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Temporal and Stoichiometric Patterns of Algal Stimulation of Litter-Associated Heterotrophic Microbial Activity

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Summary

1. Periphyton communities associated with submerged plant detritus contain interacting autotrophic and heterotrophic microbes, and are sites of extracellular enzymatic activity. The strength and nature of these interactions might be expected to change over time as microbial communities develop on plant litter. Microbial interactions and enzymatic activity can be altered by nutrient availability, suggesting that litter stoichiometry could also affect these phenomena.
2. We grew wetland plants under ambient and nutrient-enriched conditions to generate plant litter of differing nutrient content. In two experiments, we investigated: 1) the influence of algal photosynthesis on fungal and bacterial production and the activities of four extracellular enzymes throughout a 54-day period of microbial colonization and growth, and 2) the influence of litter stoichiometry on these relationships.
3. Ambient and nutrient-enriched standing-dead plant litter was collected and then submerged in wetland pools to allow for natural microbial colonization and growth. Litter samples were periodically retrieved and transported to the laboratory for experiments manipulating photosynthesis using the photosystem II inhibitor DCMU (which effectively prevents algal photosynthetic activity). Algal (^{14}C-bicarbonate), bacterial (^{3}H-leucine) and fungal (^{14}C-acetate) production, and ß-glucosidase, ß-xylosidase, leucine-aminopeptidase, and phosphatase activities (MUF- or AMC-labeled fluorogenic substrates) were measured under conditions of active and inhibited algal photosynthesis.
4. Photosynthesis stimulated overall fungal and bacterial production in both experiments, although the strength of stimulation varied amongst sampling dates. Phosphatase activity was

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stimulated by photosynthesis during the first, but not the second, experiment. No other enzymatic responses to short-term photosynthesis manipulations were observed.

5. Microbial communities on high-nutrient litter occasionally showed increased extracellular enzyme activity, fungal growth rates and bacterial production compared to communities on non-enriched litter, but algal and fungal production were not affected. Litter stoichiometry had no effects on fungal, bacterial, or enzymatic responses to photosynthesis, but the mean enzyme vector analysis angle (a measure of P- vs. N-acquiring enzyme activity) was positively correlated to litter N:P, suggesting that elevated litter N:P led to an increase in the relative activity of P-acquiring enzymes.

6. These results supported the hypothesis that algal photosynthesis strongly influences heterotrophic microbial activity on macrophyte leaf litter, especially that of fungi, throughout microbial community development. However, the strength of this photosynthetic stimulation does not generally depend on small differences in litter nutrient content.

7. Stimulation of microbial heterotrophs by algal photosynthesis could drive diurnal shifts in periphyton community and aquatic ecosystem function, as well as linking “green” (photoautotroph-based) and “brown” (detrital-based) food webs.

Introduction

Periphyton is the community of microorganisms that grow upon surfaces within aquatic ecosystems (Wetzel, 2001). These communities can include algae, bacteria, fungi, and protists, all of which may interact with each other. In many instances, the interactions among these microbial groups are simply specific examples of near-universal organismal interactions. For example, the existence of trophic (Carpenter, 1897; Bott, 1996) and pathogenic (Stewart, 1988; Peterson, Dudley, Hoagland, & Johnson, 1993) interactions are well-established.

Rapid photosynthetic stimulation of heterotrophic microbial activity is another interaction which can occur in periphyton. Algal photosynthesis can result in near-instantaneous stimulation of bacterial (Neely & Wetzel, 1995; Scott, Back, Taylor, & King, 2008; Kuehn, Francoeur, Findlay, & Neely, 2014) and fungal (Kuehn et al., 2014; Halvorson et al., 2019a) production and growth rates in periphyton. Photosynthesis may also cause similar rapid increases in the activity of carbon (C)-acquiring (e.g., ß-glucosidase, ß-xylosidase), nitrogen (N)-acquiring (e.g., leucine-aminopeptidase), and phosphorus (P)-acquiring (phosphatase) extracellular enzymes produced

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wholly or partially by microbial heterotrophs (Francoeur and Wetzel, 2003; Francoeur, Schaecher, Neely, & Kuehn, 2006; Rier, Kuehn, & Francoeur, 2007). When such stimulation occurs in periphyton communities inhabiting (and potentially decomposing) plant litter, it is a likely precursor to the priming effect (Danger et al., 2013; Kuehn et al., 2014; Halvorson et al., 2019a). In a broader context, rapid heterotrophic responses to algal photosynthesis could drive diurnal shifts in the functioning of periphyton communities and aquatic ecosystems, as well as being a linkage between "green" (photoautotroph-based) and "brown" (detrital-based) food webs.

The biomass and composition of periphyton changes greatly as communities develop from initial microbial colonization of bare surfaces to thick, mature biofilms (e.g., Biggs, 1996; Jackson, Churchill, & Roden, 2001; Vermaat, 2005; Kuehn, 2016), suggesting that the strength of photosynthetic stimulation of heterotrophic microbial production and enzyme activity could also change during community development. There is little information regarding the dynamics of these interactions over the course of periphyton community development. Several studies have compared enzymatic and microbial activities in algae-rich (i.e., light-grown) vs. algae-free (i.e., dark-grown) periphyton communities throughout several weeks of community development (e.g., Halvorson, Scott, Entrekin, Evans-White, & Scott, 2016; Wagner, Bengtsson, Findlay, Battin, & Ulseth, 2017; Halvorson et al., 2019a), but by their nature, such studies contrast the performance of algae-rich and algae-free periphyton communities though time, rather than investigating how the strength of photosynthetic stimulation varies during the development of algae-rich periphyton. A few previous studies have examined the strength of rapid photosynthetic stimulation at two dates during algae-rich periphyton community development. Two studies (Francoeur et al., 2006; Rier et al., 2007) have reported at least some significant differences in enzymatic responses to photosynthesis between sampling dates. In contrast, two other experiments found that photosynthetically-mediated stimulation of bacterial and fungal production remained relatively similar between two sampling dates (Kuehn et al., 2014; Halvorson et al., 2019a). A study employing multiple sampling times throughout the course of community development is needed to better refine our understanding of how the strength of photosynthetic stimulation of enzymatic and heterotrophic activity varies during periphyon community development.

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Nutrient (i.e., N and P) availability can regulate the biomass and production of algae, fungi, and bacteria (Gulis & Suberkropp, 2003a; 2003b; Scott, Doyle, & Filstrup, 2005; Whorley & Francoeur, 2013; Cooper, Costello, Francoeur, & Lamberti, 2016; Gulis et al., 2017), and affect the decomposition of plant litter (Neely & Davis, 1985; Ferreira et al., 2014; Manning et al., 2016). High-nutrient plant litter decomposes faster than low-nutrient litter, and the stimulatory effect of dissolved nutrients on litter decomposition rates is lower for high-nutrient litter (Manning et al., 2016), indicating that litter itself can be an important source of nutrients for decomposers.

Ecological stoichiometric theory (Sterner & Elser, 2002) predicts that N and P sufficiency should increase the importance of labile C supply for heterotrophic microbial production. Thus, it may be expected that high litter N and P content would increase the linkage of algae and heterotrophic microbes, as there could be greater microbial biomass, and the importance of labile C supply by algae (one likely interaction mechanism) should be increased. This series of interactions between fertilization, algal C exudation, and stimulation of bacterial biomass and respiration were observed in an Alaskan Peatland (Wyatt & Turetsky, 2015). However, Scott & Doyle (2006) observed weaker linkage of wetland algal and bacterial production under high N conditions, perhaps because of increased competition for P or reduced algal requirements for bacterially-regenerated nutrients (Scott et al., 2008). The influence of stoichiometry on fungal ecology (Danger, Gessner, & Bärlocher, 2016) and on microbial interactions requires additional study.

The purposes of this investigation were to: 1) examine temporal patterns in the strength of photosynthetically-mediated interactions within periphyton communities during a sequence of colonization and community development, and 2) investigate the influence of litter stoichiometry on the strength of rapid photosynthetic stimulation of heterotrophic production and enzyme activity.

Methods

Study site

This study was conducted in a small (18.2 ha) inland emergent marsh wetland, located in

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southeast Michigan (N42°12.971 W083°37.181). The study wetland is a created retention basin wetland formed more than 30 years ago. It receives water from the surrounding Paint Creek watershed and is dominated by the emergent macrophytes *Typha angustifolia*, *Schoenoplectus acutus*, and *Phragmites australis*. The experiments were situated in two separate pools in the southeastern corner of the wetland. Periodically during the study, surface water samples from the pools were collected in acid-washed bottles for analysis of total phosphorus (TP), soluble reactive phosphorus (SRP), ammonium (NH_4^+), nitrate + nitrite (NO_3+NO_2), dissolved organic carbon (DOC), pH, and alkalinity. Surface water pH was measured using a MP220 bench-top pH meter (Mettler-Toledo, Columbus, OH, USA). Alkalinity was determined by titration to pH 4.5 (APHA 1992). Samples for SRP, NH_4^+ and NO_3+NO_2 were filtered (0.2-μm pore size) then frozen (-10 ºC) until analysis, and TP samples were frozen without filtration. All N and P concentrations were measured using a Lachat AP300 discrete analyzer (Hach, Loveland, CO, USA). Persulfate digestion, followed by molybdate/ascorbic acid reduction was used for TP analyses (Hebert and Green 2005). Similarly, SRP was determined according to the molybdate/ascorbic acid method in Hebert (2005). Ammonium was assayed using the phenol/nitroprusside/hypochlorite method for unpreserved samples described in Hebert (2004). Cadmium reduction was used to determine nitrate-nitrite concentrations (Harbridge, 2005). DOC samples were filtered through pre-ashed glass-fiber filters (0.7 μm pore-size), then were preserved with concentrated phosphoric acid and stored at 4°C until analyzed for DOC using a TOC-500 analyzer (Shimadzu, Kyoto, Japan). Specific conductance was measured in the field using a YSI 63 pH/conductivity meter (YSI Inc., Yellow Springs, OH, USA). Water temperature in the first experimental wetland pool was recorded every 30 min throughout the study duration by an Onset logger (Onsett Computer Corp. Bourne, MA, USA); temperature was not monitored in the second pool.

Experiment 1. Colonization sequence.

Litter generation and microbial community colonization.

