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Kevin A. Kuehn

University of Southern Mississippi, kevin.kuehn@usm.edu

Steven N. Francoeur

Eastern Michigan University, steve.francoeur@emich.edu

Robert H. Findlay

University of Alabama

Robert K. Neely

Eastern Michigan University

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Priming in the microbial landscape: periphytic algal stimulation of litter-associated microbial decomposers

KEVIN A. KUEHN,^{1,4} STEVEN N. FRANCOEUR,² ROBERT H. FINDLAY,³ AND ROBERT K. NEELY^{2,5}

¹Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, Mississippi 39406 USA

²Center for Aquatic Microbial Ecology, Biology Department, Eastern Michigan University, Ypsilanti, Michigan 48197 USA

³Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487 USA

Abstract. Microbial communities associated with submerged detritus in aquatic ecosystems often comprise a diverse mixture of autotrophic and heterotrophic microbes, including algae, bacteria, protozoa, and fungi. Recent studies have documented increased rates of plant litter mass loss when periphytic algae are present. We conducted laboratory and field experiments to assess potential metabolic interactions between natural autotrophic and heterotrophic microbial communities inhabiting submerged decaying plant litter of *Typha angustifolia* and *Schoenoplectus acutus*. In the field, submerged plant litter was either exposed to natural sunlight or placed under experimental canopies that manipulated light availability and growth of periphytic algae. Litter was collected and returned to the laboratory, where algal photosynthesis was manipulated (light/dark incubation), while rates of bacterial and fungal growth and productivity were simultaneously quantified. Bacteria and fungi were rapidly stimulated by exposure to light, thus establishing the potential for algal priming of microbial heterotrophic decay activities. Experimental incubations of decaying litter with ¹⁴C- and ¹³C-bicarbonate established that inorganic C fixed by algal photosynthesis was rapidly transferred to and assimilated by heterotrophic microbial decomposers. Periphytic algal stimulation of microbial heterotrophs, especially fungal decomposers, is an important and largely unrecognized interaction within the detrital microbial landscape, which may transform our current conceptual understanding of microbial secondary production and organic matter decomposition in aquatic ecosystems.

Key words: algae; bacteria; decomposition; fungi; metabolic interactions; periphyton; photosynthesis; plant litter; priming effect; protozoa.

INTRODUCTION

In aquatic ecosystems, decaying plant litter often harbors a diverse community of autotrophic and heterotrophic microorganisms, which may include species of algae, bacteria, fungi, and protists. These microbial communities often form complex biofilms (Battin et al. 2007) upon the litter substrata they inhabit (hereafter detrital periphyton complex), and in the case of filamentous fungi and some bacteria, also grow pervasively within the litter substratum itself. In aquatic ecosystems exposed to sufficient light, periphytic algae (including cyanobacteria) frequently develop on the surfaces of decaying plant litter, where they can reach high cell densities and biovolumes (Suberkropp and Klug 1974, Meulemans and Roos 1985, Neely and Wetzel 1997). As a result, algal communities associated with the surfaces of living and dead plant matter and mineral substrata can be a major contributor to primary

production within aquatic ecosystems (Wetzel 1990, Goldsborough et al. 2005), and can significantly affect the uptake and immobilization of nutrients (Mulholland and Webster 2010).

The close spatial proximity of diverse microbial groups on and within detrital periphyton suggests the potential for interactions among specific microbial inhabitants. In the absence of periphytic algae, several researchers have experimentally demonstrated antagonistic interactions between litter-associated bacteria and fungi (Wohl and McArthur 2001, Gulis and Suberkropp 2003, Mille-Lindblom and Tranvik 2003, Mille-Lindblom et al. 2006, Romani et al. 2006, Baschien et al. 2009), whereas others have observed either positive (Bengtsson 1992, Romani et al. 2006) or neutral interactions (Das et al. 2012). Many of these studies were conducted within controlled laboratory microcosms using only a limited number of interacting heterotrophic microbial species. To date, few studies have assessed interactions among naturally developed microbial communities on decaying plant litter.

In the presence of algae, several interactions have been observed within periphytic microbial communities. For example, periphytic algae can provide a greater surface area for bacterial colonization (Rier and Steven-

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⁴ E-mail: kevin.kuehn@usm.edu

⁵ Present address: Provost and Vice President for Academic Affairs, Texas Women's University, Denton, Texas 76201 USA.

son 2001, Carr et al. 2005). Furthermore, prior research has demonstrated that rates of bacterial growth are enhanced by algal photosynthesis in laboratory biofilm cultures (Murray et al. 1987), natural periphyton on both artificial and natural rock substrata (Neely and Wetzel 1995, Scott et al. 2008), and natural floating mats of algae and bacteria (Scott and Doyle 2006), which is consistent with the widely established influence of phytoplankton on pelagic bacterial growth and production (Wetzel 2001).

More recently, several studies have reported that the activities of heterotrophic extracellular degradative enzymes in periphyton are rapidly increased by light availability (Francoeur and Wetzel 2003, Francoeur et al. 2006, Rier et al. 2007, Ylla et al. 2009). We observed that both short-term (diel fluctuations) and long-term (experimental shading) variation in photosynthetically active radiation (PAR) influenced algal biomass and heterotrophic extracellular hydrolytic and oxidative enzyme activities in periphytic microbial communities associated with inorganic substrata and decaying *Populus tremuloides* leaf litter (see Rier et al. 2007). Long-term experimental shading of *P. tremuloides* leaf litter resulted in a twofold decrease in the litter decomposition rate when compared to leaf litter exposed to light, suggesting that light availability and corresponding algal photosynthesis may facilitate litter decay processes through algal-mediated stimulation of litter-associated microbial decomposers. Similar findings have also been reported by other researchers (Franken et al. 2005, Lagrue et al. 2011, Danger et al. 2013, but see Albariño et al. 2008), where algal presence and/or light availability positively influenced litter processing rates through its impact on multiple trophic levels.

Observations of increased plant litter mass loss in the presence of algae suggest that algal photosynthetic activities might enhance or possibly prime microbial carbon and nutrient mineralization processes. Well documented in terrestrial ecosystems (Blagodatsky et al. 2010, Kuzyakov 2010), the priming effect describes the stimulatory influence of labile carbon additions (e.g., plant root exudates) on the microbially mediated decomposition and mineralization of recalcitrant soil organic matter. Such priming effects may also be relevant in aquatic ecosystems (Guenet et al. 2010, Bianchi 2011), where periphytic algal exudates within the litter microbial landscape could stimulate the ability of heterotrophic decomposers to process and mineralize detrital organic matter (Danger et al. 2013).

This study was conducted to assess whether periphytic algae influence heterotrophic microbial activities on and within natural submerged decaying plant detritus (i.e., a detrital periphyton biofilm complex). Throughout this study we viewed the detrital periphyton complex as a microbial landscape. This ecological concept, originating with Battin et al. (2007), states that biofilms are landscapes with spatially explicit dimensions, biodiversity, and ecosystem function; like other macroecological

landscapes, their composition, structure, and function can be influenced by scale. Unlike other landscapes, where the appropriate scales range from meters to kilometers, critical scales for microbial landscapes can fall within the micron to centimeter range. This concept provides a unifying theoretical framework for testing ecological theory at appropriate scales (Levin 1992), and understanding its attendant consequences for critical ecological processes ranging from carbon mineralization and sequestration (e.g., Suberkropp et al. 2010, Clemmensen et al. 2013) to ecological stoichiometry and its impacts on food web dynamics and nutrient cycling (e.g., Hessen et al. 2004, Cross et al. 2005). In this study, we examined whether autotrophs (algae) influenced the growth rates of heterotrophic microbial decomposers (fungi and bacteria) at the microbial landscape scale. In addition, we sought to investigate whether stimulation of heterotrophic microbial decomposers was consistent with the production, exudation, and assimilation of photosynthetically derived labile dissolved organic carbon (DOC) from co-occurring microalgal communities.

