Journal of
Plankton Research

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*J. Plankton Res.* (2014) 36(2): 364–377. First published online October 17, 2013 doi:10.1093/plankt/fbt105

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# Experimental blooms of the cyanobacterium *Gloeotrichia echinulata* increase phytoplankton biomass, richness and diversity in an oligotrophic lake

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Received June 26, 2013; accepted September 21, 2013

Corresponding editor: Beatrix E. Beisner

Cyanobacterial blooms are increasing in lakes, both eutrophic and oligotrophic, in many parts of the world. Freshwater cyanobacteria generally have negative effects on eukaryotic phytoplankton in eutrophic systems because of their ability to form dense surface aggregations (scums) that reduce light availability. However, less is known about the effects of cyanobacteria on other phytoplankton in oligotrophic lakes. Because *Gloeotrichia echinulata*, a large colonial cyanobacterium, has been increasingly observed in low-nutrient lakes in the northeastern USA and Canada, we investigated its effects on phytoplankton biomass and community structure. In field and laboratory experiments, high densities of *Gloeotrichia* had significant positive effects on the biomass of small phytoplankton (<30 μm, typically considered edible to zooplankton) relative to no-*Gloeotrichia* controls. Interestingly, *Gloeotrichia* also increased phytoplankton taxa richness and Shannon diversity, primarily by stimulating the richness and biovolume of Bacillariophyta (diatoms) and Chlorophyta (green

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algae). Our laboratory experiment further suggests that at high densities, *Gloeotrichia* may have stimulated the other phytoplankton by leaking nitrogen and phosphorus. Thus, this study suggests that continued increases in *Gloeotrichia* in low-nutrient lakes are likely to increase phytoplankton biomass and alter community structure in these systems.

KEYWORDS: algae; community structure; cyanobacteria; facilitation; food web

## INTRODUCTION

Cyanobacterial blooms are increasing in lakes in many parts of the world, threatening the quality of water used for drinking, recreation and food production (Paerl and Huisman, 2009; Paerl and Paul, 2012; Sinha *et al.*, 2012). While the majority of the bloom increases have been observed in eutrophic systems (Hallegraeff, 1993; Van Dolah, 2000; Anderson *et al.*, 2002; Paerl and Huisman, 2008, 2009), cyanobacteria are also increasing in oligotrophic and mesotrophic systems (Boyer, 2008; Ernst *et al.*, 2009; Winter *et al.*, 2011; Carey *et al.*, 2012). Cyanobacterial blooms in low-nutrient lakes are of particular interest because in comparison with eutrophic lakes, relatively little is known about how they may affect phytoplankton community structure (Carey *et al.*, 2012). For example, in eutrophic lakes, cyanobacteria tend to have inhibitory effects on other phytoplankton because they form surface aggregations (scums) that decrease light, a major factor limiting growth in turbid waters (Mur *et al.*, 1978; Reynolds *et al.*, 1987; Huisman *et al.*, 1999). However, phytoplankton in oligotrophic lakes, which tend to be limited more by nutrients than light, may be stimulated by cyanobacterial blooms if they leak nutrients from live, healthy colonies (Shi *et al.*, 2004; Agawin *et al.*, 2007) or decomposing colonies (Foree and McCarty, 1970; Jewell and McCarty, 1971; Depinto and Verhoff, 1977; Chuai *et al.*, 2011), or if nutrients are released by zooplankton grazing (Schaffner *et al.*, 1994; Fey *et al.*, 2010).

*Gloeotrichia echinulata*, a large colonial cyanobacterium, has been increasingly observed in low-nutrient systems in the northeastern USA and Canada (Carey *et al.*, 2008, 2009, 2012; Winter *et al.*, 2011). In a survey of 36 oligotrophic and mesotrophic lakes in the northeastern USA during 2006–2010, we observed surface scums with densities up to 250 colonies $L^{-1}$, although mean densities were typically low (Carey *et al.*, 2012). Subsequent sampling in 2011 and 2012 of the lakes monitored in Carey *et al.* (Carey *et al.*, 2012) indicates that densities of 50 colonies $L^{-1}$ have been observed in at least three low-nutrient lakes (Lake Auburn, Panther Pond, and Long Pond, all in south central Maine, USA), with a maximum observed littoral density of 450 colonies $L^{-1}$ in Panther Pond in 2011 (H.A.E. *et al.*, unpublished data; see Carey *et al.*, 2012 for more information on those lakes). The cause of the recent increase is unknown (Carey *et al.*, 2008, 2009), but may be related to warmer water temperatures (Karlsson-Elfgren *et al.*, 2004). *Gloeotrichia* has been well studied in high-nutrient systems, especially eutrophic Lake Erken, Sweden, where blooms regularly occur at densities of $<50$ colonies $L^{-1}$ but can reach up to $\geq 5000$ colonies $L^{-1}$ (Hyenstrand *et al.*, 2001; Karlsson-Elfgren *et al.*, 2003; Eiler *et al.*, 2006). However, much less is known about the dynamics and effects of *Gloeotrichia* blooms in low-nutrient systems.

*Gloeotrichia* has several life history characteristics that may cause it to have positive effects on other phytoplankton in low-nutrient lakes. First, *Gloeotrichia* has been shown to stimulate a diverse array of phytoplankton taxa, especially diatoms, in laboratory microcosms (Carey and Rengefors, 2010), indicating that it could potentially stimulate phytoplankton in natural systems. Second, *Gloeotrichia* is similar to other, more well-studied taxa (including *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Cylindrospermopsis raciborskii*) in that it may be able to stimulate other phytoplankton by fixing nitrogen (N; Stewart *et al.*, 1967; Roelofs and Oglesby, 1970) and transporting phosphorus (P) from the sediments into the water column during recruitment from its benthic dormant stages (Istvánovics *et al.*, 1993; Pettersson *et al.*, 1993). In eutrophic Lake Erken, Sweden and Green Lake, Washington, USA, *Gloeotrichia* recruitment from the sediments can contribute up to two-thirds of the total summer internal P load (Barbiero and Welch, 1992; Istvánovics *et al.*, 1993). Similar to other cyanobacteria (Ray and Bagchi, 2001; Wetzel, 2001; Agawin *et al.*, 2007), *Gloeotrichia* may be able to release both N and P to the water column in available forms, thereby facilitating other phytoplankton if nutrients are limiting (Pitois *et al.*, 1997; Nõges *et al.*, 2004).

Here, we conducted two experiments to examine the effects of experimental *Gloeotrichia* blooms on natural phytoplankton communities in oligotrophic lakes in the absence of large grazing zooplankton. In an *in situ* mesocosm experiment, we specifically assessed whether we



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could detect any stimulation of phytoplankton by *Gloeotrichia*, and focused on biotic response variables. In a laboratory microcosm experiment, we examined whether *Gloeotrichia* could stimulate other phytoplankton by increasing N and P concentrations. In both experiments, we chose bloom treatments that span the range of currently observed *Gloeotrichia* densities in low-nutrient lakes, as well as higher densities that represent potential future bloom scenarios.

