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Invasive *Myriophyllum spicatum* and nutrients interact to influence algal assemblages

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ABSTRACT

Eutrophication and invasive species are widespread stressors that have the potential to interact and alter the biodiversity and productivity of aquatic ecosystems. We conducted a two-way factorial mesocosm experiment testing interacting effects of nutrient loading and presence of the invasive macrophyte *Myriophyllum spicatum* on the composition, biomass, and productivity of attached and suspended algal assemblages. Chlorophyll *a* concentrations, a proxy for suspended algal biomass, were an average of 75% higher on all sampling dates in high nutrient treatments than in low nutrient treatments. By the end of the 30-day experiment, biovolume of attached algae suspended in the water column (e.g., *Bulbochaete* sp. and *Cladophora* sp.) was 35 × higher in treatments containing *M. spicatum* than in those that did not and 11 × higher in high nutrient treatments than in low nutrient treatments. This increase in attached algae was associated with changes in ecosystem productivity, including a 10% increase in dissolved oxygen saturation during the day and 1.3–2.4 × higher rates of gross primary production in high nutrient treatments with *M. spicatum*. NMDS analysis revealed major taxonomic shifts in the algal assemblage as the experiment progressed, including a loss of large diatoms and an increase in cryptomonads, planktonic chlorophytes, and attached chlorophytes. Nutrient enrichment may be critical in affecting rates of overall ecosystem productivity, but the structure of the assemblage contributing to that productivity may be influenced by interactions between nutrient enrichment and the presence of rooted macrophytes.

1. Introduction

Vascular macrophytes are widely recognized as structuring members of lake littoral zones because they create habitat for attached algae, invertebrates, and fish, and influence levels of dissolved gasses and nutrients in the water column (Carpenter and Lodge, 1986; Scheffer, 1998). There are many mechanisms by which macrophytes may facilitate or compete with algal assemblages in lakes. For example, macrophytes may stimulate the growth of attached algae by providing physical locations for attachment high in the water column and by translocating nutrients from the sediments via their root systems, then releasing them into the water column by leaking or during senescence (Cattaneo and Kalff, 1979; Kelly and Hawes, 2005). On the other hand, macrophytes may suppress planktonic algae by shading, competing for water-column nutrients, and promoting food webs with high abundances of planktonic grazers (Sand-Jensen and Borum, 1991; Scheffer, 1998). The interplay between macrophytes and algae is integral to the study of alternate clear and turbid water states in lakes, which has had broad influence on ecological theory and lake management (Scheffer et al., 1993; Jeppsen et al., 1998; Scheffer, 1998). Therefore, the interactions among macrophytes and algae, both attached and planktonic, are key to understanding how freshwater ecosystems may respond to changes caused by a variety of stressors.

Two major stressors affecting aquatic ecosystems are the proliferation of invasive species (Strayer, 2010) and eutrophication (Smith et al., 1999). Eurasian watermilfoil (*Myriophyllum spicatum*) is a submerged vascular macrophyte native to Europe, Asia, and Northern Africa (Smith and Barko, 1990). Since its introduction in the USA in the 1880s, *M. spicatum* and its hybrids with native northern watermilfoil, *M. sibericum*, have become the most aggressively managed aquatic weeds in the United States (Moody and Les, 2007). The capacity of *M. spicatum* to rapidly grow and form dense surface mats can have undesirable ecosystem consequences including exclusion or replacement of native plants, a decrease in sunlight available to other primary producers, and

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reduced water circulation (Schultz and Dibble, 2012). Moreover, *M. spicatum* tends to invade aquatic systems that are already experiencing other types of disturbance, particularly eutrophication. Madsen (1998) observed a positive relationship between nutrient status and relative abundance of *M. spicatum* in lakes in the United States and Canada. Understanding the interaction between eutrophication and invasion of *M. spicatum* is vital because invasion of *M. spicatum* is often facilitated by nutrient loading, yet these stressors may have counteracting or synergistic effects on algal assemblages.

Nutrient availability can control the composition and productivity of algal assemblages in aquatic ecosystems, yet the specific direction and magnitude of these responses is likely to vary depending on the presence of macrophytes such as *M. spicatum*. Generally, eutrophication is associated with large increases in the standing crop of algae and rates of primary productivity (Schindler, 2006). Shifts in dominant taxa of an algal assemblage can also occur with an increase in productivity (Schindler, 2006), yet whether nutrient enrichments lead to large increases in planktonic algae may depend in part on the presence of macrophytes (Scheffer et al., 1993; Bakker et al., 2010), which can directly compete for nutrients and also provide habitat for attached algae, microbes, and grazing zooplankton (Kelly and Hawes, 2005; Ferreiro et al., 2013; Grutters et al., 2017a). These attached algae may be even better competitors for nutrients than macrophytes themselves (Wetzel and Søndergaard, 1998). Moreover, planktonic cyanobacteria may prosper in the nutrient-rich conditions associated with eutrophication (Carmichael, 2001), but it has also been demonstrated that *M. spicatum* can inhibit the growth of cyanobacteria via allelopathic mechanisms (e.g., Gross et al., 1996; Nakai et al., 2000; Körner and Nicklisch, 2002). Finally, very high standing crops of planktonic algae can suppress the growth of both macrophytes and attached algae, primarily through light limitation (Scheffer et al., 1993; Grutters et al., 2017b), further complicating interactions among primary producers.

To unravel interacting effects of non-native plants and nutrient enrichment on assemblages of primary producers, we conducted an outdoor mesocosm experiment to simulate various nutrient loading and *M. spicatum* invasion scenarios. We hypothesized that nutrient additions would alter biomass, productivity, and assemblage composition of primary producers, with predicted increases in the biomass of planktonic and attached algae as well as macrophytes, and in overall rates of primary productivity. Secondly, we hypothesized that *M. spicatum* presence would alter the biomass and composition of primary producers; we predicted that the presence of *M. spicatum* would favor attached taxa by providing habitat (i.e., attachment structure) while suppressing the growth of planktonic primary producers via resource competition.

