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Periphytic algae decouple fungal activity from leaf litter decomposition via negative priming

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Abstract

1. Well-documented in terrestrial settings, priming effects describe stimulated heterotrophic microbial activity and decomposition of recalcitrant carbon by additions of labile carbon. In aquatic settings, algae produce labile exudates which may elicit priming during organic matter decomposition, yet the directions and mechanisms of aquatic priming effects remain poorly tested.

2. We tested algal-induced priming during decomposition of two leaf species of contrasting recalcitrance, *Liriodendron tulipifera* and *Quercus nigra*, in experimental streams under light or dark conditions. We measured litter-associated algal, bacterial, and fungal biomass and activity, stoichiometry, and litter decomposition rates over 43 days.

3. Light increased algal biomass and production rates and increased bacterial abundance 141–733% and fungal production rates 20–157%. Incubations with a photosynthesis inhibitor established that algal activity directly stimulated fungal production rates in the short-term.

4. Algal-stimulated fungal production rates on both leaf species were not coupled to long-term increases in fungal biomass accrual or litter decomposition rates, which were 154–157% and 164–455% greater in the dark, respectively. The similar patterns on fast- vs. slow-decomposing *L. tulipifera* and *Q. nigra*, respectively, indicated that substrate recalcitrance may not mediate priming strength or direction.

5. In this example of negative priming, periphytic algae decoupled fungal activity from decomposition, likely by providing labile carbon invested toward greater fungal growth and reproduction instead of recalcitrant carbon degradation. If common, algal-induced negative

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Authorship contributions

KAK and JRB conceived the study design and conducted the study. JRB, HMH, MBL, and SNF conducted sample analysis and HMH and RHF conducted statistical analyses. All authors contributed to writing the manuscript and provided editorial input.

Data accessibility

Data included in this manuscript may be found in the Dryad Digital Repository (doi:10.5061/dryad.8kc1n09; Halvorson et al. 2018).

priming could stimulate heterotrophy reliant on labile carbon yet suppress decomposition of recalcitrant carbon, modifying energy and nutrients available to upper trophic levels and enhancing organic carbon storage or export in well-lit aquatic habitats.

Graphical Abstract

Keywords

bacteria; detritus; ecological stoichiometry; light; microbial heterotrophs; periphyton; priming effects; streams

Introduction

Heterotrophic microbes drive organic matter breakdown across terrestrial and aquatic ecosystems and link environmental factors to major ecosystem functions including carbon (C) storage and processing (Moore et al., 2004; Hagen et al., 2012). Upon colonizing organic matter such as plant litter, microbial heterotrophs assimilate and mineralize organic C and nutrients, driving decomposition (Gessner et al., 2010). Fungi are especially adapted to break down recalcitrant C associated with compounds resistant to breakdown, such as cellulose and lignin (Romaní, Fischer, Mille-Lindblom & Tranvik, 2006; Schneider et al., 2012). Heterotrophs degrading recalcitrant C can be limited by the availability of labile C, such as acetate or glucose, which is comparatively easy to assimilate and enhances growth (Garcia-Pausas & Paterson, 2011). Indeed, heterotrophic microbes respond strongly to labile C additions, with many such additions eliciting positive ‘priming effects’ by increasing

heterotrophic decomposition of recalcitrant C (Kuzyakov, Friedel & Stahr, 2000; Guenet, Danger, Abbadie & Lacroix, 2010; Danger et al., 2013; Rousk, Hill & Jones 2015). The significance of priming is particularly well-documented in terrestrial soils, where labile C additions can increase decomposition of recalcitrant C by 67% to as much as 382% due to positive priming (Cheng et al. 2014; Rousk et al., 2015; Luo, Wang & Sun, 2016).

Though likely important for the global C cycle, priming effects and their mechanisms remain poorly studied in aquatic systems (Cole et al., 2007; Guenet et al., 2010; Bengtsson, Attermeyer & Catalán, 2018). Some studies have reported positive priming (increased decomposition rate) with additions of labile glucose, leachates, or algal exudates on breakdown of recalcitrant dissolved or particulate C (Danger et al., 2013; Hotchkiss, Hall, Baker, Rosi-Marshall & Tank, 2014; Bianchi et al., 2015), whereas others have reported no or negative priming (decreased decomposition rates; Bengtsson et al., 2015; Catalán, Kellerman, Peter, Carmona & Tranvik, 2015). Under positive priming, heterotrophs use labile C to invest in C- or nutrient-mining enzymes, stimulating decomposition (Guenet et al., 2010; Kuzyakov, 2010). Under no or negative priming, labile C may stimulate heterotrophic decomposer activity, yet this stimulation is not coupled to increased recalcitrant C turnover because microbial heterotrophs likely allocate labile C toward growth, respiration, or reproduction instead of degradative enzymes and decomposition (Kuzyakov, 2010; Catalán et al., 2015). Priming strength in aquatic systems may depend on the relative size of labile and recalcitrant C pools (Danger et al., 2013; Halvorson, Scott, Entrekin, Evans-White & Scott, 2016; Wagner, Bengtsson, Findlay, Battin & Ulseth, 2017). However, additional tests of priming are needed, especially those extending beyond closed micro- and mesocosm studies to flow-through conditions of streams and rivers (e.g., Fabian et al., 2018), where there also is a pressing need to quantify the microbial interactions that determine mechanisms and directions of priming (Guenet et al., 2010; Catalán et al., 2015).

Widespread and present even in relatively shaded aquatic systems (Greenwood & Rosemond, 2005; Roberts, Mulholland & Hill, 2007), periphytic algae may be major drivers of aquatic priming, because algae exude upwards of 33% of production as labile C available to heterotrophic microbes (Ziegler & Lyon, 2010; Kuehn, Francoeur, Findlay & Neely, 2014; Wyatt & Turetsky, 2015). Increased light availability enhances C lability through photolytic (ultraviolet-induced) degradation of recalcitrant C compounds (e.g. humic acids) into fatty acids and carbohydrate monomers (Wetzel, Hatcher & Bianchi, 1995; King, Brandt & Adair, 2012), but considerably less emphasis has been placed on the potential for light-mediated effects via algal growth and C exudation and its subsequent stimulation of heterotrophic decomposers (Danger et al., 2013; Kuehn et al., 2014). On leaf litter, active periphytic algae can double bacterial and fungal growth rates (Kuehn et al., 2014), enhance C- and nitrogen (N)-acquiring enzyme activities (Rier, Kuehn & Francoeur, 2007), and speed decomposition by 20 to 126% (Lagrue et al., 2011; Danger et al., 2013; Halvorson et al., 2016). Algae can also increase overall microbial biomass in the litter-periphyton complex, and because algae are N- and phosphorus (P)-rich relative to litter, this increases nutrient uptake and reduces C:N and C:P ratios (Danger et al., 2013; Halvorson et al., 2016). Algae also add essential polyunsaturated fatty acids that may translate to enhanced detritivore feeding and growth (Crenier et al., 2017). These algal-mediated interactions may be a missing link to understanding decomposition and other aquatic ecosystem processes, especially as riparian

canopy openness varies seasonally and spatially, increases under anthropogenic influence, and alters energy and nutrient transfer through aquatic food webs (Allan, 2004; Bechtold, Rosi, Warren & Keeton, 2016; Warren et al., 2016; Norman et al., 2017).

A second but rarely-tested factor influencing the strength and direction of aquatic priming may be the characteristics of the recalcitrant C pool. The degree of litter recalcitrance varies across plant species and plant tissues (e.g., wood versus leaves), leading to contrasting decomposition rates (Webster & Benfield, 1986; Pietsch et al., 2014). Generally, priming should be positive and stronger on recalcitrant, slow-decomposing litter compared to labile, fast-decomposing litter where heterotrophs are not as strongly limited by labile C availability, as has been proposed for terrestrial soils (Hamer & Marschner, 2005). Leaf species may therefore be an important variable influencing the strength of priming in aquatic ecosystems. However, existing tests of algal-induced priming have not compared priming across litters of varying recalcitrance. The potential role of litter recalcitrance as a mediator of priming is a research priority to connect riparian composition to broader structure and function of stream ecosystems (Kominoski, Marczak & Richardson, 2011).

