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Seasonal and scale-dependent variability in nutrient- and allelopathy-mediated macrophyte–phytoplankton interactions

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ABSTRACT

Key-words: *Ceratophyllum demersum, Elodea canadensis, nutrients, allelopathy, alternate stable states in shallow lakes*

macrophyte–phytoplankton interactions were investigated using a dual laboratory and field approach during a growing season, with responses quantified as changes in biomass. Short-term, close-range interactions in laboratory microcosms always led to mutual exclusion of macrophytes (*Elodea canadensis* or *Ceratophyllum demersum*) and algae (*Raphidocelis subcapitata*, *Fistulifera pelliculosa*) or cyanobacteria (*Synechococcus leopoliensis*), suggesting regulation by positive feedback mechanisms, progressively establishing and reinforcing a "stable state". Laboratory results suggest that close-range regulation of *R. subcapitata* and *F. pelliculosa* by macrophytes was primarily *via* nutrient (N, P) mediation. Sprig-produced allelochemicals may have contributed to inhibition of *S. leopoliensis* in *C. demersum* presence, while *S. leopoliensis* was apparently enhanced by nutrients leaked by subhealthy (discolored leaves; biomass loss) *E. canadensis*. Seasonal changes in algal growth suppression were correlated with sprig growth. Marginal differences in *in situ* phytoplankton patterns inside and outside monospecific macrophyte stands suggest that the nutrient- and/or allelopathy-mediated close-range mechanisms observed in the laboratory did not propagate at the macrophyte-stand scale. Factors operating at a larger scale (*e.g.*, lake trophic state, extent of submerged vegetation coverage) appear to override *in situ* macrophyte–phytoplankton close-range interactions.

RÉSUMÉ

Variabilité saisonnière et dépendante de l'échelle dans les interactions macrophyte-phytoplancton liées aux nutriments et à des relations d'allélopathie

Mots-clés : *Ceratophyllum demersum, Elodea canadensis,*

Les interactions macrophytes-phytoplancton ont été étudiées à l'aide d'une approche double de laboratoire et de terrain au cours d'une saison de croissance, avec des réponses quantifiées comme les changements dans la biomasse. Les interactions à court terme et à courte portée dans des microcosmes de laboratoire ont toujours conduit à l'exclusion mutuelle entre des macrophytes (*Elodea canadensis* ou *Ceratophyllum demersum*) et des algues (*Raphidocelis subcapitata*,

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nutriments, allélopathie, états stables alternatifs en lacs peu profonds

Fistulifera pelliculosa) ou cyanobactéries (*Synechococcus leopoliensis*), suggérant une régulation par des mécanismes de rétroaction positive, s'établissant progressivement et renforçant un « état stable ». Les résultats de laboratoire suggèrent que la régulation à courte portée de *R. subcapitata* et *F. pelliculosa* par les macrophytes était principalement due aux éléments nutritifs (N, P). Les composés allélochimiques produits par les brins de macrophyte peuvent avoir contribué à l'inhibition de *S. leopoliensis* en présence de *C. demersum*, tandis que *S. leopoliensis* a apparemment été renforcée par les éléments nutritifs produits par *E. canadensis* en mauvais état (feuilles décolorées, perte de la biomasse). Les changements saisonniers comme la suppression de la croissance des algues ont été corrélés avec la croissance du brin. Des différences marginales *in situ* dans les dynamiques du phytoplancton à l'intérieur et à l'extérieur des peuplements monospécifiques de macrophytes suggèrent que les mécanismes rapprochés liés aux nutriments et/ou à l'allélopathie observés en laboratoire ne se développent pas à l'échelle des formations de macrophytes. Les facteurs intervenant à plus grande échelle (par exemple, l'état trophique du lac, l'étendue de la couverture de végétation submergée) semblent l'emporter sur les interactions à courte portée macrophytes- phytoplancton *in situ*.

INTRODUCTION

Mutual exclusion between submerged macrophytes and phytoplankton is at the core of the "alternate stable states" theory for shallow lakes, according to which such lakes exist in one of two states: either clear-water and macrophyte-dominated, or turbid and phytoplankton-dominated, over a wide range of nutrient concentrations (Scheffer, 1998). Though several mechanisms have been proposed and successfully tested in controlled experiments to explain mutual exclusion of macrophytes and phytoplankton, their relative importance and true *in situ* effectiveness remain open to debate. For example, macrophytes may not be as effective in absorbing water-column nutrients in the field as they are in typical short-term laboratory experiments (Lombardo and Cooke, 2003), and direct competition for water-column nutrients between well-established (*i.e.*, midsummer) *in situ* macrophytes and phytoplankton is unlikely (Lehmann *et al.*, 1994; Schulz *et al.*, 2003). Also, though proven in the laboratory (*e.g.*, Körner and Nicklisch, 2002; Gross *et al.*, 2003) or in controlled field experiments (Jasser, 1995; Hilt *et al.*, 2006), and suspected as a cofactor in maintaining high water transparency in richly vegetated lakes (Blindow *et al.*, 2002; Hilt and Gross, 2008), *in situ* macrophyte allelopathy against phytoplankton is particularly difficult to prove (Gross *et al.*, 2007).

Further complications arise from potential interactions among such mechanisms and between such mechanisms and environmental conditions, such as light availability, lake area, or trophic state (Gross, 2003; van Geest *et al.*, 2003). The extent of sediment coverage by submerged vegetation for the macrophyte-dominated, clear-water state to become stable also is topic of discussion (Jeppesen *et al.*, 1990; Mjelde and Faafeng, 1997; Blindow *et al.*, 2002), and even less is known about how much macrophyte coverage may influence the *in situ* effectiveness of the mechanisms regulating macrophyte–phytoplankton interactions.

As both macrophytes and phytoplankton follow seasonal growth and metabolic cycles (*e.g.*, Pip and Philipp, 1990), plant–algae interactions may change during the course of a growing season. Possible seasonal changes in close-range macrophyte–algae interactions may have implications at the ecosystem scale. For example, if phytoplankton inhibition by macrophytes is stronger at the beginning of the growing season, year-long lake state (clear-water or turbid) may depend on spring conditions. Knowledge about seasonal cycles in plant–algae/cyanobacteria ecology may prove useful in management decisions, assisting lake managers in choosing not only what techniques to use for the problem at hand, but also when to apply such techniques most effectively. Yet, studies addressing seasonal aspects have started to appear only recently in the literature (*e.g.*, Blindow *et al.*, 2002; Hilt *et al.*, 2006).

We have studied macrophyte–phytoplankton interactions during a growing season (June–September) using a dual laboratory and field approach. Our intent was to shed some light on 1. as yet incompletely understood (*e.g.*, Gross *et al.*, 2007; Hilt and Lombardo, 2010) close-range, allelochemical- and nutrient-mediated macrophyte–phytoplankton interactions; 2. how such mechanisms may propagate at the scale of *in situ* macrophyte stands; and 3. temporal changes in such interactions along a growing season, also little understood as of yet. Laboratory experiments using freshly field-collected macrophytes focused on the potential roles of allelopathy and nutrient (N, P) dynamics in shaping close-range, species-specific macrophyte–cyanobacteria/algae interactions. Field data from inside and just outside monospecific macrophyte stands were discussed comprehensively with the laboratory results and with lake ecosystem characteristics to assess the relative importance of the most plausible mechanisms in shaping macrophyte–phytoplankton interactions at three different spatial scales: microhabitat (close-range), macrophyte stand (medium-range), and whole lake (large-scale).

METHODS

> DESCRIPTION OF FIELD SITES

Ceratophyllum demersum L. was collected in Spiradammen ("Pond Spira"), a small (A = 0.3 ha) and shallow ($\bar{z}$ = 1.3 m; z_{max} ~ 2.5 m), mesotrophic and richly vegetated clear-water lake (Secchi depth to bottom). *Elodea canadensis* Michx. originated from the outlet area of Østensjøvatn ("Lake Østen"), an equally shallow but larger (A = 3.1 ha; $\bar{z}$ = 1.9 m; z_{max} = 3.2 m), nutrient-rich and turbid lake. Both lakes are located in southeastern Norway close to the Oslofjord coastline (Figure A-1); Spiradammen (59°50′9"N, 10°29′52"E) is located near the city of Asker, while Østensjøvatn (59°53′40" N, 10°49′49"E) is located in the SE suburbs of Oslo. Østensjøvatn is periodically flushed with nutrient-poor water as part of management efforts aimed at curbing eutrophication effects (Gabestad, 2001). Each plant species dominated in its origin lake, though the abundance of *E. canadensis* in Østensjøvatn is variable, and was particularly low during the sampling year (2003), with no stands reaching the surface. Plant stands were not sufficiently large for a meaningful analysis in mid May, but they had reached a sufficient size by mid June. The submerged vegetation covered ~15–20% of the sediment area of Østensjøvatn and virtually all Spiradammen surface area during peak biomass (July–August).

> LABORATORY EXPERIMENTS

Four identical laboratory experiments were run at 4–5-week intervals, spanning a growing season. Initial trial dates were 11 June, 14 July, 24 August, and 29 September 2003; each trial lasted 4 d. Growth of test organisms (unicellular algae and cyanobacteria) was monitored daily in the presence or absence of macrophyte sprigs. Each trial used freshly field-collected macrophytes and inocula from long-term algal/cyanobacterial cultures under controlled, identical laboratory conditions, so that between-trial differences (if any) could be ascribed to differences in macrophyte condition.

Laboratory experiments adapted standard methods for growth inhibition bioassays using unicellular autotrophic test organisms (OECD, 2011). Test organisms were chosen among those listed in OECD (2011) to represent "typical" chlorophytes [*Raphidocelis* (=*Pseudokirchneriella*) *subcapitata* (Korshikov) G. Nygaard, J. Komárek, J. Kristiansen & O.M. Skulberg (=*Selenastrum capricornutum* Printz)], diatoms [*Fistulifera pelliculosa* (Brébisson) Lange-Bertalot (=*Navicula pelliculosa* (Brébisson ex Kützing) Hilse)], and cyanobacteria [*Synechococcus leopoliensis* (Raciborski) Komrek]. Inocula were taken from preexisting long-term monospecific, nonaxenic, clone laboratory cultures at NIVA's Algal Laboratory. Such cultures were originally isolated from natural populations, and are periodically reseeded to avoid

cellular overgrowth and medium exhaustion. Natural populations of *R. subcapitata* and *S. leopoliensis* are typically planktonic, while *F. pelliculosa* may be found also as a benthic form.

Laboratory experiments used 300 mL of OECD TG 201 (=ISO 8692) liquid algal growth medium (ISO, 2004; OECD, 2011) in clear-glass 1-L Erlenmeyer flasks. The OECD TG 201 growth medium has a pH of 8.1, is rich in HCO_3^- to buffer possible photosynthesis-driven changes in pH, and provides high quantities of nutrients in readily available form for both algae and plants (364 $\mu g \cdot L^{-1}$ of P, supplied as $PO_4^{\equiv}$, and 3928 $\mu g \cdot L^{-1}$ of N, supplied as NH_4^+). The medium was supplemented with $Na_2SiO_3 \cdot 9H_2O$ for tests with *F. pelliculosa* to obtain a Si concentration of 1400 $\mu g \cdot L^{-1}$. Such nutrient concentrations are designed to allow unrestricted exponential growth within the standard 3-day test duration (OECD, 2011). The complete recipe for the OECD TG 201 growth medium is in OECD (2011). Flasks were acid-washed before use.

Growth of *R. subcapitata*, *F. pelliculosa*, and *S. leopoliensis* was monitored in separate flasks in the presence or absence of *C. demersum* or *E. canadensis* (Figure A-2). Each experimental condition was carried out in triplicate. Flasks were inoculated with small quantities of algae ($\sim 5-10 \times 10^6$ cells$\cdot L^{-1}$) or cyanobacteria ($\sim 20 \times 10^6$ cells$\cdot L^{-1}$, corresponding to ~20 fluorescence units). Different initial cell densities were chosen to standardize initial biomass across the differently sized organisms. The OECD-recommended test duration of 72 h was extended to 96 h (=4 d), to allow more time for plant–algae/cyanobacteria interactions to develop, while growth of tests organisms in plant-devoid control flasks was still expected to be exponential.