METHODS

Study site

This study was conducted in an ~18-ha freshwater marsh located in southeast Michigan, USA (42°12'58" N 83°37'11" W). The study site is a created wetland complex formed more than 20 yr ago that receives water from the Paint Creek watershed. The study was situated in the southeastern corner of the wetland, which is dominated by the emergent macrophytes *Typha angustifolia*, *Schoenoplectus acutus*, and *Phragmites australis*.

Laboratory and field procedures

We conducted both laboratory and field manipulation experiments to examine metabolic interactions between naturally occurring autotrophic and heterotrophic microbial communities inhabiting submerged plant litter of *T. angustifolia* and *S. acutus*. During the initial experiment, overwintered standing dead leaf litter of *T. angustifolia* was collected from the marsh, returned to the laboratory, air dried, and stored at ambient lab temperatures until used. Dried *T. angustifolia* leaf blades were cut into ~16 cm long sections, placed into wire mesh trays (see Francoeur et al. 2006), and submerged in the marsh surface waters under natural August sunlight conditions. Five replicate litter trays were retrieved after 10 and 29 d. Litter was carefully removed from trays, enclosed in clean plastic containers with wetland water, placed on ice in a cooler, and returned to the laboratory within 30 min. In the laboratory, leaf litter from replicate trays was sectioned into 1.7 cm long pieces (~3.2 cm²), and the growth and production rates of litter-associated algae (¹⁴C bicarbonate incorporation, *n* = 3 replicate trays), bacteria ([³H]-leucine incorporation, *n* = 5 replicate trays), and fungi (¹⁴C-acetate incorporation, *n* = 5 replicate trays) were simultaneously quantified from randomly selected litter pieces incubated

under ultraviolet (UV)-free light ($400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR) and dark conditions. In addition, randomly selected litter pieces from replicate trays were preserved for analysis of algal community composition and relative abundance, and litter-associated biomass of algae, bacteria, and fungi ($n = 4\text{--}5$ replicate trays).

A subsequent experiment involved direct in situ manipulation of light availability to decaying leaf litter to test if long-term light exclusion influenced periphyton community development (i.e., algal colonization), and thus the potential strength of autotrophic-heterotrophic interactions. During these experiments, standing dead leaf litter of *T. angustifolia* and *S. acutus* was collected from the marsh, dried, stored, and sectioned. Litter was placed into open-top wire mesh baskets and submerged in the marsh surface waters in August under experimental canopies that varied in light availability to decomposing litter.

Five replicate experimental canopies were constructed of black Acrylite (Evonik Industries AG, Parsippany New Jersey, USA) that excluded light (hereafter opaque canopies), and five canopies were constructed of Acrylite OP4, which allowed for the passage of light (PAR+UV; hereafter transparent canopies). Each canopy was tent shaped (total area $\sim 2 \text{ m}^2$), consisting of two wooden $1.2 \times 1.2 \text{ m}$ frames covered with Acrylite, connected at a 90° angle, with two triangular Acrylite endpieces. Canopies were placed in the marsh with the open bottom of the tent just below the air-water interface. Acrylite OP4 canopies allowed the passage of 77% and 94% of ambient UV and PAR, respectively. Black Acrylite canopies excluded >99% of UV and PAR.

After 35 d of submergence, one litter basket from each canopy was retrieved and immediately returned to the laboratory. In the laboratory, leaf litter from each replicate was sectioned into 1.7 cm long pieces (*T. angustifolia* $\sim 3.2 \text{ cm}^2$, *S. acutus* $\sim 1.7 \text{ cm}^2$) and the growth and production rates ($n = 5$ replicate trays) of algae, bacteria, and fungi were simultaneously quantified under UV-free light ($400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR) and dark conditions (litter from OP4 transparent canopy treatment only). Additional litter pieces were preserved for the analysis of algal community composition and relative abundance, and biomass of algae, bacteria, and fungi ($n = 5$ replicate trays).

Water temperatures outside and inside the canopy treatments were continuously monitored every 30 min throughout the entire study period using Onset Stow-Away data loggers (Onset Computer Corp., Bourne, Massachusetts, USA). In addition, water samples were collected during sampling periods for determination of pH, alkalinity, and nutrient concentrations; dissolved inorganic nitrogen (DIN): $\text{NO}_2\text{-NO}_3\text{+NH}_4$; and total phosphorus (TP).

Algal biomass, community composition, and production

Algal biomass associated with litter samples was estimated from chlorophyll *a* concentrations. Two litter

pieces from each replicate were placed into sterile 15-mL polypropylene conical tubes, and stored frozen (-20°C , in darkness) until analyzed. Chlorophyll *a* was extracted using the hot ethanol technique, and quantified spectrophotometrically with acidification to correct for phaeopigments (Francoeur et al. 2013). Chlorophyll content was converted to algal C assuming a conversion factor of $30 \mu\text{g}$ chlorophyll *a*/mg algal C, which is approximately the midpoint of the range of algal chlorophyll : C ratios reported by Cloern et al. (1995). Algal community composition and relative abundance was determined using bright field microscopy. One litter piece from each replicate was placed in a 20-mL plastic scintillation vial containing 5–10 mL of 2.5% (v/v) glutaraldehyde and stored (4°C) until analyzed. Algae were removed from litter by scraping and brushing, then identified and enumerated ($400\times$ magnification, ≥ 100 total cells per sample) using the taxonomy of Wehr and Sheath (2003) for diatoms and Prescott (1973) for all other algae.

Algal primary productivity was estimated using ^{14}C -bicarbonate incorporation. Two litter pieces from each replicate were placed into sterile 20-mL glass scintillation vials containing 5 mL of filtered ($0.22\text{-}\mu\text{m}$ pore size) wetland water and $0.0185 \text{ MBq H}^{14}\text{CO}_3^-$. Vials were placed on their sides in a Percival E-36HO plant growth chamber (Percival, Inc., Perry, Iowa, USA) and incubated for 2 h at 20°C under light ($400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR, no UV) and dark conditions. Killed control samples ($n = 1\text{--}2$ samples) containing formalin (3% v/v) were also incubated under light and dark conditions to correct for nonbiological ^{14}C incorporation. Inorganic C pools were estimated by measuring the alkalinity of water used for incorporation assays. After incubation, samples were killed with formalin (3% v/v final concentration), filtered (except day 10 initial experiments), and litter and filters stored frozen (-20°C) until analyzed. Samples were later acid fumed, extracted, and radioassayed, and algal production was estimated following protocols described in Francoeur et al. (2006).

Bacterial biomass, growth, and production

Bacterial abundance and biomass were determined by direct count epifluorescence microscopy after staining with SYBR Green I (Molecular Probes Inc., Eugene, Oregon, USA; Buesing 2005), as detailed in Francoeur et al. (2006). Two litter pieces per replicate tray were placed in a sterile 20-mL glass scintillation vial containing 10 mL of 2% (v/v) phosphate buffered (sodium pyrophosphate, 0.1% w/v) formalin. Bacterial cells were detached from litter using probe ultrasonication (1 min on ice), and subsamples ($30\text{--}200 \mu\text{L}$) were vacuum filtered (25 mm, $0.2 \mu\text{m}$ Anodisc-supported Whatman filters; Whatman plc, Maidstone, Kent, UK) and stained. Bacterial cells were enumerated and assigned into categories according to size and shape. Biovolume estimates (V , μm^3) for each size class were calculated from length (l) and width (w) measurements using the formula: $V = w^2/4 \times (l - w) \times \pi + w^3/6 \times \pi$.

Biovolume estimates were converted to bacterial carbon (fg C) using the formula: bacterial dry mass (fg) = $435 \times V^{0.86}$ (Loferer-Kröbächer et al. 1998), assuming 50% C in bacterial dry mass.