We investigated the effects of light exposure and periphytic algae on microbial biomass and production, nutrient content, and decomposition of two leaf species of contrasting C recalcitrance, *Liriodendron tulipifera* (tulip poplar) and *Quercus nigra* (water oak) in experimental streams. We predicted that, due to positive priming induced by periphytic algae, (1) light exposure would increase litter fungal and bacterial biomass and production rates, driving faster decomposition compared to dark-incubated litter (Danger et al., 2013; Kuehn et al., 2014); (2) the stimulatory effects of light on autotrophic and heterotrophic microbial biomass would reduce bulk (i.e., litter and associated microbiota) C:N and C:P during decomposition (Danger et al., 2013; Halvorson et al., 2016); and (3) the stimulatory effects of light would be stronger on slower-decomposing, recalcitrant oak litter compared to faster-decomposing poplar litter.

Material and Methods

Experimental set-up

This study was conducted during the summer of 2013 (June-July) in outdoor experimental streams located at The University of Southern Mississippi Lake Thoreau Environmental Center mesocosm facility. In the Fall of 2012, newly-abscised leaves of *Liriodendron tulipifera* (tulip poplar) and *Quercus nigra* (water oak), two leaf species of comparatively low and high recalcitrance respectively, were collected at Lake Thoreau Environmental Center. Litter was initially air dried at 23°C, leached overnight to soften in tap water, and cut into 13.5 mm diameter disks. This leaching caused some loss of soluble compounds and increased litter molar C:N from 59.3 and 54.1 to 66.4 and 60.8 among tulip poplar and water oak, respectively. After cutting, leaf discs were dried at 30°C and stored in a desiccator. Disks were individually mounted with insect pins onto 3 mm diameter corks inserted into holes within 8 × 30 cm Plexiglas plates (Grattan and Suberkropp, 2001). Ten plates (5 each per leaf species) were placed randomly in each of eight experimental streams constructed using vinyl rain gutters lined with river rock (Fig. S1, Supporting Information). All streams received water from recirculating cattle troughs to achieve water velocity of ~0.004 m s^{-1},

with cattle troughs receiving continual well water inputs to maintain temperatures. New water inputs were balanced via outputs from a spigot in each cattle trough, allowing complete water turnover four times per day. Four of the eight replicate streams were fully shaded using opaque black plastic sheeting (photosynthetically active [PAR] and ultraviolet [UV] radiation below detection), and the other four were exposed to natural daylight, shaded only by a light mesh canopy (51% PAR and 23% UV transmittance) to reduce solar heating and UV. Two streams of each treatment were equipped with Onset StowAway temperature loggers to monitor water temperatures. A fine mesh bag containing conditioned *L. tulipifera* and *Q. nigra* litter from an unnamed forested tributary of Cross Creek at Lake Thoreau Environmental Center was placed at the head of each stream to provide microbial inoculum.

On 0, 2, 6, 10, 20, 31, and 43 days into the study, we collected leaf disks from each stream and immediately returned them to the laboratory to quantify biomass and production rates of litter-associated algae, bacteria, and fungi (see below). On each sampling date, two leaf disks of each species in each stream were used to estimate mass loss and C, N, and P contents. Disks were freeze-dried (lyophilized), weighed to the nearest 0.01 mg, and stored dry. Litter subsamples were subsequently weighed and measured for C and N contents using a Costech Elemental Analzser (Costech Analytical Technologies, Valencia, CA) and P contents by combustion, digestion in hot hydrochloric acid, and measurement of $P\text{-}PO_4$ using a SEAL Autoanalyzer 3 (SEAL Analytical, Milwaukee, WI). On days 20 and 31 of the study, we collected and froze one leaf disk from each replicate to determine algal taxonomic composition. After thawing, algae were removed from leaf disks by scraping with a razor blade and rinsing with water, then identified and enumerated using brightfield microscopy (400×; ≥100 cells [mean=188] total cells per sample; Francoeur, Rier & Whorley, 2013) using the taxonomy of Wehr & Sheath (2003). On each date, water samples were also collected at the outlets of light and dark streams to determine pH, alkalinity, and conductivity. Water samples were also frozen, thawed and filtered to measure $N\text{-}[NO_3+NO_2]$, $N\text{-}NH_4$, and $P\text{-}PO_4$ using a SEAL AutoAnalyzer 3.

Algal biomass and assimilation

Algal biomass was estimated using chlorophyll-a. On each sampling date, one disk from each replicate was collected and stored frozen (−20°C, in darkness). Chlorophyll-a was extracted in 90% ethanol (80°C, 5 min), steeped overnight (4°C, darkness), and quantified using high performance liquid chromatography (HPLC; Meyns, Illi & Ribi, 1994).

Accrual of algal biomass as chlorophyll-a was used to estimate algal C-assimilation rates on each sampling date. We converted chlorophyll-a to standing algal C using a conversion of 11.1 Chl-a mg^{-1} algal C, derived from a survey of 21 publications on periphyton C and chlorophyll-a contents (see Appendices S1, S2). We then calculated rates of algal C-assimilation on each day based on measured gains in algal C g^{-1} detrital C since the preceding date, assuming algae grew only during 16 hrs daylight each day.

Bacterial abundance and production

On each date, two disks from each replicate were preserved for bacterial abundance analysis in 10 mL 2% (v/v) sodium pyrophosphate (0.1% w/v) buffered formalin and stored at 4°C.

All samples were sonicated on ice using a Branson 150 sonifier at setting 4 for 4 × 20 s intervals. Subsamples (0.5 mL) were sieved through 70 μm strainers (Miltenyi Biotec, Cologne, Germany) to remove coarse debris, then diluted with 4.5 mL phosphate-buffered saline. Diluted samples were vortexed, bacterial cell stain and microbeads added using the Invitrogen bacteria counting kit for flow cytometry (Thermo Fisher, Waltham, MA), and analyzed using a BD LSRFortessa Cell Analyzer (flow rate = 400 events s^{-1}; fluorescence measured using a fluorescein (FITC) channel with a 530 nm bandpass filter). Based on dyed controls containing only microbeads, we counted bacterial cells as those with fluorescence above microbeads (FITC < 10^3); we also excluded any cells larger than microbeads (diameter 6 μm; forward scatter > 2×10^2). We converted from cells mL^{-1} to cells g^{-1} detrital C based on average leaf disk dry mass and C content. Ten bacterial abundance samples were lost prior to analysis.

Bacterial production rates were estimated using incorporation of [^{3}H]-leucine into bacterial protein (Gillies, Kuehn, Francoeur & Neely, 2006). On each date, two disks from each replicate were incubated in 20 mL sterile glass scintillation vials containing 4 mL filtered (0.22-μm pore) well water and 2.5 μM [4,5-^{3}H]-leucine (specific activity = 586 mCi $mmol^{-1}$). Vials were placed on their side in a Conviron plant growth chamber (Conviron, Winnipeg, Canada) and incubated (30 min, 20°C, 300 μmol quanta $m^{-2}s^{-1}$). Killed controls (5% v/v trichloroacetic acid (TCA)) corrected for non-biological ^{3}H-leucine incorporation. Leucine incorporation was stopped by TCA addition (5% v/v final concentration), followed by heating (80°C, 30 min). Samples were subsequently processed and radioassayed following protocols outlined in Gillies et al., (2006); instead of filtering samples, we employed centrifugation and removed the supernatant after each centrifugation. Bacterial production was calculated as μg bacterial C g^{-1} detrital C hr^{-1} using the conversion factors of 1.44 kg C produced $mole^{-1}$ leucine incorporated (Buesing & Marxsen, 2005).