## METHOD

### Mesocosm experiment

We deployed *in situ* mesocosms in July 2008 in a sheltered cove of oligotrophic Lake Sunapee, NH, USA (43°24′N, 72°20′W; for a complete lake description, see Carey *et al.*, 2008). We specifically focused on near-shore phytoplankton in this experiment because buoyant *Gloeotrichia* colonies accumulate in downwind shallow coves due to wind and currents (Carey *et al.*, 2008, 2012). We suspended 16 clear polyethylene bags (~50 L water volume, 0.7 m deep) from two 4.9-m long wooden floating frames anchored in the littoral zone. The tops of the enclosures were covered with 1.5 mm mesh to prevent zooplankton immigration via birds and were situated 0.2 m above the lake surface to prevent waves from overtopping the mesocosm rims. We filled the mesocosms with unfiltered water obtained from the upper 0.3 m of the lake and let them equilibrate for 24 h prior to adding *Gloeotrichia*. Our mesocosms included only small zooplankton (rotifers and nauplii) because we filled them at midday with surface water 12 days after the summer solstice, when incident and ultraviolet (UV) light were near maximum levels; most large zooplankton seek refuge at depth during the day from visual predators and UV radiation (Hairston, 1980; Lampert, 1989; Leech and Williamson, 2001). To confirm that only small zooplankton were present in the mesocosms, we sampled zooplankton densities at the beginning and end of the experiment (see below).

We randomly assigned four *Gloeotrichia* density treatments: 0 (control), 25, 50 and 400 colonies $L^{-1}$, with four replicates each and blocked the treatments by frame. These treatments bracket the range of *Gloeotrichia* densities (0–450 colonies $L^{-1}$) that we have observed in low-nutrient lakes in the northeastern USA (H.A.E *et al.*, unpublished data; Carey *et al.*, 2012). In designing this experiment, we quantified the *Gloeotrichia* treatments in units of colonies $L^{-1}$, rather than biovolume, to follow the precedent of earlier experimental and monitoring studies on *Gloeotrichia* (e.g. Roelofs and Oglesby, 1970; Barbiero and Welch, 1992; Hyenstrand *et al.*, 2001; Karlsson-Elfgren *et al.*, 2005), and because it was logistically not possible to estimate the biovolume of each of the ~95 000 colonies used in the experiment. We calculated the total number of colonies needed for each mesocosm by multiplying the bag volume by the treatment density.

Two days before the experiment began, we collected *Gloeotrichia* colonies from Lake Sunapee with a plankton net (0.5 m diameter, mesh size 100 μm). Colonies were gently rinsed into 1 L bottles and transported to the laboratory, where they were rinsed three times with filtered (Whatman GF/C, 1.2-μm pore size) Lake Sunapee water. After rinsing, the colonies were individually inspected with a dissecting microscope to manually remove any adhered debris and plankton with micro-scalpels and probes. Only the largest buoyant colonies with all of their trichomes intact were chosen for the experiment. We cleaned the colonies in aliquots of 100 with a dissecting microscope, and haphazardly assigned aliquots to treatments.

Until the experiment started, we kept the colonies at low densities to prevent light limitation in 1 L bottles filled with Whatman GF/C-filtered Lake Sunapee water. The bottles were placed in incubators set at 20°C on a 14:10 h light:dark cycle that approximated natural conditions and were swirled every 12 h. We monitored the health of the colonies throughout this period to determine whether the cleaning procedure affected their survival. Less than 1% of the colonies senesced during this time, as indicated by a loss of green coloration and buoyancy.

The day of the experiment, the Gloeotrichia were kept in the bottles in shade onshore during pre-treatment mesocosm sampling; they were then added to the mesocosms to create the four treatments. We sampled each mesocosm plus two littoral sites adjacent to the frames 24 h after *Gloeotrichia* addition, and then every 4–6 days for 20 days. The littoral sites provided a reference for mesocosm effects on response variables.

On each sampling day, we measured nutrients and plankton. We monitored dissolved oxygen and temperature at the water surface and at 0.5 m depth with a 556 MPS meter (YSI, Inc., Yellow Springs, OH, USA). With an integrated tube sampler (0.5 m long, 5.1 cm diameter), we collected 1 L from five locations within each mesocosm and pooled the 5 L in a clean bucket. We retained 1 L of this water for chlorophyll *a* filtering, 250 mL for phytoplankton and 500 mL for nutrients and returned the unused water to the mesocosm. Water for phytoplankton analyses was collected on each sampling day except the last. We immediately preserved the phytoplankton in opaque bottles with Lugol's iodine solution and stored the bottles in darkness until analysis. On the majority of sampling dates, we then filtered 3 L of water through 80 μm mesh for samples of *Gloeotrichia* density (preserved with Lugol's iodine solution) and returned the

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filtrate to the mesocosms. However, on the first and last sampling day, we collected an additional 5 L from each mesocosm and filtered it through 80 μm mesh for samples of both *Gloeotrichia* and zooplankton density; samples were immediately preserved with 70% ethanol and the filtrate was returned to the mesocosms.

We processed the chlorophyll *a*, nutrient and plankton samples according to standard protocols immediately upon returning to the laboratory. We measured both total and <30 μm [pre-filtered through a Nitex mesh; Cottingham *et al.*, 2004; hereafter, referred to as 'small-sized' (ss)] chlorophyll *a* by vacuum-filtering each sample onto Whatman GF/C filters, extracting them in methanol for 24 h and determining the chlorophyll *a* concentration using a fluorometer (Turner Designs TD 700, Sunnyvale, CA, USA) according to Arar and Collins (Arar and Collins, 1997). As *Gloeotrichia* colonies are 1−3 mm in diameter in Lake Sunapee (Carey *et al.*, 2008), they were excluded from the ss-chlorophyll *a* fraction, which generally represents a phytoplankton size fraction that zooplankton are able to efficiently graze (Lampert *et al.*, 1986; Cyr, 1998; Meyer *et al.*, 2006).

Of the 500 mL we collected for nutrient analyses, we retained 125 mL for total nutrients (total N and total P; hereafter, TN and TP), and filtered the remaining water through 0.7-μm pore size (Whatman GF/F) filters for ammonium ($NH_4^+$), nitrate ($NO_3^-$) and soluble reactive P (SRP) analyses. All soluble and total nutrient samples were frozen until analysis. TN samples were analyzed with spectrophotometric methods after basic persulfate digestion [method detection limit (MDL) = 5.3 μM]. $NO_3^-$ and $NH_4^+$ samples were analyzed on a Lachat QuikChem 8000 (Lachat Instruments, Loveland, CO, USA) according to the QuikChem Phenate method #10-107-106-1-J and QuikChem Cadmium Reduction method #10-107-04-1-A, respectively, in the Cary Institute of Ecosystem Studies' Analytical Laboratory ($NO_3^-$ MDL = 0.16 μM; $NH_4^+$ MDL = 0.54 μM). Both TP and SRP were analyzed according to Van Veldhoven and Mannaerts (Van Veldhoven and Mannaerts, 1987) by colorimetric analysis on a plate reader, with an acidic persulfate digestion for TP samples. Plate reader failure during analysis resulted in a high MDL for both fractions of P (0.25 μM); thus, we report here only the TP samples above the MDL, which essentially indicates samples in the mesotrophic range and above, given that the threshold for the oligotrophic and mesotrophic transition is ~0.32 μM (Nürnberg, 1996). Throughout the experiment, SRP, $NO_3^-$ and $NH_4^+$ concentrations were consistently below their MDL and will not be reported here.

We settled and concentrated 50 mL of each phytoplankton sample for 3 days and identified and enumerated the cells and colonies to genus at ×400 magnification according to Utermöhl (Utermöhl, 1958) on an inverted microscope. We calculated non-*Gloeotrichia* phytoplankton biovolume ($\mu m^3\ mL^{-1}$) by approximating the cells to known geometric shapes using measured linear dimensions (Olrik *et al.*, 1998). We determined taxon richness, Shannon diversity (Shannon, 1948) and evenness for the entire non-*Gloeotrichia* phytoplankton assemblage within each mesocosm on every sampling day, as well as biovolume and richness within each of the phytoplankton divisions. In addition, we counted *Gloeotrichia* colonies and identified zooplankton to genus with a dissecting microscope.