Bacterial growth and production rates were estimated using [^3H]-leucine incorporation into bacterial protein (Gillies et al. 2006). Two litter pieces per replicate tray were incubated in sterile glass scintillation vials containing 4 mL of filtered (0.22- μm pore size) wetland water and 2.5 $\mu\text{mol/L}$ [4,5- ^3H]-leucine (specific activity of 220 GBq/mmol). Vials were placed on their sides in a plant growth chamber and incubated for 30 min at 20°C under light and dark conditions. Killed control samples ($n = 1-2$ samples) containing trichloroacetic acid (TCA, 5% v/v) were also incubated to correct for nonbiological [^3H]-leucine incorporation. Incorporation of radiolabelled leucine was stopped by the addition of TCA (5% v/v final concentration), followed by heating samples for 30 min at 80°C. Samples were then cooled, filtered, rinsed, and radioassayed as described in Gillies et al. (2006). Using this [^3H]-leucine method, the signal:noise ratio between samples and killed controls ranged from 5 to 9 and 4 to 5 for light and dark incubated samples, respectively. Bacterial production was calculated as μg bacterial C produced per g detrital C per h using the conversion factor of 1.44 kg C produced per mole leucine incorporated (Buesing and Marxsen 2005). Bacterial growth rates (BGR) were calculated using the formula: $\text{BGR} = \ln(1 + \text{production/biomass ratio})$.

Fungal biomass, growth, and production

Ergosterol concentrations (Gessner 2005) and rates of [1- ^{14}C]-acetate incorporation into ergosterol (Suberkropp and Gessner 2005) were used to estimate the biomass and growth rates, respectively, of fungal decomposers. Two litter pieces per replicate tray were incubated for 5 h under light and dark conditions in sterile glass scintillation vials containing 4 mL of filtered (0.22- μm pore size) wetland water and 5 mmol/L Na[1- ^{14}C]-acetate (specific activity of 48 MBq/mmol). Killed control samples containing formalin (2% v/v; $n = 1$ sample) were also incubated to correct for nonbiological ^{14}C -acetate incorporation. Incorporation of [1- ^{14}C]-acetate was stopped by placing vials on ice and immediately filtering (1.2- μm pore size) the contents. Filters and litter pieces were washed twice with filtered (0.22- μm pore size) wetland water, and stored in glass scintillation vials at -20°C. Samples were lyophilized to dryness, weighed, and ergosterol was extracted and quantified by high pressure liquid chromatography (HPLC), following the protocols described in Gessner (2005). Ergosterol fractions eluting from the HPLC were collected in 20-mL glass scintillation vials, mixed with 10 mL of scintillation fluid (Ecolume; MP Biomedicals, Santa Ana, California, USA), and radioactivity assayed using a Beckman LS6500 scintillation counter (Beckman Coulter, Indianapolis, Indiana, USA), corrected for quenching and radioactivity in killed controls. As some

green algae are known to contain trace amounts of ergosterol (as a minor sterol), we conducted additional measurements to ascertain if any of the predominant green algal taxa in detrital periphyton samples contained ergosterol. No ergosterol was detected in these algae.

Fungal biomass was calculated using a conversion factor of 10 μg ergosterol/mg fungal C, assuming 43% C in fungal dry mass. Fungal growth rates (μ) were calculated using a conversion factor of 12.6 μg fungal biomass/nmole acetate incorporated. Fungal production was calculated by multiplying the fungal growth rate (μ) by fungal biomass (B).

Algal carbon flow into fungal ergosterol and microbial phospholipid fatty acids

The potential flow of algal-derived labile organic carbon into litter-associated microbial heterotrophs was examined by tracking the flow and incorporation of ^{14}C - and ^{13}C -bicarbonate into the fungal sterol ergosterol and microbial phospholipid fatty acids (PLFAs), respectively. For ^{14}C -bicarbonate assays, naturally occurring submerged *Typha* detritus and its associated periphyton (unknown age) was randomly collected from wetland surface waters and returned to the laboratory. In the laboratory, litter was sectioned, and randomly selected litter pieces (two per vial) were incubated at 20°C for 5 and 10 h under light conditions in sterile 20-mL glass scintillation vials containing 4 mL of filtered (0.22 μm pore size) wetland water and 9.25 MBq of $\text{H}^{14}\text{CO}_3^-$. A total of four replicate vials and one killed control (2% formalin v/v) per incubation time were spiked with the photosystem inhibitor DCMU (3-[3, 4-dichlorophenyl]-1, 1-dimethyl urea, 20 $\mu\text{mol/L}$ final concentration), while another four replicate vials plus killed control contained no DCMU. Prior studies confirmed that DCMU halts periphytic algal photosynthesis, but has no direct nontarget effects on bacterial or fungal growth (Francoeur et al. 2007). Incorporation of ^{14}C was stopped by placing vials on ice and immediately filtering (1.2 μm) and washing the contents. Ergosterol extraction and radioassay were conducted as described for fungal biomass, growth, and production.

For ^{13}C -bicarbonate assays, standing dead *Typha* leaf litter was collected, placed into wire-mesh trays, and submerged in the Paint Creek wetland. *Typha* litter and its associated periphyton was collected after 42 d, returned to the laboratory and sectioned. Randomly selected litter pieces were then incubated in sterile 20 mL-glass scintillation vials (two per vial) containing 4 mL of filtered (0.22 μm) wetland water and 5 g/L of $\text{NaH}^{13}\text{CO}_3$ (Sigma-Aldrich, St. Louis, Missouri, USA). A total of four replicate vials were spiked with the inhibitor DCMU (20 $\mu\text{mol/L}$ final concentration), while another four replicate vials contained no DCMU. Additional control incubations were also conducted, where two randomly selected litter pieces were incubated in sterile 20 mL-glass scintillation vials containing only 4 mL of filtered (0.22 μm pore size) wetland water, or in

vials containing 4 mL of filtered (0.22 μm pore size) wetland water plus unlabelled NaHCO_3 (5g/L; $n = 4$ vials for each treatment). All vials were placed on their sides and incubated at 20°C for 7 h in light conditions. Following incubation, the contents of each vial (litter and any dislodged material) were filtered (0.8 μm nitrocellulose), rinsed two times with filtered wetland water, placed in individual plastic scintillation vials, and stored frozen (−20°C) until analysis.

Microbial PLFAs from litter samples were determined following methods described in Findlay (2004). Frozen *Typha* samples were lyophilized and total cellular lipids were extracted using a modified (dichloromethane-methanol-water) lipid extraction. Phospholipids were partitioned from the total lipid pool using silicic acid column chromatography and phospholipid fatty acids converted to fatty acid methyl esters (FAME) by basic methanolic transesterification. The quantities of PLFAs were determined by analyzing FAMES on a gas chromatograph (GC; Agilent 6890) equipped with a 60 m DB-1 capillary column (Agilent Technologies, Santa Clara, California, USA) and flame ionization detector. The $\delta^{13}\text{C}$ of individual PLFAs were determined separately by analyzing FAMES on a GC (Agilent 6890; using 60 m DB-1 and DB-23 capillary columns) interfaced with a GC/CIII (ThermoFinnigan; Thermo Fisher Scientific, West Palm Beach, Florida, USA) to an isotope ratio mass spectrometer (IRMS; ThermoFinnigan Delta+). Previous studies have shown that there is no isotope fractionation during transmethylation (P. Ostrom and R. Findlay, *unpublished manuscript*) and that addition of the methyl C during derivatization ($\delta^{13}\text{C}$ of methanol used was −42.9‰) decreased, on average, $\delta^{13}\text{C}$ composition of the FAME by −0.8‰. Stable isotope ratios were measured relative to high-purity reference gas and expressed relative to the international Pee Dee River belemnite standard, v-PDB, as $\delta^{13}\text{C} = [(R_{\text{SAMPLE}}/R_{\text{PDB}}) - 1] \times 1000$, where R is $^{13}\text{C}/^{12}\text{C}$. The analytical precision for fatty acids with $\delta^{13}\text{C}$ in the natural abundance range (between −32 and −25‰) was 0.5‰ ($n = 11$ ratios) and for those with $\delta^{13}\text{C} > 250$ ‰ the analytical precision was 6‰ ($n = 10$ ratios).