Standing dead *Typha angustifolia* leaf litter was collected from the Paint Creek wetland, returned to the laboratory, air dried, and stored at ambient room temperature until used. Dried litter was cut into ~16 cm-long sections and encased within floating wire mesh trays (0.635 cm

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mesh). Sixty mesh trays were submersed in a grid pattern within a single wetland pool (approximately 1 m between trays, litter held at ca. 1-2 cm depth) on 8 July and retrieved after 5, 12, 19, 26, 40, and 54 days. On each sampling date, all litter sections were carefully removed from 10 trays, enclosed in 10 clean resealable plastic containers with wetland water, placed on ice in a cooler, and returned to the laboratory (< 30 min). In the laboratory, litter was subsectioned into 1.7 cm-long pieces for subsequent production and biomass analyses, or 0.85 cm-long pieces for enzymatic analyses. Algal biomass (chlorophyll a), algal community composition (brightfield microscopy), and fungal biomass (ergosterol) were measured as described in Francoeur et al. (2006). Bacteria were removed from litter by sonication, then bacterial abundance and biomass was measured by flow cytometry (Halvorson et al., 2019a), with counts corrected using an empirically-determined conversion for cell counts by flow cytometry relative to epifluorescence microscopy (see also Frossard, Hammes, & Gessner, 2016), and using an average cell conversion factor of 95.5 fg C per cell based on measured cell area and perimeter converted to biovolume (Bjørnsen, 1986) and biomass (Loferer-Krößbacher, Klima, & Psenner, 1998). A subset of bacterial samples was assessed for abundance and biovolume using epifluorescence direct count microscopy (as described in Francoeur et al., 2006); this relationship was then extrapolated to estimate bacterial biomass for all samples based on flow cytometry data.

Microbial activity assays

Rates of ^{14}C bicarbonate incorporation into attached algae were measured to quantify the effects of light exposure on algal photosynthesis. Paired litter sections from each tray were placed into autoclaved glass scintillation vials with 5 ml of filtered, autoclaved wetland water and 0.5 μCi $H^{14}CO_3^-$. One vial from each tray was spiked with 20 μM DCMU (final concentration) 10 min prior to addition of ^{14}C, while the other vial remained unexposed to DCMU. Prior experiments indicated that this DCMU treatment effectively halts photosynthesis, but does not directly affect bacterial production, fungal production, or extracellular enzyme activity (Francoeur, Johnson, Kuehn, & Neely, 2007). All vials were placed on their sides and incubated for 2 h in a growth chamber (400 μmol m^{-2} s^{-1} PAR, 20 ºC). Killed controls (3% v/v formalin) were used to correct for non-biological ^{14}C incorporation. Inorganic C pools were

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1 estimated by measuring alkalinity of water used for incorporation assays (titration to pH 4.5,
2 APHA 1992). After incubation, attached algae were killed with 3% formalin (final
3 concentration), filtered, and litter and filters stored frozen (-20 ºC). Samples were later fumed
4 with HCl (10 min) to remove residual inorganic ^{14}C, and dissolved in 0.5 M NaOH (80 ºC, 1 h).
5 Aliquots (100 µl) were then cleared with an equal volume of 50% H_2O_2 and mixed with 10 ml of
6 scintillation fluid (Ecolume, MP Biomedicals Inc., Santa Anna, CA, USA). Radioactivity was
7 assayed by using an LS 6500 scintillation counter (Beckman Coulter Inc., Fullerton, CA, USA),
8 corrected for quenching. Algal production was calculated following the method of Wetzel &
9 Likens (2000).

10

11 Bacterial production was assessed using the ^3H-leucine incorporation technique of Gillies,
12 Kuehn, Francoeur, & Neely (2006). Individual litter sections (paired by tray) were placed into
13 autoclaved glass scintillation vials with 3.88 ml of 0.2 µm filtered wetland water. One vial from
14 each tray was spiked with 20 µM DCMU (final concentration), while the other vial remained
15 unexposed to DCMU. After 10 min, 120 µl of ^3H-leucine stock solution was added to each vial,
16 resulting in a final leucine concentration of 2500 nM (specific activity = 220 GBq mmol^{-1}). All
17 vials were placed on their sides and incubated for 30 min in a growth chamber (400 µmol m^{-2} s^{-1}
18 PAR, 20 ºC). Trichloroacetic acid (TCA) killed controls (5% v/v final concentration) were used
19 to correct for non-biological leucine incorporation.

20

21 After incubation, leucine incorporation was stopped by the addition of 440 µl of 50%
22 TCA (5% v/v final concentration). Sample vials were incubated in a circulating water bath at 80
23 °C for 30 minutes, cooled to room temperature, and placed on ice for 30 minutes to precipitate
24 proteins. The resultant precipitated proteins were vacuum filtered through mixed nitrocellulose
25 filters (25 mm, 0.22 µm, Merck KgaA, Darmstadt, Germany), using a 0.8 µm mixed
26 nitrocellulose backing filter. Filters and litter pieces were washed 3 times each with 4 ml of cold
27 5% TCA, and then washed 2 times each with 4 ml of cold 80% ethanol. Filters and litter pieces
28 were then washed 2 times each with 4 ml of cold sterile distilled water and then placed into 15
29 ml polypropylene conical centrifuge tubes (Corning, Inc., Corning, NY, USA). Ten ml of
30 alkaline extractant (0.3N NaOH, 0.1% SDS, 25mM EDTA) was added to each tube and these

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1 tubes were incubated at 80 °C in a dry-block heater for 60 min. Tubes were removed and cooled
2 to room temperature. Sample aliquots (500 µl) of extracts were placed into sterile 1.8 ml plastic
3 microcentrifuge tubes and adjusted to a pH ~7 with the addition of HCl. Sample aliquots (100 µl)
4 of this solution were placed into individual wells of a Microdialyser System 100 (Pierce
5 Chemical Inc., Dallas, TX, USA) and dialyzed overnight (≥12h) against an ammonium
6 bicarbonate buffer (0.2M NH_4HCO_3, 0.1% SDS, 25mM EDTA, 0.1M NaCl) through a 500
7 MWCO dialysis membrane (Spectrum Chemical Mfg. Corp., New Brunswick, NJ, USA). After
8 dialysis, liquid samples were removed, mixed with 100 µl of 50% H_2O_2 in 20 ml glass
9 scintillation vials, and incubated overnight to clear samples of humic coloration. Subsequently,
10 10 ml of scintillation fluid (Ecolume) were added to each vial, mixed, and allowed to sit for >24
11 h before radioactivity was determined with a Beckman LS6500 scintillation counter. Bacterial
12 production was calculated using the conversion factors of 1.44 kg C produced mole^{-1} leucine
13 incorporated (Buesing & Marxsen, 2005).
14
15 Fungal biomass and instantaneous growth rates of fungi were determined by
16 quantifying [1-^{14}C]-acetate incorporation into ergosterol (Suberkropp & Gessner, 2005). Litter
17 sections (paired by tray) were placed into autoclaved glass scintillation vials with 3.95 ml of 0.2
18 µm filtered wetland water and 50 µl Na[1-^{14}C]-acetate (5 mM final concentration, specific
19 activity = 37 MBq mmol^{-1}). One vial from each tray was spiked with 20 µM DCMU (final
20 concentration), while the other vial remained unexposed to DCMU. Litter samples were
21 incubated for 5 h, as previous described. Incorporation of [^{14}C]-acetate label was stopped by
22 placing vials on ice and immediately filtering (1.2-µm pore size) the contents. Filters and litter
23 pieces were washed twice with filtered (0.7-µm pore size) wetland water, and stored at -20 °C
24 until extracted. Frozen samples were lyophilized, weighed, and ergosterol extracted in alcoholic
25 KOH (0.8% KOH in HPLC grade methanol, total extraction volume 10 ml) for 30 min at 80°C in
26 tightly-capped digestion tubes with constant stirring. The resultant crude litter extract was
27 cleaned by solid phase extraction (Gessner & Schmitt, 1996), then ergosterol was purified and
28 quantified by High Pressure Liquid Chromatography (HPLC) (see Su, Lohner, Kuehn,
29 Sinsabaugh, & Neely, 2007). Ergosterol fractions eluting from the HPLC column were collected
30 using an automated SF-3120 fraction collector system (Advantec MFS Inc., Dublin, CA, USA),
31 mixed with 10 ml of scintillation fluid (Ecolume), and radioactivity assayed by using a Beckman

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1 LS 6500 scintillation counter, corrected for quenching. For determination of fungal biomass,
2 litter ergosterol concentrations were converted to fungal carbon assuming a conversion factor of
3 10 µg ergosterol mg^{-1} fungal C, and 50% C in fungal dry mass (Suberkropp & Gessner, 2005).
4 Rates of acetate incorporation were converted to fungal growth rates (μ) using the conversion
5 factor of 12.6 µg fungal biomass $nmole^{-1}$ acetate incorporated (Suberkropp & Gessner, 2005).
6 Rates of fungal production were calculated by multiplying the daily growth rate (μ) by the litter-
7 associated fungal biomass (Suberkropp & Gessner, 2005).
8
9 *Enzyme activity*
10 Paired litter sections for each tray were placed into sterile glass scintillation vials with
11 660 µM solutions (3 ml, autoclaved wetland water as the solute) of the appropriate fluorogenic
12 substrate (4-methylumbelliferyl β-D-glucopyranoside, 4-methylumbelliferyl β-D-
13 xylopyranoside, L-leucine 7-amido-4-methylcoumarin hydrochloride, or 4-methylumbelliferyl
14 phosphate disodium salt) to measure the activity of βGase, βXase, LAMPase, and Pase,
15 respectively. One vial from each tray was spiked with 20 µM DCMU (final concentration), while
16 the other vial remained unexposed to DCMU. Litter sections destined for the DCMU-spiked
17 vials were incubated in a 20 µM DCMU solution for 10 min prior placement in vials. Litter-free
18 vials served as controls for non-enzymatically produced fluorescence. All vials were placed on
19 their sides and incubated for 30 min in a growth chamber (400 µmol m^{-2} s^{-1} PAR, 20 ºC). These
20 incubation conditions are saturating for Paint Creek wetland periphyton communities (Francoeur
21 et al., 2006). A 100 µl aliquot from each vial was immediately added to 100 µl of pH 10
22 carbonate/bicarbonate buffer (pHydrion) in individual wells of a black 96-well plate and
23 fluorescence measured using a Fluoroskan Ascent plate reader (ex: 355 nm, em: 460 nm,
24 ThermoFisher Scientific, Waltham, MA, USA). The addition of pH 10 buffer to the 96-well plate
25 prevented pH-mediated differences in the fluorescence yield of methlyumbelliferone (Chrost &
26 Krambeck, 1986) from affecting the fluorescence measurements, while allowing the incubation
27 vials to undergo photosynthesis-mediated shifts in pH. Methlyumbelliferone sodium salt and 7-
28 amino-4-methylcoumarin standards were included on all plates to allow for conversion of raw
29 fluorescence to concentrations.
30

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Statistical analyses

Vector analysis was used to summarize patterns in the relative activity of C-, N- and P-acquiring enzymes. We followed the approach of Moorhead, Sinsabaugh, Hill, & Weintraub (2016), with the exceptions that: C-acquiring enzymes were represented by the sum of ß-glucosidase and ß-xylosidase activities, and 2) N-acquiring enzymes were represented only by leucine-aminopeptidase. Thus, our calculated values for vector length and angle are not directly comparable to those of Moorhead et al. (2016), but the overall interpretation of differences in vector length (i.e., activity of C-acquiring enzymes vs. N- and P-acquiring enzymes) and vector angle (i.e., relative activity of P-acquiring vs. N-acquiring enzymes) remain the same.