We determined the effects of the *Gloeotrichia* treatments on chlorophyll *a*, phytoplankton community structure and nutrient concentrations after *Gloeotrichia* addition with one-way repeated measures (RM) ANOVA using SAS PROC GLM (SAS version 9.2, SAS Institute, Cary, NC, USA; Wolfinger and Chang, 1999) with the Greenhouse-Geisser correction to meet assumptions of compound symmetry, and assessed significance at $\alpha = 0.05$. We interpreted *Gloeotrichia* effects from both the main effect of treatment (*Gloeotrichia*) effects and the time × treatment interaction. We compared the differences between the three +*Gloeotrichia* treatments (400, 50 and 25 colonies $L^{-1}$) and the no-*Gloeotrichia* control with Bonferroni-corrected linear contrasts for each response variable, averaged across the time series (Maxwell, 1980).

Two-thirds of the TP concentrations were below the MDL (i.e. in the oligotrophic range), which prevented the use of RM ANOVA. As a result, for TP only, we calculated for each mesocosm the proportion of samples after *Gloeotrichia* addition that were above the MDL. We then analyzed the effect of *Gloeotrichia* density on the mean proportion of samples in a given treatment above the MDL using one-way ANOVA (JMP 8.0, SAS Institute).

We analyzed the change in zooplankton density from the initial to the final sampling (last sample − first sample) for each mesocosm with one-way ANOVA (JMP 8.0).

The littoral reference sites were excluded from the statistical analyses described above to maintain a balanced design. To assess any potential effects of the mesocosms, we compared differences in all of the nutrient and phytoplankton response variables between the 0 colonies $L^{-1}$ and lake reference treatments with separate one-way RM ANOVA. We used a Welch *t*-test to evaluate differences in zooplankton density between the 0 colonies $L^{-1}$ and lake reference treatments (JMP 8.0).

## Laboratory experiment

To determine whether *Gloeotrichia* influenced nutrient concentrations and phytoplankton biomass in oligotrophic lake water across a very broad range of *Gloeotrichia*



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densities, we tested the effect of a gradient of 10 *Gloeotrichia* densities on ss phytoplankton (<30 μm chlorophyll *a*), TN and TP in Erlenmeyer flasks. The treatments included *Gloeotrichia* densities currently measured in oligotrophic and mesotrophic lakes (up to 450 colonies $L^{-1}$), as well as much higher densities to represent potential future bloom scenarios if *Gloeotrichia* densities increase to levels observed in eutrophic lakes.

We collected 20 L of unfiltered water with a 4 L Van Dorn sampler (Wildlife Supply Co., Saginaw, MI, USA) from 0.5 m depth in Lake Sunapee and homogenized the water in a carboy. We brought the carboy back to the laboratory and kept it in the dark at room temperature for ~12 h until the beginning of the experiment. Because of low *Gloeotrichia* densities in Lake Sunapee at the time of this study (July 2010), we collected colonies as described previously but from mesotrophic Lake Morey (43°55′N, 72°8′W, Fairlee, VT, USA; 2.21 $km^2$ total surface area; 13 m max depth; Vermont Dept. of Environmental Conservation). Thus, we set up the experiment using oligotrophic Lake Sunapee water and Lake Morey colonies. We do not think that the origin of the colonies affected the experiment because colonies from both lakes appeared identical in density of trichomes, colony size and coloration when examined at ×160 magnification under a dissecting microscope. In addition, all of the Morey colonies were thoroughly cleaned as described above in filtered oligotrophic Sunapee water before the experiment began.

At the beginning of the experiment, we collected 3 L of water from the carboy, filtered it through 30 μm mesh, and used the filtrate to determine background TN, TP and <30 μm chlorophyll *a* (hereafter, ss-TN, TP and chlorophyll *a*). We filled thirty 500 mL acid-washed Erlenmeyer flasks with 400 mL of the water and randomly assigned treatments of 0, 25, 50, 100, 200, 400, 800, 1600, 3200 and 6400 colonies $L^{-1}$ with three replicates each. We prepared the *Gloeotrichia* treatments as described previously, and calculated how many colonies were needed for each flask by multiplying the water volume by the density treatment. After *Gloeotrichia* addition, the flasks were kept in an incubator at 25°C for 5 days under a 14:10 h light: dark cycle and swirled twice daily. We monitored the condition of the *Gloeotrichia* colonies by checking if any colonies senesced throughout the experiment, as indicated by the loss of green coloration and buoyancy.

At the end of the experiment, we filtered the contents of each flask through 30 μm mesh to remove all *Gloeotrichia* colonies and used the filtrate for ss-chlorophyll *a*, ss-TN and ss-TP analyses. Ss-chlorophyll *a* and ss-TN were analyzed as described above, whereas ss-TP was measured with a higher resolution method spectrophotometerically (MDL = 0.036 μM, Method 4500-P; APHA, 1980). We assessed whether *Gloeotrichia* increased total nutrients in the fraction of the flask contents that were <30 μm because soluble nutrient concentrations were likely below detection. Although this method did not directly measure *Gloeotrichia* nutrient release, we expected that nutrient uptake by phytoplankton and microbes in the flasks would be extremely rapid (Hutchinson and Bowen, 1950; Lean, 1973; Hudson *et al.*, 2000) and thus any released nutrients would be quickly incorporated into biomass and measurable in the total nutrient fraction.

We $\log_e$-transformed our response variables to equalize variance and used model selection in R 2.15.2 (R Development Core Team, 2012) to determine the best-fitting regression model predicting ss-chlorophyll *a*, ss-TN and ss-TP from *Gloeotrichia* density. We found maximum likelihood estimates for the parameters in four models often used to describe the relationship between phytoplankton and nutrient uptake: mean, linear and Monod with and without an intercept term (Monod, 1949) using a simulating annealing algorithm (R package nlme) with 10 000 iterations and a normally distributed error term; we chose the best model using the corrected Akaike Information Criterion (AICc; Burnham and Anderson, 2002).

Finally, to examine the density threshold at which the effect of *Gloeotrichia* on the response variables was significantly different from the no-*Gloeotrichia* controls, we treated *Gloeotrichia* density as a categorical variable in a one-way ANOVA with Tukey's Honestly Significant Difference tests and used linear regression to test whether ordinal density treatments predicted responses (JMP 8.0).

## RESULTS

### Mesocosm experiment

Prior to *Gloeotrichia* addition, there were no significant differences in *Gloeotrichia* density; total and ss-chlorophyll *a*; non-*Gloeotrichia* phytoplankton biovolume, richness, Shannon diversity and evenness; TN or non-*Gloeotrichia* phytoplankton division biovolume and richness among the treatments (one-way ANOVA, all $P \geq 0.14$). All TP concentrations prior to *Gloeotrichia* addition were in the oligotrophic range and below the MDL. Throughout the experiment, temperature and dissolved oxygen in the mesocosms stayed fairly constant: measured temperatures ranged from 21.8 to 24.3°C (mean = 23.4 ± 0.06°C; 1 SE) and measured dissolved oxygen concentrations ranged from 7.0 to 8.5 mg $L^{-1}$ (mean = 7.7 ± 0.02 mg $L^{-1}$). Mesocosm effects (as judged from comparison of the 0 colonies $L^{-1}$ treatment and the lake reference) were not statistically significant throughout the experiment



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for all response variables except for TN ($F_{1,4} = 12.81$, $P = 0.02$; all other variables had $P > 0.11$). TN tended to be lower (on average, by $<3.6$ μM) outside than inside the mesocosms (Fig. 1; Supplementary data, Table SI), but this difference was likely not biologically meaningful given the MDL of the TN samples.

