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Shifts in algal dominance in freshwater experimental ponds across differing levels of macrophytes and nutrients

Josephine C. Iacarella,[1,4,]† Jennifer L. Barrow,[1] Alessandra Giani,[2] Beatrix E. Beisner,[3] and Irene Gregory-Eaves[1]

[1]*Department of Biology, McGill University, 1205 Docteur Penfield Avenue, Montreal, Quebec H3A 1B1 Canada*
[2]*Department of Botany, Universidade Federal de Minas Gerais, 6627 Av. Pres. Antônio Carlos, Belo Horizonte 31270-901 Brazil*
[3]*Department of Biology, University of Quebec at Montreal, 405 Rue Sainte-Catherine E., Montreal, Quebec H2L 2C4 Canada*

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Abstract. Excess nutrient loading into ponds and shallow lakes can lead to undesirable algal growth and a shift to a turbid state. Previous work has suggested that such an ecosystem transition may be mediated by the biotic constituents of the habitat and food web; however, earlier experiments have been conducted at coarse temporal resolution and have typically used a single initial density of macrophytes, a key structural component of ponds and shallow lakes. To address these gaps, we tested the hypotheses that experimental ponds with lower macrophyte densities and more rapid increases in nutrient loading would shift to phytoplankton dominance, whereas higher macrophyte densities and slower, lower concentration nutrient inputs would maintain a clear state. Ponds containing plankton and juvenile fish were assigned to treatments with none, low, or high macrophyte densities, and weekly, high or biweekly (i.e., fortnightly), low nutrient inputs. Using additive mixed-effects models, we demonstrated that temporal trajectories of phytoplankton biomass were explained by macrophyte density in interaction with biomass of important zooplankton grazers (Bosminidae, Sididae, and Daphniidae), as well as with pH and time. Phytoplankton biomass followed a convex unimodal trajectory in ponds with no or low macrophytes (muted in the latter), and minimal increases in high macrophyte treatments. Declines in phytoplankton attributable to top-down control likely freed resources for periphyton and metaphyton, which subsequently became abundant in ponds without and with macrophytes, respectively. Our results demonstrate that high densities of macrophytes, combined with herbivory and competition for light between phytoplankton and metaphyton, enhance resilience of the clear water state to the undesirable effects associated with eutrophication.

Key words: chlorophyll-*a;* experimental pond; macrophytes; nutrients; phytoplankton; zooplankton.

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[4] Present address: Fisheries and Oceans Canada, Institute of Ocean Sciences, 9860 W. Saanich Rd., Sidney, BC V8L 4B2 Canada.

† **E-mail:** josie.iacarella@gmail.com

Introduction

Sudden, ecosystem-wide shifts in response to modest changes in the environment (i.e., regime changes) have captured the interest of many researchers and managers over the past few decades. Ponds and shallow lakes are ideal model systems for studying regime changes (Scheffer et al. 2009) and have been the focus of concerted study since the publication of seminal works (Phillips et al. 1978, Scheffer et al. 1993). Clear progress has been made in predicting transitions using high-resolution time series (Scheffer et al. 2009, Carpenter et al. 2011, Dakos et al. 2014), and theory explaining such transitions is well developed (Scheffer et al. 1993, Dent et al. 2002, Scheffer and

Carpenter 2003). However, a recent review has demonstrated that despite a wealth of freshwater studies on the topic (i.e., 135 papers identified since 1967), relatively few empirical field surveys either identify a regime change or examine the mechanisms that mediate such change (Capon et al. 2015). Many pond experiments have also been conducted, but often these studies are performed at coarse temporal resolution (i.e., phytoplankton samples collected once every two weeks or less frequently) and typically do not consider in their experimental designs the importance of submerged macrophytes densities—key structural elements of shallow ecosystems (McKee et al. 2003, Feuchtmayr et al. 2009, Olsen et al. 2015).

Field survey and experimental studies have nonetheless resulted in important advancements in our understanding of the mechanisms that facilitate regime changes in ponds and shallow lakes. Positive feedback loops reinforce macrophyte-dominated states via mechanisms that maintain water clarity, including provision of refuges for zooplankton, reduction in nutrient availability and particle suspension, and release of allelopathic chemicals (Phillips et al. 1978, Scheffer et al. 1993). However, state transitions may occur when a combination of insufficient invertebrate grazer control and excess nutrient inputs leads to an overgrowth of periphyton, subsequent declines in macrophytes, and growth of phytoplankton (Phillips et al. 1978, Jones and Sayer 2003). Once established, the turbid state tends to be maintained despite external nutrient load reductions because of new positive feedbacks including the resuspension of nutrient-rich sediments in the absence of macrophytes (Scheffer et al. 1993). Turbid states are associated with reduced ecosystem functions and services compared to clear water ponds and shallow lakes, the extent of which is not yet fully understood (Hilt et al. 2017).

Transitions from clear to turbid water states are often attributed to nutrient loading, although the response of phytoplankton biomass appears to depend on food web composition (Jeppesen et al. 1999, Beisner et al. 2003). For example, when benthic and pelagic invertebrates also responded positively to nutrient additions, a macrophyte-dominated state was maintained (McKee et al. 2003). Subsequent studies have not consistently detected a transition from macrophyte to phytoplankton dominance either, owing to food web interactions. Feuchtmayr et al. (2009) applied high levels of nutrient loading to clear water experimental ponds and found that the submerged plant community remained intact, in part owing to high gastropod abundance following fish declines. More recently, Olsen et al. (2015) conducted a similar experiment and did detect a transition to phytoplankton dominance after a prolonged exposure to high nitrogen loading whereby filamentous algae played a role in shading out macrophyte growth. Invertebrate grazer control on phytoplankton biomass may vary along gradients of macrophyte densities. Increasing macrophyte cover can enable augmented zooplankton biomass through provision of refuges at low to intermediate planktivorous fish densities; however, low macrophyte cover or high fish densities reduce the effectiveness of such refuges (Schriver et al. 1995). Thus, greater water clarity is associated with reductions in planktivorous fish through trophic cascades and with reductions in benthivorous fish because of less sediment and nutrient resuspension (Meijer et al. 1999, Nolby et al. 2015). In the absence of strong fish predation, or potentially in the presence of dense macrophytes, grazer invertebrates can suppress phytoplankton (Bakker et al. 2010) and periphyton growth (Jones and Sayer 2003, Feuchtmayr et al. 2009).