Microbial activity data were expressed per g of litter C and analyzed using 2-way, split-plot ANOVA, with day as the whole-plot effect and photosynthesis (DCMU exposure) as the within-plot factor (n = 10). Within-date comparisons of +/- photosynthesis treatments were made using paired (by tray) t-tests (n = 10). When necessary, data were log (X+1) transformed to reduce heteroscedasticity prior to ANOVA. To avoid the elevated Type I error rate inherent in ANOVAs comprised of multiple F-tests, we applied a Bonferroni-Holm correction to the results of each ANOVA (Cramer et al., 2016). All calculations were performed using Systat 10.2 (Systat Software Inc., San Jose, CA, USA). Magnitude of +/-photosynthesis effects were expressed by the photosynthesis active : photosynthesis inhibited response ratio (i.e., -DCMU treatment ÷ +DCMU treatment). Pearson correlations of ln-transformed response ratios were calculated to investigate patterns in heterotrophic responses to manipulation of photosynthesis.

Experiment 2. Litter stoichiometry.

Litter generation.

Eight circular open-bottom mesocosms (~1.8 m diameter, 0.45 m wall height) were placed into a single pool in the Paint Creek wetland in early spring. Each mesocosm enclosed a known stand of *S. acutus* or *T. angustifolia*. These two species were used because they possess different lignin content and decay characteristics (e.g., Nelson, 2011), and were common at the study site. Four mesocosms received eight approximately bi-weekly additions of N and P (~3.5 gN/m^2 and ~3 g P/m^2, as controlled-release isobutylidenediurea fertilizer and K_2HPO_4) during

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1 the growing season (May-August), while the other mesocosms received no nutrient additions.
2 Sediment pore water nutrient concentrations in the mesocoms were monitored throughout the
3 growing to assess the efficacy of the fertilization treatment (see Supplemental information). At
4 the end of the growing season, standing dead *Schoenoplectus* and *Typha* were harvested from
5 each mesocom, air-dried, and stored at room temperature in the laboratory for subsequent use the
6 following summer.
7
8 *Microbial community colonization, laboratory experiment, and quantification*
9 Wire-mesh trays were filled with sections of either nutrient-enriched or ambient nutrient
10 *Schoenoplectus* or *Typha* litter, as previously described. All trays were placed into a single
11 wetland pool (July), where natural microbial colonization and growth was allowed to proceed.
12 After 11 and 39 or 12 and 41 days (*Schoenoplectus* or *Typha*, respectively) trays were retrieved
13 and microbial biomass and composition were assessed as described for Experiment 1. In the
14 laboratory, photosynthetic activity of litter-associated microbial communities was manipulated as
15 in Experiment 1. Microbial and enzymatic activity responses to experimental manipulations were
16 quantified as in Experiment 1, with the exception that bacterial biomass was not measured, due
17 to loss of samples. The remaining litter from each tray was dried, ground with a Wiley mill, and
18 analyzed for C and N and P content as described by Kuehn, Ohsowski, Francoeur, & Neely
19 (2011). Uncolonized (Day 0) *Schoenoplectus* litter was also analyzed for C and N and P content,
20 due to loss of uncolonized *Typha* litter samples from unfertilized mesoscoms, nutrient content of
21 *Typha* litter under ambient conditions was estimated from contemporaneous-collected samples of
22 standing dead *Typha* litter from adjacent transects within the Paint Creek wetland (Ohsowski,
23 2008).
24
25 *Statistical analysis*
26 Microbial activity data were analyzed using split-plot ANOVA with 2 whole-plot factors
27 (litter nutrient level and day) and one within-plot factor (photosynthesis) (Myers & Well, 2003).
28 The influence of sampling day and litter nutrient on microbial biomass was assessed using 2-way
29 ANOVA. Responses of microbial communities from *Schoenoplectus* and *Typha* litter were
30 analyzed separately. Bonferroni-Holm corrections were applied to the results of each ANOVA,

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and, when necessary, data were log (X+1) transformed to reduce heteroscedasticity prior to ANOVA. Differences of individual elemental ratios between enrichment types of a litter species on a specific date were assessed using two-tailed separate variance t-tests. The relationship of litter stoichiometry to extracellular enzyme activities were investigated by calculating the mean extracellular enzyme activity vector lengths and vector angles, and the litter stoichiometry for each unique combination of litter species, litter enrichment, and sampling date. The relationship of relevant enzymatic vector properties and elemental ratios (i.e., vector length and C:N, vector length and C:P, vector angle and N:P, see Moorhead et al., 2016) was then assessed using Pearson's correlation coefficient. Magnitude of +/-photosynthesis effects were expressed by the photosynthesis active : photosynthesis inhibited response ratio (i.e., -DCMU treatment ÷ +DCMU treatment). All calculations were performed using Systat 10.2.

Results

Experiment 1. Colonization sequence.

Study Site and Conditions

During the study period, this Paint Creek wetland pool was slightly alkaline (pH 7.82 ± 0.25, mean ± 1 SD, n = 6), and moderately hard (alkalinity 161 ± 3 mg $CaCO_3$/l, n = 6; specific conductance 301.0 ± 98.6 µS/cm, n = 7). Water column phosphorus was available at relatively consistent concentrations, mainly as SRP (TP 37.1 ± 0.01 µg/l, n = 7; SRP 24.0 ±0.01 µg/l, n = 7). Nitrate and ammonium were present in roughly equal, but highly variable, concentrations (NO_3+NO_2 = 35.4 ± 70.7 µg/l, n = 7; NH_4^+ 38 ± 27 µg/l, n = 7). DOC was also relatively abundant (22.4 ± 4.2 mg C/l). Water temperature ranged from 10.9 to 38.2 °C, with a mean of 24.1 ± 4.8 °C.

Algal Community Composition

Algal communities consisted of Chlorophyta, Cyanophyta, Eugenlophyta and diatom Heterokontophyta. Communities shifted from initial dominance by Chlorophyta (46%) and Cyanophyta (47%), to dominance by Cyanophyta (40%) and diatoms (47%). Euglenophytes were always rare (<1%, Table 1).

Microbial biomass

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1 As expected, algal biomass increased quickly, reaching a plateau after approximately 11
2 days. Bacterial biomass exhibited high variability, but also increased relatively quickly. Fungal
3 biomass increased more slowly through the first 40 days, then doubled between day 40 and day
4 54 (Fig. 1).
5
6 *Microbial activity*
7 Photosynthesis followed approximately the same temporal pattern as algal biomass, with
8 significant differences among sampling dates ($p < 0.001$) and much greater photosynthetic
9 activity occurring after 11 days. DCMU effectively inhibited photosynthesis (mean overall
10 response ratio = 74.7) throughout the study ($p < 0.001$). Although photosynthesis in the DCMU-
11 free treatment was significantly greater than that in the DCMU-exposed treatment at every
12 individual sampling date (p always < 0.025), the magnitude of this difference varied between
13 dates (Date × DCMU interaction, $p = 0.001$), with mean date-specific response ratios ranging
14 from 20-231.8 (Fig. 2).
15
16 Bacterial ($p < 0.001$) and fungal ($p < 0.001$) production also differed significantly among
17 sampling dates, displaying increasing trends over time. When integrated over the course of the
18 study, both bacterial ($p = 0.025$) and fungal ($p < 0.001$) production were significantly greater
19 when photosynthesis was active. Photosynthesis stimulated fungal production (overall mean
20 response ratio = 2.2) to a much larger extent than bacterial production (overall mean response
21 ratio = 1.2). The strength of photosynthetic stimulation (Date × Photosynthesis interaction)
22 varied significantly among sample dates for both bacteria ($p = 0.022$) and fungi ($p = 0.004$).
23 Bacterial production in the photosynthesis treatment was significantly greater than that in the
24 DCMU-exposed treatment on only the 2 initial sampling dates, but fungal production was
25 significantly stimulated by photosynthesis at 5 of 6 individual sampling dates. Mean within-date
26 response ratios ranged from 0.9-1.5 for bacterial production and from 1.4-3.1 for fungal
27 production (Fig. 2).
28
29 *Enzyme activity*
30 βGase ($p < 0.001$) and βXase ($p < 0.001$) activities differed significantly among sampling
31 dates, with generally increasing trends through time, but neither LAMPase ($p = 0.146$) nor Pase

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1 (p = 0.106) activities differed significantly among sampling dates. Neither βGase (p =0.568) nor
2 βXase (p =0.787) was consistently greater when photosynthesis was active, but, Pase (p < 0.001;
3 mean overall response ratio = 1.3) was significantly greater when photosynthesis was active.
4 LAMPase showed a nonsignificant trend towards being stimulated by photosynthesis (mean
5 overall response ratio = 1.2). The strength of photosynthetic stimulation of Pase (p = 0.414) did
6 not vary significantly among sample dates. (Fig. 3). Vector analysis of extracellular enzymes
7 combined the patterns observed in analysis of individual enzymes. Vector length (i.e., activity of
8 C-acquiring enzymes vs. N- and P-acquiring enzymes) differed significantly among sampling
9 dates (p < 0.001) and was significantly reduced by photosynthesis (p < 0.001). The strength of
10 the vector length response to photosynthesis also varied among sampling dates (p = 0.019).
11 There was no significant effect of sampling day, photosynthesis, or their interaction on vector
12 angle (i.e., relative activity of P-acquiring vs. N-acquiring enzymes) (p always > 0.05) (Fig. 3).
13 There were no significant bivariate correlations between the magnitudes of heterotrophic
14 microbial activity responses to photosynthesis (i.e., ln response ratios of bacterial and fungal
15 production; βGase, βXase, LAMPase, and Pase activities; p always > 0.05).
16
17 **Experiment 2. Litter stoichiometry.**
18 *Study Site and Conditions*
19 The Paint Creek wetland pool used in this experiment was also slightly alkaline (pH 8.09
20 ± 0.10, mean ± 1 SD, n = 4), and moderately hard (alkalinity 150 ± 12 mg $CaCO_3$/l, n = 4;
21 specific conductance 277.7 ± 57 µS/cm, n = 5). Water column phosphorus was available at
22 slightly greater and much more variable concentrations (TP 79.4 ± 42 µg/l, n = 5; SRP 26.8 ± 11
23 µg/l, n = 5). Nitrate and ammonium were present at high and highly variable, concentrations
24 (NO_3+NO_2 = 1832 ± 491 µg/l, n = 5; NH_4^+ 107 ± 81 µg/l, n = 5). DOC was also relatively
25 abundant and highly variable (35.6 ± 42 mg C/l).
26
27 Litter from plants grown under nutrient-enriched conditions had significantly lower C:P
28 (*Schoenoplectus*, 50% reduction, p = 0.020; *Typha*, 73% reduction, p = 0.007) and N:P
29 (*Schoenoplectus*, 53% reduction, p < 0.001; *Typha*, 69% reduction, p < 0.001) content than litter
30 from plants grown under ambient nutrient exposure (Table 2). Litter stoichiometry changed over