*Gloeotrichia* densities in the mesocosms reflected the colony addition treatments; effects of *Gloeotrichia*, time and their interaction were significant (each had $P < 0.0001$; Fig. 1A and B; Table I). Further, addition of *Gloeotrichia* resulted in surface scums in all of the 50 and 400 colonies $L^{-1}$ mesocosms, which lasted for ~12 days. All of the added *Gloeotrichia* senesced and sank to the bottom of the mesocosms after 13 days.

Not surprisingly, *Gloeotrichia* addition had significant positive effects on total chlorophyll *a*, which included *Gloeotrichia* as well as other phytoplankton ($P < 0.0001$; Fig. 1C and D; Table I). The *Gloeotrichia* effect was mediated by time due to fluctuations in the 400 colonies $L^{-1}$ treatment. Total chlorophyll *a* concentrations were 260% higher in the 400 colonies $L^{-1}$ treatment than in the no-*Gloeotrichia* control at the end of the experiment, even after the *Gloeotrichia* density decreased.

Importantly, we also observed that *Gloeotrichia* addition had strong positive effects on the biomass and biovolume of other phytoplankton taxa within 24 h, well before the *Gloeotrichia* colonies senesced and sank to the bottom of the mesocosms. Both ss-chlorophyll *a* ($<30$ μm, which excluded *Gloeotrichia* and other large taxa) and non-*Gloeotrichia* phytoplankton biovolume responded significantly to *Gloeotrichia* addition (both $P \leq 0.0003$; Fig. 1E–H; Table I); the phytoplankton biovolume response was mediated by an interaction with time ($P = 0.02$). Both ss-chlorophyll *a* and non-*Gloeotrichia* phytoplankton biovolume exhibited significantly higher (up to 180 and 2500%, respectively) concentrations in the 400 colonies $L^{-1}$ treatment in comparison with the no-*Gloeotrichia* control (linear contrast: both $P < 0.0001$).

Microscopic examination of the phytoplankton community also showed that treatments with high *Gloeotrichia* density had an altered phytoplankton community composition, as indicated by significantly greater richness and diversity (both $P \leq 0.005$; Fig. 2; Table I). Richness, Shannon diversity and evenness were 48, 39 and 24% higher, respectively, in the 400 colonies $L^{-1}$ treatment than the no-*Gloeotrichia* control after *Gloeotrichia* addition. While we did not detect effects of *Gloeotrichia* on Shannon diversity and evenness in the 25 colonies $L^{-1}$ or 50 colonies $L^{-1}$ treatments, the 50 colonies $L^{-1}$ treatment did exhibit significantly higher richness than the non-*Gloeotrichia* control ($P = 0.002$).

The increase in biovolume, richness and diversity in the non-*Gloeotrichia* phytoplankton community was primarily driven by increases in the biovolume and richness of Bacillariophyta (diatoms) and Chlorophyta (green algae) in the 400 colonies $L^{-1}$ *Gloeotrichia* treatment (Fig. 3, Supplementary data, Table SI). At the end of the experiment, the biovolumes of Bacillariophyta and Chlorophyta were >5800 and 6400% higher, respectively, in the 400 colonies $L^{-1}$ treatment relative to the no-*Gloeotrichia* control, and the richnesses of Bacillariophyta and Chlorophyta were >60 and 140% higher, respectively. The 50 colonies $L^{-1}$ treatment also exhibited significantly higher Chlorophyta richness (by 140%) than the control ($P = 0.01$).

*Gloeotrichia* also increased the richness, but not biovolume, of Chrysophyta (brown algae) and Dinophyta (dinoflagellates), and the biovolume, but not richness, of Cryptophyta (cryptophytes) and Euglenophyta (euglenoids), with the Chrysophyta and Dinophyta effects mediated by time (Supplementary data, Table SI). The effects of *Gloeotrichia* on Bacillariophyta, Dinophyta and Euglenophyta biovolume, and Chrysophyta and Dinophyta richness, were mediated by time (all $P \leq 0.05$). Neither the richness nor biovolume of non-*Gloeotrichia* Cyanobacteria responded to the *Gloeotrichia* treatments (all $P \geq 0.09$), though there were significant changes through time (both $P \leq 0.003$). Importantly, no division declined in biovolume or richness after *Gloeotrichia* addition.

Data for the highest *Gloeotrichia* density treatment suggest that *Gloeotrichia* may have stimulated other phytoplankton by increasing nutrients. *Gloeotrichia* additions significantly altered TN ($P < 0.0001$; Fig. 1I and J): the 400 colonies $L^{-1}$ treatment exhibited 74 ± 17% (1 SE) higher TN concentrations than the no-*Gloeotrichia* treatment immediately after *Gloeotrichia* addition (linear contrast $P < 0.0001$). *Gloeotrichia* additions also altered the proportion of TP samples that were above the MDL (one-way ANOVA, $F_{3,12} = 9.63$, $P = 0.002$; Supplementary data, Fig. SI): 80 ± 8% of all TP samples in the 400 colonies $L^{-1}$ treatment were above the MDL and in the mesotrophic range throughout the experiment (reaching 0.77 μM), significantly higher than the 25 ± 10% observed in the no-*Gloeotrichia* control (Tukey's HSD test, $P = 0.006$).

Finally, we observed no significant differences among treatments in total zooplankton, rotifer, copepod or cladoceran density during the experiment (all $P \geq 0.33$).

## Laboratory experiment

Ss-chlorophyll *a*, ss-TN and ss-TP in the $<30$ μm water fraction increased non-linearly with *Gloeotrichia* density (Fig. 4). All three variables were strongly correlated ($r >$ 0.84 for $\log_e$-transformed data) and were described best by a Monod model with an intercept term (Supplementary



