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Effects of desiccation and rewetting on the release and decomposition of dissolved organic carbon from benthic macroalgae

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SUMMARY

1. Dissolved organic carbon (DOC) released by benthic algae is an important energy source in shallow aquatic ecosystems. Here, we evaluated the amount, composition and decomposition of DOC released by the benthic macroalga, *Cladophora glomerata*, during constant hydration and upon rewetting after drying for 0.5, 1, 2, 3 or 6 h on the shoreline of Lake Michigan, U.S.A.

2. Brief desiccation (0.5 h) significantly reduced gross primary productivity (mg C g dry mass^{-1} h^{-1}) to 0.43 ± 0.13 from 5.66 ± 0.35 for continuously wet algae.

3. When continuously wet, *Cladophora* filaments released 1.71 ± 0.26 mg DOC g DM^{-1} h^{-1} into the surrounding water, representing 30% of carbon fixation; release increased to 7.64 ± 1.08 after 1 h and peaked at 12.18 ± 0.57 after 6 h of drying. Percent extracellular release exceeded carbon fixation upon rewetting after 0.5-h desiccation and remained above 100% for each time interval thereafter.

4. The composition of exudates changed with time of desiccation. Carbohydrate release increased from 0.84 ± 0.13 to 3.0 ± 0.14 mg g DM^{-1} h^{-1} from 0 to 6 h desiccation, but declined as a percentage of total DOC from 49 to 25% over the same time. This reflected the much greater increase in protein yield from 0.40 ± 0.06 mg g DM^{-1} h^{-1} for wet algae to 7.70 ± 0.36 at 6-h desiccation (24 and 63% of total DOC release, respectively). The proportion of carbohydrates to proteins decreased with desiccation time, from 2 : 1 at 0 h to 1 : 2.5 at 6 h.

5. Exudates from constantly hydrated algae (DOC_0) and upon rewetting after 6-h desiccation (DOC_6) were degraded by more than 20% over 48 h of incubation inside dark bottles inoculated with the natural microbial community (10% lake water). Both treatments supported an exponential increase in bacterial density, but a greater proportion of DOC_0 was used by bacteria after 12 days. Bacterial density increased from 0.09 ± 0.001 to 3.30 ± 0.11 10^6 cells mL^{-1} between 0- and 12-day incubation in the DOC_0 treatment and from 0.09 ± 0.008 to 2.64 ± 0.10 during the same time period in the DOC_6 treatment.

6. Short-term desiccation results in a substantial release of DOC from *Cladophora* that is used rapidly by bacteria in the overlying water column. The drying–rewetting cycle could be important for organic matter cycling in freshwater ecosystems where benthic macroalgae occur.

Keywords: algal exudates, bacteria, *Cladophora*, decomposition, drought, Laurentian Great Lakes

Introduction

Dissolved organic carbon (DOC) released by benthic algae is an important source of energy for heterotrophic metabolism in aquatic ecosystems. In shallow water, such as the littoral of lakes, total primary production by benthic algae can be similar, or even exceed, that of the phytoplankton (Vadeboncoeur *et al.*, 2003; Malkin *et al.*, 2010). In addition to carbon that is used for growth, algae release photosynthate into the overlying water

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column as DOC (Cook *et al*., 2007; Wyatt, Stevenson & Turetsky, 2010; Wyatt *et al*., 2014), a portion of which is comprised of carbohydrates and proteins (Bertilsson & Jones, 2003). These compounds support bacterial production and, consequently, are important for the microbial loop of aquatic ecosystems (Rodríguez *et al*., 2013). Although their importance for ecosystem metabolism is becoming increasingly apparent (Hecky & Hesslein, 1995; Karlsson & Byström, 2005; Ask *et al*., 2009), we know relatively little about how environmental conditions influence the release of carbon exudates from benthic algae. Consequently, there is a gap in our ability to predict changes in organic matter cycling and energy flow associated with environmental disturbance.