Disentangling the effects of food web interactions and nutrient inputs on phytoplankton dominance has been an ongoing challenge in both freshwater (Beisner et al. 2003, Jones and Sayer 2003, Feuchtmayr et al. 2009, Kratina et al. 2012) and marine ecosystems (Heck and Valentine 2007, Eriksson et al. 2009, Hughes et al. 2013, Duffy et al. 2015). Clearly, a better understanding of these top-down and bottom-up processes, and the levels at which they begin to cause ecosystem transitions, would help guide management of food web alteration (e.g., fishing) and nutrient inputs. We designed a mesocosm experiment to assess the effect of macrophyte density and nutrient loading on phytoplankton biomass. In particular, we were interested in quantifying the contribution of macrophytes, grazers, nutrient loading, and their interactions as drivers of phytoplankton dynamics in experimental ponds. Phytoplankton responses to nutrient additions in mesocosms, ranging from two orders of magnitude smaller and three orders larger than ours,

revealed weak and idiosyncratic relationships with water volume, supporting the use of experimental ponds to make larger scale inferences to natural ponds and shallow lakes (Spivak et al. 2011). Our ponds had one of three levels of macrophyte densities and contained phytoplankton, zooplankton, and zooplanktivorous fish. To each pond, we applied press nutrient loading at either a weekly or biweekly (i.e., fortnightly) frequency, such that the two nutrient treatments had progressively disparate concentrations. To identify high-frequency changes in phytoplankton biomass, we measured total chlorophyll-*a* daily throughout the experiment. We predicted a shift toward phytoplankton dominance in ponds with no and low macrophyte treatments receiving weekly nutrient addition, as we hypothesized that the positive feedback effects of macrophytes and zooplankton would be absent or insufficient in these ponds. Conversely, ponds with high macrophyte treatments and biweekly nutrient inputs were hypothesized to maintain a clear state owing to competitive effects of macrophytes, more zooplankton refuge, and limiting nutrient resources.

Methods

Experimental ponds were established to assess the influence of macrophyte abundance and nutrient input on phytoplankton biomass. Treatments consisted of three levels of macrophyte density (none, low, and high) and two levels of press nutrient loading (weekly, high concentrations and biweekly, low concentrations) with three replicates each; all ponds contained one zooplanktivorous fish individual. The 18 ponds consisted of 568 L (1.47 m long, 0.99 m high, 0.64 m wide) black Rubbermaid stock tanks arranged in a line, with a random assignment of macrophyte treatment levels. Nutrient levels were assigned based on pre-experiment chlorophyll-*a* and total zooplankton measurements in an effort to begin with equivalent plankton concentrations across treatments. All ponds were shaded with high-density polyethylene agricultural cloth that provided 60% shade; this emulated tree cover of ponds and shallow lakes in our focal region, the Mixedwood Plains, and minimized high water temperatures in an area that otherwise had direct sunlight. The experiment was conducted for 83 d from 2 July to 22 September 2015.

Experimental pond setup

To begin the experimental setup, the ponds were filled with 350 L of tap water and allowed to dechlorinate for one week. Sediment, phytoplankton, and zooplankton were then collected from Lake Gale, Quebec (49.08, −78.51) throughout May and June 2015 to seed the ponds. Sediment was collected with bottom grab samplers, sieved over 2 mm mesh, and then 3 L was placed each into 90, 6-L plastic tubs (30.5 × 16.5 × 11.5 cm). Five plastic tubs with sediment were lowered into each pond for a total of 15 L of sediment per pond. Phytoplankton were collected by filtering 10 L of lake water per pond to retain a size range of 36–63 μm, and zooplankton were collected by taking vertical hauls using Wisconsin 80 μm mesh nets. Macrophytes, *Elodea canadensis*, purchased from an aquarium supplier were arranged in bundles of three and weighed down into the sediment tubs with two or eight bundles per tub, and five tubs per pond, for a total of 30 (3 stems × 2 bundles × 5 tubs) and 120 plants (3 stems × 8 bundles × 5 tubs) in the low and high macrophyte treatments, respectively. Macrophyte cover was measured as percent volume infested (PVI) using ImageJ (Schneider et al. 2012) following methods by Schriver et al. (1995). Initial cover was 22.08% ± 1.59% (±1 SE) in low macrophyte ponds and 45.69% ± 1.49% in high macrophyte ponds (see Appendix S1: Fig. S1 for photographs of pond treatments). Macrophyte densities were chosen to approximate cover at and above the threshold (15–20%) that can significantly reduce zooplankton predation by fish (Schriver et al. 1995).

The sediment, phytoplankton, and zooplankton were added in succession to allow for some growth of the populations before beginning the experiment. Phytoplankton and macrophytes were added to the ponds approximately one week after the sediment, and zooplankton were added three and five weeks later. Water was also mixed between the ponds (4 L from each pond combined and redistributed) prior to adding the zooplankton and four days before the experiment began to maintain similar starting concentrations of plankton.

Individual juvenile rock bass, *Ambloplites rupestris*, were added to each pond as a common invertebrate predator (George and Hadley 1979). Fish were collected from Lake Hertel, Quebec (45.55, −73.15), held for two days to ensure their

health, and then added to the ponds two weeks after the initial zooplankton addition. The use of fish in the experiments was approved by McGill University's Animal Care Committee (Protocol no. 2015-7689), and a permit for collection was obtained from the government of Quebec. No fish died or appeared unhealthy during the experiments. The day fish were added marked the first day of the experiment.

Nutrient addition

Phosphorus (P) and nitrogen (N) were initially added to all ponds two days prior to the first day of the experiment. All subsequent additions were made weekly or biweekly. Solutions of KH_2PO_4 and $NaNO_3$ were dissolved in 200 mL of deionized water and stirred into the ponds; the same volume of deionized water was added to the biweekly treatments on weeks when nutrients were not added. The weekly nutrient load consisted of 0.0154 g KH_2PO_4 and 0.4247 g $NaNO_3$, equivalent to adding 10 and 200 μg/L of P and N, respectively. The biweekly nutrient load was selected based on a progressively increasing ratio of weekly : biweekly nutrient inputs, with an approximate starting concentration of 10 μg/L P and 200 μg/L N in all ponds: 1:1 to start, 2.5:1 after 55 d, and 2.9:1 at the end of the experiment after 83 d.

