THE UTILITY OF GROWTH FORM FOR PREDICTING AND EVALUATING AQUATIC PLANT NUTRIENT RELATIONS

by

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Abstract

My dissertation focuses on the role of agricultural nutrient enrichment in structuring aquatic plant communities in Southern Manitoba, Canada, and changes in nutrient cycling resulting from changes in vegetation structure. Built around the “holy grail” framework of community ecology, which describes connections between the environment and ecosystem services or function as mediated by the traits of the biotic community, I explore the linkages between nutrient enrichment and aquatic plants of prairie streams focusing on species growth form (morphology) as a proxy for a suite of co-varying individual traits. I found that plant morphology interacted with environmental factors to determine which growth forms predominated in a stream: plant community shifts from being dominated by species with a submerged morphology to a community dominated by an emergent morphology as nutrient concentrations increase. I show how this pattern allows plant growth form to be used as an indicator of stream nutrient status. Further, I found that plants with similar growth forms share similar physiological features. Emergent plants have lower tissue nutrient concentrations per unit biomass and thus transfer fewer nutrients per unit biomass from the sediment to the water column than submerged plants. My work also includes a phylogenetic thread that brings novel insight: species at sites with higher nutrients all tend to be clustered in a few branches of the plant phylogeny whereas stream sites with lower nutrients have species from a diverse mixture of phylogenetic lineages. I used plant phylogeny to examine whether evolutionary history is related to tissue nutrient concentration and found the influence is mostly attributable to phenotype. These findings hint at the possibility of alternative stable states for prairie stream vegetation: a high nutrient emergent community and a

low nutrient submerged community. These alternative states are comparable to those found in shallow lakes, where high nutrient conditions are dominated by algal growth and bring about turbid water, whereas lower nutrients are characterized by clear water and abundant macrophyte growth. The nutrient transferring functionality of these two vegetation states should also differ, but specific quantities transferred would dependent on the proportion of biomass of each growth form.

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I have made an attempt to acknowledge those who have helped me in my endeavor, but the work represented by this (or any other) dissertation is a detailed and complicated affair. I apologize to anyone who has provided me with guidance and is absent from this list, it is a fault of my memory and not of your importance.

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Chapter 1 - General Introduction

1.1 Background

Hungarian physiologist Albert Szent-Györgyi (1893–1986) is quoted as saying "there is no life without water" and yet, the inherent qualities of water lend irony to its relationship with life. Water's physical property of fluidity and chemical property of solvency enable natural waterbodies to collect human-produced substances that disrupt their natural ecology. Water has been called the universal solvent because it can dissolve an array of hydrophilic substances. This attribute is one of the factors that make known life possible, viz., most biochemical processes occur in an aqueous medium. Water is also axiomatically said to flow downhill and, when it does, it carries other substances dissolved and suspended within it. Eventually, water will pool and evaporate, concentrating its dissolved and suspended load. The phenomena of universal solvency and downhill flow, combined with the fallacious dictum "the solution to pollution is dilution", have led to the use of our lakes, rivers and oceans as humanity's dumping grounds.

Nutrients—molecules or elements essential for growth and maintenance of life—are one major group of dissolved and suspended materials transported by rivers and streams. Of special concern are the macronutrients nitrogen (N) and phosphorus (P). In aquatic environments, these elements are found in numerous compounds and take a variety of forms: organic, inorganic, dissolved, particulate, etc. Nitrogen's pervasiveness in living systems may stem from its ubiquity, abundance and multiple naturally occurring oxidation states in the environment. Along with carbon, N is the unifying element in

amino acids and, consequently, proteins. Nitrogen is also a necessary constituent of nucleotides, the building blocks of heredity. Nucleotides are linked together in nucleic acid strands by P-containing phosphate groups and form RNA, DNA and their intermediates. Phosphorus is further required by cells for containment, protection (phospholipid bilayer membranes) and energy transport (adenosine di/triphosphate; ADP/ATP). Like N, P is a multivalent element, but unlike N it is rarely found in an elemental form and is almost always bound with oxygen as phosphate because of its high reactivity. The global reservoirs for each of these elements differ. N is drawn from the atmospheric N_2 pool, whereas P is primarily weathered from rock. Both occur commonly across the Earth (albeit P is distributed less evenly than N), but neither reservoir is readily available to plants and the rate of conversion into biologically active forms varies for each.

The nutrient enrichment of aquatic ecosystems, and its consequences, is the general motivation for my dissertation, but the plight of Lake Winnipeg (Manitoba, Canada) is the specific instigator of the research. Lake Winnipeg is the 10th largest body of freshwater on Earth by surface area, and holds approximately 294 cubic kilometers of water. Winnipeg is a relatively shallow lake, having a mean depth of only 12 m, but a surface area of 24,500 km^2. It is part of a series of large lakes that arc across North America from the five Laurentian Great Lakes in the east up to Great Bear and Great Slave Lakes in the north central part of the continent (Figure 1.1). Lake Winnipeg is positioned in the centre of this arc and is situated on the northeastern boundary of the North American prairie region, which comprises about 650,000 km^2 of the lake's catchment area. The North America prairies have suffered significant human impacts over the past 150 years, particularly conversion of land from native grassland to row-

crop and livestock agriculture (Samson and Knopf 1994). Historically, southern Manitoba was vegetated by a mixture of tall-grass prairie and wetland ecosystems (Hanuta 2001). The landscape is now dominated by a diversity of agriculture, but is principally sown in small grains and canola or used for swine farm operations. Consequently, nutrient inputs are a dominating ecological problem for Lake Winnipeg and it now receives an estimated 7,600 tonnes of P and 90,700 tonnes of N per year (Environment Canada and Manitoba Water Stewardship 2011) compared to the pre-1970 estimate of 4,600 tonnes P per year (McCullough et al. 2012). Two rivers, the Red River (of the North) and the Assiniboine River, merge in the City of Winnipeg and together are the single major contributor of nutrients to Lake Winnipeg. More than half (68%) of the P load enters the lake through the Red-Assiniboine system (Environment Canada and Manitoba Water Stewardship 2011). These added nutrients can have dramatic effects on aquatic primary producers.

Aquatic primary producers are generally separated into macroscopic aquatic plants and microscopic algae. Large plants growing in or associated with a water medium are variously referred to as water plants, hydrophytes, aquatic macrophytes or, simply, macrophytes. Macrophytes have been the subject of scientific investigation for over 125 years and records of their cultural significance (Vedic texts of ancient India) date to at least 500 B.C.E. (Sculthorpe 1967). The aquatic plants do not represent a discrete phylogenetic group; rather they are a collection of species from a diverse array of green plant lineages including green algae (Chlorophytes), mosses and moss allies (Bryophytes), ferns (Pteridophytes), and the flowering plants (Angiosperms). The term macrophyte literally means “large plant,” and is an ecological and functional term of convenience with an elusive technical definition. The use of the word arose to distinguish the large aquatic primary producers from the single celled and colonial microscopic

primary producers (algae/phytoplankton) common in aquatic ecosystems. Here, I use macrophyte to mean macroscopic (large enough to be seen with the naked eye), hydrophytic (growing in water) or phreatophytic (rooted in water) streptophyte (a clade of photosynthetic organisms with sterile “jacket” cells around their oocytes). This definition is a collection of verbal filters that sets size, habitat and evolutionary boundaries on the target study organisms. Written another way, my definition of macrophyte is: an algae, moss, liverwort, fern or flowering plant that has some or all of its above- or below-ground parts growing submerged in water or in saturated soil or sediment.

The aquatic environment imposes greater physical forces (such as drag and light attenuation) and biochemical constraints (low gaseous oxygen and carbon concentrations, lower diffusion rates, thick laminar boundary layers) upon plants (or portions of plants) that are submerged. These strong pressures have selected for various aquatic adaptations and morphological convergences. Similarity in gross physiognomy among aquatic plants is one of the earliest themes in macrophyte investigations (Schenck 1886) and the basis of growth-form classification schemes (Arber 1920; Luther 1949; Penfound 1952; Den Hartog and Segal 1964; Sculthorpe 1967). Yet, no classification has proven universal for all species because phenotypic variation, and ontogenic changes often lead to ambiguity. The simplest morphological classification is based on the position of a plant’s actively growing photosynthetic tissues relative to the water surface: submerged below, floating on, or emergent from a permanent or periodic water surface. A plant’s growth form determines which resource compartments—sediments, surface water, and/or atmosphere—it can access. For example, rooted macrophytes take up nutrients from the sediments, but can supplement with nutrient uptake from the water-column (Nichols and Keeney 1976; Pelton et al.

1998). Conversely, non-rooted species, having no contact with the substrate, can only access nutrients in the water-column. Macrophytes with aerial tissues are able to acquire CO_2, O_2 and light from both the atmosphere and water, while subsurface forms must extract or receive these from the water-column where they are much less abundant (Westlake 1975). Here, I use six general growth form groups (free-floating, emergent, floating leaf, submerged caulescent, submerged rosette, and submerged decumbent) modified from Sculthorpe (1967) that denote the rooting condition and the environmental compartment where the majority of a mature individual's photosynthetic surfaces actively grow (Table 1.1)

Growth form phenotype is a synoptic trait, having been shown to co-vary with a suite of other traits (Arber 1920; Sculthorpe 1967; Hutchinson 1975). A trait is a well-defined, quantifiable attribute of an organism, usually measured at the level of individuals and used for species level comparisons (McGill et al. 2006). Lavorel and Garnier (2002) provide a conceptual framework for investigating relationships between environmental determinants, species and ecosystem functions using traits (Figure 1.2). Their model incorporates filter theory (Keddy 1992), which states that local community structure (composition, richness, and abundance) is the result of environmental factors sorting and filtering the regional species pool based on the traits of the constituent species. According to Lavorel and Garnier's framework, the properties and relative occurrence of the species in the local assemblage determine the local ecosystem services and functions. The outcomes of these functions may then alter the environmental factors in a progressive cycle (e.g., facilitation or inhibition).

Aquatic plants are important components of freshwater ecosystems. They provide vertical structure that creates habitat for invertebrates and fish, mitigate physical

forces caused by waves and currents, and play a role in the biogeochemistry of lakes and rivers (Carpenter and Lodge 1986). Macrophyte community structure and individual physiology also respond to ecosystem changes. The aquatic plant flora of southern Manitoba, for example, has been shown to vary with water P concentration and other environmental parameters (Pip 1987, 1988). Other studies have shown vegetation tissue nutrient content (Willby et al. 2001) and the N isotope ratio (Kohzu et al. 2008) are altered by the water nutrient status. Luxuriant plant growth, however, is often undesirable to recreational and industrial users of aquatic systems. Increased nutrient input to aquatic ecosystems is one of the major causes of this excess growth (Carr et al. 1998). A detailed understanding of how nutrients affect aquatic vegetation and how the vegetation, in turn, changes nutrients is vital to efficient and effective management.

1.2 Objectives

My dissertation reflects the broad scope and thematic diversity of my personal research background and goals. I have drawn ideas and tools from different biological backgrounds and used a combination of extensive observational and intensive experimental approaches. The general question that drives this body of research is: what factors and mechanisms determine aquatic and wetland plant diversity, abundance and distribution? This is a very broad question that must be subdivided to make it tractable. Here, I investigate the environmental drivers of aquatic plant community structure and certain ecosystem functions affected by community composition, with special focus on prairie streams of southern Manitoba. I set out to examine three main questions: (1) Do environmental factors influence macrophyte community composition and abundance? (2) Can a trait-based aquatic plant metric predict waterway trophic status? and (3) How are

nutrient dynamics influenced by aquatic plant traits? Each question is the focus of one of the following chapters where I further subdivide each objective into specific aims.

1.3 Structure of the Dissertation

My dissertation broadly examines the role and utility of growth form in the nutrient ecology of aquatic plant communities. It consists of this introductory material, three primary data chapters, and general conclusions. Because the data chapters are to be submitted as stand-alone manuscripts, it was unavoidable that some methods among these chapters are repeated. My research plan followed the framework of Lavorel and Garnier (2002, Figure 1.2). This framework seeks to relate the environment to plant communities and plant communities to ecosystem function.

This logical chain is one of the fundamental concerns of plant ecology (the “holy grail” as the authors call it). Lavorel and Garnier, among others, propose that the interactions in the environment-biota-function paradigm are mediated by traits—measurable attributes of species—and not species identity. Chapters 2 and 3 of my dissertation address the environment-biota linkage of the framework and examine it in different directions and at different scales. In chapter 2, I investigate whether aquatic plant community structure can be predicted from environmental conditions. This question is assessed at a regional scale using field-data collected from agro-prairie watercourses in southern Manitoba, Canada. I then reverse the direction of this question in chapter 3, and take a cross-continental approach, using Canadian and European empirical data to examine the calibration question, "can aquatic plant community structure be used to assess environmental condition?" Finally, chapter 4 addresses the biota-function linkage and I examine the question, "what is the functional role of aquatic plant community structure in ecosystem nutrient dynamics?" For this work, I use a fine-scale experiment

to quantify nutrient uptake by heterophyllous species of aquatic plants. Each data chapter fits together within the Lavorel and Garnier (2002) framework.

Chapter 2 explores the major environmental drivers on the landscape of southern Manitoba. I predicted that occurrence of macrophyte species varies with habitat exposure to human activity. Ecological filtering will select (sort) the species of the regional species pool to determine the observed assemblage based on the traits of those species. The traits that are advantageous in the dominant environment at a site will pass through the filter and those that are disadvantageous will not. I expect macrophyte composition to vary along a gradient of human activity, but be more similar at sites with similar levels of activity. To test these predictions, I applied a comprehensive suite of multivariate analyses to physical, chemical, GIS, and vegetation data I collected from southern Manitoba in 2010 and 2011. I confirmed that the greatest environmental variation is in nutrient parameters, but found this was poorly associated with human influences. I concluded that, despite having the greatest variation, nutrients were a weak driver of macrophyte community structure and unmeasured factors, such as hydrology, may play a bigger role. I detail my evidence and a discussion of the implications for bioassessment.

Chapter 3 links the rich macrophyte literature of Europe to the advancing investigations of macrophytes as bioindicators in North America. Macrophyte metrics have been used to indicate surface water quality in Europe for many years (Kohler 1975). In contrast, only a few (but growing number) of such studies have been carried out in North America (Dennison et al. 1993; Small et al. 1996; Carr et al. 2003; Benson et al. 2008). Direct application of the European metrics to North America is not possible because only a small percentage of indicator species overlap between the two

continents. Thus, I sought to generalize the European indicators of surface water quality using a ubiquitous trait, rather than taxonomy. I selected gross physiognomy (morphology) as the candidate feature because aquatic plants can be naturally classified into growth forms and these growth forms tend to be associated with a suite of similar physiological properties. For example, plants in habitats of greater productivity (nutrient availability) are often limited by light due to shading (Grime 2002) and plants compete for light by increasing height (Gaudet and Keddy 1995). Aquatic plants must compete for light not only from neighboring macrophytes, but also epiphytes and phytoplankton. To overcome this competition, they must reach or exceed the water's surface. If macrophyte growth forms are arranged into increasing height relative to the water surface (Table 1.1), I predicted habitats with greater productivity to have a greater occurrence of taller (emergent) and surface floating forms, whereas habitats of lesser productivity to have predominance of shorter (submersed) growth forms. My third chapter explores the use of growth form as an indicator of water trophic status, applies this approach to Canada and tests its efficacy by validating the growth form metric against actual water chemistry.

Chapter 4 tackles a question of ecosystem services: do different growth forms differentially transfer nutrients out of the substrate and into the tissues? Rooted aquatic plants act as biological nutrient pumps, transferring nutrients from the sediments to their tissues and then leaching a portion of those nutrients to the water column upon senescence. The quantity that is transferred has been shown to directly correlate with tissue nutrient concentration (Qui et al. 2002). This led me to perform a detailed correlative investigation of the relationship between growth form, species identity and tissue nutrient concentration. The difficulty in attributing nutrient composition to either growth form or species identity is that species and morphology have a strong association

and may also have a shared evolutionary history. To answer this, I experimentally manipulated macrophyte growth form of three species using the plant hormone abscisic acid. This allowed me to understand the independent role of each of these factors in determining tissue nutrient concentrations.

Finally, in the general conclusions, I address the feedback between macrophyte-driven nutrient cycling and the nutrient concentrations that structure the macrophyte community. This work is only a starting point for further study, nevertheless, I theorize on scenarios of nutrient enrichment, plant community response and potential consequent nutrient transfer.

1.4 Significance

Connecting biota and diversity to ecosystem function has long been a pursuit of ecologists. Two of the most fundamental questions in ecology are: what factors determine which species coexist together, and how does the composition and biodiversity of communities affect the basic functioning of the ecosystem? Despite decades of research, these questions are still active and relevant for terrestrial vegetation, and are poorly understood or virtually unknown for aquatic plants. These questions encompass a broad arena of research and my work confronts three foci of this research as it relates to aquatic plants. A large proportion of the macrophyte ecology literature in the 1980's and 1990's focused on two questions: (1) what is the relative importance of roots versus shoots in supplying mineral nutrition for rooted taxa (Nichols and Keeney 1976)? and (2) can the response of macrophyte abundance (biomass) to nutrient enrichment be predicted (Carr et al. 1998)? Definitive answers remained elusive, but there appears to be consensus that roots supply the majority of nutrients unless concentrations in the water are high (Chambers et al. 1989; Robach et al. 1995) and

abundance increases with nutrients, but this trend can be decreased by direct and indirect physical factors such as light and current velocity (Sand-Jensen and Borum 1991; Madsen et al. 2001). In the 2000s, these lines of research gave way to investigations that developed water quality indices (e.g., Holmes et al. 1999) using the affinity of certain macrophyte taxa to pristine conditions. The efficacy of these metrics is dependent on both abundance and community composition. More recent work is focusing on macrophyte functional ecology (e.g., Riis et al. 2013). I have attempted to unite elements of macrophyte research from the past 35 years by examining community composition, abundance and growth form in relation to nutrients (different nutrient sources for differing growth forms; chapter 2), applying the growth form—nutrient source relationship to improve macrophyte-based metrics (chapter 3) and quantifying the functional role of macrophytes in ecosystem nutrient cycling (chapter 4).

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Tables and Figures

Table 1.1: Six general classes of macrophyte growth form (morphology) denoting the rooting condition and the environmental compartment where the majority of a mature individual's photosynthetic surfaces actively grow. Growth forms modified from Sculthorpe (1967) and are roughly arranged in order of decreasing stature.

Definition	Example Taxa
Free-floating Plants with or without roots (but the roots not requiring anchoring in the substrate for growth and maturation) with the majority of an individual's photosynthetic surfaces growing on top of or below the surface of the water,	Coonstail (*Ceratophyllum*), Duckweed (*Lemna*), Bladderwort (*Utricularia*)
Emergent Rooted plants with the majority of an individual's photosynthetic surfaces growing above the surface of the water.	Spikerush (*Eleocharis*), Bulrush (*Schoenplectus*), Cattail (*Typha*)
Floating leaf Rooted plants with the majority of an individual's photosynthetic surfaces growing at or on top of the surface of the water.	Watershield (*Brassenia*), Pondlily (*Nuphar*), Waterlily (*Nymphaea*)
Submerged caulescent Rooted plants with the majority of an individual's photosynthetic surfaces growing below the surface, forming a canopy high in the water-column.	Most Pondweeds (*Potamogeton*)
Submerged rosette Rooted plants with the majority of an individual's photosynthetic surfaces growing below the surface, forming a meadow low in the water-column,	Quillwort (*Isoetes*)
Submerged decumbent Rooted plants with the majority of an individual's photosynthetic surfaces growing below the surface, lying or creeping along the sediment.	Brook moss (*Fontinalis*)

Figure 1.1: Map of the North American landmass overlaid with its large waterbodies (light gray). The five Laurentian lakes are generally considered the “Great Lakes” of North America, but these are the southeastern terminus of an arc of large lakes that stretches northwest along the edge of the Canadian Shield. Lake Winnipeg is in the centre of this arc and drains to Hudson Bay via the Nelson River (catchment area, the bulk of which flows through Lake Winnipeg, is outlined in white).

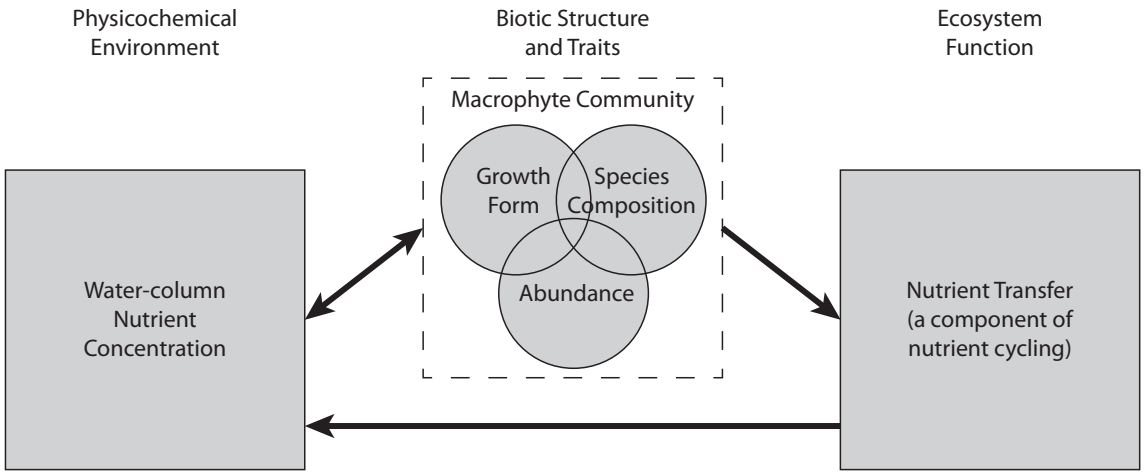

Figure 1.2: Diagrammatic representation of the "Holy Grail" framework proposed by Lavorel and Garnier (2002) linking the physicochemical environment to ecosystem function mediated by the traits of the biota. Here, I have modified and adapted the framework to show the factors explicit to my dissertation. The bi-directional arrow between nutrient concentration (environment) and macrophyte community (biota) represents chapters 2 and 3, and the arrow leading from macrophyte community to nutrient transfer (function) illustrates the connection for chapter 4. The arrow between nutrient transfer and nutrient concentration represents a feedback pathway (e.g., facilitation) that is briefly discussed in the general conclusions.

Chapter 2 - Aquatic plant community response to nutrient-producing human activities in southern Manitoba, Canada

Abstract

The drainage network to Lake Winnipeg (Manitoba, Canada), the 10th largest freshwater lake on Earth, receives elevated nutrient loads that contribute to lake eutrophication, including harmful and noxious algal blooms. To evaluate the potential for the aquatic vegetation in the tributaries of southern Manitoba to serve as bioindicators of nutrient-producing activities, I used multivariate analyses to investigate the response of the plant community to human activities that emit nutrients and in-stream environmental factors for 29 sites located on 22 tributaries of the Red River. Total nitrogen (TN) and total phosphorus (TP) concentrations accounted for the majority of measured environmental differences among these streams, yet stream-water nutrients did not correlate well with nutrient-emitting human activity assessed from geospatial data. Stream vegetative composition (particularly richness), was only weakly associated with stream-water TP and, overall, the aquatic plant assemblages in S. Manitoba were mostly insensitive to nutrient concentrations, thus failing to meet this key requirement of a nutrient bioindicator.

2.1 Introduction

Biological metrics, including morphological characteristics, behavioral attributes, composition, abundance and biochemical processes of species, genera or communities, have long been used for monitoring changes in the environment (Kolkwitz & Marsson

1908). An underlying assumption of bioassessment is that target stressors cause environmental changes and these stressors are influenced by human activities, ideally in a known way. Management actions can then be applied and assessed for their value in mitigating or remediating detrimental human activities before catastrophic damage to the ecosystem (Karr & Chu 1997). Various organisms and taxonomic groups already serve as bioindicators. In North America, macroinvertebrates are primarily used for evaluating stream quality (Rosenberg & Resh 1993) and have been adopted by the U.S. Wadeable Streams Assessment (USEPA 2004) and Canadian Aquatic Biomonitoring Network (CABIN; Carter & Pappas 2011) programs. The European Water Framework Directive (Directive 2000/60/EC & 2008/32/EC), a comprehensive body of legislation aimed at improving surface water quality, outlines the use of fish, macroinvertebrates, and both microscopic- and macroscopic-aquatic plants for biomonitoring. The use of macroscopic plants (macrophytes) for aquatic assessment is more widely applied and has a longer history in Europe than North America (cf. Haslam 1982, Nichols et al. 2000). Several aquatic plant bioassessment methods, however, have been developed in both the U.S.A. and Canada in recent years (e.g., Beck et al. 2010, Rooney & Bayley 2012).

Macrophytes are proposed to perform well as bioindicators because they: (1) can be found in a wide variety of habitats and are ecologically important (Cronk & Fennessy 2001); (2) have easily quantifiable attributes that negatively respond to degraded environmental conditions (Nichols 1999); (3) are usually sessile allowing them to serve as temporal integrators of environment change at a specific location (Adams & Sand-Jensen 1991); (4) can be identified to species with relative ease and precision compared to macroinvertebrates and algae; and (5) can be sampled quickly and with minimal costs (Fassett 1957, Crow & Hellquist 2000). The use of macrophytes as bioindicators has,

however, been criticized. Demars et al. (2012) suggest that macrophytes cannot be used to ascribe ecological condition to specific sites because the variability in the stressor-biota relationships is high, but agree that aquatic plant-based indices may still be useful for detecting human pressures at sites spanning a large gradient.

The prairie region of North America has experienced significant human impacts over the past 150 years, particularly conversion of land from native prairie to row-crop and livestock agriculture, and the loss of wetland habitat (Samson & Knopf 1994). Lake Winnipeg, the 10th largest freshwater lake on earth, is situated on the northeastern boundary of the North American prairies and drains about 650,000 km^2 of this biome. In addition to agriculture, the prairie portion of the catchment also contains several large urban centres and numerous small communities (Wassenaar and Rao 2012). Consequently, nutrient inputs are a dominating ecological problem, with the lake receiving estimated inputs of 7,600 tonnes of P and 90,700 tonnes of N per year. More than half (68%) of these loads enter the lake from the Red River, with TP export from the land drained by tributaries of the Red River being 0.13–0.33 kg/ha/y (EC/MWS 2011). Because the impact of human-derived nutrients is great in southern Manitoba and the aquatic plant flora of the region has been shown to vary in composition with P (Pip 1987; Pip 1988), the macrophyte assemblages of the region may serve as potentially informative nutrient indicators.

