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Effects of nutrient limitation on the release and use of dissolved organic carbon from benthic algae in Lake Michigan

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Abstract: Compared to phytoplankton, relatively little information exists about the importance of benthic algae as a source of dissolved organic C (DOC) in lakes. We enriched outdoor tanks with N and P in a full factorial design (unenriched, +N, +P, N+P) to evaluate effects of nutrients on the release, composition, and decomposition of DOC from *Cladophora glomerata* (L.) Kütz. in Lake Michigan. After 10 d of enrichment, biomass-specific rates of gross primary productivity (GPP) were significantly greater in the N+P treatment than in the +N or +P treatments. The fraction of fixed C released as DOC was ~37% of GPP in the N+P treatment, which was similar to material collected from the lake (initial samples). Biomass-specific rates of DOC release did not vary significantly with treatment, whereas GPP declined in the nutrient-limited treatments (+N, +P, and unenriched). Consequently, DOC release represented a higher percentage of GPP (>100%) under severe nutrient limitation. Specific ultraviolet (UV) absorbance, an indicator of aromatic compounds, indicated that DOC from material grown in the N+P treatment (DOC_{N+P}) was more labile than DOC from material grown in the +N treatment (DOC_{+N}). Both DOC_{N+P} and DOC_{+N} supported an exponential increase in bacterial density during a 19-d incubation, but DOC_{N+P} was used more rapidly and supported higher bacterial density than DOC_{+N}. *Cladophora* has become a major contributor to primary production in the shallow nearshore areas of the lower Laurentian Great Lakes, and our data suggest that ≥⅓ of this productivity is available for the benthic microbial loop as DOC. The amount of DOC released remains relatively constant as nutrient limitation reduces productivity, but the nutrient status of the alga affects the quality of DOC as a substrate for heterotrophic bacteria.
Key words: algal exudates, *Cladophora*, Laurentian Great Lakes, nutrients, primary production

Algae release organic compounds into the water column during photosynthesis (Myklestad 1995). In phytoplankton, these compounds typically account for 5 to 50% of net primary production (Baines and Pace 1991). These algae are important sources of autochthonous dissolved organic C (DOC) in aquatic ecosystems (Bertilsson and Jones 2003) and have an important role in supporting microbial communities (Cole et al. 1982, Søndergaard et al. 1995, Giroldo et al. 2007). The importance of phytoplankton as a source of DOC has been well documented in marine and freshwater ecosystems (Baines and Pace 1991, Bertilsson and Jones 2003), but relatively little information exists for freshwater benthic algae. This lack of information is an important gap in our knowledge of C cycling in aquatic ecosystems, especially in lakes, where production by benthic algae can exceed that of phytoplankton in shallow nearshore waters (Malkin et al. 2010).

The mechanisms of DOC release by algae are largely unknown, but the amount of release, which often is reported as a percentage of primary production (percent extracellular release [PER]), appears to depend on environmental conditions (Myklestad 1995). One hypothesis is that exudate release is an overflow mechanism to prevent damage to the photosynthetic apparatus (Smith and Underwood 2000, Perkins et al. 2001) and occurs in conditions where light levels support rates of photosynthesis that exceed the capacity of the algae to use carbohydrates in growth because of nutrient limitation (Fogg et al. 1965, Myklestad and Haug 1972,

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Lancelot 1983). Release of C exudates by algae may, in turn, alleviate nutrient-limitation through mineralization of essential nutrients by the associated microbial community (Fogg 1983, Bratbak and Thingstad 1985, Wood and Van Valen 1990), which uses organic compounds in a boundary layer around the algal cell (Bell and Sakshaug 1980).

Benthic algae have high rates of primary productivity (Vadeboncoeur et al. 2002, Higgins et al. 2008, Malkin et al. 2010), so changes in the rate or chemical composition of exudate release by benthic algae may have important consequences for the microbial loop in shallow lakes or in shallow regions of large lakes dominated by benthic algae. Assuming that benthic algae respond to disturbances in nutrient supply in a manner similar to phytoplankton (e.g., Myklestad 1995), we would expect to find higher exudate release by benthic algae in oligotrophic than in eutrophic waters. Furthermore, changes in the composition of exudates with nutrient status could make some exudates more labile for heterotrophic metabolism than others (Obernosterer and Herndl 1995). Thus, a better understanding of exudate release by benthic algae, including how nutrient stress affects the quantity and quality of C exudates, would increase our understanding of energy flow in lake ecosystems.

The goal of our study was to evaluate the release of DOC by the filamentous green macroalga, *Cladophora glomerata* (L.) Kütz., in Lake Michigan. This alga is widely distributed throughout the lower Laurentian Great Lakes (Michigan, Erie, and Ontario) (Higgins et al. 2008) and is among the most common algae in freshwaters of the temperate northern hemisphere (Whitton 1970, Dodds and Gudder 1992). *Cladophora* often is associated with eutrophication and is regarded as a sentinel of eutrophication in nearshore benthic ecosystems (Dodds and Gudder 1992). In the lower Laurentian Great Lakes, *Cladophora* is largely responsible for a recent increase in benthic primary production (Auer et al. 2010), termed benthification (Zhu et al. 2006), that has occurred as a consequence of greater water clarity and nutrient availability associated with the establishment of invasive dreissenid mussels (Lowe and Pillsbury 1995, Hecky et al. 2004, Malkin et al. 2008, Ozersky et al. 2009). Authors of recent studies report rates of net benthic production on *Cladophora*-dominated substrata >400 mg C m^{-2} d^{-1}, surpassing those of phytoplankton near the shoreline during the growing season (Malkin et al. 2010).