2. Methods

2.1. Experimental design

We conducted a two-way factorial experiment to test effects of nutrient loading and presence of *M. spicatum* on algal assemblages. We used twelve 1470 L round plastic stock tanks (2 m diameter × 0.6 m height; Pride of the Farm, Houghton, Iowa) in the outdoor experimental mesocosm facility at the Great Lakes Research Center at Michigan Technological University, Houghton, Michigan, USA. Mesocosms were filled with unfiltered lake water pumped from the Keweenaw waterway (47° 7' N, 88° 32' W) and leached for three months prior to draining and refilling. The Keweenaw waterway connects to Lake Superior on the west and east coasts of the Keweenaw Peninsula in the Upper Peninsula of Michigan, and includes Portage Lake and a canal. The waterway has very high exchange with Lake Superior, with the overall waterbody containing over 50% Lake Superior water depending on wind-driven currents and mixing patterns (Churchill et al., 2004). First identified in the Keweenaw waterway in 2012, *M. spicatum* is actively managed as a nuisance species in several bays (Juneau, Huckins and Marcarelli, unpublished data). We randomly assigned mesocosms to four treatment groups; three mesocosms received additions of high concentrations of nutrients relative to concentrations typically observed in the Keweenaw waterway (20 µg / L SRP and 145 µg / L DIN), three received additions of potted *M. spicatum*, three received additions of both high nutrients and *M. spicatum*, and three served as controls without additions of *M. spicatum* or high nutrients. Mesocosms did not have sediments on the bottom, and were situated under a 50% shade cloth, which was elevated 2 m above their surface. Intensities of photosynthetically active radiation measured in the mesocosms under the shade cloth over the entire experiment ranged from 49 to 1307 µmol / (m^2 * s) depending on the weather and height of reading (surface or bottom of mesocosm), which are similar to those measured in the upper 5 m of the Keweenaw waterway during August. We conducted the experiment over 30 consecutive days (5 August – 4 September 2014).

2.2. Collection and preparation of primary producers

We collected fragments of *M. spicatum* from Pike Bay of the Keweenaw waterway, Chassell, Michigan (47° 1′ N, 88° 31′ W) two months prior to the start of the experiment. *M. spicatum* and its hybrids with native Northern watermilfoil (*M. spicatum* × *M. sibiricum*) commonly occur throughout northern Michigan (Moody and Les, 2007). For confirmation of identification, we sent five fragments to the Robert B. Annis Water Resources Institute, Grand Valley State University, Muskegon, Michigan for genetic analysis; two fragments were identified as *M. spicatum* while the other three were identified as hybrids with native Northern watermilfoil (*M. spicatum* × *M. sibiricum*). Therefore, the fragments used in our experiment were likely a combination of pure *M. spicatum* and hybrid individuals. Fragments (12 cm in length; $n = 864$) were individually planted in propagation trays filled with sand and reared outdoors in Living Streams (Frigid Units Inc., Toledo, Ohio) filled with unfiltered lake water. We clipped flowers from growing *M. spicatum* regularly to prevent plants from sexually reproducing and subsequently triggering autofragmentation. After one month, we replanted 12-cm long fragments into 3.7 L round plastic pots filled with sand ($n = 20$ in each pot) and replaced them into the Living Streams, allowing them to stabilize after transplant and establish roots.

The Keweenaw waterway has low nutrient concentrations and algal standing crops as a result of currents driving exchange with Lake Superior (Marcarelli, unpublished data). Therefore, we prepared an inoculum to establish algal assemblages within the mesocosms at the start of the experiment. The day prior to experimental initiation, we filtered water from Pike Bay through a 180 µm sieve to remove large-bodied zooplankton, then through a 10 µm sieve to concentrate the algae into a single sample (~1.5 L) that was used as the inoculum.

2.3. Experiment initiation

On day zero, we filled mesocosms with unfiltered lake water and added eight pots of *M. spicatum* to every *M. spicatum* treatment (160 plants per mesocosm). 100 mL of the algal inoculum was added to each mesocosm. Because nutrient levels are low in the Keweenaw waterway, we enriched mesocosms that did not receive a high nutrient treatment (controls and *M. spicatum* alone, hereafter "low nutrient treatments") with 2 µg / L soluble reactive phosphorus (SRP, added as KH_2PO_4), a level observed in Pike Bay during autumn 2013 (Ortiz and Marcarelli, unpublished data), and 14.5 µg / L dissolved inorganic nitrogen (DIN, added as NH_4NO_3) to reflect a 16 : 1 M N : P ratio (Redfield, 1958). High nutrient treatments were enriched with concentrations that were 10 × higher than the low nutrient treatments, but still at a 16 : 1 M ratio (20 µg / L SRP and 145 µg / L DIN). We expected that nutrient uptake would occur rapidly in the mesocosms (confirmed by measurements of SRP and ammonium-nitrogen (NH_4^+-N) on day 7); therefore, to maintain a state of increased nutrient levels, we repeated nutrient enrichments of both low and high levels of SRP and DIN to all

mesocosms on days 10 and 20. This resulted in a total nutrient load of 0.52 g N / m^2 and 0.028 g P / m^2 to the high nutrient treatments over the 30 day duration of the experiment.

2.4. Experiment monitoring

We monitored environmental characteristics throughout the experiment. All measurements of environmental characteristics and sample collection occurred between the hours of 15:00 – 17:00. We measured light availability daily at the surface and bottom of each mesocosm using a LI-COR LI193SA spherical underwater quantum sensor with a LI-1400 datalogger (LI-C;1;OR Inc., Lincoln, Nebraska) to calculate light transmittance through the water column. Light measurements were conducted in open areas of each mesocosm to ensure plants themselves were not contributing to shading. In addition, we used a YSI 6920 V2 multiparameter sonde (YSI Inc., Yellow Springs, Ohio) to measure temperature, dissolved oxygen (% saturation and mg / L), conductivity, and pH daily.

On days zero, 15, and 30 of the experiment, we collected water from each mesocosm for nutrient analysis. We filtered water through precombusted 0.7 µm glass fiber filters into 60 mL Nalgene bottles and froze samples at −10 °C until analysis for SRP, total dissolved nitrogen (TDN), and dissolved organic carbon (DOC). On the day of collection, we analyzed additional filtered water samples for NH_4^+-N using fluorometric analysis (Holmes et al., 1999; following modifications by Taylor et al., 2007) with a Trilogy® fluorometer (Turner Designs, Sunnyvale, California). SRP was determined via ascorbic-acid colorimetry (APHA, 2005), and DOC and TDN were determined following acidification with hydrochloric acid with a Shimadzu TOC-V_{CSN} analyzer with a total nitrogen module TNM-1 (Shimadzu Scientific Instruments, Columbia, Maryland).