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1 time as litter-associated microbial communities developed, with enriched and unenriched litter
2 generally becoming more similar, especially for *Typha* (Table 2).
3
4 *Algal Community Composition*
5 Algal communities were broadly similar to those observed in Experiment 1, and were
6 dominated by Chlorophyta, Cyanophyta, and diatom Heterokontophyta. Euglenophytes and
7 Dinophytes were always rare (<1%, Table 1).
8
9 *Microbial biomass*
10 Algal biomass significantly increased over time on both *Schoenoplectus* ($p = 0.010$) and
11 *Typha* ($p < 0.001$) litter. Algal biomass was significantly increased by elevated nutrient content
12 in *Schoenoplectus* ($p = 0.003$), but not *Typha* ($p = 0.303$) litter (Fig. 4). Fungal biomass
13 associated with *Schoenoplectus* litter did not differ significantly between sampling dates (p =
14 0.816), but was significantly lower on nutrient-enriched litter ($p < 0.001$). In contrast, fungal
15 biomass on *Typha* litter increased through time ($p < 0.001$), but was not affected by litter nutrient
16 content ($p = 0.756$) (Fig. 4).
17
18 *Effect of litter stoichiometry and photosynthesis on microbial and enzyme activities*
19 DCMU effectively (~ 97% reduction) halted photosynthesis on both *Schoenoplectus* (p <
20 0.001) and *Typha* litter ($p < 0.001$). Photosynthetic activity on *Schoenoplectus* ($p = 0.055$) and
21 *Typha* litter ($p = 0.270$) did not vary with respect to litter nutrient level, and litter nutrient level
22 did not alter the effect of DCMU exposure in either *Schoenoplectus* ($p = 0.639$) or *Typha* litter (p
23 = 0.143) (Fig 5).
24
25 Litter nutrient level had no effect on fungal production on either *Schoenoplectus* (p =
26 0.169) or *Typha* litter ($p = 0.137$). Fungal production was significantly stimulated by
27 photosynthesis (*Schoenoplectus* litter, $p = 0.002$, RR = 3.6; *Typha* litter, $p = 0.001$, RR = 1.8).
28 Litter nutrient level did not alter the strength of photosynthetic stimulation in either
29 *Schoenoplectus* ($p = 0.836$) or *Typha* litter ($p = 0.073$) (Fig. 5).
30

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In contrast to fungal production, the effect of experimental manipulations on fungal growth rates varied substantially between litter types. Litter nutrient level did not affect fungal growth rates on *Typha* litter ($p = 0.181$), but significantly increased fungal growth rates on *Schoenoplectus* ($p < 0.001$, RR = 3.4). Fungal growth rates on *Schoenoplectus* litter declined over time ($p < 0.001$). Photosynthesis did not affect fungal growth rates on *Typha* litter ($p > 0.070$), but significantly stimulated fungal growth rates on *Schoenoplectus* litter ($p < 0.001$, RR = 3.3), and the strength of this photosynthetic stimulation also declined over time (Day x Photosynthesis interaction, $p = 0.006$) (Fig. 5).

Nutrient-enrichment of litter significantly increased bacterial production on only *Schoenoplectus* litter ($p < 0.001$, RR = 1.4), but photosynthesis significantly increased bacterial production on only *Typha* litter ($p < 0.001$, RR = 1.2). The strength of photosynthetic stimulation was not affected by litter nutrient content (Nutrient x Photosynthesis interaction, *Typha* litter $p = 0.677$; *Schoenoplectus* litter $p = 0.548$) (Fig. 5).

Litter nutrient enrichment sometimes increased enzyme activity (*Schoenoplectus*: βGase RR = 2.2 $p < 0.001$, LAMPase RR = 1.9 $p < 0.001$; *Typha*: βGase RR = 1.6 $p = 0.005$) (Fig. 6), and activities of some enzymes increased over time (*Schoenoplectus* LAMPase $p < 0.001$; *Typha* βGase $p = 0.004$). Photosynthesis did not significantly affect the activity of any enzyme (p always > 0.05), and no significant interactions of enzymatic responses to nutrients and photosynthesis were observed. Vector analysis of enzyme activities on either *Schoenoplectus* or *Typha* litter found no statistically significant response to any experimental factor.

When both litter species and both sampling dates were considered together, the mean vector angle was correlated to litter N:P ($r = 0.850$, $p < 0.010$) (Fig. 7), but mean vector length was not strongly related to litter C:N ($r = -0.218$, $p > 0.5$) or C:P ($r = -0.093$, $p > 0.5$), respectively. However, relationships of mean vector length to litter C:N (Day 11/13 $r = -0.996$, $p < 0.01$; Day 39/41 $r = -0.948$, $p < 0.1$) and C:P (Day 11/13 $r = -0.842$, $p < 0.2$; Day 39/41 $r = -0.707$, $p > 0.2$) were generally stronger on individual sampling dates.

Discussion

Photosynthesis consistently stimulated fungal and bacterial production. In both experiments in our study, fungal and bacterial production were usually significantly greater when

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photosynthesis was active. The strength of stimulation frequently varied among sampling dates, but there were no consistent temporal patterns with respect to the degree of photosynthetic stimulation. Furthermore, fungal production was always more strongly stimulated (typically doubled) by photosynthesis than was bacterial production (~20% increase). Our data substantiate the preliminary findings of Kuehn et al. (2014), which showed photosynthesis to cause both an approximate doubling of fungal production and a smaller increase of bacterial production in microbial communities attached to submerged *Typha* litter. Similar quantification of litter-associated fungal and bacterial production across a temperature gradient has also found much stronger photosynthetic stimulation of fungi than bacteria (Pope et al., in review, this issue). Taken together, these results suggest that: 1) algal photosynthetic stimulation of fungal and bacterial production on submerged plant litter is commonplace, 2) although strength of this stimulation varies through time, it can occur over the entire sequence of microbial colonization and growth, and 3) rates of fungal production are generally very strongly stimulated by algal photosynthesis.

Consistent strong stimulation of fungal production suggests that photosynthesis likely affects litter decomposition, but does not necessarily indicate the direction of the effect. Active algal photosynthesis may increase rates of litter decomposition via positive priming effects (Lagrue et al., 2011; Danger et al., 2013). In contrast, photosynthesis can also reduce litter decomposition rates via negative priming effects (Halvorson et al., 2019a), possibly as a result of fungal decomposers shifting C acquisition from litter to photosynthetically-derived labile organic C. It is possible that identity of the nutrient in shortest supply might regulate the direction of the priming effect. For example, a recent meta-analysis found that increased DIN:SRP ratios were associated with a switch from negative to positive priming of litter decomposition (Halvorson, Francoeur, Findlay, & Kuehn, 2019b).

Regardless of the potential impact on decomposition, strong photosynthetic stimulation of fungal production may have other potential implications for aquatic ecosystems, including driving diversity in fungal communities (as fungal taxa potentially specialize on either algal-derived or litter C) and inducing diurnal patterns in respiration. If strong photosynthetic stimulation of fungal production is commonplace, then the timing (day vs. night) and incubation

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1 conditions (ambient light vs. darkness) for fungal production assays may become an important
2 methodological concern.
3
4 In contrast to the consistent photosynthetic stimulation observed for fungal and bacterial
5 production, enzymatic responses to photosynthesis in this study were inconsistent. In Experiment
6 1, Pase (but not βGase, βXase, or LAMPase) was significantly stimulated (~30%) by
7 photosynthesis. Such a pattern is consistent with the hypothesis that algal-derived C rapidly
8 increased heterotrophic demand for P when photosynthesis was active, but other explanations for
9 this pattern are also possible (see below). However, in Experiment 2, no extracellular enzyme
10 activities were stimulated by short-term photosynthesis manipulations. Previous experiments
11 have also found no (e.g., βGase or βXase, Pope et al., in review, this issue) or inconsistent
12 enzymatic responses to short-term (minutes to hours) photosynthesis manipulation. For example,
13 βGase (but not βXase or LAMPase) activity on submerged *Typha* litter was stimulated by
14 photosynthesis, and strong temporal differences in the strength of photosynthetic stimulation of
15 Pase were observed in a previous experiment at this site (Francoeur et al., 2006). Similarly,
16 studies on lotic (Rier et al., 2007) and lentic (Francoeur & Wetzel, 2003) periphyton
17 communities have observed diurnal (presumably photosynthetically-mediated) changes in
18 activities of some (LAMPase and Pase) but not all (βGase, phenol oxidase) extracellular
19 enzymes. In addition to differences in the strength of photosynthetic stimulation among enzymes,
20 the influence of photosynthetic stimulation on activity of a single enzyme type can vary strongly
21 over time. Temporal differences in stimulation of Pase by photosynthesis were noted by
22 Francoeur et al. (2006), and the response of wetland periphytic LAMPase activity to
23 photosynthesis ranged from none to strong (~ 40%) stimulation, and changed from month to
24 month (Francoeur & Wetzel, 2003).
25
26 The lack of correlation amongst the magnitudes of heterotrophic microbial activity and
27 enzymatic responses to photosynthesis during Experiment 1 suggests that these responses were at
28 least partially regulated by different processes. It is well-established that bulk activities of
29 microbial extracellular enzymes correspond to differential resource (C, N, P) scarcity
30 (Sinsabaugh, VanHorn, Follstad-Shah, & Findlay, 2010; Sinsabaugh & Follstad-Shah, 2012;
31 Sinsabaugh et al., 2014), thus photosynthetically-mediated changes in the relative scarcity of