Data collection

Variables were measured either daily or weekly in the experimental ponds. Daily measures included total chlorophyll-*a* concentration (chl*a*), dissolved oxygen, specific conductivity, and pH; temperature loggers (Onset HOBO) were kept in each pond for continuous measurements. Prior to daily measurements, the sides of the ponds were scraped to remove any algal wall growth to minimize the effect of this artificial vertical surface; this growth was not removed from the ponds, but was very minimal and often not visible. The water in the ponds was then gently mixed to evenly distribute phytoplankton for sampling while avoiding sediment resuspension. Then, three, 1 L samples were taken at different locations within the pond, combined, and mixed for chl*a*. A 30 mL subsample was collected in a dark bottle and kept in a cooler for 15 min, following which total chl*a* was estimated using a laboratory-based BBE Fluoroprobe (bbe Moldaenke GmbH, Schwentinental, Germany). Subsamples used for estimating yellow substances were also collected weekly. These were filtered using a 0.45-μm PES-membrane syringe filter and measured on the Fluoroprobe; yellow substance values were used to correct all chl*a* measures for different levels of yellow substances in each pond, and over time.

Additional estimates of phytoplankton biomass were made to verify the Fluoroprobe total chl*a* estimates, as well as to determine biomass changes in metaphyton (i.e., filamentous green algae). Periphyton found growing on the surface sediments was only assessed at the end of the experiment because of the difficulty in sampling without mixing sediments and dispersing periphyton into the water column (see *Methods* below). Phytoplankton and metaphyton communities were analyzed only in the weekly nutrient addition treatment (on experimental days 1, 20, 27, 41, 62, and 76) to provide estimates of the maximal growth responses. Water samples were collected on a weekly basis using the same sampling method described for chl*a* (i.e., taken from 3 × 1 L bottles of water, combined and mixed), except that 250 mL subsamples were collected in amber glass bottles and preserved with Lugol's iodine (1% final concentration). For phytoplankton and metaphyton quantification, samples were analyzed under a Leica DM2500 compound microscope (Leica Microsystems, Wetzlar, Germany), using modified settling chambers (Hamilton et al. 2001). A minimum of 400 phytoplankton taxonomic units were counted for each sample. Algal biovolume was calculated based on single cell measurements made on six individuals of each taxa separately for each macrophyte treatment, with the exception of four rare taxa in which the median measurement was selected across macrophyte treatments (Hillebrand et al. 1999, Sun and Liu 2003). Phytoplankton taxa were assigned to one of four pigment groups based on the structure of the peripheral antennae viewed under the microscope (Longhi and Beisner 2010): greens (chl*a*, chl*b*, xanthophyll), cyanobacteria (phycocyanin), golden-browns (chl*a*, chl*c*, xanthophyll [fucoxanthin or peridinin]), or mixed (chl*a*, chl*c*, phycoerythrin). The proportion of these pigment groups in a sample were then calculated ("FD" in R; Laliberté et al. 2014). For samples with dense mats of metaphyton, we applied sonication to the subsample prior to pouring it into the settling chamber with the aim to break up long

chains of metaphyton and allow for more accurate cell counting. Samples were sonicated at 25% amplitude for 30–60 s.

Zooplankton were collected separately using the same 3 × 1 L sampling method, with the sampled water then filtered over a 63 μm mesh. Zooplankton on the filter were either counted live (during the first two full weeks of the experiment, followed by every other week) and returned to the ponds or were preserved for later counting (the first experiment day, and every other week starting the third week); all filtered water was returned to the ponds. To preserve zooplankton, filters were placed in carbonated water, then 95% ethanol, and lastly 70% ethanol for 15 s each, with final preservation in 70% ethanol (Black and Dodson 2011). Zooplankton counts were conducted on a grid plate (six rows and columns) so that the plate could be subsampled when densities of a single zooplankton group were too high to ensure accurate counts. Following the first week, biweekly live counts were subsampled so that if 100 or more individuals of a group were counted from grid rows 1 and 3, or 1, 3, and 5, then that group would no longer be counted (i.e., the count would be multiplied based on the number of remaining rows for an estimated total), whereas the rest of the groups would be counted for the remainder of the plate. Zooplankton were identified as Bosminidae, Sididae, *Ceriodaphnia* spp., *Daphnia* spp., *Scapholeberis* spp., Chydoridae, and Copepoda adults. Counts were transformed to estimates of biomass (μg) per liter using published length-weight regressions and measured lengths on a minimum of 30 individuals (Appendix S1: Table S1).

Weekly water samples were taken for total phosphorus (TP) and nitrogen (TN; μg/L) analysis at the same time and using the same 3 L of collected water as for the weekly algal samples. Samples were stored in acid-washed bottles that had been triple rinsed with deionized water. Samples were analyzed by the GRIL—University of Quebec at Montreal Aquatic Analytical Laboratory (GRIL—UQAM, Montreal, Quebec, Canada). Total phosphorus was measured spectrophotometrically by the molybdenum blue method after persulfate digestion (Griesbach and Peters 1991), and TN was measured as nitrate after persulfate digestion on an autoanalyzer (ALPKEM FS100; OI Analytical, College Station, TX, USA). Total dissolved phosphorus and nitrogen (μg/L) were also analyzed from water samples taken the week before final experiment shutdown to determine phosphorus and nitrogen concentrations in the water, exclusive of phytoplankton and zooplankton.

We terminated the experiment in September 2015, when the water temperatures dropped below 16°C—with an average of 17.9°C during the last week, and 21.1° and 20.4°C in the previous two weeks—to avoid loss of algal biomass before final measurements could be made. At the end of the experiment, all macrophytes were removed and metaphyton growing on the macrophytes were collected so that both macrophytes and metaphyton could be weighed (total wet and dry weight per pond, respectively). Three researchers then independently estimated periphyton cover (%; Griggs et al. 2015) for each of the sediment tubs located in ponds with no macrophytes; no periphyton was observed on sediments in the low and high macrophyte treatments. Periphytic algal species were identified by creating wet mounts, and then, relative abundances of different taxa were visually estimated under the microscope at 400×. Finally, the standard lengths of the fish were measured (46.4 ± 0.5 mm, $n = 18$) on the last day of the experiment. Final macrophyte cover (PVI) was 54.22% ± 4.31% and 70.76% ± 5.56% in low and high macrophyte ponds, respectively, representing an average overall increase of 28.6% ± 3.8% (across treatments). Fish also grew over the course of the experiment by 29.5% ± 1.3%. Change in macrophyte cover and fish growth was not significantly different across treatments (Appendix S2: Fig. S1).