2.5. Primary producer biomass

We collected and filtered 1 L of water through 0.7 µm glass fiber filters every 5 days for analysis of chlorophyll *a* (hereafter chl *a*) as a proxy for suspended algal biomass. Because these were grab samples of water from the mesocosms, they likely included planktonic along with attached algae that may have been dislodged and suspended in the water column. Filters were frozen at −10 °C until analysis. Chlorophyll was extracted in 95% ethanol and analyzed spectrophotometrically, correcting for pheophytin (APHA, 2005; Nusch, 1980). We analyzed chl *a* concentrations with repeated measures analysis of variance (RM-ANOVA); chl *a* concentration (ln transformed) was analyzed as the dependent variable, and *M. spicatum* (presence or absence) and nutrient addition (high vs. low) were analyzed as independent variables. We conducted this and all following RM-ANOVAs with PROC MIXED in SAS version 9.2 (SAS Institute, Cary, North Carolina) with $\alpha = 0.05$. When necessary, dependent variables were ln transformed to satisfy assumptions of analysis of variance (ANOVA). When conducting RM-ANOVAs, we first fit ten different covariance structures to our experimental data to determine which was most appropriate; for all responses a heterogeneous compound symmetry model provided the best fit for our data, as indicated by lowest fit statistics (Littell et al., 2000). Therefore, we applied this model to all of our analyses. A consequence of this model is that it results in decimal degrees of freedom estimates for all RM-ANOVA analyses presented.

To estimate change in *M. spicatum* biomass over the course of the experiment, we collected whole *M. spicatum* from each mesocosm at the end of the experiment and separated them to determine biomass of plant roots and shoots. We measured biomass as ash-free dry mass (AFDM), which was estimated by drying roots and shoots at 60 °C for 12 h, weighing, combusting at 550 °C for 4 h, rewetting with deionized water, drying, and reweighing (APHA, 2005). Final biomass (dependent variable) among treatments was analyzed with a *t*-test between the two treatments (high and low nutrient) that contained *M. spicatum*.

We collected attached algae at the conclusion of the experiment to determine its biomass as AFDM, following the same methods used to process *M. spicatum* biomass. We separated attached algae from *M. spicatum* by hand by pulling filaments from macrophyte fronds, using forceps as necessary. Algae that was attached to mesocosm walls was also collected by scraping all mesocosm surfaces. We combined attached algae collected from both plant and mesocosm surfaces into one composite sample per mesocosm. Treatment effects on the dependent variable AFDM of attached algae were analyzed with two-way ANOVA. This and all following two-way ANOVAs utilized *M. spicatum* (presence or absence) and nutrient addition (high vs. low) as independent variables with $\alpha = 0.05$, and were conducted in R version 3.3.1 (R Core Team, 2016).

2.6. Primary production and respiration

Changes in productivity were assessed in two ways. First, we observed daily concentrations of dissolved oxygen in each mesocosm using multiparameter sondes; those data were ln transformed and analyzed with RM-ANOVA with *M. spicatum* presence and nutrient addition as independent variables. Secondly, we analyzed primary production and respiration from diel-oxygen cycles in mesocosms of different treatments. To accomplish this, two YSI 6920 V2 multiparameter sondes were intermittently deployed in two mesocosms for 2–4 day durations to record measurements of dissolved oxygen and temperature at 10 min intervals. This resulted in six separate occasions where rates were simultaneously measured in two mesocosms from different treatments.

We used a one-station metabolism model to estimate gross primary production (GPP), ecosystem respiration (ER), and air–water exchange following the approach of Hotchkiss and Hall (2015) using the following equation from Van de Bogert (2007):

$$O_{(i)} = O_{(i-t)} + \left(\frac{GPP}{z} \times \frac{PPFD_i}{\sum PPFD}\right) + \frac{ER \times t}{z} + Kt(O_s - O_{(i-t)})$$

where GPP and ER are positive and negative rates of O_2 production, respectively (g O_2 / (m^2 * d)), O is the O_2 concentration (g / m^3), z is mean depth (m), t is time between measurements (d), K is temperature-corrected O_2 gas exchange rate (/ d), and O_s is O_2 saturation concentration (g / m^3). Photosynthetic photon flux density (PPFD; µmol / (m^2 * s)) was modeled using methods described by Hotchkiss and Hall (2015). Posterior probability distributions of GPP, ER, and K were simulated using Bayesian parameter estimation with informative priors for K (mean 0.003 ± standard error 0.05 / d; estimated by measuring wind speed and using a regression developed by Boyd and Coddington (1992) for small ponds) and uninformative priors for GPP and ER (0 ± 5 and -1 ± 5 g / (m^2 * d); respectively) via a random walk Metropolis algorithm and Markov chain Monte Carlo (MCMC) using RSTAN (STAN Development Team, 2016) in R version 3.3.1 (R Core Team, 2016). Both GPP and ER were statistically analyzed and are presented as positive values, although GPP represents positive fluxes of O_2 and ER represents negative fluxes of O_2 in the metabolism equation. We used a multiple imputation function ("amelia" in R, Honaker et al., 2011) to estimate metabolism values for treatments on days when no measurements were made and analyzed these data using a two-way ANOVA with *M. spicatum* (presence or absence) and nutrient addition (high vs. low) as independent variables. We excluded day in this analysis due to low replication.

2.7. Algal assemblage: biovolume and composition

We collected 250 mL of water from each mesocosm on days zero, 15, and 30 of our experiment for analysis of algal assemblages. Water was preserved in amber HDPE bottles with Lugol's solution. During analysis, we filtered water through 1.2 µm Millipore filters, cleared

filters with 50% glutaraldehyde, and set coverslips with Permount solution. We enumerated algae at 400 × with a compound microscope; a minimum of 200 cells per slide were identified to genus and categorized as planktonic or attached (Cox, 1996; Prescott, 1978; Weber, 1971). When filamentous or colonial taxa were encountered, cells were enumerated and measured individually. Cells were counted whenever they could be confidently distinguished from detritus; this occasionally included the enumeration of frustule fragments or dead cells. Samples included both planktonic taxa and chains of attached algal taxa that had been suspended in the water column. We estimated biovolume by applying measured dimensions of observed organisms to geometric models (Hillebrand et al., 1999). Five to ten individuals of each represented taxon were measured in each sample to estimate biovolume; when this was not possible due to either rarity of taxon or cell placement on the mount, averages of measurements from other samples were used. Differences in total algal biovolume (dependent variable) between treatment groups were ln transformed and analyzed with RM-ANOVA with *M. spicatum* (presence or absence) and nutrient addition (high vs. low) as independent variables.