My objective was to establish whether macrophytes are useful as bioindicators of nutrient status for streams in southern Manitoba. The study streams drain to Lake Winnipeg where nutrient-load reduction efforts are being implemented in order to improve water quality and ecological health of the lake and its basin for present and future generations. The research described here aims to determine whether measurable

attributes of the macrophyte community respond to the stressors associated with human nutrient-producing activities in southern Manitoba. Specific aims included: (1) examining relationships between the macrophyte community composition and nutrient and non-nutrient environmental variables (pH, conductivity, depth, velocity, etc.), and (2) determining if community summary metrics (richness, evenness, ecological- and phylogenetic-diversity) increase, decrease or equivocate with human-caused nutrient-emitting activities. Focusing on these specific aims enabled me to assess macrophyte community responses to nutrient-producing human activities in the context of background levels of nutrients and other environmental variables.

2.2 Methods

2.2.1 Study sites

Historically, the landscape of southern Manitoba, Canada, was predominantly a mixture of tallgrass prairie and wetland ecosystems (Hanuta 2001). The landscape is now dominated by a diversity of row-crop and livestock agriculture, but is principally sown in small grains and canola, or used for swine farm operations. The Red and Assiniboine rivers drain the region from the south and west, respectively. These rivers merge and flow north into the southern basin of Lake Winnipeg. The Red-Assiniboine river system is the single major contributor of nutrients to Lake Winnipeg. The nutrients in this system are primarily derived from the agriculturally-dominated tributary streams but with main channel urban centres also making contributions (Schindler et al. 2012).

I selected 29 study sites, 15 sampled in 2010 and 14 sampled in 2011, on 22 tributaries of the Red River (Table 2.1, Figure 2.1). I use the term ‘site’ to indicate the area between banks (across the wetted width *sensu* Biggs & Kilroy 2000) along a 100 m

stretch of stream channel. Sites were selected to span a gradient of human nutrient-producing activities in their subcatchment, while stratifying across the fine and very-fine soil texture types typical of southern Manitoba. Sites were placed at least 100 m upstream or 300 m downstream of obvious artificial influences (e.g., bridges, road crossings, etc.). All sites were "wadeable" in late summer (0.3—0.9 m deep) with channel widths of 2.5—13 m and subcatchments upstream of each site ranging in size from about 4 to 900 km^2 (Table 2.1). Consistent with watercourses throughout southern Manitoba, all of the study streams had been altered, to some degree, through channel straightening, dredging and levee construction to serve as drains in an agricultural landscape. The riparian vegetation consists mainly of native and introduced terrestrial grasses and herbs.

2.2.2 GIS data

I used the methodology described in Yates et al. (2012) and Yates & Bailey (2010a) to generate a human activity gradient (HAG) that characterizes the extent of nutrient emissions within the subcatchment upstream of my sampling sites. Subcatchments were delineated using the Hydrology Spatial Analysis Tool in ArcGIS 10 and a digital elevation model provided by Natural Resources Canada (http://geogratis.gc.ca). Agricultural and population census data acquired from Statistics Canada, Natural Resources Canada and the Manitoba Agricultural Services Corporation were intersected with the subcatchment delineations to gather data for each site. These data were arcsin- or log-transformed as per Yates et al. (2012; Appendix A). The HAG was produced using two rounds of principal components analysis (PCA). The first round consisted of three separate unscaled (covariance) PCAs, one each for row-crop (areas of grains, legumes, and other crops), livestock (animal nutrient unit equivalents per area

for pigs, cattle, etc.) and wastewater (population density served by septic, municipal sewerage, etc.) variables. The second round used a correlation matrix of loading scores from the three first-round PCAs as input for the second-round PCA. The axes of this second round PCA were used to represent the gradient. The double PCA procedure was performed to ensure the HAG generated here was compatible with the results of Yates et al. (2012). The HAG was based solely on agricultural census and remotely sensed geospatial information that was intersected within the outline of the subcatchment upstream from each plant sampling location.

Ten physicochemical ("environmental") variables were measured *in situ* at each site. Water samples for total nitrogen (TN) and total phosphorus (TP) analysis were collected biweekly between May and August; concentrations were averaged prior to statistical analysis. Previous work in southern Manitoba demonstrated the need to collect at least 10 samples in order to achieve average TN and TP concentrations with standard errors less than 20% (Corriveau & Chambers unpublished). Point measurements of conductivity, temperature, pH and turbidity were collected mid-morning at the centre of each sampling site at the time of plant sampling using a handheld multiparameter meter (556 MPS; YSI, Inc. Yellow Springs, OH, USA) and infrared turbidimeter (Orbeco-Hellige; Sarasota, FL, USA). Depth and velocity were measured at each of 30 randomly placed quadrats within the site (see plant community section below) and pooled to create a site average. Although more frequent measurements of the other environmental variables would have been preferred, logistical considerations prevented this. Frequency distributions for each variable were plotted and normality was assessed with an Anderson-Darling test. Non-normal variables (turbidity, velocity, width, TN, TP, N:P) were log-transformed to better approximate a normal distribution.

At each site, aquatic plant identity and percent cover (to the nearest 5%) were recorded in late July or early August (the season corresponding to maximum biomass). Initially, a species list was generated for each site by systematically walking around the area. Surveys were then performed in 30 0.1 m^2 quadrats distributed across the site. Placement of quadrats was intended to be unbiased: beginning from the downstream end of the survey reach, a quadrat was cast backward (so that thrower could not see a target). If the quadrat landed outside of the wetted area of the site, the quadrat was retrieved and the procedure was repeated. From a successful quadrat landing, subsequent throws were made moving upstream. If the upstream boundary was reached before 30 throws, the quadrat was tossed from the middle of the reach and the orientation was determined by looking at the seconds place on a digital watch (even = upstream, odd = downstream). Percent cover of each species present in every quadrat was recorded; a 10 cm x 10 cm square was visualized as the area of the palm of the observer's hand and used as a reference for 10% of the quadrat. Percent covers were recorded to the nearest 2.5% by visually subdividing the 10 x 10 cm area into four sections. Taxonomic voucher specimens of each species were collected to confirm identification and deposited in the Connell Memorial Herbarium (UNB) at the University of New Brunswick. Species were identified and confirmed using Crow & Hellquist (2000), FNA (1993+), Gleason & Cronquist (1991), Budd et al. (1987) and GPFA 1986. Species that were found at fewer than 4 sites were excluded from the dataset for analyses.

2.2.3 Statistical Analyses

Measured water nutrient concentrations were assumed to be influenced by both human and background (e.g., geologic or biotic) nutrient sources. To determine how much of the water chemistry is attributable to human activity versus background nutrient

levels, average measured water nutrients for each site were regressed onto the sum of the nutrient estimates from the GIS layers used to develop the HAG. The regression models used were: $TN_{meas} = b_0 + b_1 * TN_{GIS} + error$ and $TP_{meas} = b_0 + b_1 * TP_{GIS} + error,$ where TN_{meas} and TP_{meas} are measured total nitrogen or phosphorus, and TN_{GIS} and TP_{GIS} = estimated total nutrient emissions from human activities based on geospatial data. Correlation coefficients from these models were used to assess the association between the GIS estimates and summer water nutrient concentrations. Residuals from regressions were assumed to represent the background nutrient concentrations at each site.

Species percent covers were used as a proxy for abundance. Common community metrics were calculated for each site using the species abundance values: richness (number of taxa); Simpson diversity; Simpson evenness; and relative dominance (Berger-Parker 1970). Faith's (1992) phylogenetic diversity was also calculated for each site from a phylogeny of all taxa found at all sites. The phylogeny was generated using the Phylomatic ver. 3 webserver (Webb & Donoghue 2005). Non-angiosperm groups (ferns, bryophytes and charophytes) were grafted to the R20120829 tree of Phylocom taken from the topology in Qui et al. (2007). There was only one representative each of liverworts, mosses and macroalga in the dataset. Phylogenetic resolution below the family level was incorporated using the Integrated Taxonomic Information Service (www.itis.gov) taxonomic structure (subfamilies, tribes, sections, etc.) for ferns and flowering plants. Despite these efforts, a few clades were still unresolved and remained in a polytomy. Within genera (*Salix*, *Callitriche*, *Typha*, and *Sagittaria*), a maximum of three taxa were involved in polytomies but at least one taxon was a composite of specimens that were not identifiable below the rank of genus

because they lacked key characters. Thus, I was not concerned about the effect of these polytomies on my results. The largest (involving four branches) and possibly most influential polytomy occurred in the "BEP" clade of the grass family. The effect of this lack of resolution was not assessed. The resultant tree is provided in Appendix B. Branch lengths were assigned with the Bladj module of Phylocom ver. 4.2 (Webb et al. 2008). Node ages for calibration (Appendix C) were derived from published estimates or ages provided by the Angiosperm Phylogeny Website (www.mobot.org/MOBOT/research/APweb/).

I evaluated whether individual species abundances responded to the environmental or GIS variables as a linear or unimodal function to determine whether redundancy analysis (RDA; linear-based method) or canonical correspondence analysis (CCA; unimodal-based method) was the most appropriate procedure. Species abundances were fit to two competing models: (1) species percent cover as a linear function of the environment scores, $cover = b_0 + b_1 * predictor + error$, and (2) percent cover as a quadratic function of the environment scores, $cover = b_0 + b_1 * predictor + b_2 * predictor^2 + error$ using generalized linear regression. Errors were assumed to have a normal distribution with zero mean and equal variance. Model goodness-of-fit was evaluated using the Akaike's information criterion (AIC). A linear model was a better fit to the data overwhelmingly more often than unimodal (results presented in Appendix E), therefore I selected RDA for the multivariate analyses.

Ordination of the community abundances constrained to a multiple regression with the environmental and GIS variables was performed with RDA (Rao 1964, Wollenberg 1977). The model used in the RDA was:

$$[V] = TN_{GIS} + TP_{GIS} + TN_{meas} + TP_{meas} + Tb + C + NP + D + W + V + pH + Tm$$

(Equ. 2.1)

where [V] is the species abundance matrix, TN_{GIS} = GIS-estimated total nitrogen, TP_{GIS} GIS-estimated total phosphorus, TN_{meas} measured total nitrogen, TP_{meas} = measured total phosphorus, Tb = turbidity, C = conductivity, NP = total nitrogen:total phosphorus ratio, D = depth, W = width, V = velocity, and Tm = temperature. Coefficients are not shown in this model for brevity. Prior to analysis, the variables were centered on their respective means and divided by their standard deviations. Because stream width is related to subcatchment area and had very a strong influence on the vegetation, I performed subsequent analyses using partial-RDA (Legendre & Legendre 1998) to remove the effect of width and potential reveal relationships that may have been masked. Here, the species abundance matrix was first regressed on width (the conditioning variable) and then an RDA was carried out on the residual abundance matrix using the same model as above except without the W (width) explanatory term.

The relationships between community metrics and abiotic variables were assessed with linear models constructed, for each metric, in the form:

$$C = TN_{GIS} + TP_{GIS} + TN_{meas} + TP_{meas} + Tb + C + NP + D + W + V + pH + Tm$$

(Equ. 2.2)

where C is the community metric and the remaining terms as for the RDA model above. Reduced models focusing on stream nutrients only were also constructed to evaluate the relative influences of human-derived vs. background nutrients:

$$C = B_0 + B_1 * N_{GIS} + B_2 * N_{meas} + B_3 * N_{GIS} * N_{meas} + error$$

(Equ. 2.3)

where C is the community metric, N_{GIS} is the summed nutrient (TN or TP) estimates from human activities and N_{meas} is the average measured nutrient concentration. Models were built for TN and TP separately because of the strong correlation between the GIS-estimated nutrients. These were fit using generalized linear modeling (GLM). All analyses were carried out in R (R Development Core Team 2012).

2.3 Results

2.3.1 GIS analysis of nutrient emitting activities

A total of 27 GIS variables, equally divided among 3 categories (row-crop, livestock and wastewater), were used to describe anthropogenic nutrient emitting activities in the 29 study watersheds (Table 2.2). Density of swine and cattle (based on nutrient unit equivalents, e.g., quantity of N and P in manure from 1 pig = 1.3 cows), were the two most variable parameters overall, with maxima of 28.5 and 32.3 nutrient units per km^2, respectively (Table 2.2). This contrasts with the estimated nutrient inputs from wastewater sources, which had maxima of 1.6 per km^2 for septic and 1.1 per km^2 for sewage lagoon TN. Small grain crops (such as rye, hemp and buckwheat) contributed the bulk of the row-crop nutrients (0.784 nutrient units per km^2) in the region.

In the first of the two-step HAG calculation, three separate PCAs were performed, each one representing a different human activity sector (row-crop agriculture, livestock agriculture and wastewater treatment). In each case, only the first principal

component (PC1) from the first step was used for the second step of the HAG calculation. For row-crops, PC1 explained about 65% of the variance and was mostly correlated with nutrient application to small grain crops. PC1 of livestock explained about 59% of total variation and was overwhelmingly driven by a combination of swine and cattle nutrient units. The wastewater PC1 explained 50% of the variation in the data and was mostly driven by estimated TN and TP released from septic and TN released from lagoon systems (Table 2.2).

In the second step of the HAG calculation, another PCA was performed using the scores of each site projected onto the first principal components from the step one PCAs (called "standardize factor scores" by Yates et al. 2012). This resulted in two explanatory principal components. PC1 (hereafter called HAG1) explained 49% of total variation and represented a combination of rowcrop and livestock farming practices. PC2 (HAG2) explained 38% more of the variation (HAG1 and HAG2 together explained 87% of total variation) and primarily responded to wastewater activities.

2.3.2 Site surveys

In-stream nutrients (TP and TN) among the 29 sites ranged from 0.01–2.25 mg/L TP and 0.8–8.4 mg/L TN with average concentrations of 0.585 mg/L and 2.59 mg/L respectively (Table 2.1). TN, TP and their ratio (N:P) were all significantly correlated (Table 2.3). Land-based nutrient emissions (summed across various GIS data layers) for each site ranged from 7,587–1,789,013 P units/km^2 and 24,708–5,398,568 N units/km^2 (Table 2.1), averaging 408,300 P units/km^2 and 1,173,000 N units/km^2. GIS-derived TN and TP estimates were highly correlated (R^2 = 0.993). Comparison of N:P ratios showed that minimum and maximum in-stream ratios differed by a factor of 34 times (2.1–70.5; median = 5.2) for environmental data whereas land-based ratios were generally low,

ranging from 2.25–3.33 (1.5 X difference) with a median value of 3.02. Regression of the summed GIS nutrient estimates against *in-situ* measured nutrient concentrations revealed that anthropogenic nutrient emissions did not predict stream-water nutrient concentrations; the slopes of the relationships were not significantly different from zero and the correlations were weak (R^2 = 0.013 TN; R^2 = 0.029 TP).

Comparison of the environmental parameters measured at each sampling location showed that velocity, average annual TP, depth and conductivity had the highest coefficients of variation (in descending order), whereas temperature and pH had the lowest. In addition to the significant correlations between TN, TP and N:P, measures of both TN and TP were correlated with depth and TN with velocity (Table 2.3). Velocities were moderate (max = 0.36 m/s) to non-existent (min = 0 m/s).

Among all 29 sites, I recorded 58 aquatic macrophyte taxa. Site richness varied from 6-22 with a median richness of 14 taxa (Table 2.4). The species consisted of five distinct morphologies: submerged-decumbent, submerged-caulescent, floating-leaf, emergent and free-floating. The most common growth forms were emergent (35 species) and submerged-caulescent (13 species) and least common was submerged-decumbent (1 species; Table 2.4). Species frequencies followed an L-shaped curve (few common and many rare species). Two common taxa, *Sagittaria cuneata* and *Schoenoplectus tabernaemontani*, were found at > 75% of sites while 26 species were rare, occurring at only 1 site (Table 2.5). *Stuckenia pectinata* is the modal dominant species, occurring as the most abundant species at 4 of 13 (out of 29) sites.

2.3.3 Biotic-abiotic relationships

In general, species relative abundances (expressed as percent cover) did not show significant correlations with either the GIS-derived or environmental variables (Equ. 2.1) using redundancy analysis (RDA). An overall test of significance (an ANOVA-like permutation test of the RDA results; Legendre et al. 2011) showed that the constrained relationship between the abiotic matrix and the biotic community matrix was insignificant ($p = 0.49$ after 999 permutations). The summed GIS-estimated TN and TP variables, however, were very highly correlated (0.997), causing them to have high variance inflation factors (VIF). VIFs greater than 10 signify that there is collinearity among the variables in the constrained ordination. I therefore removed TN from the RDA because, generally, TP is the limiting nutrient in aquatic systems. The RDA results with and without TN were not drastically different (confirmed by examining the correlations among the environmental and GIS variables, Table 2.3), but the VIF for the GIS-based TP lowered substantially (from 510 to 2.5).

The final RDA (without GIS TN; Fig. 2.2) showed less than 40% of the variance in the species abundance matrix was explained by the abiotic variables. Using stepwise model selection, the best model is one with intercept only. Backward selection found the best model to be one with width as the only explanatory term. Because width is intimately connected to catchment area and area can influence species richness, I performed a partial RDA whereby width was regressed on the community matrix and the RDA was performed on the residual, "width-less" community matrix axes to remove the size effect. When this was done, the environmental and GIS variables explained less than 33% of the remaining variance.

Multiple regressions of the calculated community metrics against the various abiotic variables (Equ. 2.2) revealed some weak patterns. Richness, evenness and

phylogenetic diversity each had five explanatory terms that were significantly correlated with the metric (Table 2.6). Total nitrogen, N:P ratio and temperature were found to be significant with all three of these metrics. Reduced regression models that included only nutrient variable (Equ. 2.3) were further examined (Fig. 2.3). Temperature was not included in these reduced models because (1) it was not a target stressor variable and (2) it was only measured once and therefore less reliable than the nutrient measurements. Overall, the reduced regressions showed very little association between the community metrics and either nitrogen or phosphorus (Table 2.7). For example, the low delta-AIC values show there was no support for either nutrient relating to dominance better than the other. Additionally, phylogenetic diversity-nitrogen was one of the only models with coefficients significantly greater or lesser than zero, but the nitrogen model had a higher AIC value then its insignificant phosphorus partner model (Table 2.7).

2.4 Discussion

Analysis of macrophyte relative abundance and community composition showed at best weak associations with measured and GIS-derived environmental variables, including nutrients, for 29 sites in southern Manitoba. Despite variability among sub-catchments of >100-fold in stream-water TN and TP concentrations (0.01–2.25 mg/L TP; 0.8–8.4 mg/L TN) and land-based nutrient emissions (24,708–5,398,568 N units/km^2; 7,587–1,789,013 P units/km^2), relative abundance of aquatic plant communities did not show strong association with nutrients. The role of nutrients in governing the abundance and composition of aquatic macrophyte communities has long been debated. Macrophyte communities should, in theory, respond to nutrients (Haslam 1982) and field-based empirical evidence almost always finds macrophytes respond to nutrients; however, nutrient variables can be confounded with other factors (e.g., Demars and

Trémolières 2009). For example, Dodkins et al. (2005) found macrophyte communities in Northern Ireland's rivers responded to chemical stressors particularly nitrate, but Demars et al. (2012) argued that alkalinity likely had a confounding influence on the macrophyte assemblages in the Dodkins' et al. (2005) study as well as several other investigations of macrophyte-nutrient associations. In southern Manitoba, pH was not correlated with nutrient concentrations, and did not seem to influence macrophyte community structure. It is therefore unlikely that my results were confounded by differences in alkalinity. The lack of response in the Manitoba macrophyte community to the abiotic stressors measured may be due to the effect of unmeasured variables (hydrology, for example) having a stronger influence on shaping the communities.

The only community metrics with unequivocal associations to TP and TN concentrations or the N:P ratio were richness, evenness and phylogentic diversity. Elevated concentrations of both TP and TN were associated with greater richness and diversity while evenness was correlated with TN only. The similar response of richness and phylogenetic diversity is not surprising because adding species increases phylogenetic diversity, though not linearly (Vellend et al. 2011). Both richness and phylogenetic diversity showed a marked decrease when the optimal range of N:P ratios (10—24; cf. Duarte 1992) for aquatic plants was exceeded (Fig 2.2). Decreasing richness with increasing nutrients is consistent with resource-competition theory (Tilman 1982), where richness is expected to track limiting resources (increases in nutrients decrease resources that are limiting). Grime's (2001) C-S-R triangle theory predicts evenness to also decrease at higher nutrient levels because the community is expected to shift toward fewer, dominant taxa that are the best resource competitors. Thus, high nutrients should result in a decrease in richness and evenness, which should

consequently result in an overall decrease in diversity. I found richness, however, to increase at high nutrient sites in southern Manitoba. This contradictory result may be caused by the low N:P ratios at high TP sites. The theoretical predictions are likely only valid within the species' physiological optima, suggesting that composite metrics calculated using richness (such as diversity) might be ineffective when nutrient ratios are skewed outside of the optimal range for the bioindicator organism.

Though community metric associations to nutrients were ambiguous, individual species found to be associated with increased nutrients had high frequencies and abundances in southern Manitoba. For example, Seddon (1972) found a collection of species (*Stuckenia pectinata, Myriophyllum spicatum, Lemna trisulca, Ceratophyllum demersum, Lemna minor, Ranunculus aquatilis, Elodea nuttallii* and *Potamogeton berchtoldii* [= *P. foliosus* in North America]) to be associated with eutrophic conditions in Welsh ponds. Swindale and Curtis (1957) also found a group of macrophytes very similar to Seddon's (Joint Occurrence Group 4) in Wisconsin lakes to associate with high conductivity, high substrate organic matter and nitrate. In contrast, in my RDAs, these same species or their congeners from Manitoba (namely *Stuckenia pectinata, Myriophyllum spicatum, Ceratophyllum demersum, Ranunculus trichophyllus* and *Potamogeton richardsonii*) were only weakly associated with the highest nutrient conditions. Seddon (1972) also found *Potamogeton natans, Nuphar lutea* and *Nymphaea alba* to be tolerant of a wide variety of water chemistry conditions, but not strongly associated with higher trophic levels. In Manitoba, congeners of these three species were associated with the lowest TP and highest N:P sites. The RDA results are in-line with the plant community I would expect find on a shortened, high nutrient level gradient, i.e., the species associated with high nutrients are widespread and thus not

associated with the nutrient axis and the generalist taxa are detected at the lower nutrient levels where the widespread nutrient tolerate species are less well suited to survive.

While community and species associations with measured nutrients ranged from insignificant (abundance) to significant (richness, phylogenetic diversity), associations between macrophyte assemblages and nutrient emitting activities were, at best, uniformly weak. These weak associations may be attributable to: (1) poor assessment of land-based nutrient emissions, (2) lack of a relationship between land-based and stream nutrients, or (3) too many sites at the high end of the nutrient gradient. The HAG generated here agreed with a similar HAG for this region by Yates et al. (2012): both studies identified the same activities (namely, row-crop agriculture to the west of the Red River and livestock agriculture to the east) as the predominant sources of human-produced nutrients. Thus, the weak associations I observed between macrophytes and land-based nutrients emissions were unlikely a result of poor assessment of nutrient-emitting activities. The lack of positive correlation I observed between stream nutrients and the GIS-estimated nutrient emissions undoubtedly contributed to the weak association between macrophytes and nutrient-emitting activities. This lack of correlation between measured and GIS-derived nutrients may be due to a disconnect in the transfer of nutrients between land and water, as also reported by Yates et al. (2014). However, the most influential factor affecting associations (or lack thereof) between macrophytes and nutrients in southern Manitoba is likely gradient length. Lougheed et al. (2001) found the range of TP that influenced aquatic plant community structure in the Great Lakes region, the biome immediately east-southeast of my study area, was 16–670 μg/l. The TP gradient in my study extended from 12–2245 μg/l. The lower-end ranges are similar,

however, my upper range is much higher than Lougheed et al. (2001). Further, the mean P concentration here (585 μg/l) is five times greater than Lougheed et al.'s (101 μg/l). Thus, the gradient I sampled was shorter than and on the high end of the plant species' fundamental niches (physiological optima), making detection of a stressor-response relationship difficult. Future work should examine the relationship between land-based and stream nutrients by sampling overland and subsurface flows from fields and livestock operations with known nutrient application and monitoring stream nutrients above and below the discharge point. In addition, the macrophytes at sites with and without nutrient emission-reducing best management practices (BMP) should be monitored to record if lower nutrient specialist species return to sites with BMPs relative to sites without.

In conclusion, I was unable to detect any primary factors associated with aquatic plant community composition in southern Manitoba. The conflation of agricultural types from east to west and the diffuse influence of wastewater in the basin preclude untangling the impacts of row-crop agriculture, livestock agriculture or wastewater from each other. Further, my analyses suggest that the nutrient gradient on the fine and very fine soil types in the Red River basin is too short and of too high a concentration to have a stronger influence on macrophyte community structure than stochastic processes. The properties of the macrophyte community (i.e., individual species abundances and summative community metrics) are, at best, weakly correlated with nutrient enrichment in southern Manitoba. Strong biotic-abiotic associations are needed to effectively use organisms as bioindicators. The evidence I have provided here shows these measurements of the vegetation fail to satisfy this key requirement of a bioindicator and casts doubt on the efficacy of macrophyte abundance or community metrics for use as

bioindicators of stream water TN and TP in the Red River basin. This echoes recent sentiment from Demars et. al (2012) of the failing of macrophytes as nutrient metrics in Europe. My findings collectively indicate the macrophyte flora of southern Manitoba has already been altered to a community dominated by generalists and competitive species adapted to high nutrient conditions. Efforts like the Lake Winnipeg Basin Initiative will hopefully lead to reduced nutrient inputs and improved water quality. If there is a future for macrophytes as bioindicators in southern Manitoba, it will be for use in detecting these reductions by the presumed subsequent return of low nutrient specialist taxa.

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Tables and Figures

Table 2.1: Streams sampled in southern Manitoba along with their physical characteristics, average nutrient concentrations and most influential GIS variables, Area = basin area upstream of site, TN = average water column total nitrogen concentration (mg/L), TP = average water column total phosphorus concentration (mg/L), N:P = ratio of average water column TN to TP, Sum TN; Sum TP = one-ten thousandth (1/10000) of the sum of all GIS-estimated land-based N or P activities in units of equivalent nutrient emission (units/km^2).