Statistical analyses

Phytoplankton chlorophyll-*a* (measured with the Fluoroprobe in μg/L) and zooplankton biomasses (μg) per liter were analyzed over the first 55 d of the experiment. From day 55 to 83, phytoplankton samples were too contaminated with metaphyton to obtain accurate Fluoroprobe estimates, as determined by our visual observations of floating strands of metaphyton in the water samples. As such, we could not use the Fluoroprobe measurements to isolate the phytoplankton chlorophyll-*a* from that associated with the metaphyton. Phytoplankton and metaphyton cell counts were continued through day 76, but were used solely for qualitative comparison owing to

the lower sample size. Biomasses of *Ceriodaphnia*, *Daphnia*, and *Scapholeberis* were combined for the analyses as they have the same functional characteristics (i.e., filtration type and habitat preferences; Barnett et al. 2013) and are often preferred prey items of zooplanktivorous fishes (Schindler 2006). Other zooplankton groups were treated separately in the analysis as they have different functional characteristics (Barnett et al. 2013) and tended to respond differently to treatments over time. Zooplankton biomass estimates were $\log_{10}(x + 1)$-transformed, and corresponding weekly measures of total chl*a* were $\log_{10}$-transformed to improve normality. All continuous predictor variables, including zooplankton biomass estimates, were then grand mean centered to improve comparability of variables.

Plankton biomass—model selection

Additive mixed-effects models (AMMs) estimated with penalized thin plate regression splines ("mgcv" in R; Wood 2004) were used to assess the effect of treatments and covariates on weekly measures of total chl*a* biomass. A full AMM was first run with linear fixed effects of macrophyte density (none, low, high), nutrient level (weekly, biweekly), average daily temperature, pH, and measures of zooplankton biomass separated into five taxonomic categories (Bosminidae, Sididae, Daphniidae, Chydoridae, and Copepoda). Interactions were included between each covariate and categorical factors of macrophytes and nutrients, excluding three-way interactions. Dissolved oxygen and conductivity were not included in the model as they were both strongly correlated with pH ($r = 0.80$). A nonlinear term to represent time (experiment day) was included in all AMMs, with smooth interactions estimated for each combined level of macrophytes and nutrients in initial full models. All mixed-effects models included a random effect of experimental pond to account for repeated measures.

Backward stepwise multiple regression with analysis of variance likelihood ratio tests were initially used to determine variable retention. Model terms were excluded if they did not significantly improve the model fit ($P < 0.05$). Following this exclusion procedure, we used Akaike information criterion for small sample sizes (AIC_c; Bolker et al. 2009, "MuMIn" in R; Bartoń 2015) to compare models with all remaining combinations of linear terms and their interactions; all model comparisons for this step included the nonlinear term experiment day with smooth fit for each macrophyte level. Backward stepwise selection was initially used to reduce the large set of model parameters, whereas AIC_c comparisons were used following model reduction because all remaining terms showed significant model improvement, but some had a small effect on the AIC_c value ($\Delta < 3$). Post hoc analyses of single interaction term AMMs were conducted to test the significance of each factor level.

Other experimental pond constituents—model selection

Final periphyton cover and metaphyton dry weight were compared statistically across macrophyte and nutrient treatments to further assess all forms of algal growth in the ponds (i.e., maximum likelihood [ML] linear mixed-effects model [LME] for periphyton ["lme4" in R; Bates et al. 2015], and ML linear fixed-effects model for metaphyton dry weight with one measure per pond). Total phosphorus and nitrogen were also compared across treatments to determine whether the concentration of nutrients in the ponds was affected by the presence and growth of macrophytes, periphyton, and metaphyton over time; a preliminary AMM showed no significant nonlinear fits, so LME was used instead. Initial full models contained all possible interactions, and terms were reduced using backward stepwise multiple regression; best fit models were verified with AIC_c model comparisons, as described previously. All analyses were done in R (R Core Team 2015).

Results

Phytoplankton biomass followed a unimodal trajectory over time, with the highest biomass occurring in the no macrophyte, weekly nutrient treatment, and peaking after approximately 28 d of nutrient addition (Figs. 1, 2a). A similar, but lower magnitude pattern was observed in ponds with low macrophytes, whereas phytoplankton remained consistently very low in ponds with high macrophytes. Phytoplankton standing stock (based on Fluoroprobe total chl*a*) also tended to be higher in weekly nutrient treatments than in biweekly treatments. Many of the zooplankton groups increased over time, reaching highest