Algal and cyanobacterial densities were determined daily for each flask, except for day 1 (d_1) in September, when determination was prevented by technical problems. *R. subcapitata* and *F. pelliculosa* densities were determined using an electronic particle counter (Beckman Coulter Multisizer® M3, Miami, FL). This technique was not appropriate for small-sized *S. leopoliensis* because of interference from particulate material and possible sprig-associated bacteria. *S. leopoliensis* density was determined as DCMU-enhanced chlorophyll fluorescence (measured at 665 nm after excitation at 530 nm) using a Micropore Cytofluor plate scanner.

Field-collected *C. demersum* and *E. canadensis* sprigs were stored overnight in the dark at 4 °C before each trial. The short acclimatization period was dictated by the need to use freshly collected plants to preserve sprig natural conditions at the time of collection. The next day, healthy, apical ~6–8-cm long sprigs were acclimated to laboratory temperature and light, rinsed, their biomass individually determined as wet weight on an electronic precision balance (instrument resolution = 0.1 mg) following Lombardo and Cooke's (2003) methods, and placed in the flasks according to the scheme in Appendix Figure A-2. Final sprig biomass was determined as for initial biomass. Changes in sprig biomass were normalized as percent departures from initial values. Changes >100% represented net biomass accrual, and changes <100% net biomass loss.

Flasks were randomly placed on a shaking table under continuous illumination from white fluorescent lamps providing 70 $\mu E \cdot m^{-2} \cdot s^{-1}$ of effective photosynthetic radiation. Such a value allows for rapid exponential growth of test organisms (OECD, 2011), is not limiting for plants (Sand-Jensen and Madsen, 1991) or algae (Jørgensen, 1969; Geider *et al.*, 1986), and prevents photorespiration (*e.g.*, Bouterfas *et al.*, 2002). Final (d_4) densities of test organisms was exponential in all control flasks except in *S. leopoliensis* June flasks. However, final *S. leopoliensis* density in such flasks was safely above the required minimum 16 × increase from initial values for the test to be considered valid (OECD, 2011), and results from this trial were retained. The unobstructed light source, small sprig:flask size and continuous stirring allowed to exclude the involvement of shading and cell/particle trapping within the physical "filter" of sprig stems and leaves, as also argued for similar small-scale, laboratory setups (*e.g.*, Lürling *et al.*, 2006). The observed plant–algae/cyanobacteria interaction patterns therefore could be ascribed to the two remaining known possible mechanisms: allelopathy and/or competition for nutrients.

Nutrient dynamics were quantified in one subset of flasks for each monthly trial. Total dissolved phosphorus (TDP) and total dissolved nitrogen concentrations (TDN) at d_4 were determined in *R. subcapitata* flasks applying Faafeng and Hessen's (1993) methods to filtered

water samples (filter pore size = 0.45 μm). Nutrient determinations were limited to *R. subcapitata* flasks after TDP and TDN concentrations in *R. subcapitata* and *F. pelliculosa* flasks were found to be statistically similar in June (two-tailed *t*-tests: $t_{TDP} = 0.206$, $p = 0.839$; $t_{TDN} = 0.628$, $p = 0.539$; $n_{R.sub} = n_{F.pel} = 9$ and $df = 16$ for both). Sprig net nutrient (N, P) uptake rates were estimated using Hilt and Lombardo's (2010) concentration-based mass balance approach. Because of the *force majeure* coarse estimate and impossibility to apply replicate-based statistics, nutrient uptake estimates are not treated in detail. An outline of the estimation method is reported in Appendix.

Because potential allelochemicals were not identified, allelopathy was assumed to be a factor when algal growth inhibition occurred in the absence of nutrient effects (Hilt and Lombardo, 2010). Even when nutrient effects were observed, *sensu strictu* competition for nutrients was unlikely, due to the overall high nutrient availability. Therefore, as in Hilt and Lombardo (2010), a more general nutrient mediation or influence (*i.e.*, *sensu latu* competition for nutrients) in plant–algae interactions was assumed to occur in such cases.

> IN SITU PHYTOPLANKTON SURVEY

Phytoplankton at the sites and times of sprig collection was analyzed for biovolume and taxonomic composition. Two 100-mL grab water samples (depth ~20 cm) were collected inside (or above if macrophyte stands were too dense) and just outside macrophyte stands. Distance between inside and outside grab samples was ~2–5 m, depending on habitat conditions (the shorter and more distinct the habitat transition, the shorter the distance between samples). Care was taken to minimize water disturbance to avoid inclusion of epiphyton from nearby plants.

Phytoplankton samples were immediately fixed in acidified Lugol's solution (Olrik *et al.*, 1998). Taxonomic analysis was carried out at the lowest possible level, usually genus or species. Phytoplankton biovolume was estimated from cell densities (calculated according to Olrik *et al.*, 1998). *In situ* phytoplankton sampling was carried out without replication after variance for June biovolume triplicates was deemed sufficiently small ($\chi^2 \leqslant 3.71$ and $p \geqslant 0.16$ for χ^2 tests of variance with H_0 = variance ⩽ 10% of average, for total phytoplankton and all major classes; $df = 2$ for each test).

Additional samples were collected for nutrient (TDP, TDN) concentration determination, immediately stored in ice and processed within 24 h from collection following the same procedure as for laboratory nutrient samples. Water transparency was measured *in situ* as Secchi depth. Nutrient concentrations and water transparency were determined as single samples or measurements at each subsite (inside and outside macrophyte stands) at each visit. Field sampling and related sprig collection were carried out under "dry weather" conditions (*i.e.*, at least 72 h after a major storm) to avoid direct influence of runoff (*e.g.*, increased water turbulence and nutrient/silt inputs) on collected samples.

> STATISTICAL ANALYSIS

Differences among laboratory experimental conditions were tested within each monthly trial using repeated-measures ANOVAs followed by Tukey's honestly significant difference (HSD) multiple-comparison tests in case of significant plant effects within each experimental day. The HSD test effectively controls the family-wise Type I error to no more than the nominal level (Quinn and Keough, 2002), with no need for Bonferroni corrections. Because of the forced absence of within-month replication, two- or paired-sample two-tailed *t*-tests were run for specific subsets of field data.

Data departure from normality could not be assessed quantitatively because of small sample size ($n = 3$); however, data were likely not normally distributed. *In situ* phytoplankton data were square-root-transformed using Anscombe's formula [$x' = \sqrt{x + 3/8}$], percent relative abundances were arcsine-transformed using Anscombe's formula [$x' = \arcsin \sqrt{(x + 3/8) \times 4/7}$],

and all other data were log-transformed using Bartlett's formula [$x' = \log_{10}(x+1)$] before statistical analysis (Zar, 2009). Correlations and regressions were performed for selected datasets using original, untransformed data because of analysis reliability when nonnormality is not extreme (Zar, 2009). Best-fitting curves are reported for simple correlations and regressions; multiple correlations remained linear.

Significance threshold for all tests was set *a priori* at $p \leqslant 0.05$. Statistical analyses were performed with Addinsoft® XLSTAT® v. 2012.6.09 and employed a gradient of standard ($p \leqslant 0.05$) to strong ($p \leqslant 0.01$) and very strong ($p \leqslant 0.0001$) significance levels.

In situ phytoplankton assemblages were compared by means of Whittaker's (1952) similarity index expressed in percent format (%PSC):

$$\%\ \mathrm{PSC} = 100 \times \left\{ 1 - 1/2 \left[\sum_h \left| \frac{n_A}{N_A} - \frac{n_B}{N_B} \right| \right] \right\}$$

where n_A = biovolume of taxon i in assemblage (sample) A, n_B = biovolume of taxon i in assemblage B, and N_A and N_B = total biovolumes in assemblages A and B respectively, each containing *h* taxa. Two assemblages with identical species distribution have %PSC=100%.

RESULTS

> LABORATORY EXPERIMENTS

Growth of *R. subcapitata* and *F. pelliculosa* was significantly lower than controls in sprig-containing flasks in June and August, except in *E. canadensis* flasks in June, while control-*vs.*-plant differences in algal growth were nonsignificant in July and September (Figure 1; Appendix Table A-I). Growth of *R. subcapitata* remained (*quasi*) exponential through d_4 in all flasks, while *F. pelliculosa* appeared to reach a growth peak or plateau at d_3 (Figure 1). Despite always having reached d_4 density at >16× as required by the OECD (2011) test, *S. leopoliensis* did not enter a truly exponential growth in June and September control flasks (Figure 1). Except in September, growth of *S. leopoliensis* never entered the exponential phase in *C. demersum* flasks either, while cyanobacterial densities reached significant (HSD separations at $p \leqslant 0.05$: Appendix Table A-I) higher-than-control levels in *E. canadensis* flasks starting from d_1 or d_2 in all monthly trials (Figure 1). The only instance of significant lower-than-control *S. leopoliensis* d_4 density in *E. canadensis* flasks was in August, following an apparent progressive decline in cell density after a significant (HSD: $p \leqslant 0.05$) "jump start" on d_1 (Figure 1).

C. demersum sprigs grew significantly in June in all flasks, while growth was lower and more variable in July and August, and became negative in September (Figure 2; Appendix Table A-II). The only instance of significant net growth for *E. canadensis* sprigs was in *R. subcapitata* flasks in August (Figure 2); however, when data from all algal/cyanobacterial flasks were pooled, *E. canadensis* as a whole did not grow significantly in August (Table I). Though growth of *C. demersum* remained nonsignificant in *F. pelliculosa* and *S. leopoliensis* presence in August (Figure 2), *C. demersum* sprigs in general did grow in August (Table I). *C. demersum* consistently grew more or lost less biomass than *E. canadensis*, except in August, when growth patterns were similar ($p = 0.282$ for the between-species comparison in Figure 2). However, *C. demersum* never lost biomass except in September, and *E. canadensis* always lost biomass except in August (Table I). Average biomass loss of *E. canadensis* in September was twice as much as *C. demersum* 's (Table I).

When data from all monthly trials were pooled, chlorophyte or diatom growth was negatively correlated with sprig growth in an exponential fashion (Figure 3). The plant–cell growth relationship remained closer for *R. subcapitata* than for *F. pelliculosa* (qualitative comparison of r^2 and *p* values in Figure 3). Though still following negative exponential paths, the trends for *S. leopoliensis* remained not significant ($r^2 \leqslant 0.239$, $p \geqslant 0.107$; *n* and *df* as in Figure 3).

Final *R. subcapitata* cell density in macrophyte absence was positively correlated with TDP but not with TDN concentration, while growth in sprig presence was correlated with concentrations of both nutrients (Figure 4). Final (d_4) nutrient (TDP, TDN) concentrations in *R.*

