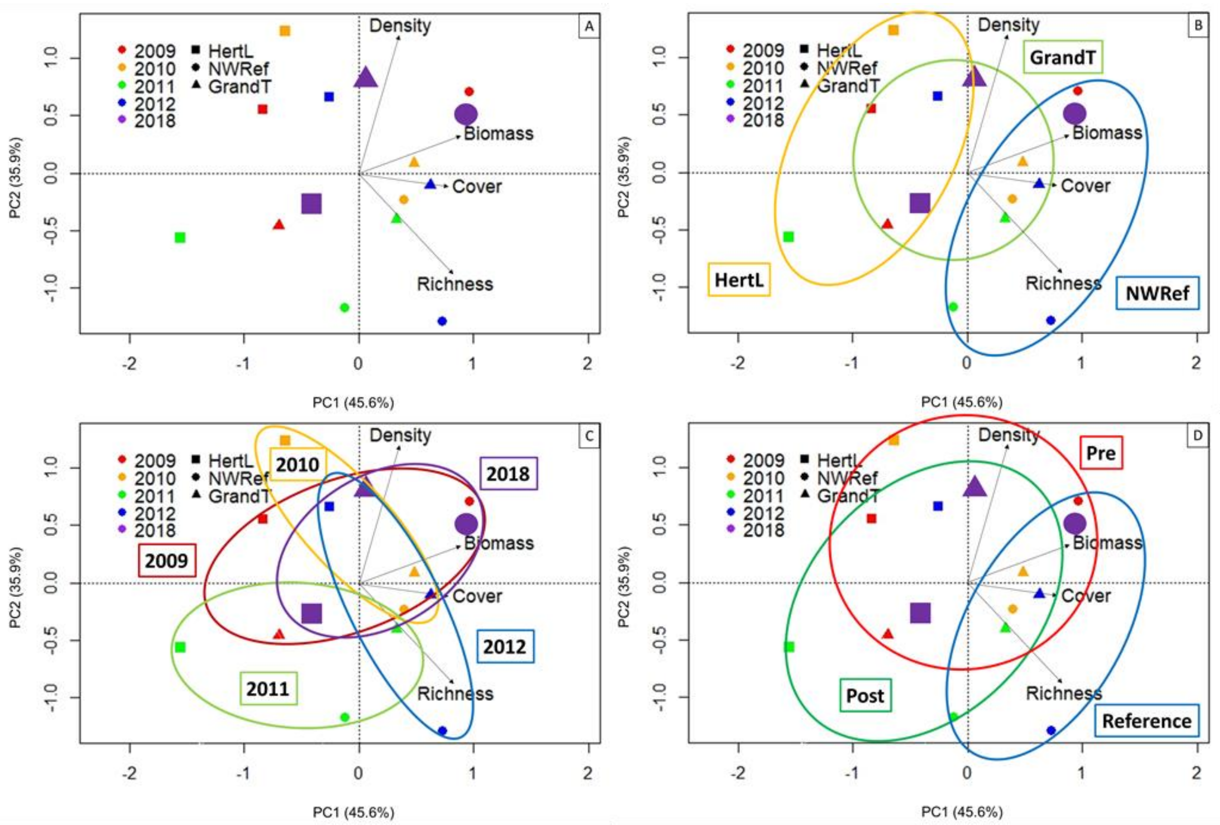

Figure 2.4: (A) A PCA biplot of biological data (macrophyte cover, total density, total biomass, and total richness) where 45.6% and 35.9% of the data are explained by PC1 and PC2, respectively. Symbol shapes represent the different transects (NWRef, HertL, and GrandT) and colors represent the sampling years pre- (2009 and 2010) and post-restoration (2011, 2012, and 2018). Each symbol represents one transect per survey year. Vector length is positively correlated with each variable's explanatory power in the dataset. (B) Biological data clustered by transect (blue = NWRef, yellow = HertL, and green = GrandT). (C) Biological data clustered by survey years (red = 2009, orange = 2010, green = 2011, blue = 2012, and purple = 2018). (D) Biological data clustered by restoration state (blue = reference transect, red = pre-restoration, and green = post-restoration). 2018 symbols (purple) intentionally increased in size to differentiate from other years.

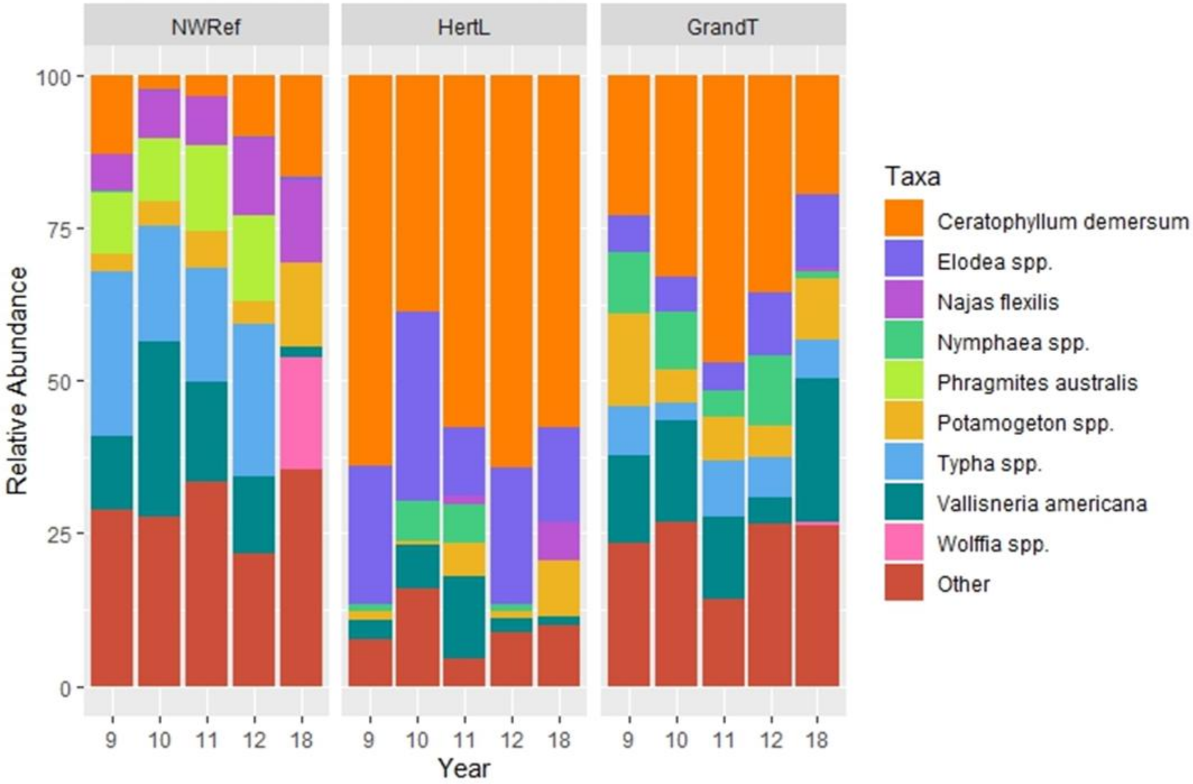

Figure 22.5: A stacked bar plot of macrophyte taxa weighted relative abundance changes within the five-year survey among all three transects. Each stacked bar represents the average relative abundance of represented macrophyte species at sites along each transect per survey year (NWRef, n = 140; HertL, n = 64; GrandT, n = 111).

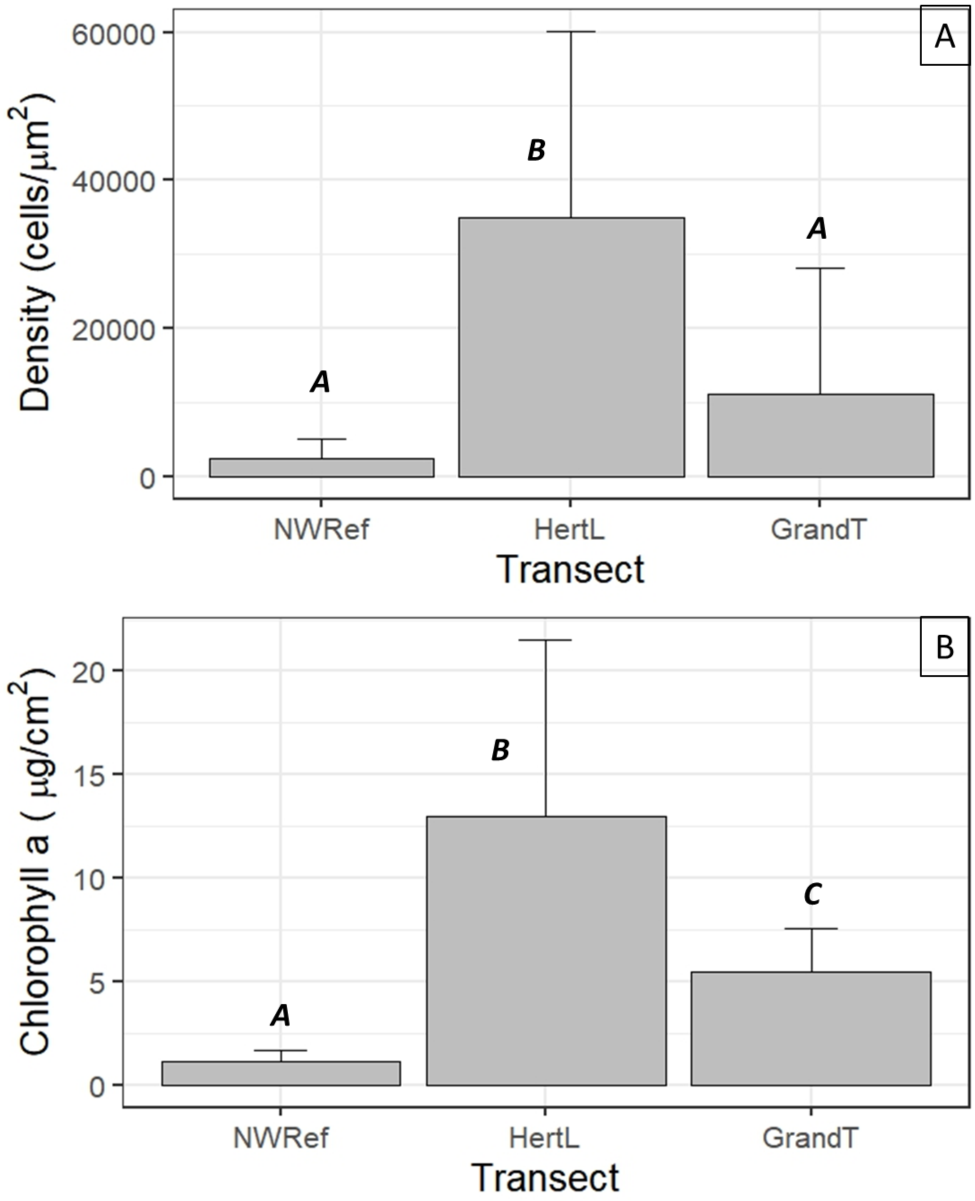

Figure 2.6: Epiphytic algae biological variables at each of the three transects in 2018. One standard deviation above the mean is provided. Letters above transects represent statistically significant differences among transects ($p<0.001$, Kruskal Wallis). (A) Mean epiphytic algae chlorophyll-a (µg/cm^2) concentrations (n = 30). (B) Mean epiphytic algae density (cells/mm^2) (n = 30).

Appendix A. Supplementary Material

Table 2.A.1: Total precipitation (cm), mean (±SD) air temperature (°C), and change in Lake Michigan water level (m) relative to the long-term Lake Michigan mean for all five survey years during the growing season (April through August).

Variable	2009	2010	2011	2012	2018
Total Precipitation (cm)	36.5	38.15	53.52	30.18	47.19
Air Temperature (°C)	16.0 (6.6)	19.1 (7.0)	17.8 (7.5)	18.8 (7.6)	17.6 (8.7)
Change in Lake Michigan Water Level (m)	-0.09	-0.27	-0.30	-0.43	0.47

Table 2.A.2: Mean (±SD) coefficient of conservatism for each survey year among all three transects. To examine restoration states, mean (±SD) C-values are provided for the reference transect, pre-restoration, and post-restoration.

Year	NWRef	HertL	GrandT
2009	3.63 (2.67)	3.77 (2.35)	3.75 (2.77)
2010	4.45 (3.10)	3.58 (2.47)	3.96 (2.76)
2011	3.90 (2.79)	3.67 (2.50)	3.38 (2.78)
2012	4.21 (2.66)	3.13 (2.56)	3.93 (2.69)
2018	4.22 (2.71)	3.56 (2.39)	4.19 (2.73)

Reference	**Pre-Restoration**	**Post-Restoration**
4.08 (2.78)	3.70 (2.61)	3.71 (2.54)

Table 2.A.3: Physical and chemical variables collected at each of the three transects in correspondence with the 2018 epiphytic algae survey that occurred between 2:00 and 4:00 pm on July 16th, 2018 for Heritage Landing, July 19th, 2018 for Northwest Reference, and July 23rd, 2018 for Grand Trunk. Mean (±SD) turbidity was determined from all sites along each transect. Abbreviations include dissolved oxygen (DO), redox potential (ORP), total phosphorus (TP), photosynthetically active radiation (PAR), nitrate (NO_3), and total kjeldahl nitrogen (TKN).

Variable	**NWRef**	**HertL**	**GrandT**
DO (mg/L)	9.64	12.33	9.02
ORP (mV)	63.3	69.4	34.2
pH	8.83	9.04	8.40
Temperature (°C)	25.88	30.08	24.29
Turbidity (NTU)	16.63 (33.40)	3.90 (2.70)	11.85 (33.79)
TP (mg/L)	0.022	0.023	0.020
PAR (µmol/s/m^2)	122.0	961.1	161.7
Light Extinction	5.76	1.78	2.49
Wind Index	317	45	117
NO_3^- (mg/L)	0.104	0.110	0.15
TKN (mg/L)	0.748	0.728	1.104
Specific Conductance (µS/cm)	341	351	355
Depth (m)	0.8	0.9	1.2

Chapter 3

Response of *Vallisneria americana* and Its Epiphytic Algal Community to Different Sources of Nutrient Enrichment

Acknowledgments

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Abstract

Nitrogen (N) and phosphorus (P) are essential for macrophyte and epiphytic algal growth and influence community composition changes. Primary producer group responses to nutrients may be influenced by direct or indirect access to a nutrient source, impacting resource competition among primary producer groups. To assess the importance of different nutrient sources, a macrophyte (*Vallisneria americana* [Michx.]) and its epiphytic algae were experimentally exposed to different sources enriched with N and P during a one-month indoor mesocosm experiment, and their interactions and biotic responses were measured. Nutrients were manipulated to create four different treatments: 1) addition to sediment porewater (S); 2) addition to water column (WC); 3) addition to both the sediment and water column (B); and 4) no nutrient additions--control (C). We hypothesized that the greatest macrophyte and epiphytic algal biomass increases would occur in the S and WC treatments, respectively, and that both groups' biomass would be greater in the non-control treatments compared to the control.

Unexpected phytoplankton biomass increases, presumably due to water column enrichment, altered light availability and our predicted macrophyte-epiphyte responses; *V. americana*'s biomass was greatest in the C treatment, where there was less phytoplankton and hence more light availability for the macrophyte, and epiphytic algal biomass did not vary significantly among treatments. Enriched porewater was as equally important for sustaining macrophyte and epiphytic algal biomass as water column nutrients, especially when phytoplankton presence decreased light availability and elevated nutrient competition. Even with an indirect negative association between phytoplankton and epiphyte biomass and density, epiphytic algae still displayed an adverse impact on their host macrophyte.