Fungal biomass and production

Litter-associated fungal biomass and production were determined using ergosterol and rates of [1-^{14}C]-acetate incorporation into ergosterol, respectively (Suberkropp & Gessner, 2005). On each date, two disks from each replicate were placed in 20 mL sterile glass scintillation vials containing 4 mL filtered (0.22-μm pore) well water and 5 mM Na[1-^{14}C]-acetate (specific activity = 1.31 mCi $mmol^{-1}$), and incubated in the growth chamber (5h, 20°C, 300 μmol $m^{-2}s^{-1}$). Non-biological ^{14}C-acetate incorporation was determined using killed-controls containing formalin (2% v/v). Incorporation of [1-^{14}C]-acetate was stopped by placing the vials on ice and immediately filtering (1.2-μm pore). Filters and litter pieces were rinsed twice with 4 mL filtered well water and stored frozen (−20°C) until extraction. Samples were lyophilized, weighed, and ergosterol extracted in methanolic KOH (8 g L^{-1} KOH, HPLC-grade methanol, extraction volume 10 ml) for 30 min at 80°C. The resultant extract was cleaned by solid phase extraction and ergosterol quantified by HPLC following methods of Gessner (2005). Ergosterol fractions eluting from the HPLC were collected in scintillation vials, mixed with 10 mL scintillation fluid (Ecolume, MP Biomedicals, Santa Ana, CA), and radioactivity assayed using a Beckman LS6500 Scintillation Counter, corrected for quenching and radioactivity in killed controls. We converted ergosterol concentrations to fungal C assuming 5 μg ergosterol mg^{-1} fungal dry mass and 43% fungal

C (Gessner & Newell, 2002; Findlay, Dye & Kuehn, 2002; Kuehn et al., 2014). Rates of ^{14}C-acetate incorporation were converted to fungal growth rates (μ) using the conversion factor 12.6 μg fungal biomass nmol^{-1} acetate incorporated (Gessner & Newell, 2002). Rates of fungal production were calculated by multiplying fungal growth rate by fungal biomass.

Incubations with the photosynthesis inhibitor DCMU

On days 20 and 31, we conducted short-term litter microbial production assays in the presence or absence of the photosystem II inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU, see Francoeur, Johnson, Kuehn & Neely, 2007). At least 5 minutes prior to assays, duplicate-collected leaf disks from each replicate were placed into scintillation vials containing filtered well water with either 20 μM DCMU in 0.01% v/v acetone or the corresponding volume of acetone without DCMU. We measured instantaneous algal C-assimilation rates using ^{14}C-bicarbonate incorporation to verify DCMU inhibited algal photosynthesis (see Appendix S1, Fig. S2). We measured bacterial and fungal production rates as above (including appropriate killed-controls) in the presence and absence of DCMU. On each date, we determined the impact of inhibiting photosynthesis on fungal and bacterial production rates for each leaf species and light treatment combination, calculated as microbial production rates (μg C g^{-1} detrital C hr^{-1}) in the absence of DCMU minus production rates in DCMU presence.

Litter decomposition rates and cumulative microbial production

Using bulk leaf disk dry mass collected for mass loss, fungal production, and algal assimilation over time in each stream, we calculated litter dry mass decomposition rates k (d^{-1}) based on the exponential decay model (Bärlocher, 2005)

$$M_t = M_0 \times e^{-kt}$$

where M_t is bulk leaf disk dry mass (mg) at time t (days), and k is the exponential decay coefficient (d^{-1}). We determined k from iterative fitting using nonlinear least squares. We similarly estimated litter-specific C decomposition rates k based on bulk litter disk C on each date, calculated as disk dry mass multiplied by measured %C content. For this calculation, from bulk litter C we subtracted measured fungal biomass C and converted bacterial abundances to bacterial biomass to subtract bacterial biomass C (see Appendix S1). We also subtracted algal biomass C by converting chlorophyll-a to algal C using a conversion of 11.1 μg Chl-a mg^{-1} algal C (Appendix S1).

We also used measured microbial production rates on each date to estimate cumulative algal, bacterial, and fungal production per leaf disk throughout the study, converting to mg microbial C g^{-1} initial litter C. Details on these calculations may be found in Appendix S1.

Statistical analysis

We used repeated-measures split-plot ANOVA to test effects of time (repeated measures), leaf species (split plots within streams), and light treatment (across streams) on biomass and production rates of litter-associated algae, fungi, and bacteria, as well as litter molar C:N and

C:P, during the study (see Table S1, Fig. S1). From the production assays using DCMU manipulations, we used model II major axis regression (R package lmodel2; Legendre, 2018) to test relationships between mean algal assimilation rates and fungal and bacterial responses to DCMU across all treatments and dates. For these regressions, we used algal assimilation rates estimated from date-to-date algal chlorophyll-a accrual, instead of rates based on ^{14}C-bicarbonate incorporation, because the latter underestimated algal production rates inferred from chlorophyll-a accrual (see Appendix S1). Finally, we employed split-plot ANOVA to test the effects of leaf species and light treatment on dry mass and litter C decomposition rates. Response variables were square-root or $\log_{10}$-transformed where necessary to improve equality of variances and normality. We employed Bonferroni correction within related analyses to reduce family-wise error rates for multiple tests. All statistical analyses were conducted using R version 3.3.1 (2016, R Foundation for Statistical Computing).

Results

Light treatments differed in transmittance of PAR and UV light, but did not differ in temperature, conductivity, pH, or alkalinity (Table S2). Water collected from the outlet of light and dark streams in the study ranged from 20–30 μg L^{-1} N-NH_4, 2–30 μg L^{-1} N-[NO_3+NO_2], and >300 μg L^{-1} P-PO_4, and dark treatment outlet water was higher in P-PO_4 and N-[NO_3+NO_2] concentrations compared to light treatment water (Table S2). Algal communities inhabiting light-exposed litter were similar between leaf species on days 20 and 31. Communities were dominated by Chlorophytes (e.g., *Oocystis*, *Oedogonium*, and *Characium*) and Heterokonts (exclusively diatoms, such as *Gomphonema* and *Nitzschia*), with Cyanophytes (e.g., *Chroococcus*, *Oscillatoria*) also common (Table S3).

As expected, under light exposure algal biomass increased early, and was significantly greater in the light than the dark treatment (which showed negligible accrual of algae) (Table 1, $P<0.001$; Fig. 1a,b). Bacterial abundance generally increased during the experiment and was also greater in the light compared to the dark treatment ($P<0.001$; Table 1, Fig. 1c,d). Fungal biomass exhibited distinct temporal patterns across treatments, increasing steadily over time in the light, but peaking earlier in the dark and earlier on poplar compared to oak litter (Day × Light × Species interaction, $P<0.001$; Fig. 1e). Fungal biomass was significantly greater on dark-incubated compared to light-incubated litter ($P<0.001$, Fig. 1f).

Algal C-assimilation rates varied over time, but were more than 10-fold higher on light-incubated litter compared to dark-incubated litter ($P<0.001$; Fig. 2a,b) and did not differ between leaf species (Table 1). Bacterial production rates did not differ between light treatments, but bacterial production was higher on poplar compared to oak litter ($P<0.001$; Table 1, Fig. 2c,d) and showed temporal variation that differed between leaf species during decomposition ($P<0.001$; Table 1). Fungal production rates increased early to peak by day 6 or 10 and declined later, and similar to fungal biomass, there was a significant Day × Light × Species interaction ($P<0.001$; Fig. 2e). Fungal production rates were significantly higher on poplar compared to oak litter ($P<0.001$), as well as on light treatment compared to dark treatment litter ($P<0.001$; Table 1). In addition, there was a weak but notable Light × Species

interaction (P=0.019) reflecting stronger light stimulation of fungal production rates on oak litter (Fig. 2f).

The photoinhibitor DCMU effectively stopped instantaneous algal C-assimilation (Fig. S2) and DCMU consistently reduced fungal but not bacterial production (Fig. 3). Model II major axis regression indicated the magnitude of fungal production decrease with DCMU presence was positively related to algal assimilation rates (slope=2.66, P=0.001, R^2=0.84; Fig. 3a). In contrast, bacterial responses to DCMU were not related to algal C-assimilation (slope= −0.11, P=0.338, R^2=0.04; Fig. 3b).

Bulk litter C:N and C:P declined rapidly during the first 10 days (Fig. S3). Bulk C:N did not differ across leaf species or light treatments, but during the first 6 days, C:N was higher on light-incubated litter, especially poplar, and declined earlier on dark-incubated compared to light-incubated litter (Day × Light interaction; P<0.001; Table 1; Fig. S3). Bulk C:P also declined earlier in the dark, especially for poplar litter (Day × Light interaction; P<0.001), and although light effects were not significant, C:P of oak litter was higher than C:P of poplar throughout decomposition (P<0.001; Table 1; Fig. S3).