The amount and composition of carbon transferred from algae to the surrounding water varies across aquatic ecosystems (Baines & Pace, 1991) and probably depends on environmental conditions (Bertilsson & Jones, 2003). Exudate release is thought to operate as an overflow mechanism to prevent damage to the photosynthetic apparatus when photosynthesis is active, but carbon assimilation is interrupted, often by limiting resources (Smith & Underwood, 2000). Several studies have found support for this hypothesis, mainly from pelagic environments, where algae typically release large amount of carbohydrate when nutrients are limited (Myklestad, 1995; Giroldo & Vieira, 2002). The release of carbon exudates can be decoupled from photosynthesis when cells become unhealthy and leak organic material, including proteins, across the cell membrane or following cell lysis (Gardner *et al*., 1987; Sundh, 1992; Agusti *et al*., 1998). By altering the ability of algae to construct cellular material, environmental conditions probably play an important role in determining the availability of organic matter for bacteria in the surrounding water (Obernosterer & Herndl, 1995).

In shallow habitats, benthic algae are exposed to desiccation during drying-rewetting cycles (e.g. Gottlieb, Richards & Gaiser, 2005) and taxa that can withstand drying are prominent (Rober *et al*., 2013). In particular, filamentous green algae are common where drying is fairly frequent such as in intermittent wetlands (Wyatt *et al*., 2012) and in rivers regulated by dams (Blinn *et al*., 1995). In recent years, the filamentous green macroalga, *Cladophora glomerata*, has become increasingly abundant in the lower Laurentian Great Lakes of North America (Michigan, Erie and Ontario) where it is exposed to air during seasonal changes in lake level and following the displacement of water during seiche events (Csanady, 1967). Following periods of rapid growth, *Cladophora* can detach from the bottom and wash onto shore where it is exposed to drying–rewetting episodes by successive wave action. Currently, little is known about how drying–rewetting episodes influence the release of carbon exudates from this, as well as other, macroalgae that occur in aquatic environments with periodic desiccation.

In general, estimates of exudate release from benthic algae have focussed on actively growing algal material and are reported as a function of photosynthesis. Drying may halt photosynthesis and result in leaching of accumulated photosynthate into the overlying water column. This mechanism of exudate release may be especially important nearshore and explain why the density of heterotrophic bacteria is often high when benthic macroalgae are present (Whitman *et al*., 2003; Olapade *et al*., 2006). The goal of this study was to assess the importance of periodic desiccation on the release of carbon exudates and subsequent uptake by bacteria in areas of the Laurentian Great Lakes where benthic macroalgae are abundant. To do this, we evaluated the amount, composition and decomposition of carbon exudates released during constant hydration and upon rewetting following short-term desiccation. We hypothesised that (i) in conditions of constant hydration, photosynthate is used for growth and is not released as DOC, (ii) desiccation stress negatively influences photosynthesis and promotes the release of DOC and (iii) desiccation affects the composition of released DOC and thus its importance as an energy source for heterotrophic bacteria.

Methods

This study was conducted in July 2011 at the Central Michigan University Biological Station, located on the eastern shore of Beaver Island in northern Lake Michigan, U.S.A. (latitude 45°42′N, longitude 85°30′W). *Cladophora* was collected from a depth of 30–50 cm along a 100-m reach of rocky shoreline, placed in a dark cooler filled with lake water and transported to the CMU Biological Station (*c*. 500 m away). *Cladophora* material at this site had been constantly submerged since its establishment *c*. 3 weeks before the project began. Filaments had <10% epiphyte cover and were bright green when collected.

To simulate the effects of drying over the course of 1 day, we placed tufts of *Cladophora* material (*c*. 4 cm wide, 10 cm long) onto wet sand inside a clear open-top plastic container (50 × 70 × 20 cm) in direct sunlight for five time intervals (0.5, 1, 2, 3, 6 h); $n = 12$ for each time interval. Sand was collected from the shoreline adjacent to the CMU Biological Station and homogenised before adding to a depth of 10 cm inside the container.

The sand was covered with filter paper to prevent sand granules from attaching to algae while drying. Sand was kept wet by adding lake water to a 500-mL polyethylene bottle connected to the container by plastic tubing (Fig. 1). The experiment was conducted on a wooden platform located *c.* 20 m from the lake shore, and drying was conducted between 10:00 and 16:00 hours. Photosynthetically active radiation (PAR) was measured with a LI-COR quantum sensor and LI-250 light meter (LI-COR, Lincoln, NE, U.S.A.) and ranged from 1132 to 1492 μmol photons m^{-2} s^{-1} during the drying process. Mean relative atmospheric humidity ranged from 68 to 73% and air temperature from 21.1 to 25.0 °C. The goal was to simulate the natural drying process as material washes onto shore or is uncovered by a seiche. Percentage water loss was used as a basis for evaluating algal parameters in response to drying (see below).