Keywords: macrophytes, epiphytic algae, nutrient source, nitrogen and phosphorus, mesocosm experiment, Muskegon Lake

Introduction

Macrophytes are essential for maintaining high water quality conditions in shallow water ecosystems, partially through substrate stabilization and nutrient cycling (Allen, 1971; Wigand et al., 2000), which can be especially important for mitigating phytoplankton growth (Phillips et al., 1978; Phillips et al., 2016). Additionally, macrophyte presence is critical for aquatic organisms, as they provide habitat for epiphytic algae, which help sustain the food web’s energy base, contribute to nutrient cycling, and fix carbon (Allen, 1971; Dodds, 2003; Vadeboncoeur and Steinman, 2002).

Epiphytic algal proximity to their host macrophyte forms a physical barrier between the water column and plant; this may reduce macrophyte oxygen and carbon uptake, obstruct dissolved nutrient transport, and shade the macrophyte’s surface (Sand-Jensen and Borum, 1991; Schneider et al., 2012a). Macrophyte influences on epiphytic algae may be positive or negative; the host plant may release nutrients available for epiphytic algae or release harmful allelopathic chemicals (Burkholder, 1996; Erhard and Gross, 2006). These interactions therefore could impact the success of both primary producer groups, especially when they are in competition for nutrients and light (Allen, 1971; Eminson and Moss, 1980).

Unlike terrestrial ecosystems (Vitousek et al., 2010) where primary producers obtain a majority of their nutrients such as nitrogen (N) and phosphorus (P) (Elser et al., 2007) from the soil, aquatic primary producers obtain a majority of their nutrients from the sediment and/or the water column. Indirect or direct access to a nutrient source may stimulate resource competition in environments where multiple primary producer groups have direct access to the same source (Wear et al., 1999). Conversely, access to multiple sources may result in higher cumulative autotrophic biomass and diversity due to complementary use of nutrients from direct and indirect sources by differing groups and species, irrespective of ecosystem type (cf. Bracken and Stachowicz, 2006; Gross et al., 2007; Passy and Larson, 2019). Additionally, aquatic primary producers (algae or macrophytes) located higher in the periphyton matrix or macrophyte canopy may have preferential access to water column nutrients, subsequently reducing light availability to organisms located beneath them or outcompeting understory organisms for light (Sand-Jensen and Borum, 1991).

Direct access to water column enrichment may stimulate macrophyte and epiphytic algal growth; however, phytoplankton resource interception resulting in shading of epiphytic algae and macrophytes, along with epiphytic algal resource interception resulting in shading of the macrophyte, may counteract this positive response (Madsen and Cedergreen, 2002; Chambers et al., 1989). Where sediment nutrients are enriched, rooted macrophytes have the competitive advantage, since epiphytic algae only indirectly access sediment nutrients leaching from the macrophyte host or through diffusion across the sediment-water interface (Burkholder, 1996; Périllon and Hilt, 2019; Vadeboncoeur and Steinman, 2002). Since sediment is not a direct nutrient source for epiphytic algae, studies rarely include this location when examining macrophyte-epiphytic algal responses to elevated nutrient concentrations (Song et al., 2015).

Macrophytes and epiphytic algal responses to nutrient enrichment make them commonly used bioindicators of ecosystem health and trophic level (Blanco et al., 2004; Schneider and Lindstrøm, 2011; Uzarski et al., 2017). In Muskegon Lake, MI, macrophytes were included in a littoral restoration project (2010-2011); the recovery and improvement of macrophyte communities were evaluated through pre-restoration (2009 and 2010) and post-restoration surveys (2011, 2012, and 2018) (Ogdahl and Steinman, 2015). Physical habitat variables and hydrologic conditions were measured alongside the macrophyte community to assess the variation in site responses to restoration. However, the interactions between macrophytes and their epiphytic algae were not examined. Since these interactions have been shown to influence both individual macrophyte and community success, a separate survey of both macrophytes and their epiphytic algae took place in 2018.

To complement the Muskegon Lake 2018 survey, a controlled experiment was designed to better understand how macrophyte and epiphytic algae proximity influences both primary producer groups' responses to direct and indirect nutrient sources. The main objective was to determine how *Vallisneria americana* (Michx.) and its epiphytic algal community respond to four treatments containing different sources of nutrient enrichment: 1) addition to the sediment porewater (S); 2) addition to the water column (WC); 3) additions to both the water column and sediment (B); 4) a control with no nutrient additions (C).

We hypothesized that both *V. americana* and epiphytic algal biomass would increase in the nutrient-enriched treatments compared to the C treatment. We also predicted that *V. americana* biomass increase would be greatest in the S treatment due to the macrophyte's competitive advantage for sediment nutrients coupled with reduced epiphytic algal loads, allowing higher light levels to reach leaf surfaces (Barko et al., 1991; Burkholder and Wetzel, 1990; Sand-Jensen and Borum, 1991). In contrast, the greatest epiphytic algal biomass was hypothesized to occur in the WC treatment where the epiphytic algae have direct nutrient access, resulting in less light and nutrients reaching the host macrophyte (Dodds, 2003; Sand-Jensen and Borum, 1991).

Since we anticipated a rapid turnover of epiphytic algal species in response to changing environmental conditions during the experiment, we hypothesized that treatments with nutrient enrichment, especially the WC and B treatments, would result in community shifts to genera with higher nutrient optima and filamentous growth forms, which have greater access to nutrients outside the epiphytic algal boundary layer (Steinman et al. 1992). Epiphytic algal community structure in all four treatments was also compared with the community structure found on *V. americana* growing naturally in Muskegon Lake.

Methods

Mesocosm set-up

This experiment was conducted using a complete randomized block design (Figure 3.1) and the four nutrient treatments (WC, S, B, and C) were randomly assigned to twelve indoor, 1325 L mesocosm tanks, three mesocosms per treatment type. For nutrient enrichment, N as sodium nitrate ($NaNO_3$) and P as potassium phosphate (KH_2PO_4) were added at 10x the ambient concentrations found within Muskegon Lake (Table 3.1). Mesocosm water column nutrient concentrations were based on July 2016 and 2017 Muskegon Lake long-term monitoring data; ambient concentrations were 0.016 mg/L of soluble reactive P (SRP) (10x concentration increase: 0.16 mg/L of SRP) and 0.15 mg/L of nitrate (NO_3^--N) (10x concentration increase: 1.5 mg/L of NO_3^--N). Mesocosm sediment nutrient concentrations were based on three replicate sediment cores (4 cm diameter, 10 cm deep) taken at all three transects (Figure 3.A1) during the 2018 Muskegon Lake survey, using a hand-held gravity corer (Davis and Steinman, 1998); ambient sediment concentrations were 0.041 mg/L of SRP (10x concentration increase: 0.41 mg/L of SRP) and 0.21 mg/L of NO_3^--N (10x concentration increase: 2.1 mg/L of NO_3^--N).

Each mesocosm was populated with eight buckets, a single macrophyte planted per bucket. Four buckets contained a single live *V. americana* and the other four contained a single artificial aquarium plant resembling *V. americana*; the artificial plants served as structural controls for epiphytic algae. *V. americana* macrophytes were purchased from the Carolina

Biological Supply Company and buckets were randomly placed into mesocosms to limit possible bias. Each live and artificial plant was potted in a 7.6 L bucket containing homogenized, 2 mm sieved sediment collected from the Heritage Landing transect in Muskegon Lake (Figure 3.A1), and autoclaved at 121 °C for 40 min. One submersible pump per mesocosm facilitated water circulation and aeration during the experiment. Each mesocosm was lit with a single metal halide bulb, set on a 15/9 L:D photoperiod cycle, and room temperature was maintained at 22 °C. Mesocosms were filled with ~950 L of Muskegon Lake water that was filtered through a 300 µm-mesh to remove zooplankton and large debris.

Mesocosm experiment

V. americana were received on July 11th, 2018, immediately placed in mesocosms containing submersible pumps, and given two weeks to recover from transport and acclimate to new environmental conditions. Next, epiphytic algae were removed from a variety of Muskegon Lake macrophyte taxa using a toothbrush to produce a large volume of inoculum. 5 L of homogenized epiphytic algae inoculum was added into each mesocosm along with artificial plants with pumps in operation; two weeks were given for epiphytic algal colonization. The mesocosm experiment ran for one month starting on September 15th, 2018 and ending on October 15th, 2018. All mesocosm surfaces, sediment surfaces, and bucket sides were cleaned weekly of periphyton and 90% of each mesocosm's water volume was replaced with filtered Muskegon Lake water to mitigate phytoplankton growth. Water column nutrients were surface broadcasted into the WC and B treatments as a liquid solution on day 1 of the experiment and restocked weekly after mesocosms had been refilled with new Muskegon Lake water. 15 mL perforated centrifuge tubes containing N- and P-enriched agar were inserted into the sediment for sediment nutrient enrichment; agar was created in the lab to ensure N- and P-enrichment. One nutrient-filled centrifuge tube was placed in the sediment adjacent to each plant in the S and B treatments on experiment day 1. Unamended centrifuge tubes also were added to the C and WC treatments to control for any tube-induced effects in the S and B treatments.

Water quality variables (dissolved oxygen [DO], turbidity [NTU], pH, temperature [T], specific conductance [SC], redox potential [ORP], and total dissolved solids [TDS]) were measured daily at approximately 1700 hrs with a YSI 6600 sonde. 1 L water samples were collected from each mesocosm every week to determine concentrations of water column SRP, total phosphorus (TP), total kjeldahl nitrogen (TKN), and NO_3^-, after which the removed water sample volume was replaced in the mesocosm. Light intensity of photosynthetically active radiation (PAR) was measured once a week in each mesocosm using a Li-Cor quantum sensor. PAR measurements at different mesocosm depths were used to quantify the light extinction coefficient. After visually observing high phytoplankton accumulation in certain mesocosms, phytoplankton chlorophyll-*a* (Chl-*a*) concentrations were measured weekly (see below) for the last three weeks of the experiment. At the end of the experiment, one sediment core was collected from each macrophyte bucket to determine porewater SRP, porewater NO_3^-, sediment TP, and sediment TKN. Entire macrophytes were removed and used for processing.

Muskegon Lake survey

A full description of Muskegon Lake as a study site and associated shoreline restoration activities can be found in Ogdahl and Steinman (2015). Briefly, Muskegon Lake is a drowned river mouth lake located in central West Michigan that connects directly to Lake Michigan (Figure 3.A1). Three littoral habitats were chosen for epiphytic algae surveys: two restored habitats along the south shoreline (Heritage Landing and Grand Trunk) and one reference habitat (Northwest Reference) along the north shoreline. *V. americana*, a subdominant macrophyte in Muskegon Lake, was selected for collection due to its ribbon-like leaf blades and simple morphology that made full removal of the epiphytic algae community possible.

Surveys took place between 1400 and 1600 hrs at Heritage Landing on July 16th, 2018, at Northwest Reference on July 19th, 2018, and at Grand Trunk on July 23rd, 2018. At each habitat, the transect to be sampled was separated into sites starting from shore. One site along each transect with a depth of ~1 m was chosen for *V. americana* collection where ten individual plants were randomly sampled from a 10 m-width area, approximately 5 m in any direction from the boat. The top 20 cm of each macrophyte was removed and placed on ice for transport to the lab for epiphytic algae removal; a proportion of each *V. americana* was used to limit epiphytic algae processing time. Water quality variables were measured with a YSI 6600 sonde and irradiance was measured using a Li-Cor quantum sensor at each site. A 1 L water sample was collected for SRP, TP, NO_3^-, and TKN analysis. Random macrophytes also were collected along the entire transect and used to create the mesocosm experiment's algae inoculum.

Laboratory processing

Macrophytes, sediment cores, and water samples were refrigerated until processing. Macrophyte fragments from Muskegon Lake and whole plants from the mesocosm experiment were scrubbed with a toothbrush to remove epiphytic algae, and toothbrush bristles and leaf blades were rinsed with distilled water. One toothbrush was used per macrophyte to limit epiphytic algal contamination between samples. Blades were microscopically examined to confirm removal of adnately attached algae. Leaf blade photos were taken to determine macrophyte surface area for calculating epiphytic algae density using ImageJ software (Schneider et al., 2012b). Live *V. americana* segments and whole plants were then dried for 96 hours at 85 ˚C and weighed to determine plant biomass (g).

Sediment samples were homogenized and centrifuged for 20 min at 3600 rpm to separate the supernatant, and porewater was filtered through a 0.45 µm acid washed filter for porewater SRP and NO_3^- analysis. Remaining sediments were then dried for 24 hours at 105 ˚C, subsampled for sediment TKN analysis, ashed at 550˚C for 4 hours, and then used to determine sediment TP and sediment organic matter (OM) content. Sediment OM (%) was expressed as the difference between pre-and post-combustion weights. Water column SRP, TKN, and NO_3^- subsamples were filtered through 0.45 µm acid washed filters. Water column TP was digested with persulfate and stored at 4 °C. All SRP, TP, and TKN samples were analyzed using a SEAL AQ2 discrete automated analyzer (APHA, 1998) and NO_3^- was analyzed with ion chromatography on a Dionex ICS-2100 (APHA, 1998).

An aliquot of the epiphytic algal samples from Muskegon Lake and the mesocosm experiment was used to determine chlorophyll-*a* (Chl-*a*) concentrations by filtering the sample through a 0.45 µm GF/F filter (Whatman®) and freezing at −18 °C. Within 30 days of freezing, filters were ground and steeped in 90% buffered acetone for 24 hours in the dark. After centrifuging, Chl-*a* was analyzed using a Shimadzu UV-1601 spectrophotometer (Steinman et al., 2017). A 50 mL subsample of epiphytic algae was preserved with 1% Lugols solution and used for non-diatom algae identification in a Palmer-Maloney nanoplankton counting chamber. Permanent slides for diatom identification were created by: boiling 10 mL of the 50 mL subsample in 30% hydrogen peroxide with potassium permanganate for 1 hour; performing a series of distilled water rinses to remove oxidation byproducts; evaporating the sample onto a coverslip; and mounting coverslips onto a microscope slide using heat and Naphrax©. All algal samples were identified to genus using a Nikon H550L Eclipse 80i light microscope.

Data analyses

Differences in environmental variables (SRP, TKN, TP, NO_3^-, pH, DO, light extinction [Light], NTU, T, ORP, SC, TDS, porewater SRP [PoreSRP], porewater NO_3^- [PoreNO3], sediment TP [SedTP], sediment TKN [SedTKN], and phytoplankton Chl-*a* [Wchla]) among the four treatments were tested using Kruskal-Wallis. When significance was detected, a Wilcoxon test post-hoc with Bonferroni correction was used. Data were log-square root transformed, normality was tested using Shapiro-Wilk, and variance was tested using Levene's Test of equal variance.

Almost all live *V. americana* in the mesocosm experiment produced ramets attached to the original plant through horizontal rhizome growth; ramets are hereby referred to as propagules. Therefore, the original live *V. americana* macrophyte used for epiphytic algae removal was weighed by itself and then all propagules per original plant were weighed together for a total of two biomass (g) values: individual *V. americana* biomass and total *V. americana* biomass including the weight of all propagules. Measuring the individual *V. americana* biomass separate from the total *V. americana* biomass (including propagules) allowed for the later comparison of individual *V. americana* biomass with its epiphytic algae community's biological variables through regression analyses. The number of propagules per live *V. americana* also was included in the analyses as propagule number. Total length (cm) of the original *V. americana* was measured from the end of the root system to the longest leaf blade. The lengths of

V.americana roots and shoots were measured separately, then divided to determine macrophyte length root:shoot ratio.