Bulk litter dry mass loss rates were on average 2.9-fold faster on dark-incubated compared to light-incubated litter (P=0.006) and were also faster among poplar compared to oak litter (P=0.001; Fig. 4a), but showed no Light × Species interaction (Table S4). In comparison, light treatment differences in litter-specific C decomposition rates were smaller, but poplar still exhibited greater C loss rates compared to oak (P<0.001; Fig. 4b). Over the 43-day study, dark-incubated litter lost on average 53.9% (poplar) and 18.6% (oak) of initial dry mass compared to 28.1% (poplar) and 6.9% (oak) losses among light-incubated litter (Fig. S4).

Reflecting the above contrasts in decomposition and microbial activity, cumulative litter-specific C mass loss and algal and fungal production differed across leaf species and treatments (Table 2, Fig. 5). Cumulative bacterial production was higher on poplar litter, but did not differ strongly between light treatments. Compared to dark-incubated litter, light-incubated litter exhibited 37% (poplar) and 23% (oak) lower cumulative litter-specific C loss, contrasted with 73% (poplar) and 147% (oak) greater cumulative fungal production (Fig. 5).

Discussion

Our study suggests broad implications of negative priming in aquatic systems by demonstrating how algal photosynthesis can simultaneously stimulate heterotrophic activity while inhibiting heterotrophic biomass accrual and leaf litter decomposition. The results support our prediction of algal-stimulated fungal activity on decomposing litter, consistent with previous studies (Kuehn et al., 2014; Soares, Kritzberg & Rousk, 2017). Although fungal stimulation would be expected to increase decomposition rates, the lack of concurrent increases in fungal biomass or litter decomposition rates did not support our hypothesis of a positive priming effect. Instead, we observed negative priming, in which the labile C provided by algae increased growth rates of microbial heterotrophs (i.e., fungi), but inhibited

the breakdown of recalcitrant C, perhaps due to preferential substrate use (Kuzyakov, 2010; Guenet et al., 2010). Although poplar decomposed faster than oak litter, similar algal-induced negative priming on both leaf species also did not support our hypothesis that substrate recalcitrance would mediate priming strength. Complemented by quantitative assessment of the underlying biological mechanisms, our study expands the spectrum of priming effects documented in aquatic settings – especially in flow-through conditions that are poorly characterized (Lagrue et al., 2011) – pointing to a larger need to understand the microbial interactions underlying organic matter processing across the breadth of aquatic ecosystems (Guenet et al., 2010).

Our experiment provides empirical evidence of negative priming because algae increased fungal production but suppressed leaf litter dry mass loss rates – a notable decoupling, since aquatic fungi (i.e., hyphomycetes which dominate in flowing environments) are considered major drivers of plant litter decomposition in stream ecosystems (Suberkropp & Chauvet, 1995; Romaní et al., 2006; Gessner et al., 2010; Kuehn, 2016). At a mechanistic level, algae may suppress litter decomposition through two effects, one apparent and one actual: 1) accrual of new algal biomass could counterbalance mass lost due to heterotrophic degradation of litter C, thereby reducing apparent decomposition, and 2) preferential substrate use of algal-derived labile C substrates by heterotrophs could reduce actual heterotrophic decomposition of litter (Guenet et al., 2010, Halvorson et al., 2016). Both mechanisms occurred in our experiment. For example, on the last day of our study, bulk litter C mass loss was 103 and 304 mg C g^{-1} initial C lower in the light-exposed oak and poplar litter, respectively. Of this difference, algal biomass had slowed bulk litter C mass loss in the light treatments by accruing 57 and 75 mg C g^{-1} initial C (Table 2). Removing the contribution of microbial biomass and considering only litter-specific mass loss gives a truer estimate of mass loss due to decomposition. In our study, bulk litter mass loss underestimated the true mass loss in the light by 44 and 25%, mainly due to mass addition from algae. The difference between litter-specific C mass loss in the light and dark treatments (59 and 218 mg C g^{-1} initial C for oak and poplar, respectively) thus represents mass loss attributable to heterotrophic preferential substrate use of algal-derived C (i.e., true negative priming). Elevated fungal growth rates in light treatments must have been supported by a non-litter C source, likely labile algal exudates, because algal-stimulated fungal production rates were not coupled to increased litter mass loss and hence enhanced fungal acquisition of litter C (Kuehn et al., 2014; Soares et al., 2017).

As an additional indicator that algae suppressed heterotrophic degradation of litter C, increased fungal production rates under light did not translate to greater fungal biomass accrual. This suggests fungi did not invest algal-derived C into new hyphal growth and/or degradative enzyme production to acquire litter substrate C. Given that fungal growth was not invested in biomass, production was likely channeled to an alternate pathway – plausibly spore production, which can account for as much as 80% of production in some hyphomycetes (Suberkropp, 1991; Kuehn, 2016). We did not quantify reproductive spore production in this study, but this remains an important question because a previous study found no significant effect of algae on fungal sporulation in a positive priming scenario (Danger et al., 2013). Low fungal biomass, countered with elevated algal biomass, could explain the similarity of litter C:N and C:P in light and dark treatments. The earlier declines

of litter C:N and C:P in the dark compared to light treatments may be attributable to earlier fungal relative to algal colonization. Because we observed negative priming on two leaf species of differing recalcitrance, our study suggests algal-driven decoupling of fungal activity from decomposition may occur independent of underlying substrate recalcitrance. Since algal stimulation of heterotroph production did not stimulate heterotroph biomass accrual or litter decomposition, our study also highlights, at a methodological level, the importance of coupled measures of microbial activity, biomass accrual, and substrate decomposition to accurately test priming effects and their mechanisms.

An important question regarding priming is the quantitative link between labile C addition and stimulated heterotrophic activity (Kuzyakov, 2010). We showed that light stimulated long-term fungal (but not bacterial) production rates; our photosynthesis manipulations using DCMU also demonstrated direct short-term algal simulation of fungal but not bacterial production rates. These DCMU incubations confirmed algal photosynthesis as the primary driver of long-term fungal stimulation by light, because DCMU consistently reduced short-term fungal production by similar magnitudes as the long-term difference between light vs. dark treatments (Fig. S5). By enhancing the lability of dissolved organic C (DOC), UV photolysis could explain long-term stimulation of heterotrophic activity by light (Wetzel et al. 1995; King et al., 2012); however, UV photolysis cannot explain short-term stimulation, because short-term algal stimulation of fungi during DCMU manipulations occurred under exclusively PAR (no UV) in the laboratory. UV photolysis should also increase long-term litter breakdown rates, but we observed the opposite effect in light vs. dark comparisons of decomposition. Instead, algal addition of labile C is the most probable mechanism for algae to stimulate fungi, but indirect effects of algal photosynthetic activity, such as increases in periphyton O_2 concentrations or pH, may also be responsible (Rier et al., 2007; Kuehn et al., 2014). We also note that DCMU does not inhibit photosynthesis in cyanobacterial heterocysts (strictly photosystem I), but we show photosynthesis was minimal in the presence of DCMU, and heterocystous cyanobacteria were rare, comprising <2% of the algal community. If algal supply of new labile C is the primary mechanism stimulating fungi, yet fungi do not degrade additional litter C (Fig. 5), then the magnitude of fungal stimulation should not exceed rates of algal C production. However, fungal stimulation exceeded algal C-assimilation, which points to an unmeasured C source supporting fungal stimulation by algae.