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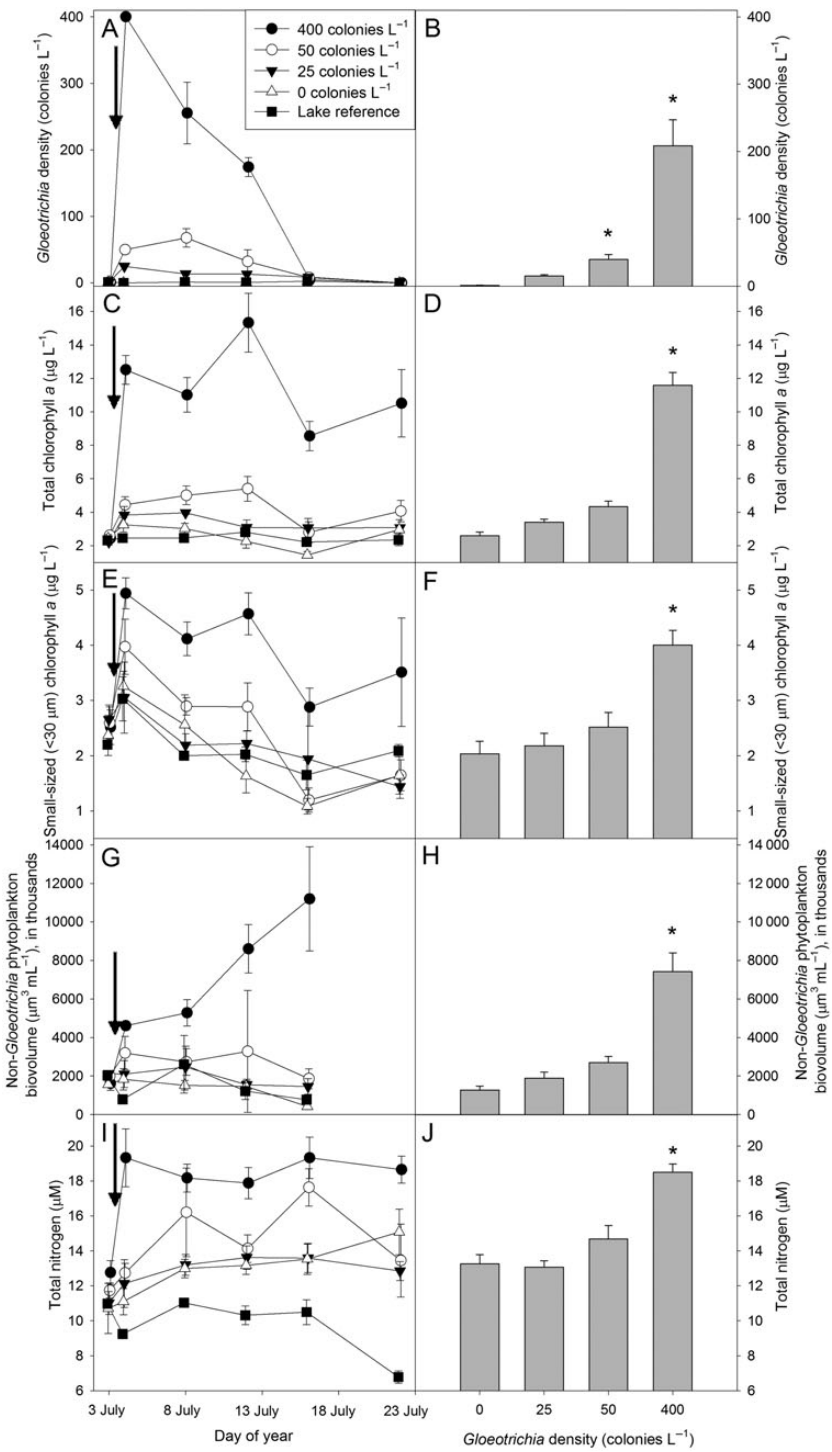


**Fig. 1.** The effect of the *Gloeotrichia* treatments on: (**A**) *Gloeotrichia* density (± 1 SE); (**C**) total chlorophyll *a;* (**E**) ss-chlorophyll *a*; (**G**) non-*Gloeotrichia* phytoplankton biovolume and (**I**) total nitrogen concentrations in the mesocosms over time. The 400, 50, 25 and 0 colonies $L^{-1}$ treatments were manipulated experimentally within the mesocosms; the lake reference treatment refers to the lake outside the mesocosms. Small-sized chlorophyll *a* refers to the <30 µm fraction, and the arrow indicates the day of *Gloeotrichia* addition. Total and ss-chlorophyll *a* were determined with fluorometry; the non-*Gloeotrichia* phytoplankton biovolume was determined with microscopy. Phytoplankton samples were not collected on 22 July. The mean (± 1 SE) effect of *Gloeotrichia* in the 0, 25, 50 and 400 colonies $L^{-1}$ treatments across all sampling dates after *Gloeotrichia* addition on: (**B**) *Gloeotrichia* density; (**D**) total chlorophyll *a*; (**F**) ss-chlorophyll *a*; (**H**) non-*Gloeotrichia* phytoplankton biovolume and (**J**) total nitrogen concentration. The asterisks denote treatments that were significantly different from the control using linear contrasts.

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*Table I: Statistical results for the mesocosm experiment in Lake Sunapee, NH, USA: repeated measures one-way ANOVA testing the effects of the* Gloeotrichia *treatments, time, and a* Gloeotrichia *treatment × time interaction on the response variables after* Gloeotrichia *addition*

| Response Variable | Factor | DF | *F*-value | *P*-value |
|---|---|---|---|---|
| *Gloeotrichia* density | *Gloeotrichia* | **3.12** | **286.64** | **<0.0001** |
| | 0 vs. 400 colonies $L^{-1}$ contrast | **1.12** | **664.84** | **<0.0001** |
| | 0 vs. 50 coonies $L^{-1}$contrast | **1.12** | **22.74** | **0.0005** |
| | 0 vs. 25 colonies $L^{-1}$ contrast | 1.12 | 3.01 | 0.11 |
| | Time | **4.48** | **67.50** | **<0.0001** |
| | *Gloeotrichia* × Time | **12.48** | **42.76** | **<0.0001** |
| Total chlorophyll *a* | *Gloeotrichia* | **3.12** | **63.45** | **<0.0001** |
| | 0 vs. 400 colonies $L^{-1}$ contrast | **1.12** | **150.34** | **<0.0001** |
| | 0 vs. 50 colonies $L^{-1}$ contrast | 1.12 | 5.73 | 0.03 |
| | 0 vs. 25 colonies $L^{-1}$ contrast | 1.12 | 1.25 | 0.29 |
| | Time | **4.48** | **7.77** | **0.002** |
| | *Gloeotrichia* × Time | **12.48** | **2.91** | **0.008** |
| Small-sized chlorophyll *a* (<30 μm) | *Gloeotrichia* | **3.11** | **15.97** | **0.0003** |
| | 0 vs. 400 colonies $L^{-1}$ contrast | **1.11** | **39.57** | **<0.0001** |
| | 0 vs. 50 colonies $L^{-1}$ contrast | 1.11 | 2.39 | 0.15 |
| | 0 vs. 25 colonies $L^{-1}$ contrast | 1.11 | 0.29 | 0.60 |
| | Time | **4.44** | **13.03** | **<0.0001** |
| | *Gloeotrichia* × Time | 12.44 | 0.83 | 0.58 |
| Non-*Gloeotrichia* phytoplankton biovolume | *Gloeotrichia* | **3.9** | **21.43** | **0.0002** |
| | 0 vs. 400 colonies $L^{-1}$ contrast | **1.9** | **46.77** | **<0.0001** |
| | 0 vs. 50 colonies $L^{-1}$ contrast | 1.9 | 2.17 | 0.17 |
| | 0 vs. 25 colonies $L^{-1}$ contrast | 1.9 | 0.12 | 0.74 |
| | Time | 3.27 | 0.92 | 0.40 |
| | *Gloeotrichia* × Time | **9.27** | **3.80** | **0.02** |
| Total nitrogen | *Gloeotrichia* | **3.9** | **28.29** | **<0.0001** |
| | 0 vs. 400 colonies $L^{-1}$ contrast | **1.9** | **61.45** | **<0.0001** |
| | 0 vs. 50 colonies $L^{-1}$ contrast | 1.9 | 1.19 | 0.30 |
| | 0 vs. 25 colonies $L^{-1}$ contrast | 1.9 | 0.27 | 0.62 |
| | Time | 4.36 | 1.78 | 0.19 |
| | *Gloeotrichia* × Time | 12.36 | 1.64 | 0.18 |
| Non-*Gloeotrichia* taxa richness | *Gloeotrichia* | **3.9** | **20.65** | **0.0002** |
| | 0 vs. 400 colonies $L^{-1}$ contrast | **1.9** | **57.04** | **0.0009** |
| | 0 vs. 50 colonies $L^{-1}$ contrast | **1.9** | **17.41** | **0.002** |
| | 0 vs. 25 colonies $L^{-1}$ contrast | 1.9 | 4.81 | 0.07 |
| | Time | **3.27** | **16.84** | **<0.0001** |
| | *Gloeotrichia* × Time | 9.27 | 1.31 | 0.30 |
| Non-*Gloeotrichia* Shannon diversity | *Gloeotrichia* | **3.9** | **8.53** | **0.005** |
| | 0 vs. 400 colonies $L^{-1}$ contrast | **1.9** | **24.27** | **0.0008** |
| | 0 vs. 50 colonies $L^{-1}$ contrast | 1.9 | 5.83 | 0.04 |
| | 0 vs. 25 colonies $L^{-1}$ contrast | 1.9 | 2.77 | 0.13 |
| | Time | **3.27** | **26.14** | **<0.0001** |
| | *Gloeotrichia* × Time | 9.27 | 1.26 | 0.32 |
| Non-*Gloeotrichia* taxa evenness | *Gloeotrichia* | **3.9** | **4.20** | **0.04** |
| | 0 vs. 400 colonies $L^{-1}$ contrast | **1.9** | **12.08** | **0.007** |
| | 0 vs. 50 colonies $L^{-1}$ contrast | 1.9 | 3.44 | 0.097 |
| | 0 vs. 25 colonies $L^{-1}$ contrast | 1.9 | 1.64 | 0.23 |
| | Time | **3.27** | **33.61** | **<0.0001** |
| | *Gloeotrichia* × Time | 9.27 | 1.29 | 0.30 |