We further investigated shifts in the algal assemblage composition among mesocosms on days zero, 15, and 30 with non-metric multidimensional scaling ordination (NMDS). All 36 genera identified in the experiment were included in the main matrix and ten environmental variables were included in the secondary matrix. The environmental variables were selected to describe water chemistry and physical properties and their relationships with the algal assemblage. We conducted this analysis in auto-pilot mode (slow and thorough) of PC-ORD version 6 with Sørenson's (Bray-Curtis) distance measure and a random starting configuration (McCune and Mefford, 2011). Runs (n = 500) were completed, 250 with real data and 250 with randomized data to determine dimensionality.

3. Results

3.1. Environmental characteristics

Temperature, conductivity, and light transmittance were not statistically different among mesocosms of all treatment groups (two-way ANOVA, Table 1). The pH ranged from approximately 8.6 in low nutrient treatments to approximately 9.1 in high nutrient treatments, with the most elevated pH observed in treatments with high levels of nutrients and *M. spicatum* (Table 1). Concentrations of SRP, TDN, and DOC varied little between treatments on days 15 and 30 (two-way ANOVA, Table 1). Concentrations of NH_4^+-N ranged from 2.03 to 11.26 µg / L, with the lowest concentrations observed in high nutrient treatments containing *M. spicatum* and highest concentrations observed in low nutrient treatments without *M. spicatum*. Concentrations of NH_4^+-N were 2.2–2.3 × higher when *M. spicatum* was absent, irrespective of nutrient treatment (Table 1).

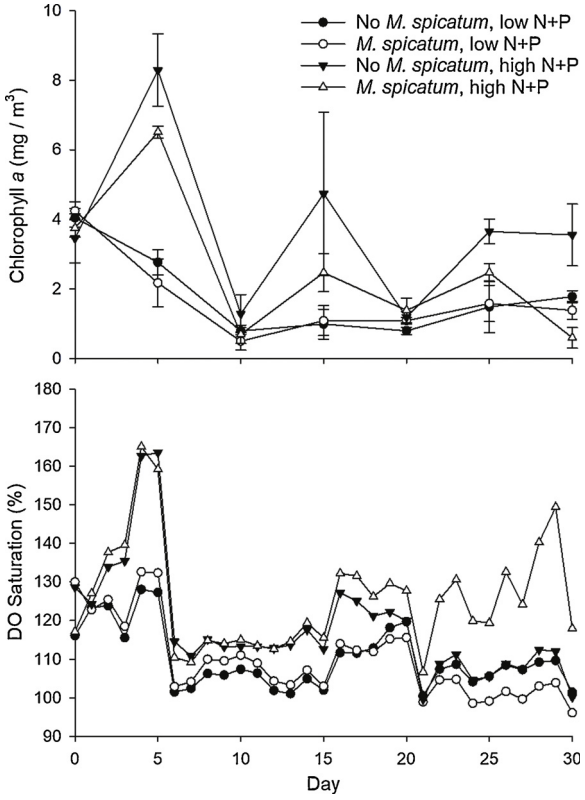

Fig. 1. (Top) Water column chlorophyll *a* in five day increments throughout the duration of the 30 day experiment and (bottom) daily dissolved oxygen saturation measured in the water column of each mesocosm between 15:00–17:00. Data points represent treatment means (n = 3 for each point) on that day ± standard error.

3.2. Primary producer biomass

Water column chl *a* concentration was an average of 75% higher in high nutrient treatments, regardless of the presence of *M. spicatum* (Fig. 1). This pattern of higher chl *a* concentrations in high nutrient treatments was significant over time (RM-ANOVA, Table 2). Presence of *M. spicatum* also affected chl *a* concentrations significantly over time (RM-ANOVA, Table 2). Because nutrients were added on days zero, 10, and 20, we observed somewhat predictable declines in chl *a* as nutrients were depleted (Fig. 1). On day 30 of the experiment, water column chl *a* concentrations were an average of 63% lower in treatments containing *M. spicatum*, regardless of nutrient treatment. Concentrations were highest in the high nutrient treatment without *M. spicatum*—an abrupt decrease in chl *a* occurred in the high nutrient treatments that

Table 1
Means ± standard error of physical and chemical environmental conditions among mesocosms of different treatments.

Treatment	Low N + P		High N + P	
	No *M. spicatum*	*M. spicatum*	No *M. spicatum*	*M. spicatum*
Temperature (°C)*	19.99 ± 0.09	19.87 ± 0.11	19.96 ± 0.18	20.01 ± 0.04
Conductivity (µS/cm)+	110 ± 0.2	110 ± 0.6	110 ± 0.4	110 ± 0.3
pH+	8.57 ± 0.09	8.56 ± 0.02	8.96 ± 0.03	9.27 ± 0.03
Light (% of surface light reaching bottom)*	78.7 ± 0.5	78.1 ± 0.3	75.7 ± 0.5	77.2 ± 0.8
SRP (µg/L)§	1.41 ± 0.17	1.41 ± 0.17	1.34 ± 0.39	1.24 ± 0.30
NH_4^+-N (µg/L)§	11.26 ± 0.92	5.08 ± 0.52	4.64 ± 0.65	2.03 ± 0.36
TDN (µg/L)§	227.73 ± 9.3	204.72 ± 2.6	251.50 ± 19.2	257.43 ± 41.2
DOC (mg/L)§	4.22 ± 0.014	4.27 ± 0.021	4.74 ± 0.192	4.53 ± 0.076

* Means of daily measurements over 30 day sampling period.
\+ Means of intermittent sampling (n = 23 for conductivity, n = 21 for pH).
§ Means of measurements on days 15 and 30.