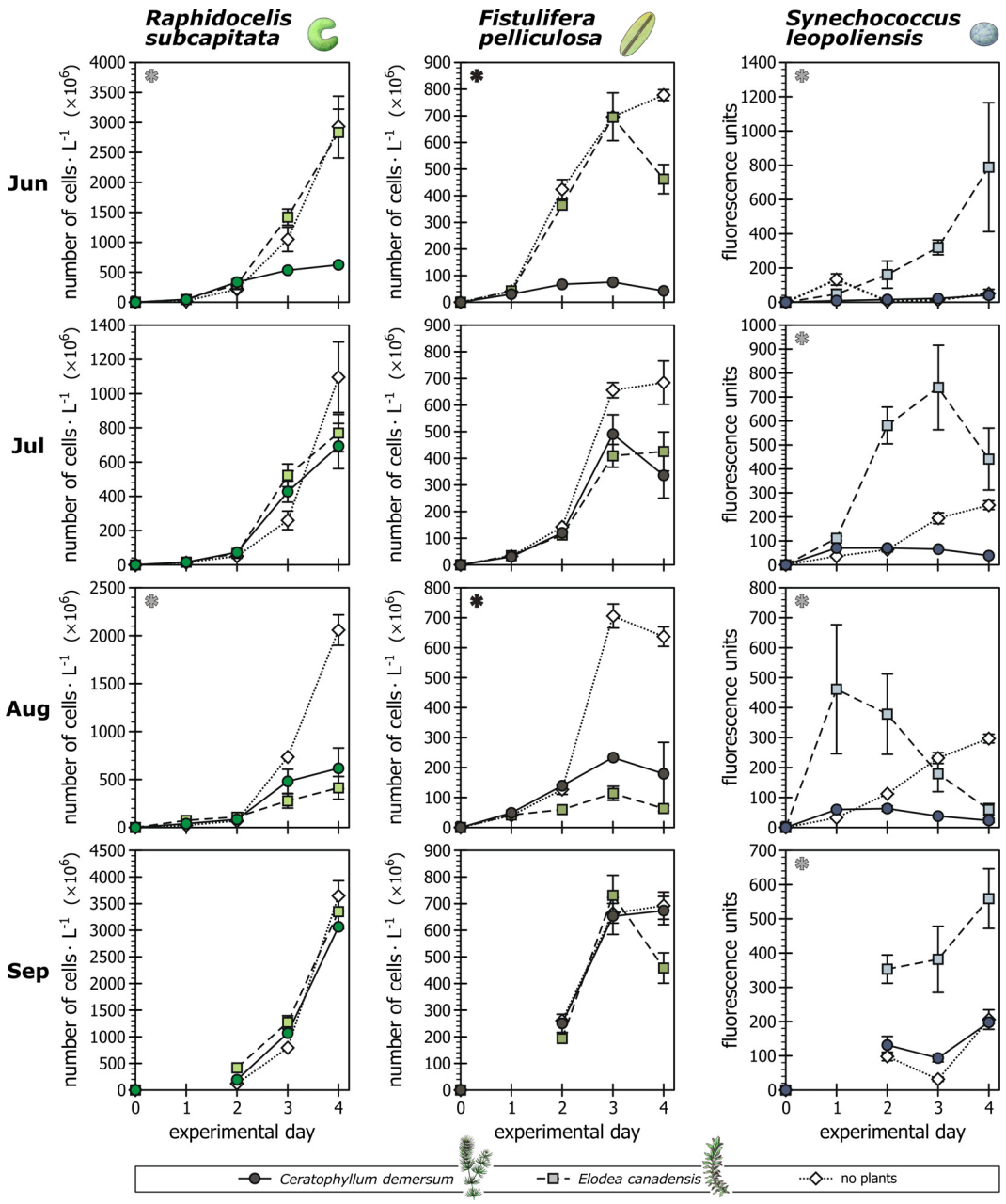

Figure 1

Density of algae and cyanobacteria (as cell counts or fluorescence) in laboratory flasks in the presence or absence of C. demersum or E. canadensis sprigs; average ± standard error (n = 3 for each). Asterisks in each panel represent the significance level of differences in plant treatments for repeated-measures ANOVAs (✲ $p \leqslant 0.05$; ✲ $p \leqslant 0.01$; ✲ $p \leqslant 0.0001$; no asterisk: n.s.); differences in time were always significant at $p \leqslant 0.0001$. Complete ANOVA and accompanying HSD test results are in Appendix Table A-I. Please note the different y axis scales.

subcapitata flasks were always significantly lower than initial conditions in sprig absence and in *C. demersum* presence ($t \geqslant 2.788$, $p \leqslant 0.049$ for initial-*vs.*-final *t*-tests with $n_{init} = n_{fin} = 3$ and $df = 4$). TDN concentration in *E. canadensis* presence remained significantly lower than in no-plant flasks until September ($t \geqslant 3.600$, $p \leqslant 0.023$), when it surged to significantly higher-than-control values ($t = -4.613$, $p = 0.010$) along with TDP concentration ($t = -6.989$, $p = 0.002$). TP concentration in *E. canadensis* presence was lower than in control flasks only in July ($t = 4.736$, $p = 0.009$).

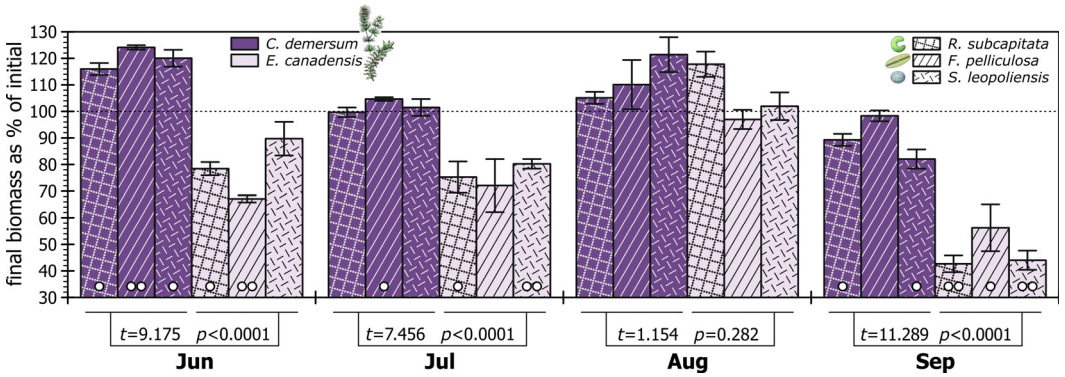

Figure 2

Net change in sprig live biomass (wet weight) for C. demersum or E. canadensis at d_4 in laboratory flasks in algal/cyanobacterial presence; average ± standard error (n = 3 for each). Horizontal dotted line represents no change; biomass gain is above and biomass loss below the line. White dots represent significant biomass gain or loss according to paired-sample t-tests (n = 3 and df = 4 for each), with significance thresholds at p ⩽ 0.05 (○), p ⩽ 0.01 (○○), and p ⩽ 0.0001 (○ ○ ○); complete results are in Table A-II. Two-tailed t-tests within each month ($n_{C.dem} = n_{E.can} = 9$; df = 16) compare C. demersum-vs.-E. canadensis biomass changes regardless of test organism.

Table I

Net changes in sprig biomass during the 4-d flask experiment by month (average ± standard error), with final biomass expressed as percent departure from initial condition (initial biomass =100%, biomass accrual >100% and biomass loss <100%), and associated paired-sample two-tailed t-tests (performed on arcsine-transformed percent data), pooling data across phytoplankton taxa. For each comparison, n = 9 and df = 8. Negative t values represent biomass loss from initial condition, with significant biomass accrual or loss for p ⩽ 0.05. Significance levels are highlighted with ○ (p ⩽ 0.05), ○○ (p ⩽ 0.01), and ○ ○ ○ (p ⩽ 0.0001).

	Ceratophyllum demersum		*Elodea canadensis*	
	Final biomass as % of initial	Initial-*vs.*-final comparison	Final biomass as % of initial	Initial-*vs.*-final comparison
Jun	120.1 ± 1.6	t =10.577 $p < 0.0001^{○○○}$	78.4 ± 3.8	t =-5.808 $p = 0.0004^{○○}$
Jul	101.9 ± 1.3	t =1.541 p =0.162	75.8 ± 3.6	t = −7.311 $p < 0.0001^{○○○}$
Aug	112.2 ± 4.1	t =2.841 $p = 0.022^{○}$	105.6 ± 3.8	t =1.538 p =0.163
Sep	90.0 ± 2.7	t = −3.739 $p = 0.006^{○○}$	47.8 ± 3.6	t =-15.281 $p < 0.0001^{○○○}$

The strength of TDP–growth relationship was similar in sprig-absent controls and in *C. demersum*- and *E. canadensis*-present flasks [χ^2_P = 3.467, p = 0.177 for Paul's (1988) multiple comparison of r^2 values; details in Appendix Table A-III]. However, the slopes for the TDP–growth correlations in Figure 4 were significantly different ($p < 0.0001$) in *C. demersum* and *E. canadensis* presence (Appendix Table A-III). The TDN–growth relationship was significantly highest in *C. demersum* flasks, followed by *E. canadensis* and control flasks, in this order [χ^2_P = 17.131, p = 0.0002 for Paul's (1988) multiple comparison of r^2 values followed by Zar's (2009) Tukey-type test: Appendix Table A-III]. The three slopes for the TDN–growth correlations also were significantly different (Appendix Table A-III). The closest *R. subcapitata*–nutrient relationship occurred for TDN in the presence of *C. demersum* ($r^2_{TDN-C.dem}$ = 0.935) (Figure 4). The strength of the *R. subcapitata* –TDN correlation in *C. demersum* flasks was significantly higher than the corresponding *R. subcapitata* –TDP correlation (Z = 2.882, p = 0.004: Appendix Table A-III), while the opposite occurred in control flasks

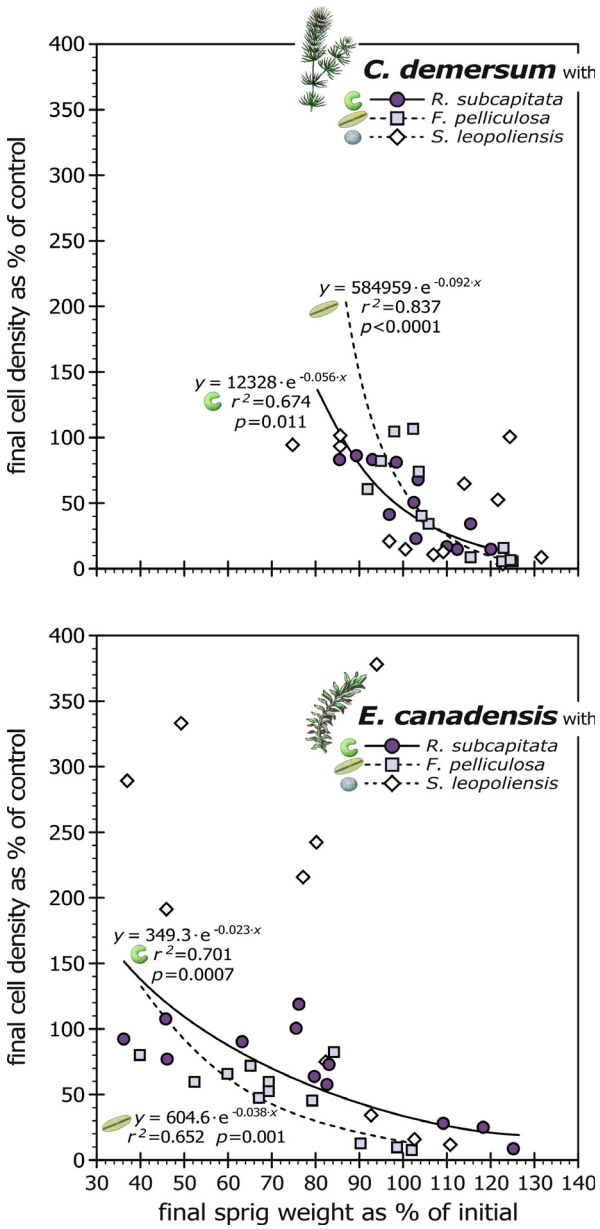

Figure 3
Relationships between algal/cyanobacterial density (as percent of corresponding controls; 100% = same as in controls) and sprig biomass change (as percent of initial live weight; 100% = no change) in C. demersum- or E. canadensis-present flasks at d_4. For all regressions, n = 12 and df = 10. Two E. canadensis–S. leopoliensis outliers (final cell density >400% of control) are excluded from graphical representation but included in regression analysis. S. leopoliensis regressions were not significant ($r^2_{C.dem}$ = 0.239, p = 0.107; $r^2_{E.can}$ = 0.153, p = 0.209).

(Z = 3.353, p = 0.0008). The TDP and TDN correlations had similar strength in *E. canadensis* flasks (Z = 0.929, p = 0.353: Appendix Table A-III).