Differences in mean total biomass, mean individual biomass, length, and propagule number for the live *V. americana* among the four treatments (control, sediment, water column, and both) were tested using a one-way ANOVA with the aov() function in R. When statistical significance was detected, a pairwise t-test with Bonferroni correction was used. Differences in *V. americana* length root:shoot ratios among the four treatments were tested using a Kruskal-Wallis test and if significant, a post-hoc Wilcoxon test with a Bonferroni correction was used. Normality was tested using Shapiro-Wilk, variance was tested using Lavene's Test of equal variance, and all data were log transformed.

Relative abundance (%) of each epiphytic algae genus and total epiphytic algal richness were determined for each live *V. americana* and artificial macrophyte in all four treatments. Epiphytic algae cell density (cells/mm^2) was determined using algal counts and the volume of the Palmer Maloney nanoplankton counting chamber. Epiphyton diversity was measured using the Shannon diversity index:

$$H' = -\sum p_i * \ln (p_i) \quad (2)$$

Equation 3.1: H' is the Shannon diversity index and p_i is the proportion of the community composed of species *i*.

Differences in the epiphytic algae biological variables (Chl-*a,* density, richness, and diversity) among the four treatments (control, sediment, water column, and both) and between plant types (live *V. americana* and artificial macrophyte) were tested using a two-way nested

ANOVA with the aov() function in R. When significance was detected, a pairwise t-test with Bonferroni correction was used. Normality was tested using Shapiro-Wilk, variance was tested using Levene's Test of equal variance, and all data were log transformed.

A non-metric multidimensional scaling (NMDS) ordination was run to examine clustering among treatments (control, sediment, water column, and both) using epiphytic algae genera relative abundance. Environmental data (SRP, TKN, TP, NO_3^-, pH, DO, Light, NTU, T, ORP, SC, TDS, PoreSRP, PoreNO3, SedTP, SedTKN, and Wchla) were overlaid onto the NMDS using the envfit() function, a part of the vegan package in R (Oskanen et al., 2018). Adonis was used to evaluate significant differences among treatments and between plant types. A SIMPER post-hoc determined which epiphytic algae genera contributed to the greatest amount of variation among the four treatments.

Relationships between live *V. americana* biological variables (total biomass, individual biomass, total length, length root:shoot ratio, and propagule number) and epiphytic algae biological variables (Chl-*a,* density, richness, and diversity) on the live *V. americana* for the mesocosm experiment were evaluated using regression analysis. Relationships between live *V. americana* biological variables (total biomass, individual biomass, total length, length root:shoot ratio, and propagule number) and phytoplankton Chl-*a* along with all environmental data also were evaluated with regression analysis. Relationships of epiphytic algae biological variables on both plant types (Chl-*a,* density, richness, and diversity) with phytoplankton Chl-*a* and all environmental data were evaluated with regression analysis.

All four epiphytic algae biological variables (Chl-*a*, density, richness, and diversity) on Muskegon Lake's *V. americana* were determined for each of the three sampled transects

(Northwest Reference, Heritage Landing, and Grand Trunk). Differences in the epiphytic algae total richness and diversity among transects were tested using a one-way ANOVA and a pairwise t-test post-hoc with Bonferroni correction. Chl-*a* concentrations and cell density (square-root transformed but not normally distributed) were tested using Kruskal-Wallis and a Wilcoxon test post-hoc with Bonferroni correction. Normality was tested using Shapiro-Wilk and variance was tested using Levene's Test of equal variance. All statistical analyses were performed using R version 3.5.2 (R Core Team, 2017).

Results

Mesocosm physical and chemical variables

Water temperature was similar among most treatments but slightly higher in the B treatment compared to the other treatments ($p < 0.001$, Kruskal-Wallis, Table 3.2). However, environmental conditions in treatments receiving nutrients through the water column (i.e., WC and B) were very different than those in the S and C treatments. Phytoplankton Chl-*a* was greater in the WC and B treatments compared to the S and C treatments, likely contributing to higher NTU, light extinction coefficient, DO concentrations, and pH in the WC and B treatments ($p < 0.001$, Kruskal-Wallis). In contrast, TDS and SC were greater in the C and S treatments than in the WC and B treatments ($p < 0.001$, Kruskal-Wallis). As anticipated, water column SRP, TP, NO_3^-, and TKN were all greater in the WC and B treatments compared to the S and C treatments ($p < 0.001$, Kruskal-Wallis). Porewater SRP was greater in the B treatment than in the C ($p = 0.02$, Kruskal-Wallis), and porewater NO_3^- was greater in the WC and B treatments than in the S and C, at the time of sampling ($p < 0.001$, Kruskal-Wallis).

Macrophyte biological variables

Total live *V. americana* biomass ($p = 0.004$, 1-way ANOVA, Figure 3.2a) and individual live *V. americana* biomass (i.e., no propagules) ($p = 0.07$, 1-way ANOVA, Figure 3.2b) were greater in the C treatment than the WC treatment, although individual biomass was not significant. *V. americana* length root:shoot ratio also was greatest in the C treatment but lowest in the B treatment ($p = 0.04$, Kruskal-Wallis, Figure 3.2c). Total *V. americana* length ($p = 0.40$, 1-way ANOVA, Figure 3.2d) and propagule number ($p = 0.10$, 1-way ANOVA, Figure 3.2e) were greater in the B and WC treatments compared to the S and C, although not statistically significant.

Epiphytic algae biological variables and community structure

No evident trends for epiphytic algal Chl-*a* occurred among treatments or plant types ($p_{Treatment} = 0.21$, $p_{Type} = 0.83$, 2-way nested ANOVA, Figure 3.3a). Epiphytic algal density was greater on artificial macrophytes compared to live *V. americana*, although this difference was driven largely by the C treatment ($p_{Treatment} = 0.13$, $p_{Type} = 0.002$, 2-way nested ANOVA, Figure 3.3b). Epiphytic algal richness was greater in the C and S treatments compared to the B treatment ($p_{Treatment} < 0.001$, $p_{Type} = 0.22$, 2-way nested ANOVA, Figure 3.3c). Epiphytic algal diversity was greatest in the S treatment and lowest in the B treatment ($p_{Treatment} < 0.001$, $p_{Type} = 0.46$, 2-way nested ANOVA, Figure 3.3d).

85 epiphytic algal genera were identified from both plant types and all four treatments (Figure 3.4). Most genera were from the Bacillariophyta (diatom) phylum (58%) followed by Chlorophyta (21%), Cyanobacteria (15%), Euglenophyta (3%), Cryptophyta (2%), and Charophyta (1%). Epiphytic algae relative abundances varied among the four treatments:

cyanobacteria *Phormidium* and *Cylindrospermum* dominated the C treatment; the S treatment contained the greatest relative abundance of chlorophyte *Mougeotia*; the WC treatment contained the greatest proportions of diatoms, specifically *Fragilaria* and *Achnanthidium*; and the B treatment also was dominated by *Phormidium* (Figure 3.4). Within each treatment, epiphytic algae community structure was relatively similar between live *V. americana* and artificial macrophytes.

Within the NMDS biplot, epiphytic algae relative abundance was ordinated in a horizontal gradient (Figure 3.5a). Treatment clusters suggested differences in epiphytic algae community structure (p = 0.001, F = 8.28, Adonis, Figure 3.5b). The C and S treatments overlapped and were associated with increased sediment nutrients as well as ORP, TDS, and SC. The WC and B treatments were more closely associated with high water column nutrients, DO, pH, phytoplankton Chl-*a*, NTU, and light extinction. Clustering by plant type was not significant (p = 0.78, F = 0.52, Adonis).

Nine epiphytic algal genera explained ~90% of the variation among treatments (SIMPER, Table 3.3). *Lyngbya* was the main driver of dissimilarity across treatments, with the highest relative abundance in the B treatment followed by the C, WC, and S (Table 3.3). High relative abundance of *Cylindrospermum* in the C treatment, *Mougeotia* in the S treatment, *Achnanthidium* and *Fragilaria* in the WC treatment, and *Fragilaria* in the B treatment also were strong contributors to the dissimilarity of each treatment from all other treatments.

Biological and environmental interactions

Most regressions between live *V. americana* and epiphytic algae vs. environmental variables were statistically significant but with low explanatory power (Table 3.A1). Water column nutrient enrichment was positively associated with individual *V. americana* biomass (SRP: $R^2 = 0.13$; $p < 0.05$) and propagule number (e.g., NO_3^-: $R^2 = 0.19$; $p < 0.05$; SRP: $R^2 = 0.10$; $p < 0.05$). In contrast, *V. americana* root:shoot ratio was negatively correlated with both nutrient enrichment sources (e.g., PoreNO_3^-: $R^2 = -0.20$; $p < 0.005$; SRP: $R^2 = -0.20$; $p < 0.005$). Light extinction was positively associated with propagule number but negatively associated with length root:shoot ratio. Total *V. americana* biomass was negatively associated with NTU. Sediment nutrient enrichment was positively but weakly correlated with epiphytic algal Chl-*a* (e.g., PoreNO_3^-: $R^2 = 0.05$; $p < 0.05$) and density (e.g., PoreSRP: $R^2 = 0.07$; $p = 0.01$; PoreNO_3^-: $R^2 = 0.05$; $p < 0.05$). Epiphytic algal diversity (e.g., PoreNO_3^-: $R^2 = -0.21$; $p < 0.001$) and richness (e.g., NO_3^- and SRP: $R^2 = -0.20$; $p < 0.001$) were negatively correlated with both nutrient enrichment sources and light extinction. In general, most regressions involving phytoplankton Chl-*a* had much higher R^2 values compared to the regressions involving macrophytes and epiphytic algae (Table 3.A1). Phytoplankton Chl-*a* was positively correlated with both nutrient enrichment sources (e.g., NO_3^-: $R^2 = 0.81$; $p < 0.001$), light extinction ($R^2 = 0.96$; $p < 0.001$), and NTU.

In terms of biological regressions, epiphytic algal density was positively correlated with individual *V. americana* biomass ($R^2 = 0.13$; $p < 0.05$) and negatively correlated with total length ($R^2 = -0.13$; $p < 0.05$), and length root:shoot ratio ($R^2 = -0.20$; $p < 0.01$). Epiphytic algal richness and diversity were negatively associated with total *V. americana* length and positively associated with *V. americana* length root:shoot ratio. Phytoplankton Chl-*a* was negatively associated with *V. americana* length root:shoot ratio, epiphytic algal diversity, and epiphytic algal richness, whereas it was positively associated with propagule number ($R^2 = 0.18$; $p < 0.01$).

Muskegon Lake environmental variables and epiphytic algae

In Muskegon Lake, DO, ORP, pH, T, NTU and TP were greatest at Heritage Landing followed by Northwest Reference and then Grand Trunk (Table 3.4). Light extinction was greatest at Northwest Reference and lowest at Heritage Landing. NO_3^- and TKN were greatest at Grand Trunk and lowest at Heritage Landing. Water depth and SC also were greatest at Grand Trunk but lowest at Northwest Reference. A cumulative 1 m water level rise in Lake Michigan from 2012 to 2018 increased water depth in Muskegon Lake, subsequently increasing this variable's contribution to in-lake community changes.

Epiphytic algal Chl-*a* ($p < 0.001$, Kruskal-Wallis, Figure 3.6a) and density ($p < 0.001$, Kruskal-Wallis, Figure 3.6b) on *V. americana* were greatest at Heritage Landing followed by Grand Trunk, then Northwest Reference. Epiphytic algal diversity ($p < 0.001$, 1-way ANOVA, Figure 3.6c) and richness ($p = 0.03$, 1-way ANOVA, Figure 3.6d) were greatest at Grand Trunk compared to the other two transects; richness was significant but pairwise post-hoc results were not.

68 algae genera on *V. americana* were identified from all three Muskegon Lake transects, with most from the Bacillariophyta phylum (56%) followed by Chlorophyta (23%), Cyanobacteria (14%), and Charophyta (4%). Most epiphytic algae present at the Muskegon Lake transects were also present in the four treatments. However, the dominant Muskegon Lake genera were different from those in the mesocosms (Figure 3.7). For example, *Cocconeis* and *Bulbochaete* were abundant at the Muskegon Lake transects but not in the mesocosm treatments, whereas *Achnanthidium* and *Mougeotia* were abundant in the mesocosm

treatments but not at the Muskegon Lake transects. *Lyngbya*, *Phormidium*, and *Pseudanabaena* were present at the Muskegon Lake transects and in most mesocosm treatments.

Epiphytic algae comparisons

Epiphytic algal Chl-*a* (10.01-17.20 µg/cm^2, Figure 3.3a) and density (Figure 3.3b) were greater in the mesocosm experiment compared to Muskegon Lake (Chl-*a*: 1.11-12.98 µg/cm^2, Figure 3.6a; Figure 3.6b). Epiphytic algal richness (17-22, Figure 3.3c) and diversity (1.18-1.83, Figure 3.3d) on *V. americana* were slightly lower in the mesocosm experiment compared to Muskegon Lake (Richness: 18-24, Figure 3.6d; Diversity: 1.65-2.33, Figure 3.6c). In general, the mesocosms' environmental conditions and epiphytic algal community, particularly in the WC and B treatments, were most similar to those at the Heritage Landing transect, containing greater NTU, DO, pH, and epiphytic algal Chl-*a* and density. Epiphytic algal diversity and richness were highest in the S and C treatments for the mesocosm experiment, but lower than Grand Trunk's values, which were highest among the three Muskegon Lake transects. Lastly, the mesocosm experiment had a higher dominance of cyanobacteria taxa (Figure 3.4), while Muskegon Lake was dominated more by diatoms (Figure 3.7).

Discussion

Mesocosm conditions

Observed nutrient concentrations generally matched the expected concentrations for each treatment, except porewater SRP and NO_3^-. Autoclaving can increase the rate of sediment N and P release (Southwell et al., 2010) and inhibit microbial activity; however, microbial communities may reform over time, influencing nutrient release through mineralization or by changing environmental conditions such as increasing pH or DO (Tuominen et al., 1994).

Therefore, warmer water temperatures and oxygenated conditions along with microbial uptake could have lowered porewater SRP (Huang et al., 2011; Tuominen et al., 1994), and potential nitrifying bacteria could have elevated porewater NO_3^- (Henriksen et al., 1981). The possible presence of P-adsorbing metals such as iron oxide and manganese oxide could have influenced low porewater SRP (House and Denison, 2000; Li et al., 2010). Agar N and P concentrations for enrichment also may have been slightly off, influencing the porewater nutrient results.

Macrophyte and epiphytic algal responses

The original proposed hypotheses for *V. americana* and epiphytic algal biomass responses to nutrient sources were based off the assumptions that autotrophic growth form and physical location relative to the nutrient source would drive their competitive interactions, resulting in a macrophyte advantage when the enriched nutrient source was sediments but an epiphytic algal advantage when the enriched source was the water column. The unforeseen positive response of phytoplankton to water column nutrient enrichment likely had the dual effects of 1) reducing nutrient availability to macrophytes and their attached epiphytic algae due to phytoplankton uptake; and 2) reducing light availability to the macrophyte-epiphytic algal community (Sand-Jensen and Borum, 1991).