Table 2
RM-ANOVA results for concentration of chl *a*, total suspended algal biovolume, and dissolved oxygen. *F* values are included with degrees of freedom (df) as subscripts. Significant effects and interactions between factors are displayed in bold. All data was ln transformed as needed to meet statistical assumptions of ANOVA.

	Chlorophyll *a* (mg/m^3)		Biovolume of Suspended Algae ($\mu m^3/ml$)		Dissolved Oxygen (mg/L)	
Effect	F_{df}	*p*	F_{df}	*p*	F_{df}	*p*
M. spicatum	$3.17_{1,8.4}$	0.1111	$1.14_{1,9.86}$	0.3119	$\mathbf{27.81_{1,8.49}}$	**0.0006**
Nutrients	$\mathbf{11.99_{1,8.4}}$	**0.0079**	$\mathbf{29.75_{1,9.86}}$	**0.0003**	$\mathbf{313.41_{1,8.49}}$	**< 0.0001**
Day	$\mathbf{51.12_{6,20.1}}$	**< 0.0001**	$\mathbf{70.68_{2,10.2}}$	**< 0.0001**	$\mathbf{196.53_{30,62.3}}$	**< 0.0001**
*M. spicatum**Nutrients	$1.68_{1,8.4}$	0.2290	$0.45_{1,9.86}$	0.5169	$\mathbf{37.00_{1,8.49}}$	**0.0002**
*M. spicatum**Day	$\mathbf{3.50_{6,20.1}}$	**0.0156**	$3.19_{2,10.2}$	0.0838	$\mathbf{3.21_{30,62.3}}$	**< 0.0001**
Nutrients*Day	$\mathbf{10.17_{6,20.1}}$	**< 0.0001**	$\mathbf{11.62_{2,10.2}}$	**0.0023**	$\mathbf{39.41_{30,62.3}}$	**< 0.0001**
*M. spicatum**Nutrient*Day	$1.53_{6,20.1}$	0.2192	$0.64_{2,10.2}$	0.5469	$\mathbf{16.38_{30,62.3}}$	**< 0.0001**

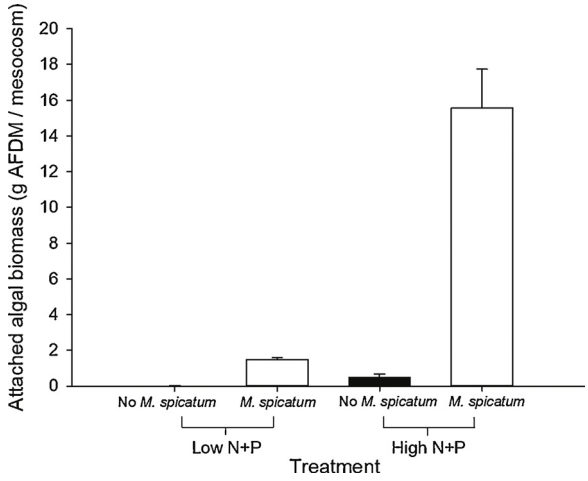

Fig. 2. Means (n = 3 for each bar) ± standard error (SE) of final biomass of attached algae in each mesocosm treatment group measured as ash-free dry mass (AFDM) on experimental day 30. Note that the mean for the low nutrient treatment without *M. spicatum* is too small to appear at the scale of this figure (0.015 ± 0.005 g AFDM / mesocosm).

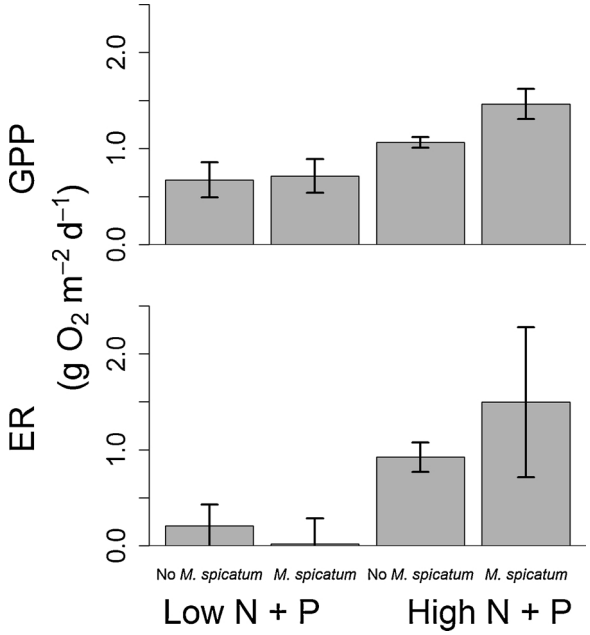

Fig. 3. Average rates of gross primary production (GPP) and ecosystem respiration (ER) in each mesocosm treatment group (n = 6 for each bar; bars represent 95% confidence intervals). These averages incorporate paired measurements recorded throughout the 30 day experiment. GPP was significantly different between treatments (two-way ANOVA, $p < 0.001$). ER showed significant interaction between factors which precluded analysis of the main effects.

contained *M. spicatum* between days 25 and 30, where concentrations dropped below even the low nutrient treatment groups by 57–67 % (Fig. 1). This response was coincident with observations of substantial growth of attached algae on *M. spicatum* in the high nutrient treatments as the experiment progressed. On day 30, attached algae biomass, quantified as AFDM, was an average of 35 × higher in mesocosm treatments containing *M. spicatum* than those without *M. spicatum* (two-way ANOVA, $F_{3,8} = 58.2$, $p < 0.001$; Fig. 2) and was 11 × higher in high nutrient treatments than the low nutrient treatments (two-way ANOVA, $F_{3,8} = 44.8$, $p < 0.001$; Fig. 2). Additionally, there was a significant interaction of nutrient level and presence of *M. spicatum* on AFDM of attached algae (two-way ANOVA, $F_{3,8} = 39.3$, $p < 0.001$). The total final biomass of *M. spicatum* did not differ between nutrient treatments (mean ± SE of low and high nutrient treatments were 7.2 ± 1.1 g and 5.9 ± 0.5 g, respectively), and there was also no statistical differences in root or shoot biomass between treatments.

3.3. Primary production and metabolism

Dissolved oxygen concentrations measured in the afternoon were 4–20 % higher in the high nutrient treatments until day 15; during the second half of the experiment DO declined in the high nutrient treatments without *M. spicatum*, but remained high in the treatments with both high nutrients and *M. spicatum* (Fig. 1). Nutrient addition, *M. spicatum*, and sampling day all interacted to affect dissolved oxygen concentrations throughout the experiment (RM-ANOVA, Table 2).