We collected and rehydrated replicate samples of algal material within each respective treatment (i.e. different time intervals) to evaluate the importance of drying–rewetting episodes on biomass-specific algal photosynthesis, exudate release and exudate composition. Twelve pieces of algal material (1 g wet weight each) were collected at random from the sand at each time period and weighed to determine desiccated mass. Each replicate sample (i.e. a piece of algal material) was placed into a separate clear biological oxygen demand bottle filled with 0.2-μm-filtered lake water to measure net primary production (NPP) for 1 h in the light and then wrapped with aluminium foil to measure oxygen consumption in the dark (dark respiration, R_d) for an additional 1 h. Oxygen concentrations were measured using a luminescent DO probe (Hach Company, Loveland, CO, U.S.A.). Bottles were placed in a 115-L clear tank with recirculating water pumped from near the lake bed offshore to maintain constant water temperature (range 20.8–22.7 °C) during incubations. PAR varied during the incubation, but minimum PAR (range from 1034 to 1363 μmol m^{-2} s^{-1}) exceeded values reported to be saturating for *Cladophora* photosynthesis (*c.* 500 μmol m^{-2} s^{-1}) in other studies (Higgins *et al.*, 2008). Following light incubation, a 60-mL aliquot of water was collected from each bottle using a syringe fitted with a rubber tube and filtered into a 60-mL acid-washed polyethylene bottle using a 0.45 μm filter (Millipore, Bedford, MA, U.S.A.). Water samples were kept cool in the dark and later analysed for biomass-specific DOC, total carbohydrate and protein concentration (all in mg g DM^{-1} h^{-1}). Algal material was blotted dry and weighed for rehydrated mass and then dried at 105 °C for 24 h for estimating dry mass (DM; APHA, 1998). Percentage water loss was calculated using the formula: (rehydrated mass − dry mass) − (desiccated mass − dry mass)/(rehydrated mass − dry mass). At the same time as the desiccation experiment was started, we measured gross primary productivity (GPP) and DOC release from 12 pieces of fully hydrated algal material (i.e. without desiccation stress, termed 0 h in treatment descriptions). In a concurrent study (Wyatt *et al.*, 2014), we documented constant exudate release from fully hydrated algal material over the course of a day with temperature and PAR values similar to the present study. GPP was calculated using NPP and R_d, and GPP values were converted to carbon units based on a C : O molar ratio of 0.375 and a photosynthetic quotient of 1.2 (Wetzel & Likens, 2000). Samples were analysed for DOC using a Shimadzu TOC-V analyser (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.). Carbohydrates were measured following the phenol sulphuric method (Dubois *et al.*, 1956), and protein concentration was measured following the Bradford method using bovine serum albumin as a standard (Bradford, 1976) with a Cary 60 UV-vis spectrophotometer (Agilent Technologies, Santa Clara, CA, U.S.A.). GPP and exudate release (measured as DOC concentration) were expressed as carbon evolution per unit DM, and exudate release was calculated as a percentage of GPP.

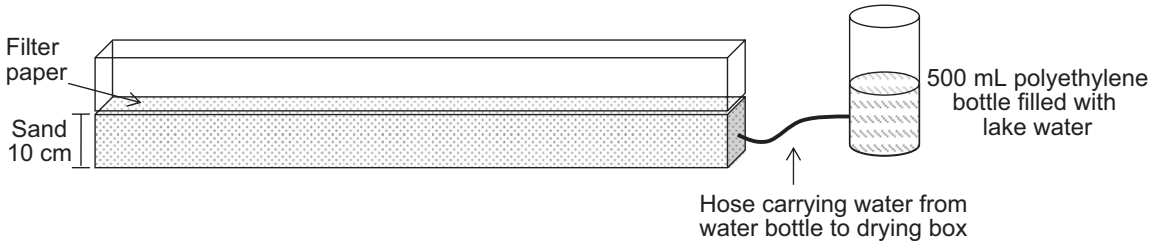

Fig. 1 Clear, open-top container (50 × 70 × 20 cm) used to dry algal material. The container was filled with wet sand to a depth of 10 cm. The top layer of sand was covered with a 50 × 70 sheet of filter paper to prevent sand granules from attaching to the algal material during the drying process. The sand was kept saturated by adding water to an external container connected to the drying box by a plastic hose.