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1 resources (e.g., photosynthetic provision of labile C; e.g., Wyatt, Stevenson, & Turetsky, 2010;
2 Wyatt, Tellez, Woodke, Bidner, & Davison, 2014) could result in differential up- or down-
3 regulation of enzymatic synthesis. Furthermore, relative scarcity of N or P might interact with C
4 supply to influence microbial capacity to produce extracellular enzymes (see Halvorson et al.,
5 2019b). Individual optima of extracellular enzymes with respect to environmental conditions
6 (e.g., pH, Espeland & Wetzel, 2001; Francoeur & Wetzel, 2003), coupled to photosynthetically-
7 mediated shifts in these environmental conditions (e.g., Jones, Eaton, & Hardwick, 2000;
8 Espeland & Wetzel, 2001) could add another layer of complexity to enzymatic responses to
9 photosynthesis. Similarly, periphytic fungal and bacterial production may be partially modulated
10 by multiple factors, such as differential requirements for nutrients (Gulis & Suberkropp, 2003a),
11 differential resource use efficiency (Romaní, Fischer, Mille-Lindblom, & Tranvik, 2006; Wyatt
12 et al., 2019), and both antagonistic (Mille-Lindbom & Tranvik, 2003; Gulis & Suberkropp,
13 2003c) and synergistic (Romaní, et al., 2006) biotic interactions. Mechanistic experiments
14 manipulating factors likely to be altered by photosynthesis (e.g., labile organic C, pH, and
15 dissolved O_2) will be required to better understand the importance of the various proximate
16 driving factors for heterotrophic and enzymatic responses to photosynthesis.
17
18 In our study, all production and enzyme assays were begun concurrently with the
19 initiation of photosynthesis in the photosynthesis-active treatment, in order to mimic a simple
20 night-day transition. However, the need to achieve sufficient radiolabeling of fungal tissue
21 required longer incubation periods (5 h) for fungal production than bacterial production or
22 enzyme assays (30 min). It is possible that shorter incubations contributed to relatively small and
23 inconsistent photosynthetic stimulation of bacterial production and enzyme activities in our
24 experiments. However, Espeland, Francoeur, & Wetzel (2001) found that pre-incubation times <
25 8 h did not affect the strength of photosynthetic stimulation of bacterial production or enzyme
26 activities in wetland periphyton. Nevertheless, additional experiments initiating bacterial
27 production and enzyme assays after 4.5 h of pre-incubation photosynthesis manipulation would
28 be required to fully address this possibility. In any case, substantial and consistent photosynthetic
29 stimulation of fungal production remains a robust finding.
30

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1 Litter nutrient content did not affect the strength of photosynthetic stimulation of
2 bacterial production, fungal production, or enzyme activities. The relatively small changes in
3 litter stoichiometry (C:N range 50-64, C:P range 1009-5,219) resulting from our plant
4 fertilization protocol might have contributed to this lack of response. In comparison, Manning et
5 al. (2016) found strong relationships between litter stoichiometry and decomposition rate, but
6 their study employed several different litter species, encompassing much wider ranges of C:N
7 (ca. 60-180) and C:P (ca. 200-10,000). It is also possible that litter nutrients supplied a relatively
8 small proportion of heterotrophic microbial nutrient demand in our experiment, given the
9 abundant dissolved nutrients at the study site. However, several longer-term biotic responses to
10 altered litter stoichiometry were observed (see below), suggesting that litter nutrient content was
11 biologically relevant. Another possible explanation for the general lack of relationship is that the
12 interaction between litter stoichiometry and photosynthetic stimulation was too subtle to be
13 reliably detected at the conclusion of a short photosynthesis manipulation assay. This seems
14 especially likely for enzymatic responses, in which enzymes produced prior to the imposition of
15 experimental conditions could be contributing a substantial amount of activity measured during
16 the assay.
17
18 Manipulation of litter stoichiometry had more of an effect on overall microbial biomass,
19 activity, and extracellular enzyme activity. The effects of nutrient content often differed between
20 the two litter species. Microbial biomass was unaffected by nutrient content of *Typha* litter,
21 whereas nutrient-enhanced *Schoenoplectus* litter supported greater algal, but less fungal,
22 biomass. On both litter types, algal and fungal production per gram of litter C were unaffected by
23 nutrient enhancement. However, fungal growth rates on *Schoenoplectus* litter were greatly
24 stimulated by nutrient enhancement, which partially offset the lower fungal biomass and
25 prevented a nutrient-mediated reduction in fungal production on *Schoenoplectus* litter. Bacterial
26 production showed opposite responses to nutrient enhancement of *Typha* (decrease) and
27 *Schoenoplectus* (increase) litter, suggesting a litter-specific difference in bacterial response to
28 stoichiometric changes. Some of these litter-specific responses might be linked to intrinsic
29 differences between *Typha* and *Schoenoplectus* litter such as the lower lignin content of
30 *Schoenoplectus* litter (Nelson, 2011), or could possibly be the result of more complex
31 interactions within the microbial community (e.g., greater algal biomass and algal-derived C on

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1 nutrient-enriched *Schoenoplectus* litter possibly favoring a fungal life history strategy of rapid
2 spore production instead of hyphal biomass accrual). Exploration of the factors contributing to
3 such litter-specific responses could be of considerable interest.
4
5 Alteration of litter stoichiometry affected extracellular enzyme activity in ways broadly
6 congruent with predictions based upon litter stoichiometry and the resulting relative scarcity of
7 C, N and P within litter. Nutrient-enhanced *Schoenoplectus* litter displayed elevated βGase and
8 LAMPase activity, consistent with expectations of how the activities of these enzymes would
9 change under conditions in which the relative abundance of P was increased. Similarly, nutrient-
10 enhanced *Typha* litter displayed elevated βGase activity, which could be interpreted as an
11 enzymatic response to a relative scarcity of C compared to N and P. Across both litter species
12 and sampling dates, enzyme vector angles were strongly related to litter N:P, suggesting that
13 elevated litter N:P led to increased activity of P-acquiring enzymes, at the expense of N-
14 acquiring enzyme activity. Although sample sizes were small, the relationship of vector length to
15 litter C:N and C:P at individual sampling dates is consistent with the expectation that increased
16 litter N and P content would result in reduced activity of N- and P-acquiring enzymes, relative to
17 C-acquiring enzymes.
18
19 In conclusion, photosynthesis consistently stimulated bacterial and fungal production on
20 submerged plant litter; fungal production was especially strongly stimulated (typically doubled)
21 by photosynthesis. Photosynthetic stimulation of extracellular enzyme activity was weaker and
22 less consistent among enzymes and across experiments. Strong photosynthetic stimulation of
23 heterotrophic production coupled with weak effects on extracellular enzyme activity suggests
24 that photosynthetic stimulation may not always lead to increased litter decomposition. A
25 comprehensive understanding of how photosynthetic stimulation of heterotrophs affects
26 ecosystem function is needed, in order to expand scientific knowledge and provide a basis for
27 ecosystem management. Experimental manipulation of litter stoichiometry via plant fertilization
28 had less effect on the strength of photosynthetic stimulation, but frequently altered microbial
29 biomass, production and extracellular enzyme activity. Additional research encompassing a
30 wider range of litter and environmental nutrient availability is needed to fully understand these
31 potential relationships.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author (Steven Francoeur; steve.francoeur@emich.edu) upon reasonable request.

Authorship Statement

SNF co-designed the study, collected and analyzed data, and led manuscript drafting. RKN co-designed the study and edited manuscript drafts. SU collected data and assisted with drafting the manuscript. KAK co-designed the study, collected and analyzed data, and edited manuscript drafts.

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10 Figure legends
11
12 Figure 1. Mean algal (a), fungal (b), and bacterial (c) biomass per gram litter C in experiment 1.
13 Bars are ± 1 SE.
14
15 Figure 2. Mean algal (a), fungal (b), and bacterial (c) production under photosynthesis-active
16 (open bars) and photosynthesis-inhibited (black bars) conditions in experiment 1. Statistically-
17 significant experimental factors are indicated within each panel. * indicates a significant
18 difference between photosynthesis-active and photosynthesis-inhibited conditions on a particular
19 sampling date. Bars are ± 1 SE.
20
21 Figure 3. Mean enzyme activity (V_{max}, nmol gC^{-1} h^{-1}) for βGase (a), βXase (b), LAMPase (c),
22 and Pase (d) under photosynthesis-active (open bars or symbols) and photosynthesis-inhibited
23 (black bars or symbols) conditions in experiment 1, and vector representation plot of all
24 experiment 1 enzyme data (e). (Day 5 = circles, Day 12 = point-up triangles,
25 Day 19 = point-down triangles, Day 26 = squares, Day 40 = stars, and Day 54 = diamonds).
26 Statistically-significant experimental factors are indicated within each panel. * indicates a
27 significant difference between photosynthesis-active and photosynthesis-inhibited enzyme
28 activity on a particular sampling date. Bars are ± 1 SE.
29

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1 Figure 4. Mean algal (a, b) and fungal (c, d) biomass on nutrient-enriched (open bars) and
2 unenriched (cross-hatched bars) *Schoenoplectus* and *Typha* litter during experiment 2.
3 Statistically-significant experimental factors are indicated within each panel. Bars are ± 1 SE.
4
5 Figure 5. Mean algal (a, b), bacterial (c, d), and fungal (e, f) production, and fungal growth rates
6 (g, h) on photosynthesis-active (open or cross-hatched bars) and photosynthesis-inhibited (black
7 or grey bars), nutrient-enriched (open or black bars) and unenriched (cross-hatched or grey bars),
8 *Schoenoplectus* and *Typha* litter during experiment 2. Statistically-significant experimental
9 factors are indicated within each panel. Bars are ± 1 SE.
10
11 Figure 6. Mean βGase (a, b), βXase (c, d), LAMPase (e, f), and Pase (g, h) activities (V_{max}) on
12 photosynthesis-active (open or cross-hatched bars) and photosynthesis-inhibited (black or grey
13 bars), nutrient-enriched (open or black bars) and unenriched (cross-hatched or grey bars),
14 *Schoenoplectus* and *Typha* litter during experiment 2. Statistically-significant experimental
15 factors are indicated within each panel. Bars are ± 1 SE.
16
17 Figure 7. Relationships of enzymatic vector analysis mean length to litter C:N (a) and
18 C:P (b), and of vector analysis mean angle to litter N:P (c). Data are mean values for each
19 individual combination of litter species, litter nutrient treatment, and sampling date. Squares
20 = *Schoenoplectus* litter, circles = *Typha* litter, open symbols = Day 11/13, black symbols =
21 Day 39/41. Bars indicate ± 1 SD of data used to calculate means for correlation analysis.
22
23
24
25 Supporting Information
26
27 Additional supporting information can be found online in the Supporting Information section at
28 the end of the article.
29 **Appendix S1.**
30 Methods for measuring sediment pore water nutrient content in mesocosms.
31 **Appendix S2.**

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1 Table S1. Sediment pore water nutrient content (mean ± 1 SD).