	Physical Variables			Stream Nutrients			GIS Variables		
Sub-catchment	Area (km^2)	Velocity (cm/s)	Depth (cm)	TN	TP	N:P	Sum TN	Sum TP	N:P
Arnaud Drain	4.4	0.00	4.2	6.4	2.25	3	2.7	0.83	3
Buffalo Creek	637.9	0.02	61.3	3.1	0.55	6	428.4	170.1	3
Crooked Lake Channel	180.5	0.00	68.4	1.6	0.12	13	47.6	16.9	3
Dufrost Drain	54.7	0.00	15.0	5.0	0.97	5	41.6	14.5	3
Edie Creek	110.0	0.30	6.1	1.3	0.06	22	13.0	4.0	3
Elm Creek Channel	757.2	0.10	77.4	2.35	0.32	7	316.5	110.3	3
Elm River	127.8	0.12	57.2	1.3	0.31	4	56.0	24.9	2
11-A Drain	323.2	0.00	3.0	8.4	0.99	8	151.6	56.0	3
Forrester Drain	48.4	0.10	34.6	4.6	2.07	2	33.8	11.0	3
Gamby Drain	28.7	0.03	18.9	2.2	0.52	4	13.7	4.3	3
Graham Creek	178.2	0.00	46.2	3.15	0.55	6	95.8	31.7	3
Kirk Drain	65.7	0.36	63.7	2.7	0.59	5	56.9	19.2	3
Kronsgart Drain	74.9	0.00	83.6	1.5	0.56	3	53.8	16.9	3
Elm River	116.7	0.33	57.2	1.3	0.50	3	54.5	24.2	2
Rat River	166.3	0.10	75.0	0.8	0.02	40	2.5	0.76	3
Little Morris River	908.9	0.01	32.5	2.6	0.22	12	539.9	178.9	3
Tobacco Creek	646.0	0.00	50.0	2.96	0.23	13	54.4	24.2	3
Morris River	468.8	0.03	28.0	2.8	0.59	5	253.3	90.6	3
North Shannon Creek	187.3	0.02	19.6	3.05	0.88	3	91.2	32.5	3
Oak Bluff Drain	7.7	0.00	16.0	1.5	0.46	3	5.2	1.6	3
Elm River	105.5	0.15	75.7	1.1	0.37	3	40.8	18.0	2
Omands Creek	56.6	0.00	15.2	2.7	0.59	5	28.7	8.6	3
Tobacco Creek	362.5	0.10	43.3	1.86	0.18	10	218.2	66.0	3
Rat River	831.9	0.10	75.0	0.8	0.01	80	35.9	11.0	3
Rosehiem Drain	219.8	0.00	33.7	2.8	1.10	3	128.5	48.6	3
Rat River	438.6	0.30	75.0	0.8	0.03	27	14.8	4.5	3
Tobacco Creek	383.1	0.20	43.3	2.96	0.24	12	225.4	68.9	3
Rat River	710.9	0.20	50.0	0.8	0.03	27	27.2	8.3	3
W Branch LaSalle River	58.0	0.01	18.5	3.0	1.51	2	43.0	16.8	3

Table 2.2: Descriptive statistics for the landscape-based data used to generate the human activity gradients as well as results of principal components analysis. Values are in standardized nutrient units per km^2. For livestock, the values have been transformed to make each type of animal equivalent on a nutrient basis (http://www.omafra.gov.on.ca/english/nm/regs/nmpro/nmpro03-jun03.htm).

	Descriptive Statistics				PC1
	Min	Median	Max	Std dev	Loadings
Row Crop					
canola	0	0.253	0.39	0.112	0.375
maize	0	0.139	0.337	0.106	0.233
flax	0	0.114	0.357	0.076	0.164
forage crops	0	0.045	0.174	0.047	0.052
potatoes	0	0.036	0.218	0.063	0.076
seed crops	0	0.044	0.227	0.047	0.027
legumes	0	0.172	0.38	0.1	0.28
sunflower	0	0.103	0.377	0.074	0.165
small grains	0	0.59	0.784	0.205	0.815
Livestock					
bison	0	0.118	0.574	0.169	0.006
goats	0.002	0.01	0.035	0.008	0.0003
chickens	0.032	0.278	3.434	0.573	0.062
horses	0.026	0.371	0.827	0.202	0.01
other poultry	0	0.007	0.105	0.019	0.002
swine	0.728	7.526	28.496	5.027	0.723
sheep	0.011	0.07	0.127	0.037	0.002
cattle	0.772	5.307	32.31	5.009	0.688
turkey	0.001	0.006	0.279	0.078	0.009
Wastewater					
septic density	0.107	0.318	0.838	0.185	0.121
septic TN	0.017	0.562	1.621	0.454	0.749
septic TP	0.004	0.152	0.871	0.257	0.423
lagoon land	0	0	0.262	0.102	0.031
lagoon land TN	0	0	0.57	0.23	0.081
lagoon land TP	0	0	0.131	0.05	0.014
lagoon stream	0	0	0.967	0.229	0.214
lagoon stream TN	0	0	1.058	0.365	0.424
lagoon stream TP	0	0	0.371	0.108	0.109

Table 2.3: Correlations among environmental variables. Pairings with slopes significantly different from zero ($p < 0.05$) are indicated in bold. Cond. = conductivity, N:P = nitrogen to phosphorus ratio, Temp. = temperature, TN = total nitrogen, TP = total phosphorus.

	pH	Temp.	Cond.	Velocity	Depth	Width	TN	TP	N:P
Turbidity	-0.015	-0.083	-0.054	-0.161	-0.299	-0.176	0.234	0.000	0.320
pH		0.301	-0.123	0.058	-0.057	-0.254	0.173	0.113	0.160
Temp.			0.255	-0.098	-0.300	-0.074	0.372	0.164	-0.012
Cond.				-0.357	-0.251	-0.164	0.176	-0.046	-0.020
Velocity					0.308	0.172	**-0.482**	-0.364	0.289
Depth						0.309	**-0.607**	**-0.534**	0.329
Width							-0.237	-0.059	-0.055
TN								**0.784**	**-0.493**
TP									**-0.767**

Table 2.4: Streams sampled in southern Manitoba along with macrophyte richness (Rich.), the number of species falling within each of six growth forms: submerged-decumbant (Dec.), submerged-caulescent (Caul.), floating leaved (Float.), emergent (Emer.) and free floating (Free), and the dominant species (Dom. Spp.). Species codes follow Appendix E. There were no species in the submerged-rosette growth form category.

		Growth Forms					
	Rich.	Dec.	Caul.	Float.	Emer.	Free	Dom. Spp.
Arnaud Drain	11	0	1	0	8	2	pot.fol
Buffalo Creek	15	0	4	1	9	1	pot.ric
Crooked Lake Channel	17	0	5	0	8	4	stu.pec
Dufrost Drain	15	0	2	0	12	1	pot.fol
Edie Creek	16	0	2	0	12	2	stu.pec
Elm Creek Channel	14	1	0	0	12	1	spa.eur
Elm River	10	0	3	0	5	2	pot.fol
11-A Drain	15	0	2	0	11	2	sag.cun
Forrester Drain	14	1	5	0	7	1	stu.pec
Gamby Drain	11	0	1	0	9	1	typ.lat
Graham Creek	20	1	6	0	11	2	sag.spp
Kirk Drain	14	0	3	0	9	2	spa.eur
Kronsgart Drain	17	0	1	0	13	3	lem.tri
Elm River	22	0	7	1	9	5	ziz.pal
Rat River	9	1	3	1	3	1	sag.cun
Little Morris River	14	0	2	1	11	0	spa.ang
Tobacco Creek	9	1	1	0	7	0	bol.flu
Morris River	8	0	0	0	6	2	bol.flu
North Shannon Creek	20	1	6	0	10	3	pha.aru
Oak Bluff Drain	8	1	0	0	6	1	ele.spp
Elm River	14	0	4	1	5	4	ziz.pal
Omands Creek	12	0	2	0	9	1	lem.min
Tobacco Creek	6	0	1	0	14	1	typ.lat
Rat River	6	0	2	2	2	0	hip.vul
Rosehiem Drain	22	1	6	0	12	3	stu.pec
Rat River	8	0	2	1	3	2	elo.can
Tobacco Creek	9	0	0	0	9	0	sag.cun
Rat River	8	1	3	2	2	0	pot.ric
W Branch LaSalle River	20	0	3	0	15	2	lem.min

Table 2.5: Macrophyte species occurrence in 29 streams in southern Manitoba. Occurrence (given in square brackets) is the number of streams in which the growth form, and the various species representing the growth form, were observed. Growth forms are submerged-decumbant (Decumb.); submerged-caulescent (Caul.); floating leaved (Float.); emergent (Emer.) and free floating (Free) Species codes are the first three letters of the genus, a period, and the first three letters of the species of taxon. Full species names are in Appendix E.

Growth Form				
Decumb. [9]	Caul. [25]	Float. [8]	Emer. [29]	Free [24]
Bry.spp [9]	*Call.het* [9]	*Nup.var* [1]	*Ali.tri* [16]	*Cer.dem* [9]
	*Cal.*spp [6]	*Pot.nat* [3]	*Ast.lan* [1]	*Lem.min* [19]
	Cha.glo [1]	*Spa.ang* [6]	*Bec.syz* [4]	*Lem.tri* [11]
	Elo.can [5]		*Bol.flu* [16]	*Ric.*spp [1]
	Myr.sib [7]		*Car.aqu* [6]	*Spi.pol* [3]
	Myr.ver [2]		*Car.*spp [1]	*Utr.vul* [6]
	Pot.amp [2]		*Ele.aci* [5]	
	Pot.fol [18]		*Ele.mac* [19]	
	Pot.ric [8]		*Equ.flu* [3]	
	Ran.aqu [2]		*Gly.*spp [3]	
	Ran.fla [1]		*Hip.vul* [7]	
	Stu.pec [13]		*Jun.*spp [1]	
	Zan.pal [3]		*Leer.ory* [1]	
			Lyt.sal [4]	
			Men.are [5]	
			Per.amp [10]	
			Pha.aru [13]	
			Phra.aus [1]	
			Poa.spp [3]	
			Rum.occ [3]	
			Sag.cun [24]	
			Sag.lat [1]	
			*Sag.*spp [7]	
			Sal.eri [2]	
			Sal.ser [1]	
			*Sal.*spp [9]	
			Sch.tab [22]	
			Siu.sua [13]	
			Spa.eur [15]	
			*Spa.*spp [1]	
			Spa.pec [10]	
			Typ.ang [9]	
			Typ.lat [8]	
			*Typ.*spp [1]	
			Ziz.pal [4]	

Table 2.6: Summary of correlations between macrophyte community metrics and environmental factors. Bolding indicates significance at 0.05 level.

Variable	Richness	Simpson Diversity	Simpson Evenness	Relative Dominance	Phylo. Diversity
Turbidity	**-0.39**	-0.36	0.21	0.20	**-0.37**
pH	0.03	0.01	0.01	0.02	-0.02
Temp	**0.48**	-0.07	**-0.53**	0.14	**0.43**
Cond.	0.29	-0.17	**-0.37**	0.32	0.25
Vel.	-0.15	-0.27	0.07	0.22	-0.10
Depth	-0.26	0.13	**0.37**	-0.14	-0.31
Width	0.11	-0.16	-0.02	0.13	0.09
TN	**0.39**	0.31	**-0.38**	-0.27	**0.44**
TP	**0.38**	0.28	-0.35	-0.30	**0.45**
N:P	**-0.48**	-0.35	**0.49**	0.34	**-0.55**
GIS TP	0.34	0.12	-0.29	0.00	0.29
GIS TN	0.31	0.11	-0.27	0.00	0.25

Table 2.7: Summary of reduced models describing macrophyte community metrics as a linear function of GIS-estimated human nutrient (total phosphorus [TP] and total nitrogen [TN]) inputs and field-measured nutrient concentrations. Each model includes an interaction term that is unwritten. Even = Simpson evenness, D = Simpson diversity, Dom = relative dominance, R = richness, PD = Faith phylogenetic diversity.

Model	Intercept (B_0)	GIS (B_1)	Meas (B_2)	Interaction (B_3)	ΔAICc	Adj. R^2
Even = TP_{GIS} + TP_{meas}	0.09	0	0.20	-0.05	0.00	0.174
Even = TN_{GIS} + TN_{meas}	0.08	0	0.04	-0.02	2.80	-0.007
D = TN_{GIS} + TN_{meas}	0.20	0.07	0.82	-0.11	0.00	0.003
D = TP_{GIS} + TP_{meas}	0.44	0.04	0.63	-0.08	0.28	-0.007
Dom = TN_{GIS} + TN_{meas}	1.13	-0.10	-1.40	0.21	0.00	-0.001
Dom = TP_{GIS} + TP_{meas}	0.69	-0.03	-1.14	0.17	0.09	-0.004
R = TP_{GIS} + TP_{meas}	6.50	0.51	-41.80	11.13	0.00	0.250
R = TN_{GIS} + TN_{meas}	0.89	1.26	4.85	0.50	5.49	0.020
PD = TP_{GIS} + TP_{meas}	995.96	53.98	-2321.1	727.91	0.00	0.256
PD = TN_{GIS} + TN_{meas}	134.30	180.2	1709.4	-168.90	4.57	0.129

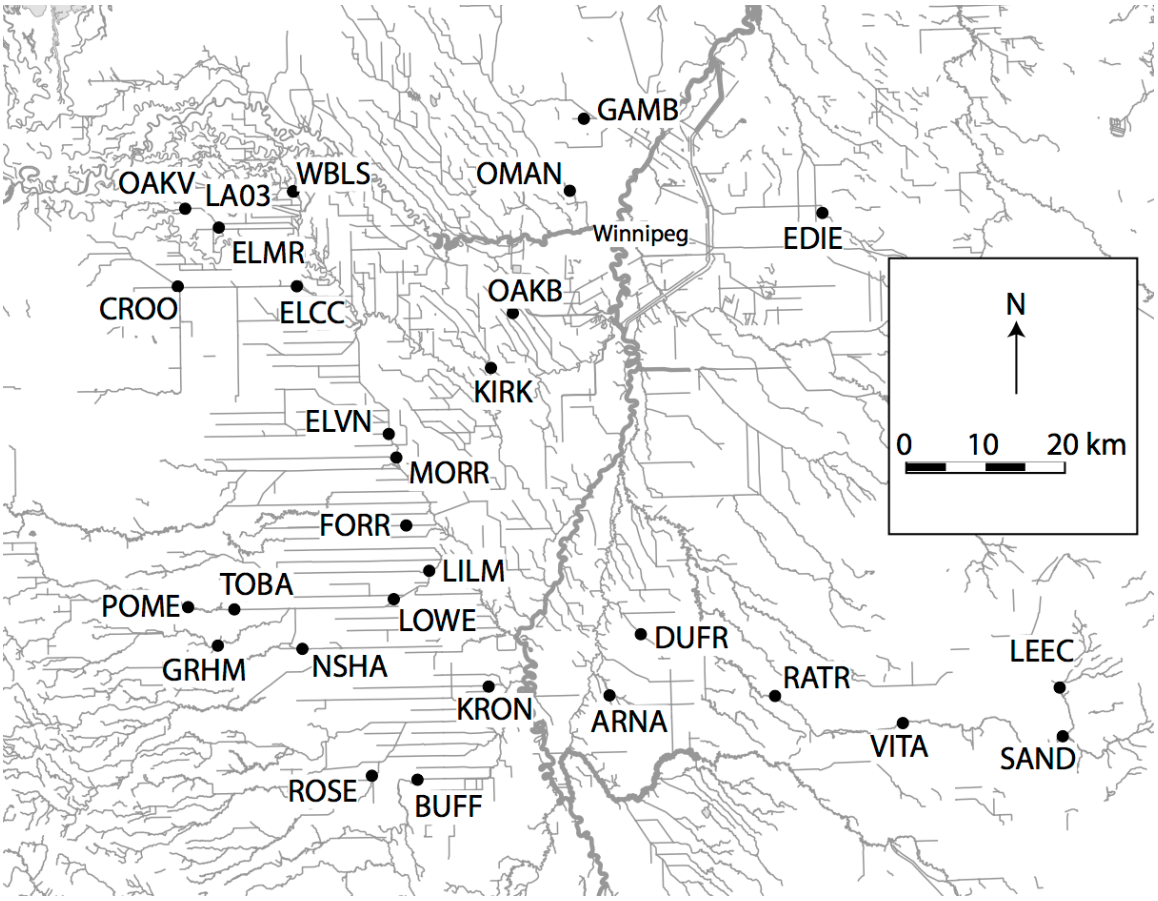

Figure 2.1: Map of southern Manitoba, Canada drainage network and macrophyte sampling sites. The Red River flows south to north through the centre of the figure. Table 2.1 gives site designations and tributaries.

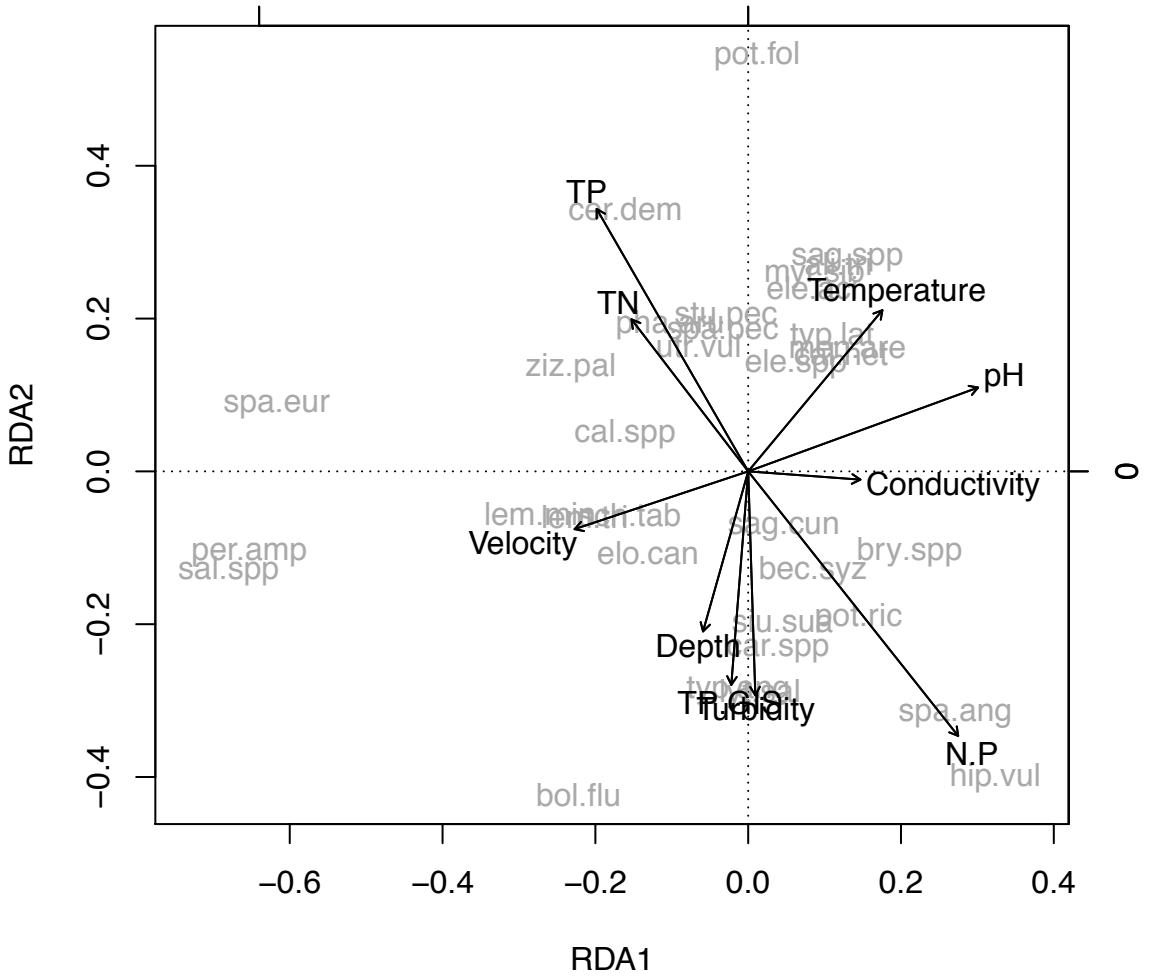

Figure 2.2: Redundancy Analysis (RDA) biplot diagram. Abbreviated species names are shown in red at the position along each RDA axis where they have average abundance. Constraining (environmental) factors are shown as arrows. Arrow length indicates the strength of the factor in the linear combination comprising the axes and direction the relative correlation with each axis.

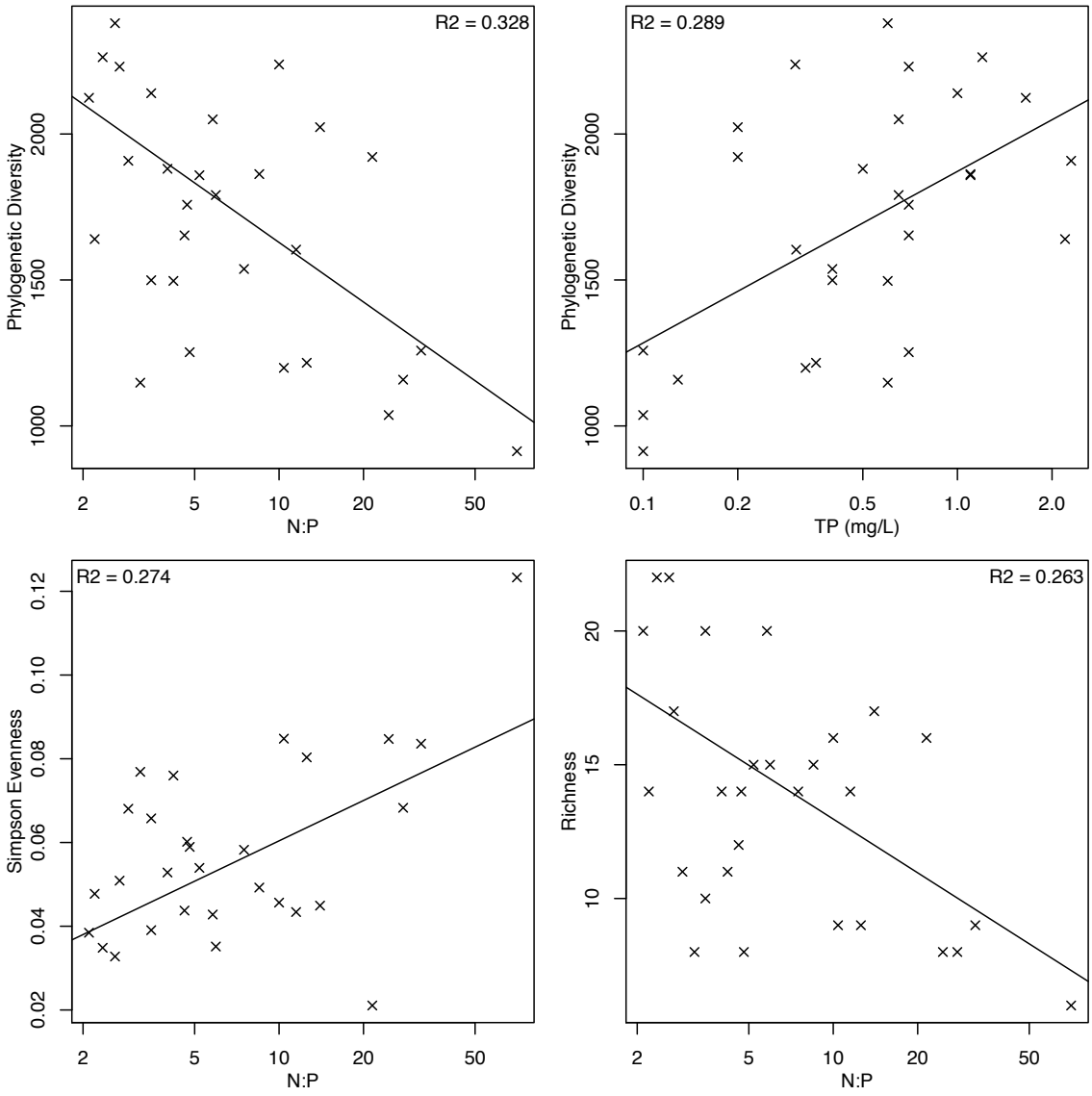

Figure 2.3: Community metrics (phylogenetic diversity, Simpson evenness and richness) as a function of environmental variables (total phosphorus, TP, and nitrogen to phosphorus ratio, N:P) for macrophytes in tributaries of the Red River in southern Manitoba, Canada. Only associations with correlation test p-values less than 0.01 are shown.

Chapter 3 - Using traits to generalize European aquatic plant trophic indices for use in Canada

Abstract

Water quality in lakes and rivers can be estimated using aquatic macrophyte trophic indices. Index algorithms incorporate species composition at a site with nutrient affinity values assigned to each species. Development of these indices is costly, time-consuming and has tended to be limited to regional application. Inter-regional application and comparison is made difficult because lack of taxonomic overlap results in missing affinity values. Here, I used a traits-based approach to expand the geographic scope of existing aquatic plant trophic indices. I generalized affinity values using the hypothetical response of plant growth form to a light-nutrient gradient and estimated values for previously unassigned species. My generalized affinity values were used to adapt European plant trophic indices to Canada; I then evaluated the performance of the method by validating Canadian site scores against actual water nutrient concentrations. Trophic affinity scores for European indices and field collected data from Canada agreed: free floating and emergent growth forms are associated with enriched waters (> 0.2 mg/L total phosphorus, TP, concentration) whereas rosette forms are associated with oligotrophic conditions (< 0.05 mg/L TP). The response was consistent across longitudes. Calculated site scores using different indices were highly collinear. Site scores using growth form-based affinity values were more strongly correlated with TP than those using species-based values (0.42–0.56 versus 0.008–0.25), validating my hypothesis that aquatic plant growth form is related to nutrient status. I verified a general, traits-based, ecological relationship whereby increased surface water nutrient

enrichment correlates with dominance of particular aquatic plant growth forms. Using this relationship, I demonstrated an approach for adapting species-based water trophic indices to allow broader geographic application and potentially simpler data collection. This method may lead to a simple, easy to apply traits-based method of assessing water chemistry.

3.1 Introduction

Primary productivity in freshwaters is frequently limited by phosphorus (P) or, occasionally, nitrogen (N) (Kalff 2001; Allan & Castillo 2007). Limitation by these nutrients affects abundance and composition of phytoplankton and, in some cases, macroscopic aquatic plant (macrophyte) communities (Moss et al. 2013). Many studies have examined the source of nutrients for uptake by macrophytes, and a limited consensus has formed that most rooted aquatic plants obtain nutrients from the substrate but can also uptake nutrients directly from the water through their above-ground tissues when water column nutrient concentrations are high (Nichols & Keeney 1976). Although the relationship between water column and sediment nutrient concentrations is indirect and complex (Clarke & Wharton 2001), aquatic plant community composition has been shown to change with surface water nutrient concentration and trophic status (Carbiener et al. 1990).

Consideration of the role of nutrients in structuring aquatic plant communities has led to the development of numerous aquatic plant trophic state indices, particularly in Europe (Holmes et al. 1999; Schneider & Melzer 2003; Haury et al. 2006; Willby et al. 2009). The indices vary in their computation, but all are calculated based on species composition at a site and nutrient affinity values assigned *a priori* to those species. These affinity values indicate the putative optimal nutrient condition for a species. Affinity

values for the constituent taxa of an index are often empirically derived by extensive sampling in the region where the index will be applied and assigning the affinity value based on modal occurrence of each species along the nutrient gradient (e.g., Willby et al. 2009). This regional nature makes construction of indices in new areas costly and time-consuming. A lower cost alternative is to assign affinity values based on expert consensus (e.g., Haury et al. 2006), but this method may lack accuracy and is constrained by the availability of local experts.