Laboratory incubations

We collected exudates from algal material following 0-h (DOC_0) and 6-h (DOC_6) desiccation, for use in laboratory incubations of bacteria decomposition. Algal material from each time interval (0 and 6 h) was placed in four replicate beakers each containing 500 mL 0.2-μm-filtered lake water and kept under constant light (500 μmol m^{-2} s^{-1}) and at room temperature (*c.* 21 °C) for 36 h. The incubation process was not sterile, but bacterial density was monitored and remained $<1 \times 10^4$ cells L^{-1}, which has been shown to have low impact on the composition of exudates in other studies (Giroldo, Ortolano & Vieira, 2007). Algal material was removed from beakers using forceps, and exudates were filtered through a 0.2-μm VacuCap filter (Pall Life Sciences, Ann Arbor, MI, U.S.A.) into an acid-washed flask, and solutions from each treatment were diluted to approximately the same DOC concentration (65 mg C L^{-1}). A bacterial inoculum was prepared by filtering lake water through a 0.7-μm filter (GF/F filter; Whatman, Springfield Mill, U.K.). Other studies have shown that *c.* 80% of bacterial cells pass through 0.7-μm-pore-size filters (Giroldo *et al.*, 2007). The bacterial inoculum was kept at a constant temperature (*c.* 21 °C) until the start of the experiment (2 h).

We quantified bacterial degradation of DOC_0 and DOC_6 to evaluate the importance of desiccation period on energy available for bacteria in the water column. This experimental design allowed us to evaluate degradation patterns between treatments at each end of the desiccation spectrum and with the potential for the greatest differences in chemical composition. A bacterial inoculate (50 mL of 0.7-μm-filtered lake water) was pipetted into six Erlenmeyer flasks each containing 450 mL of DOC_0 or DOC_6; $n = 3$ for each treatment. Flasks were enriched with KH_2PO_4 and KNO_3 to a final concentration of 5 μmol P and 50 μmol N, respectively, to prevent nutrient limitation of bacteria (Fagerbakke, Heldal & Norland, 1996). Our goal was to test the hypothesis that bacteria use algal exudates as a carbon source without the confounding variable of nutrient limitation of bacterial growth. Flasks were incubated in the dark for 12 days, and temperature was kept constant throughout the experiment (*c.* 21 °C). Flasks were sampled at 0, 2, 4, 8 and 12 days for measures of specific UV absorbance at $\lambda = 254$ nm ($SUVA_{254}$), DOC concentration and bacterial abundance. Water samples were analysed for UV-vis absorption at $\lambda = 254$ nm using a Beckman Coulter model DU 800 spectrophotometer (Beckman Coulter, Inc., Brea, CA, U.S.A.). The proportion of DOC used in each time interval (% DOC use) was calculated using the formula: $[(DOC_{initial} - DOC_{final})/(DOC_{initial})]$ (100), where $DOC_{initial}$ is the starting concentration and DOC_{final} is the concentration at each time interval. $SUVA_{254}$ was calculated by dividing UV-vis absorbance at $\lambda = 254$ nm by DOC concentration. $SUVA_{254}$ gives an 'average' molar absorptivity for all the molecules contributing to the DOC in a sample (Chin, Aiken & O'Loughlin, 1994). Subsamples for bacterial growth were preserved with formaldehyde and later stained with DAPI (4′,6-diamino-2-phenylindole) and filtered onto 0.2-μm black filters (OSMONIC INC., Livermore, CA, U.S.A.; Porter & Feig, 1980). Bacterial growth was evaluated by direct counts using a Leica DM 4000 microscope with fluorescence (Leica Microsystems, Wetzlar, Germany). At least 300 bacteria or 25 fields were counted per filter.

Statistical analyses

Gross primary productivity, DOC, total carbohydrate and protein yield were compared between time intervals using one-way ANOVAs and Tukey's test for *post hoc* comparison of means. Differences in DOC concentration, $SUVA_{254}$ and bacterial abundance between treatments during the degradation experiment were analysed with *t*-tests. All statistical analyses were performed with SYSTAT (version 11; SYSTAT Software Inc., Point Richmond, CA, U.S.A.). The distributions of variables were $\log(x + 1)$-transformed if necessary to correct for non-normal distribution and unequal variances among treatments prior to analysis.