In light of the recent transfer of primary production from pelagic to benthic pathways, we were interested in measuring: 1) the percentage of fixed C that is released into the water column by *Cladophora* during normal growth and development, 2) the effect of nutrient limitation on DOC release, and 3) the role of DOC from *Cladophora* as an energy source for heterotrophic bacteria. Based on the available literature (i.e., Obernosterer and Herndl 1995), we hypothesized that: 1) PER would be greatest in conditions of nutrient-limited primary production and 2) exudates released by *Cladophora* under nutrient-limited conditions would be used by bacteria less efficiently than DOC from material grown with a balanced nutrient supply.

METHODS

Tank experiment

We collected algae in July 2011 from a 100-m reach of rocky shoreline ~500 m north of the Central Michigan University Biological Station on Beaver Island in northern Lake Michigan, USA (lat 45°42′N, long 85°30′W). The timing of *Cladophora* establishment along this area of shoreline is variable, and dense stands of *Cladophora* can persist as late as mid-September. *Cladophora* used in our study appeared on the shoreline in mid-June, a few weeks before the initiation of our study, and was bright green at the time of collection. We collected *Cladophora* tufts (~10 cm in length) attached to nonliving zebra mussel (*Dreissena polymorpha*) shells by hand from a depth of 20 to 50 cm (photosynthetically active radiation [PAR] ~1000 μmol m^{-2} s^{-1}), placed them in dark coolers filled with lake water, and transported them to the Central Michigan University Biological Station where they were kept in the dark until the start of the experiment (within 4 h of collection). We agitated each algal tuft before removing it from the cooler to dislodge particles of sediment and macroinvertebrates and then placed tufts in large, open, glass tanks filled with 115 L of filtered water pumped directly from Lake Michigan. The tanks were on a level wooden platform behind a low sand dune ~20 m from the lake shore and exposed to ambient light and temperature. We aerated the tanks vigorously to provide mixing and to facilitate gas exchange with the atmosphere. We spaced 8 tufts evenly in each tank and anchored them to the bottom with metal weights (stainless steel nuts) attached to nylon line. We added nutrients from a stock solution of KNO_3 and KPO_4 to achieve concentrations of 1000 μg N/L and 100 μg P/L in a full factorial design (+N, +P, N+P). We assumed these concentrations would saturate algal growth rates because they exceeded those reported to be limiting for benthic algae in studies reviewed by Borchardt (1996). We also established a treatment with lake water but without nutrient additions (unenriched [UE]). Nutrient concentrations in lake water were below the limits of detection (see Results), so we expected algal material in the UE treatment to be N and P limited. We used 6 replicate tanks for each treatment, for a total of 24 experimental tanks. We repeated nutrient amendments every 2 d for 8 d from 9–16 July 2011. We ended the experiment on day 10, 2 d after the last nutrient addition. We expected that this length of time would provide the greatest time possible for treat-

ment effects to occur without development of confounding enclosure effects.

When we started the nutrient enrichment experiment, we collected an additional 24 samples randomly from substrata along the 100-m reach of rocky shoreline. We used these samples to measure initial rates of photosynthesis and DOC release from material representative of that used in the tank experiment. We collected samples beginning on the south end of the 100-m reach and worked our way north along the shoreline placing each algal sample in an individually numbered 120-mL polyethylene bottle filled with lake water. We grouped sequential samples into clusters of 4 to represent 6 different collection zones along the shoreline. We treated the mean of each group of 4 samples as single replicate in statistical analyses. We measured photosynthesis and DOC release of the initial material in situ as described below.

We monitored water temperature, dissolved O_2 (DO), and pH in each tank in the morning every 2 d with a Hydrolab Multi-Probe Surveyor 4 (HACH Hydromet, Loveland, Colorado). We collected water for nutrient analysis immediately after nutrient additions on days 0, 4, and 8. We filtered water samples through a 0.45-μm filter and stored them frozen in acid-rinsed polyethylene bottles until nutrient analysis. We measured dissolved inorganic N (DIN) as NO_3 + NO_2 by the Cd-reduction method with a Bran + Luebbe QuAAtro auto-analyzer (SPX, Norderstedt, Germany) and for soluble reactive P (SRP) by the ascorbic acid colorimetric method on a Beckman–Coulter model DU 800 spectrophotometer (Beckman–Coulter Inc., Indianapolis, Indiana) (APHA 1998).