Data analysis

Statistical analyses were performed using SYSTAT software (version 13), with differences considered significant at the $P < 0.05$ level. When necessary, data were log transformed prior to analysis to reduce heteroscedasticity. Biomass, growth, and production data (algal, bacterial, and fungal) during each experiment were analyzed using independent Student's t tests. Because of multicollinearity of individual PLFAs, concentrations of microbial PLFAs among incubation treatments (i.e., $\text{NaH}^{13}\text{CO}_3$, $\text{NaH}^{13}\text{CO}_3 + \text{DCMU}$, and controls) were first analyzed using principal component analysis (PCA), where data were summarized into component factor loadings. Factor scores from the first two principal components (corresponding to 89.3% of

total variance) were then analyzed using a MANOVA. Isotopic signatures ($\delta^{13}\text{C}$) of microbial PLFAs among incubation treatments were analyzed using a one-way ANOVA. Values and variation in the text, tables, and figures are mean \pm SE unless otherwise noted.

RESULTS

Environmental conditions

Paint Creek wetland surface waters were slightly alkaline (pH 7.8 ± 0.1), hard water (alkalinity 166 ± 21 mg CaCO_3/L), and mesotrophic (DIN 244 ± 87 and TP 44 ± 10 $\mu\text{g}/\text{L}$) during the litter field incubations ($n = 4$ samples). Water temperatures varied little between open surface waters and surface waters under experimental canopies (mean daily temperature \pm SD; open water $20.6^\circ\text{C} \pm 1.5^\circ\text{C}$, OP4 transparent canopy $22.3^\circ\text{C} \pm 2.9^\circ\text{C}$, opaque canopy $21.3^\circ\text{C} \pm 2.9^\circ\text{C}$).

Initial laboratory experiments

During in situ incubation within the marsh, *T. angustifolia* litter was rapidly colonized by periphytic algae under natural sunlight conditions. Algal biomass increased, although not significantly ($P = 0.16$), between the two collection dates (Table 1). Algal communities inhabiting decaying *Typha* litter were similar on both collection dates, and were dominated by cyanobacteria and diatom assemblages (Table 2). As expected, short-term, laboratory production assays of litter-associated algae (^{14}C -bicarbonate incorporation) were significantly ($P < 0.001$) influenced by incubation in the light vs. dark, with litter samples having negligible rates of algal production when samples were incubated in the dark (Fig. 1).

Light availability also significantly ($P < 0.05$) influenced the short-term growth and production rates of litter-associated bacterial and fungal decomposers. When collected litter samples were incubated under lighted conditions, growth rates (μ) of bacteria (^3H -leucine incorporation) and fungi (^{14}C -acetate incorporation) were $\sim 60\%$ and 66–138% higher, respectively, compared to corresponding samples that were incubated in the dark (Fig. 2A, B). Production rates of litter-associated bacteria and fungi followed a similar pattern (data not shown), with rates being $\sim 61\%$ and 76–123% higher when incubated in the light, respectively. No significant differences in litter-associated bacterial ($P = 0.65$) or fungal biomass ($P = 0.92$) were observed between the two collection dates (Table 1). Fungal biomass was > 20 times greater than corresponding bacterial biomass, accounting for $\geq 95\%$ of total heterotrophic microbial biomass.

Field manipulation experiments

Field manipulation of light had a major impact on the development patterns of detrital periphyton communities. As expected, algal biomass on decaying *T. angustifolia* and *S. acutus* litter decreased significantly ($P < 0.01$) when litter was submerged under shaded

TABLE 1. Biomass of microbial communities associated with decaying *Typha angustifolia* and *Schoenoplectus acutus* leaf litter under natural conditions, and under field manipulation experiments using opaque (dark) and transparent (photosynthetically active radiation [PAR] + ultraviolet [UV]) canopies.

Experiment and species	Day	Algal biomass (mg C/g detrital C)	Bacterial biomass (mg C/g detrital C)	Fungal biomass (mg C/g detrital C)
Natural decay (full light)				
<i>T. angustifolia</i>	10	6.3 ± 1.1	1.8 ± 0.3	37.9 ± 3.0
	29	8.6 ± 0.9	1.9 ± 0.2	38.3 ± 3.6
Field manipulation (canopies)				
Transparent				
<i>T. angustifolia</i>	35	5.0 ^a ± 0.9	1.7 ± 0.1	21.7 ± 2.5
<i>S. acutus</i>	35	6.4 ^a ± 1.8	1.2 ± 0.9	38.6 ± 4.0
Opaque				
<i>T. angustifolia</i>	35	1.2 ^b ± 0.3	1.3 ± 0.3	30.4 ± 8.8
<i>S. acutus</i>	35	1.0 ^b ± 0.4	1.0 ± 0.1	49.3 ± 5.8

Notes: Values are the mean and SE ($n = 5$ samples; occasionally 4 due to lost samples). Values with different superscript letters within a species indicate significant differences ($P < 0.01$).

conditions (Table 1). Visual examination of shaded litter samples (day 35) revealed very few attached algae (data not shown), indicating that the opaque canopies greatly reduced algal presence. In contrast, periphytic algae rapidly colonized submerged *T. angustifolia* and *S. acutus* litter under light transparent canopies, with algal biomass concentrations similar to those observed earlier on *T. angustifolia* litter exposed to natural sunlight conditions (Table 1). Algal communities inhabiting *T. angustifolia* and *S. acutus* litter under transparent canopies displayed some differences, with diatoms dominating on *Typha* litter and cyanobacteria dominating on *Schoenoplectus* litter (Table 2). These field treatments produced two detrital periphyton complexes; +algae (transparent canopies) and -algae (opaque canopies).

Consistent with patterns of algal colonization, rates of algal production on decaying plant litter were also significantly influenced ($P < 0.001$) by canopy treatment. Algal productivity in short-term laboratory production assays was >10 times higher on *T. angustifolia* and *S. acutus* litter collected from transparent canopies (+algae) compared to litter collected from opaque canopies (-algae; Fig. 3A, B). Likewise, when decaying *T. angustifolia* and *S. acutus* litter from transparent canopies (+algae) was assayed under dark

laboratory conditions, rates of algal production decreased significantly ($P < 0.001$).

Plant litter incubated under transparent and opaque canopies displayed mixed effects on the short-term growth and production rates of bacteria and fungi. Bacterial growth and production assays conducted under constant light exposure revealed no significant differences in bacterial growth (Fig. 4A, B) or production rates (data not shown) between canopy treatments, suggesting that litter-associated bacteria grew equally well in the presence or absence of algae. However, when litter from transparent canopies (+algae) was assayed under both light and dark conditions, rates of bacterial growth (*Typha* only; Fig. 4A) and production (data not shown) increased significantly ($P < 0.05$) when litter was incubated in light, implying that bacterial activity was influenced by algal photosynthetic activity when algae were present. No significant difference in bacterial biomass was observed on *T. angustifolia* ($P = 0.27$) or *S. acutus* ($P = 0.19$) litter incubated under the different canopy treatments (Table 1).

In contrast to bacteria, fungi exhibited a more pronounced response to canopy treatments, with litter from transparent (+algae) canopies supporting significantly ($P < 0.05$) higher rates of fungal growth than litter from opaque (-algae) canopies (Fig. 4C, D). Fungal growth rates associated with *T. angustifolia*

TABLE 2. Mean relative abundance (percentage of cells) of algal divisions associated with decaying *T. angustifolia* and *S. acutus* leaf litter under natural conditions, and under field manipulation experiments using transparent (PAR + UV) canopies.

Experiment and species	Day	Heterokontophyta (%)	Chlorophyta (%)	Cyanophyta (%)	Other (%)
Natural decay (full light)					
<i>T. angustifolia</i>	10	29.4 ± 4.2	17.2 ± 2.6	53.0 ± 2.8	0.4 ± 0.3
<i>T. angustifolia</i>	29	43.7 ± 4.2	17.1 ± 4.9	38.7 ± 5.7	0.4 ± 0.2
Field manipulation (transparent canopies)					
<i>T. angustifolia</i>	35	46.9 ± 14.4	24.3 ± 16.6	25.5 ± 3.3	3.3 ± 1.2
<i>S. acutus</i>	35	9.1 ± 20.8	26.9 ± 16.8	63.2 ± 33.3	0.5 ± 2.0

Note: Values are mean and SE ($n = 5$ samples), except *S. acutus* ($n = 3$, due to lost samples).