Results

As expected, water loss of algal material increased with drying time. Algal material was fully hydrated at 0 h (by definition, 0% water loss). Loss was *c.* 10% after 0.5 h, 40% after 1 h and 80% after 2 h, and algae were fully dehydrated (100% water loss) after 6 h.

Gross primary productivity decreased with desiccation time. GPP (mg C g Dry $Mass^{-1}$ h^{-1}) at 0.5 h (0.43 ± 0.13) was significantly lower than without desiccation (5.66 ± 0.35; ANOVA, $F_{5,66} = 209.5$, $P < 0.0001$). GPP decreased in each time interval following 0.5 h, reaching 0 mg C g DM^{-1} h^{-1} after 3 h. There were no significant differences in GPP between time intervals after 0.5-h desiccation ($P > 0.05$).

Dissolved organic carbon release increased upon rewetting after each desiccation time interval (Table 1). DOC

Table 1 Exudate composition released by algae following each of five drying intervals (0.5, 1, 2, 3 and 6 h) and with no drying (0 h)

	Drying time (h)					
Exudate composition (mg g DM^{-1} h^{-1})	0	0.5	1	2	3	6
Carbohydrates	0.83 ± 0.13^{a}	0.56 ± 0.07^{a}	2.08 ± 0.29^{b}	2.83 ± 0.26^{c}	2.89 ± 0.12^{c}	3.00 ± 0.14^{c}
Protein	0.40 ± 0.06^{a}	0.43 ± 0.05^{a}	2.11 ± 0.30^{b}	3.22 ± 0.30^{c}	5.94 ± 0.25^{d}	7.71 ± 0.36^{e}
Dissolved organic carbon	1.71 ± 0.26^{a}	1.67 ± 0.20^{a}	7.64 ± 1.08^{b}	10.6 ± 0.98^{c}	11.3 ± 0.47^{c}	12.2 ± 0.57^{c}

Values are mean ± 1 SE, $n = 12$. Significant differences are indicated by different letters.

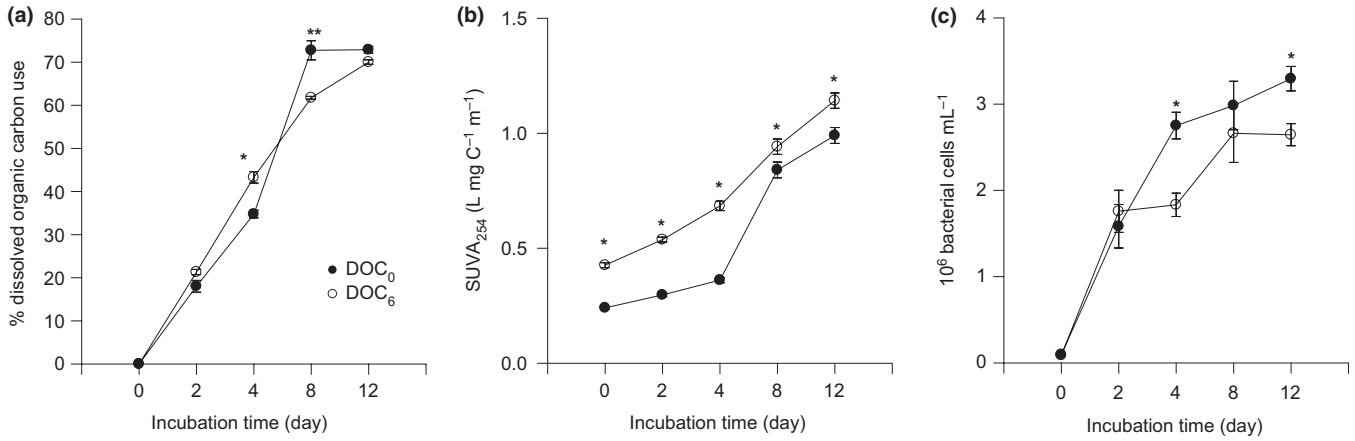

Fig. 2 Comparison of (a) percentage of dissolved organic carbon (DOC) use, (b) $SUVA_{254}$ (L mg C^{-1} m^{-1}) and (c) 10^6 bacterial cells mL^{-1} between the DOC_0 and DOC_6 treatments during a 12-day incubation period. The asterisks indicate significant differences between DOC_0 and DOC_6 treatments (Student's *t*-test, $P < 0.05$). Points are mean ± 1 SE, $n = 3$ for each treatment.