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Table 1. Division-level algal community composition (mean relative abundance as % cells ± 1 SD) on experimental substrata over the course of the study.

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Experiment 1

	Relative abundance					
Division	12 Jul. (Day 5)	19 Jul. (Day 12)	26 Jul. (Day 19)	2 Aug. (Day 26)	16 Aug. (Day 40)	30 Aug. (Day 54)
Chlorophyta	46 ± 5	37 ± 7	48 ± 7	29 ± 5	20 ± 4	13 ± 2
Cyanophyta	47 ± 5	50 ± 8	38 ± 6	40 ± 9	45 ± 6	40 ± 9
Euglenophyta	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1
Heterokontophyta	7 ± 3	13 ± 2	14 ± 4	30 ± 7	34 ± 7	47 ± 9

Experiment 2

	Relative abundance							
	Schoenoplectus litter				*Typha* litter			
	9 Aug. (Day 11)		6 Sept. (Day 39)		11 Aug. (Day 13)		8 Sept. (Day 41)	
Division	Unenriched	Enriched	Unenriched	Enriched	Unenriched	Enriched	Unenriched	Enriched
Chlorophyta	56 ± 19	55 ± 16	17 ± 10	15 ± 11	63 ± 16	70 ± 8	34 ± 25	20 ± 10
Cyanophyta	32 ± 18	15 ± 16	60 ± 27	70 ± 22	22 ± 16	14 ± 8	42 ± 29	19 ± 25
Dinophyta	0	0	<1 ± <1	0	0	0	0	0
Euglenophyta	<1 ± 1	5 ± 4	<1 ± <1	2 ± 1	3 ± 2	3 ± <1	< 1 ± 1	< 1 ± 1

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Heterokontophyta	11 ± 6	25 ± 11	22 ± 22	13 ± 12	12 ± 8	13 ± 5	23 ± 30	61 ± 23

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Table 2. Litter stoichiometry (molar elemental ratios, mean ± 1 SD) and elemental content in experiment 2. Day 0 values are for litter uncolonized by microbes (n = 6 Unenriched *Typha*, 8 for all others), Day 11 (*Schoenoplectus*) / 13 (*Typha*) (n = 9/8) and Day 39 (*Schoenoplectus*) / 41 (*Typha*) (n = 9/8) values represent the stoichiometry of the litter-periphyton complex at specific sampling dates. Superscript symbols denote significant differences of an individual elemental ratio between enrichment types of a litter species on a specific date: * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$

	Day 0			Day 11/13			Day 39/41		
Litter Type	C:N	C:P*	N:P‡	C:N‡	C:P‡	N:P‡	C:N†	C:P†	N:P†
Unenriched *Schoenoplectus*	52 ± 6	2004 ± 250	38 ± 1	47 ± 4	3954 ± 795	85 ± 18	30 ± 4	1885 ± 454	62 ± 9
Enriched *Schoenoplectus*	50 ± 17	1009 ± 935	18 ± 9	34 ± 3	1757 ± 149	52 ± 3	23 ± 4	1161 ± 246	49 ± 4

Litter Type	C:N	C:P†	N:P‡	C:N†	C:P*	N:P	C:N	C:P	N:P
Unenriched *Typha*	64 ± 15	5219 ± 2160	81 ± 20	69 ± 10	4133 ± 1086	59 ± 7	38 ± 9	1923 ± 637	50 ± 9
Enriched *Typha*	56 ± 6	1392 ± 279	25 ± 4	56 ± 8	3193 ± 806	57 ± 8	30 ± 5	1337 ± 323	45 ± 4

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	Day 0			Day 11/13			Day 39/41		
Litter Type	%C	%N	%P	%C	%N	%P	%C	%N	%P
Unenriched *Schoenoplectus*	46.6 ± 0.7	1.1 ± 0.1	0.06 ± 0.01	40.4 ± 1.5	1.0 ± 0.1	0.03 ± 0.00	35.0 ± 3.2	1.4 ± 0.2	0.05 ± 0.01
Enriched *Schoenoplectus*	46.5 ± 0.7	1.2 ± 0.3	0.19 ± 0.09	41.5 ± 1.3	1.4 ± 0.1	0.06 ± 0.01	30.8 ± 2.2	1.6 ± 0.2	0.07 ± 0.01

Litter Type	%C	%N	%P	%C	%N	%P	%C	%N	%P
Unenriched *Typha*	47.1 ± 0.5	0.9 ± 0.2	0.03 ± 0.01	38.3 ± 2.2	0.7 ± 0.1	0.02 ± 0.00	33.0 ± 3.3	1.1 ± 0.2	0.05 ± 0.02
Enriched *Typha*	49.2 ± 0.5	1.0 ± 0.1	0.10 ± 0.02	39.4 ± 1.8	0.8 ± 0.1	0.03 ± 0.01	30.9 ± 2.3	1.2 ± 0.2	0.06 ± 0.01

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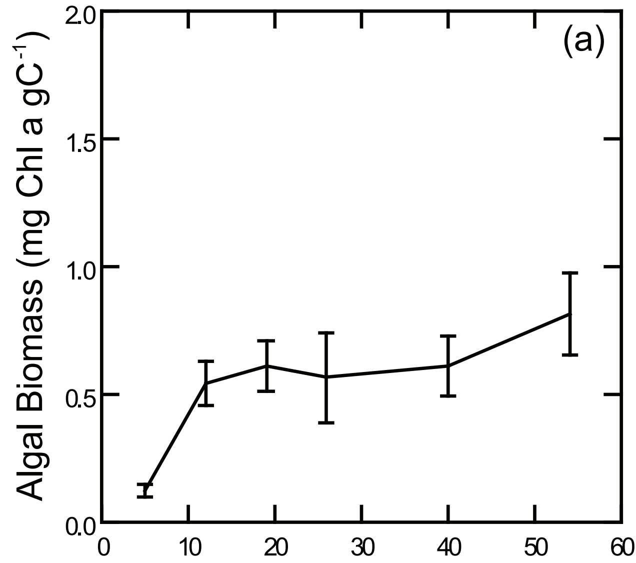

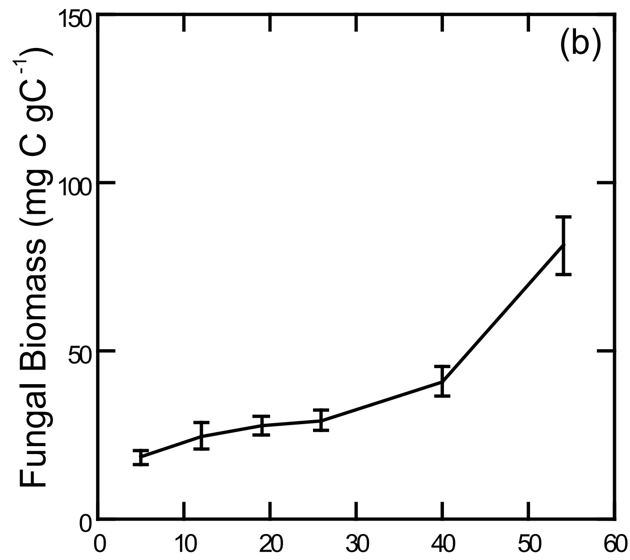

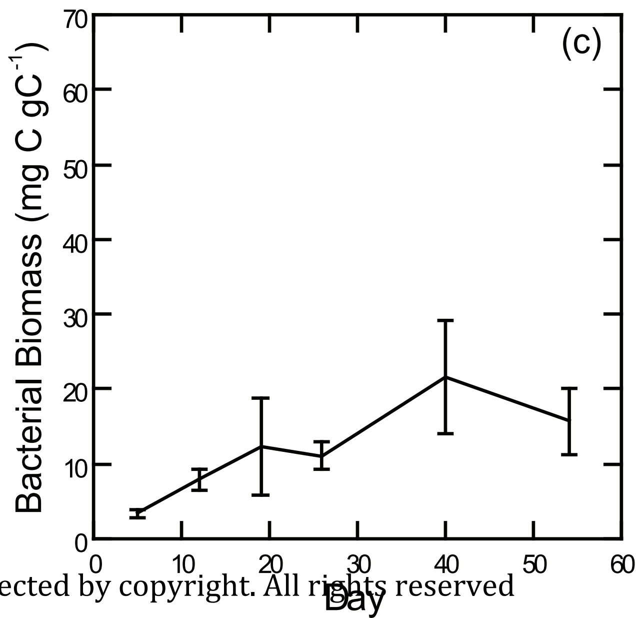

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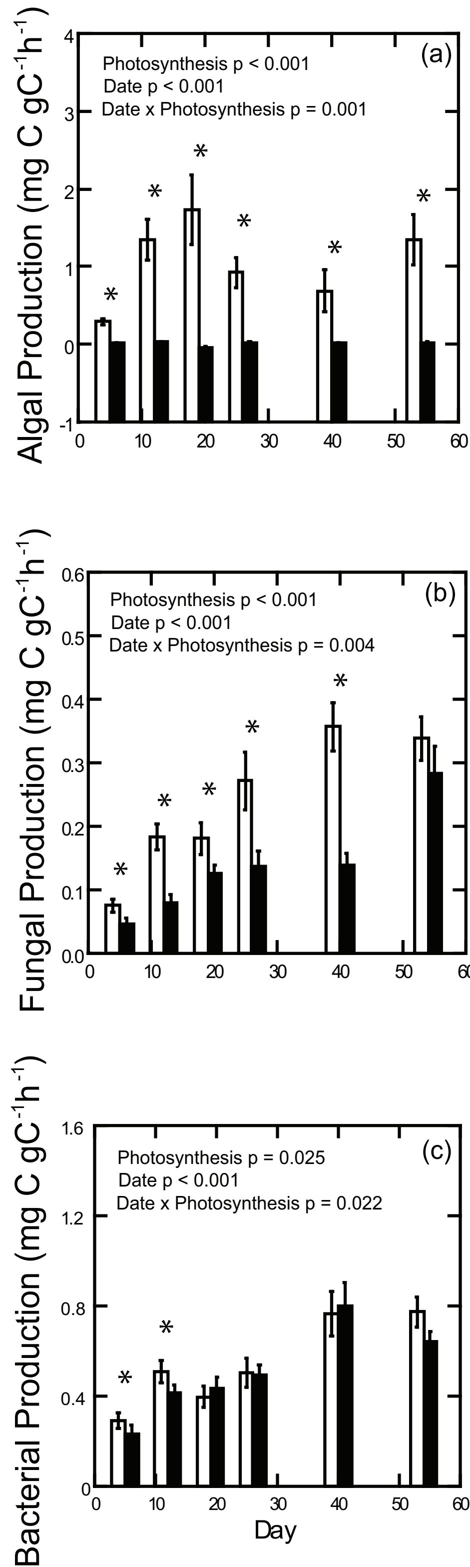

Figure 2. Mean algal (a), fungal (b), and bacterial (c) production under photosynthesis-active (open bars) and photosynthesis-inhibited (black bars) conditions in experiment 1. Statistically-significant experimental factors are indicated within each panel. * indicates a significant difference between photosynthesis-active and photosynthesis-inhibited conditions on a particular sampling date. Bars are +/- 1 SE.