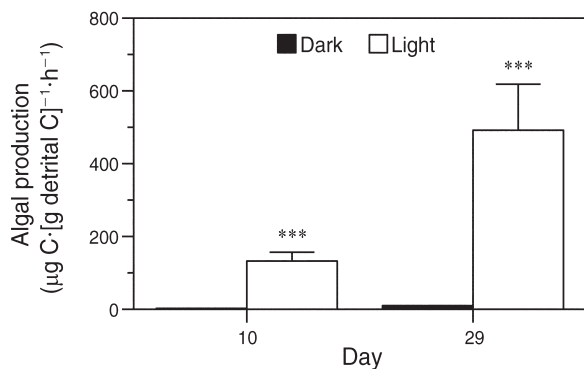


FIG. 1. Rates of periphytic algal production (as measured by ¹⁴C-bicarbonate incorporation) in natural *Typha angustifolia* detrital periphyton incubated in the laboratory for 2 h in light (400 µmol·m⁻²·s⁻¹ photosynthetically active radiation [PAR], ultraviolet [UV] free) or dark. *T. angustifolia* detrital periphyton was collected 10 and 29 d after litter submergence. Values are means + SE (*n* = 3 samples). Asterisks indicate significant differences between light and dark incubated samples (*P* < 0.001).
*** *P* < 0.001.

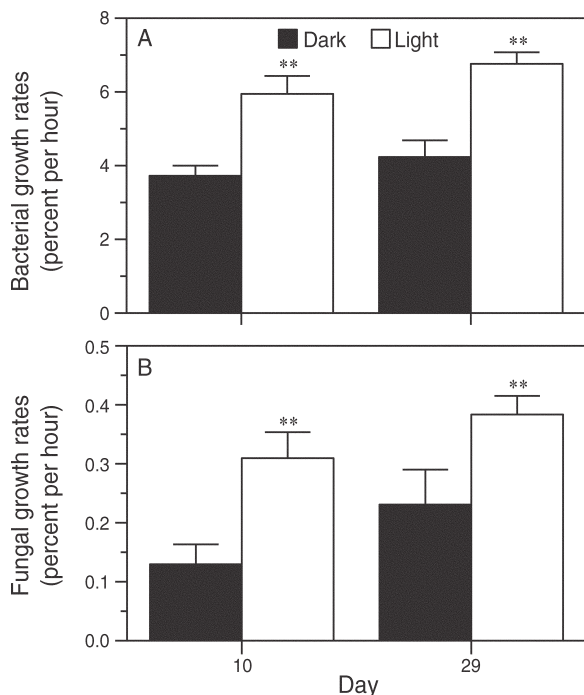


FIG. 2. Growth rates of (A) bacteria (as measured by [³H]-leucine incorporation) and (B) fungi (as measured by ¹⁴C-acetate incorporation) in natural *T. angustifolia* detrital periphyton incubated in the laboratory for 30 min in light (400 µmol·m⁻²·s⁻¹ PAR, UV free) or dark. *T. angustifolia* detrital periphyton was collected 10 and 29 d after litter submergence. Values are means + SE (*n* = 5 samples). Asterisks indicate significant differences between light and dark incubated samples.
** *P* < 0.01.

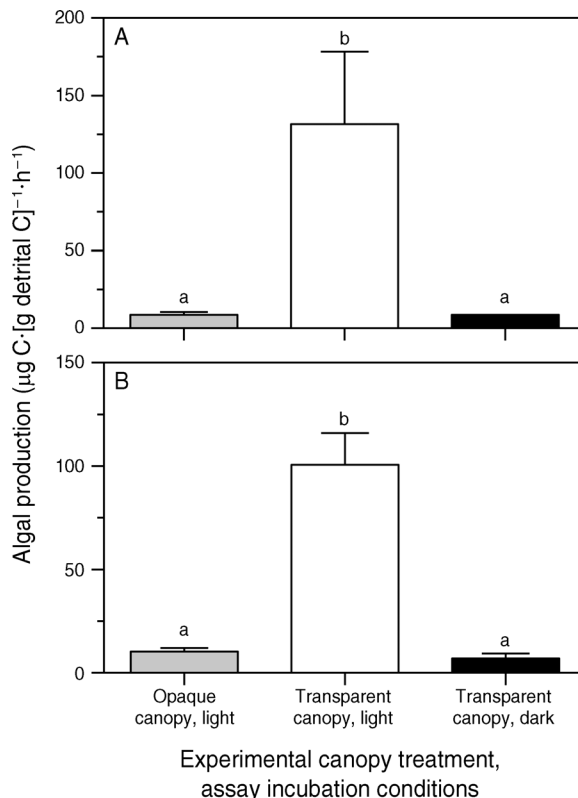


FIG. 3. Rates of periphytic algal production (¹⁴C-bicarbonate incorporation) in (A) natural *T. angustifolia* and (B) *Schoenoplectus acutus* detrital periphyton incubated in the laboratory for 2 h in light (400 µmol·m⁻²·s⁻¹ PAR, UV free) or dark. Litter was collected 35 d after submergence under opaque or transparent canopies. Values are means + SE (*n* = 5 samples). Different letters above the plotted bar indicate significant differences between light and dark incubated samples (*P* < 0.001).

and *S. acutus* litter were ~114% and 40% higher, respectively, when algae were present. Corresponding rates of fungal production followed a similar pattern (data not shown).

A more striking response in fungal growth and production was observed when *T. angustifolia* and *S. acutus* litter incubated under transparent canopies (+ algae) was assayed under both light and dark conditions. Similar to bacteria, rates of fungal growth (Fig. 4C, D) and production (data not shown) were significantly greater (*P* < 0.01) when litter samples were incubated in the light, providing additional evidence that when present, algae and their photosynthetic activities can influence fungal activities within decaying plant litter.

Algal labile carbon transfer to microbial heterotrophs

Short-term, laboratory experimental incubations of natural decaying *T. angustifolia* litter with ¹⁴C-bicarbonate established that ¹⁴C was transferred to and incorporated by fungal decomposers. This was most

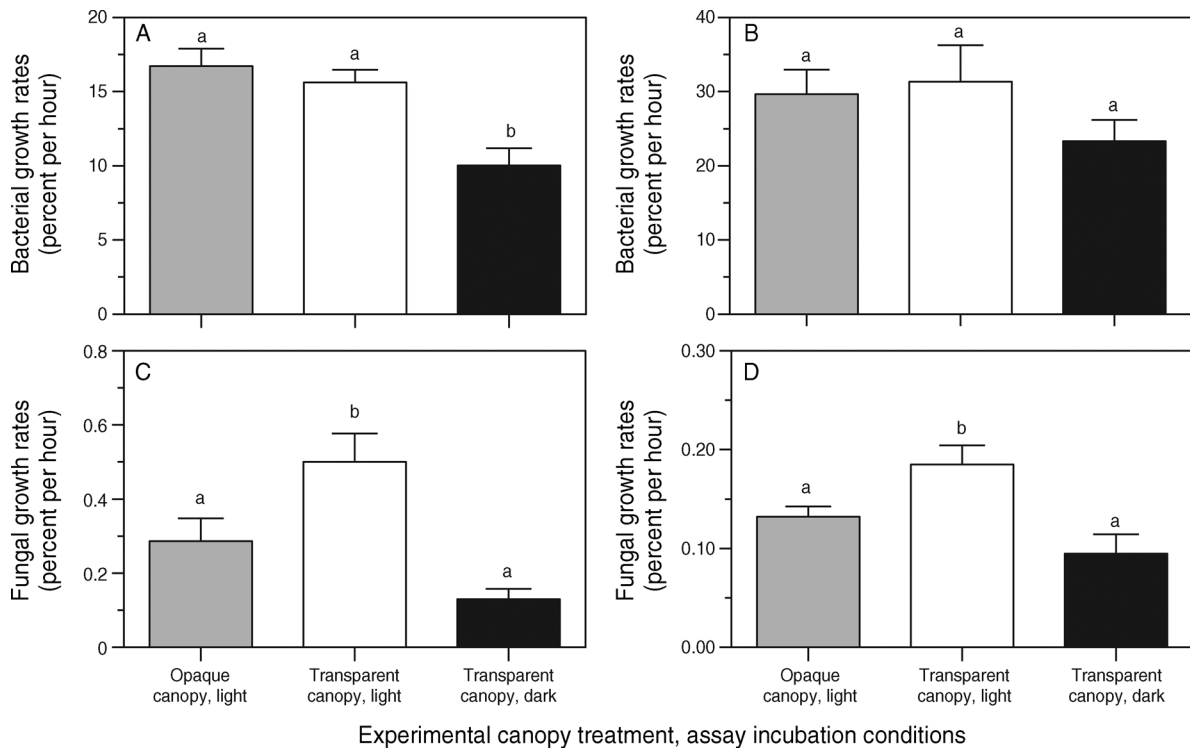


FIG. 4. (A and B) Bacterial and (C and D) fungal growth rates of (A and C) *T. angustifolia* and (B and D) *S. acutus* detrital periphyton complexes, respectively, incubated in the laboratory for 30 min in light ($400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR, UV free) or dark (transparent canopies only). Litter was collected 35 d after submergence under opaque or transparent canopies. Values are means \pm SE ($n = 5$ samples). Different letters above the plotted bar indicate significant differences ($P < 0.05$).