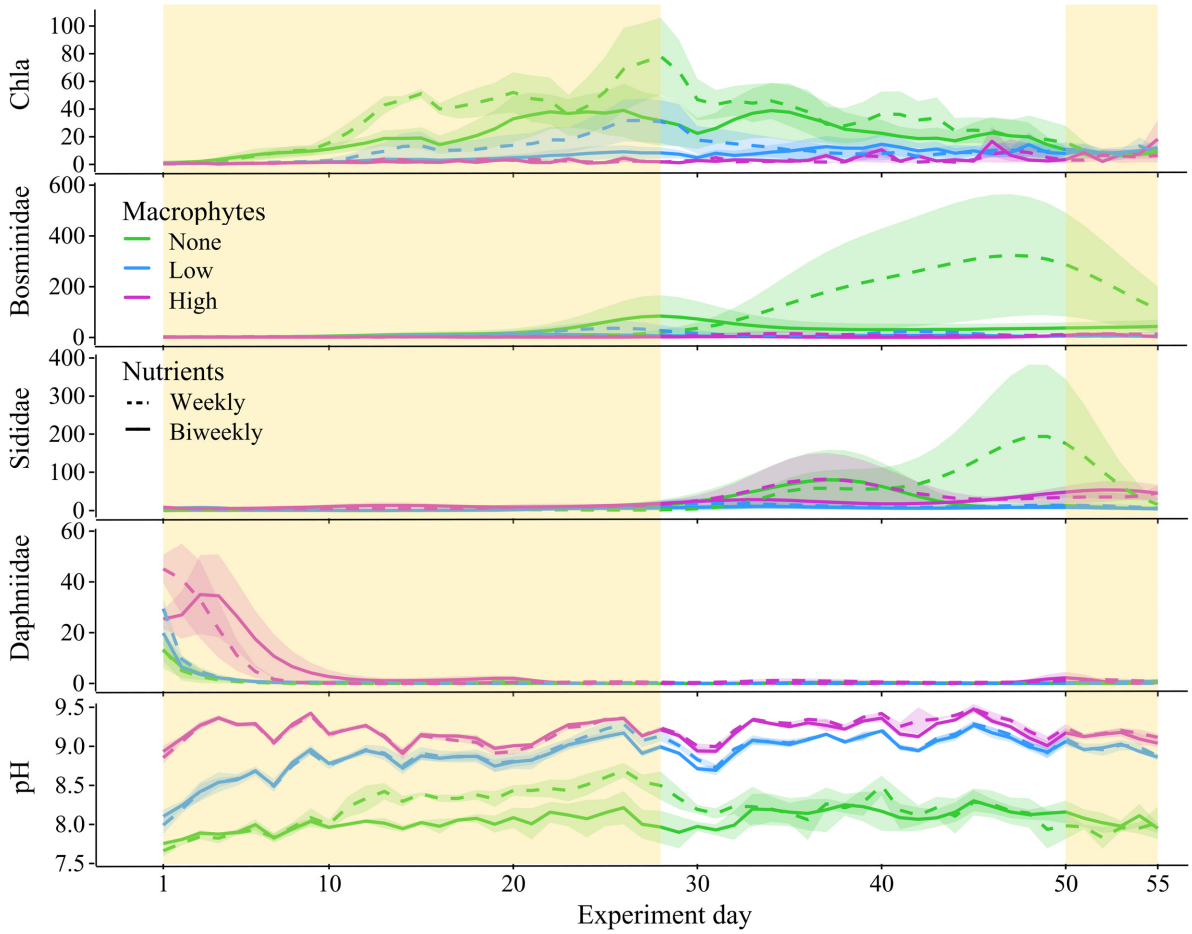

Fig. 1. Experimental ponds with different macrophyte densities (none [green], low [blue], high [pink]) and nutrient inputs (weekly [dashed line], biweekly [solid line]) showed phases (orange/white shading) of dominance transitioning from phytoplankton–zooplankton–periphyton/metaphyton. Phytoplankton (Fluoroprobe measure of total chlorophyll-*a* [chl*a*], μg/L) and pH were measured daily, and zooplankton biomasses (μg) per liter were interpolated from weekly counts for visualization. Shaded areas around mean fit lines are ±1 SE. Note different *y*-axis scales.

biomasses between days 28 and 50 and showing a tendency to decline afterward. Both Bosminidae and Sididae increased primarily in the no macrophyte, weekly nutrient treatment. Daphniidae was the only group that did not increase over time (Fig. 1); all treatments had very low Daphniidae levels after 10 experimental days, although their biomasses were maintained for several days longer in the high macrophyte treatments. Finally, pH increased with macrophyte density, but showed little difference through time or between nutrient treatments (Fig. 1).

Phytoplankton biomass, measured as Fluoroprobe total chlorophyll-*a*, was best explained by interactions of macrophyte treatment with biomass estimates of Bosminidae, Sididae, and Daphniidae, as well as with pH and experiment day (best fit model, adj. $r^2 = 0.80$; Fig. 3; see Appendix S2: Table S1 for model comparisons). Weekly vs. biweekly nutrient addition was not found to significantly affect phytoplankton biomass (likelihood ratio comparisons, $P > 0.05$), although there were a few brief periods where the standard error bars between the weekly and biweekly treatments from the same macrophyte group did not overlap (e.g., between days 12 and 20 in the no macrophyte ponds and between days 25 and 30 in the low macrophyte ponds; Fig. 1). Phytoplankton declined with increasing zooplankton biomass; significant negative relationships were particularly

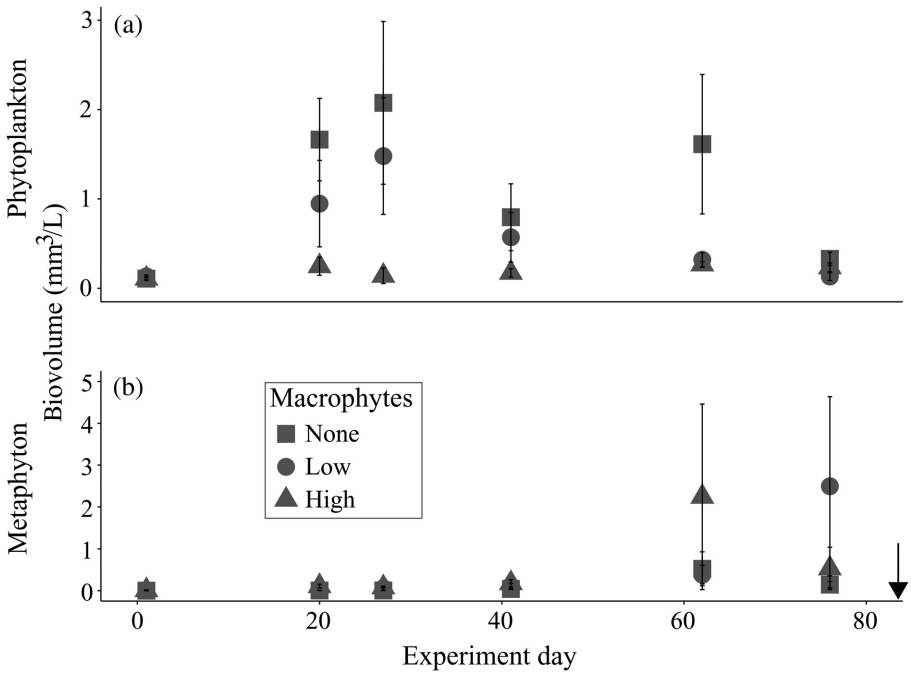

Fig. 2. Phytoplankton (a) and metaphyton biovolume (mm^3/L) (b) estimates measured over the course of the experiment from ponds with different macrophyte densities (none, low, and high) and treated weekly with nutrient inputs. Error bars are mean ± 1 SE. The arrow at 83 d indicates when samples were collected to quantify metaphyton dry mass.

consistent for Bosminidae ($t = -2.08$, $P = 0.04$), Sididae ($t = -3.43$, $P < 0.001$), and Daphniidae ($t = -3.10$, $P = 0.002$) in ponds with no macrophytes (Fig. 3). A positive relationship was found between phytoplankton and pH in no ($t = 4.42$, $P < 0.001$) and low ($t = 5.01$, $P < 0.001$) macrophyte treatments. Smooth fits for experiment day showed that phytoplankton followed a unimodal pattern over time in the no ($F = 11.33$, est. df = 1.95, $P < 0.001$) and low ($F = 21.29$, est. df = 1.96, $P < 0.001$) macrophyte treatments and a linear increase in the high macrophyte treatment ($F = 15.98$, est. df = 1.0, $P < 0.001$). Phytoplankton biovolume supported the trend found in Fluoroprobe total chl*a* (days 0–55) and also showed an additional, smaller peak at day 62 of the experiment (Fig. 2a). The initial peaks in phytoplankton biomass were caused by an increase in green algae in the no and low macrophyte ponds (Appendix S2: Table S2, Fig. S2). There was markedly less change over time among phytoplankton groups in the high macrophyte ponds than in the no and low macrophyte ponds.