Except for TDP in *C. demersum* flasks, final nutrient concentrations and sprig growth were inversely correlated (Table II). Daily sprig (fresh weight) nutrient uptake rates remained in the –40–6 $\mu g \cdot g^{-1} \cdot d^{-1}$ range for P and in the –270–104 $\mu g \cdot g^{-1} \cdot d^{-1}$ range for N (Appendix Table A-IV). Estimated daily nutrient uptake rates were always positive for *C. demersum*, except in July when sprigs leaked 1.1 $\mu g \cdot P \cdot g^{-1} \cdot d^{-1}$. Phosphorus uptake rates were always negative for *E. canadensis* except in August, when sprigs sequestered 3.8 $\mu g \cdot P \cdot g^{-1} \cdot d^{-1}$, but N uptake rates remained positive until September, when *E. canadensis* leaked 269.3 $\mu g \cdot N \cdot g^{-1} \cdot d^{-1}$

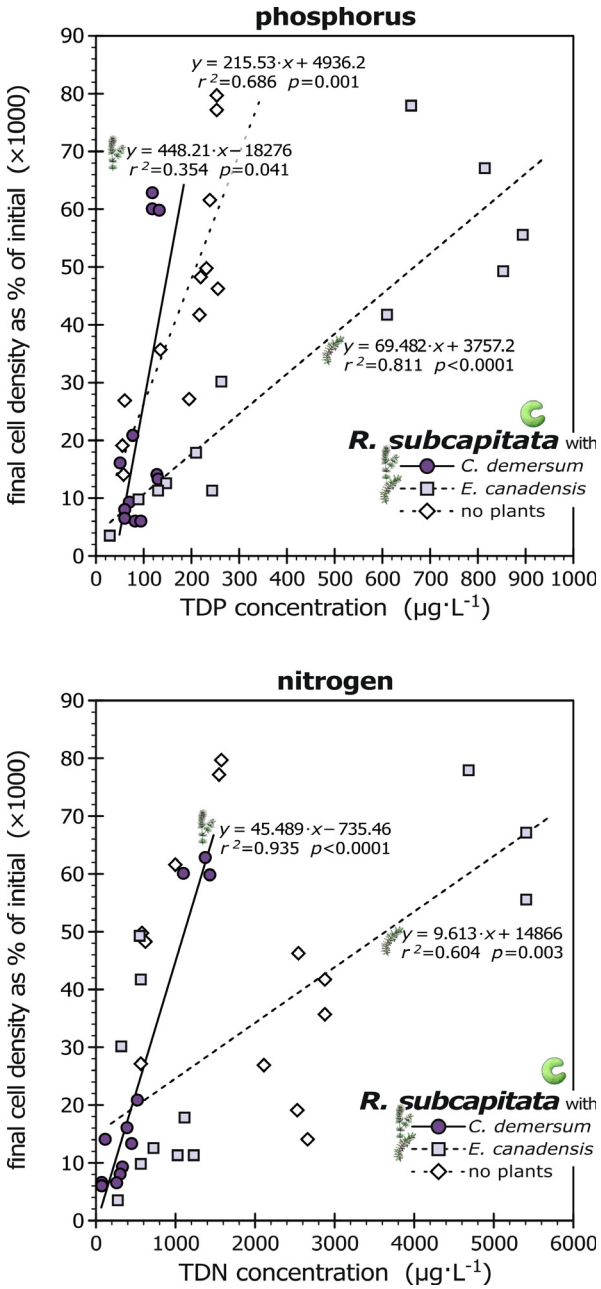

Figure 4
Final R. subcapitata density (as percent of initial; 100% = no change) in relation to d_4-nutrient concentrations in C. demersum-, E. canadensis-, or plant-devoid laboratory flasks. For all regressions, $n = 12$ and $df = 10$. All regressions were significant ($p \leqslant 0.05$) except for the TDN relationship in plant absence ($r^2 = 0.143$, $p = 0.225$).

(Appendix Table A-IV). Significant inhibition of *R. subcapitata* growth occurred only when sprig nutrient uptake rates were positive for both P and N (Appendix Table A-IV). Estimated daily nutrient uptake rates were correlated with sprig growth for *E. canadensis* (multiple linear correlation: $r^2 = 0.740$, $df = 9$, $p = 0.002$: % change in biomass $= 91.58 - 0.76 \times \text{TDP} - 7.12 \times 10^{-2} \times \text{TDN}$, with nutrient uptake rates as $\mu g \cdot g^{-1} \cdot d^{-1}$) but not for *C. demersum* ($r^2 = 0.022$, $df = 9$, $p = 0.903$).

Table II

Correlations between net sprig growth and total dissolved phosphorus or total dissolved nitrogen concentrations (TDP and TDN) at t_F in C. demersum- or E. canadensis-containing flasks (June–September data pooled; n =12 and df = 11 for all correlations). Net sprig growth as percent departure from initial biomass; nutrient concentrations in μg·L^{-1}. Correlations are linear (lin) or exponential (exp); negative r values represent negative slopes for either correlation type. Significance levels are highlighted with ○ ($p \leqslant 0.05$), ○○ ($p \leqslant 0.01$), and ○○○ ($p \leqslant 0.0001$).

	C. demersum			*E. canadensis*		
	type	*r*	*p*	type	*r*	*p*
TDP	lin	–0.209	0.495	lin	–0.755	0.003○○
TDN	lin	–0.839	0.0005○○	exp	–0.864	0.0002○○

> IN SITU PHYTOPLANKTON SURVEY

In situ total phytoplankton biovolume fluctuated widely in *E. canadensis*-dominated Østensjøvatn (Figure 5), but biovolume remained significantly lower inside than outside the *E. canadensis* stand (paired-sample two-tailed *t*-test on pooled monthly data: $t = -3.321$, $df = 3$, $p = 0.045$; Appendix Table A-V). Total phytoplankton biovolume in *C. demersum*-dominated Spiradammen remained at low values throughout the sampling period (Figure 5). Total phytoplankton biovolume was significantly lower in Spiradammen (paired-sample, two-tailed *t*-test on pooled monthly data: $t = 5.471$, $p = 0.0009$, $n = 8$ and $df = 7$).

Biovolumes of major phytoplankton groups were similar inside and outside macrophyte stands (Appendix Table A-V). Cyanobacterial, diatom, and cryptophyte biovolumes were significantly lower in Spiradammen (paired-sample, two-tailed *t*-tests on pooled monthly data: $t_{cyano} = 2.949$, $p = 0.021$; $t_{diatom} = 8.882$, $p < 0.0001$; $t_{crypto} = 4.373$, $p = 0.003$; $n = 8$ and $df = 7$ for all); differences for other groups remained not significant ($p > 0.05$).

Total number of phytoplankton taxa was similar inside and outside macrophyte stands (paired-sample, two-tailed *t*-tests on pooled monthly data: $t_{Spi} = 0.507$, $p = 0.647$; $t_{Øst} = 0.269$, $p = 0.805$; $n = 4$ and $df = 3$ for both), with most taxa common to both subhabitats in either lake (Figure 5). Number of phytoplankton taxa was significantly higher in Østensjøvatn (paired-sample, two-tailed *t*-test on pooled monthly data: $t = 6.289$, $p = 0.0004$, $n = 8$ and $df = 7$). Inside- and outside-stand assemblages within each lake had similar taxonomic structures, with %PSC never <50% in either lake (Figure 5).

The cryptophyte *Rhodomonas lacustris* was the only major phytoplankton taxon common to the two lakes. *R. lacustris* codominated the Spiradammen phytoplankton with the chlorophytes *Raphidocelis subcapitata* and *Botryococcus braunii*. The only cyanobacterium present in appreciable density in Spiradammen was *Woronichinia naegeliana* (~4–10% of total biovolume in June and July). Østensjøvatn phytoplankton was codominated by several *Dolichospermum* (=*Anabaena*) species and by a diverse assemblage of diatoms (*Diatoma tenuis*, *Asterionella formosa*, *Fragilaria* spp., *Stephanodiscus hantzschii*, *Aulacoseira* spp.). Phytoplankton maxima in Østensjøvatn were dominated by *Dolichospermum lemmermanni* (~50% of June total biovolume) and *D. planctonicum* (~60–70% of August total biovolume). The cyanobacterium *Microcystis* was observed only in Østensjøvatn, where it remained at low numbers (0–5% by volume). Relative abundance of all common phytoplankton taxa was similar inside and outside macrophyte beds in either lake (Appendix Table A-VI).

Water transparency followed the same temporal trend in both lakes, with qualitatively (no replication) lower transparency inside macrophyte stands in June followed by equal inside-*vs.*-outside values afterwards (Figure 5). Outside-stand Secchi depth in Spiradammen was always to bottom (sediment patches were always visible from the boat) from July on. Water transparency was significantly higher in Spiradammen (paired-sample, two-tailed *t*-test on pooled monthly data: $t = 3.628$, $p = 0.008$, $n = 8$ and $df = 7$). In-lake nutrient concentrations were always higher in Østensjøvatn (Figure 5), resulting in a significant between-lake difference (paired-sample, two-tailed *t*-tests on pooled monthly data: $t_{TDP} = 4.286$, $p = 0.004$; $t_{TDN} = 4.769$, $p = 0.002$; $n = 8$ and $df = 7$ for both). Total phytoplankton biovolume was not correlated with TDP or TDN concentrations in Spiradammen, but it was in Østensjøvatn (Table III).

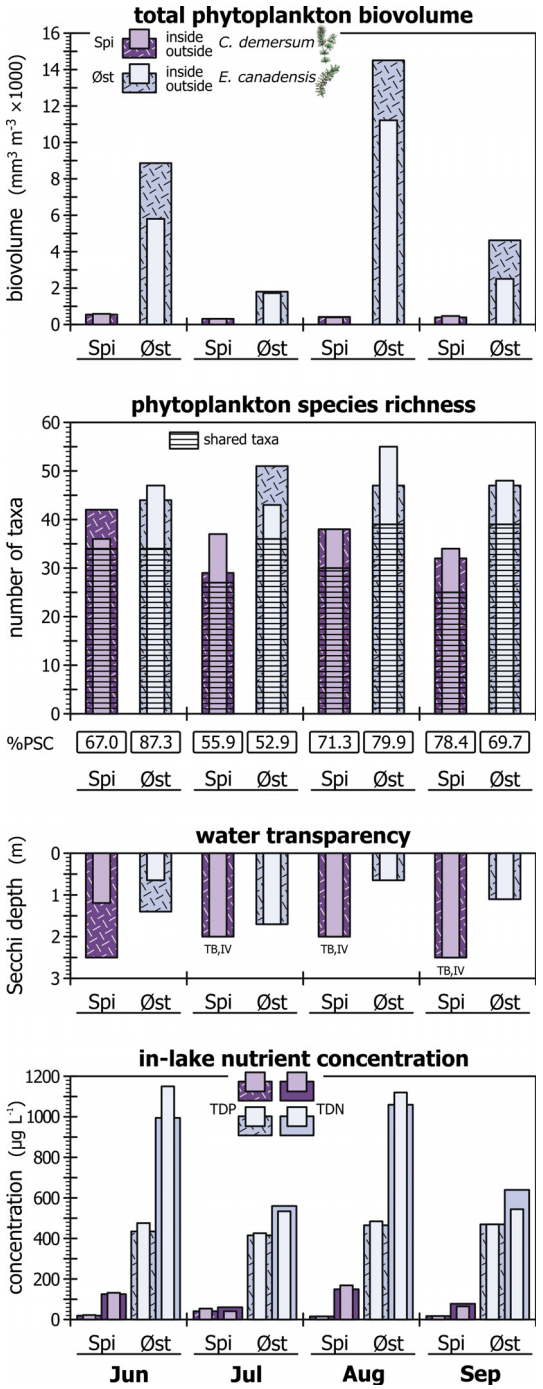

Figure 5

Comparison of inside- vs. outside-macrophyte stand phytoplankton (top panels) and physico-chemical conditions (bottom panels) in C. demersum-dominated Spiradammen (Spi) and E. canadensis-dominated Østensjøvatn (Øst) at the time of macrophyte sprig collection. The number of taxa common to inside and corresponding outside phytoplankton assemblages is represented by horizontal line shading. Taxonomic similarity of inside-vs.-outside stand phytoplankton assemblages is quantified by the %PSC index (0% = no taxonomic overlap; 100% = complete overlap). TB = Secchi depth to bottom; IV = Secchi disc disappeared in the vegetation (sediments visible in unvegetated patches nearby). For all variables, n =1 within each sampling month and subhabitat (inside or outside macrophyte stands).

Table III
Correlations between total phytoplankton biovolume (in $mm^3 \cdot m^{-3}$) and total dissolved phosphorus or total dissolved nitrogen concentrations (TDP and TDN, in $\mu g \cdot L^{-1}$) inside and outside the C. demersum (Spiradammen) or E. canadensis stands (Østensjøvatn) that supplied sprigs for laboratory experiments (June–September data pooled; n = 4 and df = 3 for all correlations). Correlations are linear (lin) or exponential (exp); negative r values represent negative slopes. Significance levels are highlighted with ○ ($p \leqslant 0.05$), ○○ ($p \leqslant 0.01$), and ○○○ ($p \leqslant 0.0001$).