likely driven by photosynthesis and labile dissolved organic carbon (DOC) exudation from co-occurring periphytic algae within the microbial landscape. Assimilation of ^{14}C into fungal biomass (ergosterol) was significantly ($P < 0.001$) reduced in light-incubated litter samples exposed to the photosynthesis inhibitor DCMU (Fig. 5).

Similar patterns were also observed when *Typha* litter samples were incubated with ^{13}C -bicarbonate. Significantly greater amounts of ^{13}C were detected in microbial PLFAs ($F_{3,11} = 36.12\text{--}798.82$ [range], $P < 0.0001$, ANOVA) when *Typha* litter samples were incubated with ^{13}C -bicarbonate in the light vs. corresponding litter samples that were incubated in the light with ^{13}C -bicarbonate and DCMU (Figs. 6 and 7). As expected, assimilation of ^{13}C was observed in PLFAs that are commonly associated with phototrophic microeukaryotes (Fig. 6, Table 3). However, assimilation of ^{13}C was also noted in PLFAs that are only found in heterotrophic microbes (e.g., bacteria and protozoa; Figs. 6 and 7, Table 3), providing additional support for utilization of labile algal exudates by litter microbial heterotrophs. Total PLFAs concentrations among experimental treatments were not significantly different (Wilks' lambda $F_{2,13} = 0.058$, $P = 0.94$, MANOVA), ranging from 655 ± 45 to 802 ± 102 ng PLFA/mg detrital C (Table 3).

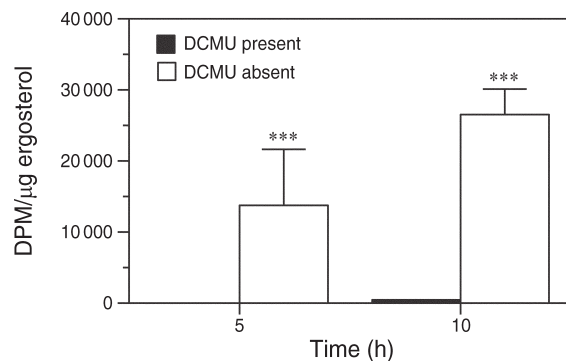


FIG. 5. Carbon source tracking experiment showing the transfer and incorporation of ^{14}C into fungal ergosterol, as disintegrations per minute (DPM), during 5 and 10 h experimental light ($400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR, UV free) incubations of natural *T. angustifolia* detrital periphyton with ^{14}C -bicarbonate. Incubations were conducted in the presence and absence of the photosynthesis inhibitor DCMU (3-[3, 4-dichlorophenyl]-1, 1-dimethyl urea, $20 \mu\text{mol/L}$ final concentration). Values are means \pm SE ($n = 4$ samples). Asterisks indicate significant differences between light and dark incubated samples at each sampling period.

*** $P < 0.001$.

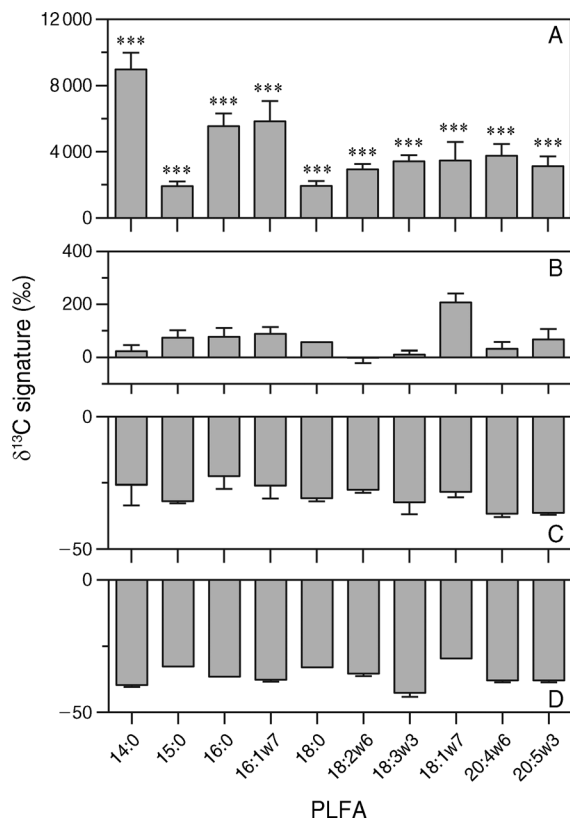


FIG. 6. The $\delta^{13}\text{C}$ of widely distributed and microeukaryotic-specific PLFAs (phospholipid fatty acids) following 7 h light ($400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR, UV free) incubations of natural *T. angustifolia* detrital periphyton with (A) wetland water containing ^{13}C -bicarbonate, (B) wetland water containing ^{13}C -bicarbonate and the photosynthesis inhibitor DCMU, (C) wetland water only, or (D) wetland water and non-labeled bicarbonate. Values are means and SE ($n = 4$ samples). Asterisks indicate a significant difference between the incubation treatments for each individual PLFA.

*** $P < 0.001$.

DISCUSSION

Plant litter decomposition is a key ecosystem process in aquatic and terrestrial habitats (Hagen et al. 2012), which has been examined as a function of numerous physical, chemical, and biological factors (Graça et al. 2005). Light-mediated decomposition of both dissolved and particulate organic matter (photodegradation) is widely accepted as an important abiotic process (King et al. 2012), and a potential mechanism for stimulating microbial heterotrophic activities. Photolysis of recalcitrant dissolved organic matter (DOM) can lead to the production of organic molecules that are more readily assimilated by heterotrophic microorganisms (Wetzel et al. 1995, Paul et al. 2012). In contrast, light-mediated biotic decomposition processes via algal stimulation of litter-associated microbial heterotrophs have only recently been identified (Neely 1994, Neely and Wetzel 1997, Francoeur et al. 2006, Rier et al. 2007, Danger et

al. 2013), yet the underlying stimulatory mechanism(s) and its overall significance for carbon and nutrient cycling in aquatic ecosystems remain poorly understood.