For *V. americana*, light limitation likely outweighed the predicted stimulatory effect of nutrient enrichment on *V. americana* biomass; phytoplankton Chl-*a* had a stronger positive correlation with nutrient enrichment than *V. americana* biomass, reinforcing the negative impacts of phytoplankton. Phytoplankton-induced light reductions also likely accounted for a greater total macrophyte biomass in the C treatment compared to the nutrient-enriched treatments. Low-light, especially in the WC and B treatments, may have induced *V. americana* propagule production to increase macrophyte survival rate in a disturbed habitat (Li et al., 2018); this could explain why there is a positive association between propagule number and water column enrichment when usually, propagule production decreases with elevated nutrients (Grace et al., 1993). For epiphytic algae, a lack of biomass (as Chl-*a*) and density variation among treatments was a potential product of phytoplankton's dual effects, however the positive association of epiphytic algal biomass and density with elevated nutrients supports the basis of our hypothesis (Carrick et al., 1988; Fairchild et al., 1985).

The influence of source of nutrient enrichment on *V. americana* was more evident when examining length root:shoot ratio. Macrophytes usually respond to nutrient increases by decreasing root:shoot ratios (Barko et al., 1991; Madsen and Cedergreen, 2001); all nutrient-enriched treatment length root:shoot ratios were lower than the C treatment, and ratios were negatively correlated with both nutrient sources. Increased length root:shoot ratios in the C treatment may be the result of root system biomass and surface area expansion to increase nutrient uptake efficiency (Barko et al., 1988; Barko et al., 1991; Madsen and Cedergreen, 2001). Water column enrichment (Dülger et al., 2017; O'Connell et al., 2015) and lower light availability (Cronin and Lodge, 2003) could have stimulated *V. americana* energy redirection towards shoot growth, decreasing length root:shoot ratios in the WC and B treatments compared to the S treatment.

Sediment enrichment served as an important nutrient source for both epiphytes and the macrophyte, regardless of direct or indirect access. Despite the water column nutrient addition in the B treatment, which also resulted in high phytoplankton growth and turbidity, the sediment nutrient addition was apparently sufficient to overcome potential light limitation for

V. americana (Barko and James, 1998), as *V. americana* biomass was not significantly different than the control. The compensating role of sediment as a nutrient source for *V. americana* was confirmed in the S treatment, where similar to B, biomass was not significantly different than the control. However, both the length root:shoot ratio and propagule number did vary between the S and B treatments, albeit not significantly, suggesting the role of sediments as a nutrient source is idiosyncratic, and may vary depending on the response variable, other nutrient sources, and environmental conditions (Chambers et al., 1989; Madsen and Cedergreen, 2002).

Sediment enrichment was considered an accessory nutrient source for epiphytic algae since sediment enrichment on its own was not enough to increase epiphytic algal biomass; even with comparable light availability, biomass was slightly lower in the S treatment than the C treatment. Enrichment at both sources in the B treatment may have compensated for the negative impacts of phytoplankton nutrient competition and light limitation on epiphytic algal biomass seen in the WC treatment; epiphytic algal biomass in the B treatment was greater than the C, WC, and S treatments.

Epiphytic algal communities were predicted to shift in algal growth form in the nutrient-enriched treatments to increase nutrient uptake efficiency; however, cell-stacking growth forms were dominant in all treatments. Both high-light availability and nutrient enrichment can increase filamentous or chain-forming algae (Steinman and McIntire, 1987; Steinman et al., 1989), accounting for this growth form in the C treatment where light was abundant. Nutrient-enriched treatment epiphytic algal communities shifted towards competitive genera with higher nutrient affinities, like *Phormidium* (Loza et al., 2014), and algal richness and diversity declined due to a decrease in habitat suitability for genera with lower nutrient optima like

Cylindrospermum (Carrick et al., 1988; Van der Grinten, 2004), which was found only in the C treatment. Light availability also influenced algal composition; low-light and nutrient-favoring *Achnanthidium* and *Fragilaria* (Berthon et al., 2011; Steinman et al., 1992) were most abundant in the WC treatment where turbidity was greatest, while high-light preferring *Mougeotia* was most abundant in the S treatment, where light requirements could be met (Lowe et al., 1986).

When examining macrophyte-epiphytic algal interactions, a lack of variation in algal biomass, richness, and diversity between plant types suggested *V. americana* was not actively influencing epiphytic algae (Cattaneo and Kalff, 1979; Grutters et al., 2017). Differences in epiphytic algal density between plant types was likely a function of surface area: live *V. americana* growth potentially reduced overall epiphytic algal density, whereas the static surface area of artificial macrophytes promoted a more dense, mature epiphytic algal coverage. Additionally, *V. americana* leaves experienced more physical drag due to water movement than artificial macrophytes, potentially facilitating epiphytic algal sloughing, which could have lowered algal density (Strand and Weisner, 1996).

Unexpectedly, *V. americana* biomass was significantly positively correlated with epiphytic algal density; however, we speculate again that this was likely due to phytoplankton response to nutrients. *V. americana*'s shoot growth response to low-light would bring attached epiphytic algae closer to the water's surface, ultimately benefiting epiphytic algal growth. An increase in epiphytic algal density could in turn negatively impact the host macrophyte (Sand-Jensen and Søndergaard, 1981), as seen with the negative association between both *V. americana* length and length ratio, vs. epiphytic algal density.

Muskegon Lake and experiment comparisons

Although similar to Muskegon Lake, epiphytic algal Chl-*a* and density were greater in the mesocosms, possibility due to greater physical abrasion or herbivory occurring in the lake. Lower epiphytic algal richness and diversity in the mesocosms may have resulted from controlled environmental conditions preventing colonization of new species from nearby habitats, as well as decreasing habitat heterogeneity among treatments. A greater abundance of cyanobacteria in the mesocosm experiment compared to the Muskegon Lake survey was likely influenced by the use of lake water from September-October when phytoplankton communities have seasonally shifted from spring diatom dominance to summer cyanobacteria dominance (Gillett and Steinman, 2011). Cyanobacteria also favor turbid and nutrient-rich conditions, which was reflected in the greater relative abundance of this phylum in certain treatments (Havens et al., 2003).

Conclusions and ecological implications

Overall, our anticipated macrophyte-epiphytic algal responses to source of nutrient enrichment did not conform to reality due to nutrient-induced phytoplankton growth, increasing the importance of light and turbidity as drivers of *V. americana* and epiphytic algae biological variables. Although phytoplankton presence hindered the ability to fully address the effects of direct vs. indirect nutrient sources on macrophytes and epiphytic algae, some conclusions could be made. Where water column nutrients were scarce or in high demand, porewater N and P were most beneficial for increasing *V. americana* biomass and its epiphytic algal biomass and density. Therefore, nutrient enrichment at a directly accessible source may not guarantee a biomass increase for a particular primary producer group; indirect nutrient availability may be needed to elicit a biomass increase under stressed conditions.

For macrophytes with a basal meristem, like *Vallisneria* spp., indirect sediment nutrient availability for epiphytic algae should be considered when examining nutrients and primary producer interactions (Périllon and Hilt, 2019). Epiphytic algae on meadow-forming macrophytes, with a greater proportion of plant biomass closer to the sediment (Madsen et al., 2001; Wigand et al., 2000), may become more reliant on porewater nutrients when phytoplankton biomass is elevated. Epiphytic algae's closer proximity to the sediment than phytoplankton may give epiphytes a competitive advantage over sediment nutrients (Sand-Jensen and Borum, 1991; Vadeboncoeur et al., 2001), dependent on meroplankton movement in the vertical water column (Schelske et al., 1995). Since epiphytic algal existence is contingent on macrophyte host survival, *V. americana*'s adaptability to nutrient-rich environments is critical. *Vallisneria*'s growth form is less adept to competing in nutrient-rich and turbid conditions than canopy-forming macrophytes (Chambers and Kalff, 1987; Madsen et al., 2001; Tang et al., 2019); however, its physiological adaptations to low-light may compensate for its disadvantageous morphology, allowing this genus to exist in both nutrient-rich and -poor habitats (Song et al., 2015; Titus and Adams, 1979).

Further research concerning macrophyte and epiphytic algal responses to nutrient enrichment is recommended, including treatments with and without phytoplankton, to more definitively detect macrophyte-epiphytic algal interactions. If littoral habitat restoration is to occur where light and nutrients are the main drivers of community change, all nutrient pools should be investigated, since indirect nutrient sources can be just as important for primary producers as direct sources. In extreme circumstances, epiphytic and planktonic growth, as a response to elevated nutrients, has facilitated total macrophyte community loss (Phillips et al., 1978; Phillips et al., 2016); epiphytic algal mitigation should be incorporated into restoration efforts, especially if phytoplankton is decreasing light availability. Choosing restoration locations with greater physical disturbance may control epiphytic algal biomass while promoting high algal richness and diversity, assuming hydrologic exposure is not limiting macrophyte colonization. A balance between habitat characteristics that favor macrophyte growth and lessen epiphytic algal stress is therefore critical for successful macrophyte community rejuvenation and the recovery of associated ecosystem services.

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Table 3.1: Mean (±SD) ambient SRP and NO_3^--N concentrations in the water column(n per nutrient = 12) and porewater (n per nutrient = 9) of Muskegon Lake along with the mean expected (ambient and 10x ambient nutrient concentrations anticipated for each respective treatment) (±SD) and observed SRP and NO_3^--N concentrations in the water column (n per nutrient = 15) and porewater (n = 1) for each of the four mesocosm treatments.

	Muskegon Lake	**Control (C)**		**Sediment (S)**		**Water Column (WC)**		**Both (B)**	
Variable	**Ambient**	**Expected**	**Observed**	**Expected**	**Observed**	**Expected**	**Observed**	**Expected**	**Observed**
Water Column SRP (mg/L)	0.016 (0.009)	0.016 (0.009)	0.006 (0.001)	0.016 (0.009)	0.006 (0.001)	0.16 (0.009)	0.07 (0.05)	0.16 (0.009)	0.09 (0.06)
Water Column NO_3^--N (mg/L)	0.15 (0.10)	0.15 (0.10)	0.16 (0.12)	0.15 (0.10)	0.15 (0.12)	1.50 (0.10)	1.38 (0.96)	1.50 (0.10)	1.40 (0.94)
Porewater SRP (mg/L)	0.041 (0.02)	0.041 (0.02)	0.010	0.41 (0.02)	0.01	0.041 (0.02)	0.01	0.41 (0.02)	0.01
Porewater NO_3^--N (mg/L)	0.21 (0.09)	0.21 (0.09)	0.82	2.10 (0.09)	0.86	0.21 (0.09)	4.09	2.10 (0.09)	4.84

Table 3.2: Mean (±SD) values of physical and chemical variables collected in each of the four treatments. Asterisks on variables indicates Kruskal-Wallis significance among treatments with a p-value < 0.05. Superscripts on variable values indicate significant similarities or differences among treatments.

Variable	Control (C)	Sediment (S)	Water Column (WC)	Both (B)
Temperature* (°C)	22.89 (1.47)^{a}	22.89 (1.52)^{a}	22.61 (1.34)^{a}	23.56 (1.45)^{b}
DO* (mg/L)	10.32 (1.60)^{a}	10.05 (1.37)^{a}	14.95 (4.58)^{b}	13.70 (3.57)^{b}
pH*	8.92 (0.33)^{a}	8.89 (0.31)^{a}	9.38 (0.51)^{b}	9.26 (0.45)^{b}
ORP* (mV)	71.51 (14.84)^{a}	71.05 (13.26)^{a}	55.60 (18.67)^{b}	60.28 (25.18)^{b}
Turbidity* (NTU)	1.14 (1.53)^{ab}	0.89 (1.45)^{a}	2.65 (1.97)^{c}	1.41 (2.04)^{bc}
Phytoplankton Chlorophyll-*a (mg/L)**	0.03 (0.03)^{ab}	0.01 (0.004)^{a}	0.14 (0.11)^{c}	0.15 (0.24)^{bc}
Light Extinction*	1.32 (0.45)^{a}	1.12 (0.14)^{a}	2.03 (0.79)^{b}	1.96 (0.66)^{b}
TDS* (g/L)	0.26 (0.01)^{a}	0.26 (0.01)^{a}	0.24 (0.03)^{b}	0.24 (0.02)^{b}
Specific Conductivity* (µS/cm)	392.47 (17.70)^{a}	398.64 (14.53)^{a}	365.00 (42.47)^{b}	375.49 (36.19)^{b}
SRP* (mg/L)	0.006 (<0.001)^{a}	0.007 (<0.001)^{a}	0.07 (0.05)^{b}	0.11 (0.04)^{b}
TP* (mg/L)	0.03 (0.006)^{a}	0.03 (0.01)^{a}	0.16 (0.05)^{b}	0.18 (0.07)^{b}
NO_3^-* (mg/L)	0.16 (0.12)^{a}	0.15 (0.12)^{a}	1.38 (0.95)^{b}	1.49 (0.89)^{b}
TKN* (mg/L)	0.74 (0.33)^{a}	0.64 (0.23)^{a}	1.12 (0.32)^{b}	1.83 (2.27)^{b}
Porewater SRP* (mg/L)	0.01 (0.001)^{a}	0.01 (0.003)^{ab}	0.01 (0.001)^{ab}	0.01 (0.004)^{b}
Sediment TP (mg/L)	0.07 (0.03)	0.07 (0.03)	0.07 (0.04)	0.08 (0.02)
Porewater NO_3^-* (mg/L)	0.82 (1.22)^{a}	0.86 (1.24)^{a}	4.09 (1.88)^{b}	4.84 (3.30)^{b}
Sediment TKN (mg/L)	0.22 (0.09)	0.19 (0.05)	0.24 (0.08)	0.20 (0.06)

Table 3.3: SIMPER post-hoc results for the NMDS clustering by treatment. Mean dissimilarity (%) for each treatment indicates which genera contributed the most to the separation among treatments. Genera present for each treatment contribute to ~90% of the cumulative mean dissimilarity. Genera mean relative abundance (%) (as Abd.) within each of the four treatments is provided. A dash indicates that a genus did not substantially contribute to the dissimilarity within a treatment and was not a major contributor to the separation in clusters for that treatment.

		Control (C)		**Sediment (S)**		**Water Column (WC)**		**Both (B)**	
Genus	**Growth Form**	**Dissimilarity**	**Abd.**	**Dissimilarity**	**Abd.**	**Dissimilarity**	**Abd.**	**Dissimilarity**	**Abd.**
Cylindrospermum	Filamentous	16.3	21.7	5.8	0.0	5.1	0.1	5.4	0.3
Mougeotia	Filamentous	9.4	10.7	15.8	22.4	8.4	1.6	8.7	1.5
Pseudanabaena	Filamentous	8.7	8.0	11.8	14.8	12.8	9.6	9.2	7.1
Achnanthidium	Stalked	10.7	5.3	9.6	12.5	12.9	24.3	11.7	19.6
Fragilaria	Colonial	12.7	13.0	12.5	14.8	14.2	21.7	15.6	21.6
Lyngbya	Filamentous	19.1	19.9	20.2	14.5	21.7	16.2	28.1	40.4
Phormidium	Filamentous	8.6	10.6	7.9	8.3	4.6	0.0	4.8	0.1
Kirchneriella	Colonial	3.4	3.1	2.6	1.4	5.1	6.5	2.6	0.4
Scenedesmus	Colonial	1.3	0.7	1.5	0.5	4.4	6.0	1.6	0.8
Nitzschia	Motile	1.2	2.1	1.9	3.1	2.3	4.1	1.4	2.0
Coelastrum	Colonial	-	-	1.1	0.4	1.5	2.1	1.4	1.8

Table 3.4: Physical and chemical variables collected at each of the Muskegon Lake transects in correspondence with the 2018 epiphytic algae survey.