release after 0 h (1.71 ± 0.26 mg g DM^{-1} h^{-1}) was 30% of carbon fixation and similar to the value after 0.5-h desiccation ($P > 0.05$). Upon rewetting after 0.5-h desiccation, DOC release exceeded carbon fixation and percentage extracellular release remained above 100% in each drying time interval thereafter (Table 1). Release of DOC upon rewetting increased to 7.64 ± 1.08 mg g DM^{-1} h^{-1} after 1 h and peaked after 6 h of desiccation (12.18 ± 0.57). DOC release upon rewetting after 2-, 3- and 6-h desiccation was significantly greater than after 0-, 0.5- and 1-h desiccation and after 1 h was significantly greater than after 0- and 0.5-h desiccation (ANOVA, $F_{5,66} = 54.02$, $P < 0.0001$); DOC release was similar upon rewetting after 2, 3 and 6 h of desiccation ($P > 0.05$).

The composition of exudates changed over time of desiccation (Table 1). Carbohydrate yield increased from 0.84 ± 0.13 to 3.0 ± 0.14 mg g DM^{-1} h^{-1} and represented 49 and 25% of total exudate production, upon rewetting after 0- and 6-h desiccation, respectively (Table 1). Protein yield (mg g DM^{-1} h^{-1}) increased from 0.40 ± 0.06 to 7.70 ± 0.36 and represented 24 and 63% of total exudate production, after 0- and 6-h desiccation, respectively (Table 1). The proportion of carbohydrates to proteins decreased with desiccation time, from 2 : 1 at 0 h to 1 : 2.5 after 6-h desiccation (Table 1). Carbohydrate yield after 2-, 3- and 6-h desiccation was significantly greater than after 0, 0.5 and 1 h, and carbohydrate and protein release after 1-h desiccation was significantly greater than after 0- and 0.5-h desiccation (ANOVA, $F_{5,66} = 40.5$, $P < 0.0001$). Carbohydrate yield was similar after 2-, 3- and 6-h desiccation ($P > 0.05$). Protein yield was similar after 0- and 0.5-h desiccation but increased significantly after each successive time interval thereafter (ANOVA, $F_{5,66} = 146.99$, $P < 0.0001$; Table 1).

Degradation of algal exudates

Algal exudates were labile during biodegradability assays. DOC_0 decreased from 65.52 ± 0.17 to 42.37 ± 0.33 mg C L^{-1} and DOC_6 from 65.91 ± 0.37 to 37.41 ± 0.81 mg C L^{-1}, representing 34 and 43% DOC loss in each treatment, respectively, after 4-day incubation. After 12-day incubation, DOC_0 had decreased to 17.83 ± 0.45 and DOC_6 to 9.78 ± 0.36 mg C L^{-1}, representing a similar 73 and 70% DOC use in each treatment, respectively (Fig. 2a). Initial $SUVA_{254}$ of DOC_0 was significantly lower than that of DOC_6 (Fig. 2b).

$SUVA_{254}$ increased with decomposition in both treatments, but remained significantly higher in the DOC_6 treatment than in the DOC_0 treatment throughout the incubation period (Fig. 2b). Bacterial density increased with decomposition, increasing from 0.09 ± 0.001 to 3.30 ± 0.11 10^6 cells mL^{-1} in the DOC_0 treatment and from 0.09 ± 0.008 to 2.64 ± 0.10 in the DOC_6 treatment (Fig. 2c).