	Spiradammen					
	Inside *C. demersum*			Outside *C. demersum*		
	Type	*r*	*p*	Type	*r*	*p*
TDP	lin	–0.506	0.494	lin	–0.663	0.337
TDN	lin	0.467	0.533	lin	0.197	0.803

	Østensjøvatn					
	Inside *E. canadensis*			Outside *E. canadensis*		
	Type	*r*	*p*	Type	*r*	*p*
TDP	exp	0.997	0.0002○○	exp	0.964	0.008○○
TDN	exp	0.919	0.027○	exp	0.943	0.016○

DISCUSSION

> LABORATORY EXPERIMENTS

Both *C. demersum* and *E. canadensis* exhibited some ability to suppress algal growth in laboratory microcosms until late August (Figure 1), consistent with earlier laboratory observations (Gross *et al.*, 2003; Lürling *et al.*, 2006). Except in September, *S. leopoliensis* never grew in *C. demersum* flasks (Figure 1), though cyanobacterial inhibition by *C. demersum* remained not significant in June due to uncharacteristic low cyanobacterial growth in control flasks (Appendix Table A-I). Growth of *S. leopoliensis* instead was almost always enhanced in *E. canadensis* presence (Figure 1; Appendix Table A-I). Although our quantitative (Figure 1) and qualitative results (consistent observations of crystal-clear medium in *C. demersum* flasks through d_4) align with earlier observations of cyanobacterial growth inhibition by *C. demersum* (Wium-Andersen, 1987; Jasser, 1995; Nakai *et al.*, 1999; Gross *et al.*, 2003; Hilt and Gross, 2008), cyanobacterial enhancement by *E. canadensis* does not (Erhard and Gross, 2006; Hilt, 2006). Though overlapping on some aspects, seasonal dynamics in plant–algae and plant–cyanobacteria interactions varied with macrophyte species (Figure 1), suggesting that the observed patterns may have been determined by a combination of species-specific plant–algae interactions and seasonal changes in macrophyte condition, as also argued elsewhere (Jasser, 1995; Hilt *et al.*, 2006).

The experimental setup restricts the list of the possible mechanisms involved in the observed plant–algae interactions to allelopathy and competition for nutrients. Both *C. demersum* and *E. canadensis* produce allelochemicals capable of inhibiting algae and/or cyanobacteria (*e.g.*, Gross *et al.*, 2003; Erhard and Gross, 2006). Inhibition of algal photosynthetic pathways, a more direct measure of allelopathic interference than is population growth, was specifically observed in the physical presence of *C. demersum* (Körner and Nicklisch, 2002) and *E. canadensis* (Lürling *et al.*, 2006), indicating that allelopathy by both *C. demersum* and *E. canadensis* could have been involved in mediating algal/cyanobacterial growth. However, the close association of *R. subcapitata* growth patterns with nutrient concentrations (Figure 4) strongly suggests nutrient (co)mediation of plant–chlorophyte interactions, as also found elsewhere (Lürling *et al.*, 2006; Takeda *et al.*, 2008; Hilt and Lombardo, 2010).

Regression analysis (Figure 4; Appendix Table A-III) suggests that *R. subcapitata* growth shifted from being P regulated in sprig absence to being P and N coregulated in *E. canadensis* presence, and N regulated in *C. demersum* presence. N-mediated (co)regulation of phytoplankton patterns was observed also *in situ* in richly vegetated, *C. demersum*-dominated

shallow Norwegian lakes (Mjelde and Faafeng, 1997), as well as in other, similar ecosystems (Gligora *et al.*, 2007). Direct, positive *R. subcapitata*-nutrient relationships changing strength and/or direction in sprig presence (Figure 4 and Appendix Table A-III) and inverse sprig–nutrient relationships (Table II) suggest that nutrient dynamics in the flasks were driven by sprigs, as also found by Lürling *et al.* (2006) and Hilt and Lombardo (2010) in similar-purpose experiments, with growth of *R. subcapitata* being inhibited when growing sprigs were able to sequester nutrients, or enhanced when withering sprigs leaked nutrients into the medium.

However, estimated daily nutrient uptake rates by sprigs (Appendix Table A-IV) remained in the lower end of the known range from similar short-term experiments (*e.g.*, Pelton *et al.*, 1998; Lombardo and Cooke, 2003; Hilt and Lombardo, 2010), and quantitative inhibition of *R. subcapitata* apparently was not related to sprig nutrient uptakes (Appendix Table A-IV). Also, nutrient uptake rates were not correlated with *C. demersum* growth ($r^2 = 0.022$, $p = 0.903$), suggesting that the *total* quantity of nutrient removed by sprigs (with larger sprigs sequestering more nutrients) was the basis for the competition for nutrients between *C. demersum* and *R. subcapitata*. The significant sprig growth–nutrient uptake correlation for *E. canadensis* ($r^2 = 0.740$, $p = 0.002$) might have been driven by the wider range of biomass change for *E. canadensis* than *C. demersum* (Table I), suggesting that total nutrient removal (or leaking) might have been a nonnegligible cofactor in *E. canadensis* – *R. subcapitata* interactions as well. Despite the coarse estimation basis for nutrient uptake rates, our results suggest that sprigs inhibited algal growth by preemptively sequestering nutrients before algae can do so, supporting Craine *et al.* 's (2005) view of availability- and not uptake-driven competition for nutrients by plants. However, we cannot go beyond a generally stated involvement of competition for nutrients between macrophyte sprigs and algae, as 1. nutrient availability remained overall high; 2. nutrient effects cannot be separated satisfactorily with current methodology (*e.g.*, Lürling *et al.*, 2006; Gross *et al.*, 2007); 3. both macrophyte and algal metabolism quickly adapt to substrate availability (Touchette and Burkholder, 2001; Collos *et al.*, 2005); and 4. competition remains one of the most difficult ecological mechanisms to demonstrate empirically (*e.g.*, Connell, 1980, 1983; Goldberg and Scheiner, 2001).

Absence of evidence of *R. subcapitata* nutrient-independent inhibition by *C. demersum* and absence of *R. subcapitata* growth inhibition by *C. demersum* exudates in a separate trial (unpublished data) support earlier observations of absence of allelopathy against chlorophytes by *C. demersum* (Jasser, 1995). The possible involvement of allelopathy by *E. canadensis* against *R. subcapitata* may be more difficult to extrapolate, as earlier investigations targeting *E. canadensis* allelopathy against chlorophytes yielded contradicting results (*e.g.*, Erhard and Gross, 2006 *vs.* Lürling *et al.*, 2006). The apparent absence of nutrient-independent action by *E. canadensis* against *R. subcapitata* may stem from species-specific insensitivity to *E. canadensis* potential allelochemicals by *R. subcapitata* (whose susceptibility to *E. canadensis* allelochemicals was not specifically tested in earlier studies), and/or from absent or insufficient allelochemical production by the Østensjøvatn population of *E. canadensis*. However, even if occurring, allelopathic inhibition of *R. subcapitata* by *E. canadensis* seems to have been subordinate to *sensu latu* competition for nutrients.

Growth patterns of *F. pelliculosa* closely resembled those of *R. subcapitata*. Though nutrient concentrations were not determined in *F. pelliculosa* flasks, similar chlorophyte and diatom growth patterns (Figure 1) and similar nutrient patterns in June *R. subcapitata* and *F. pelliculosa* flasks ($p \geqslant 0.539$ for *t*-tests) suggest the possibility of similar macrophyte–chlorophyte and macrophyte–diatom interactions, including an important role played by nutrients. Alternatively or additionally, *F. pelliculosa* may have been (co)limited by Si despite the high initial availability, as d_4 collapses in *F. pelliculosa* density occurred in many flasks, regardless of sprig presence or absence (Figure 1). Such d_4 collapses were not observed in sprig-devoid *R. subcapitata* or *S. leopoliensis* flasks, which do not require Si for growth. Production of anti-diatom allelochemicals has been demonstrated for both *C. demersum* (Gross, 1995) and *E. canadensis* (Wium-Andersen, 1987), and diatoms seem to be particularly sensitive to allelochemicals from a number of macrophyte species (Gross, 1995; Körner and Nicklisch, 2002; Hilt, 2006), suggesting a possible involvement (albeit not quantifiable) of

allelopathic inhibition of *F. pelliculosa* by *C. demersum* and/or *E. canadensis*. Weakly significant inhibition of *F. pelliculosa* but not *R. subcapitata* growth by *C. demersum* exudates in a separate experiment (T. Källqvist, unpublished data) supports the hypothesis of some involvement of allelopathy at least in *C. demersum*–diatom interactions.

Regardless of the underlying mechanism(s), inhibition of chlorophyte and diatom growth occurred when sprigs accrued biomass (Figure 3). Takeda *et al.* (2011) similarly found strong inhibiting effects on the cyanobacterium *Microcystis aeruginosa* (Kützing) Kützing by extracts from *Potamogeton pusillus* L. taken from aquarium-cultivated plants which grew 10× during the assays, mirroring Gross's (2003) patterns for *Myriophyllum spicatum* L. In a rare study that quantified both plant and cyanobacterial growth response to coexistence, Li *et al.* (2009) found an inhibitory action of high-density *Microcystis* on the growth and photosynthetic rate of *Ceratophyllum oryzetorum* Kom., but also an inhibitory effect of actively growing *C. oryzetorum* on low-density *Microcystis*. The observed inverse relationship between sprig and algal growth (Figure 3) is consistent with higher nutrient and/or allelochemical concentrations in sites of new growth such as plant apical meristems or stem apices (Goulder and Boatman, 1971; Gross *et al.*, 1996; Gross, 2000). In particular, Goulder and Boatman's (1971) observation of N accumulation in the apical 2 cm of field-collected *C. demersum* shoots is highly consistent with our own observation of sprig-driven, N-dependent *R. subcapitata* growth in *C. demersum* flasks (Figure 3 and Appendix Table A-III). Thiébaut (2005) found higher ability to incorporate ambient P by faster-growing plant species, including *Elodea* spp., similarly suggesting that plant ability to incorporate nutrients may be related to active growth.

Additionally, the observed temporal pattern in *C. demersum* net biomass change (Figure 2) mirrors *in situ* bimodal seasonal fluctuations in standing crop and metabolite production in temperate-climate natural *C. demersum* populations (Goulder, 1969; Pip and Philipp, 1990). Seasonal growth patterns for *E. canadensis* are highly variable, with late-starting populations seldom reaching full stand size and/or full-strength *in situ* phytoplankton inhibition (Ozimek and Balcerzak, 1976; Rørslett *et al.*, 1986). Delayed, stunted *E. canadensis* growth was observed in Østensjøvatn and other nearby locations during the study year, probably because of a long period of low light availability associated with an unusual high spring water level during the study year (authors' personal observation). Allelochemical production seems to be closely associated with light availability (Gross, 2000, 2003; Hilt *et al.*, 2006), possibly explaining *E. canadensis*'s "poor performance" against target algae in our experiments. However, significant algal growth suppression by *E. canadensis* in August (Figure 1) was associated with a short-lived but evident *in situ* new growth from old, "brown" shoots, further supporting the hypothesis that inhibition of target algae, whether allelochemical- or nutrient-mediated, is strongly dependent on plant growth and/or health condition.

Different growth patterns for *S. leopoliensis* suggest different mechanisms in macrophyte–cyanobacteria than in macrophyte–algae interactions. Except for September, when sprig general inhibiting capabilities declined (Figure 1), growth patterns for *S. leopoliensis* did not follow seasonal trends, with significant inhibition in *C. demersum* presence in July and August and consistent enhancement in *E. canadensis* presence (Figure 1 and Appendix Table A-I). All *C. demersum*-containing June–August flasks remained clear through d_4, and absence of significant *S. leopoliensis* inhibition in June was due more to uncharacteristic low growth in control flasks than to high growth in *C. demersum* flasks. Though nutrient concentrations in *S. leopoliensis* flasks were not determined, consistent absence of *S. leopoliensis* growth in *C. demersum* flasks suggests a looser association of cyanobacterial than algal growth with nutrient availability, and hence a likely nonnegligible (co)involvement of allelopathy by *C. demersum* against *S. leopoliensis*. This hypothesis is supported by demonstrated higher cyanobacterial (including *Synechococcus*) sensitivity to selective and/or strong allelopathic action by *C. demersum* and other macrophytes (Jasser, 1995; Gross *et al.*, 1996; Nakai *et al.*, 1999; Gross *et al.*, 2003; Hilt and Lombardo, 2010). An apparent strong anti-cyanobacterial allelopathic effect of *C. demersum* may have prevented *S. leopoliensis* from developing, thus allowing *C. demersum* to take up the available nutrients at leisure and become established. Well-established, healthy *C. demersum* sprigs in turn may have rendered

nutrients unavailable and/or produced full-strength anti-cyanobacterial allelochemicals, reinforcing sprig dominance.