The geographical limitation of macrophyte indices results in a lack of taxonomic overlap and possible methodological discord between indices, making inter-regional application or comparison difficult (Birk & Willby 2010). Further, when a species does occur in two or more indices, the assigned affinity values are often incongruent: for example, *Elodea nuttallii* is accorded an affinity value of 9.44 out of 10 for the River Macrophyte Nutrient Index (Willby et al. 2009) compared to 2.75 out of 4 for the Trophic Index of Macrophytes (Schneider & Melzer 2003). Attempts have been made to adapt pre-existing indices to new regions by adding local taxa to an index from another region (Szoszkiewicz et al. 2002); however, trophic affinity values for any new taxa still need to be generated. A large-scale effort within the European Union to harmonize and inter-calibrate various indices (Birk et al. 2006) yielded some success, but involved still greater time and effort.

During the past decade, ecological studies have emphasized organism traits over species composition (i.e., traits versus taxonomy) for their theoretical and predictive value (Shipley 2010). McGill et al. (2006) argue that traits support mechanistic interpretations and are therefore more generalizable than simply a taxonomic-based approach. For instance, Ali et al. (1999) examined the response of various aquatic plant

traits to habitat productivity and suggested that functional (trait-based) models have the potential to predict water P conditions to the same precision or better than existing species assemblage-based methods (such as Holmes et al. 1999). Traits may also eliminate problems such as intra-species variability arising from ecotypes. Considering the universal adaptive strategy theory (i.e., C-S-R trigonal model; Grime 2002; Grime & Pierce 2012), which predicts that plants with a competitive growth strategy have a greater advantage at low levels of disturbance, in combination with ecosystem models that predict that nutrients and light are the primary drivers of aquatic plant competitive success (e.g., Hilton et al. 2006), I argue that the trophic status of an aquatic ecosystem is predictable from the growth form structure of the aquatic plant community.

High nutrient availability in surface water provides more resources for macrophyte growth but also promotes sestonic and epiphytic algal growth that reduces light transmittance to macrophytes. This dynamic has been shown to underlie the transition between macrophyte- or algal-dominated alternative stable states in shallow water systems (Scheffer et al. 2003). Among the aquatic plants in the macrophyte-dominated state, greater competitive advantage would be conveyed on growth forms with their photosynthetic tissue at or above the water surface (if light is limiting), or those most capable of using water column nutrients (if nutrients are in abundance). Conversely, low nutrient availability in surface water leads to greater light transmission and allows submerged growth forms to dominate the aquatic plant community. Because growth form is not region-specific like taxonomy, basing trophic affinity on growth form could allow surface water trophic status to be estimated for any aquatic plant community, regardless of taxonomic composition.

Here, I investigate the potential for traits to expand the geographic scope of existing plant trophic indices by enabling estimates of trophic affinity values for as yet unassigned species. I use a potential mechanism linking aquatic plant growth form and nutrient conditions to generate and generalize affinity values, and use this relationship to predict water trophic status from aquatic vegetation. Specifically, I investigate (1) whether growth forms have differential patterns of trophic affinity in the European metrics and the field-surveys of Canadian aquatic plants and associated water TP. (2) Based on the pattern, I devise an approach to estimate trophic affinity values for growth forms instead of species. I apply these growth form affinity values to the Canadian field-survey data and calculate trophic index scores for each site. (3) I then evaluate the performance of my method by validating the growth-form-based site scores against actual water nutrient concentrations. Together, these analyses allowed me to devise an approach for transforming European taxonomy-based water trophic indices to trait-based indices that allowed broader geographic application and potentially simplify data collection.

3.2 Methods

3.2.1 Data sources

I assembled two data matrices, one of data collated from published European aquatic plant trophic metrics and the other of field data collected at sites across Canada. Six regional European aquatic plant trophic indices were examined (River Macrophyte Nutrient Index [RMNI; Willby et al. 2009]; Mean Trophic Rank [MTR; Holmes et al. 1999]; Macrophyte Biological Index for Rivers [IBMR; Haury et al. 2006]; Finnish Oligotrophy Score [OTS; Kanninen 2009; Leka et al. 2008]; Trophic Index of Macrophytes [TIM; Schneider & Melzer 2003]; Macrophyte Nutrient Index for Ponds [M-NIP; Sager &

Lachavanne 2009]) and two vegetation classification schemes (Ellenberg Nitrogen Index [Ellenberg et al. 1992]; Newbold-Palmer Trophic Rank [Newbold & Palmer 1979]). All eight metrics were developed in Western Europe. Vegetation classification is solely taxonomic, and trophic status is mostly based on water column P or N (Table 3.1).

Each index or classification scheme includes a species list with a trophic component assigned to each taxon that I call the trophic affinity value (T). The T value putatively represents the optimal nutrient condition for a given species along a gradient of enrichment. These values have been derived either through field collected data (e.g., MTR, TIM, M-NIP), or both empirical data and expert opinion (e.g., IBMR, RMNI). Most of the indices or classification schemes base this value, directly or indirectly, on water column P, with the exception of the Ellenberg index which is based on N affinity. Although several metrics include filamentous algae in their species lists, I limited the scope of my work to include only the Charales (macroscopic charaphycean algae) and Embryophytes ("land plant" lineages, e.g. bryophytes, ferns and seed plants).

Canadian aquatic plant species abundance data and water-column nutrient concentrations were collated from 12 published sources, 5 previously unpublished datasets and 31 newly sampled sites first presented here (Appendix D). These data come from both lotic and lentic waters, ranging across Canada (45–55°N, 72–123°W) and were recorded between 1979–2011. Of the many published sources I considered, this dataset represents only those sites with compatible plant estimation methods and TP measurements. TP was selected because it was the most commonly available nutrient parameter among the studies.

Plant survey methods for newly sampled sites follow the protocol outlined in Holmes et al. (1999) and conform to the standards of the European Union Water

Framework Directive (WFD). Surveys were performed in late July through late August, the local season of fruit set and peak aquatic plant biomass. Surveys of the five previously unpublished datasets (Chambers & Kalff, unpublished; Appendix D) were carried out following a similar protocol.

3.2.2 Taxonomic identity, growth form and trophic affinity

Before analysis, taxon names for the species lists associated with the European metrics and Canadian dataset were harmonized to follow those listed in the Flora of North America (Flora of North America Editorial Committee, 1993+) volumes 2–5, 7–8, 19–26, and the Integrated Taxonomic Information System database (http://www.itis.gov).

All species included in the European metrics and the Canadian dataset were categorized by their mature physiognomy into one of six growth form categories based on those outlined in Sculthorpe (1967). This simple schema accounts for rooting condition and the environmental compartment where the majority of an individual plant's photosynthetic surfaces actively grow (water, atmosphere, or both). I have modified this classification into six general growth form classes (described in Table 1.1 of this dissertation).

Despite ensuring taxonomic congruency among species included in the European metrics and species present at the Canadian sites, there remained a number of Canadian species with no ascribed trophic affinities. Unknown trophic affinity values for Canadian taxa were estimated using a traits approach based on species growth form. I assumed growth form had a univariate, Gaussian response (sensu Gauch & Whittaker 1972) to nutrient concentration or trophic affinity and used measures of the centre and distribution of this response to determine unknown affinity values. The central trophic

affinity value (the mean for continuous scale values and median for ordinal scale values) for each European index or classification scheme, was calculated for every growth form based on the individual trophic affinity values for all species exhibiting that growth form. These calculated centrality values were then assigned to Canadian taxa of the appropriate growth form. Estimates of ecological amplitude were derived from the inverse standard deviations of each growth form for each metric.

3.2.3 Site scores

I calculated a site score using each European index for every site in the Canadian dataset. Each index uses a slightly different algorithm, but they all fit a general formula for calculating site score:

$$S = c\frac{\sum A_i W_i T_i}{\sum A_i W_i} \qquad \text{(Equ. 3-1)}$$

where *S* is the site score; *c* is a scaling constant; *A* is the abundance or similar measure of quantity of occurrence (presence/absence, percent cover, biomass, etc.) of taxon *i* at the site; *T* is the trophic affinity value for that taxon; and *W* is a weighting factor (such as a measure of ecological amplitude or tolerance) for the taxon. In essence, the site score is a weighted average of the trophic affinity values for each taxon adjusted by a measure of occurrence and a weighting factor, scaled by a constant. Some indices do not use weighting factors or a scaling constant in which case these parameters can be set to unity and regarded as arbitrary. In the case of the Ellenberg and Newbold-Palmer ranks, which do not specify an equation to calculate a site score, I use the general equation (3.1) with relative abundance and the parameters specified in Table 3.2.

I partitioned the taxa in the Canadian dataset into two lists: the complete list and a partial list of only those species common to both the European metrics and the Canadian dataset. For the latter, three trophic affinity calculations were employed: 1) the actual trophic affinity value ("species affinity value") assigned to each species by the European metrics, 2) the mean or median affinity value calculated for that taxon's growth form ("growth form affinity value" as described in section 2.3), and 3) the mean or median calculated in (2) and multiplied by the amplitude weighting.

In the case of the complete list of taxa in the Canadian dataset, there were species that did not occur in any European metric and thus had no ascribed affinity values. For this list, site scores were calculated based on: 1) trophic affinity values assigned to species by the European metrics or, for unascribed species, values calculated using only the growth form mean or median value (i.e., a "mixed" approach); 2) affinity values calculated using only the growth form mean or median value (i.e., growth form only approach); and 3) affinity values calculated using only growth form affinity values with amplitude weighting (i.e., weighted growth-form approach).

Thus, a total of six possible site scores were generated for each index at each site in the Canadian dataset: 1) partial taxon list with unweighted species values; 2) partial taxon list with unweighted growth form affinity values; 3) partial taxon list with weighted growth form affinity values; 4) complete taxon list with mixed, unweighted values; 5) complete taxon list with unweighted growth form affinity values; and 6) complete taxon list with weighted growth form affinity values. When the European metrics were missing representatives of a growth form, no growth form affinity value could be inferred. For these metrics, site scores based on growth form affinity values

were calculated excluding the taxa of the missing forms. This occurred in 4 of 8 cases (TIM, M-NIP, Ellenberg, and Newbold-Palmer).

To evaluate inter-metric trends, calculated site scores were standardized from 0 to 1 based on the range limits of the original European metric. To directly compare the relative score assigned by all metrics for each site, the scores for IBMR, MTR, and OTS were also reversed ($\min[x] + \max[x] - x_i$) because these metrics use a quality scale (higher values mean better water quality) rather than the impairment scale (higher values mean poorer water quality) used by the other metrics.

3.2.4 Analyses

To determine if the growth forms of the community change with increasing nutrient concentration, I tested for differences in the trophic affinity values of the species lists for the European metrics with growth form as the factor. For the Canadian dataset, I used observed TP concentrations in place of trophic affinity value. Trophic indices with rational T-values (RMNI, TIM and M-NIP) and the Canadian data were compared using one-way ANOVA. Metrics with ordinal T-values (MTR, IBMR, OTS, Ellenberg and Newbold-Palmer) were analyzed with a non-parametric Kruskal-Wallis test. For ANOVA or Kruskal-Wallis tests with significant differences, post-hoc multiple comparisons were performed. Tukey honest significant difference was used when tests were parametric and, for Kruskal-Wallis tests, I used the nonparametric Tukey-type multiple comparison of Zar (1999).

To evaluate the performance of each index against actual water chemistry for the Canadian sites, I calculated Spearman rank correlations between the site scores and log base-10 transformed TP concentrations. Correlations were compared among the three

possible site scores based on the partial taxon list and the three possible site scores based on the complete taxon list for each metric. Each correlation was normalized to a bias corrected Fisher's z (Zar 1999) and tested with a χ^2 (Chi-square) test. The correlations between transformed TP and site scores generated with the various taxa lists and trophic values within each metric were compared by a Tukey-like multiple comparison (Zar 1999). All analyses were carried out in R (R Development Core Team 2012).

3.3 Results

3.3.1 Canadian and European datasets

The data sources from Europe and Canada are comparable in their chemical and biological parameters. The RMNI index has the greatest number of taxa in common with the Canadian data (n = 54) whereas TIM has the least (n = 17; Table 3.1). Proportionally, however, the shared taxa represent 22% and 34% of the European taxa listed for the respective index. RMNI and MTR span the greatest trophic range, but trophy is measured as soluble reactive phosphorus (SRP) whereas the Canadian data set reports TP. M-NIP and TIM are the only two of the seven P-based metrics used here that employ TP instead of SRP. Even assuming SRP composed the majority of TP, these two indices do not encompass the full trophic range found in RMNI, MTR, or the Canadian dataset (Table 3.1).

3.3.2 Growth form-trophic affinity relationship

When growth forms are arranged roughly by position relative to the water surface (Figure 3.1), comparison of species affinity scores for the European macrophyte trophic indices and classification schemes showed that forms with the majority of

photosynthetic tissue at (floating leaf and free floating) or above (emergent) the water surface were associated with enriched waters. RMNI and Ellenberg showed increasing species affinity values from rosette/decumbant to free-floating forms while IBMR, MTR, and OTS show a trend of decreasing affinity value with growth form stature. This difference in trend direction is due to the design of each index: species affinity values in the latter three indices are inverted relative to the former (i.e., the best quality sites have values close to 20 for IBMR compared to values close to 1 for RMNI).

Four metrics, namely RMNI (F: 24.5 p-value: < 0.0001), MTR (F: 10.4 p-value: < 0.0001), IBMR (F: 14.5 p-value: < 0.0001) and Ellenberg rank (KW: 14.3 p-value: 0.0064), have at least one growth form group that differs significantly in mean or median species affinity value from other growth forms. In the case of RMNI and IBMR, growth forms separate into three significantly different post-hoc groups: submerged-rosette and submerged-decumbent cluster into a low affinity value group while submerged-caulescent and emergent compose a mid-affinity value group with floating-leaved forms straddling the low and mid-range groups, and free floating having the highest affinity values. The remaining significant metrics separate into two post-hoc groups. MTR and Ellenberg metrics both show a separation between high and low value groups, but they differ slightly in which forms are included in each group. OTS does not demonstrate growth form groups trending with species affinity value, but significant differences among forms are still detected. The TIM, M-NIP, Newbold-Palmer and Ellenberg metrics are each missing representatives from one or more growth forms. In all these cases, decumbent is not included and, in the case of TIM, rosette forms are also lacking.

Analysis of the Canadian aquatic plant dataset, grouped by growth form, also showed a trend of submerged bottom-dwelling plants in low nutrient waters compared

with emergent and free-floating plants in P-rich waters (Figure 3.2). Dominance by macrophyte growth form differed with TP concentration ($p < 0.001$; ANOVA), with post-hoc comparison identifying three groups: submerged-rosettes associated with low (< 0.05 mg/L) TP concentrations; submerged-decumbent, submerged-caulescent and floating leaved forms predominating in moderate (0.05–0.2 mg/L) TP concentrations; and emergent and free floating forms dominating under high (> 0.2 mg/L) TP concentrations.

For the Canadian dataset, testing the relationship between TP and growth form with longitude as a covariate showed no effect of geographic location on the change in growth form with TP (*F*: 0.99, *p*-value: 0.462; ANCOVA). Similarly, flow type (lotic versus lentic) showed no significant interactive effect (*F*: 0.66, *p*-value: 0.654; Two-way ANOVA) on the growth form relationship. TP, however, did vary significantly with both longitude and flow type independently, albeit sample sizes for flow type were unbalanced (lentic = 21; lotic = 75).

3.3.3 Unknown trophic affinity estimates

To estimate affinity values for the 137–174 (depending on index) Canadian macrophyte species that do not occur in pre-existing European metrics, I employed the trait-based growth form relationship from the European metrics (Figure 3.1). Mean species affinity values for RMNI, TIM and M-NIP (rational scaled indices) and median species affinity values for MTR, IBMR, OTS, Ellenberg and Newbold-Palmer (ordinal scaled metrics) of all species exhibiting each growth form resulted in growth form affinity values that varied by 0.05–6 fold for the eight European metrics (Table 3.3). For each metric, the range in estimated values was narrower than the potential scale of the metric. Standard deviations were smallest among rosette and free-floating forms and greatest for submerged-caulescent and emergent forms.

Applying the growth form approach to estimate affinity values for Canadian species not recognized in the European metrics resulted in values for an additional 137–174 species. In the case where the European metrics were missing representatives of specific growth forms, I was unable to estimate an affinity value. In addition, MTR, IBMR, TIM and Newbold-Palmer each had too few individual representatives of rosette and decumbent growth forms resulting in small standard deviations and amplitude weighting estimates (s^{-1}) approaching infinity or undefined. For calculation of site scores, these amplitudes were manually set to the lowest, positive, non-zero value ($1E^{-15}$).

3.3.4 Canadian Site Scores

Trophic metric scores were calculated for each Canadian site using the six combinations of taxa lists (only Canadian species present in European metrics versus all Canadian species), affinity values (species versus growth form) and ecological amplitude weighting (with or without). Site scores using the partial list of taxa common to both the European metrics and the Canadian dataset and the actual European affinity values for each species varied from 0.008–9.55 for RMNI (metric with greatest range, when standardized to maximum) to 0.002–2.77 in M-NIP (metric with the smallest range, when standardized to maximum). Results from the same list, but using growth form inferred affinity values, showed the greatest variation in RMNI from (3.79–9.00) to the least in OTS (1.00–2.25). For comparison, M-NIP with growth form based values ranged 1.24–2.81 and OTS with species-based values ranged 0.003–3.72.

Site scores were also calculated using the complete list of taxa in the Canadian dataset. In this list, site scores were based on a mixture of actual trophic affinity values assigned by the European metrics; for species without affinity values, the growth form estimated trophic affinity values were substituted. These site scores had ranges (3.44–

9.58 in RMNI and 0.103–3.05 in M-NIP) similar to those calculated using growth forms in the partial list. Site scores based on the complete list using only the growth form estimated values, with and without weighting, differed little from the mixed list. Without amplitude weighting, growth form-only site scores ranged from 3.88–8.94 (RMNI) to 1.02–2.77 (OTS) and 0.10–2.80 (M-NIP).

Inter-comparison of metrics (Figure 3.3) calculated using growth form affinity values for only those species in common between the European metrics and Canadian dataset (i.e., the partial list) revealed that the highest correlated metrics were Ellenberg with MTR (r = 1.00), RMNI with IBMR, MTR with TIM, and TIM with Ellenberg (all r = 0.99). OTS had the lowest correlations with all other metrics. I also calculated correlations using species affinity values. These revealed M-NIP with Newbold Palmer (r = 0.94) and Ellenberg with RMNI (r = 0.93) to have the highest correlations whereas OTS, again, had the lowest (e.g., with M-NIP r = 0.33).

3.3.5 Validation of site scores against total phosphorus

The performance of each metric was evaluated by correlating Canadian site scores with measured TP at each site. Overall, the Spearman rank correlations for all metrics were stronger when using growth form (0.19–0.56) compared to species affinity (0.008–0.49) values. In most cases, including additional information about the estimated ecological amplitude of each growth form did not improve the site score correlation with TP (Figure 3.4).

Site scores calculated using the complete list of taxa with the mixed (species and form) values had higher correlations (0.12–0.49; Figure 3.4 upper panel) than those calculated using the reduced list with species values only (0.008–0.25; Figure 3.4 lower

panel). Site scores based on the complete list also had lower correlations with amplitude weighting (0.19–0.47) than those without (0.33–0.53).

3.4 Discussion

Comparison of macrophyte species, grouped by growth form in relation to nutrient concentrations, showed that macrophyte growth form changed with surface water enrichment at a continental scale based on field data from across Canada and meta-data from European metrics. Arranging the growth forms by position relative to the water surface showed that bottom-dwelling growth forms (such as submerged-rosette and submerged-decumbent) have greater relative abundance in oligotrophic waters, whereas forms with the majority of photosynthetic tissue at (floating leaf and free floating) or above (emergent) the water surface increase in abundance with increasing trophy. This pattern is clear regardless of regional differences in species identity. For European data, five of the eight regional macrophyte trophic indices or classification schemes showed emergent forms associated with enriched conditions. Moreover, for Canada, this relationship was consistent despite longitudinal changes in biomes (from mountains to grassland to forest) and varying background nutrient concentrations (decreasing from west to east). Although my analysis is the first to examine macrophyte growth form distribution in relation to nutrients at this large scale, others have observed similar patterns at local scales. For example, Lougheed et al. (2001) found a fringe of emergent species were indicative of turbid, nutrient-rich wetlands while a high density of submerged forms indicated higher wetland quality. Likewise, Chambers (1987) found a shift from meadow-forming growth forms (such as rosette) at sites with low nutrient input, to canopy-forming forms (i.e., caulescent) at sites with higher nutrient input. This change in growth form dominance with nutrients is likely caused by light limitation attributable to

epiphytic or planktonic algal response to higher nutrients, thereby conferring an advantage to growth forms with photosynthetic tissue near or above the water surface. My finding, that patterns of macrophyte growth forms differ with increasing surface water nutrients holds true on two north-temperate continents, suggests that macrophyte growth form may be a widely applicable bioindicator of water trophic status.

Application of eight European macrophyte indices and classification schemes to Canadian waters showed that site scores calculated using growth form affinity values had higher correlation with measured water chemistry than scores calculated using European species affinity values. Further, the growth form affinity values were robust, showing greater correlation with actual water chemistry even when calculated based only on taxa common to both Canada and the European metrics (i.e., when 72–91% of Canada taxa were not included in metric calculations). In nearly all cases, the addition of amplitude weighting using imputed ecological amplitudes performed more poorly than unweighted growth form affinity scores, but better than species affinity scores.

The rosette and decumbent forms often comprised a group related to significantly lower trophic values. The site scores generated from metrics missing representatives of these growth forms (M-NIP, TIM: missing rosette and decumbent; Newbold-Palmer missing decumbent) are likely less reliable than metrics with all forms represented. These influential growth forms (rosette and decumbent) were also infrequent in the Canadian dataset possibly resulting in higher site scores at sites with low total phosphorus.

The European metrics were each developed using associations with various measures of water trophy. RMNI, MTR, and IBMR use water-column soluble reactive P whereas TIM and M-NIP use water and sediment TP. M-NIP is based on TIM so the

species affinity values of these two indices are likely not independent of each other as is the case for the other metrics. Newbold-Palmer rank is based on wastewater pollution and, presumably, is a proxy for elevated N, P, and biological oxygen demand. The Ellenberg indicator is used for both terrestrial and aquatic ecosystems, and is indicative of substrate N (Ellenberg et al. 1992). The N-based Ellenberg indicator and the various P-based metrics show similar trends, namely bottom-dwelling growth forms dominating oligotrophic waters and free floating forms common in eutrophic waters, suggesting the relationship is a function of nutrients in general and not specifically N or P. Because most of the metrics were highly correlated, the choice of one over another for use in Canada is arbitrary. My results show, however, that most metrics perform better in Canada when using growth form affinity values rather than species affinity values. Although the use of growth form affinity values should be validated using data from Europe where the indices originated, the data used here enable me to show that a general trophic index using plant trait data can indicate water trophy across a broad geographic scale, as suggested by Ali et al. (1999). The traits over taxonomy in this approach has both predictive power and mechanistic explanation because organism function (i.e. phenotypic expression of the genetic material), not species identity, is actually interacting with the environment.

The European trophic indices are often distinguished or stratified based on physical site characteristics. Separation of flow types (lotic versus lentic), for example, is commonly practiced for most of the European taxonomy-based protocols. Macrophyte Nutrient Index for Ponds (MNIP) was designed for use in shallow lentic systems, whereas RMNI and IBMR were intended for use in lotic systems, as were MTR and TIM. The sites in my Canadian dataset represent a mixture of both lotic and lentic habitats.

The use of this mixed flow type data with the European affinity values violates their flow-specific assumption. My growth form-nutrient relationship, however, was consistent among lotic and lentic waters, suggesting this technique is robust to differences in flow. Nevertheless, greater investigation into the growth form-flow relationship may lead to refinement of the trait-based metric.

In conclusion, directly adopting European macrophyte metrics to Canada using the species specific trophic affinity values determined in the metric's originating country or region showed a poor relationship with actual water TP concentration for a large Canadian dataset. By adapting the metrics using a key plant trait, namely growth form, instead of species identity, the relationship between metric score and actual water TP concentration improved. This growth-form based metric is explicitly based on a mechanism of increased light competition with water nutrient enrichment. A mechanistic index such as this is an improvement over empirical, taxonomy-based indices in terms of reducing identification errors or omissions by simplifying data collection, broadening the geographic scope of existing aquatic plant trophic indices to include the Holarctic region, and offers the ability to make inter-regional and even inter-continental comparisons.

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Tables and Figures

Table 3.1: Richness (R), shared taxa, trophic range and region where developed for six aquatic plant trophic indices (RMNI: River Macrophyte Nutrient Index; MTR: Mean Trophic Rank; IBMR: Macrophyte Biological Index for Rivers; OTS: Finnish Oligotrophy Score; TIM: Trophic Index of Macrophytes; M-NIP: Macrophyte Nutrient Index for Ponds) and two vegetation classification schemes (Ellenberg Nitrogen Index; Newbold-Palmer Trophic Rank) compared to meta-data collated from sources in Canada. SRP=soluble reactive phosphorus, TP=total phosphorus.

Metric	R	Shared taxa	Trophic range (mg/L)	Region
RMNI	241	54 (22%)	0.001–8 (SRP)	UK
MTR	122	30 (25%)	0.02–4 (SRP)	UK
IBMR	169	33 (20%)	0.005–0.185 (SRP)	France
OTS	159	47 (30%)	ca. 0-0.09 (TP)	Finland
TIM	50	17 (34%)	0.005–0.567 (TP)	Germany
M-NIP	108	30 (28%)	0–0.611 (TP)	Switzerland
Ellenberg N	149	37 (25%)	Relative, (Nitrogen)	Europe
Newbold-Palmer	93	27 (29%)	not specified	UK
Canadian	191	–	0.02–2.25 (TP)	Canada

Table 3.2: Parameter name and range of values for components of six aquatic plant trophic indices and two vegetation classification schemes as they relate to equation 3.1 for calculating trophic score for a site. c = scaling constant, A = abundance measure, W = weighting factor, T = trophic indicator value.

Parameter	RMNI	MTR	IBMR	OTS	TIM	M-NIP	Ellenb.	Newbold-Palmer
c	1	10	1	1	1	1	1	1
A	% Rel. Cover	% Rel. Cover	% Rel. Cover	% Rel. Cover	% Rel. Cover	% Rel. Cover	% Rel. Cover	% Rel. Cover
W	Weighted	1	Weighted	1	Weighted	Weighted	1	1
T	1—10	10—0	20—0	1—5	1—4	1—4	1—9	1—150

Table 3.3: Mean (RMNI, TIM and M-NIP) or median (MTR, IBMR, OTS, Ellenberg and Newbold-Palmer) trophic affinity values for six aquatic plant trophic indices and two vegetation classification schemes partitioned by aquatic plant growth form. '–' indicates the metric was lacking representatives of this growth form.