The contrasts refer to linear contrasts between the no-*Gloeotrichia* control and the 400, 50 and 25 colonies $L^{-1}$ treatments, which were Bonferroni corrected ($\alpha = 0.0167$). DF denotes degrees of freedom (numerator, denominator), and significant effects are in bold.

data, Table SII). We did not observe any mortality of *Gloeotrichia* colonies (assessed by the loss of green coloration and buoyancy) during the 5-day experiment.

Finally, the *Gloeotrichia* density at which the ss-chl *a*, ss-TN and ss-TP were significantly different from the no-*Gloeotrichia* control occurred between 400 and 800 colonies $L^{-1}$ for ss-chl *a* and ss-TP, and between 800 and 1600 colonies $L^{-1}$ for ss-TN (Tukey's HSD test). All three response variables exhibited a significant increasing trend (ordinal regression $P < 0.0001$).

## DISCUSSION

In the absence of large grazing zooplankton, high densities of *Gloeotrichia* increased the biomass of other phytoplankton in both our field and laboratory experiments, as



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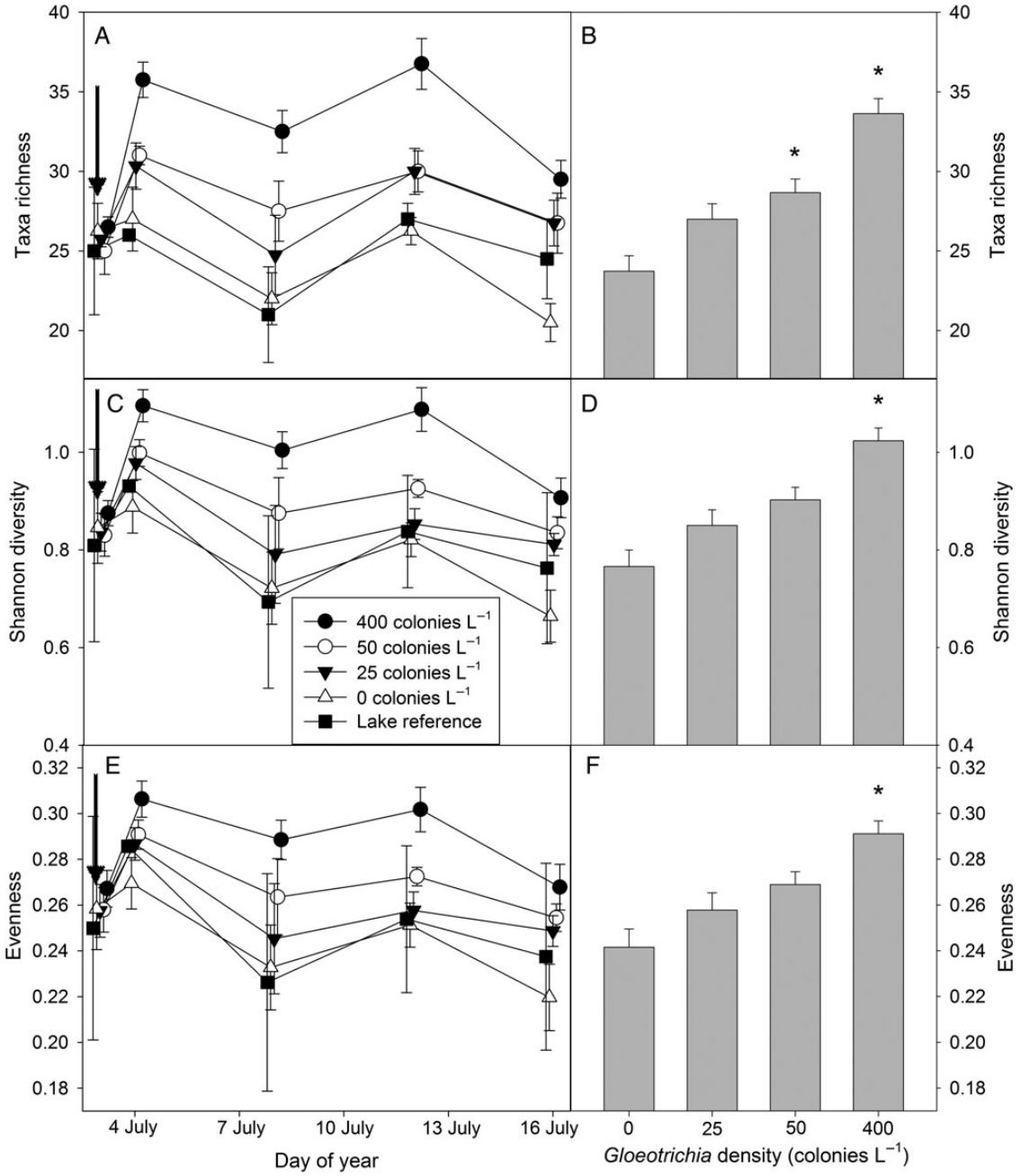


**Fig. 2.** The effect of the *Gloeotrichia* treatments on the (**A**) taxa richness (±1 SE); (**C**) Shannon diversity and (**E**) evenness of the non-*Gloeotrichia* phytoplankton community over time. The 400, 50, 25 and 0 colonies $L^{-1}$ treatments were manipulated experimentally within the mesocosms; the lake reference treatment refers to the lake outside the mesocosms. The arrow indicates the day of *Gloeotrichia* addition. The mean (±1 SE) effect of *Gloeotrichia* in the 0, 25, 50 and 400 colonies $L^{-1}$ treatments across all sampling dates after *Gloeotrichia* addition on: (**B**) taxa richness; (**D**) Shannon diversity and (**F**) evenness. The asterisks denote treatments that were significantly different from the control using linear contrasts.