After 10 d in experimental conditions, we removed algal material from 4 substrata inside each tank with forceps. We analyzed each subsample separately and used the mean of 4 subsamples collected from each tank as a single replicate in statistical analyses. To measure net primary production (NPP), we incubated ~1 g of algal material in a 300-mL clear biological O_2 demand (BOD) bottle filled with 0.2-μm-filtered lake water for 1 h in the light. We measured DO before and after each incubation with a luminescent DO probe (HACH Hydromet), and we used a syringe fitted with a rubber tube (10 cm long) to collect a 60-mL sample from each bottle immediately after incubation. We removed the tube from the syringe and filtered the liquid into an acid-washed polyethylene bottle through a syringe-driven 0.45-μm filter unit (Millipore, Bedford, Massachusetts) for subsequent measurement of UV absorbance at λ = 254 nm ($SUVA_{254}$) and DOC (mg/L) concentration within 1 h of collection (see below). Meanwhile, we refilled each BOD bottle with 0.2-μm-filtered lake water, wrapped the bottle with aluminum foil, and incubated the bottle for 1 h in the dark to measure O_2 consumption (dark respiration [R_d]). We conducted incubations between 1000 and 1400 h on a sunny day with an average PAR of 1100 μmol m^{-2} s^{-1} at incubation depth. We removed algal material at the end of the incubation and dried it at 105°C for 24 h to measure dry mass. We calculated gross primary productivity (GPP) from NPP and R_d and converted GPP values to units of C based on a C : O molar ratio of 0.375 and a photosynthetic quotient of 1.2 (Wetzel and Likens 2000). We calculated exudate release as the difference between initial DOC concentration of incubation water and DOC concentration after light-bottle incubation. We expressed productivity rates as C evolution per unit dry mass and exudate release as PER.

We separated a subsample of algal material in the laboratory and froze it until we measured chlorophyll *a* concentration, total P (TP), and total N (TN). We measured chlorophyll *a* on a Beckman–Coulter model DU 800 spectrophotometer after extraction with ethanol and correction for phaeophytin (APHA 1998). We dried 2 subsamples of algal material at 105°C for 24 h and weighed them. We measured their TN and TP content by oxidizing the particulate matter with persulfate and then analyzing SRP following the ascorbic-acid method and NO_3 following the 2^{nd}-derivative UV-spectroscopy method (APHA 1998). We calculated the proportion of P and N in each sample by dividing the mass of P and N by dry mass and reported nutrient content per unit dry mass.

We measured UV-visible (Vis) absorption of water samples at λ = 254 nm with a Beckman–Coulter model DU 800 spectrophotometer. We measured DOC with a Shimadzu TOC-V analyzer (Shimadzu Scientific Instruments, Columbia, Maryland) and calculated $SUVA_{254}$ by dividing UV-Vis absorbance at λ = 254 nm by DOC concentration. $SUVA_{254}$ gives an average molar absorptivity for all the molecules contributing to the DOC in a sample, and it has been used as a measure of DOC aromaticity (Chin et al. 1994).

Laboratory incubations

$SUVA_{254}$ values differed significantly only between the +N and N+P treatments (see Results below), so we used DOC from these 2 treatments (DOC_{+N} and DOC_{N+P}, respectively) to compare degradation rates. This experimental design offered us the opportunity to evaluate degradation patterns between treatments at each end of the nutrient spectrum and with the potential for the greatest differences in exudate composition. We collected exudates from algal material grown in the +N and N+P treatments in 4 separate beakers. Each beaker contained 500 mL of 0.2-μm-filtered lake water enriched with the same nutrient concentrations as the tanks and kept under constant light (PAR ~500 μmol m^{-2} s^{-1}) and at room temperature (~21°C) for 36 h. The incubation process was not sterile, but bacterial densities were monitored and were $<1 \times 10^4$

bacteria/L, which allowed us to achieve a highly concentrated exudate solution (~30 mg C/L). We removed algal material from beakers with forceps and filtered each solution of concentrated exudates through a 0.2-µm VacuCap filter (Pall Life Sciences, Ann Arbor, Michigan) into separate 2000-mL acid-washed flasks. We collected a subsample from each flask to measure DOC concentration and then diluted each solution (DOC_{+N} and DOC_{N+P}) to approximately the same DOC concentration (23 mg C/L) with Milli-Q water. We prepared a bacterial inoculum from filtered lake water (0.7 µm GF/F; Whatman, Springfield Mill, UK) collected from an area of rocky shoreline with dense *Cladophora* growth. About 80% of bacterial cells pass through 0.7-µm-pore-size filters (Giroldo et al. 2007). We kept the bacterial inoculum at a constant temperature (~20°C) until the start of the experiment (within 2 h of collecting the lake water).

We pipetted 50 mL of the bacterial inoculate (0.7-µm-filtered lake water) into 6 Erlenmeyer flasks and added 450 mL of DOC_{+N} or DOC_{N+P} (n = 3 for each treatment). To prevent nutrient limitation of bacteria (Fagerbakke et al. 1996), we brought each flask to a final concentration of 5 µM P and 50 µM N by adding KH_2PO_4 and KNO_3, respectively. Our goal was to test the hypothesis that bacteria use algal exudates as a C source without the confounding variable of nutrient limitation of bacterial growth. We incubated the cultures in the dark and kept the temperature constant throughout the experiment (~21°C). We sampled flasks at 0, 2, 4, 8, 13, and 19 d and measured $SUVA_{254}$, DOC, and bacterial abundance. We processed and analyzed samples for $SUVA_{254}$ and DOC as described above. We fixed bacterial samples with formaldehyde, stained them with 4′,6-diamino-2-phenylindole (DAPI), and filtered them onto 0.2-µm black filters (Osmonics Inc., Livermore, California) (Porter and Feig 1980). We evaluated bacterial growth from direct counts made with the aid of a Leica DM 1000 epifluorescence microscope with UV and a light source (Leica Microsystems, Buffalo Grove, Illinois). We counted ≥300 bacterial cells or 25 fields/filter.