Growth Form	RMNI	MTR	IBMR	OTS	TIM	M-NIP	Ellenberg	Newbold-Palmer
Rosette	2.72	8	15	4	–	–	1	61
Decumbent	3.78	9	15	2	–	–	–	–
Caulescent	6.26	5	11	2	2.4	2.6	5	73
Floating Leaf	5.69	6	12	3	1.7	2.56	4	41
Emergent	7.16	4	11	2	2.65	2.63	6	72
Free Floating	8.99	3	6	1	3.18	2.67	7	114

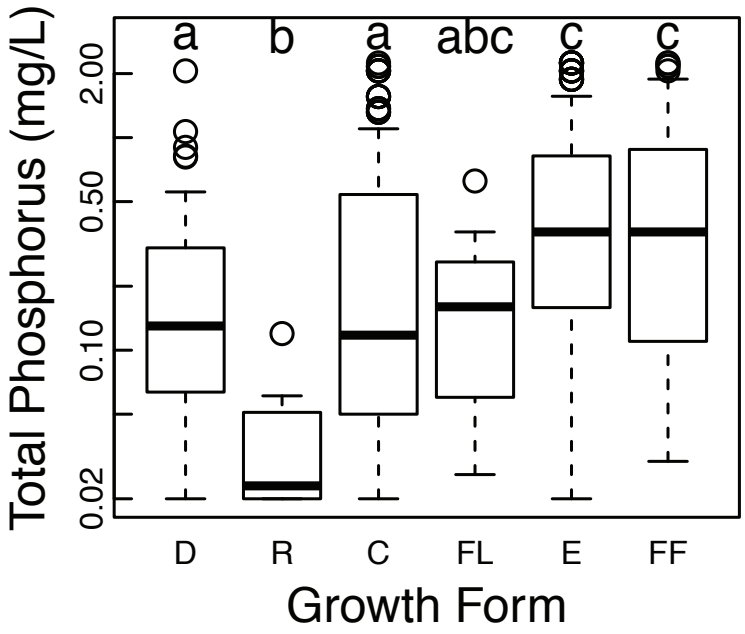

Figure 3.1: Box and whisker plot of water-column total phosphorus (mg/L) for aquatic plants found at sites across Canada, partitioned by growth form. Differences in trophic affinity values among growth forms are identified by lowercase letters based on the results of post hoc multiple comparison. C = submerged-caulescent, D = submerged-decumbent, E = emergent, FL = floating leaf, FF = free floating, R = submerged-rosette.

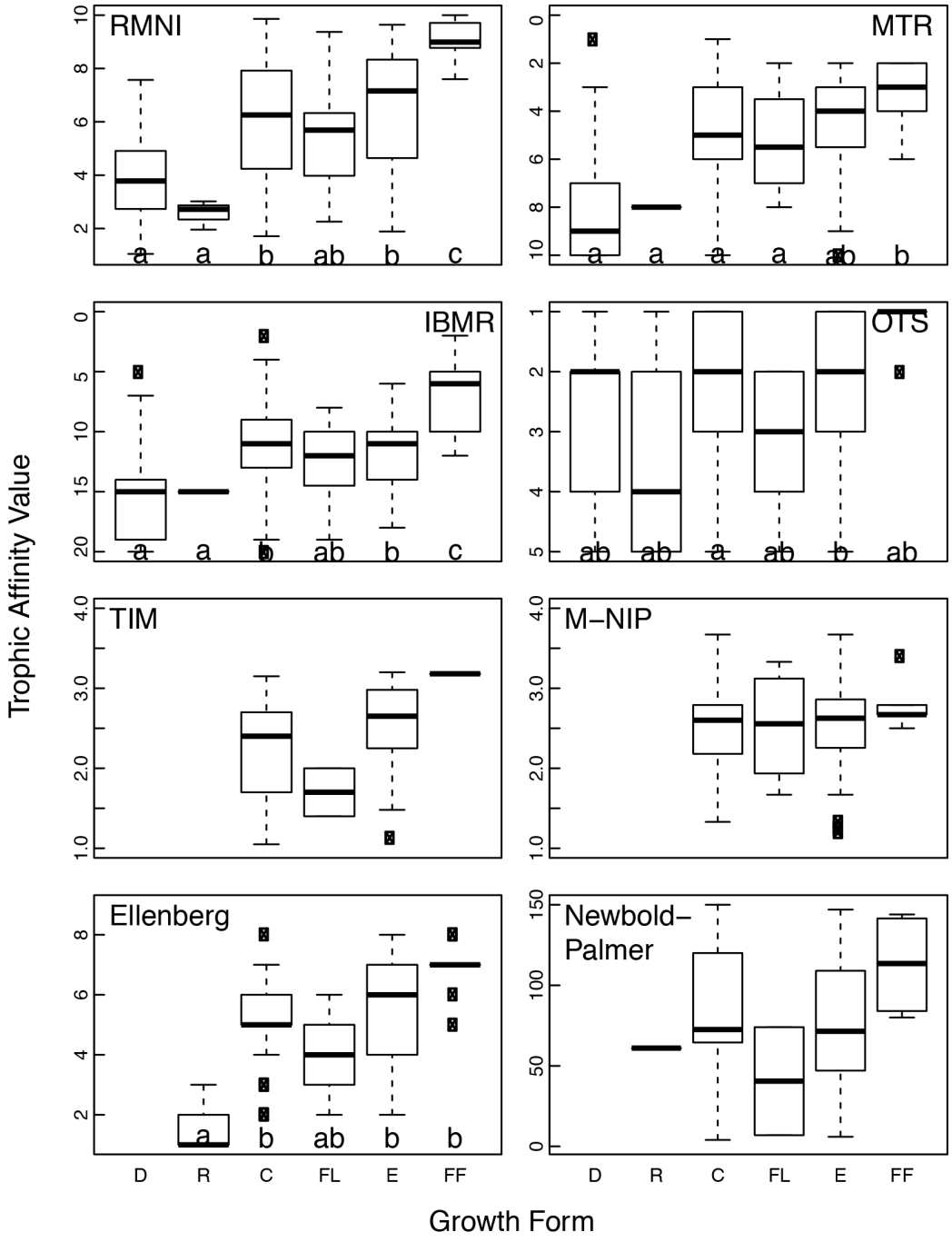

Figure 3.2: Box and whisker plot of standardized trophic affinity scores for five macrophyte trophic indices: River Macrophyte Nutrient Index (RMNI), Mean Trophic Rank (MTR), Macrophyte Biological Index for Rivers (IBMR), Finnish Oligotrophy Score (OTS), Trophic Index of Macrophytes (TIM), and Macrophyte Nutrient Index for Ponds (M-NIP); and two vegetation classification schemes: Ellenberg Nitrogen Index and Newbold-Palmer Trophic Rank, partitioned by growth form. For each metric, differences in trophic affinity values among growth forms are identified by lowercase letters based on the results of post hoc multiple comparison. C = submerged-caulescent, D = submerged-decumbent, E = emergent, FL = floating leaf, FF = free floating, R = submerged-rosette.

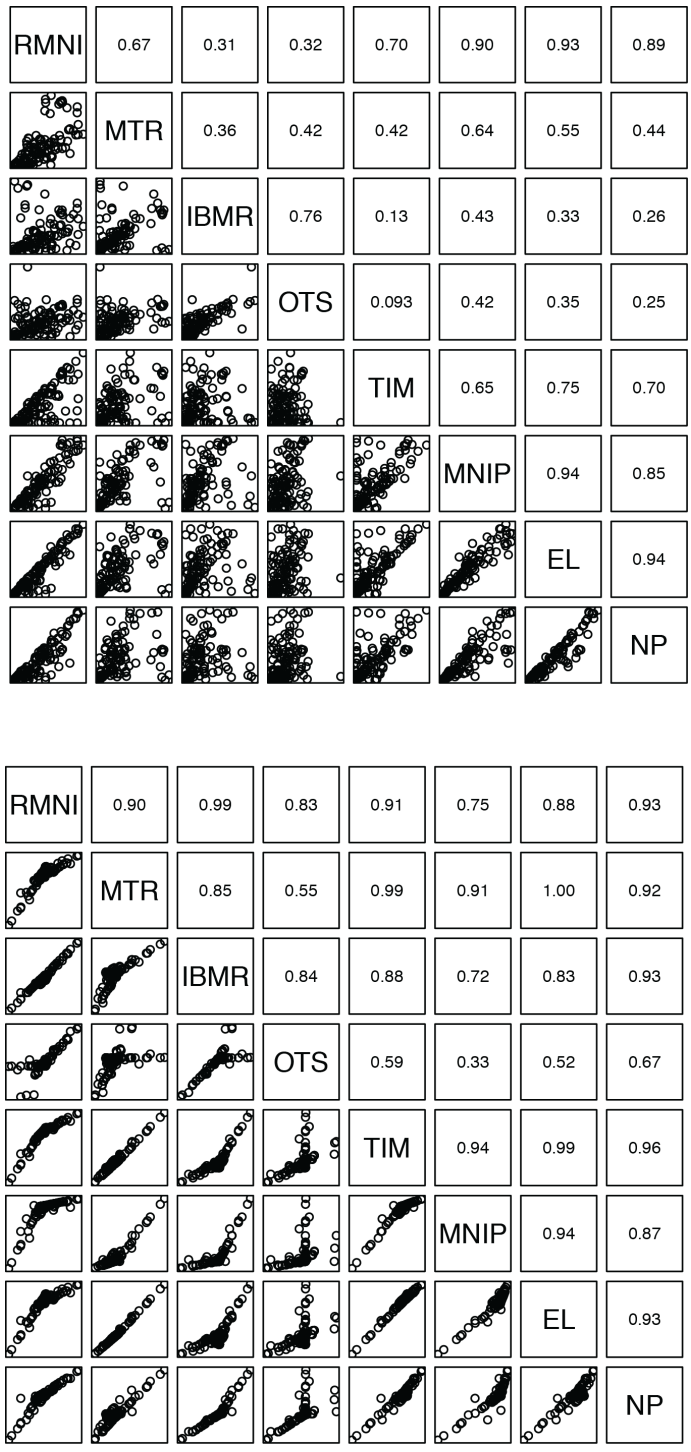

Figure 3.3: Scatterplot matrices and Pearson correlation coefficients comparing the site trophic scores calculated using species-based (upper panel) or growth form-based (lower panel) trophic affinity values on a meta-dataset of Canadian aquatic plant abundance for six aquatic plant trophic indices and two vegetation classification schemes.

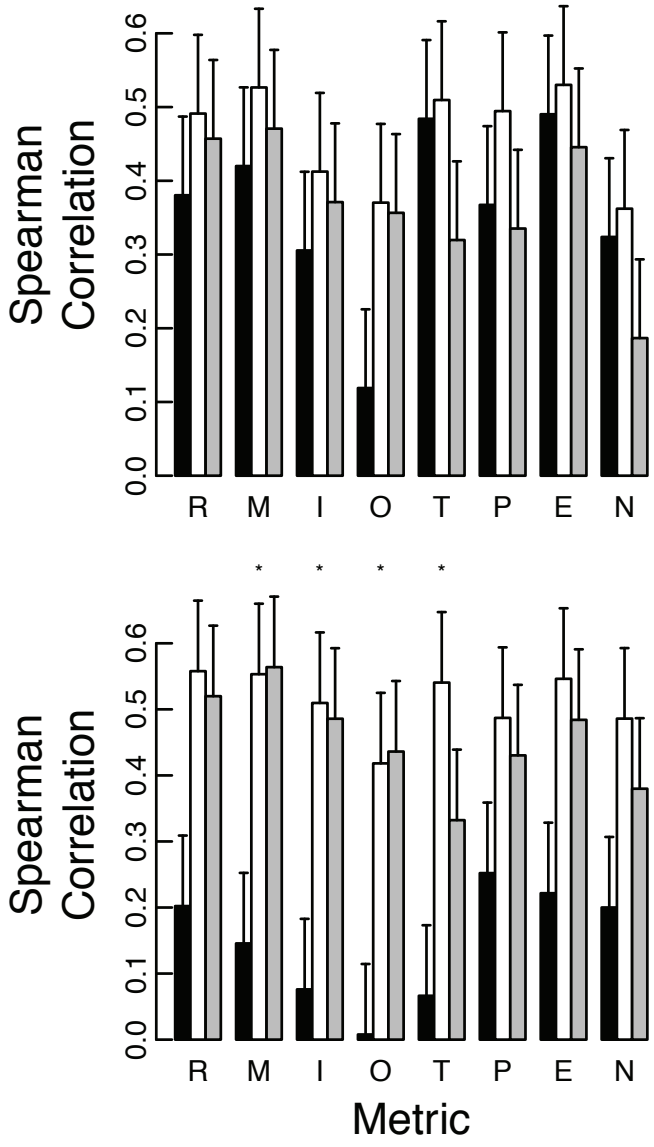

*Figure 3.4: Spearman rank correlation coefficients between site scores calculated for six aquatic plant trophic indices and two vegetation classification schemes versus total phosphorus concentration. Upper panel uses a complete list of taxa and a combination of species-based and growth-form-based affinity values (black); unweighted growth form-based affinity values (white); or growth form-based affinity values with a weighting factor (grey). Lower panel uses a partial list of taxa and species-based affinity values (black); unweighted growth-form-based affinity values (white); or growth form-based affinity values with a weighting factor (grey). Bonferroni corrected significance (α = 0.006) within a metric indicated by: *.*

Chapter 4 - Variation in aquatic plant nutrient composition with species identity and growth form

Abstract

Nitrogen and phosphorus concentrations in aquatic plants are controlled by environmental factors, species identity (evolutionarily determined) and morphology (which is a combination of genotype and phenotypic expression). A single morphology is often assigned to a species but this fails to account for variations in phenotype within a species, thereby confounding assessment of the relative importance of shared evolutionary history versus ecological effects of phenotype on plant nutrient content. Using phylogenetic and experimental approaches, I compared the role of plant morphology (i.e., growth form) and species identity in determining aquatic angiosperm tissue nutrient composition. Examination for phylogenetic signal identified significant autocorrelation in growth form but not in nutrient concentrations. Comparison of tissue nutrient concentrations with and without accounting for phylogeny showed that the emergent growth form had lower tissue nutrient concentrations than submerged forms. Experimental manipulations of growth form in three aquatic plant species revealed consistent differences in tissue nutrient concentrations among species but inconsistent differences between phenotypes. I conclude that some of the variation in tissue nutrient concentrations is attributed to overlapping and interacting phylogenetic and ecological information but the particular response differs with the scale of investigation.

4.1 Introduction

Global biogeochemical cycles involve pathways where elements move through air, water and soil, interacting with biological and geologic elements to determine material flux (Bashkin 2003). Vegetation requires macronutrients, particularly nitrogen (N) and phosphorus (P), to support growth and reproduction; however, plants also play a role in transferring or facilitating the transfer of N and P between abiotic pools (e.g., soil to water, atmosphere to soil, etc.) (Baldy et al. 2007). In the case of aquatic ecosystems, rooted aquatic plants (macrophytes) facilitate exchange of nutrients between sediment and open-water compartments, incorporating N and P from the substrate into their roots and then transferring this material to above-ground tissues. Upon senescence and subsequent decomposition, these nutrients move from the above-ground tissues into the water, generating seasonal nutrient pulses in temperate water bodies (DeMarte & Hartman 1974, Welsh & Denny 1979).

The quantity of nutrients lost from plant biomass to the water column is strongly correlated with tissue nutrient concentration, particularly for P (Qui et al. 2002). Invariably, species identity is a major factor determining variability in tissue nutrient concentrations (Boyd 1978, Waughman 1980, Hayati & Procter 1990), although several physical, chemical and biological parameters are also drivers of tissue nutrient differences. For example, macrophyte nutrient uptake and tissue nutrient concentrations are affected by biomass (Wersal & Madsen 2011), hydrology (Andersen & Mitsch 2005; Szabo et al. 2010), seasonality (Gerloff & Krombholz 1966; Nichols & Keeney 1976), water nutrient quantity (Gerloff & Krombholz 1966; Cedergreen & Madsen 2003; Cronin & Lodge 2003), substrate nutrient availability (Angelstein et al. 2009) and light (McRoy & Barsdate 1970; Cedergreen & Madsen 2003; Cronin & Lodge 2003; Lee & McNaughton

2004). Tissue nutrient concentrations also vary with plant morphology and phenotype (Demars & Edwards 2008).

Macrophyte gross physiognomy is often classified into distinct growth forms based on the position of the plant's vegetative and reproductive structures relative to the substrate and water surface (Hutchinson 1975; see also Table 1.1 in this dissertation). Some aquatic plants, however, can display phenotypic plasticity in their morphology, to the point of expressing different growth forms among and even within individuals (Wanke 2011). This plasticity is an adaptation to variable water levels and the physiological change is driven by ecological factors such as temperature (Deschamp and Cooke 1984), photoperiod (Cook 1969), and light intensity (Goliber 1989). Yet, often only one growth form is attributed to all individuals of a species based on the most common or mature morphology of that species. This linkage of growth form to species identity confounds our ability to examine the relative importance of shared evolutionary history versus the ecological effects of this trait on tissue nutrient concentration and, in turn, seasonal nutrient transfer.

My objective was to compare the influence of plant phylogeny versus growth form on the tissue nutrient composition of aquatic angiosperms. To achieve this goal, I first compiled a database of aquatic macrophyte species and associated tissue nutrient data. First I tested for phylogenetic signal (the degree to which phylogeny predicts the similarity among species and traits) in macrophyte tissue nutrient concentrations by examining the variance in nutrient concentrations among growth forms with and without accounting for shared evolutionary history. Second, I experimentally manipulated growth form of three aquatic plant species capable of expressing both submerged-caulescent and emergent forms to test for variance in tissue nutrient concentrations as related to

species identity, phylogenic relation and growth form. This integrated approach of analysis of empirical data and experimental testing allowed me to uncouple the relative influence of species identity, evolutionary history and growth form on aquatic plant nutrient composition.

4.2 Methods

4.2.1 Phylogenetic analysis of empirical data

A database of N and P concentrations in aquatic plant tissue was compiled from published journal articles (Appendix F). Data for the three species (*Hippuris vulgaris* L., *Megalodonta beckii* (Torr. ex Spreng.) Greene and *Myriophyllum heterophyllum* Michx.) examined in the experimental section of this paper were included. Methods sections of all papers were carefully reviewed to ensure data were compatible, namely: (1) tissue nutrients were reported as total N or P concentrations, not a chemical species, in units convertible to mass nutrient per dry mass plant, and were obtained through a clearly described or certified analytical protocol; and (2) when the study involved nutrient manipulation, only values from control or reference conditions were used. These criteria excluded studies where values were reported in g nutrient/g fresh mass or as N:P ratios. Most papers presented means and some measure of variation (standard error or standard deviation); when only range values were reported, I estimated mean values as the median, i.e. average of the highest and lowest values. All units were converted to mg/g dry mass. The taxonomy in the list was synonymized to follow the International Plant Name Index (www.ipni.org). Each taxon was assigned one of five growth forms categories (emergent, floating-leaved, submerged-caulescent, submerged-rosette, free-floating; Table 1.1) as defined in Sculthorpe (1967) (Sculthorpe's submerged-thalloid

form did not occur in the database). Growth forms were assigned to each species based on the mature morphology described in the paper from which tissue nutrient data were obtained or, if not described, from species accounts, figures, and taxonomic reference materials.

4.2.2 Tissue nutrients and phylogeny

A phylogeny was generated for all the taxa in the compiled database using the Phylomatic ver. 3 webserver (Webb & Donoghue 2005). The resulting topology was assigned branch lengths with the Bladj module of Phylocom ver. 4.2 (Webb et al. 2008). Node ages for calibration were derived from published estimates (Appendix C) or ages provided by the Angiosperm Phylogeny Website (www.mobot.org/MOBOT/research/APweb/). I then tested for phylogenetic signal among the mean N and P concentrations and growth form for each species following Abouheif's (1999) C_{mean} procedure as implemented in the adephylo package (Jombart & Dray 2010) for R (R Core Team 2013) and the test of standardized independent contrast variance (Blomberg and Garland, 2002; Blomberg et al., 2003) as implemented in Phylocom. Both procedures were set to run with 99,999 repetitions.

4.2.3 Tissue nutrients and growth form

I examined the variance in nutrient concentrations of the phylogenetic nodes where the descendants have different growth forms versus nodes where all the descendants share the same growth form, using phylogenetically independent contrasts (Felsenstein 2008). Contrasts were calculated for tissue nutrient concentrations using the Analysis of Traits module in Phylocom. Unresolved branches were treated as soft polytomies and contrasts were calculated as per Pagel (1992). A factor of growth form

contrasts for each node in the tree was generated based on whether descendent nodes shared the same growth form category or not. In cases where the categories of descendent nodes differed, the growth form contrast factor was assigned as “Various”. When the categories of descendent nodes were the same, the growth form contrast for that node was assigned the category of the descendent nodes. The nutrient concentration contrasts were tested for mean differences among these growth form contrasts using one-way ANOVA with Tukey honest significant difference (HSD) post-hoc comparisons. Concentration data were log transformed prior to contrast calculation to conform to distributional assumptions.

4.2.4 Experimental manipulation of growth form and species

An outdoor mesocosm experiment was conducted to examine the effect of two factors (growth form and species) on tissue N and P concentrations. Three species (*Hippuris vulgaris*, *Megalodonta beckii* and *Myriophyllum heterophyllum*) were selected because they can express both emergent and submerged growth forms, represent a range of evolutionary lineages in the eudicot clade, and were readily available. Emergent growth forms were induced in each species using abscisic acid (ABA). Treatments not receiving ABA were expected to remain in a submerged-caulescent (hereafter called “submerged”) form and those treated with ABA to express an emergent form (Wanke 2011). The design resulted in six treatments: *Hippuris*-no ABA/submerged (Hip-Sub), *Hippuris*-ABA/emergent (Hip-Emer), *Megalodonta*-no ABA/submerged (Meg-Sub), *Megalodonta*- ABA/emergent (Meg-Emer), *Myriophyllum*-no ABA/submerged (Myr-Sub), *Myriophyllum*- ABA/emergent (Myr-Emer).

Apical growing shoots of each species were field-collected in late spring and incubated in a 650 L holding tank for 14 days. Individuals of each species originated

from the same population and water body, but collection locations differed: *Hippuris* from Tobacco Creek, Pomeroy, MB Canada (49.40309° N, 98.00117° W); *Megalodonta* from Grand Lake, Flowers Cove, NB Canada (46.01929° N, 66.02628° W); and *Myriophyllum* from French Lake, Lakeville Corner, NB Canada (45.90033° N, 66.25915° W). Single shoots were each transplanted into 15 L tanks (mesocosms). Each species-growth form treatment combination was replicated 25 times, for a total of 150 independent mesocosms. The 25 replicates were chosen based on an *a priori* power analysis for power = 0.8 and alpha = 0.05, using data from J. Culp, *unpublished* and Nichols & Keeney (1976).

The mesocosms were arranged in a 6 by 10 m grid and treatments were fully randomized across this array. Mesocosms were stocked with approximately 10 dm^3 of organic sediments taken from the Sunpoke Lake dugway, Rusagonis Station, NB Canada (45.76680° N, 66.55207° W). Sediments were cleaned of roots and debris and homogenized prior to stocking. Fresh mass and length at planting were recorded for each of the 150 experiment individuals plus 30 additional randomly selected individuals from the planting stock. The 30 additional plants were dried in a 60°C oven until constant mass to obtain dry mass values. Linear regressions of dry mass on fresh mass were created for each species (*Hippuris*: slope = 8.6, R^2 = 0.93; *Megalodonta*: slope = 13.6, R^2 = 0.99; *Myriophyllum*: slope = 17, R^2 = 0.97); planting dry mass for each experimental subject was then estimated from these regressions. Mesocosms receiving hormone treatment were inoculated with ABA to create a 10 µM solution. Depth, photosynthetically active radiation (PAR), temperature, turbidity and water nutrient chemistry parameters were monitored throughout the experiment to ensure the design was randomized and to account for possible bias (Appendix G). None of these

parameters differed significantly ($\alpha = 0.05$) among treatments; turbidity, however, did increase uniformly across treatments over time despite continuous efforts to remove algal biomass.

The experiment ran for 84 days. Plants were harvested in early September, which coincided with peak biomass of natural populations. Upon harvest, roots were thoroughly washed and above-ground tissue cleaned of any algal accumulation. Individuals were then partitioned into root, submerged shoot, and emergent shoot portions. These were oven dried at 60°C until constant mass. Each portion was then individually pulverized in a ball mill grinder (Retsch MM 400) with 25 mm stainless steel balls at 27 Hz for 2 min. Total P and total N expressed as mg nutrient / g dry mass (DM) were measured on each tissue type by the Canadian Rivers Institute Environmental Chemistry Lab (Saint John, NB, Canada). The powdered plant tissue was microwave digested in a 50% hydrochloric acid solution for 10 min. The digested tissue was analyzed for total P and total N on an ICP-OES (iCAP 6500 DUO) using argon gas as a purge. Samples were run with blanks and certified reference materials (peach leaves; NIST 1547) for quality control. Due to the low tissue mass of some specimens, the lower detection limits were set at 20 mg N/g DM and 0.125 mg P/g DM.

Proportion of plants expressing an emergent growth form was tested with Pearson's chi-squared test for counts. Only plants where ABA treatment resulted in the expression of the emergent form and non-ABA treated plants that remained in submerged form were included in subsequent analyses. The reduced dataset resulted in an unbalanced design among the species and growth form factors and subsequent tests were adjusted accordingly.

Mean differences were tested with either a one-way, omnibus ANOVA (for initial values with species as the only factor) or a two-way ANOVA (for effects of species, treatment and their interaction). When interactions were non-significant, F ratios and p-values for each main effect were calculated and reported after accounting for the other main effect ("type II" sum of squares). When the interaction was significant, the results of a two-way ANOVA with orthogonal contrasts ("type III" sum of squares) were reported.

Post hoc multiple comparisons were carried out with Tukey honest significant differences tests. Variation in means is reported as standard error unless otherwise indicated. All analyses were carried out with the R statistical environment and language except power calculations, which were performed with G*Power 3.1 (Faul et al. 2007, 2009).

4.3 Results

4.3.1 Empirical database

The tissue nutrient concentration database contained 142 records comprising 69 species from 22 studies. The 69 species were divided unevenly among five growth forms, with emergent and submerged-caulescent having the greatest number of species and submerged-rosette the fewest (34, 22, and 2, respectively). Nutrient concentrations varied from 5–66.8 mg N / g DM and 0.23–13.4 mg P / g DM with means of 21.2 mg/g and 4.24 mg/g respectively (Table 4.1).