Discussion

Desiccation tolerance of algae is determined largely by the ability to restore photosynthesis and respiration after rehydration (Wiltens, Schreiber & Vidaver, 1978). Compared with benthic microalgae (i.e. sediment diatoms), which have the ability to migrate vertically within sediments during aerial exposure (McKew *et al.*, 2011), attached macroalgae are susceptible to periods of desiccation. Similar to results reported in previous studies (Usher & Blinn, 1990; Blinn *et al.*, 1995; Bergey *et al.*, 2010), our results show that drying, followed by rehydration, had a strong effect on *Cladophora* photosynthesis. Brief drying of only 0.5 h (10% water loss) decreased photosynthesis by 90%, and photosynthesis did not recover after 90% water loss, which occurred after 3 h of drying. Such rapid dehydration could be due a lack of mucilage and the high surface area of branching filaments exposed to air (Usher & Blinn, 1990; Angradi & Kubly, 1993; Blinn *et al.*, 1995). Following 100% water loss, *Cladophora* filaments became pale in colour and rigid, this remaining after rehydration. Similar results have been reported in rivers with regulated flow, where the surface of *Cladophora* mats becomes covered with a thin layer of bleached filaments with a rigid texture following aerial exposure (Bergey *et al.*, 2010). Although this result could be construed as indicating poor adaptation to desiccation stress, quick-drying outer filaments may serve as a shield to protect underlying filaments from desiccation during periods of exposure (Usher & Blinn, 1990). As the algal mat collapses during aerial exposure, rigid outer filaments may trap water and prolong hydration of internal filaments.

As a fraction of photosynthesis, DOC release from continuously hydrated *Cladophora* material was within the range of those reported for phytoplankton (Baines & Pace, 1991; Biddanda & Benner, 1997) and benthic algae (Kaplan & Bott, 1982; Wyatt *et al.*, 2012, 2014) in other studies. The production of extracellular carbon by healthy algae may be due to unbalanced metabolism, where cells release carbon in excess of their energetic requirements (Staats *et al.*, 2000). Under optimal conditions, we would expect the carbon fixed during photosynthesis to be used in growth and only a small amount to be released into the water column. Our results support this hypothesis, with about 30% of fixed carbon released to the surrounding environment when algal material was continuously hydrated. In contrast, exudate release upon rewetting increased substantially following even brief desiccation, exceeding carbon fixation after only 10% water loss. This finding is similar to those described for benthic diatoms in intermittent wetlands, where exudate release increases sharply upon rewetting following desiccation (McKew *et al.*, 2011). The release of organic carbon is thought to prevent damage to the photosynthetic apparatus in conditions where photosynthesis is active, but growth restricted (Smith & Underwood, 2000; Perkins *et al.*, 2001). However, this mechanism does not explain our data because desiccated tissue was not photosynthetically active, suggesting that DOC release was not an active process (Fogg, Nalewajko & Watt, 1965), but instead a passive process (Bjørnsen, 1988) associated with autolysis, which can occur following stress of algal material (Sundh, 1992).

While the chemical composition of dissolved organic matter derived from phytoplankton (Biddanda & Benner, 1997; Biersmith & Benner, 1998; Giroldo *et al.*, 2007) and marine benthic algae (Haas & Wild, 2010) has previously been analysed, there are few data available on the composition of organic matter released by freshwater macroalgae. Our results indicate that carbohydrates and proteins accounted for *c.* 75% of the total organic carbon released from healthy material. These results are similar to the composition of exudates of various phytoplankton (Biersmith & Benner, 1998) and indicate rather homogenous algal exudates. Both carbohydrates and proteins released upon rewetting increased with the duration of desiccation, but the increase was greater for protein. The high carbohydrate content of DOC from non-stressed material is consistent with the release of recent photosynthate from phytoplankton (Biddanda & Benner, 1997). The increase in protein content suggests a loss of cytoplasm or damage to membranes and is consistent with prior reports of cell lysis following dehydration (Rosenstock & Simon, 2001).

The rapid degradation of organic matter observed in our study suggests that exudates released by benthic macroalgae are likely to be an important energy source for heterotrophic bacteria in this lake and presumably other aquatic ecosystems, especially following stranding events. After 8 days of laboratory incubation, *c.* 70 and 60% of exudates were degraded in the DOC_0 and DOC_6 treatments, respectively. A high percentage of exudate

use in the DOC_0 treatment was not surprising given that carbohydrates are considered to be an excellent food source for bacteria (Giroldo *et al.*, 2007). Bacterial density increased in the DOC_0 treatment on day 4, just prior to an increase in decomposition on day 8. Further, $SUVA_{254}$ values, which have been used as an indicator of aromaticity (Chin *et al.*, 1994), were greater in the DOC_6 treatment compared with the DOC_0 treatment throughout the incubation period. The relative importance of proteins, which can contribute up to 50% of the heterotrophic bacterial nitrogen requirements (Tupas & Koike, 1990), may have been subdued as a consequence of nutrient enrichment during the laboratory incubation.