Statistical analyses

We used univariate repeated measures analysis of variance (rmANOVA) models with an adjusted Bonferroni significance level to test whether physical and chemical conditions (temperature, DO, pH, SRP, DIN) differed among nutrient treatments during the 10-d nutrient enrichment experiment. In instances when rmANOVA indicated significant differences among treatments, we used Tukey's Honestly Significant Difference (HSD) multiple comparison technique to calculate which treatments were significantly different. We evaluated the effects of nutrient treatments on algal responses (TN, TP, chlorophyll *a*, GPP, R_d, NPP, PER, $SUVA_{254}$) with 1-way ANOVA and post hoc comparison of means with Tukey's HSD. We used *t*-tests to evaluate differences in DOC use, $SUVA_{254}$, and bacterial growth between nutrient treatments in the degradation experiment. We completed all statistical analyses in SYSTAT (version 11.0; SYSTAT, Evanston, Illinois). We log(x + 1)-transformed variables if necessary to correct for nonnormal distribution and unequal variances among treatments prior to analysis.

RESULTS

Physical conditions and nutrient concentrations

Water temperature in outdoor tanks reflected day-to-day differences in air temperature and varied from 15 to 25°C during the 10-d study period, but water temperature was similar among nutrient treatments ($p > 0.05$). DO ($F_{3,140} = 3.43$, $p = 0.019$) and pH ($F_{3,140} = 27.58$, $p < 0.0001$) increased over time in the N+P treatment and were significantly higher in the N+P treatment than in the +N, +P, and UE treatments (Fig. 1A, B). Concentra-

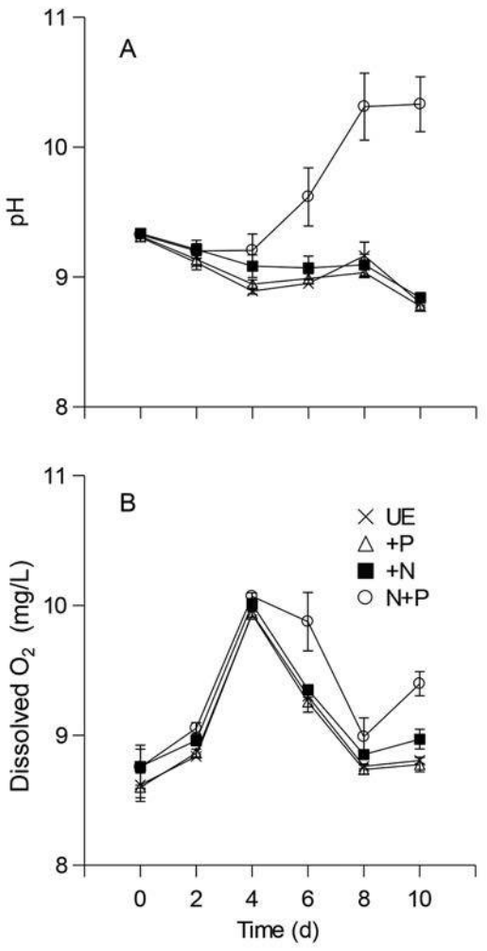

Figure 1. Mean (±1 SE, n = 6) pH (A) and dissolved O_2 (B) in outdoor tanks during a 10-d nutrient enrichment experiment. UE = unenriched.

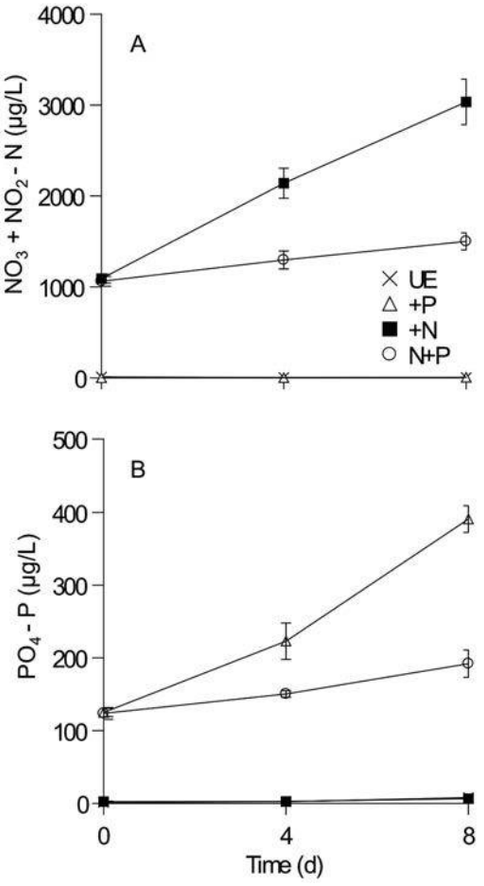

Figure 2. Mean (±1 SE, n = 6) concentrations of NO_3 + NO_2-N (A) and PO_4-P (B) in outdoor tanks after nutrient additions. UE = unenriched.

tions of DIN and SRP increased to target levels when N and P were added together but accumulated in the water column when either nutrient was added individually. DIN and SRP concentrations were 3035.0 ± 225.6 µg/L (mean ± SE) and 390.9 ± 16.55 µg/L in the +N and +P treatments, respectively, after 10 d (Fig. 2A, B). SRP and DIN concentrations remained below analytical detection in the +N and +P treatments, respectively, and in the UE treatment during the 10-d experiment.