Within the taxa in the database, specific growth forms have a tendency to characterize specific clades on the phylogeny (Figure 4.1). Floating leaf forms are largely in the Nympheales but *Potamogeton natans* is also classified as primarily floating leaf. The bulk of the free floating forms are in the Araceae (Lemnoideae) but *Eichhornia*

(Pontederiaceae) also exhibits this form. The submerged-caulescent and emergent forms have the greatest number of representatives and, overall, are spread across the tree. Yet, these forms still show a tendency to group within clades. In the monocots, emergent forms are clustered in the Alismataceae and Poales whereas the submerged-caulescent form is found within the Hydrocharitaceae (also shown by Les et al. 2006) and the majority of the Potamogetonaceae. The Pontederiaceae show a mixture of growth forms, with the aforementioned free floating *Eichhornia*, the submerged-caulescent *Zosterella dubia* and emergent *Pontederia cordata*. In the eudicots, the submerged-caulescent form is only seen grouping at the genus level in *Myriophyllum* and *Callitriche*, but it also occurs in single representatives of *Ceratophyllum*, *Ranunculus*, and *Megalodonta*. The emergent form is most consistent among the three representatives of the rosids clade, but it also arises four other times within the eudicots. Among the intermixed forms in the eudicots are the two representatives of the submerged-rosette form: *Lobelia dortmania* and *Littorella uniflora*.

4.3.2 Phylogenetic signal

High and low N and P concentrations occurred throughout the phylogenetic tree (Figure 4.1). Tests for phylogenetic signal in nutrient concentrations revealed no significant autocorrelation among species for either nutrient, but significant phylogenetic autocorrelation was displayed among growth forms (Table 4.2). Visual inspection of Figure 4.1 suggests that concentrations of both nutrients, but especially N, were lower for emergent species. This pattern is particularly evident in the grass-sedge-rush (Poales) clade in the centre of the figure. In the case of N, concentrations also tended to have greater magnitudes, but also higher variation, in the eudicot clade versus the rest of the tree (Figure 4.1).

4.3.3 Growth Form and tissue nutrients

Results of a one-way ANOVA on the literature database showed that tissue nutrient concentrations varied amongst growth forms with the effect of growth form being highly significant for P (F = 6.67, p-value < 0.0001) and marginally significant for N (F = 2.14, p-value = 0.08). Post hoc multiple comparisons revealed that the submerged-caulescent and emergent forms significantly differed from one another, with emergent having lower nutrient concentrations (Figure 4.2).

4.3.4 Phylogeny versus growth form

Because tissue nutrient concentrations varied with growth form (Figure 4.2) and specific growth forms dominated certain clades on the phylogeny (Figure 4.1), I tested for mean differences in the phylogenetic-independent contrasts of N and P. A one-way ANOVA revealed no significant difference between the phylogenetically independent N and P values (contrasts) at nodes with descendants sharing the same growth form versus those with descendants of different growth forms. Analyses of tissue nutrient concentrations among growth forms with and without accounting for phylogeny, combined with finding a lack of phylogenetic signal among these concentrations (Table 4.2), suggests nutrient concentrations respond to the joint effect between phylogeny and morphology, but the pattern in the main phylogeny effect may be too weak to detect (Figure 4.2).

4.3.5 Experimental manipulation of growth form and species

In total, 149 of 150 plants survived the 12-week mesocosm experiment; only one representative of the Hip-Sub treatment was lost. The proportion of plants expressing an emergent growth form with hormone treatment was significant (X^2 = 44.13, p-value <

0.0001), with 60 of 75 (80%) treated plants expressing emergent growth forms while 55 non-ABA treated plants (74%) remained in submerged form (Table 4.3). Of the 19 non-treated plants that expressed the emergent form, all were *Hippuris*. Given that the focus of my investigation was on relative differences between emergent and submerged forms among species, the remaining results are based on the subset of 115 plants where ABA treatment resulted in the expression of the emergent form and non-ABA treated plants remained in submerged form.

All three species increased in biomass over the duration of the experiment. Although highly variable within a species, biomass increases averaged 221% (±206% standard deviation) for *Myriophyllum*, 62% (± 148%) for *Hippuris* and 39% (± 79%) for *Megalodonta*. Final biomass pattern among the taxa mirrored the initial order with *Hippuris* remaining larger than either *Megalodonta* or *Myriophyllum* both at the start and finish of the experiment (Table 4.4). Mean final biomass also varied by tissue type (Table 4.4). The proportion of emergent biomass significantly differed among species ($F = 31.28$, p-value < 0.0001; ANOVA) with *Hippuris* having greater final emergent mass (63.5%) than either *Megalodonta* (25%) or *Myriophyllum* (15.3%), which were not differentiated. Submerged biomass of non-ABA treated plants and biomass of the submerged portions of the ABA-treated plants also differed by species ($F = 10.03$, p-value < 0.0001; two-way ANOVA), but not by treatment. Here again, *Hippuris* had greater final submerged mass than either *Megalodonta* or *Myriophyllum,* which were weakly differentiated (p-value = 0.059; Tukey HSD).

Stem length increased in all treatments over the course of the experiment but at different rates among species (data not shown). Within a species, final lengths did not differ between submerged and emergent forms. *Hippuris* was significantly longer at both

start and finish of the experiment (16.1 ± 4.3 and 47.5 ± 23.2 cm, respectively; mean ± SE) compared to both *Megalodonta* (6.8 ± 1.3 and 11.8 ± 3.2 cm, respectively) or *Myriophyllum* (8.9 ± 2.8 and 15.3 ± 6.4 cm, respectively).

Analysis of tissue P concentration at the end of the experiment revealed significant interaction and main effects (species: $F = 29.5$, p-value < 0.0001; treatment: $F = 20.0$, p-value < 0.0001; species x treatment: $F = 25.3$, p-value < 0.0001). Overall, *Megalodonta* showed the highest concentrations (3.50 +/- 0.143 mg P/g DM) and *Hippuris* the lowest (2.54 +/- 0.178 mg P/g DM). The significant interaction term related to the high tissue P concentration within emergent *Megalodonta* tissue (6.58 +/- 0.269 mg P/g DM) and low concentration in emergent *Hippuris* (2.52 +/- 0.268 mg P/g DM) (Figure 4.3).

Total quantity of P (concentration x biomass) within the above ground tissues (submerged and emergent tissues together) showed significant average differences among species, treatment and species-treatment interaction (species: $F = 7.8$, p-value = 0.0007; treatment: $F = 5.5$, p-value = 0.021; species x treatment: $F = 6.2$, p-value = 0.0029). Differences among species for submerged tissue only also showed significant interaction and main effects (species: $F = 6.0$, p-value = 0.0034; treatment: $F = 7.3$, p-value = 0.0079; species * treatment: $F = 4.5$, p-value = 0.013). The majority of this was driven by the greater quantity within *Hippuris* treatments relative to the other species (Figure 4.3).

Average final tissue N concentration showed no significant effect of species, growth form or their interaction. In contrast to tissue P, where *Megalodonta* had the highest average concentration, N concentrations in all three species are closely matched both at the start and end of the experiment. Coefficients of variability for tissue N were

greater than for tissue P, with more samples near method detection limits for N as compared to P, and this resulted in the achieved power (1 - β) of this test being 0.23 for N compared to 0.87 for P.

Total quantity of N contained within the whole plant showed significant average differences among species (F = 12.1, p-value = 0.0001), but this was largely driven by biomass differences. Comparing only the amount of N in submerged or emergent tissue among species showed a significant species effect, marginally insignificant treatment effect and non-significant interaction (species: F = 7.8, p-value < 0.001; treatment: F = 2.8, p-value = 0.099). The trends in the N data followed the pattern in P where the submerged tissue within a species contained a greater quantity of N than its emergent counterpart (Figure 4.3). Comparison of N:P ratios by species and growth form showed only emergent *Megalodonta* had a significantly lower mean N:P than the rest, which showed no differentiation.

4.4 Discussion

Analysis of literature data and contemporary experimental data showed that tissue N and P concentrations differed with growth form. The empirical dataset showed that nutrient concentrations were lower in emergent than submerged forms, but phylogenetic influence on this pattern could not be detected. Controlled experiments involving manipulation of growth form also showed lower nutrient concentrations in emergent than submerged tissues in one of three heterophyllous species for P and two of three species for N. My literature findings are consistent with those of Demars & Edwards (2008) who found significant differences in tissue nutrient concentration between helophytes and hydrophytes, with helophytes (comparable to my emergent growth form) having lower mean N and P concentrations than hydrophytes (comparable

to my submerged-caulescent growth form). Findings from analysis of both literature and experimental data showing emergents with lower nutrient concentrations than submerged species most likely relate to the greater carbon to nutrient ratio in emergent than submerged dry mass. Emergent species must support their photosynthetic apparatus in the air, thereby requiring more carbon-rich and, hence nutrient poor, structural supporting tissue than submerged plants, which are capable of using water density and buoyancy for support (Willby et al. 2001).

Although aquatic plant tissue nutrient concentrations differed among growth forms (particularly between submerged and emergent forms), my literature database showed little phylogenetic signal among these concentrations and thus no apparent evolutionary trends. Growth form, however, did show significant phylogenetic autocorrelation, and specific morphologies were associated with major clades. This result is consistent with my assertion that morphology and species identity are linked. Assuming nutrient uptake and allocation are heritable traits, the findings that tissue nutrient concentrations differ by growth form but not phylogeny is ambiguous. The simplest explanation is that the joint correlation between phylogeny and nutrient concentration is too weak to detect. This could be caused by a number of factors, for example tissue nutrient concentrations may have become isolated from growth form as a result of genetic drift (i.e., they are not a primary trait being acted upon by selective pressures). Alternatively, because nutrient concentrations are the result of various processes including uptake, allocation, and structural investment, the actual concentrations may be highly labile due to differential selection on each process, thereby masking the phylogenetic signal.

Experimental manipulation of three species capable of expressing alternate growth forms provided confirmatory results for emergent vs. submerged forms at the inter-species scale. When all individuals of a species were pooled and differences in N and P concentrations examined based on mature morphology for the species, regardless of expressed phenotype, *Hippuris* (emergent mature morphology) had lower overall N and P concentrations than *Megalodonta* and *Myriophyllum* (submerged mature morphology). This pattern is consistent with the investigations of Demars and Edwards (2008). Results at the intra-species scale were, however, inconsistent. *Hippuris* had higher N and P concentrations in emergent tissues than submerged while *Megalodonta* had the opposite; whereas *Myriophyllum* showed a differential response among nutrients. For N, the detection limits were low resulting in a low statistical power (~23%).

Because the phylogenetic analysis and growth form assignment was done at the species level, the experimental result of the emergent growth form having lower nutrient concentrations than the submerged form is consistent with the literature findings. Yet, there was still considerable unexplained variation in all results. The relationship between tissue nutrient concentration and plant uptake is complex and studies have found tissue nutrient concentrations to be both correlated with sediment nutrient availability (Shilla et al. 2006) and poorly correlated with plant nutrient uptake (Verhoeven et al. 1988). Nutrient source for macrophytes (water versus sediment) has also been extensively debated in the literature. Studies have shown that sediments provide the majority of plant nutrients (Barko and Smart 1980, Carignan and Kalff 1980, Huebert and Gorham 1983) but the water column can also provide much of the required nutrients (Chambers 1989, Pelton 1998, Robach 1995) typically when the ratio of sediment to water nutrient availability is low (Rattray et al. 1991). Emergent plants have less or no access to water

column nutrients because they often have thick cuticles that impede nutrient uptake from the water (in submerged form, however, they can lack a cuticle allowing them to absorb nutrients from the water and internally relocate them). I cannot quantify the effect of these influences in my results, but there is presumably some variation in species' ability to accessing water column nutrients.

I have shown leaf P concentrations to vary with plant growth form (amalgamated at a species level) and this variation is independent of any residual influence from shared evolutionary history. Qui et al. (2002) showed that leaf P concentration is a useful predictor of the quantity of nutrients transferred from the sediments to the above ground tissues by rooted aquatic plants. Other work has shown the majority of nutrients in above ground tissues are leached to the water column upon senescence (Nichols and Keeney 1976). I can now extend these studies and hypothesize that plant communities with different modal growth forms will differ in the overall quantity of nutrients transferred from the sediments. In particular, I predict emergent dominated communities will transfer a lower quantity per unit biomass than submerged dominated communities, and total quantity of nutrients transferred will be determined by these concentrations and total biomass of each form. Emergent portions often also have a waxy cuticle surrounding them, which may further slow or reduce nutrient leaching during decomposition.

Experimental manipulation of additional heterophyllous species will provide a more complete picture of species-independent growth form differences and ensure these trends are consistent across all angiosperm lineages. I can, however, draw some conclusions for future sampling of tissue nutrients. When the question of interest is at a species level and the sampling units are individuals, my results suggest that care should be taken to minimize or stratify across phenotypic variation in growth form if

amalgamating different tissue types. When the question of interest is at a community level and the units of investigation are species, my results suggest that classification based on the mature growth form of a species can be informative even when amalgamating samples from phenotypically different individuals, at least for the species I investigated here.

I have shown that growth form has the potential to be ecologically informative of tissue nutrient contents, but the results should be examined in the context of potential phylogenetic influence. Particular distinction may be made between submerged-caulescent versus emergent taxa. The expected differences in N and P concentration between emergent and submerged-caulescent forms vary with the scale of investigation. Pooled across species, mean concentrations of both N and P are consistently lower for emergent than submerged forms, but the ranges of the concentrations overlap. Within a species, particularly polymorphic (heterophyllic) species, however, concentrations differ among expressed forms of the individual and the quantity is not consistent with form. Thus, concentrations recorded at the scale of the individual may be more strongly related to factors other than gross morphology.

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Tables and Figures

Table 4.1: Summary of empirical database of nitrogen and phosphorus tissue concentrations collated from literature values.

Growth Form	No. of Records	No. of Species	N range (mg/g)	Mean N (mg/g)	P range (mg/g)	Mean P (mg/g)
Submerged-rosette	3	2	18.7–21.3	19.8	1.16–3.96	2.47
Submerged-caulescent	75	22	11.3–66.8	25.2	1.17–13.4	5.40
Floating leaf	6	5	15.4–29.7	22.2	1.16–5.5	2.6
Emergent	45	34	5–64.7	17.7	0.23–12.2	2.90
Free floating	13	6	12.5–39	21.7	1.92–7.5	3.99
Overall	142	69	5–66.8	21.2	0.23–13.4	4.24

Table 4.2: Tests of phylogenetic signal among nitrogen and phosphorus concentrations and species growth form using a test for serial independence (C_{mean}) and a variance of standardized independent contrasts (VarContr) test. Only growth form (bold) shows a significant signal.

Trait	C_{mean}	*p*-value	VarContr	*p*-value
N concentration	0.074	0.306	0.939	0.627
P concentration	-0.098	0.185	0.084	0.506
Growth form	0.292	**0.0003**	0.009	**< 0.0001**

Table 4.3: Counts of plants expressing submerged or emergent tissue types following treatment or no treatment with 10µm abscisic acid (ABA). Sub = submerged, Emer = emergent.

	Hippuris vulgaris		*Megalodonta beckii*		*Myriophyllum heterophyllum*	
Treatment	Sub	Emer	Sub	Emer	Sub	Emer
ABA	7	18	4	21	4	21
Non-ABA	5	19	25	0	25	0

Table 4.4: Mean tissue mass (g) +/- standard error for three aquatic plant species treated or not treated with 10µm abscisic acid (ABA) to induce an emergent growth form or retain submerged growth form respectively. Initial values were measured just prior to planting and represent submerged tissue.

	Hippuris vulgaris		*Megalodonta beckii*		*Myriophyllum heterophyllum*	
	Sub	Emer	Sub	Emer	Sub	Emer
Initial mass	0.426 (+/-0.191)	0.407 (±0.096)	0.085 (±0.017)	0.101 (±0.022)	0.090 (±0.009)	0.101 (±0.009)
Final mass						
Emergent	0 (±0)	0.214 (±0.05)	0 (±0)	0.019 (±0.004)	0 (±0)	0.013 (±0.003)
Submerged	0.142 (±0.063)	0.174 (±0.041)	0.109 (±0.022)	0.054 (±0.012)	0.105 (±0.021)	0.072 (±0.016)
Root	0.128 (±0.057)	0.396 (±0.093)	0.025 (±0.005)	0.032 (±0.007)	0.129 (±0.026)	0.087 (±0.019)
Total	0.27 (±0.121)	0.785 (±0.185)	0.134 (±0.027)	0.105 (±0.023)	0.188 (±0.038)	0.134 (±0.029)
% change	-36.5	92.9	57.7	3.9	257.5	177.6

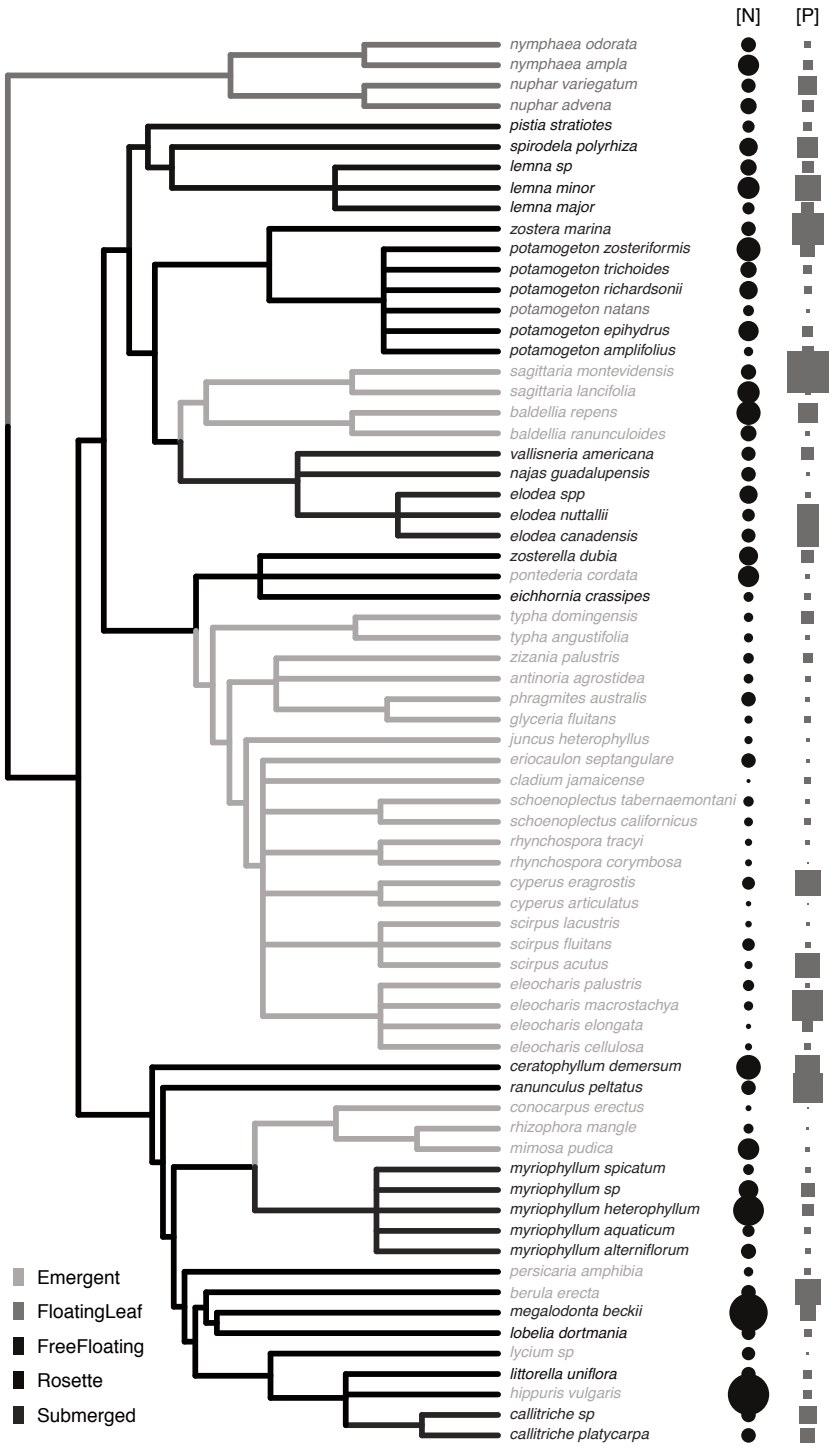

Figure 4.1: Phylogeny of 69 aquatic plant taxa found in the database of tissue nutrient concentrations gathered from the literature. Tree topology is derived from the resolved tree at the angiosperm phylogeny website (Phylomatic tree R20120829) and branch lengths are calibrated from published sources (Appendix B). The size of the green circles (total nitrogen) and orange squares (total phosphorus) illustrates the relative mean nutrient concentration for each species. Clades are colored by growth form using a parsimony rule.

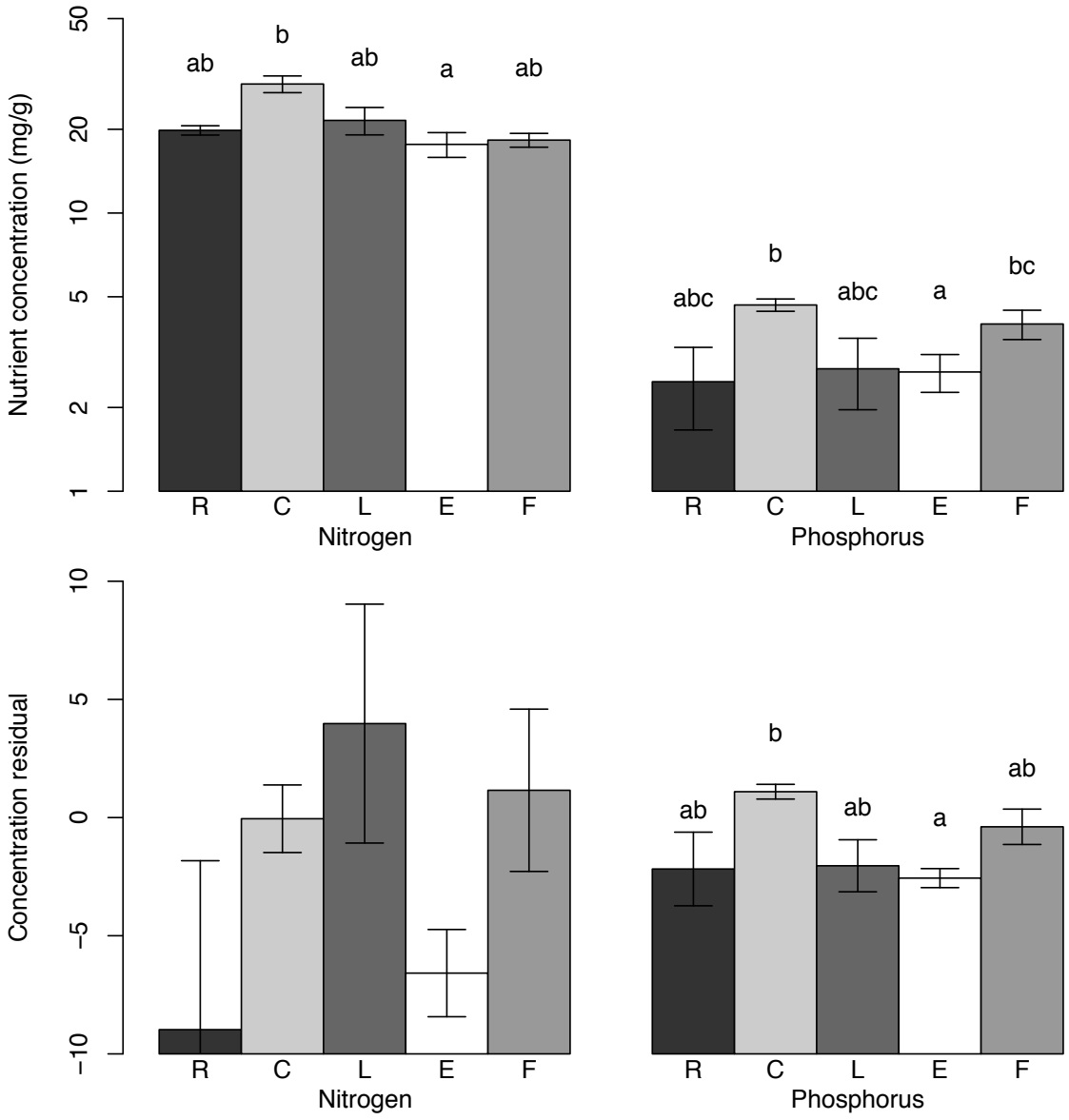

Figure 4.2: Barplots of nitrogen and phosphorus tissue concentrations for 69 aquatic plant species gathered from the literature. Values are partitioned by the growth form morphology of the species. Upper panel shows values obtained from the literature; lower panel shows residuals of literature values after accounting for the growth form clades in Figure 4.1. R = Rosette, C = Caulescent, L = Floating leaf, E = Emergent, F = Free floating. Letters above bars indicate post hoc groupings; residuals for N show no significant mean differences.