The initial increase in phytoplankton biomass was followed by growth of periphyton and metaphyton (Figs. 2b, 4). There was visible periphyton growth on sediments in the no macrophyte ponds, with the cyanobacterium *Geitlerinema splendidum* (a potentially toxic alga) identified as the dominant species (Appendix S2: Table S3). Metaphyton grew loosely attached to macrophytes in low and high macrophyte ponds, with a minimal amount observed in no macrophyte ponds; metaphyton was predominately *Oedogonium* spp., but with some representation of *Bulbochaete* spp. and *Mougeotia* spp. Periphyton cover tended to be higher with weekly nutrient inputs (Fig. 4a), whereas metaphyton dry weight was greatest in high-density macrophyte ponds (Fig. 4b); however, we did not detect a significant effect of treatments on either of these measures ($P > 0.05$). Metaphyton biovolume peaked in both low and high macrophyte densities at days 76 and 62, respectively (Fig. 2b).

Total phosphorus was affected by a slight interaction between nutrient treatment and experiment

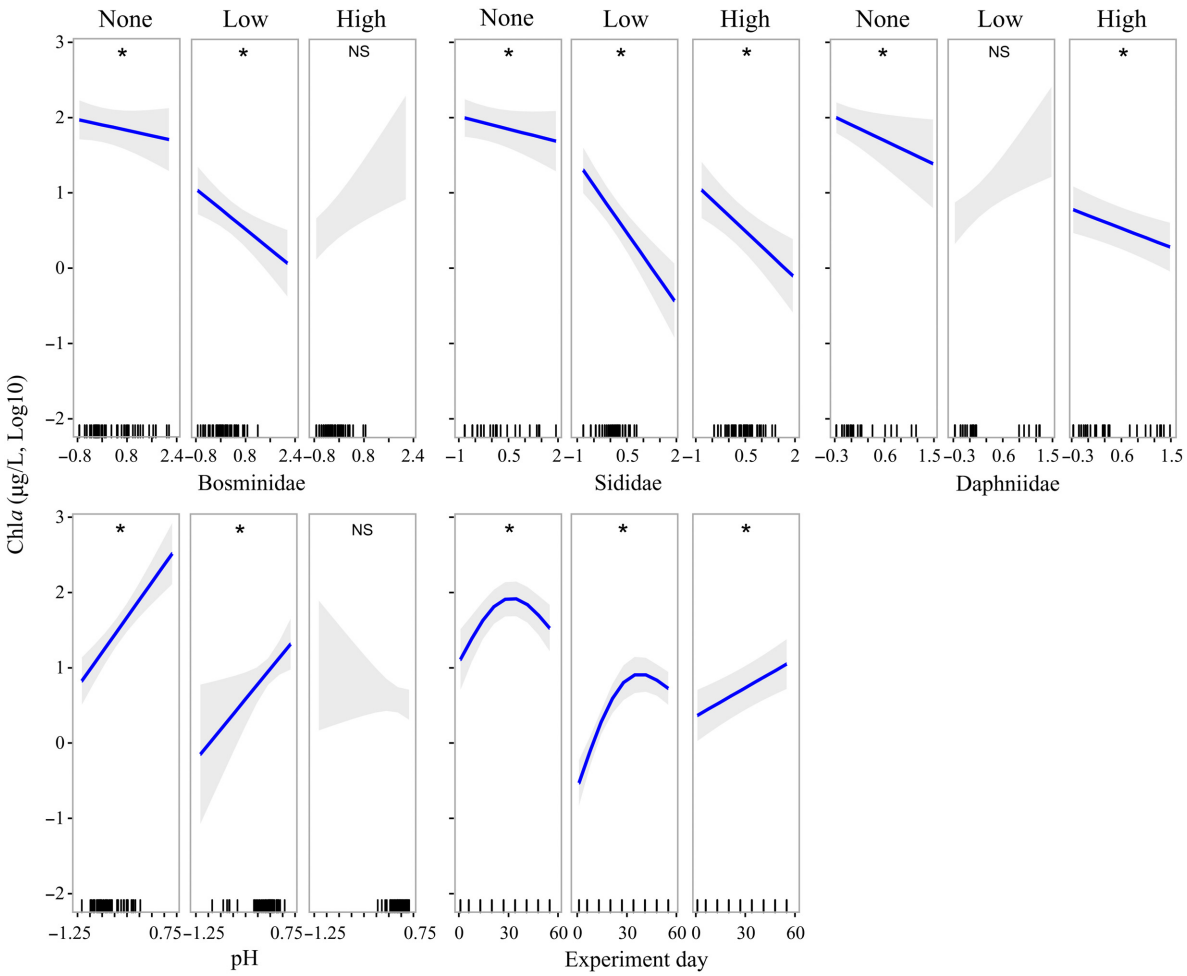

Fig. 3. Variation in phytoplankton biomass (Fluoroprobe total chlorophyll-*a*, chl*a*, µg/L, $\log_{10}$) was best explained by interacting effects of macrophyte density (none, low, high) with Bosminidae, Sididae, and Daphniidae biomass (µg) per liter ($\log_{10}$ + 1, mean centered), as well as pH (mean centered) and time (experiment day, nonlinear term). Predicted model fits (mean fit [blue line] and 95% confidence intervals [CIs, gray shaded area]) for each covariate were calculated by holding all other covariates at their mean value; raw data observations are indicated along the *x*-axes. Significant linear effects and nonlinear smooth terms ($P < 0.05$) are indicated with asterisks; non-significant (NS) slopes are depicted with CIs only.

day (model w/term vs. w/o, $\chi^2 = 5.24$, $P = 0.02$, $\Delta AIC_c = 3.12$) and tended to be higher in no macrophyte treatments ($\chi^2 = 6.36$, $P = 0.04$, $\Delta AIC_c = 2.06$; Tukey's tests for all pairwise combinations of macrophyte treatments, $P > 0.05$; Fig. 5a). Total phosphorus was higher in weekly nutrient treatments and increased over time, with a tendency to increase at a higher rate in weekly treatments. Total nitrogen was explained by a three-way interaction among macrophyte treatment, nutrient treatment, and experiment day ($\chi^2 = 24.13$, $P < 0.001$, $\Delta AIC_c = 19.57$; Fig. 5b). Total nitrogen was higher and increased over time in ponds with weekly nutrient input, but declined or remained the same over time in biweekly nutrient treatments.