Algal stoichiometry, biomass, and productivity

Algal nutrient content reflected the manipulation of water-column nutrients. Algal TN (mg/g) was significantly greater in the +N and N+P treatments and lower in the +P and UE treatments than in the initial samples ($F_{4,25}$ = 44.00, p < 0.0001; Fig. 3A). Algal TP (µg/g) was significantly greater in the +P and N+P treatments and lower in the +N and UE treatments than in the initial samples ($F_{4,25}$ = 88.86, p < 0.0001; Fig. 3B). The molar N : P ratio of algal material was significantly higher in the +N treatment (113.8) and lower in the +P treatment (3.0) than in the N+P (15.4) and UE treatments (20.0) and the initial samples (15.1) ($F_{4,25}$ = 111.26, p < 0.0001). Absolute concentrations of N and P were >2× that in algal material from the N+P than from the UE treatment, but on a molar basis, they did not differ significantly (p = 0.943).

Biomass-specific chlorophyll a concentration was significantly greater in the N+P treatment than in the +N, +P, and UE treatments ($F_{4,25}$ = 10.41, p < 0.0001; Fig. 4A). R_d in the N+P treatment (–3.67 ± 1.39 mg C g^{-1} h^{-1}; mean ± SE) was >2× that in the other treatments (range: –1.15 ± 0.13 to –1.3 ± 0.39 mg C g^{-1} h^{-1}), but these differences were not significant (p = 0.070). NPP followed the same pattern and was significantly higher in the N+P treatment (12.75 ± 3.06 mg C g^{-1} h^{-1}; $F_{4,25}$ = 9.78, p = 0.0004) than in the other treatments, which were not significantly different (range: 1.48 ± 0.22 to 3.75 ± 1.12 mg

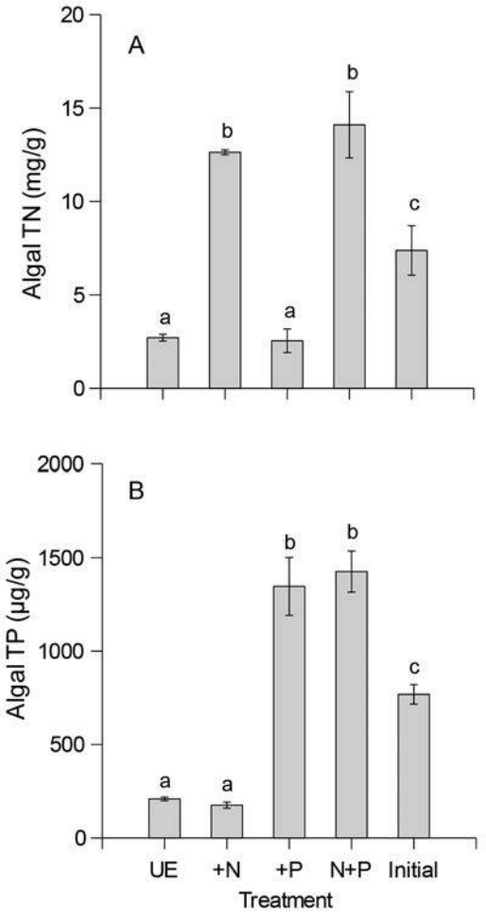

Figure 3. Mean (±1 SE, n = 6) total N (TN) (A) and total P (TP) (B) of *Cladophora* grown in outdoor tanks and collected from the field (initial samples) (n = 6). Bars with the same letters are not significantly different based on Tukey's Honestly Significant Difference tests (α = 0.05). UE = unenriched.

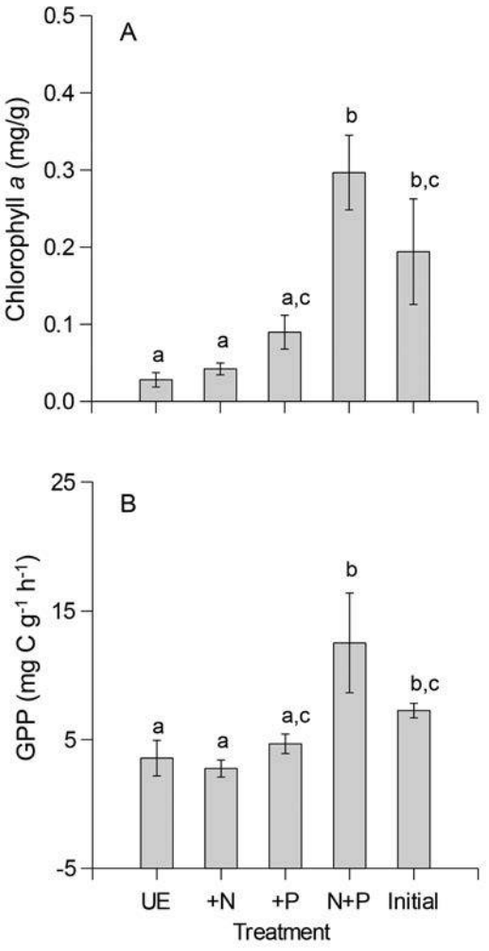

Figure 4. Mean (±1 SE, n = 6) biomass-specific algal chlorophyll *a* concentration (A) and gross primary productivity (GPP) (B) of *Cladophora* grown in outdoor tanks and collected from the field (initial samples) (n = 6). Bars with the same letters are not significantly different based on Tukey's Honestly Significant Difference tests (α = 0.05). UE = unenriched.