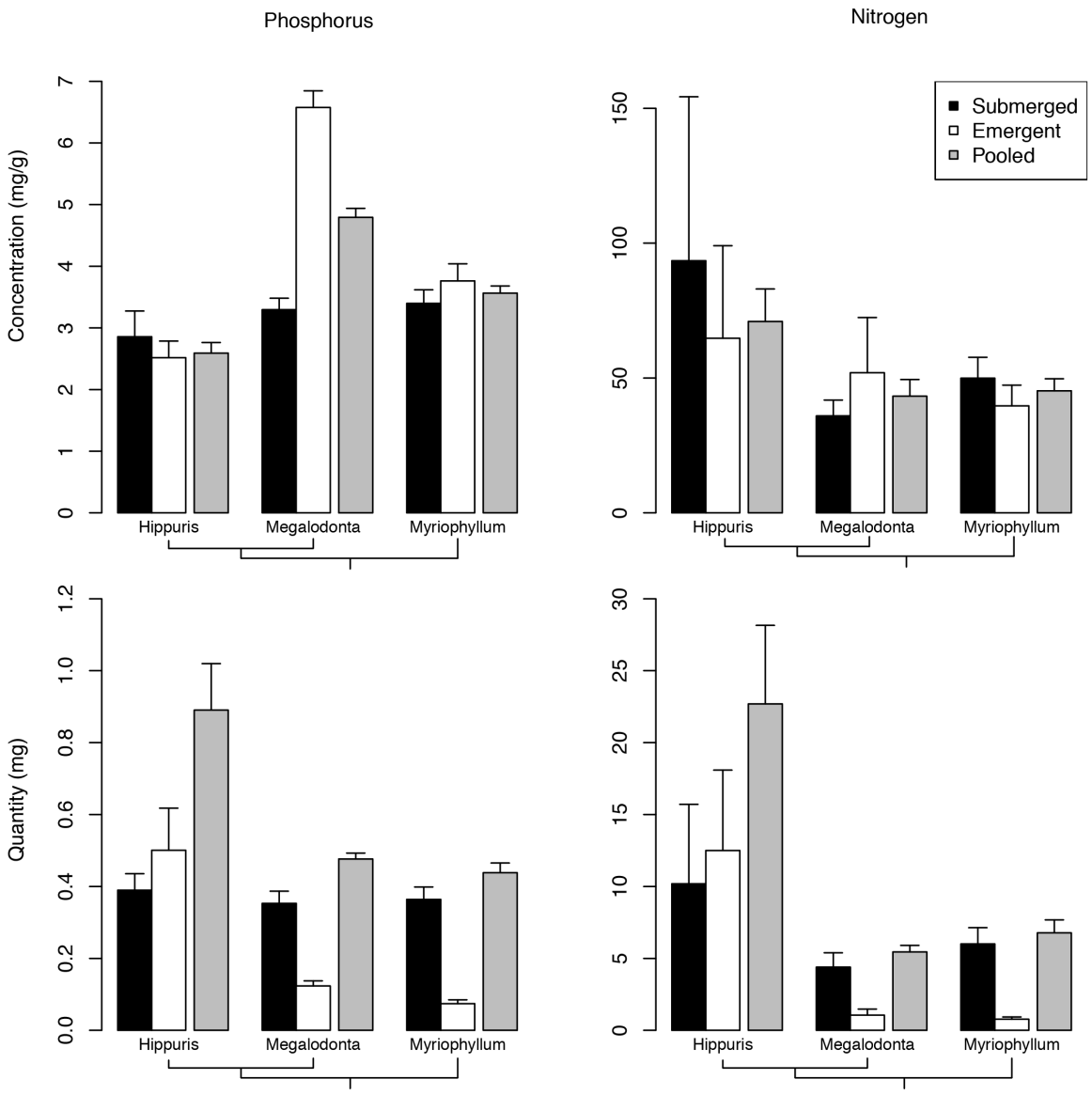

Figure 4.3: Barplots of nitrogen and phosphorus tissue concentrations and total quantities (+/- standard error) after experimental manipulation of growth form in three aquatic plant species: Hippuris vulgaris*, Megalodonta* beckii*, and* Myriophyllum heterophyllum*. Tree below names depicts phylogenetic relationship among species. Species mature morphology is emergent in* H. vulgaris *and submerged in* M. beckii *and* M. heterophyllum*.*

Chapter 5 - General Conclusion

The framework proposed by Laverol and Garnier (2002; Figure 1.2) connects environmental change to community structure and ecosystem function via the traits of species. Traits are functional and physiological attributes of species and are, or directly represent, the properties of an organism upon which the environment acts (McGill et al. 2006). Thus, studying traits rather than species has the advantage of research conclusions being generalizable to new locations that have a different complement of species but with comparable traits to those examined. I set out to investigate (1) which environmental factors most closely correlate with (and potentially predict) macrophyte community composition and abundance? (2) can a trait-based aquatic plant metric predict aquatic ecosystem trophic status? and (3) how are nutrient dynamics related to aquatic plant growth form? Each of these questions examined, in part, the role of phylogeny (species identity) versus phenotype (growth form). Plant growth form is a morphological trait that is influenced by numerous physiological and functional trade-offs (Chapin 1993); thus it represents an accumulation of ecological and evolutionary pressures. My investigations revealed that sorting macrophyte species into phenotypic categories improves prediction and assessment of ecological patterns compared to use of taxonomic identity alone. In this chapter, I place the results of my dissertation work in the context of Laverol and Garnier's (2002) framework. I then synthesize the previous chapters by examining their combined implications and conclude by discussing some of the insights that emerged from my work and proposing future research avenues.

5.1 Dissertation Findings

The second chapter of my dissertation focused on the environment—traits—community structure linkage within Laverol and Garnier's (2002) "Holy Grail" framework (Figure 5.1). In it, I sought to evaluate the potential of aquatic plant communities in southern Manitoba tributary streams to serve as bioindicators of nutrient-producing human activities, but I was not able to find any strong patterns between the macrophyte assemblages and nutrients. There was no significant correlation between total phosphorus (TP) or total nitrogen (TN) in water samples vs. human nutrient-emitting activities assessed from geospatial data. I did find a weak increasing trend between phylogenetic diversity and TP, and a negative correlation between phylogenetic diversity and N:P. N:P was also positively correlated with evenness, but negatively correlated with richness. Adding traits to the analysis—advocated as a method that is robust to the vagaries in species composition (Culp et al. 2011)—by examining growth form composition change with change in nutrients, did not reveal any strong associations between morphology and nutrients. These findings may indicate that other factors, such as hydrology, are more important for determining macrophyte community structure in high nutrient environments.

In chapter three I assessed the growth form response to nutrients by testing growth form as a metric of water trophic status across Canada. This metric used the environment—traits—community structure framework in reverse direction to imply environmental condition from community (trait) structure. I first devised generalized trophic affinity values for growth forms based on values assigned to species in pre-existing European plant trophic indices. Affinity values from the European indices corroborated results from previous studies (e.g., Chambers 1987), showing that free

floating and, additionally, emergent growth forms, are associated with enriched waters (> 0.2 mg/L TP). In contrast, the submerged rosette growth form was associated with oligotrophic conditions (< 0.05 mg/L TP). The response is consistent across longitudes, occurring in Central and Western European indices, and also in field collected data from across Canada. By using this general pattern of plant growth form change along a nutrient gradient to estimate values for species occurring in Canada but not in Europe (and thus having unassigned species affinity values), I attempted to adapt European plant trophic indices to Canada. I then tested the performance of my growth form method by validating calculated trophic scores against actual water nutrient concentrations for field-collected data from Canada. I also calculated trophic scores based on species values (incomplete due to unassigned taxa). The growth form-based trophic scores were more strongly correlated with [TP] than the species-based scores, validating my hypothesis that aquatic plant growth form can be used to coarsely indicate waterway nutrient status. Though my growth form approach showed wide variation in affinity value, it shows promise for both stand-alone application and as potential method to inter-calibrate among indices from other regions. This provides significant potential to adapt taxonomy-based water trophic indices using traits, to allow broader geographic application and potentially simplify data collection.

Chapter four examined the Holy Grail framework's linkage of traits to ecosystem function. Comparing the influence of growth form and species identity on aquatic angiosperm tissue nutrient composition using phylogenetic assessment of a literature database of tissue nutrient concentrations and a controlled experiment, helped to unravel the relative importance of shared evolutionary history versus the ecological effects of phenotype on this physiological property. I found significant phylogenetic

autocorrelation of growth form but no phylogenetic signal among nutrient concentrations in the database of literature values. By removing the influence of phylogeny from these data, I showed that growth form, independent of evolutionary history, differed in tissue nutrient concentrations—with emergent growth form species having lower concentrations than those with a submerged form. Experimentally manipulating growth form in a controlled mesocosm experiment whereby three heterophyllous species were forced to express either the submerged-caulescent or emergent growth form revealed that differences between the two growth form phenotypes were inconsistent among the three species (i.e., emergent tissues had lower concentrations than submerged in *Hippuris vulgaris,* higher than submerged in *Megalodonta beckii,* and equivocal in *Myriophyllum heterophyllum*). When pooled, both emergent and submerged tissue values within a species and classified individuals by their species' mature growth form (regardless of expressed morphology), however, tissue nutrient concentrations were consistent with the phylogenetic results. This showed, not surprisingly, that growth form is partially determined by a species' evolutionary trajectory whereas tissue nutrient concentration appears to be independent of phylogeny. Of greater interest, some of the variation in tissue nutrient concentrations is attributable to overlapping and possibly interacting ecological and evolutionary influences. The response of both growth form and tissue nutrient concentration, therefore, differs with the spatiotemporal scale of investigation. Because the quantity of nutrients transferred from the sediments to the water column by plants is correlated with tissue nutrient concentration, my findings have significant implications for the role of macrophytes in ecosystem nutrient cycling, thus linking traits to ecosystem function.

5.2 Synthesis

The objective of the Laverol and Garnier's framework is "to predict changes in ecosystem processes (such as biogeochemical cycling) by considering the role of plant traits in ecosystem structure and processes" (Laverol & Garnier 2002). The model was developed with the intent to simultaneously predict terrestrial vegetation response and changes in ecosystem function to altered global environmental factors. Here, I have interpreted the results of my phenotypic versus phylogenetic analyses within the framework (Figure 5.1) to relate aquatic macrophyte response and stream nutrient dynamics to human-influenced nutrient enrichment.

The combined results from my three chapters hint at the possibility of alternative equilibrium states for aquatic vegetation of prairie waterways. The quantity of nutrients transferred from the sediments to above ground tissues is linearly correlated with tissue nutrient concentrations (Qui et al. 2002) and the majority of the nutrients in above-ground macrophyte biomass is lost to the water-column upon senescence (Nichols and Keeney 1973; Carpenter 1980; Landers 1982). When water nutrient levels are high, abundance (and presumably, biomass) of emergent and free-floating taxa increase (chapter 3). Because nutrients per unit biomass are lower for emergent than submerged growth forms (chapter 4), a higher quantity of emergent biomass would be required to transfer the same quantity of nutrients from the sediments to the water relative to submerged biomass. Conversely, when water nutrient levels are lower, relative abundance of submerged-form taxa increases (chapter 3). The submerged growth form has higher nutrient concentrations per unit mass than emergent, and thus less submerged biomass would be required to transfer the same quantity of nutrients compared to emergent biomass. In a lentic system, the nutrients transferred to the

water-column by macrophytes would determine water nutrient availability and create a feedback loop: submerged plants transferring more nutrients which potentially shift the community toward emergent taxa whereas emergent plants transfer less nutrients shifting the community toward more submerged taxa. This scenario may arrive at an equilibrium proportion of these two growth forms or it may oscillate between forms chaotically or in dynamic equilibrium.

Alternatively, if the nutrients transferred to the water by the macrophytes were not available to feed back into the local environment—flushed downstream in a lotic system, for example—then the submerged form, because it transfers more nutrients, would eventually deplete the sediment nutrient pool without affecting local water chemistry. The emergent form may also deplete the sediment nutrient pool, but at a slower rate due to less nutrient transfer. For either scenario, if the demand rate by vegetation exceeds supply, then the sediment nutrients would be “mined” away resulting in an equilibrium state whereby the macrophyte vegetation is lost due to unproductive sediments. This is, of course, an over simplification and factors such as cumulative biomass of each growth form in the community and sediment-water column dynamics will alter the general results.

Model prediction of alternative stable states have been empirically confirmed for shallow lakes with nutrient/light interactions forcing either a clear-water, macrophyte dominated state or a turbid-water, algal dominated state (Scheffer et al. 1993). Based on a simple mechanism using only light and nutrient concentration in their model, Sheffer et al. (2003) showed that within the clear-water macrophyte state, shallow lake vegetation can exist in two further alternative states: either dominated by submerged growth forms or covered by the unrooted surface-floating form. The existence of macrophyte

alternative stable states, characterized by different macrophyte growth forms, complicates the use of morphology as an indicator of nutrients. At intermediate nutrient levels, the plant community can be in either a submerged or free floating state depending on starting conditions. Given this, a morphological index may only be an effective indicator at very high or very low nutrient concentrations where the overlap (hysteresis) in morphologies does not exist. This issue will require further attention before widespread adoption of a macrophyte morphological metric.

5.3 Insights

My work has been grounded in ecological concepts, particularly community assembly and functional ecology, but my systematics background has allowed me to include a phylogenetic component that brought some novel insights and hinted at future directions for investigation. Though not explicitly tested, in chapter 2 there appeared to be a pattern of species at sites with higher N:P ratios to be clustered in a few branches of the plant phylogeny whereas sites with lower N:P had species from a diverse mixture of phylogenetic lineages. In chapter 3, I used the plant phylogeny and niche conservatism to estimate the European-style trophic affinity values for taxa from North America that had no affinities assigned to them. In chapter 4, I examined whether evolutionary history predicted tissue nutrient concentrations or if the influence could be solely attributable to phenotype. Combining my evolutionary skill set with traditional macrophyte ecology provided my most novel additions to the scientific literature. In this work I moved from “aquatic macrophytes” as a myriad of taxa to sorting these species into phylogenetic and phenotypic groups, then used these categories to assess ecological patterns.

5.4 Future Directions

To further test the role of traits versus taxonomy, continued efforts should be made to incorporate phylogenetic techniques into ecological studies. Though this has been the case for terrestrial plant ecology for many years, inclusion of formal evolutionary ecology in macrophyte research is still in an early stage. Recent work by Puijalon et al. (2011) has demonstrated the value of this approach for macrophyte ecohydrology.

This work suggests two obvious, specific research avenues to pursue. The first centres on the synthesis presented in the general conclusions: I recommend mathematically formalizing the model depicted in Figure 5.2 and analyzing it for the nutrient—growth-form alternative states I describe. Empirical data should be collected to test the predictions of the theoretical results.

The second direction would be to improve the growth form-based metric of chapter three. This metric has the potential for general applicability, but requires further refinement before that is realized. Future work should focus on reducing the variation in growth form response to nutrients in order to increase index sensitivity. This has been carried out or is underway for several of the taxonomy-based European macrophyte metrics by adjusting affinity values for ecoregion.

5.5 Summary

Other investigators have found the relative abundance of plants with a free-floating growth form to increase with nutrient enrichment (Sheffer et al. 2003). In chapter three, I showed that relative abundance of different growth forms can estimate stream trophic status. In chapter four, I showed that growth form partially explained tissue

nutrient concentrations. Taken together, these results hint at the possibility of alternative ecosystem states dependent on initial water nutrient concentration and the feedback of vegetation to changes in water trophic status. Thus, I have broken some new ground in the “Grail quest” to understand the dynamics of macrophytes in the face of nutrient enrichment and predict shifts in plant sediment-to-water nutrient transfer. Most significantly, I have shown that using traits, specifically growth form, has value over and in tandem with taxonomy, yet can be confounded by short gradients and hysteresis. I also highlighted the utility of growth form for predicting the general response of aquatic plant to nutrient enrichment, and attempted to apply it to evaluating surface water trophic status and forecasting expected tissue nutrient concentrations.

I recommend continued use of phylogenetic analyses in macrophyte ecological studies, incorporation of traits, such as morphology, or other functional classification in macrophyte-based indices and expansion of macrophyte growth form mathematic-theoretical approaches to include processes such as nutrient transfer to advance knowledge on “traits vs taxonomy” and further development of predictive tools for nutrient monitoring and bioassessment.

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Tables and Figures

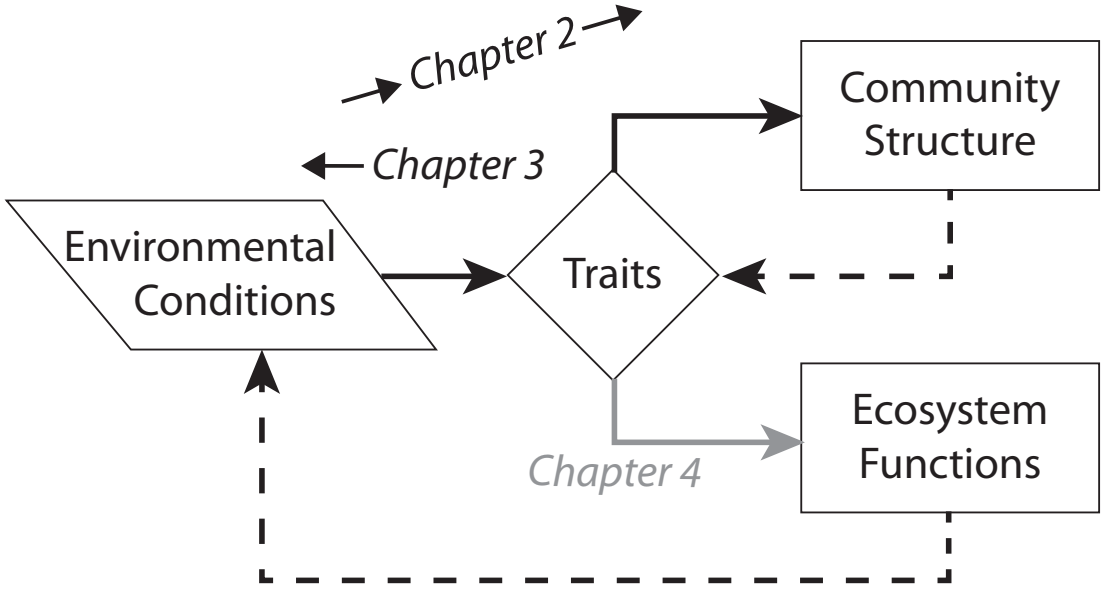


Figure 5.1: A modified illustration of Laverol and Garnier's (2002) "Holy Grail" conceptual framework for connecting environmental change, community structure and ecosystem function via species traits. Arrow colours and line type represent how my dissertation chapters apply to each linkage: solid black = chapters 2 & 3 (direction of investigation along the linkage is indicated above the pathway); solid gray = chapter 4; dashed = assumed (unevaluated).

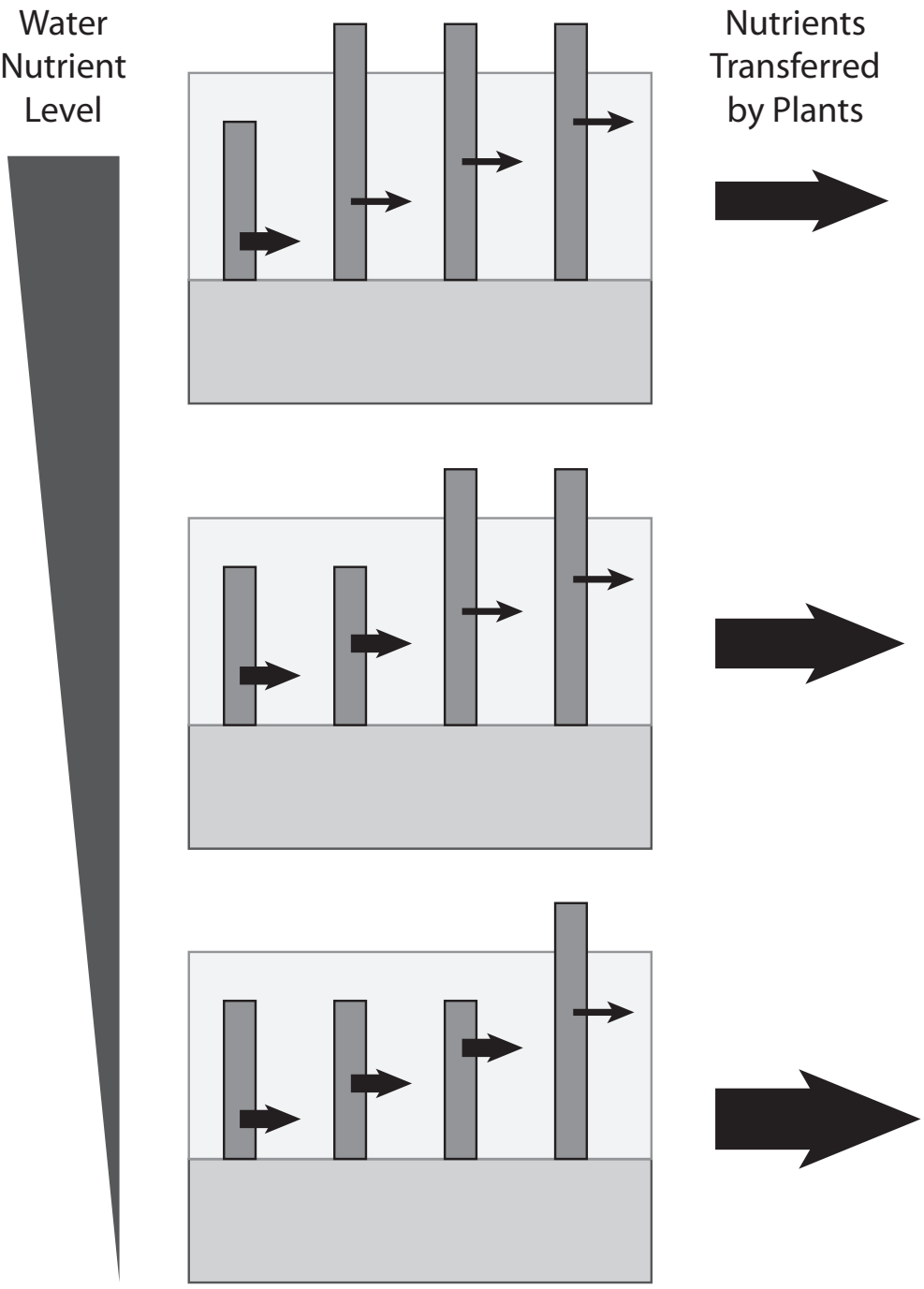


Figure 5.2. Graphical model of the combined results from my dissertation chapters. Increases in ambient water-column nutrients (gradient on left) cause changes in relative biomass of the macrophyte emergent (tall bars) and submerged (short bars) growth forms (chapters 2 & 3). Differential tissue concentrations of each growth form (central panel, small arrows; chapter 4) result in concomitant decrease in cumulative, per unit biomass quantity of nutrients transferred out of the substrate into the water column (large arrows on right) as water nutrients are enriched.

Appendices

Appendix A

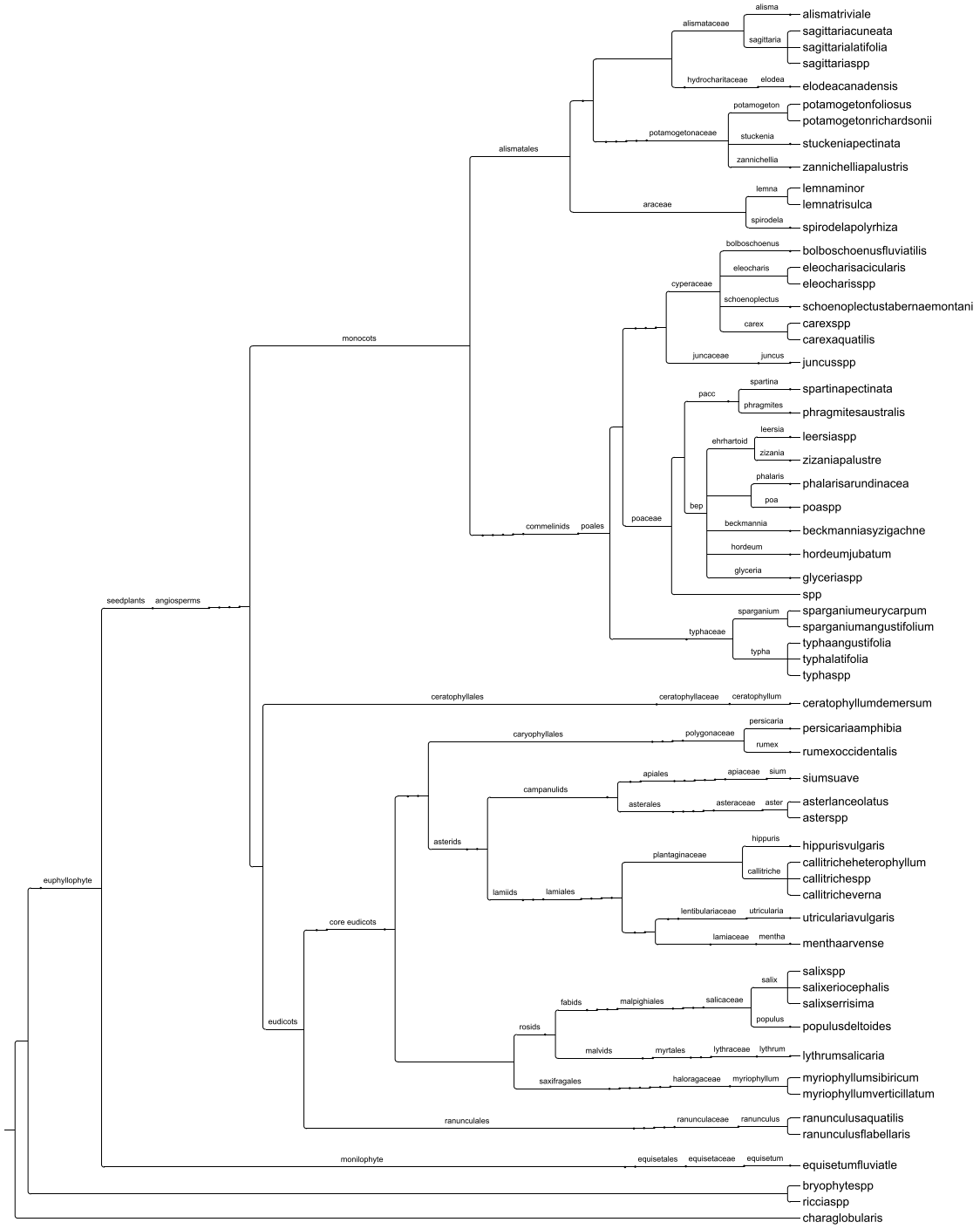
Appendix A: Specific variables and transformations used to generate a nutrient-producing human activity gradient (HAG) for southern Manitoba, Canada.

Variable	Transformation	Units
Row Crop		
Proportion total catchment area sown in canola	arcsine	m^2/m^2
Proportion total catchment area sown in maize	arcsine	m^2/m^2
Proportion total catchment area sown in flax	arcsine	m^2/m^2
Proportion total catchment area sown in forage crops	arcsine	m^2/m^2
Proportion total catchment area sown in potatoes	arcsine	m^2/m^2
Proportion total catchment area sown in seed crops	arcsine	m^2/m^2
Proportion total catchment area sown in legumes	arcsine	m^2/m^2
Proportion total catchment area sown in sunflowers	arcsine	m^2/m^2
Proportion total catchment area sown in small grains	arcsine	m^2/m^2
Livestock		
Livestock nutrient unit density of bison	$\log_{10}$	animal nutrient units/km^2
Livestock nutrient unit density of goats	$\log_{10}$	animal nutrient units/km^2
Livestock nutrient unit density of hens/chickens	$\log_{10}$	animal nutrient units/km^2
Livestock nutrient unit density of horses	$\log_{10}$	animal nutrient units/km^2
Livestock nutrient unit density of other poultry	$\log_{10}$	animal nutrient units/km^2
Livestock nutrient unit density of pigs	$\log_{10}$	animal nutrient units/km^2
Livestock nutrient unit density of sheep & lambs	$\log_{10}$	animal nutrient units/km^2
Livestock nutrient unit density of other cattle	$\log_{10}$	animal nutrient units/km^2
Livestock nutrient unit density of turkeys	$\log_{10}$	animal nutrient units/km^2
Wastewater		
Population density served by septic systems	$\log_{10}$	septic systems/ person/km^2
Estimated total nitrogen produced by septic systems	$\log_{10}$	kg/km^2
Estimated total phosphorus produced by septic systems	$\log_{10}$	kg/km^2
Population density served by sewage lagoons that discharge onto land	$\log_{10}$	lagoons/ person/km^2
Estimated total nitrogen produced by sewage lagoons that discharge onto land	$\log_{10}$	kg/km^2

Estimated total phosphorus produced by sewage lagoons that discharge onto land	$\log_{10}$	kg/km^2
Population density served by sewage lagoons that discharge into streams	$\log_{10}$	lagoons/ person/km^2
Estimated total nitrogen produced by sewage lagoons that discharge into streams	$\log_{10}$	kg/km^2
Estimated total phosphorus produced by sewage lagoons that discharge into streams	$\log_{10}$	kg/km^2

Appendix

Appendix B: Phylogeny for all taxa recorded at 29 sites in Southern Manitoba. Tree generated using Phylomatic webserver (http://phylodiversity.net/phylomatic/) with taxonomic ranks below family resolved using the generic classification in published floras.