Several possibilities may explain how fungal stimulation exceeded algal C-assimilation rates during long-term exposure to light and short-term DCMU manipulations. An earlier study showed that DCMU has no short-term toxicity to fungi (Francoeur et al., 2007), and DCMU toxicity also would not explain the similar long-term difference of fungal production between light- and dark-incubated litter (Fig. S5). We recognize that these biomass-based estimates of algal C-assimilation provide a low measure because they assume no day-to-day losses of chl-a during algal turnover, but these estimates exceeded rates measured with ^{14}C-bicarbonate incorporation, perhaps due to degassing of ^{14}C during assays (Appendix S1). Converting measured standing litter chl-a to primary production rates during assays (Morin, Lamoureux & Busnarda, 1999) indicates rates >1000 μg C g^{-1} detrital C hr^{-1} on light-incubated litter, providing algal C-assimilation rates sufficient to support fungal stimulation. Furthermore, biomass conversions quantify only algal production which is incorporated into

particular biomass, and do not include the fraction of algal production exuded as soluble labile C. Exudation rates are frequently >30% of primary production, and approach (or even slightly exceed) 100% of primary production under stressful conditions (e.g., nutrient limitation) (Ziegler & Lyon, 2010; Wyatt, Tellez, Woodke, Bidner & Davison, 2014; Wyatt & Turetsky, 2015) and exudates represent the most plausible C pool supporting fungal production (Kuehn et al., 2014). Possibly supplemented by other forms of labile C such as accumulated microbial necromass, algae clearly stimulated fungal activity on decomposing litter, but there remains a need for tests of the mechanisms and detailed accounting of C flows that determine priming effects (Kuehn et al., 2014).

In contrast to fungi, bacterial abundance increased with light exposure, but bacterial production rates did not respond to algae in the long- or short-term. While suggesting algae facilitate bacterial colonization of periphyton, perhaps by increasing space available to bacteria (Carr, Morin & Chambers, 2005), our findings contrast with some previous reports of periphytic algal stimulation of bacterial production (Kuehn et al., 2014; Wyatt & Turetsky, 2015). However, Soares et al. (2017) also found litter-associated bacterial growth responded only weakly to algae or glucose additions. Other studies of litter periphyton have shown algae decreased bacterial abundance in the presence of fungi, possibly because of fungal-bacterial antagonism (Danger et al., 2013). Weak bacterial responses may also partly reflect the ability of bacteria to use leaf-derived labile C, especially leachates early into decomposition, as well as the high P-PO_4 concentrations in our study system, which can decouple algal and bacterial production because algae are less reliant on bacterially-regenerated P (Scott, Back, Taylor & King, 2008). Given observations of strong fungal yet weak bacterial responses to algae, fungi may serve as the main recipients of algal-derived C, and therefore the primary determinants of priming during litter decomposition.

Conclusions

Our observations of negative priming point to several unanticipated effects of algal-mediated labile C addition on recalcitrant C degradation in aquatic ecosystems. Foremost, our study reiterates the question of why negative priming occurs in some settings, whereas positive priming occurs in others (Bengtsson et al., 2018). In two previous litter decomposition studies, increased algal biomass under high nutrients erased positive algal-induced priming (Danger et al., 2013; Halvorson et al., 2016). Our findings may be attributable to high nutrient availability which, combined with high light, could raise algal exudation to fully support, rather than augment, heterotrophic C-demands (Guenet et al., 2010; Wyatt et al., 2014; Wagner et al., 2017). Well water inputs ensured constant fresh nutrient influx, but the light treatment water was comparatively lower in P-PO_4 and N-[NO_3+NO_2], likely due to greater in-stream algal growth sufficient to drawdown nutrients. Still, stronger nutrient limitation in the light treatment would not fully explain our findings, because fungal activity was clearly higher in this treatment, N-NH_4 levels were non-limiting and slightly higher in the light, N-fixation was minimal based on the low proportion of cyanobacteria with heterocysts, and P-PO_4 concentrations were high and non-limiting in both treatments. Contrasting DOC levels may also have contributed to our findings; while DOC was likely higher and more labile in the light streams due to greater periphyton growth, the DCMU

incubation results support direct fungal stimulation by algal photosynthesis, not elevated streamwater DOC, as the primary driver of priming in our study.

Given the prevalence of algae in aquatic settings, the interactions revealed in our study carry broad implications for aquatic ecosystems. Our flume design simulated streamflow, but may bias biological breakdown relative to leaf physical breakdown and transport in natural forested streams (Webster et al., 1999). The interactions revealed in our study are worth further *in situ* assessment because they may be patchier and persist over shorter intervals (days to weeks) in real streams. However, under base flow and in well-lit lentic systems such as marshes, and with higher nutrient and light availability under anthropogenic land use (Allan, 2004), our study suggests algal-induced negative priming may force a heterotrophic shift from using litter C as a resource to using litter as a surface substratum for growth. This is apparent in the comparison of dry mass versus litter-specific C loss rates, showing algae suppressed decomposition both by adding new biomass to detrital periphyton, and by reducing heterotrophic use of detrital C (especially on poplar). Negative priming during litter decomposition could also slow organic matter turnover, increasing C storage, potential organic matter export downstream, and accessibility of algal and detrital C in aquatic food webs. Detrital-based systems with sufficient light may exhibit blurrier contrasts between “green” and “brown” bases of energy flow, given that fungal C may largely (based on cumulative fungal production, 42–60% of total production) derive from algal C-exudation instead of detrital C. Yet, if algal-derived C is not invested in fungal biomass, as we observe here, this labile C may ultimately transfer poorly to upper trophic levels. Future research should address how high algal yet low fungal biomass under light could affect trophic transfer to primary consumers (Guo, Kainz, Valdez, Sheldon & Bunn, 2016; Crenier et al., 2017; Norman et al., 2017). Finally, the dissimilar responses of fungal biomass vs. activity indicate labile C additions may shift competitive interactions or succession among litter-associated fungi, e.g., favouring fungi specializing on algal-derived C over recalcitrant-degrading taxa (Voříškova & Baldrian, 2013). Linkages between priming and heterotrophic community composition are a promising topic of investigation (Fabian et al., 2018), with implications for long-term, downstream microbial community composition and function. Further quantification of microbial interactions and their mechanisms will enhance understanding of the direction and ecological implications of priming effects in aquatic systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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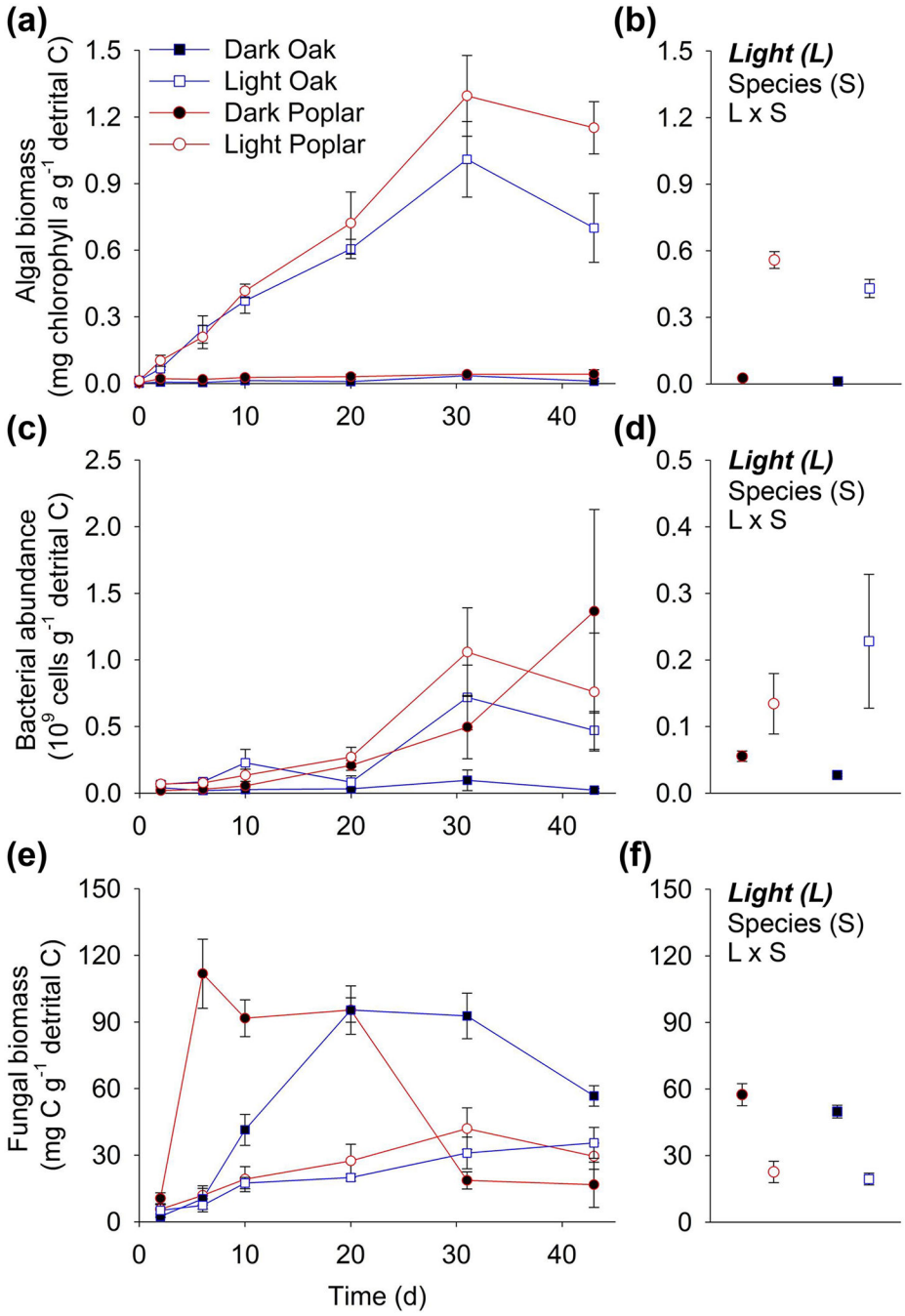