Discussion

Our experimental ponds revealed that macrophyte density and zooplankton grazer control were more important than the rate of external

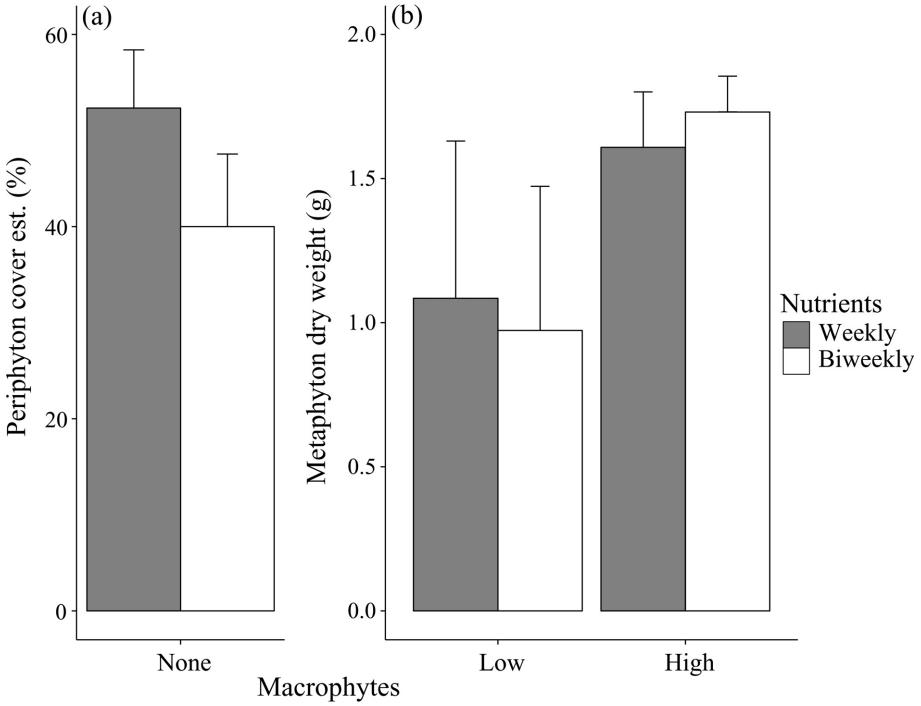

Fig. 4. Periphyton cover (%) on sediments in ponds with no macrophytes (a) and total metaphyton weight per pond (g) (b) after 83 d of weekly (gray bars) and biweekly (white bars) nutrient inputs. Error bars are mean ± 1 SE.

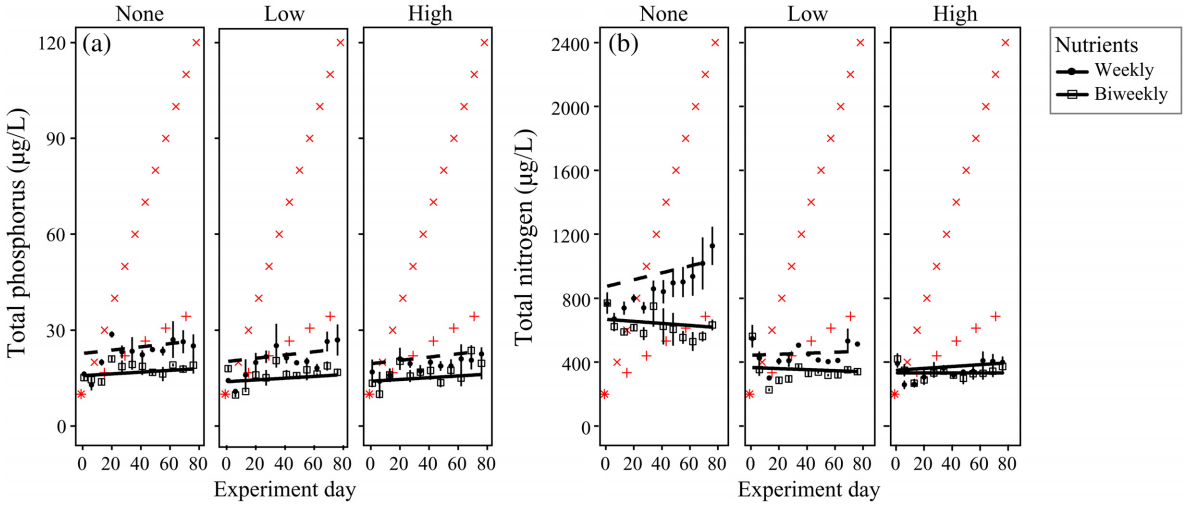

Fig. 5. Total phosphorus (a) and nitrogen concentrations (µg/L) (b) varied across macrophyte densities (none, low, and high), nutrient inputs (weekly [filled circle, dashed line], biweekly [open square, solid line]), and experiment day. Total phosphorus was affected by an interaction between nutrient inputs and time, as well as a slight additive effect of macrophytes, whereas total nitrogen was influenced by an interaction among macrophyte density, nutrient treatments, and time. The total cumulative amount of P and N added weekly (*x*) and biweekly (+) is shown in red. Lines are mean model fits, and error bars are mean ± 1 SE.

nutrient inputs in mediating changes in phytoplankton biomass. Ponds with no or low macrophytes experienced an initial increase in phytoplankton, followed by a shift in algal dominance to either periphytic algae on sediments in no macrophyte treatments or to metaphyton in ponds with macrophytes. Although we never detected a shift to a turbid state, our results support the suggestion that phytoplankton and algal dynamics in shallow aquatic ecosystems may be as strongly related to trophic interactions as to eutrophication (Jeppesen et al. 1999, Beisner et al. 2003, Feuchtmayr et al. 2009). Ponds with macrophytes appeared to be more resilient to phytoplankton blooms when supplied with nutrients, but eventually were prone to metaphyton growth. To our knowledge, only one previous experimental study has assessed trophic interactions along a gradient of macrophyte densities (Schriver et al. 1995) and was consistent with our findings that trophic relationships and algal dominance are mediated differentially across contrasting macrophyte densities.