Our study provides information about the timescale involved in microbial processing of new organic matter as it becomes available and is used by heterotrophic bacteria. Algal exudates in both DOC_0 and DOC_6 treatments, while representing a highly bioreactive component of the organic matter pool, became less reactive with microbial degradation. $SUVA_{254}$ values increased with decomposition, especially in the DOC_0 treatment, where $SUVA_{254}$ increased rapidly following the increase in carbon use after 4 days. Similarly, other studies have found that aromatic compounds tend to increase with bacterial degradation, demonstrating the ability for heterotrophic bacteria to alter exudate composition during decomposition (i.e. Giroldo, Vieira & Paulsen, 2003; Wyatt *et al.*, 2012). Collectively, these findings offer support for the size-reactivity continuum model for organic matter (Amon & Benner, 1996), which predicts that diagenetically young material is most reactive and organic matter becomes less bioreactive as it is broken down during microbial processing.

During the past few decades, *Cladophora* biomass has increased in the Laurentian Great Lakes, largely due to changes in optical properties and nutrient concentrations following the establishment of invasive dreissenid mussels (Hecky *et al.*, 2004; Ozersky *et al.*, 2009). After periods of rapid growth, *Cladophora* detaches from the bottom and accumulates in protected areas and along shorelines (Higgins *et al.*, 2008). Heterotrophic bacteria, which in some cases include human pathogens, have been found to be associated with both living and senescent *Cladophora* mats (Whitman *et al.*, 2003; Ishii *et al.*, 2006; Olapade *et al.*, 2006). Despite the potential for this and other benthic macroalgae to act as a vector for bacteria, little information exists to explain the mechanisms driving the relationships between bacteria and benthic macroalgae in near-shore areas of the Great Lakes. The rapid increase in bacterial abundance in our study suggests that one mechanism whereby actively growing and senescent *Cladophora* may support heterotrophic bacteria within the algal mat is through the release of DOC. When we extrapolate our measures of DOC release to estimates of biomass reported in the literature (Higgins *et al.*, 2005), we find that actively growing *Cladophora* has the potential to contribute up to 1.6 g C m^{-2} h^{-1} to the surrounding water; this amount increases to 11.4 g C m^{-2} h^{-1} in conditions of periodic exposure to air. Given the low carbon environment of the Great Lakes (*c.* 2 mg L^{-1} DOC; K. H. Wyatt, unpublished data), *Cladophora*, along with benthic microalgae (Lowe, 1996), is probably an important contributor to the nearshore carbon budget in areas where benthic mats occur.

This current study of algal-derived DOC in the Laurentian Great Lakes adds to a growing body of literature on the release of extracellular material by algae across a wide range of aquatic ecosystems (Smith & Underwood, 2000; Kiemle, Domozych & Gretz, 2007; Bellinger *et al.*, 2010). In particular, our work expands on previous research evaluating exudate release by benthic microalgae following periods of desiccation (McKew *et al.*, 2011) and provides new information about how drying–rewetting episodes influence organic matter cycling by macroalgae in shallow benthic environments. While this study is on Lake Michigan, our results are relevant to other freshwater ecosystems where *Cladophora* and other benthic macroalgae occur (i.e. Parker & Maberly, 2000). Benthic algae are frequently exposed to air in a range of aquatic environments, including rivers with regulated flow (Usher & Blinn, 1990; Blinn *et al.*, 1995), intermittent wetlands (Gottlieb *et al.*, 2005; Thomas *et al.*, 2006; Rober *et al.*, 2013) and intertidal areas of marine ecosystems (Davison & Pearson, 1996; Underwood & Kromkamp, 1999). More work is needed to evaluate the link between microbial community composition and exudate composition with drying–rewetting cycles across these ecosystems. Also, we maintained relatively constant water temperature during field and laboratory incubations. It is likely that seasonal changes in water temperature will influence the release of carbon exudates by algae and regulate the uptake of organic matter by bacteria. Additional study is needed to evaluate the importance temperature, as well as other proximate factors such as nutrient concentrations, on exudate release and uptake by heterotrophic bacteria within a seasonal context. This, along with research to evaluate exudate composition during microbial degradation, will provide a better understanding of the relative importance of different organic compounds for energy flow in aquatic ecosystems.

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