The metabolic balance determined as GPP : ER was net autotrophic in all mesocosms on all measurement dates, ranging from 1.3 – 15.4. GPP was 1.8 × higher in high nutrient addition treatments (two-way ANOVA, $F_{1,20} = 80.3$, $p < 0.001$; Fig. 3). Treatments with *M. spicatum* had 1.3 × higher overall GPP than those without *M. spicatum* (two-way ANOVA, $F_{1,20} = 17.3$, $p < 0.001$; Fig. 3). Although nutrient addition had an effect on ER (two-way ANOVA, $F_{1,20} = 33.2$, $p < 0.001$, Fig. 3), there was a significant interaction between this effect and presence of *M. spicatum* (two-way ANOVA, $F_{1,20} = 5.9$, $p = 0.02$), where ER was higher in the treatments with *M. spicatum* with high nutrient additions, but lower in the treatments with *M. spicatum* with low nutrient additions.

3.4. Algal assemblage: biovolume & composition

Over the course of the experiment, mesocosms with high nutrient conditions maintained higher biovolume of planktonic and attached algal taxa suspended in the water column (hereafter total algal biovolume; RM-ANOVA, Table 2). Although no significant differences occurred in total algal biovolume between treatments during days zero and 15 (two-way ANOVA, day zero: $F_{3,8} = 0.59$, p = 0.46; two-way

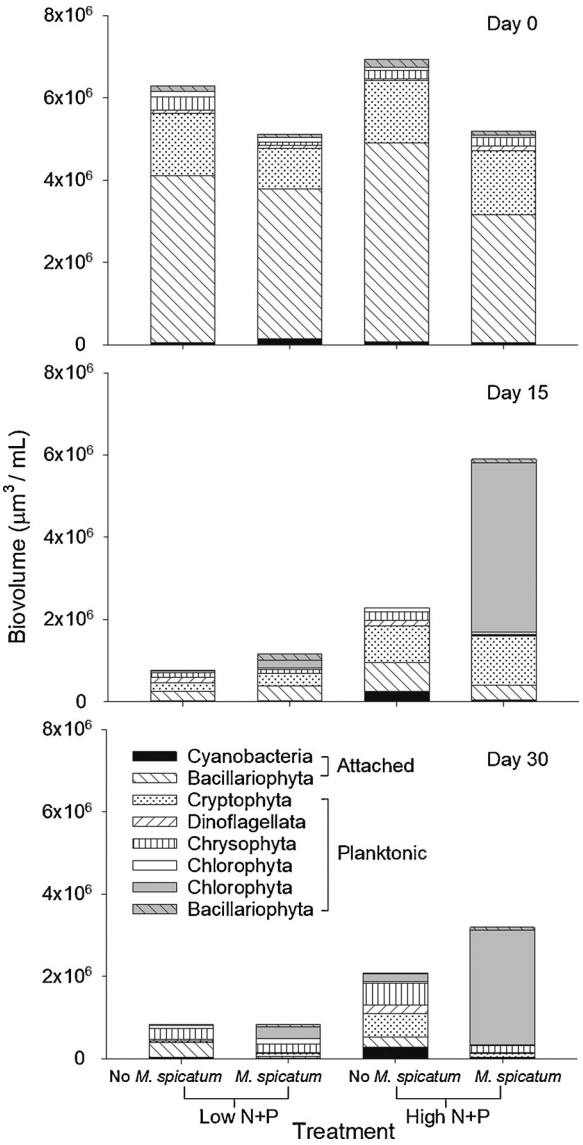

Fig. 4. Mean biovolumes (n = 3 for each bar) of observed taxonomic groups in each mesocosm treatment group on experimental days zero, 15, and 30.

ANOVA, day 15: $F_{3,8} = 2.64$, $p = 0.14$), total algal biovolume was approximately 3 × higher in the high nutrient treatments than the low nutrient treatments on day 30 (two-way ANOVA, day 30: $F_{3,8} = 25.28$, $p = 0.001$), with no significant effect of *M. spicatum* or interaction between the 2 treatments. On day 30, total algal biovolume was similar amongst low nutrient treatments regardless of the presence of *M. spicatum* ($2.78 \times 10^6 \pm 0.65 \times 10^6$ and $2.75 \times 10^6 \pm 0.70 \times 10^6$ µm³ / mL, without and with *M. spicatum* respectively; Fig. 4). In high nutrient treatments, total algal biovolume was slightly higher in treatments with *M. spicatum* ($10.65 \times 10^6 \pm 0.52 \times 10^6$ µm³ / mL; Fig. 4) than those without ($6.95 \times 10^6 \pm 1.62 \times 10^6$ µm³ / mL; Fig. 4).

There were drastic changes in the composition of the algal assemblage over time and between treatments. Large diatoms, such as *Fragilaria*, *Asterionella*, and *Tabellaria* spp. that were present at the start of the experiment were sparse by day 30 (Fig. 4, Table 3). Conversely, we observed an increase in dinoflagellates (*Gymnodinium*, *Dinococcus*, *Peredinium*, and *Ceratium* spp.) through time (Table 3). Attached algae such as *Cladophora* and *Bulbochaete* spp. dominated treatment groups containing high nutrients and *M. spicatum* in days 15 and 30 (70% and 87% of total biovolume, respectively; Fig. 4). Cyanobacteria constituted 11–13% of the total biovolume only in the high nutrient treatments with *M. spicatum*; this biovolume was predominantly composed of *Anabaena* sp., *Cylindrospermum* sp., and *Oscillatoria* sp. (Fig. 4, Table 3).

The non-metric multidimensional scaling ordination had a 2-dimensional solution with a stress of 0.127 and final instability of 0.0 after 48 iterations. The cumulative r^2 was 0.816 with axis 1 explaining the most variation ($r^2 = 0.524$) followed by axis 2 ($r^2 = 0.291$). Total nitrogen ($r^2 = 0.51$, tau = −0.47), conductivity ($r^2 = 0.46$, tau = −0.51), and light transmittance ($r^2 = 0.46$, tau = 0.51) had strong correlations with axis 1, while pH ($r^2 = 0.23$, tau = −0.21) was correlated with axis 2. Of the 36 of genera, *Cryptomonas* ($r = -0.878$) and *Fragilaria* ($r = -0.799$) displayed the strongest negative correlation with axis 1. *Crucigenia* ($r = 0.287$) and *Synura* ($r = 0.258$) were both positively correlated with axis 1 (see S1 for Supporting information). *Oscillatoria* ($r = 0.515$) and *Synedra* ($r = 0.417$) were positively correlated with axis 2, while *Cladophora* ($r = -0.690$) displayed the strongest negative correlation (see S1 for Supporting information).