Variable	NWRef	HertL	GrandT
DO (mg/L)	9.64	12.33	9.02
ORP (mV)	63.3	69.4	34.2
pH	8.83	9.04	8.40
Temperature (°C)	25.88	30.08	24.29
Turbidity (NTU)	1.2	11.3	0.7
TP (mg/L)	0.022	0.023	0.020
Light Extinction	5.76	1.78	2.49
NO_3^- (mg/L)	0.104	0.110	0.15
TKN (mg/L)	0.748	0.728	1.104
Specific Conductance (µS/cm)	341	351	355
Depth (m)	0.8	0.9	1.2

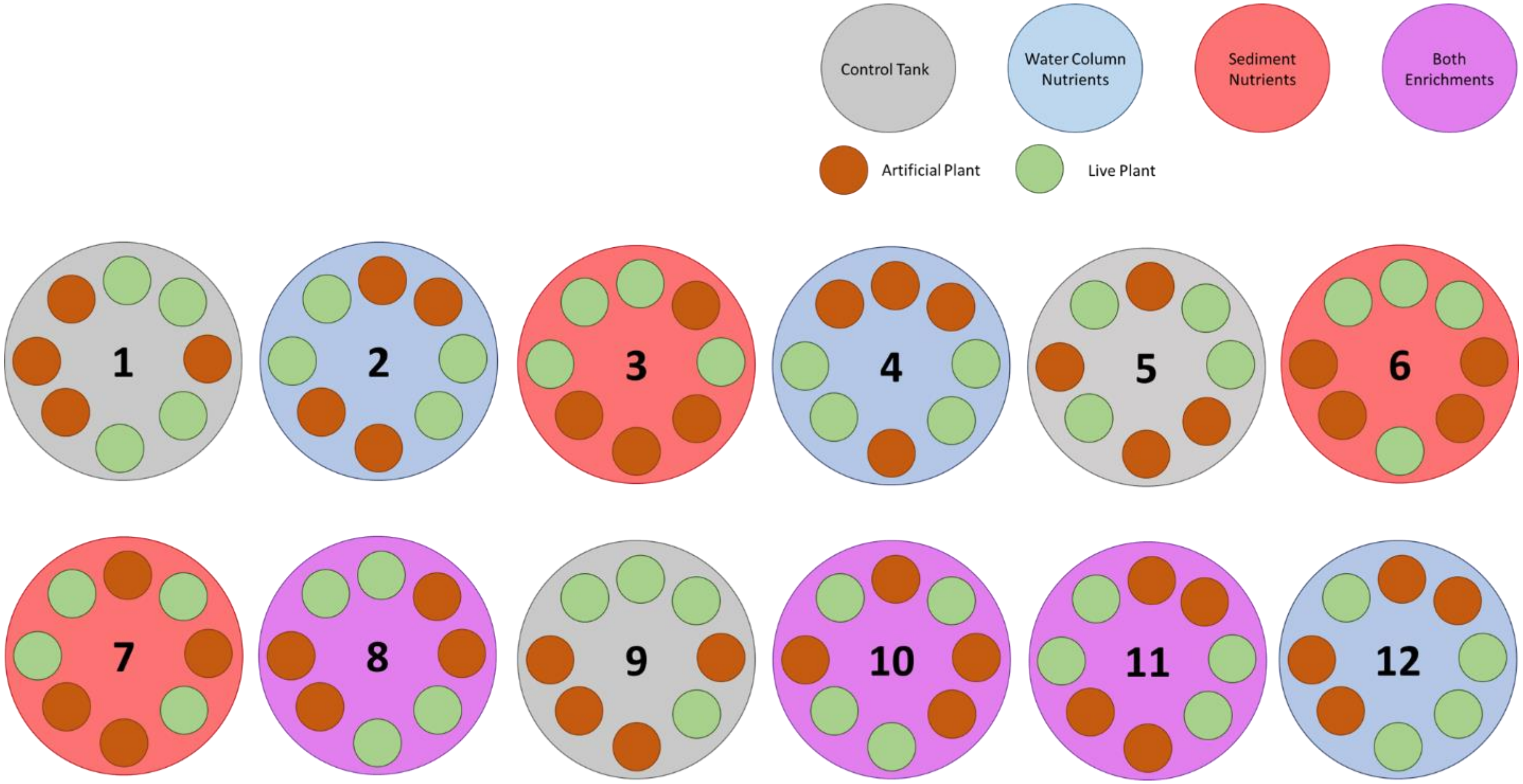

Figure 3.1: Experimental design of sediment vs. water column nutrient enrichment source. Twelve mesocosms are represented by large circles. Large circle color indicates the assigned source of nutrient enrichment treatment to each mesocosm. Smaller circles within mesocosms represent the random placement of individual live *V. americana* (green) and artificial macrophytes (orange) planted in buckets containing sediment.

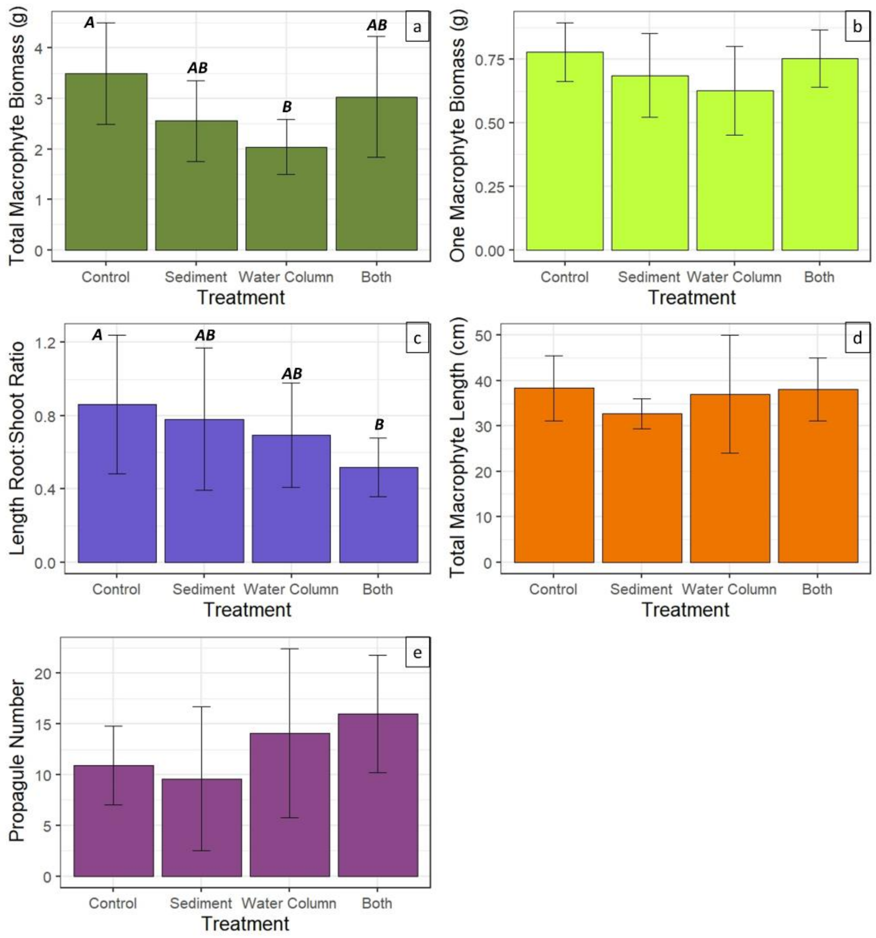

Figure 3.2: Mean live *V. americana* macrophyte biological variables in each treatment after 28 days. One standard deviation above and below the mean is provided, n = 48. Uppercase, bold letters above treatment groupings represent statistically significant differences among treatments. (a) Mean total macrophyte biomass (described as the individual *V.americana* biomass plus biomass of all propagules attached to that individual) with statistically significant differences among treatments (p=0.004). (b) Mean individual macrophyte biomass (g) (defined as the original macrophyte used for removing epiphytic algae). (c) Mean macrophyte length root:shoot ratio with statistically significant differences among treatments (p=0.04). (d) Mean total macrophyte length (cm). (e) Mean propagule number per individual macrophyte.

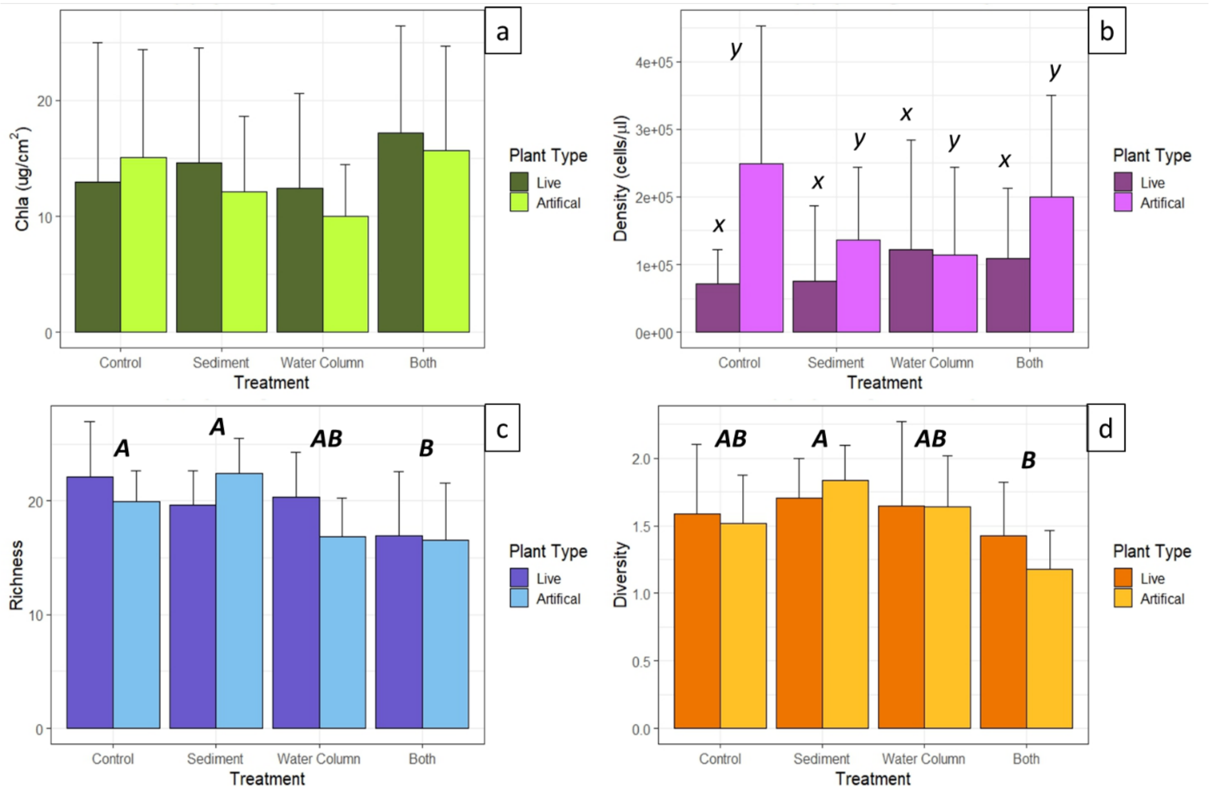

Figure 3.3: Mean epiphytic algae biological metrics on live *V. americana* and artificial macrophytes in each treatment. One standard deviation above the mean is provided. Uppercase, bold letters above treatment groupings represent statistically significant differences among treatments. Lowercase letters above individual bars represent statistically significant differences between plant types, n = 89. (a) Mean epiphytic algae chlorophyll-a (µg/cm^2). (b) Mean epiphytic algae density (cells/mm^2) with statistically significant differences between plant types (p=0.002). (c) Mean epiphytic algae genera richness with statistically significant differences among treatments (p<0.001). (d) Mean epiphytic algae diversity with statistically significant differences among treatments (p=0.006).

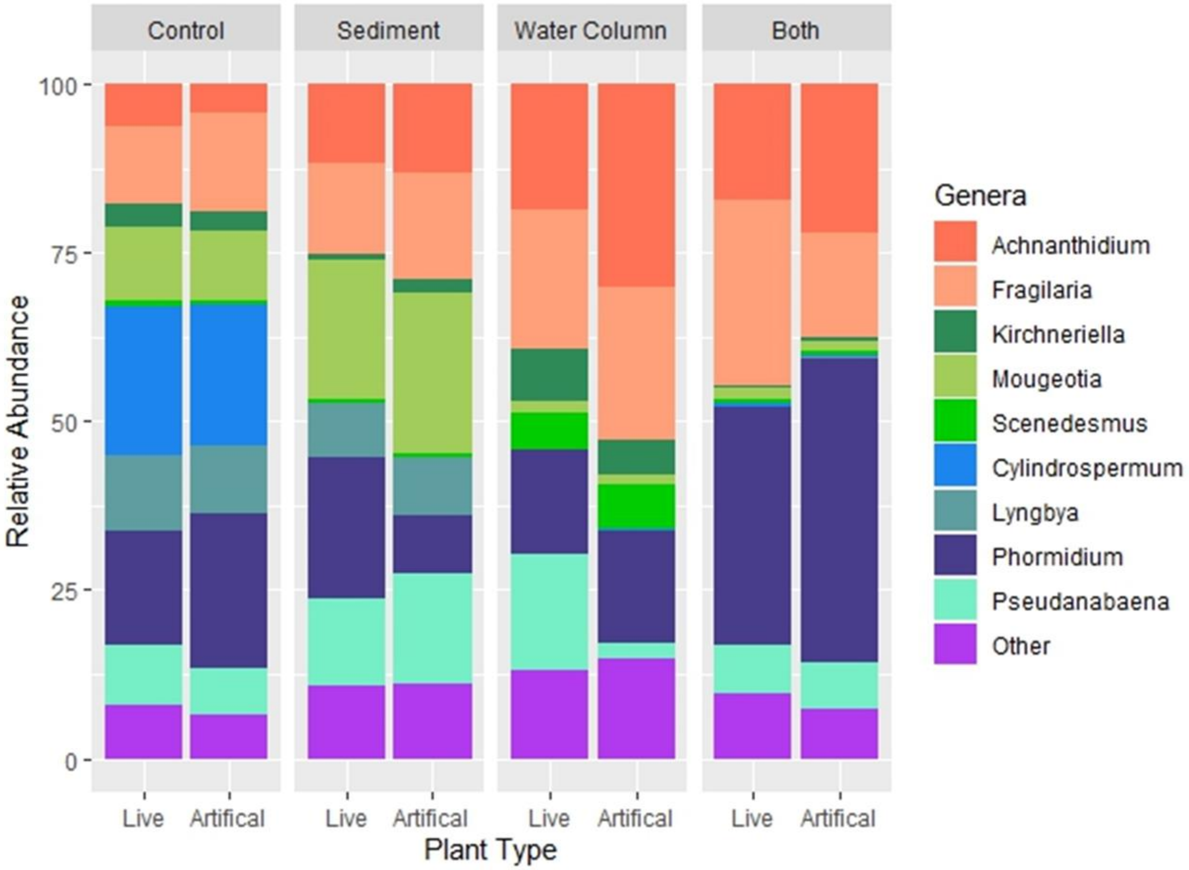

Figure 3.4: A stacked barplot of the mean relative abundances of epiphytic algal taxa growing on live *V. americana* and artificial macrophytes within all four treatments.