S. leopoliensis enhancement in *E. canadensis* presence is counterintuitive. Although it is considered a "weak" allelopathic species (Hilt and Gross, 2008), *E. canadensis* produces allelochemicals that may be particularly effective against cyanobacteria, including some *Synechococcus* strains (Erhard and Gross, 2006). *E. canadensis*'s variable seasonal or interannual growth (Ozimek and Balcerzak, 1976; Rørslett *et al.*, 1986) may lead to variable allelopathic strength against algae and cyanobacteria, with higher effectiveness during periods of higher growth, mirroring Gross's (2000) seasonal observations for *in situ M. spicatum* subjected to varied epiphytic colonization. The isolated instance of eventual *S. leopoliensis* inhibition by *E. canadensis* occurred in August, *i.e.*, at the only time of significant chlorophyte and diatom inhibition (Figure 1) coupled with sprig biomass retention (Figure 2 and Table I), supporting this hypothesis. At least some of the anti-cyanobacteria allelochemicals produced by *E. canadensis* are phenolic compounds (Erhard and Gross, 2006), which *Synechococcus* may be able to neutralize (Wurster *et al.*, 2003). *S. leopoliensis* thus may be able to neutralize the little (if any) phenolic compounds produced by subhealthy *E. canadensis*, but not the full-strength allelochemical production by actively growing *E. canadensis*. However, further studies are needed to test this hypothesis.

An apparent ineffectiveness in allelochemical production and/or nutrient sequestration by *E. canadensis* allowed *S. leopoliensis* to take an early advantage of the available nutrients, possibly further weakening *E. canadensis* and inducing nutrient leaching from withering, "leaky" sprigs. Bacteria in nonaxenic flasks may have further increased nutrient availability by transforming sprig-leaked nutrients from organic into inorganic forms (Pehlivanoglu and Sedlak, 2004). Once well established in the flasks, *S. leopoliensis* also may have further reduced P availability for *E. canadensis* by coprecipitating P with calcite (Dittrich *et al.*, 2003). Senescing macrophyte tissue also releases carbon (C) that may be rapidly converted into readily available inorganic forms (Anesio *et al.*, 1999), leading to a concerted increase in nutrient availability for *S. leopoliensis* while *E. canadensis* progressively withered. Senescing *E. canadensis* also may be more susceptible than *C. demersum* to colonization by microdecomposers (Czeczuga *et al.*, 2005), possibly enhancing nutrient leaching from withering *E. canadensis*. Favored by its minute size, high CO_2 and P affinity, and adaptive, combined ability to take up inorganic C and N at increasing availability (Ritchie *et al.*, 2001; Tandeau de Marsac *et al.*, 2001), *S. leopoliensis* may have heavily contributed to the demise of *E. canadensis* in all but the August flasks (Figure 1). The dramatic d_3–d_4 recovery by *E. canadensis*, however, suggests that sprigs were sufficiently healthy or active in August that they were able to successfully counteract *S. leopoliensis*'s initial advantage. The mechanism(s) behind such a recovery remain(s) unknown.

Sprig–algae/cyanobacteria interactions in our experimental flasks always led to dominance of either organism type, and coexistence (as both types of organisms coexisting in active growth phase) was never observed despite the short experimental duration (Figure 3). Using larger microcosms (~120 L), Li *et al.* (2009) observed that growth, photosynthesis, and cyanobacterial inhibitory capabilities of an Asian species of *Ceratophyllum* were inversely proportional to cyanobacterial density, with eventual plant biomass loss under the highest experimental cyanobacterial density, supporting our view of a two-way close-range interaction between macrophytes and phytoplankton. Such an "either–or" small-scale pattern resembles the macrophyte–phytoplankton mutual exclusion often observed in larger or natural settings (Hasler and Jones, 1949; Mjelde and Faafeng, 1997). Apparently contradicting results in preliminary trials, with inhibition of target algae by *E. canadensis* but not *C. demersum* (Mjelde and Brettum, unpublished data), and the dramatic divergence in the outcomes of *C. demersum– vs. E. canadensis*–cyanobacteria interactions, further suggests that, as in larger settings (Scheffer, 1998) or in modeled systems (Hulot and Huisman, 2004), the interaction outcome may be determined stochastically or by some initial condition progressively reinforcing and establishing the initial direction, rather than being rigidly "predetermined" *a priori*. Though to varying extent, many algal and plant species share the ability to quickly adapt to increasing

nutrient availability (Touchette and Burkholder, 2001; Collos *et al.*, 2005) and/or coprecipitate P with calcite during active photosynthesis (*e.g.*, Brammer, 1979; Hartley *et al.*, 1995), also supporting this hypothesis.

> IN SITU PHYTOPLANKTON SURVEY AND EXTRAPOLATION TO ECOSYSTEM-WIDE FEATURES

All major chlorophyte taxa and chlorophytes as a group were not influenced by macrophyte presence in the field (Appendix Tables A-V and A-VI). The chlorophyte *R. subcapitata* was even a codominant in *C. demersum*-dominated Spiradammen, in clear contrast with the growth inhibition observed in *C. demersum* presence in the laboratory (Figure 1), but in agreement with an *in situ* neutral or mildly enhancing effect on chlorophytes by *C. demersum* (Jasser, 1995). Though differences were less dramatic, a similar discrepancy between laboratory and field patterns was observed also for *E. canadensis*, suggesting that nutrient- and/or allelochemical-(co)mediated sprig effects in close-range macrophyte–chlorophyte interactions do not propagate at medium range (macrophyte stand and vicinity).

Nutrient mediation, and possibly even competition for nutrients, was the primary factor involved in chlorophyte regulation by macrophyte sprigs in the laboratory (Figure 4 and Table II), and estimated nutrient uptake rates by sprigs in flasks (Appendix Table A-IV) also were in the lower end of the typically high range for similar short-term experiments (*e.g.*, Hilt and Lombardo, 2010). However, Lombardo and Cooke (2003) found that net foliar P uptake by post-peak (*i.e.*, midsummer/autumnal) *C. demersum* and other small-leaved macrophyte species, including *Elodea* spp., approach zero for exposure periods ⩾10 weeks, suggesting that strong nutrient effects in short-term small-scale investigations may be an experimental artifact and may not transfer into natural situations – at least not at the magnitude observed in the laboratory. *In situ* observations of lower-than-expected phytoplankton biomass, but not TP concentration, in richly vegetated natural habitats (Rooney and Kalff, 2003; Lombardo, 2005) similarly support Lombardo and Cooke's (2003) hypothesis that direct competition for water-column nutrients may not contribute to low midsummer phytoplankton biomass in macrophyte-rich shallow waters, though competition may occur during macrophyte early spring growth (van Donk *et al.*, 1993). Small contributions to *in situ* total nutrient retention by well-established macrophytes was observed also in other aquatic systems (Lehmann *et al.*, 1994; Schulz *et al.*, 2003), further supporting our hypothesis.

The only clear *in situ* macrophyte effects were closer nutrient–phytoplankton associations (comparison of *p* values in Table III) coupled to season-long significantly lower total phytoplankton biovolume (Appendix Table A-V) inside than outside the *E. canadensis* stand in Østensjøvatn, suggesting that some nutrient-mediated regulation of phytoplankton biovolume by macrophytes may occur at the medium-scale (macrophyte stand) as well as the small scale (laboratory flasks). Absence of a comparable *C. demersum* effect in Spiradammen suggests that interspecific differences in plant condition, metabolism, or biomass may have played a role in the observed *in situ* patterns. For example, while nutrient uptake for the rootless *C. demersum* is exclusively foliar (Denny, 1972), the rooted *E. canadensis* may also function as a nutrient source, translocating sediment nutrients from roots to shoots, thus potentially enriching the water column (Rørslett *et al.*, 1986). Sediment-extracted nutrients are typically stored in plant tissues in healthy, dense *E. canadensis* stands (Rørslett *et al.*, 1986; D. Berge, NIVA, pers. comm.). However, subhealthy *E. canadensis* was not always able to retain nutrients (Figure 4), and the close, positive nutrient–phytoplankton association inside the *E. canadensis* stand (Table III) could have simply reflected nutrient-mediated interactions over a wider range of nutrient concentrations (thus leading to a significant correlation) than for *C. demersum*, consistent with the laboratory findings.

Increased sedimentation and/or decreased sediment/algal resuspension in macrophyte stands as tall as 20 cm (Vermaat *et al.*, 2000) suggest that, though subhealthy and stunted, the *E. canadensis* stand may have been sufficient to act as a physical trap against phytoplankton. Absence of any selective macrophyte action on phytoplankton groups and taxa

(Appendix Tables A-V and A-VI) supports this hypothesis. Sufficiently tall macrophytes also may minimize water movements within and in close proximity of the stands, creating "microstratifications" in which nutrients may become rapidly depleted, leading to strong, albeit temporary, local competition (Ondok *et al.*, 1984; Jones *et al.*, 1996; Herb and Stefan, 2004), contributing to shape the *in situ* patterns that we observed (Figure 5 and Table III).

Phytoplankton readily taking advantage of dramatic increases in nutrient availability in eutrophic, *E. canadensis*-dominated Østensjøvatn (Figure 5) suggests that any close- or medium-range and/or short-term nutrient-mediated regulation of phytoplankton growth by macrophytes may have been overridden by ecosystem-wide changes in trophic state. Such a hypothesis is supported by the observation that *in situ* phytoplankton responded quickly to the nutrient surge in August (Figure 5), despite *E. canadensis*'s relative "well-being" and close-range effectiveness against all test organisms in the laboratory (Figure 1). Between-lake differences in phytoplankton patterns were generally stronger than within-lake differences, further supporting the hypothesis that lake effects may have overridden any close-range or stand-scale effect of macrophytes. The two lakes shared only one major taxon (*Rhodomonas lacustris*), but assemblage composition was highly similar inside and outside macrophyte stands in either lake ($p \geqslant 0.650$ for paired-sample *t*-tests and %PSC >50%), with most taxa common to both subhabitats (Figure 5). Biovolumes of some major groups were significantly lower ($p \leqslant 0.021$ for paired-sample *t*-tests for cyanobacteria, diatoms and cryptophytes) in Spiradammen, while within-lake differences remained highly qualitative (Appendix Tables A-V and A-VI). The significantly higher total number of phytoplankton taxa in Østensjøvatn ($p = 0.0004$ for the paired-sample *t*-test) similarly may be ascribed to a lake effect, as species richness tends to be proportional to lake area (Jones *et al.*, 2003). Larger between- than within-lake differences occurred also for physico-chemical variables such as nutrient concentrations and water transparency (Figure 5; statistical details in Results).

Phytoplankton in *C. demersum*-dominated Spiradammen did not take advantage of fluctuating nutrient concentrations (*e.g.*, a ~2× increase in TDP in July: Figure 5), resulting in a decoupling of the nutrient–phytoplankton association (Table III). Even more than for the sparse *E. canadensis* stands in Østensjøvatn, inorganic C may have become depleted in the dense *C. demersum* stands, leading to a possible strong, local C limitation of phytoplankton in richly vegetated Spiradammen, at least during daily peaks in macrophyte photosynthesis; however, this hypothesis remains untested. *In situ* competition between *C. demersum* and phytoplankton for macronutrients other than N, P, or C also was unlikely. For example, similar abundance in Spiradammen and Østensjøvatn of other Si-requiring phytoplankton such as chrysophytes ($p > 0.05$ for between-lake *t*-tests; detailed results not shown), suggest an unlikely involvement of Si in regulating diatom dynamics in Spiradammen. As for diatoms, cyanobacterial abundance in Spiradammen was uncharacteristically low when compared to the in-lake mesotrophic nutrient level, as well as when compared with other *C. demersum*-dominated, mesotrophic shallow lakes in southeastern Norway (M. Mjelde, unpublished data). Cyanobacteria typically have high physiological requirements for P (Wetzel, 2001), and none of the taxa found in Spiradammen is known to fix atmospheric N_2, suggesting that nutrients may have regulated cyanobacterial biomass to some extent. However, cyanobacterial biovolume remained too low to be primarily or solely explained by nutrient limitation. Densely growing, canopy-forming macrophytes such as *C. demersum* may effectively shade underlying phytoplankton to the point of halting photosynthesis (Goulder, 1969; Frodge *et al.*, 1990), possibly suppressing phytoplankton locally. However, as Secchi depth was to bottom during most of the growing season (Figure 5), and *C. demersum* grew to the surface only in part of the lake, light limitation was an unlikely factor in the observed lake-wide low phytoplankton biovolume in Spiradammen. As found elsewhere, (*e.g.*, Hilt, 2006), these patterns suggest a possible involvement of *C. demersum* allelochemicals in determining *in situ* cyanobacterial patterns.