C g^{-1} h^{-1}). GPP reflected these differences and was significantly greater in the N+P treatment than in the +N, +P, and UE treatments ($F_{4,25}$ = 9.88, p < 0.001; Fig. 4B). Biomass-specific chlorophyll *a*, NPP, and GPP did not differ between initial samples and N+P or +P treatments or among +N, +P, and UE treatments (p > 0.05; Fig. 4A, B).

PER and $SUVA_{254}$ of algal exudates

Biomass-specific DOC release (mg C g^{-1} h^{-1}) was similar among nutrient treatments and the initial samples (p = 0.256; Fig. 5A). PER was significantly higher in material grown in the +N and UE treatments than in the N+P and +P treatments and the initial samples ($F_{4,25}$ = 6.32, p < 0.001; Fig. 5B). PER was similar between material grown in +N and UE treatments and among the +P and N+P treatments and the initial samples (p > 0.05). $SUVA_{254}$ values differed only between the N+P and +N treatments. Values were higher in the +N than in the N+P treatment ($F_{4,25}$ = 2.84, p = 0.04; Fig. 6).

Degradation of algal exudates

Exudates derived from algae grown in the N+P treatment (DOC_{N+P}) had a greater percent degradation and supported greater bacterial density than material grown in the +N treatment (DOC_{+N}). DOC_{N+P} decreased by 21% within the first 2 d of incubation (Fig. 7A), and $SUVA_{254}$ increased by 40% over the same period (Fig. 7B). Less than 2% of DOC_{+N} was used during the first 8 d (Fig. 7A). A spike in decomposition occurred on day 13 in both treatments, but was especially strong in the DOC_{+N} treatment where exudate use increased by 35% between days

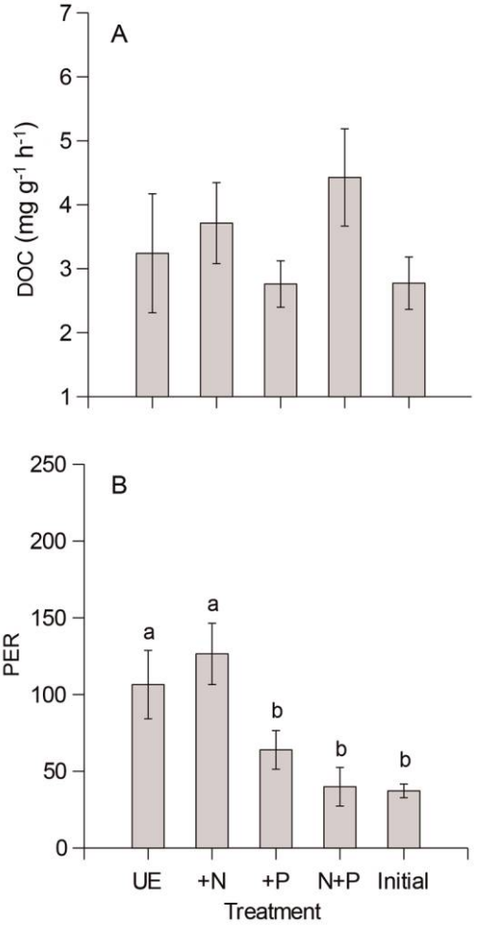

Figure 5. Mean (±1 SE, n = 6) biomass-specific dissolved organic C (DOC) release (A) and percent extracellular release (PER) (B) of *Cladophora* grown in outdoor tanks and collected from the field (initial samples) (n = 6). Bars with the same letters are not significantly different based on Tukey's Honestly Significant Difference tests (α = 0.05). UE = unenriched.

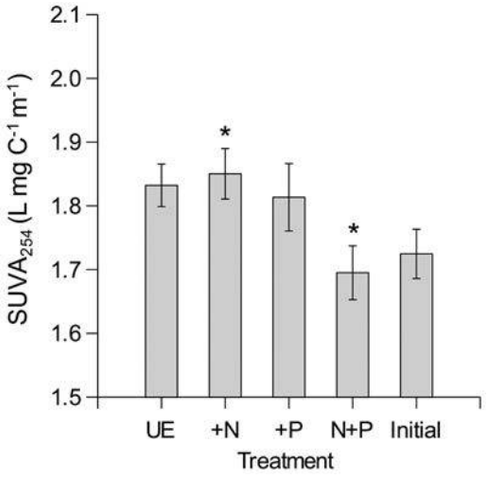

Figure 6. Mean (±1 SE, n = 6) specific ultraviolet absorption at λ = 254 nm ($SUVA_{254}$) of *Cladophora* grown in outdoor tanks and collected from the field (initial samples) (n = 6). Asterisks indicate bars that were significantly different based on Tukey's Honestly Significant Difference tests (α = 0.05). UE = unenriched.

8 and 13. Bacterial growth increased with exudate decomposition from 0.51 ± 0.45 × 10^6 (mean ± SE) to 67.08 ± 1.25 × 10^6 cells/mL in the DOC_{N+P} treatment and from 0.57 ± 0.41 × 10^6 to 43.70 ± 2.28 × 10^6 cells/mL in the DOC_{+N} treatment (Fig. 7C).