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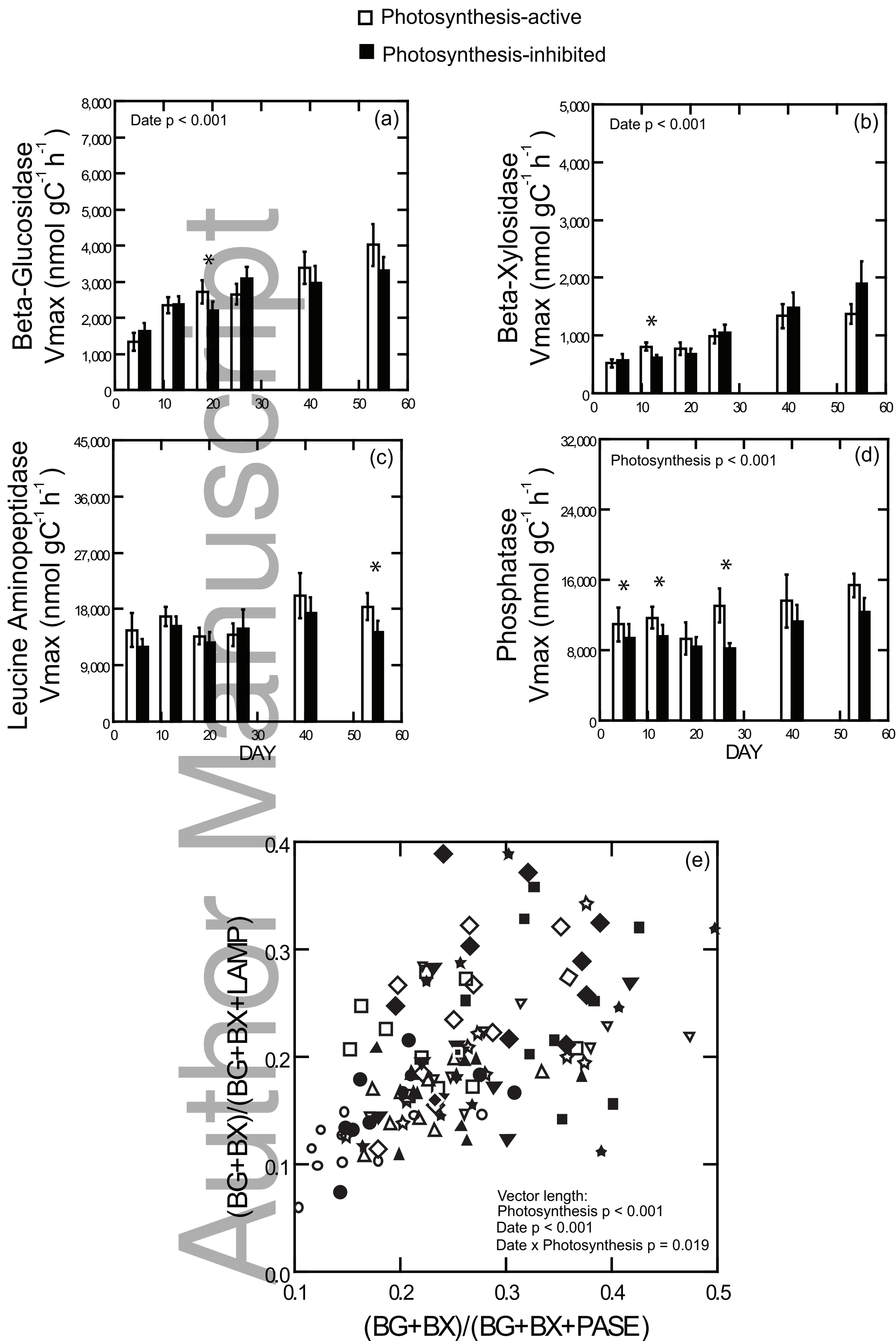

Figure 3. Enzyme activity (Vmax, nmol gC-1 h-1) for Beta-Glucosidase (a), Beta-Xylosidase (b), Leucine Aminopeptidase (c), and Phosphatase (d) under photosynthesis-active (open bars or symbols) and photosynthesis-inhibited (black bars or symbols) conditions in experiment 1, and a vector representation plot of all experiment 1 enzyme data (e) (Day 5 = circles, Day 12 = point-up triangles, Day 19 = point-down triangles, Day 26 = squares, Day 40 = stars, and Day 54 = diamonds). Statistically-significant experimental factors are indicated within each panel. * indicates a significant

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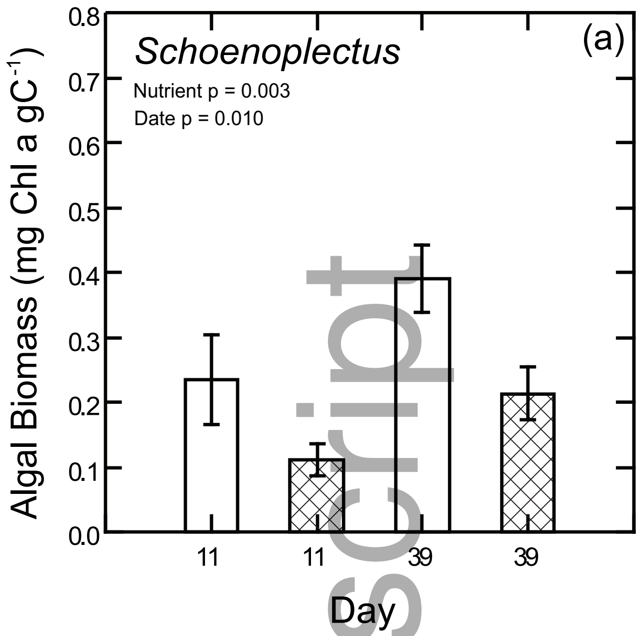

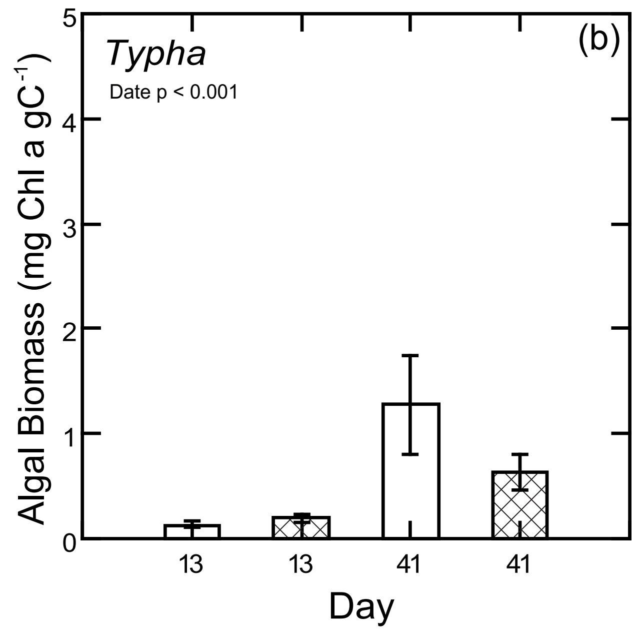

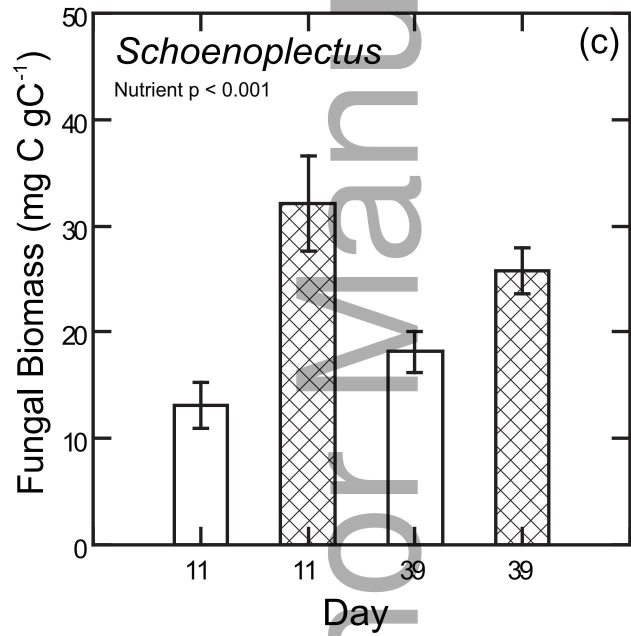

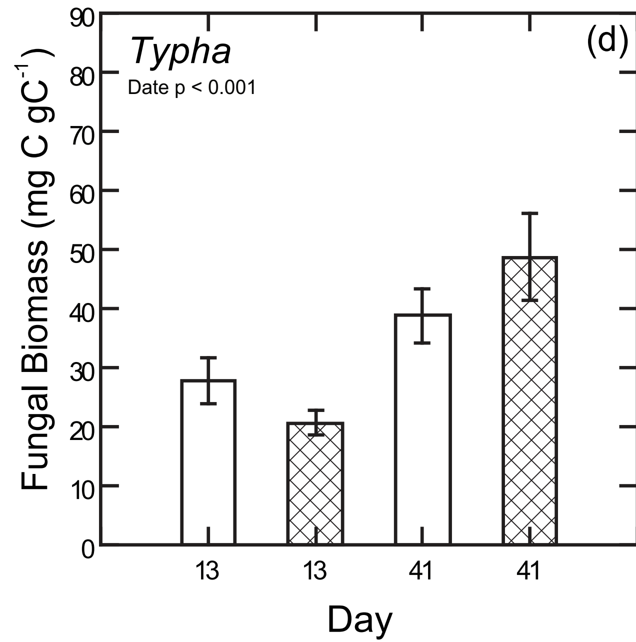

Figure 4. Algal (a, b) and fungal (c, d) biomass on nutrient-enriched (open bars) and unenriched (cross-hatched bars) *Schoenoplectus* and *Typha* litter during experiment 2. Statistically-significant experimental factors are indicated within each panel. Bars are +/- 1 SE.