supported by several lines of evidence. First, in the field mesocosm experiment, the biovolume of the entire non-*Gloeotrichia* fraction of phytoplankton (not only the <30 μm fraction) increased in response to the highest density of *Gloeotrichia* (Fig. 1G). Moreover, the total chlorophyll *a* concentration in the 400 colonies $L^{-1}$ treatment remained higher than the no-*Gloeotrichia* control throughout the experiment (Fig. 1C), despite decreases in the density of *Gloeotrichia* (Fig. 1A) and ss-chlorophyll *a* (Fig. 1E) over time, suggesting that the >30 μm fraction of non-*Gloeotrichia* phytoplankton must have increased. Second, the immediate increase in ss-chlorophyll *a* in the



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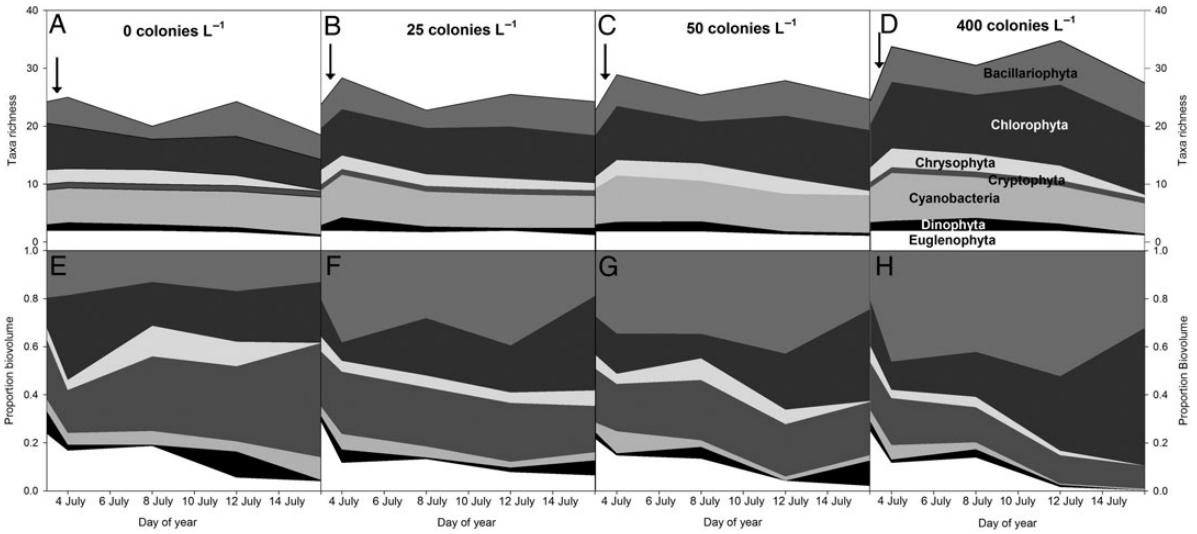


**Fig. 3.** Phytoplankton community composition in the *Gloeotrichia* treatments. The number of taxa in the non-*Gloeotrichia* phytoplankton community over time in the: (**A**) 0 colonies $L^{-1}$; (**B**) 25 colonies $L^{-1}$; (**C**) 50 colonies $L^{-1}$ and (**D**) 400 colonies $L^{-1}$ *Gloeotrichia* treatments. The shaded segments denote the taxa richness of seven different phytoplankton divisions. The proportion of the non-*Gloeotrichia* phytoplankton biovolume in each phytoplankton division in the (**E**) 0 colonies $L^{-1}$; (**F**) 25 colonies $L^{-1}$; (**G**) 50 colonies $L^{-1}$ and (**H**) 400 colonies $L^{-1}$ treatments.

400 colonies $L^{-1}$ treatment following *Gloeotrichia* addition represents true stimulation of phytoplankton growth, primarily diatoms and green algae; no detached *Gloeotrichia* trichomes were observed in the phytoplankton counts (C.C.C., personal observation). We think it unlikely that these increases were due to cells attached to *Gloeotrichia* colonies because (i) individual colonies were cleaned thoroughly in both experiments, and (ii) ss-chlorophyll *a* did not increase linearly with *Gloeotrichia* density in the laboratory experiment (Fig. 4, Supplementary data, Table SII). In addition, the increases in phytoplankton observed in the 400 colonies $L^{-1}$ mesocosms are consistent with potential growth rates for phytoplankton. At 22°C, the mesocosm temperature at the beginning of the experiment, the density of Chlorophyta cells increased at a growth rate ($r$) of 1.2 per day after *Gloeotrichia* addition. This growth rate is consistent with observed growth rates of different Chlorophyta taxa at 20°C (Reynolds, 2006).

Importantly, our work demonstrates that high densities of *Gloeotrichia* did not merely increase the biomass of primary producers: *Gloeotrichia* additions also increased the richness and diversity of other phytoplankton taxa. Our mesocosm results are consistent with previous laboratory experiments in which *Gloeotrichia* increased the growth rates of a diverse array of phytoplankton taxa, and in which some taxa responded more positively than others (Carey and Rengefors, 2010). After 96 h of co-incubation with *Gloeotrichia*, the growth rate ($r$) of the diatom *Cyclotella* sp. (Bacillariophyta) increased substantially more than any other taxon tested in those experiments, which included species in the Chrysophyta, Cryptophyta, Cyanobacteria and Dinophyta (the study did not include Chlorophyta; Carey and Rengefors, 2010). Many Bacillariophyta have been characterized as opportunistic species that can quickly respond to nutrient pulses (Kilham and Kilham, 1980) and several taxa within the Chlorophyta are considered 'invasive opportunists' for their ability to quickly increase their growth rate in response to increased resources (Reynolds, 2006). This may explain why the immediate stimulation following addition of *Gloeotrichia* occurred in the Bacillariophyta and Chlorophyta more than in any other group in the present study (Fig. 3). Although increases in nutrients are well-documented after the decomposition of cyanobacterial blooms (e.g. Engstrom-Ost *et al.*, 2002; Hambright *et al.*, 2007; Karjalainen *et al.*, 2007), the immediate shift in phytoplankton community structure and increase of TN observed in our mesocosm experiment after colony addition, while the *Gloeotrichia* were alive and forming surface scums, indicates that blooms of *Gloeotrichia* may have unique effects on plankton food webs, especially in comparison with blooms of other cyanobacterial taxa (e.g. Engstrom-Ost *et al.*, 2013). While our two experiments were conducted in the absence of large, grazing zooplankton, we hypothesize that if zooplankton were present, *Gloeotrichia*'s stimulation of eukaryotic phytoplankton could provide a subsidy to higher trophic levels.