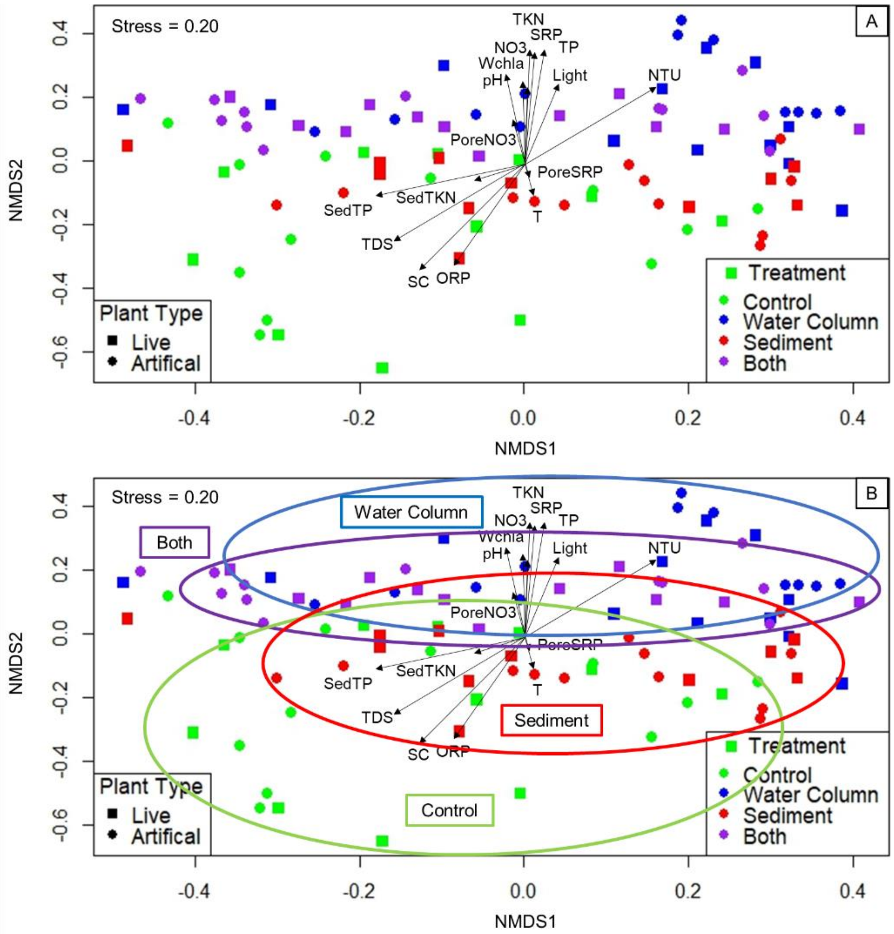

Figure 3.5: (a) An NMDS biplot with mean relative abundance data for epiphytic algal taxa and a stress value of 20%. Colors represent the different treatments (control, water column, sediment, and both) and symbol shapes represent plant type (Live and Artificial). Environmental variables are overlaid onto the plot (phytoplankton chlorophyll-*a* [Wchla], soluble reactive phosphorus [SRP], total Kjeldahl nitrogen [TKN], total phosphorus [TP], nitrate [NO_3^-], pH, dissolved oxygen [DO], light extinction [Light], turbidity [NTU], temperature [T], oxidation-reduction potential [ORP], specific conductance [SC], total dissolved solids [TDS], porewater SRP [PoreSRP], porewater NO_3^- [PoreNO3], sediment TP [SedTP], and sediment TKN [SedTKN]). Vector length is positively correlated with each variable's explanatory power in the dataset. (b) Same biplot as (a) but with algae relative abundances clustered by treatment.

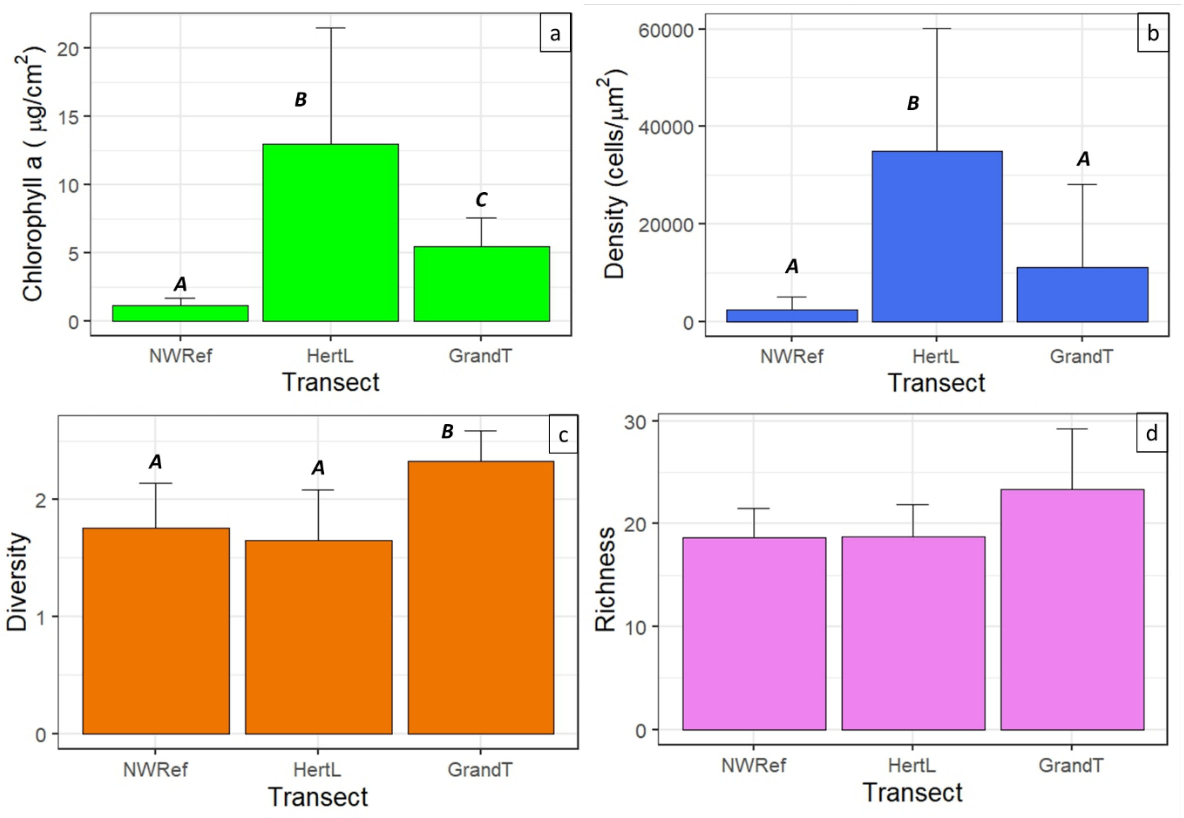

Figure 3.6: Mean epiphytic algae biological metrics at each of the three Muskegon Lake transects. One standard deviation above the mean is provided. Letters above transect groupings represents statistically significant differences among transects, n = 30. (a) Epiphytic algal chlorophyll-a (µg/cm^2) concentrations. (b) Epiphytic algal density (cells/mm^2). (c) Epiphytic algal diversity. (d) Epiphytic algal richness.

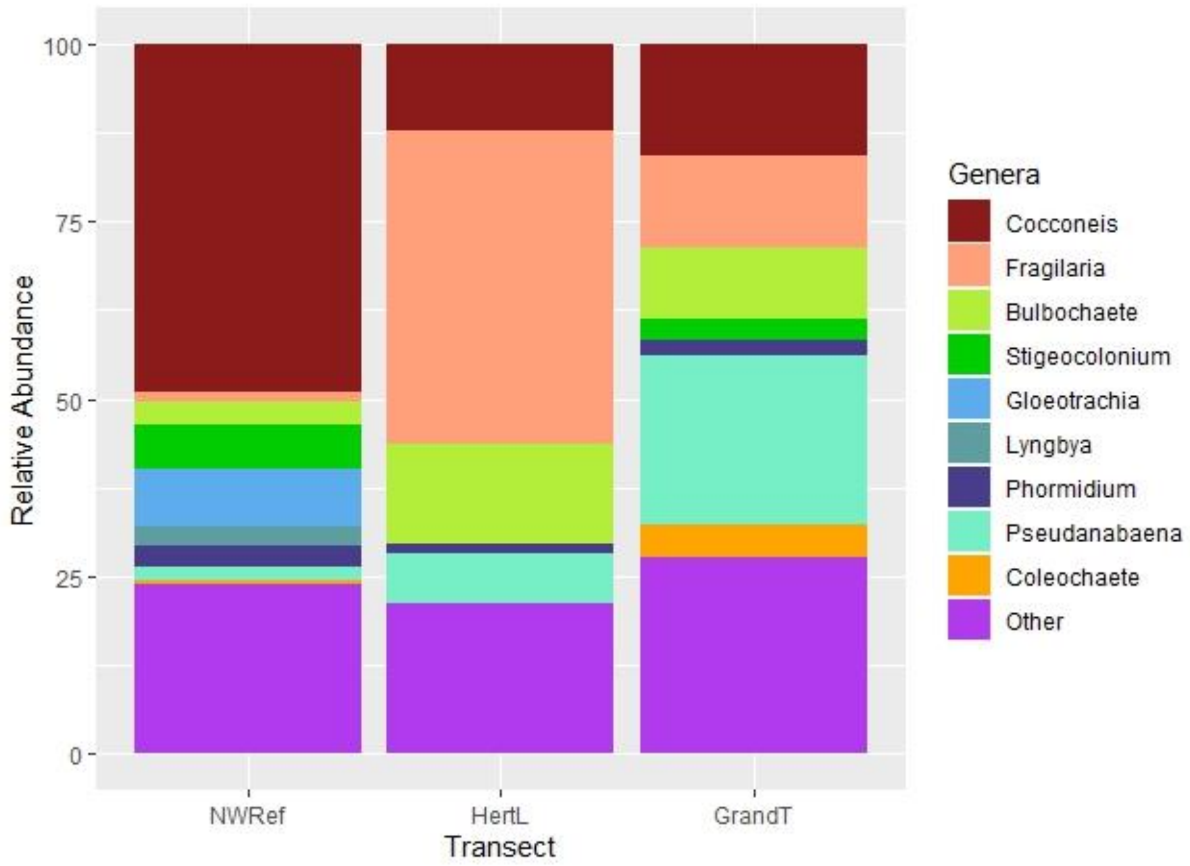

Figure 3.7: A stacked barplot of the mean relative abundances of epiphytic algal taxa growing on *V. americana* macrophytes at the three Muskegon Lake transects (Northwest Reference [NWRef], Heritage Landing [HertL], Grand Trunk [GrandT]).

Appendix A. Supplementary Material

Table 3.A1: Linear regression results for significant correlations between variables for the source of nutrient enrichment experiment. Negative signs in front of R^2 values indicate a negative correlation between the two variables.

Regression	R^2	P	F	Df
Biological vs. Environmental:				
Total Macrophyte Biomass				
Total Macrophyte Biomass vs. NTU	-0.09	0.05	4.26	42
Total Macrophyte Biomass vs. TDS	-0.13	0.02	6.42	42
Total Macrophyte Biomass vs. Temperature	0.16	0.008	7.77	42
Total Macrophyte Biomass vs. Specific Conductance	0.10	0.03	4.92	42
Individual Macrophyte Biomass				
Individual Macrophyte Biomass vs. SRP	0.13	0.02	6.08	42
Individual Macrophyte Biomass vs. Temperature	0.22	0.001	11.87	42
Macrophyte Length				
Macrophyte Length vs. Temperature	0.14	0.01	7.02	42
Macrophyte Length Root:Shoot Ratio				
Length Root:Shoot Ratio vs. DO	-0.08	0.05	3.92	42
Length Root:Shoot Ratio vs. Light	-0.13	0.02	6.02	42
Length Root:Shoot Ratio vs. pH	-0.24	<0.001	13.55	42
Length Root:Shoot Ratio vs. Temperature	-0.25	<0.001	13.65	42
Length Root:Shoot Ratio vs. Porewater NO_3^-	-0.20	0.002	10.4	42
Length Root:Shoot Ratio vs. SRP	-0.20	0.002	10.24	42
Length Root:Shoot Ratio vs. NO_3^-	-0.15	0.009	7.54	42
Length Root:Shoot Ratio vs. TKN	-0.17	0.005	8.69	42
Length Root:Shoot Ratio vs. TP	-0.17	0.005	8.63	42
Length Root:Shoot Ratio vs. ORP	0.09	0.04	4.33	42
Propagule Number				
Propagule Number vs. DO	0.12	0.02	5.80	42
Propagule Number vs. Light	0.13	0.02	6.23	42
Propagule Number vs. pH	0.10	0.04	4.45	42

Propagule Number vs. NO_3^-	0.19	0.003	9.92	42
Propagule Number vs. SRP	0.10	0.04	4.50	42
Propagule Number vs. TKN	0.10	0.04	4.47	42
Propagule Number vs. TP	0.10	0.03	4.86	42
Epiphyton Chla				
Epiphyton Chla vs. pH	0.04	0.04	4.14	89
Epiphyton Chla vs. Temperature	0.12	<0.001	12.13	89
Epiphyton Chla vs. Sediment TP	0.11	0.001	11.14	89
Epiphyton Chla vs. Porewater NO_3^-	0.06	0.02	5.67	89
Epiphyton Density				
Density vs. TDS	0.05	0.04	4.57	89
Density vs. Porewater SRP	0.07	0.01	6.55	89
Density vs. Porewater NO_3^-	0.05	0.04	4.35	89
Density vs. Sediment TP	0.05	0.02	5.05	89
Epiphyton Diversity				
Diversity vs. Porewater NO_3^-	-0.21	<0.001	23.72	89
Diversity vs. Porewater SRP	-0.11	0.001	11.50	89
Diversity vs. NO_3^-	-0.09	0.003	9.09	89
Diversity vs. SRP	-0.08	0.008	7.27	89
Diversity vs. TKN	-0.08	0.007	7.45	89
Diversity vs. TP	-0.06	0.02	5.79	89
Diversity vs. Light	-0.08	0.005	8.24	89
Diversity vs. pH	-0.12	0.007	12.28	89
Diversity vs. Temperature	-0.16	<0.001	16.84	89
Diversity vs. ORP	0.05	0.05	3.94	89
Epiphyton Richness				
Richness vs. NO_3^-	-0.20	<0.001	22.40	89
Richness vs. SRP	-0.20	<0.001	21.65	89
Richness vs. Porewater NO_3^-	-0.16	<0.001	17.45	89
Richness vs. TP	-0.18	<0.001	19.96	89
Richness vs. TKN	-0.17	<0.001	18.13	89
Richness vs. Sediment TKN	0.08	0.007	7.41	89
Richness vs. Light	-0.20	<0.001	22.13	89
Richness vs. pH	-0.13	<0.001	13.66	89
Richness vs. Temperature	-0.13	<0.001	13.58	89
Richness vs. DO	-0.11	0.001	11.02	89