The successional trajectories of the assemblages that received high nutrient additions were divergent from those with low nutrient additions. Movement in the ordination space indicates assemblages in all treatments moved along axis 1 as the populations of soft-bodied taxa grew and diatoms decreased, but diverged on axis 2. The low nutrient treatments exhibited an assemblage increasingly dominated with *Oscillatoria* through time, whereas the high nutrient treatment had less *Oscillatoria* and more *Cladophora* (see S1 for Supporting information). The successional shifts in both treatments were positively correlated to light transmittance and negatively correlated with nutrients, conductivity, and dissolved oxygen (see S1 for Supporting information). The high nutrient assemblage was more associated with an increase in pH through time than the assemblages in the low nutrient treatments.

4. Discussion

The presence of *M. spicatum* in conjunction with increased nutrients altered aquatic algal assemblages in our experimental mesocosms substantially, both in terms of composition and biovolume. Concentrations of water-column chlorophyll *a*, a proxy for biomass of suspended algae, were consistently higher in high nutrient treatments than low nutrient treatments until day 25 of the experiment, indicating those algal assemblages were bolstered by nutrient additions. Yet by day 30, chlorophyll *a* declined sharply in the high nutrient treatment with *M. spicatum*, decreasing to 57–67 % lower than the low nutrient treatments. This abrupt decline, coinciding with observed increases in attached algae and shifts in the suspended assemblage composition to include typically attached taxa such as *Cladophora* sp., may indicate possible competition between planktonic and attached algae in those treatments. Additions of nutrients also led to an increase in productivity; GPP was 1.8 × higher in high nutrient treatments than low nutrient treatments, and pH was also elevated in those treatments and associated with the presence of attached algae in the NMDS ordination. Together, our results support the controlling role of nutrients on algal assemblages and productivity in aquatic ecosystems, but suggest that macrophytes can play an important role in shifting productivity between water column and the benthos.

Nutrient enrichment clearly served as a primary control on productivity in our experiment, stimulating biomass of planktonic and attached algae and thus stimulating rates of primary production. The role of nutrients for controlling biomass and rates of primary production has long been understood by aquatic ecologists (e.g., Vollenweider, 1968), but our study highlights the important role of attached algae for responding to nutrient enrichment. Even though the nutrient loads added to the "high nutrient" treatments in our experiment are more typical of oligotrophic to mesotrophic lake conditions (e.g., Vollenweider, 1968), we observed attached algal AFDM as much as 11 × higher in high nutrient treatments than in low nutrient treatments. Attached algae may be particularly important contributors to GPP, particularly at low nutrient conditions like those in our experiment

Table 3
List of algal taxa observed on experimental days zero, 15, and 30. The number of plus symbols is indicative of the proportion of sample biovolume contributed by any given taxa: + represents taxa contributing less than 5% of biovolume, + + represents taxa contributing 5–20% of biovolume, and + + + represents taxa contributing greater than 20% of the total biovolume. Blank cells indicate that the taxa was not observed in a treatment on that day.

		Treatment					
		No *M. spicatum*			*M. spicatum*		
	Taxa	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
Attached *Bacillariophyta*	*Achnanthidium* Kützing, 1834	+	+	+	+	+	+
	Cocconeis Ehrenberg, 1838	+	+		+	+	+
	Cymbella Agardh, 1830	+				+	
	Gomphonema Agardh, 1824	+	+	+	+	+	+
Planktonic *Bacillariophyta*	*Asterionella* Hassall, 1850	+	+		+		
	Cyclotella Kützing, 1834	+ +	+	+	+ +	+	+
	Fragilaria Lyngbye, 1819	+ + +	+ +	+ +	+ + +	+ +	+
	Melosira Agardh, 1824	+					
	Navicula Bory, 1822	+	+	+	+		
	Nitzschia Hassall, 1845	+					+
	Staurosira Ehrenberg, 1843	+		+	+		
	Synedra Ehrenberg, 1830	+	+ +	+ +	+	+	+
	Tabellaria Ehrenberg, 1840	+			+		
Attached *Chlorophyta*	*Bulbochaete* Agardh, 1817					+	+
	Cladophora Kützing, 1843			+ +		+ + +	+ + +
Planktonic *Chlorophyta*	*Closterium* Nitzsch, 1817		+		+	+	+
	Cosmarium Corda, 1834						+
	Crucigenia Morren, 1830			+	+	+	+
	Scenedesmus Meyen, 1829	+	+	+	+	+	+
	Unidentified Green	+	+	+	+	+	+
Chrysophyta	*Dinobryon* Ehrenberg, 1835	+			+		
	Mallomonas Perty, 1852	+	+ +	+ + +	+	+	+ +
	Synura Ehrenberg, 1838	+	+	+	+	+	+
Cryptophyta	*Cryptomonas* Ehrenberg, 1831	+ + +	+ + +	+ + +	+ + +	+ +	+
	Rhodomonas Karsten, 1898	+	+	+	+	+	+
Cyanobacteria	*Anabaena* Bory, 1822	+	+ +		+	+	
	Aphanocapsa Nägeli, 1849	+	+	+	+	+	+
	Chroococcus Nägeli, 1849			+			+
	Cylindrospermum Kützing, 1843		+	+ +		+	
	Merismopedia Meyen, 1839			+			
	Microcystis Kützing, 1833	+			+		
	Oscillatoria Vaucher, 1803	+	+	+	+	+	+

(*continued on next page*)

Table 3 (*continued*)

		Treatment					
		No *M. spicatum*			*M. spicatum*		
	Taxa	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
Dinoflagellata	*Ceratium* Schrank, 1793		+				
	Dinococcus Fott, 1960		+	+			+
	Gymnodinium Stein, 1883	+	+	+ +	+	+	+
	Peridinium Ehrenberg, 1830		+	+			

(Vadeboncoeur et al., 2001; Brothers et al., 2013).