Taken together, the results of our studies are consistent with our initial hypothesis that *Gloeotrichia* stimulates other phytoplankton by increasing available nutrients, likely due to diffusion or metabolic processes from both live and senescent colonies, as has been observed for



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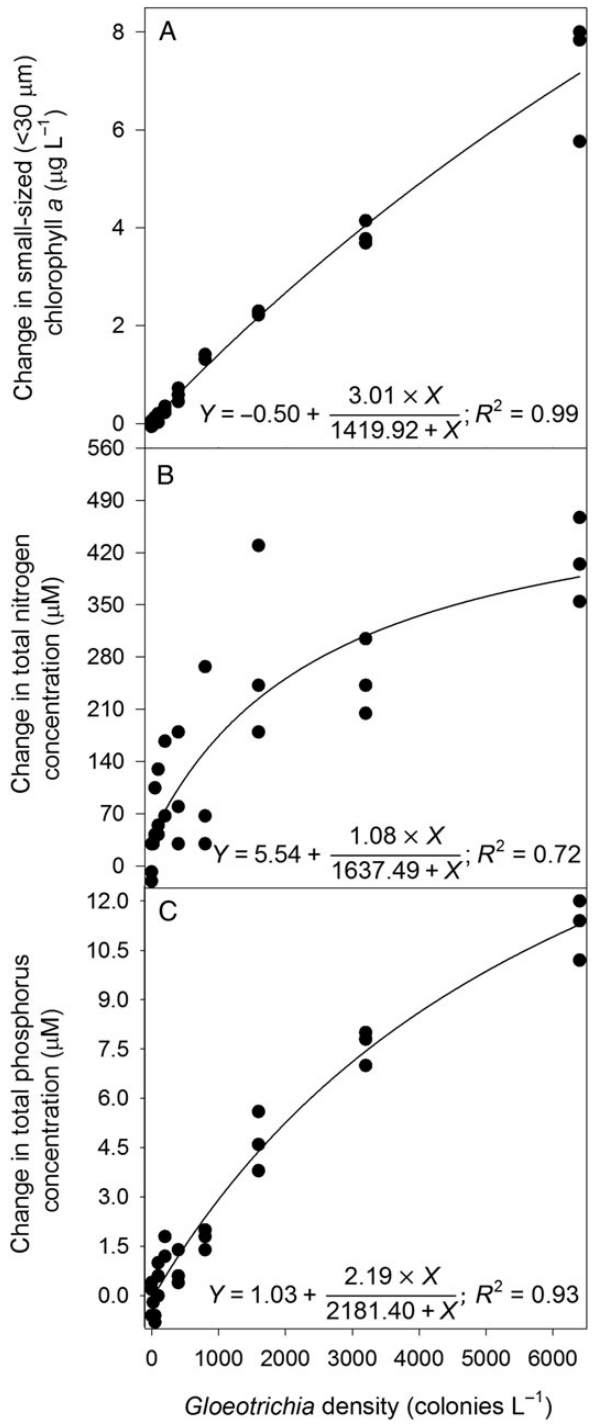


**Fig. 4.** The effect of different *Gloeotrichia* densities on (**A**) chlorophyll *a*, (**B**) total nitrogen and (**C**) total phosphorus concentrations in water passed through a 30 µm filter in the laboratory experiment. For all three response variables, a Monod model with an intercept term was the best-fitting regression model (equations on figure). Data were $\log_e$-transformed prior to model fitting, but the untransformed data are plotted. The concentrations in the no-*Gloeotrichia* control have been subtracted from all values for all variables.

other cyanobacterial taxa (Ray and Bagchi, 2001; Shi *et al.*, 2004; Agawin *et al.*, 2007). Because of the lack of soluble N and P data above the MDL in the field experiment, we are not able to definitively test this hypothesis. However, we think it is likely that any soluble N or P that may have been released would have been immediately taken up by other phytoplankton. This hypothesis is supported by the increase in ss, phytoplankton biomass within 24 h of *Gloeotrichia* addition to the 400 colonies L$^{-1}$ mesocosms, coincident with significantly higher TN concentrations as well as more TP samples above the MDL. In addition, after 5 days in the laboratory experiment, *Gloeotrichia* significantly increased TN and TP in the flask filtrate in treatments >800 and >400 colonies L$^{-1}$, respectively, which were tightly correlated with increases in ss, phytoplankton. Consistent with these observations, nutrient increases due to *Gloeotrichia* were hypothesized to stimulate other phytoplankton in Antermony Loch, Scotland (Pitois *et al.*, 1997) and Lake Peipsi, Estonia (Nõges *et al.*, 2004).

It is also possible that *Gloeotrichia* stimulated the phytoplankton community by releasing other beneficial compounds. Many cyanobacteria can emit bioactive secondary metabolites (Gross, 2003; Legrand *et al.*, 2003), which other phytoplankton may have evolved to recognize and use (Suikkanen *et al.*, 2004). For example, some phytoplankton taxa can take up dissolved organic compounds (Sanders *et al.*, 1990; Tittel and Kamjunke, 2004). *Gloeotrichia* may also produce antibacterial or antifungal compounds that benefit other phytoplankton (Legrand *et al.*, 2003). Although we did not measure these compounds in our experiments, they made have played a role. Regardless of the mechanism, it is notable that no algal group exhibited significant decreases in biovolume or richness in response to *Gloeotrichia*.

Finally, while it is possible that the stimulation of other phytoplankton was due solely to *Gloeotrichia* senescence, we think this is unlikely given the rapid increases in TN and phytoplankton (within 24 h) relative to the slower decreases in *Gloeotrichia* density in the mesocosms (Figs 1 and 2) and the fact that there was no observable mortality in the laboratory experiment over a 5-day period. Furthermore, TN did not continue to increase as the *Gloeotrichia* colonies senesced during the experiment. In addition, the bloom collapse in the mesocosm experiment over 13 days is similar to patterns of *Gloeotrichia* density seen in natural systems (Karlsson-Elfgren *et al.*, 2003; Carey *et al.*, 2008). Karlsson-Elfgren *et al.* (Karlsson-Elfgren *et al.*, 2003) estimated that pelagic *Gloeotrichia* can remain in the water column for up to 2 weeks, consistent with our data, and patterns previously

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observed in Lake Sunapee (Carey *et al.*, 2008) and other lakes (Roelofs and Oglesby, 1970; Barbiero and Welch, 1992; Nõges *et al.*, 2004). Furthermore, the removal of colonies during sampling also most likely contributed to the observed decline in *Gloeotrichia* density. Because wind and currents concentrate buoyant *Gloeotrichia* colonies in shallow, downwind coves (as also observed in Carey *et al.*, 2008, 2012), we expect that epilimnetic phytoplankton would be able to access nutrients recycled from *Gloeotrichia* colonies both during and after blooms when the colonies die and sink to the shallow sediments.

In conclusion, our results indicate that if bloom densities increase to levels observed in eutrophic lakes, *Gloeotrichia* may alter phytoplankton biomass, richness and diversity in oligotrophic lakes. The combined results of the laboratory and mesocosm experiments indicate that *Gloeotrichia* blooms at the critical threshold of ~400 colonies $L^{-1}$ can significantly alter phytoplankton community structure and may increase nutrient concentrations as well in the absence of large zooplankton. Given that these results occurred in oligotrophic lake water and that densities of *Gloeotrichia* approaching this threshold have occurred in lakes with nutrient concentrations near the oligotrophic-mesotrophic boundary (H.A.E. *et al.*, unpublished data), consideration of cyanobacteria as drivers of change in phytoplankton community structure in low-nutrient systems seems warranted.

## SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

## ACKNOWLEDGEMENTS

We thank S.C. Davis and R.Q. Thomas for field and lab assistance and gratefully acknowledge the Lake Sunapee Protective Association, M. and T. Eliassen, G. and J. Montgomery, J. Fichter, and R. Wood for access to field sites. R.Q. Thomas and A.S. Flecker provided invaluable comments.

## FUNDING

This work was supported by the National Science Foundation (a Graduate Research Fellowship to C.C.C., DEB-1010862, DEB-0749022, EF-0842267, EF-0842112, EF-0842125; CDI-0941510); Bates College; the Cornell Biogeochemistry and Biocomplexity Program; the Mellon Foundation; and the Kieckhefer Adirondack Foundation.

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