Besides trophic state, the two study lakes also differed in surface area, shoreline characteristics, and total macrophyte coverage. The observed between-lake differences may have been driven by more than one factor, possibly acting simultaneously, with varying magnitude, and

interacting in nonlinear ways. For example, the ability of macrophyte stands to trap and retain suspended particles (Ackerman and Okubo, 1993; Vermaat *et al.*, 2000) may have been much greater in Spiradammen, whose sediment surface was almost entirely covered by macrophytes. In fact, coverage rather than biovolume may be more important in determining the "filtering" capability of macrophyte stands, as even short plant beds can be effective particle traps (Vermaat *et al.*, 2000). Between-lake differences in macrophyte-based particle-trapping ability may have been exacerbated by different lake surface areas, as larger lakes (in this case, Østensjøvatn) tend to be more prone to wind-induced water/nutrient recirculation and sustained phytoplankton biovolume (Jeppesen *et al.*, 1990; Guildford *et al.*, 1994). In their extensive review, Gasith and Hoyer (1997) also argue that the lake-wide limnological and metabolic effects of submerged macrophytes decrease with increasing lake size faster than their importance in providing a structural habitat, emphasizing the importance of lake size in determining its metabolism. Differences in shoreline characteristics also may have played a role, with much of Spiradammen being protected from wind action by tall riparian trees, while most of the Østensjøvatn shoreline at the outlet (*i.e.*, sampling) area was relatively open. Though they remain largely untested, these hypotheses are consistent with the general observation that the probability of dominance by submerged macrophytes decreases with increasing lake surface area (Duarte *et al.*, 1986; Gasith and Hoyer, 1997; van Geest *et al.*, 2003).

CONCLUSIONS

Though the effects of *sensu latu* competition for nutrients and allelopathy could not be satisfactorily separated in laboratory experiments, our results suggest that 1. close-range, short-term macrophyte–phytoplankton interactions lead to dominance by either organism type, mirroring the longer-term, larger-scale mutual exclusion typically observed in natural conditions; 2. regardless of the mechanisms involved, close-range, short-term interactions are driven by macrophytes; 3. outcomes of close-range interactions may (co)depend on some initial condition (*e.g.*, macrophyte health) rather than being rigidly determined *a priori*; 4. *sensu latu* competition for nutrients is likely the primary factor involved in chlorophyte and diatom close-range regulation by *C. demersum* and *E. canadensis* under laboratory conditions; 5. allelochemicals are likely involved in close-range cyanobacterial inhibition by *C. demersum*; 6. apparently subhealthy *E. canadensis* is ineffective against otherwise susceptible cyanobacteria; 7. regardless of the mechanisms involved, macrophytes exert close-range control over phytoplankton only when actively growing; 8. competition for nutrients and/or allelopathy do(es) not seem to be primary factor(s) involved in *in situ* macrophyte–phytoplankton patterns; and 9. *in situ* macrophyte–phytoplankton patterns may be strongly influenced by ecosystem-wide factors such as trophic state, wind exposure, extent of total submerged vegetation coverage and related lake-wide sedimentation capabilities.

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APPENDIX

Figure A-1
Location of C. demersum-dominated Spiradammen and E. canadensis-dominated Østensjøvatn in southeastern Norway. Spiradammen (59°50′9"N, 10°29′52"E) is located near the city of Asker and Østensjøvatn (59°53′40"N, 10°49′49"E) is located in the southeastern suburbs of Oslo. In situ data collection in larger Østansjøvatn was restricted to the northern area to have comparable sampling surface areas in the two lakes. Maps elaborated from Google Earth images (©2013 Google & Cnes/Spot Images).

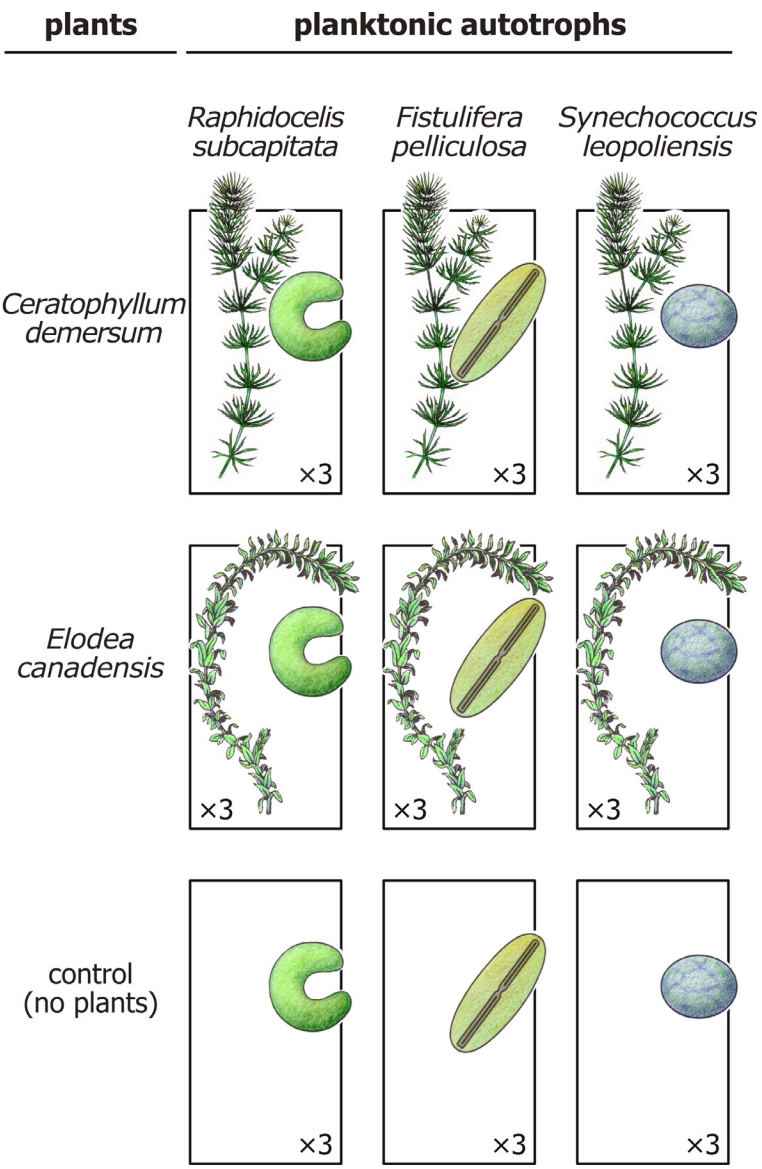

Figure A-2
Experimental design for each of four trials of the laboratory experiment using whole C. demersum or E. canadensis sprigs. Each condition was carried out in triplicate. Material is not drawn to scale.

Table A-I

Complete results for the repeated-measures-ANOVAs and a posteriori Tukey HSD tests for the short-term flask experiments described in Figure 1. Statistical tests were performed on square-root-transformed data. Degrees of freedom were 2,6, 4,24, and 8,24 for plant, time, and plant × time interaction factors, respectively, for repeated-measures-ANOVAs, except in September, when df = 3,18 and 6,18 for time and interaction. For one-way ANOVAs testing differences in plant condition within each experimental day, ctrl = control (no plants), C.dem = C. demersum, and E.can = E. canadensis; df = 2,6 for each test. ANOVAs were considered significant for $p \leqslant 0.05$. Day-specific ANOVAs and HSD tests were not performed ("–") when repeated-measures $p_{plant} > 0.05$. Day-zero one-way ANOVAs (always not significant: $F \approx 0$, $p \approx 1$) and associated HSD are not reported. For HSD tests, different letters identify significantly different average values at $p \leqslant 0.05$, with "a" = lowest and with increasing average values progressively identified alphabetically.

	Raphidocelis subcapitata									
	Repeated-measures ANOVA			**Day-specific one-way ANOVAs**			**Day-specific Tukey HSD tests**			
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Jun	plant	13.079	0.006	d_1	7.675	0.022	d_1	a	b	ab
	time	229.753	<0.0001	d_2	13.209	0.006	d_2	a	b	b
	plant × time	15.312	<0.0001	d_3	9.964	0.012	d_3	ab	a	b
				d_4	16.110	0.004	d_4	b	a	b
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Jul	plant	0.179	0.840	d_1	–	–	d_1	–	–	–
	time	295.736	<0.0001	d_2	–	–	d_2	–	–	–
	plant × time	4.765	0.001	d_3	–	–	d_3	–	–	–
				d_4	–	–	d_4	–	–	–
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Aug	plant	9.147	0.015	d_1	25.924	0.001	d_1	a	a	b
	time	175.369	<0.0001	d_2	11.970	0.008	d_2	a	a	b
	plant × time	18.789	<0.0001	d_3	7.719	0.022	d_3	b	ab	a
				d_4	17.553	0.003	d_4	b	a	a
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Sep	plant	4.596	0.062	d_1	–	–	d_1	–	–	–
	time	866.684	<0.0001	d_2	–	–	d_2	–	–	–
	plant × time	4.560	0.003	d_3	–	–	d_3	–	–	–
				d_4	–	–	d_4	–	–	–

Table A-I
Continued.

Fistulifera pelliculosa										
	Repeated-measures ANOVA			**Day-specific one-way ANOVAs**			**Day-specific Tukey HSD tests**			
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Jun	plant	449.922	<0.0001	d_1	9.129	0.015	d_1	b	a	b
	time	396.487	<0.0001	d_2	123.506	<0.0001	d_2	b	a	b
	plant × time	63.479	<0.0001	d_3	97.334	<0.0001	d_3	b	a	b
				d_4	192.546	<0.0001	d_4	c	a	b
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Jul	plant	4.892	0.055	d_1	—	—	d_1	—	—	—
	time	302.909	<0.0001	d_2	—	—	d_2	—	—	—
	plant × time	4.573	0.001	d_3	—	—	d_3	—	—	—
				d_4	—	—	d_4	—	—	—
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Aug	plant	62.618	<0.0001	d_1	2.259	0.186	d_1	—	—	—
	time	86.298	<0.0001	d_2	26.587	0.001	d_2	b	b	a
	plant × time	17.522	<0.0001	d_3	128.592	<0.0001	d_3	c	b	a
				d_4	15.820	0.004	d_4	b	a	a
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Sep	plant	4.063	0.077	d_1	—	—	d_1	—	—	—
	time	383.794	<0.0001	d_2	—	—	d_2	—	—	—
	plant × time	2.790	0.033	d_3	—	—	d_3	—	—	—
				d_4	—	—	d_4	—	—	—
Synechococcus leopoliensis										
	Repeated-measures ANOVA			**Day-specific one-way ANOVAs**			**Day-specific Tukey HSD tests**			
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Jun	plant	18.667	0.003	d_1	22.619	0.002	d_1	b	a	a
	time	11.363	<0.0001	d_2	8.869	0.016	d_2	a	a	b
	plant × time	7.248	<0.0001	d_3	81.925	<0.0001	d_3	a	a	b
				d_4	6.992	0.027	d_4	a	a	b
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Jul	plant	33.784	0.001	d_1	46.766	0.0002	d_1	a	b	c
	time	57.428	<0.0001	d_2	76.498	<0.0001	d_2	a	a	b
	plant × time	16.199	<0.0001	d_3	20.229	0.002	d_3	a	a	b
				d_4	12.666	0.007	d_4	b	a	b
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Aug	plant	20.931	0.002	d_1	7.226	0.025	d_1	a	ab	b
	time	9.011	<0.0001	d_2	7.646	0.022	d_2	ab	a	b
	plant × time	7.939	<0.0001	d_3	11.856	0.008	d_3	b	a	b
				d_4	47.669	0.0002	d_4	b	a	a
		F	*p*		*F*	*p*		ctrl	C.dem	E.can
Sep	plant	40.682	0.0003	d_1	np	np	d_1	np	np	np
	time	79.566	<0.0001	d_2	24.533	0.001	d_2	a	a	b
	plant × time	8.800	<0.0001	d_3	19.195	0.002	d_3	a	a	b
				d_4	18.888	0.003	d_4	a	a	b

np: not performed (fluorescence-based density not determined because of instrumentation failure).