Results obtained in this investigation provide compelling evidence that periphytic algae can stimulate the heterotrophic activity of bacteria and fungi within the litter microbial landscape. We consistently demonstrated significant short-term light-based metabolic stimulation of bacterial and fungal growth and production within detrital periphyton communities. In the presence of algae, growth and production rates of bacteria and fungi increased rapidly ($>60\%$) when detrital periphyton complexes were assayed under light vs. dark conditions. These findings are ecologically intriguing and significant, since they demonstrate a largely unrecognized role of autochthonous primary producers within detritus-based aquatic ecosystems that are typically viewed as being driven by heterotrophic processes. To our knowledge, this study is the first report of light-mediated stimulation of fungi. This novel phenomenon underscores the potential importance of photoautotrophic-heterotrophic interactions to ecosystem-level decomposition and nu-

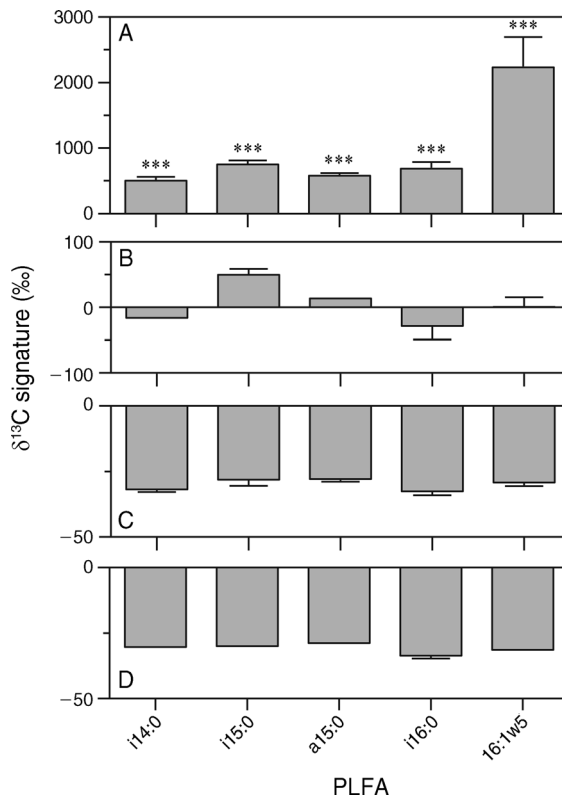


FIG. 7. The $\delta^{13}\text{C}$ of bacterial-specific PLFAs following 7 h light ($400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR, UV free) incubations of natural *T. angustifolia* detrital periphyton. Treatments are as in Fig. 6. Values are means and SE ($n = 4$ samples). Asterisks indicate a significant difference between incubation treatments for each individual PLFA.

*** $P < 0.001$.

TABLE 3. Concentrations of major phospholipid fatty acids (PLFAs; measured as ng PLFA/mg detrital C) extracted from *T. angustifolia* detrital periphyton samples.

PLFA	Functional group assignment	Treatment			
		¹³ C	¹³ C + DCMU	Control A	Control B
i14:0	B	4.7 ± 1.1	4.2 ± 0.6	4.8 ± 0.5	4.6 ± 0.4
14:0	WD; B, MU	20.7 ± 3.4	17.5 ± 1.3	18.5 ± 2.2	21.7 ± 2.6
i15:0	B	25.6 ± 5.2	22.9 ± 2.7	27.1 ± 2.1	26.1 ± 2.7
a15:0	B	6.5 ± 1.1	6.3 ± 0.7	7.7 ± 1.0	7.0 ± 0.8
15:0	WD; B, MU	5.2 ± 0.9	4.8 ± 0.5	6.0 ± 0.6	4.7 ± 0.6
16:0	WD; B, MU	172.0 ± 20.3	140.6 ± 7.1	160.9 ± 7.7	163.3 ± 22.3
i16:0	B	7.5 ± 0.9	6.5 ± 0.2	6.6 ± 0.3	6.8 ± 0.6
16:1w7c	WD; B, MU	88.3 ± 11.6	74.1 ± 4.9	88.4 ± 7.3	78.8 ± 4.6
16:1w7t	B	10.2 ± 1.3	8.7 ± 0.7	10.1 ± 0.7	9.1 ± 0.9
16:1w5c	B	32.6 ± 4.7	28.9 ± 3.0	35.3 ± 2.7	30.7 ± 1.3
18:0	WD; B, MU	44.2 ± 6.9	34.9 ± 4.1	50.5 ± 8.0	43.3 ± 12.5
18:1w7c	B, HU	94.3 ± 11.0	83.7 ± 7.6	89.7 ± 6.0	89.8 ± 11.5
18:1w7t	B	16.7 ± 2.4	12.1 ± 1.4	15.9 ± 2.0	14.7 ± 4.9
18:1w9	WD; B, MU	51.7 ± 7.4	40.5 ± 3.4	52.7 ± 8.6	48.6 ± 12.3
18:2w6	WD; B (cyanobacteria), MU	88.9 ± 9.4	60.3 ± 5.1	92.6 ± 9.2	80.8 ± 15.9
18:3w3	MU (green algae, fungi)	25.3 ± 3.4	20.9 ± 1.6	24.2 ± 4.7	24.4 ± 4.2
20:4w6	MU (predominately HU protozoa)	9.1 ± 2.0	7.3 ± 0.8	9.6 ± 2.4	10.1 ± 2.2
20:5w3	MU (predominately PU diatoms)	12.3 ± 3.6	9.9 ± 2.1	15.1 ± 5.4	12.1 ± 2.8
Other minor fatty acids (combined total)		86.4 ± 11.5	70.7 ± 4.9	85.8 ± 6.2	77.6 ± 8.2
Total		802.4 ± 101.7	655.1 ± 44.9	802.2 ± 62.2	754.3 ± 101.7

Notes: Functional groups WD (widely distributed), B (bacteria), MU (microeukaryotes), HU (heterotrophic microeukaryotes), and PU (phototrophic microeukaryotes) are from Findlay (2004), as appropriate for aquatic environments. Values are mean and SE ($n = 4$ samples). Experimental treatments included samples incubated in wetland water containing ¹³C-bicarbonate (¹³C), wetland water containing ¹³C-bicarbonate and the photosynthesis inhibitor DCMU (¹³C + DCMU), wetland water only (Control A), and wetland water containing non-labeled bicarbonate (Control B).

trient cycling, as fungal decomposers play a key role in litter decomposition in aquatic systems (Gulis et al. 2009).

Our observations of light-mediated interactions among detrital-inhabiting microbial photoautotrophs and heterotrophs are consistent with prior research examining periphytic microbial communities colonizing inert substrata (Neely and Wetzel 1995, Scott et al. 2008). Neely and Wetzel (1995) used a dual-isotopic radiolabeling assay to simultaneously quantify rates of algal and bacterial productivity in natural periphyton communities colonizing glass coverslips. In their study, rates of algal and bacterial production were positively correlated over a range of PAR flux densities (20 to 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). When exposed to 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR, rates of periphytic bacterial production increased by ~62% in comparison to bacteria exposed to low light levels (20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR). In addition, when the photosynthesis inhibitor DCMU was applied to periphyton communities, algal photosynthetic rates declined to negligible levels and bacterial production decreased concomitantly by ~46% over all light intensities. Scott and Doyle (2006) observed similar autotrophic-heterotrophic interactions in floating periphyton mats, where rates of heterotrophic bacterial production were positively correlated with rates of algal photosynthesis. Collectively, the results obtained in prior studies and the present investigation suggests that light-mediated algal stimulation of microbial heterotrophs may be a wide-

spread phenomenon on a variety of submerged substrata.

Increased availability of labile DOC is among the several mechanisms that could drive the light-mediated interactions in the detrital periphyton complex. Periphytic communities are net sinks for DOC (Romaní et al. 2004), and prior studies have documented that heterotrophic bacterial growth (Bernhardt and Likens 2002) and production (Sobczak 1996) in periphyton are stimulated by labile organic C amendments. The production and exudation of labile DOC from periphytic algae has been well documented (Jones and Cannon 1986, Ziegler et al. 2009), and likely constitutes a source of labile DOC for microbial heterotrophs within the detrital periphyton complex. In the present study, carbon source tracking experiments using ¹⁴C and ¹³C confirmed that inorganic C was transferred to and incorporated by microbial heterotrophs (bacteria, fungi, and protozoa). This process was inhibited by DCMU, which indicates that algal photosynthetic C fixation and labile DOC exudation were critical intermediate steps. These findings concur with other recent tracer studies that have demonstrated that periphytic microbial heterotrophs readily take up and assimilate algal-derived DOC (Ziegler et al. 2009, Ziegler and Lyon 2010, Risse-Buhl et al. 2012), and add further support to the hypothesis that the production and exudation of labile DOC by periphytic algae may be an important factor in stimulating heterotrophic microbial activities in detrital periphyton communities.