DISCUSSION

Evidence of nutrient limitation

Our experimental design allowed us to evaluate the effects of nutrient stress on algal productivity and exudate release across a wide range of nutrient conditions. Several lines of evidence point to nutrient colimitation in conditions without nutrient enrichment and for single limitation in individual nutrient treatments. Using the Redfield ratio of 16 as an estimate (Redfield 1958), N : P ratios were symptomatic of N-limitation with the addition of P without N and of P limitation with the addition of N without P. DIN and SRP accumulated in the water column when each respective nutrient was added individually, indicating that in the absence of N, P was not assimilated rapidly by the algae and vice versa. However, tissue nutrient concentrations did reflect nutrient additions, possibly resulting from luxury uptake (reviewed by Dodds and Gudder 1992). Last, GPP indicated that algal material grown with N+P enrichment fixed C at a greater rate than material grown in individual nutrient treatments or without nutrient additions. Higher rates of photosynthesis probably explain the increase in DO and pH in the N+P treatment, with higher rates of photosynthesis removing protons from the water and shifting the bicarbonate equilibrium to carbonate.

Nutrient controls on exudate release

In common with the literature for phytoplankton (Myklestad 1995), our results show that rates of DOC release from *Cladophora* represent a substantial percentage of GPP, and our estimate of 37% extracellular release from *Cladophora* collected from the field is within the range reported for phytoplankton (Baines and Pace 1991). PER of

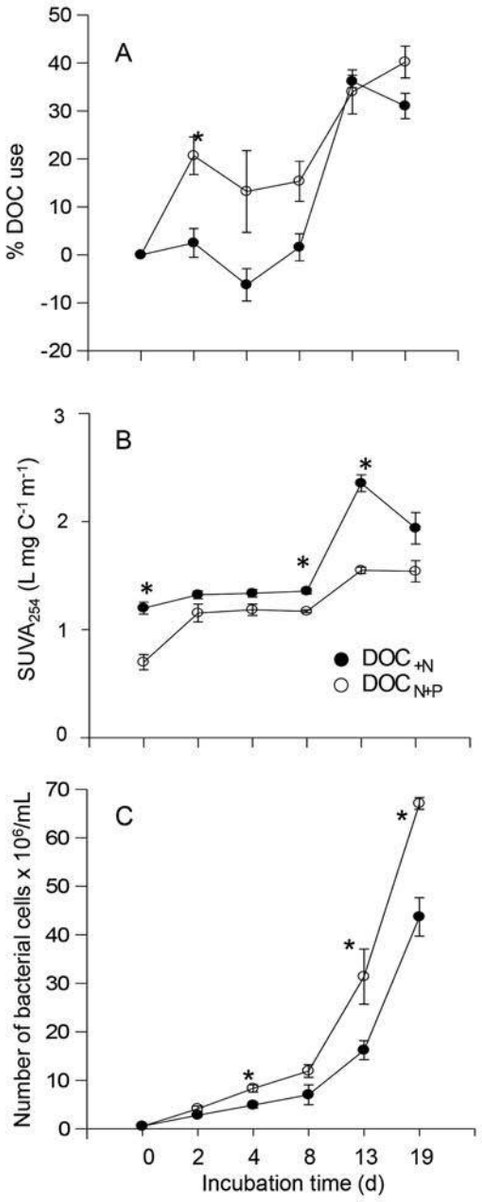

Figure 7. Mean (±1 SE, n = 3) % dissolved organic C (DOC) use (A), specific ultraviolet absorption at λ = 254 nm ($SUVA_{254}$) (B), and number of bacterial cells × 10^6/mL (C) in the DOC_{N+P} and DOC_{+N} treatments during a 19-d incubation period. Asterisks indicate significant differences between DOC_{N+P} and DOC_{+N} treatments (Student's t-test, $p < 0.05$).

algal exudates varied substantially across nutrient treatments, with the greatest PER in UE and +N (P limitation) treatments and the lowest PER in the N+P treatment. This finding is complementary to previous work showing the importance of nutrient status for the release of exudates from phytoplankton (Lancelot 1983, Obernosterer and Herndl 1995, Giroldo and Vieira 2002). Most investigators found an increase in PER when ≥1 nutrients were in low supply (e.g., Obernosterer and Herndl 1995). However, in our study, PER appeared to be driven more by differences in photosynthetic rates between treatments than by differences in release rates related to nutrient limitation. When we evaluated biomass-specific rates of exudate release, we saw relatively constant exudation in proportion to biomass when C fixation rates were altered by nutrient manipulation. Absolute exudate release differed little between N-enriched and P-enriched algal material, but P-enriched material had a higher photosynthetic rate, resulting in a higher PER compared to N-enriched material. This aspect of our work is difficult to evaluate relative to other studies because most workers have expressed exudation solely in proportion to total C fixation. Our results support the notion that algae in less productive waters probably will release a larger fraction of their photosynthate as organic compounds than will algae in eutrophic waters (i.e., Bertilsson and Jones 2003), but they also indicate that, on a biomass basis, the net effect on energy flow associated with exudates may be similar across a wide nutrient gradient. As more efforts are made to estimate the release of C exudates by benthic algae in the Laurentian Great Lakes, we suggest that ecological models be based on relatively constant biomass-specific exudate release rather than on variable PER.