Appendix

Appendix C: Node ages for calibrating phylogeny generated using Phylomatic webserver (http://phylodiversity.net/phylomatic/). Branch lengths were assigned with the Bladj module of Phylocom ver. 4.2. Ages derived from published estimates or the Angiosperm Phylogeny Website (http://www.mobot.org/MOBOT/research/APweb/).

Node	Age (MYA)	Reference
caryophyllales	115.0	Bell 2010
asterales	105.2	Janssens 2009
plantaginaceae	57.0	Bell 2010
malvids	113.0	Hengghang Wang et al. 2009
eudicots	129.4	Magallón et al. 2013
ceratophyllales-eudicot split	100.0	Dilcher & Wang 2009
poaceae	83.0	Janssen & Bremer 2004
cyperaceae	88.0	Janssen & Bremer 2004
poales	113	Janssen & Bremer 2004
pontederiaceae	89.0	Janssen & Bremer 2004
commelinids	113.0	Bell 2010
alismataceae	109.2	L.-Y. Chen 2012
hydrocharitaceae	75.0	Janssen & Bremer 2004
lemnoideae	122	Nauheimer et al. 2012
araceae	131.0	Nauheimer et al. 2012
alismatales	138.0	Bell 2010
monocot-eudicot split	157.0	Bell 2010
nymphaeaceae	100.1	Magallón et al. 2013
nymphaeales-asterales	183.4	Magallón et al. 2013
root	400.0	arbitrary

Appendix

Appendix D: Source information and attributes of compiled Canadian aquatic plant occurrence data used to calculate and validate site scores of a growth form-based index of water trophic status. Records are arranged by ascending mean total phosphorus (TP).

Locality	Province	Rich-ness	Mean TP (mg/L)	Flow	Reference
Lac Bowker	QC	7	0.02	Lentic	Chambers & Kalff, unpublished
Lac Bowker	QC	8	0.02	Lentic	Chambers & Kalff, unpublished
Wetland RC83	BC	8	0.02	Lotic	Boyd & Savard, 1987
Lac Orford	QC	8	0.02	Lentic	Chambers & Kalff, unpublished
Lac Orford	QC	9	0.02	Lentic	Chambers & Kalff, unpublished
Whitewater Lake	ON	26	0.03	Lentic	Dale & Miller, 1978
Wetland RC46	BC	9	0.03	Lotic	Boyd & Savard, 1987
Wetland RC72	BC	7	0.03	Lotic	Boyd & Savard, 1987
Southern SK network	SK	15	0.03	Lotic	Pip, 1979
Jenkins Lake	AB	9	0.03	Lentic	Prepas et al., 2001
Pigeon Lake	AB	24	0.04	Lentic	Haag & Noton, 1981a
Wetland RC17	BC	8	0.04	Lotic	Boyd & Savard, 1987
Wetland RC63	BC	4	0.04	Lotic	Boyd & Savard, 1987
Wetland RC77	BC	3	0.04	Lotic	Boyd & Savard, 1987
Bay of Quinte	ON	15	0.05	Lentic	Minns et al., 1993; Warren, 1995
Bay of Quinte	ON	21	0.05	Lentic	Minns et al., 1993; Warren, 1995
Wetland RC54	BC	5	0.05	Lotic	Boyd & Savard, 1987
Wetland RC65	BC	3	0.05	Lotic	Boyd & Savard, 1987
Baptiste Lake	AB	22	0.05	Lentic	Stockerl & Kent, 1984
Wetland RC05	BC	5	0.06	Lotic	Boyd & Savard, 1987
Lac Lovering	QC	18	0.06	Lentic	Chambers & Kalff, unpublished
Edie Creek	MB	16	0.06	Lotic	this study
Lofty Lake	AB	5	0.07	Lentic	Prepas et al., 2001
Buffalo Lake	AB	13	0.07	Lentic	Haag & Noton, 1981b
North Halfmoon Lake	AB	9	0.07	Lentic	Prepas et al., 2001
Wetland RC48	BC	4	0.08	Lotic	Boyd & Savard, 1987
Wetland RC78	BC	6	0.08	Lotic	Boyd & Savard, 1987
Wetland RC81	BC	4	0.08	Lotic	Boyd & Savard, 1987
Wetland RC51	BC	7	0.09	Lotic	Boyd & Savard, 1987
Wetland RC73	BC	3	0.09	Lotic	Boyd & Savard, 1987
Wetland RC25	BC	9	0.11	Lotic	Boyd & Savard, 1987

Wetland RC57	BC	8	0.11	Lotic	Boyd & Savard, 1987
Crooked Lake Channel	MB	17	0.12	Lotic	this study
Wetland RC47	BC	6	0.12	Lotic	Boyd & Savard, 1987
Sheerness cooling pond	AB	9	0.12	Lentic	Barton et al., 1991
Wetland RC40	BC	2	0.13	Lotic	Boyd & Savard, 1987
Wetland RC82	BC	3	0.13	Lotic	Boyd & Savard, 1987
Wetland RC52	BC	9	0.14	Lotic	Boyd & Savard, 1987
Wetland RC69	BC	3	0.14	Lotic	Boyd & Savard, 1987
Wetland RC80	BC	4	0.14	Lotic	Boyd & Savard, 1987
Tobacco Creek	MB	12	0.16	Lotic	this study
Wetland RC07	BC	6	0.16	Lotic	Boyd & Savard, 1987
Tobacco Creek	MB	6	0.17	Lotic	this study
Wetland RC36	BC	3	0.18	Lotic	Boyd & Savard, 1987
Wetland RC27	BC	6	0.19	Lotic	Boyd & Savard, 1987
Little Morris River	MB	12	0.20	Lotic	this study
Wetland RC58	BC	4	0.20	Lotic	Boyd & Savard, 1987
Wetland RC50	BC	3	0.21	Lotic	Boyd & Savard, 1987
Wetland RC10	BC	5	0.24	Lotic	Boyd & Savard, 1987
Wetland RC23	BC	7	0.24	Lotic	Boyd & Savard, 1987
Little Morris River	MB	6	0.25	Lotic	this study
Wetland RC09	BC	6	0.26	Lotic	Boyd & Savard, 1987
Southeastern MB network	MB	24	0.26	Lotic	Pip, 1987
Lac Magog	QC	4	0.27	Lentic	Chambers & Kalff, unpublished
Lac Magog	QC	14	0.27	Lentic	Chambers & Kalff, unpublished
Lake Memphremagog	QC	12	0.28	Lentic	Chambers, 1987
Elm Creek Channel	MB	6	0.29	Lotic	this study
Elm River	MB	10	0.31	Lotic	this study
Elm River	MB	22	0.35	Lotic	this study
Elm Creek Channel	MB	9	0.35	Lotic	this study
Elm River	MB	14	0.36	Lotic	this study
Wetland RC45	BC	9	0.39	Lotic	Boyd & Savard, 1987
Oak Bluff Drain	MB	8	0.40	Lotic	this study
Buffalo Lake Channel	MB	9	0.51	Lotic	this study
Wetland RC01	BC	8	0.54	Lotic	Boyd & Savard, 1987
Wetland RC19	BC	5	0.54	Lotic	Boyd & Savard, 1987
Wetland RC21	BC	7	0.54	Lotic	Boyd & Savard, 1987

Wetland RC39	BC	7	0.55	Lotic	Boyd & Savard, 1987
Graham Creek	MB	15	0.55	Lotic	this study
Graham Creek	MB	14	0.56	Lotic	this study
Morris River	MB	8	0.59	Lotic	this study
Oman's Creek	MB	12	0.59	Lotic	this study
Kirk Drain	MB	9	0.61	Lotic	this study
Buffalo Lake Channel	MB	10	0.63	Lotic	this study
Gamby Drain	MB	6	0.66	Lotic	this study
Kronsgart Drain	MB	8	0.80	Lotic	this study
Roseheim Drain	MB	18	0.81	Lotic	this study
Wetland RC03	BC	8	0.82	Lotic	Boyd & Savard, 1987
Lake Memphremagog	QC	8	0.88	Lentic	Chambers, 1987
Lake Memphremagog	QC	7	0.88	Lentic	Chambers, 1987
North Shannon Creek	MB	17	0.90	Lotic	this study
Dufrost Drain	MB	15	0.97	Lotic	this study
Lake Memphremagog	QC	4	0.97	Lentic	Chambers, 1987
North Shannon Creek	MB	14	0.98	Lotic	this study
Wetland RC12	BC	5	1.01	Lotic	Boyd & Savard, 1987
Wetland RC84	BC	5	1.05	Lotic	Boyd & Savard, 1987
Wetland RC29	BC	8	1.07	Lotic	Boyd & Savard, 1987
11-A Drain	MB	15	1.08	Lotic	this study
Wetland RC34	BC	3	1.10	Lotic	Boyd & Savard, 1987
Wetland RC08	BC	5	1.32	Lotic	Boyd & Savard, 1987
West Branch LaSalle River	MB	11	1.37	Lotic	this study
Roseheim Drain	MB	15	1.56	Lotic	this study
West Branch LaSalle River	MB	8	1.88	Lotic	this study
Forrester Drain	MB	15	2.07	Lotic	this study
Wetland RC85	BC	5	2.17	Lotic	Boyd & Savard, 1987
Arnault Drain	MB	11	2.25	Lotic	this study

Appendix E

Appendix E: Aquatic plant growth forms and species (with name codes) from 28 sites in 22 tributaries of the Red River, southern Manitoba, Canada. Also shown for each species are the environmental parameters (from 580 pairing of 58 taxa and 10 parameters) that fit a linear model with species abundance better than a unimodal based on differences in AIC values. Only AIC differences greater than or equal to 2 are shown, species with a dash (–) had no association with any environmental parameter.

Growth Form	Species	Code	No. Sites	Environmental Parameter	AIC Difference
Free floating	*Ceratophyllum demersum*	cer.dem	9	TP	6.2
				Turbidity	6.3
	Lemna minor	lem.min	19	pH	15.6
	Lemna trisulca	lem.tri	11	Temp.	14.5
	Riccia spp	ric.spp	1	Width	2.2
				Conductivity	13.2
	Spirodela polyrhiza	spi.pol	3	Depth	4.7
				Temp.	24.5
	Utricularia vulgare	utr.vul	6	–	–
Emergent	*Alisma triviale*	ali.tri	16	pH	4.2
	Aster lanceolatus	ast.lan	1	Depth	3.0
				Temp.	15.2
	Beckmannia syzigachne	bec.syz	4	–	–
	Bolboscheonis fluvialis	bol.flu	16	–	–
	Carex aquatilis	car.aqu	1	Depth	3.0
				Temp.	15.2
	Carex spp	car.spp	6	–	–
	Eleocharis acicularis	ele.aci	5	–	–
	Eleocharis spp	ele.spp	19	–	–
	Equisetum fluviatle	equ.flu	3	Depth	5.4
				Turbidity	5.8
	Glyceria spp	gly.spp	3	Depth	4.3
				Temp.	7.4
	Hippuris vulgaris	hip.vul	7	TP	2.2
				Velocity	2.5
				N.P	24.9
	Juncus spp	jun.spp	1	–	–
	Leersia oryzoides	lee.ory	1	–	–
	Lythrum salicaria	lyt.sal	4	Width	4.4
	Mentha arevense	men.are	5	–	–
	Persecaria amphibia	per.amp	10	Velocity	3.0
	Phalaris arundinacea	pha.aru	13	–	–
	Phragmites australis	phr.aus	1	–	–
	Poaceae spp	poa.spp	3	–	–
	Rumex occidentalis	rum.occ	3	–	–
	Sagittaria cuneata	sag.cun	24	TN	7.5

	Sagittaria latifolia	sag.lat	1	Depth	2.5
				TN	17.0
	Sagittaria spp	sag.spp	7	–	–
	Salix eriocephalis	sal.eri	2	Depth	5.4
				Temp.	9.1
	Salix serrisima	sal.ser	1	Depth	3.0
				Temp.	15.2
	Salix spp	sal.spp	9	Velocity	3.1
	Schoenplectus tabernaemontani	sch.tab	22	–	–
	Sium suave	siu.sua	13	–	–
	Sparganium eurycarpum	spa.eur	15	–	–
	Sparganium spp	spa.spp	1	–	–
	Spartina pectinata	spa.pec	10	Width	2.9
	Typha angustifolia	typ.ang	9	Width	4.6
	Typha latifolia	typ.lat	8	–	–
	Typha spp	typ.spp	1	Width	2.2
				Conductivity	13.2
	Zizania palustre	ziz.pal	4	–	–
Floating	*Nuphar variegata*	nup.var	1	–	–
leaved	*Potamogeton natans*	pot.nat	3	TP	2.5
				TN	2.7
				N.P	28.4
	Sparganium angustifolia	spa.ang	6	–	–
	Callitriche heterophyllum	cal.het	9	–	–
Caulescent	*Callitriche* spp	cal.spp	6	–	–
	Chara globularis	cha.glo	1	TP	4.4
				pH	7.3
	Elodea canadensis	elo.can	5	–	–
	Myriophyllum sibiricum	myr.sib	7	Turbidity	8.1
	Myriophyllum verticillatum	myr.ver	2	–	–
	Potamogeton amplifolius	pot.amp	2	N.P	26.4
	Potamogeton foliosus	pot.fol	18	Turbidity	8.5
	Potamogeton richardsonii	pot.ric	8	Conductivity	3.6
	Ranunculus aquatilis	ran.aqu	2	Turbidity	5.7
	Ranunculus flammula	ran.fla	1	–	–
	Stuckenia pectinata	stu.pec	13	pH	2.4
	Zannichellia palustris	zan.pal	3	–	–
Decumbent	Bryophyte spp	bry.spp	9	–	–

Appendix

Appendix F: Database of nitrogen (N) and phosphorus (P) tissue concentrations collated from literature sources. Dash indicates concentration was not available.

Reference	Growth Form	Species	N	P
Fernandez-Alaez et al. 1999	Emergent	*Antinoria agrostidea*	13.6	1.55
Fernandez-Alaez et al. 1999	Emergent	*Baldellia ranunculoides*	22.5	1.46
Pulido et al. 2011	Emergent	*Baldellia repens*	34.0	5.67
Baldy et al. 2007	Emergent	*Berula erecta*	—	5.25
Baldy et al. 2007	Emergent	*Berula erecta*	—	9.65
Miao & Zou 2012	Emergent	*Cladium jamaicense*	5.0	3.75
Rejmankova et al. 2011	Emergent	*Cladium jamaicense*	6.3	0.23
Rejmankova et al. 2011	Emergent	*Conocarpus erectus*	8.3	0.41
Rejmankova et al. 2011	Emergent	*Cyperus articulatus*	7.7	0.42
Rejmankova et al. 2011	Emergent	*Cyperus eragrostis*	18.2	7.66
Miao & Zou 2012	Emergent	*Eleocharis cellulosa*	10.5	3.38
Rejmankova et al. 2011	Emergent	*Eleocharis cellulosa*	9.5	0.31
Miao & Zou 2012	Emergent	*Eleocharis elongata*	7.5	3.15
Rejmankova et al. 2011	Emergent	*Eleocharis macrostachya*	13.1	9.12
Fernandez-Alaez et al. 1999	Emergent	*Eleocharis palustris*	15.8	1.26
Gerloff & Krombholz 1966	Emergent	*Eriocaulon septangulare*	20.3	1.00
Fernandez-Alaez et al. 1999	Emergent	*Glyceria fluitans*	11.4	1.87
this study	Emergent	*Hippuris vulgaris*	64.7	2.52
Fernandez-Alaez et al. 1999	Emergent	*Juncus heterophyllus*	11.4	0.93
Rejmankova et al. 2011	Emergent	*Lycium sp*	18.8	0.62
this study	Emergent	*Megalodonta beckii*	52.0	6.58
Rejmankova et al. 2011	Emergent	*Mimosa pudica*	30.2	1.32
this study	Emergent	*Myriophyllum heterophyllum*	39.7	3.76
Fernandez-Alaez et al. 1999	Emergent	*Persicaria amphibia*	13.3	1.84
Rejmankova et al. 2011	Emergent	*Phragmites australis*	20.4	1.34
Rejmankova et al. 2011	Emergent	*Pontederia cordata*	29.7	1.34
Rejmankova et al. 2011	Emergent	*Rhizophora mangle*	14.0	0.80
Rejmankova et al. 2011	Emergent	*Rhynchospora corymbosa*	9.8	0.44
Miao & Zou 2012	Emergent	*Rhynchospora tracyi*	10.2	1.33
Rejmankova et al. 2011	Emergent	*Sagittaria lancifolia*	31.4	1.68
Rejmankova et al. 2011	Emergent	*Sagittaria montevidensis*	21.5	12.20
Malecki-Brown et al. 2010	Emergent	*Schoenoplectus californicus*	12.5	2.00
Andersen & Mitsch 2005	Emergent	*Schoenoplectus tabernaemontani*	15.6	1.27

Andersen & Mitsch 2005	Emergent	*Schoenoplectus tabernaemontani*	13.6	1.52
Rejmankova et al. 2011	Emergent	*Scirpus acutus*	11.4	7.31
Fernandez-Alaez et al. 1999	Emergent	*Scirpus fluitans*	17.8	1.74
Fernandez-Alaez et al. 1999	Emergent	*Scirpus lacustris*	9.2	1.04
Andersen & Mitsch 2005	Emergent	*Typha angustifolia*	14.1	1.22
Andersen & Mitsch 2005	Emergent	*Typha angustifolia*	11.7	1.24
Malecki-Brown et al. 2010	Emergent	*Typha domingensis*	10.0	2.25
Miao & Zou 2012	Emergent	*Typha domingensis*	6.4	1.64
Rejmankova et al. 2011	Emergent	*Typha domingensis*	11.7	0.70
Rejmankova et al. 2011	Emergent	*Typha domingensis*	24.5	9.72
Lee & McNaughton 2004	Emergent	*Zizania palustris*	14.0	2.10
Lee & McNaughton 2004	Emergent	*Zizania palustris*	16.0	3.70
Cronin & Lodge 2003	FloatingLeaf	*Nuphar advena*	24.9	—
Lee & McNaughton 2004	FloatingLeaf	*Nuphar variegatum*	20.0	5.50
Rejmankova et al. 2011	FloatingLeaf	*Nymphaea ampla*	29.7	2.70
Miao & Zou 2012	FloatingLeaf	*Nymphaea odorata*	21.0	1.66
Mathews 2010	FloatingLeaf	*Nymphaea odorata*	—	2.04
Fernandez-Alaez et al. 1999	FloatingLeaf	*Potamogeton natans*	15.4	1.16
Polomski et al. 2009	FreeFloating	*Eichhornia crassipes*	14.0	1.92
James et al. 2006	FreeFloating	*Lemna major*	15.0	3.07
James et al. 2006	FreeFloating	*Lemna major*	16.2	3.49
James et al. 2006	FreeFloating	*Lemna major*	16.3	3.72
James et al. 2006	FreeFloating	*Lemna major*	19.2	3.85
James et al. 2006	FreeFloating	*Lemna major*	18.9	3.91
Steffenhagen, et al. 2012	FreeFloating	*Lemna minor*	22.0	7.50
Cedergreen & Madsen 2003	FreeFloating	*Lemna minor*	32.0	—
Cedergreen & Madsen 2003	FreeFloating	*Lemna minor*	39.0	—
Szabo et al. 2010	FreeFloating	*Lemna sp*	12.5	—
Szabo et al. 2010	FreeFloating	*Lemna sp*	33.5	—
Polomski et al. 2009	FreeFloating	*Pistia stratiotes*	17.1	2.48
Steffenhagen, et al. 2012	FreeFloating	*Spirodela polyrhiza*	26.0	6.00
Pulido et al. 2011	Rosette	*Littorella uniflora*	21.3	3.96
Fernandez-Alaez et al. 1999	Rosette	*Littorella uniflora*	18.7	1.16

Gerloff & Krombholz 1966	Rosette	*Lobelia dortmania*	19.5	2.30
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	2.15
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	2.20
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	3.02
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	3.60
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	3.92
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	4.49
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	4.60
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	5.28
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	5.46
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	6.18
Thiebaut 2005	Submerged	*Callitriche platycarpa*	—	6.68
Baldy et al. 2007	Submerged	*Callitriche sp*	—	5.30
Steffenhagen, et al. 2012	Submerged	*Ceratophyllum demersum*	32.5	10.00
Nichols & Keeney 1976	Submerged	*Ceratophyllum demersum*	16.3	—
Nichols & Keeney 1976	Submerged	*Ceratophyllum demersum*	20.5	—
Nichols & Keeney 1976	Submerged	*Ceratophyllum demersum*	22.7	—
Gerloff & Krombholz 1966	Submerged	*Ceratophyllum demersum*	34.1	7.10
Han-Feng et al. 2007	Submerged	*Ceratophyllum demersum*	49.2	5.17
Han-Feng et al. 2007	Submerged	*Ceratophyllum demersum*	66.8	6.20
Mathews 2010	Submerged	*Elodea canadensis*	—	1.34
Thiebaut 2005	Submerged	*Elodea canadensis*	—	5.13
Thiebaut 2005	Submerged	*Elodea canadensis*	—	7.78
Thiebaut 2005	Submerged	*Elodea canadensis*	—	7.94
Thiebaut 2005	Submerged	*Elodea canadensis*	—	8.77
Thiebaut 2005	Submerged	*Elodea canadensis*	—	9.25
James et al. 2006	Submerged	*Elodea canadensis*	18.3	5.66
James et al. 2006	Submerged	*Elodea canadensis*	21.1	5.66
James et al. 2006	Submerged	*Elodea canadensis*	18.3	6.11
James et al. 2006	Submerged	*Elodea canadensis*	19.3	6.30
James et al. 2006	Submerged	*Elodea canadensis*	21.0	6.30
Thiebaut 2005	Submerged	*Elodea nuttallii*	—	2.77
Thiebaut 2005	Submerged	*Elodea nuttallii*	—	4.89
Thiebaut 2005	Submerged	*Elodea nuttallii*	—	5.11
Thiebaut 2005	Submerged	*Elodea nuttallii*	—	6.04
Thiebaut 2005	Submerged	*Elodea nuttallii*	—	6.06
Thiebaut 2005	Submerged	*Elodea nuttallii*	—	6.39
Thiebaut 2005	Submerged	*Elodea nuttallii*	—	8.18
Thiebaut 2005	Submerged	*Elodea nuttallii*	—	9.16
Thiebaut 2005	Submerged	*Elodea nuttallii*	—	9.19
James et al. 2006	Submerged	*Elodea nuttallii*	15.7	4.91
James et al. 2006	Submerged	*Elodea nuttallii*	15.5	5.37
James et al. 2006	Submerged	*Elodea nuttallii*	18.1	5.50
James et al. 2006	Submerged	*Elodea nuttallii*	18.1	6.63
James et al. 2006	Submerged	*Elodea nuttallii*	21.1	7.41

Gerloff & Krombholz 1966	Submerged	*Elodea sp*	25.6	1.50
this study	Submerged	*Hippuris vulgaris*	58.0	2.86
this study	Submerged	*Megalodonta beckii*	36.0	3.30
Fernandez-Alaez et al. 1999	Submerged	*Myriophyllum alterniflorum*	21.3	1.66
Wersal & Madsen 2011	Submerged	*Myriophyllum aquaticum*	21.3	2.13
Polomski et al. 2009	Submerged	*Myriophyllum aquaticum*	12.9	1.96
this study	Submerged	*Myriophyllum heterophyllum*	49.9	3.40
Gerloff & Krombholz 1966	Submerged	*Myriophyllum* sp	27.7	4.10
Mathews 2010	Submerged	*Myriophyllum spicatum*	—	1.53
Nichols & Keeney 1976	Submerged	*Myriophyllum spicatum*	11.3	—
Nichols & Keeney 1976	Submerged	*Myriophyllum spicatum*	12.7	—
Nichols & Keeney 1976	Submerged	*Myriophyllum spicatum*	16.6	—
Nichols & Keeney 1976	Submerged	*Myriophyllum spicatum*	20.2	—
Mathews 2010	Submerged	*Najas guadalupensis*	—	1.17
Cronin & Lodge 2003	Submerged	*Nuphar advena*	20.9	—
Cronin & Lodge 2003	Submerged	*Potamogeton amplifolius*	13.1	—
Gerloff & Krombholz 1966	Submerged	*Potamogeton epihydrus*	28.4	3.00
Gerloff & Krombholz 1966	Submerged	*Potamogeton richardsonii*	25.9	2.30
Fernandez-Alaez et al. 1999	Submerged	*Potamogeton trichoides*	23.0	2.39
Gerloff & Krombholz 1966	Submerged	*Potamogeton zosteriformis*	33.7	4.40
Thiebaut 2005	Submerged	*Ranunculus peltatus*	—	4.18
Thiebaut 2005	Submerged	*Ranunculus peltatus*	—	5.52
Thiebaut 2005	Submerged	*Ranunculus peltatus*	—	8.51
Thiebaut 2005	Submerged	*Ranunculus peltatus*	—	9.30
Thiebaut 2005	Submerged	*Ranunculus peltatus*	—	10.91
Thiebaut 2005	Submerged	*Ranunculus peltatus*	—	13.42
Gerloff & Krombholz 1966	Submerged	*Vallisneria americana*	19.8	3.70
McRoy & Barsdate 1970	Submerged	*Zostera marina*	—	7.86
McRoy & Barsdate 1970	Submerged	*Zostera marina*	—	10.99
Mathews 2010	Submerged	*Zosterella dubia*	—	1.50
Gerloff & Krombholz 1966	Submerged	*Zosterella dubia*	26.7	5.80

Appendix

Appendix G: Environmental conditions of experimental mesocosm array. Values reported as mean +/- standard deviation.

Parameter	Value
Water TN (mg/L)	2.60 (+/- 0.19)
Water TP (mg/L)	0.30 (+/- 0.03)
Sediment TN (mg/g)	3.38 (+/- 0.29)
Sediment TP (mg/g)	0.55 (+/- 0.03)
Light, 400-700 nm (µmol/m^2/s)	37.1 (+/- 5%)
Water Temperature (°C)	19.8 (+/- 4.7)
Turbidity (NTU)	43.0 (+/- 3.21)
Depth (cm)	16.4 (+/- 0.27)

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