Periphytic algae on sediments have also been found to dominate primary production and maintain clear water states in macrophyte-free ponds and shallow lakes (Vadeboncoeur and Steinman 2002, Genkai-Kato et al. 2012). We observed periphyton growing on sediment in our no macrophyte ponds after zooplankton had grazed down the phytoplankton bloom, likely enabling periphyton growth. Periphyton have been found to maintain clear water states up to TP levels of 100 μg/L (Vadeboncoeur et al. 2003, Genkai-Kato et al. 2012), a concentration over three times higher than levels at the end of our experiment (despite our nutrient loading rates; Fig. 5).

Growth of filamentous algae or metaphyton on macrophytes may be an important component of the transition from macrophyte to phytoplankton dominance (Jones and Sayer 2003, Olsen et al. 2015). Filamentous algae can out-compete macrophytes for light and carbon (Phillips et al. 1978, 2016, Jones et al. 2002), but once macrophytes are reduced, phytoplankton blooms may inhibit further metaphyton growth through shading (Zhang et al. 2014). In macrophyte treatments, metaphyton began growing in the water column loosely attached to the macrophytes. Our finding that the final dry weight was greater in the high-density macrophyte treatments, whereas biovolume was greater in the low-density treatments at the end of the experiment likely results from whether metaphyton were growing more attached to the macrophytes in the former case, or mostly in the water column, in the latter (Figs. 2b, 4b). The first peak in metaphyton biovolume in the high macrophyte ponds may have subsequently sunk out of the water column. In addition, the metaphyton samples collected for final dry weight analysis were obtained one week after the last date used to quantify algal biovolume (including metaphyton; Fig. 2b).

Initial loss of Daphniidae was likely due to fish predation (Schindler 2006), as the green algae that became dominant during their decline was comprised of edible-sized taxa (Appendix S2: Table S2, Fig. S2). Loss of Daphniidae, a dominant competitor in pelagic environments with phytoplankton prey, allowed Bosminidae and Sididae biomass to increase from very low initial numbers. Bosminidae and Sididae increased in our experimental ponds following phytoplankton growth in a typical predator–prey manner, effectively suppressing further phytoplankton dominance (Fig. 1). This was particularly evident in no macrophyte treatments where phytoplankton increased the most initially. Total zooplankton biomass declined around day 50 across macrophyte densities, potentially from the reduced phytoplankton biomass or from fish predation. Sididae were a more consistent predictor of phytoplankton biomass across macrophyte densities compared to the other zooplankton taxa; this strong trophic response may be a result of their higher clearance rates than both Bosminidae and Daphniidae (Barnett et al. 2013). The overall low levels of Bosminidae and Sididae in low macrophyte ponds may be a result of both lower phytoplankton growth compared to no macrophyte ponds and less refuge compared to high macrophyte ponds. However, we did find that Chydoridae reached a biomass of up to 250 μg/L in low macrophyte ponds. Given that most Chydoridae taxa scrape epiphytic periphyton (Fryer 1968, Masclaux et al. 2012) and are associated with benthic habitats (including macrophytes; Chengalath 1982), our finding that the biomass of this group did not explain significant variation in pelagic total chl*a* was expected. While some zooplankton species (usually littoral taxa, including Chydoridae) may be able to use periphyton and

metaphyton algae as a food source (Fulton 1988, Siehoff et al. 2008), there was no evidence for suppression of these algal groups by grazing as they became abundant following the increase in zooplankton. Previously, it has been demonstrated that grazing of epiphytic periphyton by gastropods and ostracods can prevent the loss of macrophytes and help to maintain a clear water state (McKee et al. 2003, Feuchtmayr et al. 2009). In our ponds, gastropods were absent and ostracods were only present at low densities. In addition, nutrient regeneration by filtering zooplankton has been found to have a stronger effect on periphyton growth than herbivory by scraping cladocerans (Lövgren and Persson 2002). As such, phytoplankton chl*a* appeared to be strongly influenced by filtering zooplankton biomass, particularly in no macrophyte treatments, and periphyton and metaphyton algae were not controlled by scraping zooplankton.

Macrophyte density played a significant role in the ecosystem dynamics of our experimental ponds. While phosphorus assimilation by macrophytes is primarily from the sediments (Carignan and Kalff 1980), *Elodea canadensis* can also take up phosphorus (Eugelink 1998) and nitrogen (Ozimek et al. 1993) directly from the water through its roots and leaves. In our ponds, we did not detect a strong effect of macrophyte density on TP. Macrophytes did appear to reduce nitrogen concentrations (Fig. 5), as they are known to do through direct assimilation and indirect facilitation of denitrifying bacteria; denitrification rates can be equivalent or greater than assimilation rates in freshwater systems and may serve as a buffer for excess nitrate inputs (Nizzoli et al. 2014). Nitrogen assimilation by both macrophytes and metaphyton may have contributed to reduced phytoplankton growth in high macrophyte treatments; however, nitrogen did not appear to be limiting in the ponds, as total dissolved nitrogen remained high across treatments at the end of the experiment (Appendix S2: Fig. S3). *Elodea canadensis* can also suppress growth of epiphytic periphyton through production of allelopathic chemicals (Erhard and Gross 2006), but the growth of *E. canadensis* and metaphyton as the experiment progressed suggests that this was not an important factor in our experiment. It is more likely that both *E. canadensis* and metaphyton suppressed phytoplankton growth through light limitation in low and high macrophyte ponds, whereas zooplankton primarily mediated phytoplankton growth in ponds without macrophytes.

Despite a threefold difference in nutrient inputs between weekly and biweekly treatments by the end of the experiment, macrophyte densities and food web interactions were more influential in determining final algal dominance. These results add to the growing evidence that community structure and top-down effects may be equally or more important than bottom-up factors in both shallow freshwater (Carpenter et al. 1985, Jones and Sayer 2003, Feuchtmayr et al. 2009, Kratina et al. 2012) and marine systems (Eriksson et al. 2009, Duffy et al. 2015). However, managing eutrophication remains a complex problem that has not been effectively solved through singular top-down (e.g., biomanipulation; Carpenter et al. 1995) or bottom-up approaches (e.g., mitigating point and diffuse nutrient sources; see review by Schindler 2006). With respect to the maintenance of more desirable clear water states, our study indicates that systems characterized by high initial macrophyte densities and food webs in which grazing zooplankton dominate are more resilient to nutrient inputs.

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