Our data do not discriminate between DOC release from the immediate products of photosynthesis or from less transient cellular pools of carbohydrates, nor was our study designed to address questions about the mechanisms responsible for DOC release in algae, which may be a consequence of passive leakage across cell membranes (Bjørnsen 1988) or active excretion (Fogg 1983). The active-excretion hypothesis suggests that DOC release is a means to vent excess reduced organic compounds under circumstances where GPP exceeds the demand for growth or storage (Fogg 1983), thereby preventing damage to the photosynthetic apparatus (Fogg et al. 1965). An alternative explanation to active excretion is that dissolved exudates are simply released into the surrounding water by passive permeation or leakage across the cell membrane. This hypothesis is supported by studies showing constant exudation rates in phytoplankton incubated in light–dark cycles (Berman and Kaplan 1984, Smith and Underwood 2000) and when C fixation rates varied under different concentrations of dissolved inorganic C (Smith and Wiebe 1976). In our study, passive permeation should have been at least partly responsible for exudate release because: 1) constant exudation was observed in proportion to biomass when C fixation rates differed because of nutrient manipulation and 2) exudate release was greater than C fixation in conditions of nutrient limitation.

Bacterial degradation of algal exudates

Our results show that exudates released by lake benthic algae are a source of C for bacterial degradation. A review of the literature on phytoplankton indicates that, on average, 46% of the excreted dissolved organic matter is incorporated by bacteria (Baines and Pace 1991). Our results were similar, with 30 and 40% of the exudates being degraded in the DOC_{+N} and DOC_{N+P} treatments, respectively. On the other hand, a large fraction of exudates was *not* degraded during our study. This result was somewhat expected because other decomposition experiments have shown that a portion of exudates is resistant to bacterial mineralization (Wyatt et al. 2012), even after several years (Fry et al. 1996). These findings highlight the potential for algal exudates to have a significant influence on long- and short-term C cycling through the release of recalcitrant and labile C compounds, respectively.

Little information exists regarding environmental controls on the quality of exudates released by freshwater benthic macroalgae, but studies of phytoplankton have shown that nutrient status can affect exudate composition (e.g., Obernosterer and Herndl 1995). Degradation patterns in our study indicated that exudates derived from material grown in conditions of balanced nutrient supply were initially more labile for microbial decomposition than those derived from P-limited conditions. This finding was somewhat expected because it is fairly well established that nutrient availability strongly influences the composition of cellular components, with high nutrient availability resulting in more protein-rich cells (Hama and Honjo 1987). Furthermore, $SUVA_{254}$ values were lower in the DOC_{N+P} than in the DOC_{+N} treatment, a result suggesting that exudates derived from conditions of balanced nutrient supply were more labile than those derived from conditions of P limitation. In both treatments, increases in $SUVA_{254}$ values indicated that *Cladophora* exudates became more recalcitrant after degradation. This finding supports previous work showing an increase in aromatic compounds during microbial degradation (Wyatt et al. 2012) and demonstrates the ability of microbial communities to alter the composition of algal exudates during decomposition (Giroldo et al. 2003). Exudates derived from nutrient-enriched material were more labile than exudates from material grown in nutrient-limited conditions, but a large portion of exudates from both sources was degraded by the natural bacterial community and probably was an im-

portant source of energy along the shoreline where benthic algal mats occur.

Implications of exudate release for the Laurentian Great Lakes ecosystem

Disturbance associated with nutrient inputs often lead to unintended consequences in aquatic ecosystems, including increased abundance of benthic algae (Raven and Taylor 2003, Worm and Lotze 2006, Rober et al. 2011). Few places exist where this fact is more apparent than in the lower Laurentian Great Lakes where increased water clarity and nutrient availability associated with increases in the abundance of invasive dreissenid mussels have resulted in greater benthic algal biomass along the shoreline (e.g., Malkin et al. 2010). Using our estimates of biomass-specific DOC release (2.8 mg C g^{-1} h^{-1}) and maximum seasonal biomass for *Cladophora* reported in the literature (range: <1–940 g dry mass/m^2; Higgins et al. 2005), we estimate that healthy algae have the potential to contribute >2.6 g C m^{-2} h^{-1} as DOC along the shoreline during the growing season. The rapid degradation of exudates and rapid increase in bacterial density observed in our study show that a substantial portion of *Cladophora* exudates are incorporated into bacterial biomass and, therefore, are important for sustaining bacterial communities and food webs along the shoreline. Algal exudates also can form complexes with toxic metals, such as Cu and Pb (Alldredge et al. 1993, Zhou et al. 1998) and may be important for the transfer of these metals to sediments and to the food web if consumed by zooplankton or other organisms (Giroldo and Vieira 2002). Alteration of the microbial loop through changes in substrate quality and biogeochemical cycling associated with metallic complexes probably are important outcomes of the increased benthic primary production in the Laurentian Great Lakes.

Release of exudates from actively growing phytoplankton has been recognized for some time, but our study is among the few (i.e., Wyatt et al. 2010, 2012) showing that freshwater benthic algae release a substantial portion of their photosynthate into the water during normal growth. By evaluating how environmental factors affect exudate release, we now have a better understanding of the potential for altered energy flow in areas where mats of *Cladophora* and, possibly, other benthic algae occur. However, we held environmental conditions constant and enriched incubation flasks to prevent nutrient limitation of bacteria during assays. In situ conditions will vary and proximate factors, such as temperature and nutrient availability, probably influence the ability of bacteria to degrade exudate release by benthic algae along the shoreline. Further research is needed to evaluate the role of proximate factors in heterotrophic metabolism in the presence of algal exudates and in a seasonal context. Such research and a more-complete understanding of the 2-way link between microbial community composition and exudate composition will provide a better understanding of the complexity of C cycling in freshwater benthic ecosystems.

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