Alternatively, photolysis of both dissolved and particulate organic matter is a well known abiotic decomposition process (King et al. 2012), which can lead to the production of DOC more readily utilized by heterotrophic microorganisms (Wetzel et al. 1995, but see Tranvik and Bertilsson 2001). However, a recent study has indicated that most DOC mineralization in lake waters could be explained by microbial activity independent of any DOC photolysis (Koehler et al. 2012). UV light is thought to initiate the majority of DOM photolysis; however, UV light was not present in any of our laboratory assay incubations. Taken together, this suggests that our observed stimulation of heterotrophic microbial activity was not driven by DOC photolytic mechanisms.

In addition to increased DOC availability, other potential mechanisms stimulating heterotrophic microbial metabolism within detrital periphyton may result from photosynthetic alteration of the detrital milieu. Empirical evidence exists for rapid increases of extracellular hydrolytic and oxidative enzyme activities in periphytic communities exposed to light (Espeland et al. 2001, Francoeur et al. 2006, Rier et al. 2007, Ylla et al. 2009), which likely result from photosynthetically mediated shifts in pH. Algal photosynthesis can rapidly increase the pH within periphytic microbial communities from <7 to >9 (Revsbech et al. 1983, Espeland et al. 2001), which is the optimum pH for many periphytic degradative enzymes (Espeland et al. 2001, Francoeur and Wetzel 2003). Rier et al. (2007) reported increased extracellular hydrolytic and oxidative enzyme activities in natural decaying *P. tremuloides* leaf litter exposed to light vs. dark conditions within experimental stream mesocosms. These enzymes were likely produced by microbial heterotrophs, particularly fungi (Romani et al. 2006), and their increased activity implies accelerated rates of microbial carbon and nutrient acquisition from decaying litter.

The instantaneous light-mediated stimulation of, and labile algal carbon flow to, microbial heterotrophs within detrital periphyton strengthens the contention that periphytic algae may be eliciting a priming effect (PE) on the decay activities of litter-associated microbial decomposers (Danger et al. 2013). Well established in terrestrial soils, the PE describes the natural phenomenon where the mineralization rate of recalcitrant soil organic matter is enhanced by pulsed or continuous inputs of labile carbon (Blagodatsky et al. 2010, Kuzyakov 2010). In this regard, labile carbon inputs produce hotspots and hot moments of microbial activity (e.g., in the rhizosphere), where heterotrophic microbial decomposers are provided energy-rich compounds that aid in their metabolic capabilities (e.g., enzyme production) to degrade and mineralize more refractory soil organic matter. As in terrestrial ecosystems, both fungi and bacteria play a fundamental role in the breakdown and mineralization of organic matter in aquatic habitats, and their ability to process and assimilate organic matter

is intimately coupled with and influenced by their metabolic activities (e.g., growth, enzyme production, and respiration).

Prior studies have demonstrated that algal presence and/or light availability can accelerate rates of plant litter decomposition (Franken et al. 2005, Rier et al. 2007, Lagrue et al. 2011, Danger et al. 2013). Franken et al. (2005) examined the effect of light intensity on the decomposition of poplar leaves (*Populus nigra*) in the presence or absence of the invertebrate shredders *Asellus aquaticus* and *Gammarus pulex*. In the absence of invertebrates, a significant relationship was observed between leaf mass loss and algal abundance at the different light intensities. At the time, the authors speculated that algal exudates may have promoted the increased growth of fungi and bacteria, which in turn facilitated increased microbial decay of poplar leaf material. Similar findings have been recently reported by Danger et al. (2013), where they observed that periphytic algae (diatoms), in combination with heterotrophic microbial decomposers (fungi and bacteria), significantly stimulated the decomposition of alder (*Alnus glutinosa*) leaf litter. Although we did not quantify rates of plant litter mass loss in the present study, our observations of light-based stimulation of, and labile algal carbon flow to, fungal and bacterial decomposers in detrital periphyton, in combination with previous studies demonstrating increased light-based stimulation of decomposition strongly supports the likelihood of an algal-mediated PE in the detrital microbial landscape.

Recently, Guenet et al. (2010) reviewed three theoretical mechanisms that could explain the PE phenomenon, all of which centered on microbial interactions between labile organic matter (LOM) and recalcitrant organic matter (ROM) decomposers. Three competing mechanistic hypotheses were proposed: (1) LOM degrading enzymes produced by LOM decomposers will also degrade ROM, which in turn stimulates ROM decomposers but not LOM decomposers (i.e., co-metabolism), (2) LOM degradation by LOM decomposers supplies energy-rich compounds to the ROM decomposers, thus allowing ROM decomposers to produce ROM-degrading enzymes, which accelerates decay and nutrient release for both ROM and LOM decomposers, and (3) a single decomposer population produces enzymes able to degrade both LOM and ROM, and increased LOM availability and degradation provides energy for the synthesis of ROM-degrading enzymes. Given the difference in enzymes needed to degrade algal exudates (e.g., alpha glucosidase for starches, while no extracellular enzymes are required for monomeric sugars) compared to cellulose and lignin in plant litter (e.g., cellobiose oxidase, phenol oxidase, laccase, peroxidase, see Eriksson 1984), mechanistic hypothesis 1 does not seem likely in detrital periphyton communities. Mechanistic hypotheses 2 and 3 are plausible; however, testing and differentiating between these two theoretical mech-

organisms would require a much greater detailed species-level investigation. A novel fourth mechanism potentially explaining stimulation in detrital periphyton communities may also involve a combination of LOM inputs (algal photosynthate) and corresponding photosynthetic alteration of the detrital milieu. As mentioned earlier, it is well established that photosynthetic activity in periphyton communities can rapidly alter pH and oxygen availability (e.g., Revsbech et al. 1983), which can stimulate the activities of extracellular degradative enzymes (e.g., Espeland et al. 2001, Francoeur and Wetzel 2003). As a consequence, changes in environmental conditions engendered by algal photosynthetic activities may by themselves facilitate increased enzymatic activity, leading to increased LOM availability and a subsequent PE on the ROM-decomposer community. This mechanistic aspect was not examined in the present study, but does represent an area that holds promise for future research.

Guenet et al. (2010) also outlined a series of unanswered questions concerning the similarity and differences in PE between terrestrial and aquatic environments, and specific aspects of the PE in aquatic habitats. While emergent marsh plant detritus is intermediate along a ROM–LOM continuum, increased decomposition stimulated by algal exudates is clearly an example of PE within a freshwater habitat, and adds to the examples of LOM simulating ROM decomposition in aquatic ecosystems. Interpreted within the microbial landscape concept, the detrital periphyton complex serves, to the best of our knowledge, as the first example demonstrating both hotspots and hot moments of PE in aquatic systems. Each complex, once an active algal community is established, becomes a unique microbial landscape element in which the PE can stimulate microbially mediated litter decomposition. As the effect is dependent upon sunlight, each day when PAR is present becomes a hot moment for PE-stimulated litter decomposition. We have speculated that (and are looking forward to testing whether) algal exudates also stimulate decomposition of refractory components of DOM present in these systems.

The microbial diversity within the detrital periphyton complex likely serves to stimulate energy mobilization through the detrital food web. Microbes have favorable elemental stoichiometry relative to detritus, have the potential to provide essential nutrients (fatty acids, vitamins, etc.), and serve as the primary food resource for the detritus-feeding consumers (e.g., invertebrates) that dominate in these systems. Our study, combined with the findings of Danger et al. (2013), provides a basis upon which to compare the PE phenomenon in terrestrial and freshwater environments and its overall impact on ecological processes.

In summary, this study significantly extends our understanding of periphytic microbial interactions beyond previously reported algal-bacterial couplings on inert substrata. The evidence that fungi respond to

algal metabolism, and perhaps more importantly, that periphytic algae function as a photosynthetic conduit for labile carbon supply to microbial heterotrophs over very short time intervals, are important advances for understanding the functional role of both fungi and bacteria in carbon cycling processes. Given the ubiquitous nature of periphytic biofilms in any damp habitat, our results highlight the need for more sophisticated studies to discern the details of metabolic couplings within litter-associated microbial communities and their potential impact on ecosystem carbon flow and nutrient cycling pathways.

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