Table A-II

Paired-sample two-tailed t-tests comparing macrophyte sprig net growth in the presence of each tested phytoplankton taxon, reported visually in Figure 2. Tests were performed on arcsine-transformed data, with final sprig biomass expressed as percent departure from initial condition (initial biomass =100%, biomass accrual >100% and biomass loss <100%); n = 4 and df = 3 for each comparison. Negative t values represent biomass loss from initial condition. Significant t values are highlighted with ○ (p ⩽ 0.05), ○○ (p ⩽ 0.01), and ○○○ (p ⩽ 0.0001).

Ceratophyllum demersum			
	R. subcapitata	***F. pelliculosa***	***S. leopoliensis***
Jun	$t = 6.264$ $p = 0.025^{○}$	$t = 25.324$ $p = 0.002^{○○}$	$t = 5.534$ $p = 0.031^{○}$
Jul	$t = -0.176$ $p = 0.877$	$t = 6.677$ $p = 0.022^{○}$	$t = 0.532$ $p = 0.648$
Aug	$t = 2.078$ $p = 0.173$	$t = 1.174$ $p = 0.361$	$t = 2.786$ $p = 0.108$
Sep	$t = -5.056$ $p = 0.037^{○}$	$t = -0.713$ $p = 0.550$	$t = -5.237$ $p = 0.035^{○}$
Elodea canadensis			
	R. subcapitata	***F. pelliculosa***	***S. leopoliensis***
Jun	$t = -9.346$ $p = 0.011^{○}$	$t = -28.753$ $p = 0.001^{○○}$	$t = -1.660$ $p = 0.239$
Jul	$t = -4.416$ $p = 0.048^{○}$	$t = -3.031$ $p = 0.094$	$t = -12.679$ $p = 0.006^{○○}$
Aug	$t = 3.355$ $p = 0.079$	$t = -0.846$ $p = 0.487$	$t = 0.456$ $p = 0.693$
Sep	$t = -18.571$ $p = 0.003^{○○}$	$t = -5.343$ $p = 0.033^{○}$	$t = -16.065$ $p = 0.004^{○○}$

Table A-III

Pairwise (Z test after z transformation of $\sqrt{r^2}$) and multiple comparisons (Paul, 1988) of coefficients of determinations r^2, and pairwise comparisons of slopes (t-test after Zar, 2009) for the linear regressions in Figure 4. Significantly different values of r^2 according to Zar's (2009) Tukey-type test with significance threshold at p ⩽0.05, following significant Paul's (1988) χ^2-based tests, are identified by different letters ("a" = lowest). Negative values for pairwise comparisons indicate that r^2 or slope for the lefthand regression is lower than for the righthand regression. All tests are two-tailed; df for pairwise comparisons of $r^2 = +\infty$. Statistically significant differences are highlighted with ○ (p ⩽ 0.05), ○○ (p ⩽ 0.01), and ○○○ (p ⩽ 0.0001).

Nutrient-specific comparisons				
	Phosphorus		**Nitrogen**	
r^2	$\chi^2_P = 3.467$ $df = 2$ $p = 0.177$	**multiple comparison: not performed**	$\chi^2_P = 17.131$ $df = 2$	**multiple comparison:** $r^2_{C.dem}$ **c** $r^2_{E.can}$ **b** r^2_{ctrl} **a**
slope	C.dem *vs.* ctrl:	$t = 0.231$ $df = 20$ $p = 0.820$	C.dem *vs.* ctrl:	$t > 1000$ $df = 20$ $p < 0.0001^{○○○}$
	E.can *vs.* ctrl:	$t = -0.428$ $df = 20$ $p = 0.673$	E.can *vs.* ctrl:	$t = 2.730$ $df = 20$ $p = 0.013^{○}$
	C.dem *vs.* E.can:	$t > 1000$ $df = 20$ $p < 0.0001^{○○○}$	C.dem *vs.* E.can:	$t > 1000$ $df = 20$ $p < 0.0001^{○○○}$

Comparisons by experimental condition (N *vs.* P)			
	C.dem	**E.can**	**ctrl**
r^2	$Z = 2.882$ $p = 0.004^{○○}$	$Z = 0.929$ $p = 0.353$	$Z = 3.353$ $p = 0.0008^{○○}$

Table A-IV

Average net nutrient (N, P) daily uptake rate estimates (as $\mu g \cdot g^{-1} \cdot fw \cdot d^{-1}$, ± standard error; n = 3 for each) for macrophyte sprigs in R. subcapitata flasks, by month. Positive numbers indicate net nutrient uptake by sprigs, and negative numbers indicate net nutrient loss from sprigs; • = rates associated with significant algal growth inhibition, as per d_4 HSD tests in Appendix Table A-I.

	C. demersum		*E. canadensis*	
	P	N	P	N
Jun	5.5• ±0.7	24.2• ±1.3	–20.4 ±10.2	5.7 ±4.3
Jul	–1.1 ±1.3	104.6 ±3.9	–6.3 ±1.8	87.1 ±6.6
Aug	5.8• ±0.1	104.0• ±2.9	3.8• ±3.0	97.8• ±12.6
Sep	6.5 ±0.5	3.4 ±4.9	–38.4 ±5.1	–269.3 ±17.0

Table A-V

Inside-vs.-outside macrophyte stand biovolume comparisons (paired-sample two-tailed t-tests) for total phytoplankton and major groups in C. demersum-dominated Spiradammen and E. canadensis-dominated Østensjøvatn. Tests were performed on square-root-transformed, pooled monthly data (in $mm^3 \cdot m^{-3}$); n = 4 and df = 3 for each comparison. Negative t values represent average biovolumes lower at inside-stand sites. Significant t values are highlighted with ○ ($p \leqslant 0.05$), ○○ ($p \leqslant 0.01$), and ○○○ ($p \leqslant 0.0001$).

	Spiradammen	Østensjøvatn
total phytoplankton	$t = 0.659$ $p = 0.557$	$t = -3.321$ $p = 0.045$○
Cyanophyceae	$t = -0.747$ $p = 0.509$	$t = -1.701$ $p = 0.188$
Chlorophyceae	$t = -1.817$ $p = 0.167$	$t = -0.428$ $p = 0.698$
Bacillariophyceae	$t = 0.647$ $p = 0.564$	$t = -1.910$ $p = 0.152$
Chrysophyceae	$t = -0.011$ $p = 0.992$	$t = -0.622$ $p = 0.578$
Cryptophyceae	$t = -0.044$ $p = 0.968$	$t = -1.555$ $p = 0.218$
Dinophyceae	$t = 1.895$ $p = 0.154$	$t = 2.787$ $p = 0.069$

Table A-VI

Inside-vs.-outside macrophyte stand percent relative abundance comparisons (paired-sample two-tailed t-tests) for selected phytoplankton taxa in C. demersum-dominated Spiradammen and E. canadensis-dominated Østensjøvatn. Tests were performed on arcsine-transformed, pooled monthly data; n = 4 and df = 3 for each comparison. Negative t values represent relative abundances lower inside macrophyte stands. Significant differences for p ⩽ 0.05 (○), p ⩽ 0.01 (○○), and p ⩽ 0.0001 (○ ○ ○). Phytoplankton taxa are listed alphabetically; nomenclature after Guiry and Guiry (2012). Taxon groups: BAC = *bacillariophytes (diatoms),* CYA = *cyanobacteria,* CHL = *chlorophytes,* CHR = *chrysophytes,* CRY = *cryptophytes,* DIN = *dinophytes; "–" = taxon absent; "nc" = not calculable (taxon observed in <3 monthly samplings).*

Taxon	Group	Spiradammen	Østensjøvatn
Aulacoseira (=Melosira) italica* var.*tenuissima (Grunow) Simonsen	BAC	—	$t = -0.988$ $p = 0.396$
Botryococcus braunii Kützing	CHL	$t = -1.785$ $p = 0.172$	nc
Ceratium cornutum (Ehrenberg) Claparède & J.Lachmann	DIN	$t = 1.220$ $p = 0.310$	—
Cryptomonas curvata Ehrenberg	CRY	nc	$t = 1.731$ $p = 0.182$
Cryptomonas erosa[1] Ehrenberg	CRY	$t = -1.352$ $p = 0.269$	$t = -1.054$ $p = 0.369$
Dolichospermum (=Anabaena) lemmermannii (P. Richter) P. Wacklin, L. Hoffmann & J. Komárek	CYA	—	$t = -0.648$ $p = 0.563$
Dolichospermum (=Anabaena) planctonicum (Brunnth.) Wacklin, L.Hoffm. & Komárek	CYA	—	$t = -1.005$ $p = 0.389$
Dolichospermum (=Anabaena) spiroides (Kleb.) Wacklin, L.Hoffm. & Komárek	CYA	—	$t = 1.977$ $p = 0.142$
***Fragilaria* spp.**	BAC	—	$t = 0.649$ $p = 0.563$
***Gymnodinium* spp.**	DIN	$t = 0.823$ $p = 0.471$	$t = 1.601$ $p = 0.208$
Katablepharis ovalis Skuja	CRY	$t = 2.703$ $p = 0.074$	$t = 1.040$ $p = 0.375$
***Mallomonas* spp.**	CHR	nc	$t = -1.691$ $p = 0.189$
***Microcystis* spp.**	CYA	—	$t = 0.923$ $p = 0.424$
Monoraphidium (=Ankistrodesmus) contortum (Thuret) Komàrková-Legnerová	CHL	—	$t = -0.552$ $p = 0.619$
Monoraphidium (=Raphidium) minutum (Nägeli) Komárková-Legnerová	CHL	$t = -0.545$ $p = 0.624$	nc
***Peridinium* spp.**	DIN	$t = 0.875$ $p = 0.446$	$t = 1.681$ $p = 0.191$
Raphidocelis (=Pseudokirchneriella) subcapitata (Korshikov) G. Nygaard, J. Komárek, J. Kristiansen & O.M. Skulberg	CHL	$t = -1.689$ $p = 0.190$	nc
Rhodomonas lacustris[2] Pascher & Ruttner	CRY	$t = 0.035$ $p = 0.974$	$t = -1.197$ $p = 0.317$
Scenedesmus arcuatus Lemmermann	CHL	$t = 2.425$ $p = 0.094$	—
***Scenedesmus* spp.**	CHL	—	$t = 1.140$ $p = 0.337$
Stephanodiscus hantzschii[3] Grunow	BAC	—	$t = 2.040$ $p = 0.134$
Uroglenopsis (=Uroglena) americana (Calkins) Lemmermann	CHR	$t = 1.309$ $p = 0.282$	—
Woronichinia (=Gomphosphaeria) naegeliana (Unger) Elenkin	CYA	$t = -0.899$ $p = 0.435$	—

[1] includes var. *reflexa*.
[2] includes var. *nannoplanctonica*.
[3] includes var. *pusillus*.

ESTIMATION OF DAILY NUTRIENT UPTAKE BY EXPERIMENTAL SPRIGS: OUTLINE OF METHODS

Sprig net nutrient (N, P) uptake rates were estimated using Hilt and Lombardo's (2010) concentration-based mass balance approach. The average quantity of nutrients removed by *R. subcapitata* (from control flasks) was subtracted from each flask-specific total (sprig+algal) quantity of nutrients removed in each sprig-present flask. The latter was given by the difference between final (measured) and initial (nominal) concentration (e.g., Hilt and Lombardo, 2010). The quantity of removed nutrients was then divided by average sprig biomass [as (final+initial)/2] and by experimental duration (4 d) to produce daily uptake rate estimates (as μg of N or P removed daily per g of sprig fresh weight) for each plant species in each month. Although such a method does not allow to fully separate nutrient uptake by coexisting macrophytes, periphyton, and phytoplankton, the short experimental time (Lombardo and Cooke, 2003) and the higher macrophyte biomass typically lead to significantly higher total macrophyte than total microalgal uptake rates (Pelton *et al.*, 1998; Hilt and Lombardo, 2010), allowing for a coarse yet sufficiently reliable flask-based estimate of sprig nutrient uptake rates.