Richness vs. NTU	-0.13	<0.001	13.76	89
Richness vs. ORP	0.17	<0.001	17.91	89
Richness vs. Specific Conductance	0.10	0.002	9.94	89
Phytoplankton Chla				
Phytoplankton Chla vs. NO_3^-	0.81	<0.001	387.9	89
Phytoplankton Chla vs. Porewater NO_3^-	0.72	<0.001	226	89
Phytoplankton Chla vs. Porewater SRP	0.05	0.02	5.14	89
Phytoplankton Chla vs. TP	0.78	<0.001	312.1	89
Phytoplankton Chla vs. Sediment TKN	-0.18	<0.001	19.27	89
Phytoplankton Chla vs. Light	0.96	<0.001	2019	89
Phytoplankton Chla vs. NTU	0.61	<0.001	138.2	89
Phytoplankton Chla vs. pH	0.67	<0.001	179	89
Phytoplankton Chla vs. TDS	-0.17	<0.001	17.79	89
Phytoplankton Chla vs. ORP	-0.80	<0.001	355.8	89
Phytoplankton Chla vs. Specific Conductance	-0.52	<0.001	97.98	89
Biological vs. Biological:				
Macrophyte Total Biomass				
Macrophyte Total Biomass vs. Macrophyte Length	0.25	<0.001	13.67	42
Individual Macrophyte Biomass				
Individual Macrophyte Biomass vs. Density	0.13	0.02	6.05	42
Individual Macrophyte Biomass vs. Macrophyte Length	0.34	<0.001	21.74	42
Individual Macrophyte Biomass vs. Macrophyte Biomass	0.37	<0.001	25.17	42
Macrophyte Length				
Macrophyte Length vs. Density	-0.13	0.02	6.24	42
Macrophyte Length vs. Diversity	-0.11	0.03	5.00	42
Macrophyte Length vs. Richness	-0.09	0.05	4.01	42
Macrophyte Length Root:Shoot Ratio				
Macrophyte Length Root:Shoot Ratio vs. Richness	0.23	<0.001	12.84	42
Macrophyte Length Root:Shoot Ratio vs. Diversity	0.17	0.006	8.35	42
Macrophyte Length Root:Shoot Ratio vs Density	-020	0.002	10.63	42
Macrophyte Length Root:Shoot Ratio vs. Phytoplankton Chla	-0.09	0.05	3.98	42

Propagule Number				
Propagule Number vs. Phytoplankton Chla	0.18	0.004	9.27	42
Epiphyton Chla				
Epiphyton Chla vs. Diversity	-0.11	0.001	11.28	89
Epiphyton Chla vs. Richness	-0.06	0.02	6.04	89
Epiphyton Chla vs. Density	0.06	0.02	5.99	89
Epiphyton Density				
Density vs. Diversity	-0.35	<0.001	47.31	89
Density vs. Richness	-0.10	0.002	9.40	89
Epiphyton Diversity				
Diversity vs. Phytoplankton Chla	-0.07	0.01	6.82	89
Diversity vs. Richness	0.15	<0.001	16.15	89
Epiphyton Richness				
Richness vs. Phytoplankton Chla	-0.15	<0.001	15.23	89

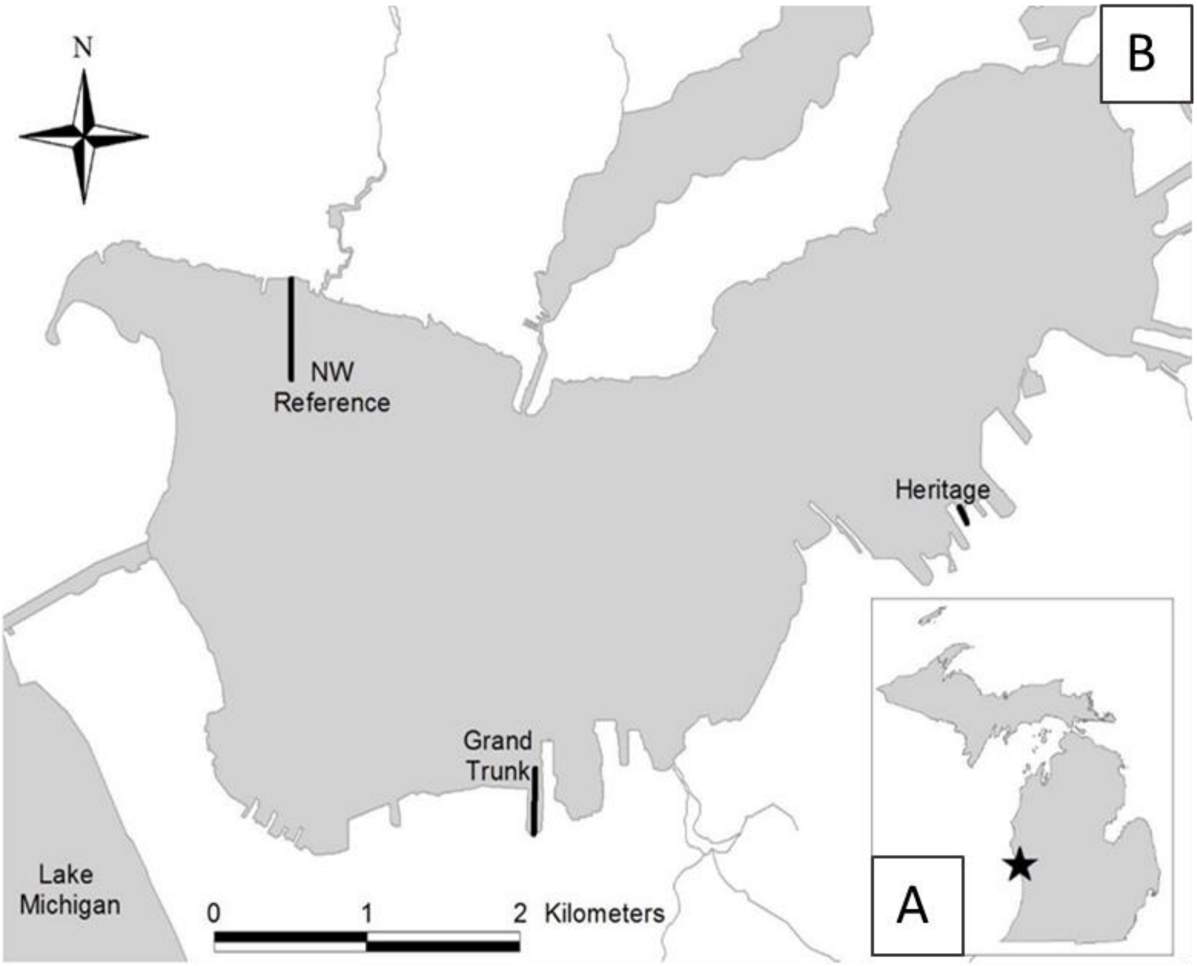

Figure 3.A1: (a) Map of the state of Michigan with the location of Muskegon Lake indicated by a black star. (b) Muskegon Lake with the three macrophyte survey transects indicated by black lines showing the length of each transect. Restored transects (Grand Trunk and Heritage Landing [Heritage]) are perpendicular to the southern shoreline and Northwest (NW) Reference is perpendicular to the northern shoreline.

Chapter 4

Synthesis and Conclusions

Introduction

More than 93% of species residing in the world's largest lakes rely on littoral habitats and 76% of those are restricted to existing within this lake zone (Vadeboncoeur et al., 2011). Despite a small proportion of habitat supporting most aquatic organisms in lakes, littoral zones are the most often disturbed due to human activities (Niemi et al., 2007; Vadeboncoeur et al., 2011). Littoral habitat degradation often leads to a decline in macrophyte community integrity and function. The loss of macrophytes would negatively impact lake ecosystems in numerous ways, including: disrupt the movement and retention of organic matter across the aquatic-terrestrial interface; decrease periphytic algae presence (Carpenter and Lathrop, 1999; Sass et al., 2006); decrease littoral habitat complexity; and alter other biological interactions in the littoral zone (Brauns et al., 2011).

Most investigations into the loss of macrophyte communities have been prompted by the human impacts associated with eutrophication (Qiu et al., 2001; Søndergaard et al., 2007), with a goal to restore littoral habitat, through macrophyte planting, in order to reduce resource availability for phytoplankton and help alleviate turbid water conditions (Phillips et al., 1978; Phillips et al., 2016). Other littoral habitat disturbances such as lakeshore development, shoreline hardening, and dredging can be just as disruptive to ecosystem dynamics but have received less attention (Elias and Meyer, 2003). Even fewer of these distressed habitats have undergone restoration (Alexander et al., 2008; Ogdahl and Steinman, 2015; Radomski and Goeman, 2011).

No matter the type of shoreline disturbance, understanding the habitat characteristics driving macrophyte community structure (i.e., richness, density, biomass, and diversity) is crucial for developing a successful restoration plan, as are biological interactions with other primary producers. Shifts in resources required by all autotrophs (i.e., nutrients and light) can directly impact macrophyte community structure and can indirectly impact macrophytes by impacting the surrounding primary producer groups' biological metrics. Therefore, usual positive macrophyte responses to growth-stimulating environmental changes, such as nutrient-enriched habitat, may be diminished by the coinciding increase in epiphytic algal growth to nutrient-rich environments.

Primary producer responses to nutrients

Interactions among macrophytes, epiphytic algae, and phytoplankton in shallow aquatic systems are most often studied when examining the alternating dominance between macrophyte-epiphytic algal communities and phytoplankton (Phillips et al., 1978; Phillips et al., 2016; Sand-Jensen and Borum, 1991). In natural ecosystems with clear water conditions, initial phytoplankton increases during nutrient enrichment can be mitigated by the dominance of dense macrophyte communities and their epiphytic algae. Water column nutrient increases are widely known to first enhance epiphytic algal growth, which then leads to a decline in macrophytes and subsequent phytoplankton dominance usually seen in eutrophic conditions (e.g., Phillips et al., 1978). In contrast to the usual order of primary producer changes, I speculate that the small population size of live *Vallisneria americana* in the mesocosms (4 individuals) for my laboratory experiment was not large enough to diminish phytoplankton growth. Additionally, abrupt nutrient pulses, combined with a week-long water residence time, could have favored phytoplankton growth (MacIntyre and Cullen 1996; Sand-Jensen and Borum, 1991). All three primary producer groups therefore responded simultaneously to water column nutrient availability, and phytoplankton became an important biological component in the mesocosm environment.

Macrophyte-epiphytic algal responses to sources of nutrient enrichment were different than my initial predicted hypotheses, as my hypotheses did not account for phytoplankton presence. Results from my experiment were, however, like macrophyte-epiphytic algal responses typically exhibited when all three primary producer groups, including phytoplankton, are exposed to water column enrichment; after water column nutrients were added into the water column (WC) and both (B) treatments, phytoplankton chlorophyll-*a* (Chl-*a*) concentrations increased ~5 fold greater than in the sediment (S) and control (C) treatments.. Similar to results in Romo et al. (2007), macrophyte biomass in my study was greatest in the control (C) treatment, where concentrations of water column and porewater total phosphorus (TP) were below 0.06 mg/L and water column nitrate (NO_3^-) was ~0.16 mg/L. Phytoplankton proliferation of nutrients and light, especially in the WC and B treatments, and persistence through the experiments duration counteracted the usual stimulatory influence of nutrient enrichment on both *V. americana* and its epiphytic algae. Despite the absence of a significant nutrient effect on epiphytic algal density, overall macrophyte length, length root:shoot ratio, and propagule number were negatively associated with epiphyton density; epiphytic algae was suggested to influence the reduction in *V. americana growth*, the similar response often documented in shallow ecosystems transitioning from clear water environments to eutrophic, phytoplankton-dominated environments (Phillips et al., 1978; Phillips et al., 2016; Romo et al., 2007).

Unlike most studies involving primary producer interactions and nutrients, my experiment addressed porewater as a source of nutrient enrichment. Porewater nutrients increased the mean biomass accumulation for both macrophytes and epiphytic algae in the B treatment. However, epiphytic algal biomass could not be sustained only with the indirect availability of porewater nutrients whereas macrophyte direct access was enough for sustaining *V. americana* biomass. In the S treatment, the combination of greater macrophyte growth due to porewater enrichment, the presence of epiphytic algae, and the absence of water column enrichment helped mitigate phytoplankton growth in this clear water environment; phytoplankton Chl-*a* concentrations (0.01 µg/L) were even lower in the S treatment compared to the C treatment (0.03 µg/L). Our documentation of epiphytic algal association with porewater enrichment suggests that the reliance of attached algae on indirect nutrient sources is dependent on the surrounding environmental conditions and biological interactions.

As mentioned in Chapter 3, the two different sources of nutrient enrichment, along with light dynamics, were the main determinants of primary producer biomass-related responses in the mesocosm experiment (Figure 4.1A). Although I had expected a minimal phytoplankton response to nutrient enrichment (Figure 4.1B), nutrients substantially increased phytoplankton accumulation, which in turn, reduced light availability for *V. americana* and epiphytic algae (Figure 4.1A). Phytoplankton presence diminished epiphytic algae's response to water column nutrients and helped emphasize the role of porewater as an accessory nutrient source for increasing epiphytic algae biomass and density. As expected, epiphytic algae negatively impacted *V. americana* possibly through the interception of nutrients and light, even despite phytoplankton lessening epiphytic algal biomass and density increases (Figure 4.1B). Although we expected *V. americana* biomass to be greatest in the S treatment, both water column and sediment nutrient enrichment elicited different responses from all macrophyte biological variables; biomass accumulation and propagule production were associated more with water column nutrients, whereas length root:shoot ratio was equally associated with water column and porewater nutrients.

Drivers of epiphytic algae community change

Even though environmental conditions were different between Muskegon Lake and the mesocosm experiment, both study locations demonstrated the negative impacts of epiphytic algal growth on macrophyte communities; epiphytic algal density in the mesocosm experiment was negatively associated with *V. americana* total length and in Muskegon Lake, the transects with the greatest epiphytic algal density and biomass had the lowest total macrophyte community biomass and density. For example, Heritage Landing contained the greatest epiphytic algal densities on *V. americana* (34881 cells/μm^2), but the lowest macrophyte community biomass (112.48 kg) among transects.

To combat epiphytic algal negative impacts, my studies indicated that increased physical disturbance and intermediate light availability may reduce the intensity of epiphytic algal disturbance on *V. americana*. In Muskegon Lake, the lowest epiphytic algal density and biomass occurred at Northwest Reference, which experienced the greatest hydrologic exposure to wind and wave action (Wind Index [WI]: 317), greatest light extinction coefficient (5.16), greatest turbidity (16.63 NTU), and lowest photosynthetically active radiation (PAR) value at 0.5 m depth

(122 μmol/s/m^2) of the three transects. Epiphytic algae responded similarly to the same environmental variables in the mesocosm experiment; current velocity may have imposed greater leaf drag on live vs. artificial macrophytes, reducing epiphytic algal density on live *V.* americana. Additionally, higher turbidity (WC: 2.65 NTU; B: 1.96 NTU) and light extinction (WC: 2.03; B: 1.96) in the WC and B treatments likely decreased epiphytic algal biomass and density.

In both my laboratory and field studies, physical disturbance regimes and light dynamics that favored epiphytic algal density and biomass increases often reduced epiphytic algal richness and diversity. High-light and low phytoplankton biomass in the S treatment helped maintain variability in microhabitat conditions on macrophyte surfaces, increasing epiphytic algal richness and diversity. In contrast, nutrient enrichment and light limitation in the WC and B treatments stressed epiphytic algal communities by narrowing resource regimes and increasing competitive exclusion, reducing richness and diversity (Carrick et al., 1988; DeNicola and Kelly, 2014; Hillebrand and Sommer, 2000). The Grand Trunk habitat in Muskegon Lake experienced intermediate hydrologic exposure and light extinction, promoting the greatest epiphytic algal diversity and richness among transects, a response often seen in ecosystems (Connell, 1978; England et al., 2008; Larned, 2010; Molino and Sabatier, 2001). NO_3^- and total kjeldahl nitrogen (TKN) concentrations also were greatest at Grand Trunk, possibly benefiting diversity and richness at this transect. A positive nutrient response in Muskegon Lake and a contrasting negative nutrient response in the mesocosm experiment displayed the importance of moderate resource availability for promoting habitat heterogeneity and preventing species monodominance. Resource saturation may therefore decrease epiphytic algal diversity and richness, as seen in the mesocosm experiment.