Figure 1.

Mean ± SE algal biomass (a,b), bacterial abundance (c,d), and fungal biomass (e,f) on leaf litter exposed to dark or light regimes during decomposition. Panels are divided into temporal trends (a,c,e) and time-pooled averages for each leaf species and light treatment combination (b,d,f). Bold italics designate significant time-pooled effects (P<0.006; Table 1).

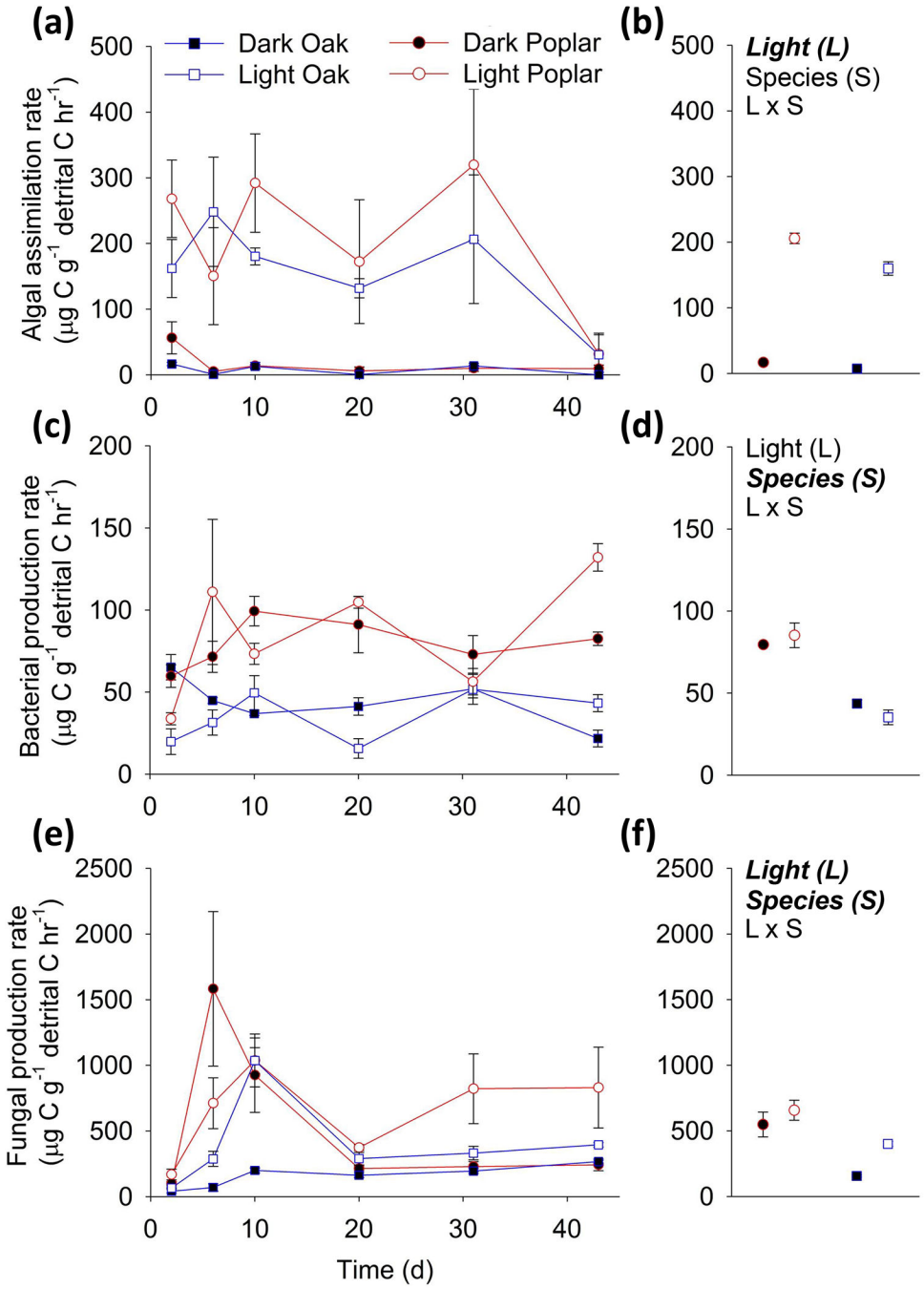

Figure 2.

Mean ± SE assimilation or production rates of algae (a,b), bacteria (c,d), and fungi (e,f) on leaf litter exposed to dark or light regimes during decomposition. Panels are divided into temporal trends (a,c,e) and time-pooled averages for each leaf species and light treatment combination (b,d,f). Bold italics designate significant time-pooled effects (P<0.006; Table 1).