Contrary to our predictions, no significant change in the biomass of *M. spicatum* occurred in either high or low nutrient treatments over the course of the experiment. This may be in part due to the short duration or timing of our experiment, which occurred later in the growing season when macrophytes typically begin to senesce (Smith and Barko, 1990). Alternately, *M. spicatum* may have suffered nutrient limitation due to our low overall nutrient loads, and the fact that we added nutrients to the water column instead of sediments. As *M. spicatum* may preferentially use nutrients from sediments under natural conditions (Barker and James, 1998), this could have amplified competition with attached algae in our experimental mesocosms, although we cannot specifically evaluate the strength of this interaction in the context of this experiment. We added nutrients repeatedly during the experiment (day zero, 10 and 20) because we observed that NH_4 concentrations were drawn down very quickly in the high nutrient mesocosms; this means that the effect of day in our study was affected by the length of time since the most recent nutrient addition (e.g., day 15 is 5 days after a nutrient addition, while day 30 is 10 days after). This lag effect of nutrient enrichment may have affected the responses of algal biomass and assemblages we observed through time, although would have similar effects on our comparisons among nutrient and macrophyte treatments.

Although we observed a stronger effect of nutrients than *M. spicatum* on algal assemblage biomass, there were notable compositional shifts coincident with the presence of *M. spicatum*. Growth of attached algal taxa (e.g., *Cladophora* sp. and *Bulbochaete* sp.) was facilitated by the presence of *M. spicatum*, likely because the plants provided surface area for attachment (Dibble et al., 1996; Ferreiro et al., 2013). The abundance of attached algal taxa may act to further increase surface area for other epiphytes (Power et al., 2009). In addition, cyanobacteria (e.g., *Anabaena* sp., *Cylindrospermum* sp., and *Merismopedia* sp.) composed 11–13% of the primary producer biovolume in the high nutrient treatments lacking *M. spicatum*, but were effectively absent in treatments that did contain *M. spicatum*. In another mesocosm experiment, Švanys et al. (2014) observed that the presence of *M. spicatum* inhibited the biomass of cyanobacteria more consistently than other planktonic taxa. Gross et al. (1996) displayed that through the release of allelopathic polyphenols, *M. spicatum* exhibited high algicidal activity towards several species of cyanobacteria (e.g., *Anabaena*, *Synechococcus*, *Synechocystis,* and *Trichormus* spp.); similarly, Nakai et al. (2000) described suppression of *Microcystis aeruginosa* as the result of four growth-inhibiting polyphenols released by *M. spicatum*. Körner and Nicklisch (2002) also report growth inhibition of *Microcystis aeruginosa* in the presence of *M. spicatum*. Further, Trochine et al. (2010) describe the potential for filamentous green algae to allelopathically suppress the growth of phytoplankton. Thus, we postulate that the shifts in algal assemblages we observed over the course of this experiment could be due to allelopathic interactions between cyanobacteria and *M. spicatum* or among algal taxa.

The artificial conditions in our mesocosms may have facilitated the increase of attached algae by excluding natural ecological feedback mechanisms such as water turbulence, invertebrate grazing, and shading. The degree of wave action in a natural system can influence the dynamic between macrophytes and their epiphytes; while macrophytes tend to thrive in areas of intermediate wave exposure, Strand and Weisner (1996) showed that epiphyte production is highest in sheltered locations. Although we did not examine whether there were benthic invertebrates in our mesocosms, we did qualitatively observe zooplankton in grab samples collected from all mesocosms at the end of the experiment and found a diverse assemblage of cladocerans (primarily *Ceriodaphnia* and *Bosmina* spp.) and copepods, including a large number of nauplii. There may also have been limitations on natural pelagic-benthic interactions in our experiment. For example, research by Vadeboncoeur et al. (2001) indicated that compensatory declines in benthic (including attached and epiphytic) algal taxa because of shading by pelagic algal assemblages occurred in response to nutrient additions to the water column at a whole-lake scale. Because our mesocosms were shallow, however, the water column was not deep enough to permit effective pelagic shading, enabling attached taxa to thrive while suppressing planktonic taxa. In natural littoral zones, the degree to which algal productivity will be partitioned between attached and suspended algae will be determined by all of these factors, along with the primary controls of nutrient availability and physical structure provided by macrophytes examined in our experiment.

We posit that the impact of a non-native macrophyte invasion on algal assemblage biomass and composition is largely dependent on whether the plant is colonizing previously uncolonized substrate or merely replacing existing native macrophytes. When invasive macrophytes replace native macrophytes but do not increase available colonization space for attached algae, there may not be a large effect on the algal assemblage or whole-system productivity. In other words, restructuring of the macrophyte assemblage may not necessarily result in restructuring of the algal assemblage. If the macrophytes vary substantially in their structural complexity, an effect on attached assemblages might be expected, but in general there does not seem to be a difference in complexity between native and non-native taxa (Schultz and Dibble, 2012), and recent findings by Grutters et al. (2017b) indicate that attached algal assemblages do not tend to vary between native and non-native macrophyte hosts. But, if an invasive macrophyte is establishing in an area where macrophyte biomass was previously low or even absent, then the invasion may serve to shift productivity from the water column to the benthos by favoring the growth of attached algae.

The results of our experiment demonstrated that macrophytes may play an important role in controlling littoral zone responses to nutrient enrichment by facilitating the growth of attached algae. In turn, this may lead to a decline in pelagic algae, facilitating water clarity and shifting productivity from the water column to the benthos, as has been demonstrated in studies of alternate stable states in lakes (Scheffer et al., 1993; Vadeboncoeur et al., 2001; Bakker et al., 2010). Nutrient enrichment may be key for controlling the establishment of *M. spicatum*

and rates of overall ecosystem productivity (e.g., Vollenweider, 1968; Madsen, 1998), but the results of our experiments suggest that which algal taxa carry out that productivity and whether it occurs in the water column or associated with the benthos is controlled by the presence of the plant itself. We posit that the potential for *M. spicatum* invasion to shift productivity in this way is greatest when it invades an area where macrophytes were not previously established, thereby providing a novel physical habitat for establishment of attached algae.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.aquabot.2019.03.003.

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