Muskegon Lake macrophyte community trajectory

Since the goals of restoration aim to improve degraded ecosystems, temporal trajectories are often developed for predicting increases in habitat function over time (Hobbs and Norton, 1996). Many models for post-restoration community changes have assumed a simple, rapid, and predictable trajectory that favors an increase in habitat quality due to restoration (e.g., Mitsch et al., 1998) (Figure 4.2A). However, this expected timeline has been considered unrealistic (Matthews et al., 2009; Zedler and Callaway, 2002). The intensity of original habitat disturbance, the effectiveness of restoration, and the degree of environmental fluctuations or constraints all could influence the rate of habitat improvement, and the direction of habitat quality movement towards or away from the reference habitat conditions (Bullock et al., 2011; Matthews et al., 2009; Zedler and Callaway, 1999). The realized complexity of restoration outcomes has led to the development of multiple trajectory options (Bullock et al., 2011).

In my Muskegon Lake macrophyte survey, macrophyte richness, density, and biomass were noticeably impacted by the intensity of hydrologic and meteorological fluctuations among survey years (Figure 4.2B), obscuring distinct responses to restoration efforts and making it difficult to predict community changes among survey years. These environmental changes could have slowed the pace of ecosystem improvement, which is partially why definitive responses to restoration are not yet visible six years after restoration occurred. If environmental conditions in Muskegon Lake were more stable, macrophyte community responses to restoration may have been more discernable and may have appeared over a shorter time period.

Along with monitoring macrophyte community changes over time at restored transects, habitat quality also was compared to a designated reference habitat. Heritage Landing and Grand Trunk's macrophyte community metrics often mimicked changes occurring at Northwest Reference, suggesting that most macrophyte responses at the restored transects could be attributed, at least in part, to annual environmental variation and not to restoration, per se. When a restored transect did diverge from the reference during post-restoration, it was due to a decrease in macrophyte habitat quality; restoration-induced increases in habitat quality at restored transects were difficult to discern.

Restored habitat improvement was, however, evident when comparing coefficient of conservatism metrics (C-values) for all three transects during post-restoration. Northwest Reference's C-values post-restoration were lowest in 2011 (3.90) and increased in 2012 (4.21), with a similar C-value (4.22) in 2018. Grand Trunk's C-value in 2011 was lower (3.19) than the reference, but increased in 2012 (3.93), and almost matched the reference's value in 2018 (4.19). Based on Grand Trunk's current trajectory, it is likely that macrophyte communities at this restored transect will reach the same quality standard as the reference transect in the near future. Additionally, the similarities between Grand Trunk and Northwest Reference physical habitats, even before restoration took place, could have eased the achievement of reference standards for Grand Trunk.

In contrast to Grand Trunk's habitat quality trajectory, Heritage Landing's C-values during post-restoration were greatest in 2011 (3.67), decreased in 2012 (3.13), and increased in 2018 (3.56), but did not reach 2011 quality. The stochastic trajectory of Heritage Landing indicates that this restored transect may take longer to obtain reference-quality macrophyte community structure, and some biological variables at this transect may never reach reference standards (Bullock et al., 2011). As mentioned in Chapter 2, it is likely that Heritage Landing's physical habitat features constrained macrophyte communities at this transect; Heritage Landing's high slope gradient and low hydrologic exposure decreased optimal growth conditions for macrophytes and increased epiphytic algal density and Chl-*a*. In addition, human pressures are greatest at this site, where festivals with up to 40,000 people take place throughout the summer, immediately adjacent to this transect. Anecdotal stories of pollutant dumping in this area, combined with heavy boating, add additional stress. These features may have inhibited the improvement of habitat quality at Heritage Landing when exposed to a high intensity of environmental fluctuation.

Other restoration monitoring projects have documented a community divergence from the anticipated trajectory, four to five years after restoration occurred (Bullock et al., 2011; Matthews and Spyreas, 2010); it would be advantageous to continue Muskegon Lake monitoring at least 10 years past initial restoration to assess long term trajectories since current monitoring has only spanned six years post-restoration. Additionally, macrophyte community resilience to environmental fluctuation is considered an important goal of restoration efforts (Lake, 2012), especially for Muskegon Lake, where temporal macrophyte community dynamics were partially driven by water level changes, precipitation accumulation, and temperature fluctuations. Climate change projections predict the severity of these events to increase in the future (O'Reilly et al., 2015), and since the endpoint of climate change is uncertain, the capacity of macrophyte resilience to this stressor will be difficult to estimate (Lake, 2012). At of 2018, only one survey year has captured macrophyte responses to increasing water levels above the long-term Lake Michigan mean. Therefore, longer-term monitoring at the restored habitats also would be critical for determining the extent of macrophyte community resistance, as a product of restoration success, to climate change (Palmer et al., 2005; Suding and Gross, 2006; Zedler and Callaway, 1999).

Incorporating reference habitats into restoration analysis has long been an effective method for standardizing the definition of a desirable habitat, and researchers are encouraged to examine multiple reference habitats during monitoring (Hobbs and Norton, 1996). The first four years of Muskegon Lake monitoring, 2009-2012, included two reference transects, but reduced funding in 2018 permitted the sampling of only one reference transect. I therefore recognize that the Northwest Reference transect is not a sole representation of all more "natural" environments in Muskegon Lake, and restored transects' comparison to only one reference location should be interpreted with care. Additionally, a mean C-value of 4.08 out of 10 at Northwest Reference for all five survey years indicated the potential presence of past or current stressors at the reference habitat. Northwest Reference had not experienced the direct influence of the industrial activities affecting the south shoreline in the 20th century, which was the reason it was selected as a reference habitat. However, it may have experienced disturbance during the lumber industry's peak in the 1880's, and may be experiencing current localized disturbance cause by human recreation. The presence of invasive emergent macrophytes at the reference transect, *Phragmites australis* and *Typha angustifolia*, also could be decreasing reference habitat quality and may be responsible for a portion of C-value fluctuation among survey years. Nevertheless, transect quality was still greater at the reference than the restored locations, providing an achievable goal for macrophyte communities undergoing restoration.

Conclusions

Overall, my results supported the post-restoration improvement of restored transect habitat quality in Muskegon Lake apart from the variation in physical habitat characteristics among transects and the change in water level and precipitation over the five survey years. Epiphytic algal surveys in Muskegon Lake were not a part of the original habitat monitoring plan devised with the shoreline restoration project, but evaluation of these communities has broadened our understanding of the lake's littoral ecosystem dynamics and supported the negative influence of epiphytic algae on their host *V. americana*. Any alteration in surrounding habitat environmental conditions (i.e., light and nutrients) could impact *V. americana* and epiphytic algae biological variables, influencing macrophyte-epiphytic algal interactions and ultimately impacting overall macrophyte community structure. Understanding the opposing macrophyte and epiphytic algal responses to the same environmental variable could be useful in choosing advantageous habitats for restoration; for example, macrophyte biomass and density in Muskegon Lake favored high hydrologic exposure, whereas epiphytic algal biomass and density favored low exposure and finally, algal richness and diversity favored intermediate exposure.

Conducting pre-restoration surveys can help determine which physical characteristics drive macrophyte spatial variability among sites and which temporal variables (e.g., precipitation or water level) drive macrophyte community structure changes among survey years. Pre-restoration results can then be used to better predict post-restoration habitat recovery and improvement trajectory, helping anticipate the time required to reach reference standards. Although epiphytic algae were only examined over a spatial gradient in Muskegon Lake, hydrologic and meteorological fluctuations between years also likely impacted epiphytic algae community structure, just as macrophyte biological variables responded to these changes. It is suggested that future restoration projects, if funding and resources allow, should evaluate both macrophytes and epiphytic algae to determine the environmental conditions best suited for mitigating algal growth on host plants while still supporting a diverse and species rich epiphytic algal community.

Finally, my research demonstrated that even if restoration is successfully implemented and properly designed, uncontrollable and unpredictable environmental changes can still occur, potentially offsetting the predicted restoration trajectory. The intensity of environmental fluctuations became increasingly visible as Muskegon Lake surveying progressed, especially in 2018, making our results useful for informing future restoration efforts in an era of global climatic shifts (Harris et al., 2006). Wetlands are considered some of the most vulnerable habitats in the face of climate change (Erwin, 2009); continued Muskegon Lake monitoring would not only help confirm restoration trajectories, but also would determine the retainment of littoral ecosystem services during climatic environmental shifts. Our research therefore contributes to the expanding literature evaluating ecological restoration and the implications of climate change (Wilby et al., 2010).

We advocate for both the reduction of preventable stressors at restored habitats to increase macrophyte community stability, even after initial restoration is implemented, and for the incorporation of resiliency into restoration designs. This may include adaptive management strategies, such as invasive species removal, to direct trajectories towards the desired habitat quality (Erwin, 2009) and shoreline development that incorporates soft features and optimizes carbon storage (Mcleod et al., 2011). A holistic awareness of littoral community structure and the main ecological drivers of community change will be imperative for executing restoration projects designed to resist predicted environmental shifts. If unexpected environmental changes do occur, this knowledge also will benefit the formation of suitable adaptive management plans to further improve habitat integrity.

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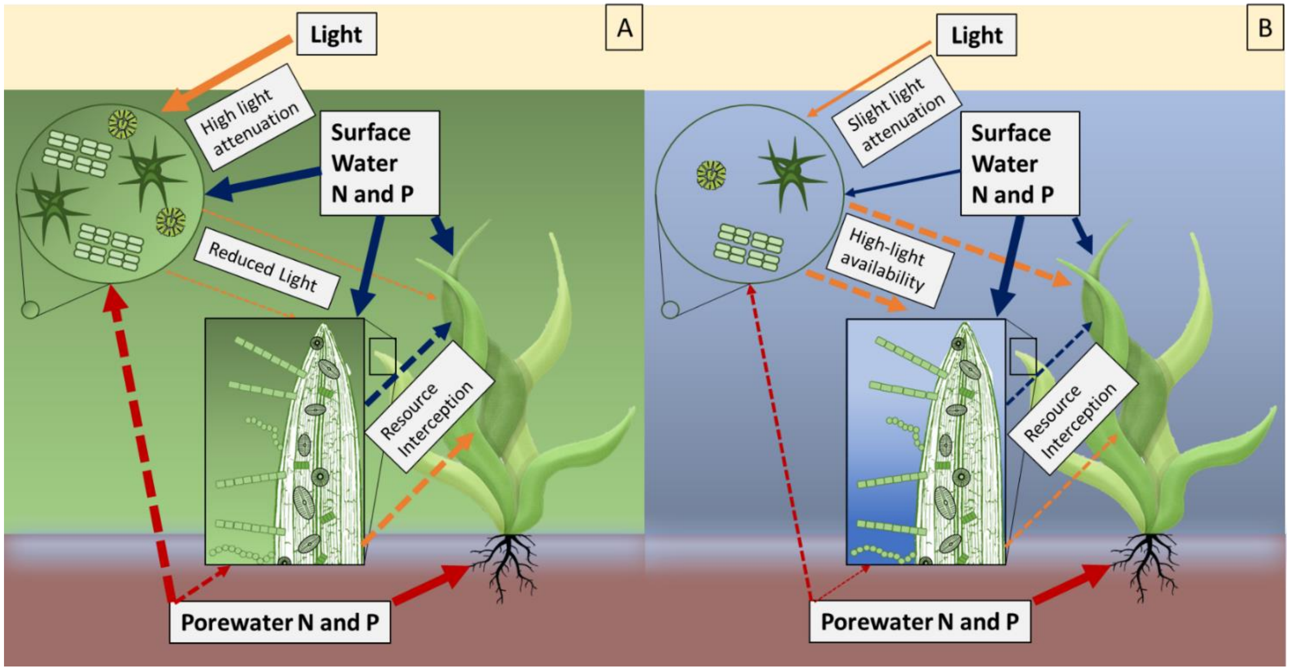

Figure 4.1: A conceptual diagram of macrophyte, epiphytic algae, and phytoplankton interactions within the controlled mesocosm experiment. Red arrows indicate porewater nutrient uptake, blue arrows indicate surface water nutrient uptake, and orange arrows indicate light attenuation. Solid arrows represent direct access to a resource and dashed arrows indicate indirect access to a resource. Orange arrow thickness indicates the amount of light availability and blue and red arrow thickness indicates the importance of that nutrient for each primary producer group. (A) Observed macrophyte, epiphytic algae, and phytoplankton interactions. (B) Expected macrophyte, epiphytic algae, and phytoplankton interactions.

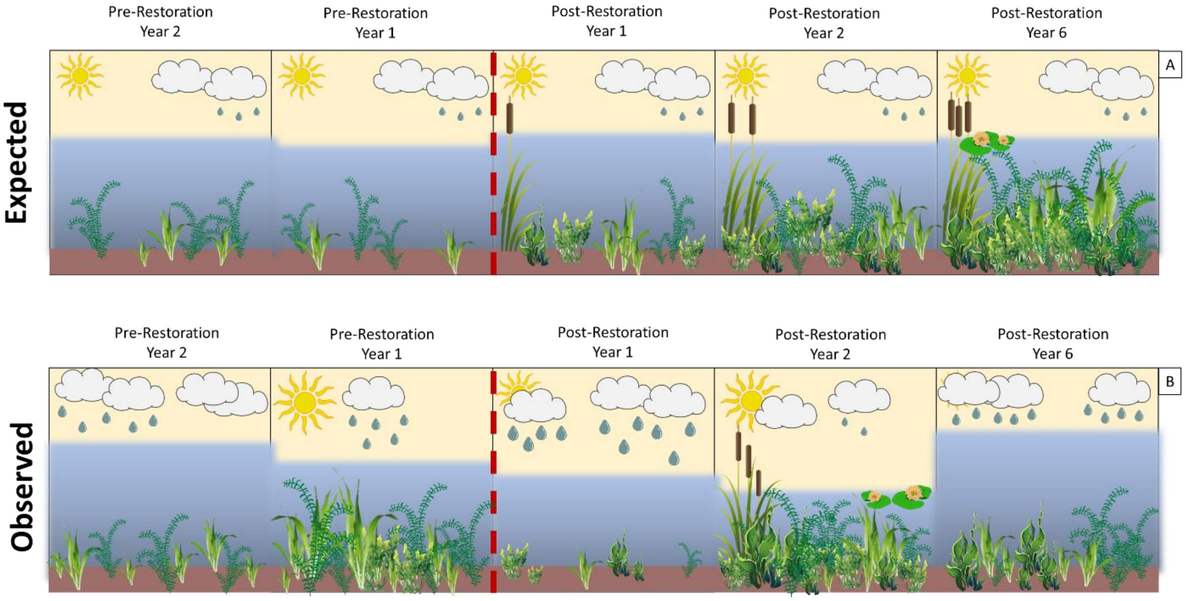

Figure 4.2: A conceptual diagram of expected and observed macrophyte community structure at restored shoreline habitats in Muskegon Lake and the varying environmental conditions among survey years. The sun represents warmer air temperatures and cloud presence represents cooler air temperatures. The number and size of raindrops indicates the amount of precipitation accumulation per survey year during the growing season. Water level is indicated by the different proportions of aquatic habitat available. Macrophyte density is represented by the number of macrophytes. Macrophyte biomass is represented by the size of macrophytes. Macrophyte richness is represented by the number of different macrophyte types. The red dotted line represents a separation between pre- and post-restoration survey years. A) Expected trajectory of Muskegon Lake shoreline restoration implementation. B) Observed trajectory of Muskegon Lake shoreline restoration implementation.