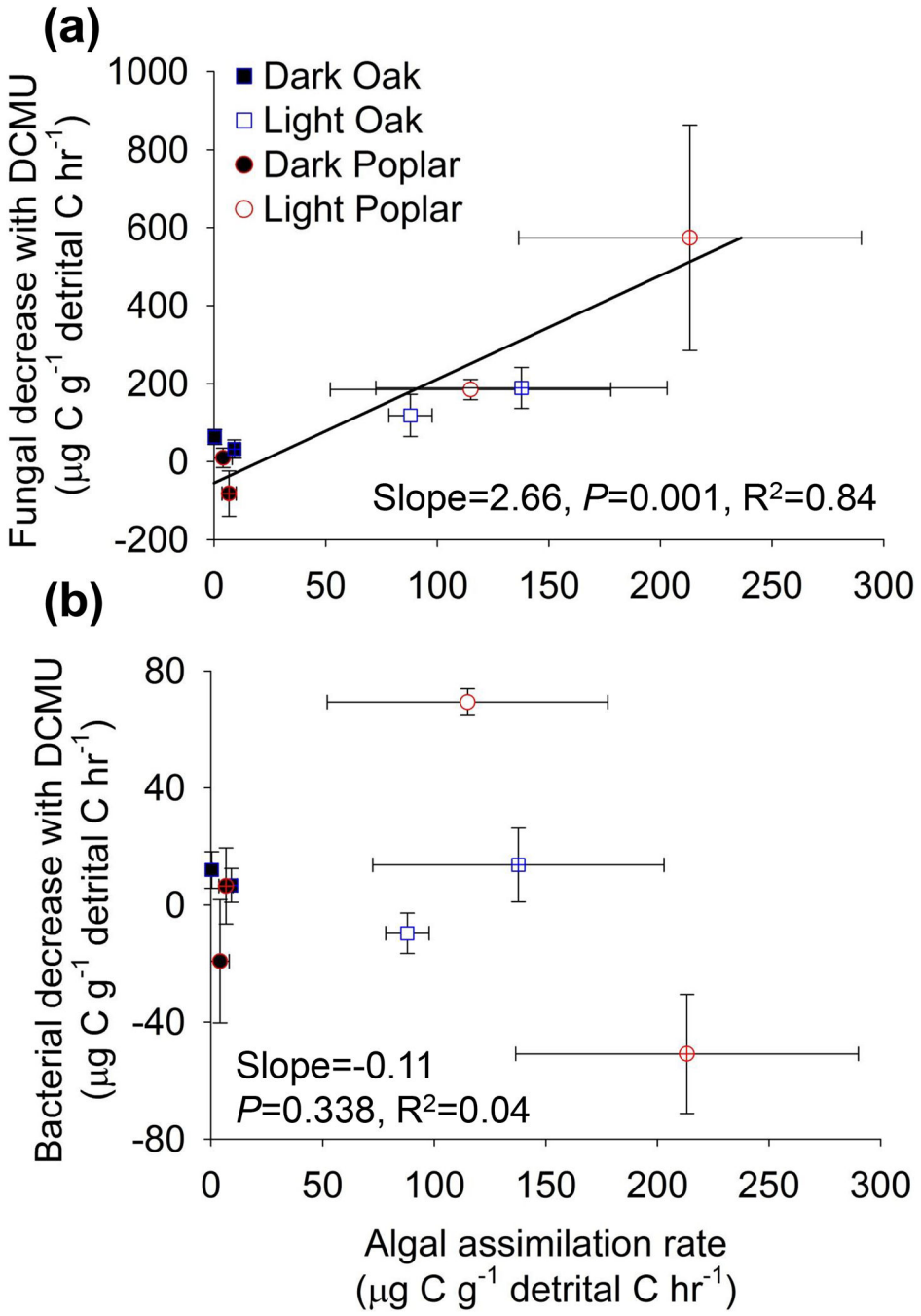

Figure 3.

Mean ± SE decreases in fungal (a) or bacterial (b) production rates in response to DCMU inhibition of photosynthesis, as a function of mean ± SE algal assimilation rates after 20 days (symbols not cross-hatched) or 31 days (symbols cross-hatched) of decomposition under dark or light conditions. Decreased production rates were calculated as [production in DCMU absence] – [production in DCMU presence]. Algal assimilation rates were determined from chlorophyll-a accrual and conversion to algal C (Appendix S1). In (a), the solid black line indicates fungal responses to DCMU presence are positively related to algal

assimilation rates based on Model II major axis regression (slope =2.66, P=0.001, R^2=0.84). Bacterial responses were not related to algal assimilation (slope=−0.11, P=0.338, R^2=0.04).

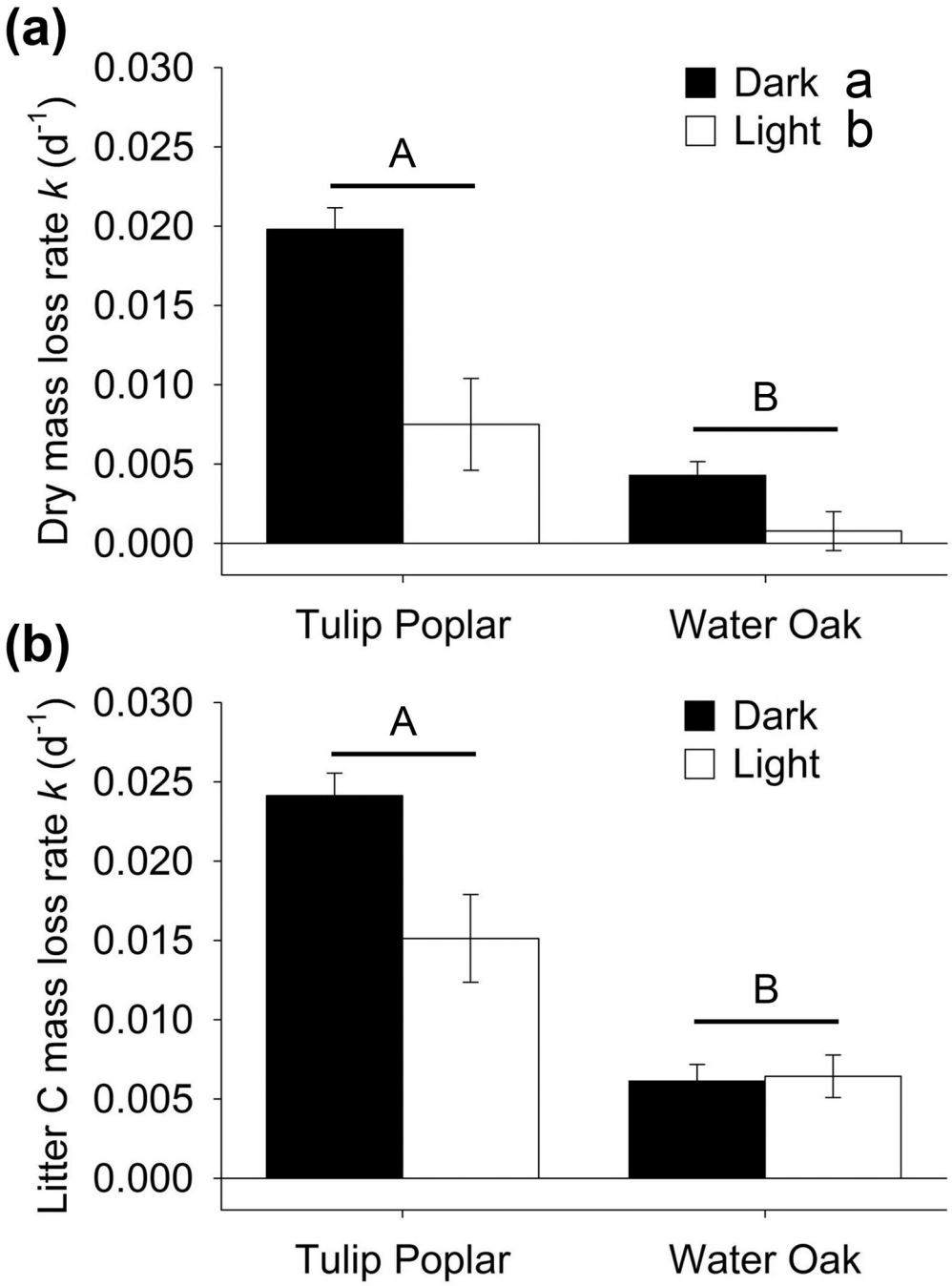

Figure 4.

Mean ± SE litter decomposition rates *k* based on dry mass loss (a) or litter-specific C mass loss (b) of tulip poplar and water oak litter under light or dark conditions. Letters designate statistically significant differences between light treatments (lower-case letters) or leaf species (upper-case letters; $P<0.025$; Table S4).

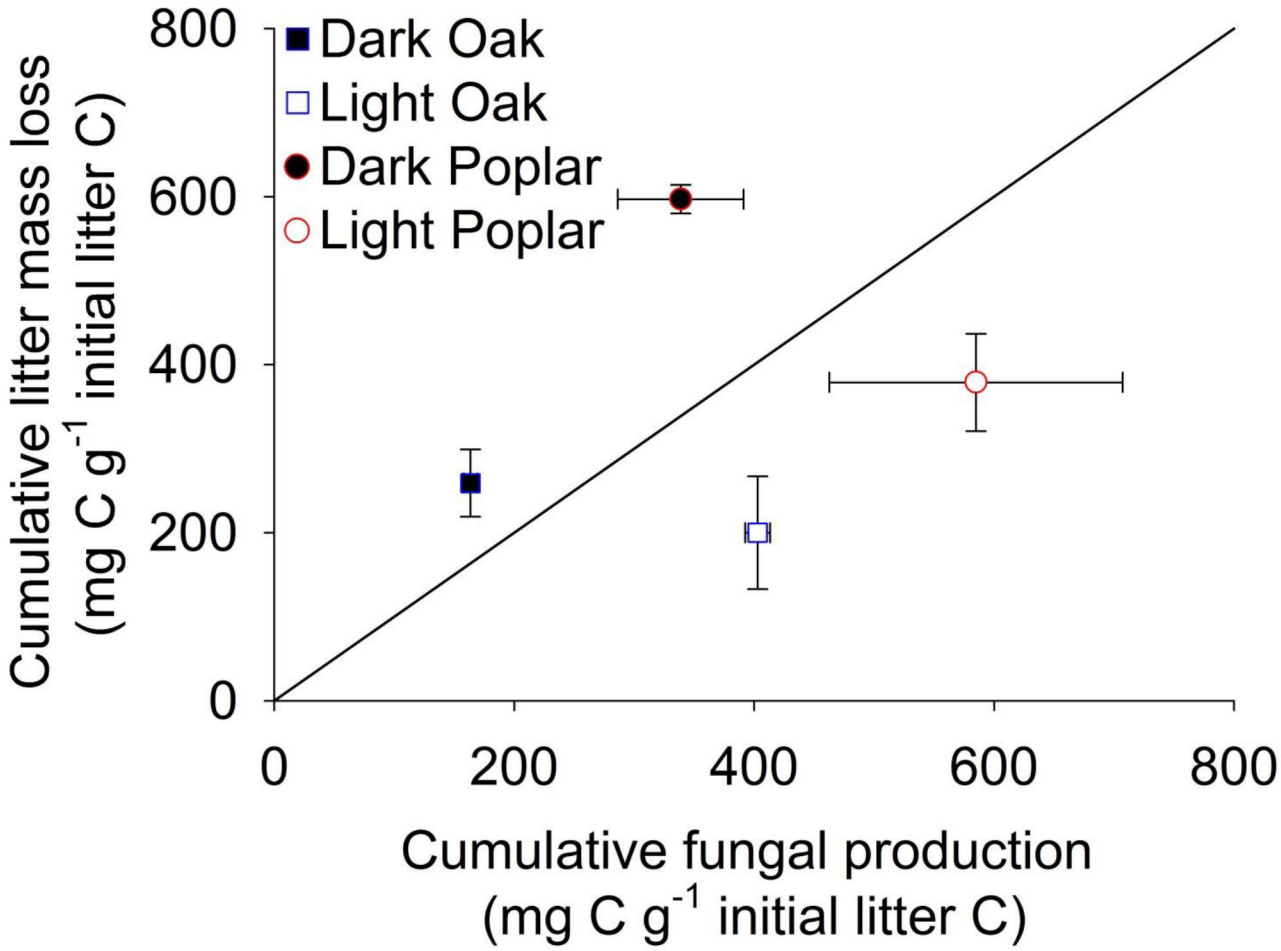

Figure 5.

Scatterplot of mean ± SE cumulative fungal C production and litter-specific C mass loss of water oak and tulip poplar litter exposed to either light or dark conditions during decomposition. The solid black line designates a 1:1 relationship. Cumulative fungal production and mass loss were determined through the last sampling date (day 43) and are expressed as mg C g^{-1} initial litter C (see also Table 2).

Table 1.

Repeated-measure split-plot ANOVA table testing effects of light treatment, leaf species, and day on algal biomass, bacterial abundance, fungal biomass, algal assimilation rates inferred from chlorophyll-a accrual, bacterial production rates, fungal production rates, and litter molar C:N and C:P during decomposition. Among the bacterial abundance within-stream results, N/A designates terms could not be tested because of insufficient sample size.

Response	Factor	F-value	P-value[a]	Factor	F-value	P-value[a]
	Within-streams, temporal effects:			**Across-streams, pooled across time:**		
Algal biomass[b]	Day (D)	$14.8_{6,36}$	**<0.001**	Light (L)	$266.5_{1,6}$	**<0.001**
	D × L	$5.1_{6,36}$	**<0.001**	Leaf species (S)	$3.8_{1,6}$	0.098
	D × S	$0.3_{6,36}$	0.908	L × S	$0.4_{1,6}$	0.533
	D × L × S	$0.8_{6,36}$	0.602			
Bacterial abundance[b,d]	Day (D)	N/A	N/A	Light (L)	$43.8_{1,6}$	**<0.001**
	D × L	N/A	N/A	Leaf species (S)	$0.1_{1,6}$	0.754
	D × S	N/A	N/A	L × S	$1.9_{1,6}$	0.219
	D × L × S	N/A	N/A			
Fungal biomass[c]	Day (D)	$50.6_{5,30}$	**<0.001**	Light (L)	$31.4_{1,6}$	**0.001**
	D × L	$19.3_{5,30}$	**<0.001**	Leaf species (S)	$1.7_{1,6}$	0.235
	D × S	$23.6_{5,30}$	**<0.001**	L × S	$0.1_{1,6}$	0.754
	D × L × S	$22.2_{5,30}$	**<0.001**			
Algal assimilation[b]	Day (D)	$11.3_{5,30}$	**<0.001**	Light (L)	$296.8_{1,6}$	**<0.001**
	D × L	$3.2_{5,30}$	0.020	Leaf species (S)	$0.1_{1,6}$	**0.781**
	D × S	$0.5_{5,30}$	0.794	L × S	$4.7_{1,6}$	**0.074**
	D × L × S	$0.5_{5,30}$	0.800			
Bacterial production	Day (D)	$2.1_{5,30}$	0.092	Light (L)	$0.1_{1,6}$	0.736
	D × L	$3.9_{5,30}$	0.008	Leaf species (S)	$69.0_{1,6}$	**<0.001**
	D × S	$6.1_{5,30}$	**<0.001**	L × S	$1.8_{1,6}$	0.223
	D × L × S	$2.2_{5,30}$	0.079			
Fungal production[b]	Day (D)	$40.3_{5,30}$	**<0.001**	Light (L)	$47.0_{1,6}$	**<0.001**
	D × L	$0.7_{5,30}$	0.645	Leaf species (S)	$131.0_{1,6}$	**<0.001**
	D × S	$10.5_{5,30}$	**<0.001**	L × S	$10.24_{1,6}$	0.019
	D × L × S	$9.0_{5,30}$	**<0.001**			
Litter C:N[b]	Day (D)	$18.0_{6,36}$	**<0.001**	Light (L)	$2.8_{1,6}$	0.145
	D × L	$4.6_{6,36}$	**0.001**	Leaf species (S)	$1.0_{1,6}$	0.358
	D × S	$2.5_{6,36}$	0.037	L × S	$0.3_{1,6}$	0.626
	D × L × S	$1.4_{6,36}$	0.226			
Litter C:P[b]	Day (D)	$69.6_{6,36}$	**<0.001**	Light (L)	$13.7_{1,6}$	0.010
	D × L	$3.6_{6,36}$	**0.006**	Leaf species (S)	$45.4_{1,6}$	**<0.001**
	D × S	$2.9_{6,36}$	0.019	L × S	$2.0_{1,6}$	0.209

Response	Factor	F-value	P-value[a]	Factor	F-value	P-value[a]
	Within-streams, temporal effects:			**Across-streams, pooled across time:**		
	D × L × S	$3.6_{6,36}$	**0.006**			

[a] Boldface indicates significant *P*-values after Bonferroni adjustment (α=0.006).

[b] Log-transformed prior to analysis.

[c] Square-root transformed prior to analysis.

[d] Due to missing samples, only between-stream effects were tested, exclusively on day 10.

Table 2.

Mean (±SE) bulk (litter + microbial) and litter-specific C mass loss and standing algal biomass on the last sampling date, as well as cumulative bacterial, algal, and fungal production estimated over 43 days of decomposition of tulip poplar and water oak litter under light or dark conditions. Litter-specific C mass loss is calculated from litter C remaining after subtracting standing algal, bacterial, and fungal biomass C from bulk litter + microbial C on the same date. See Appendix S1 for calculation methods used to determine cumulative microbial production.

Leaf species	Light treatment	Bulk C mass loss[a]	Algal biomass (day 43)[a]	Litter- specific C mass loss[a]	Cumulative algal production[a]	Cumulative bacterial production[a]	Cumulative fungal production[a]
Tulip Poplar	Dark	588 (17)	1.5 (0.7)	597 (17)	6 (1)	52 (1)	339 (52)
	Light	284 (68)	75 (13)	379 (58)	121 (16)	70 (7)	585 (122)
Water Oak	Dark	214 (40)	0.8 (0.1)	259 (40)	5 (1)	40 (2)	163 (6)
	Light	111 (77)	57 (15)	200 (67)	102 (15)	35 (5)	403 (10)

[a]All units are in mg C g^{-1} initial litter.