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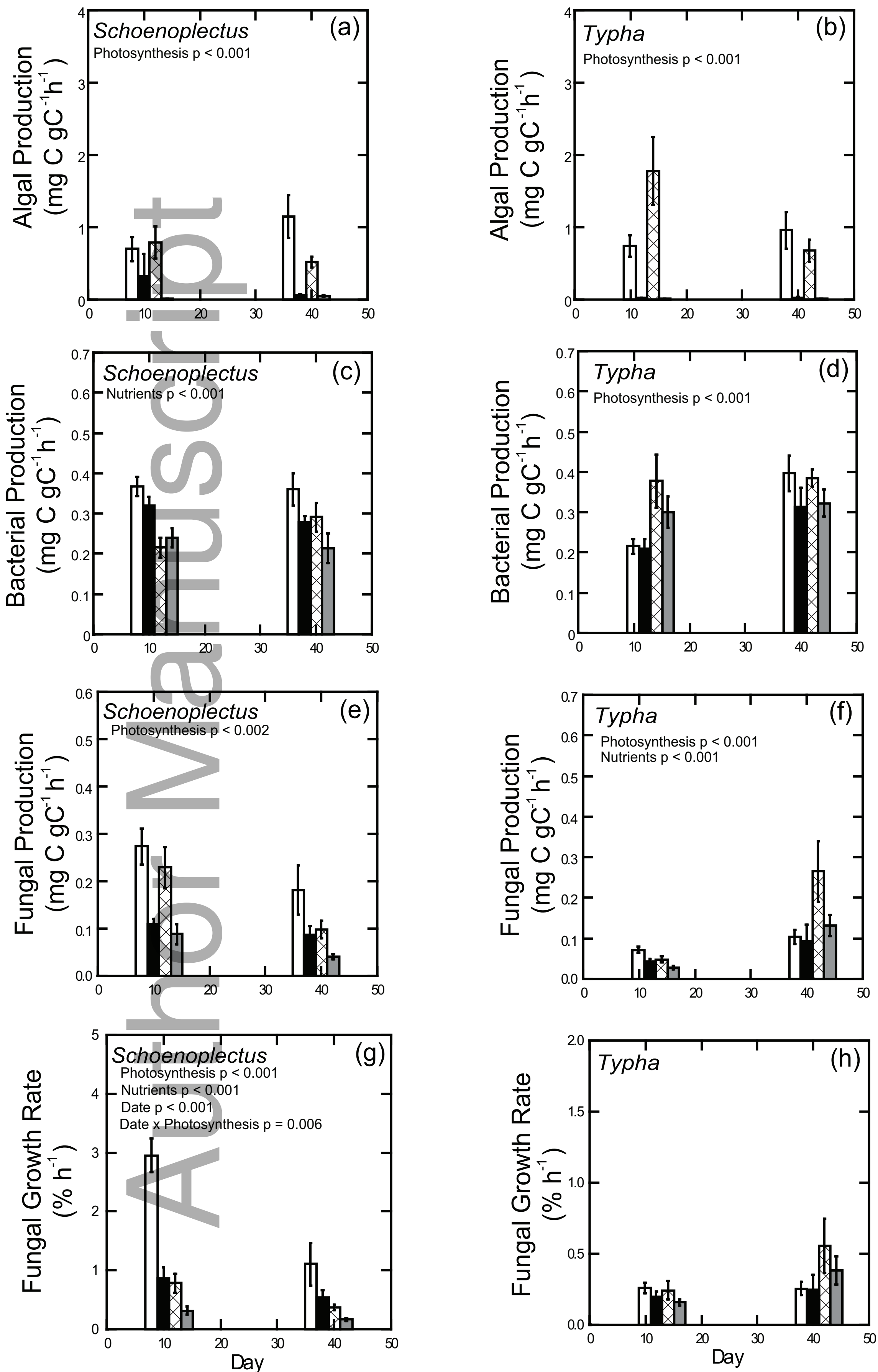

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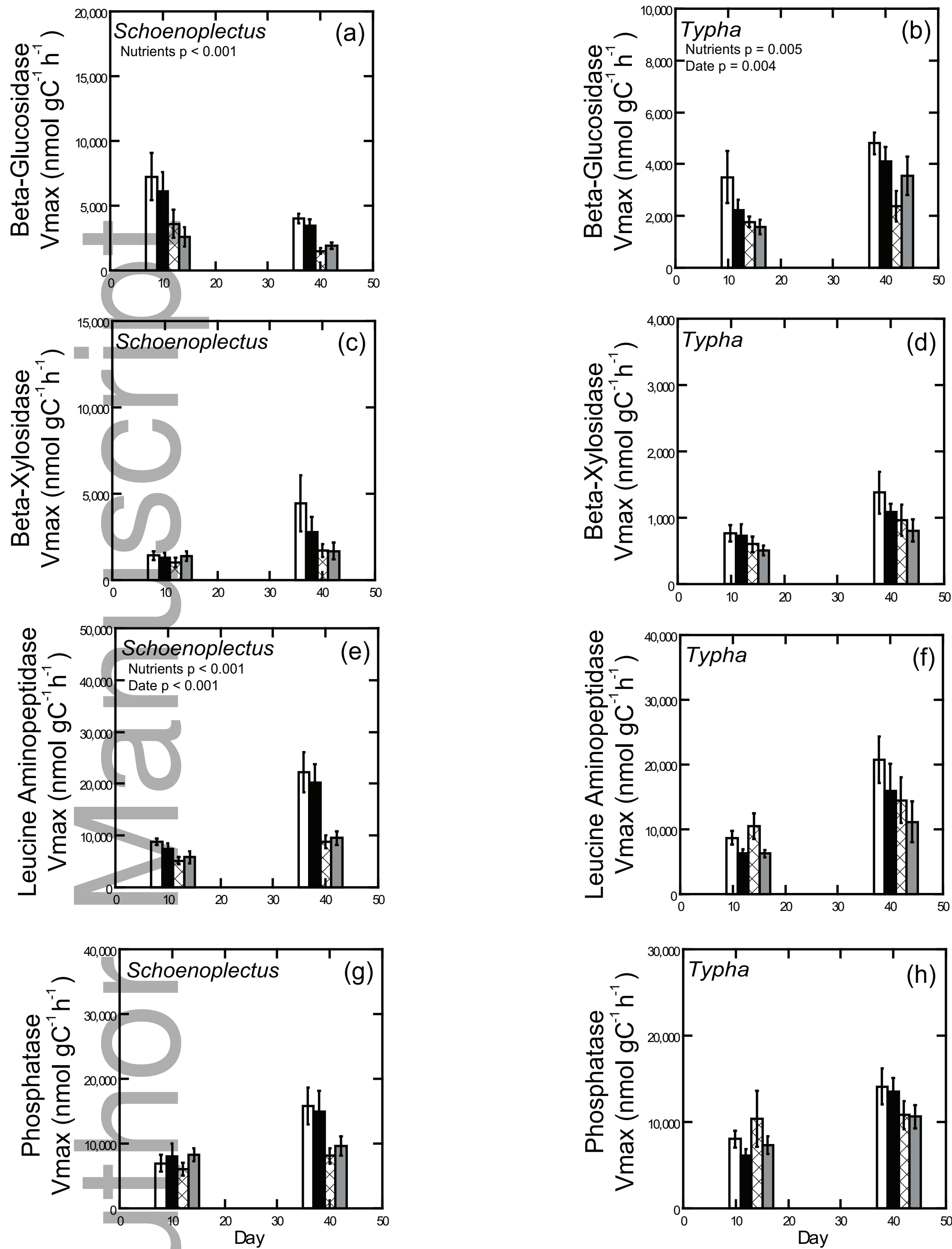
Figure 5: Algal (a, b), bacterial (c, d), and fungal (e, f) production and fungal growth rates (g, h) on photosynthesis-active (open or cross-hatched bars) and photosynthesis-inhibited (black or grey bars), nutrient-enriched (open or black bars)



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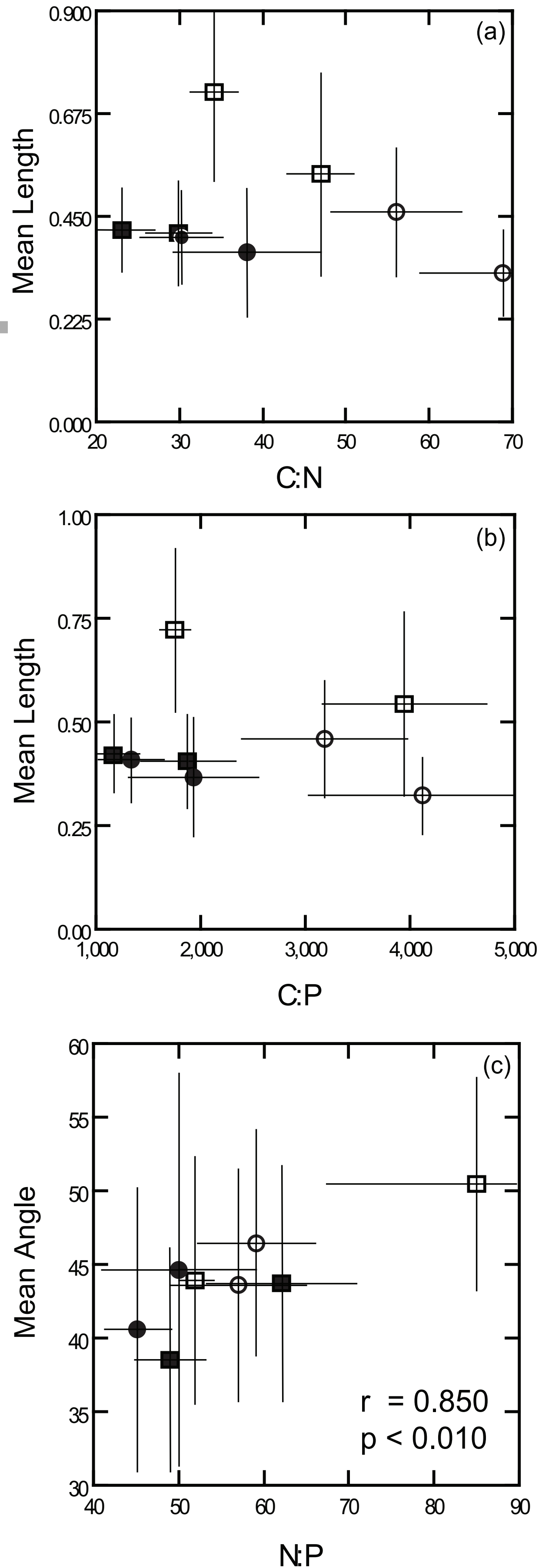

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Figure 7. Relationships of enzymatic vector analysis mean length to litter C:N (a) and C:P (b), and of vector analysis mean angle to litter N:P (c). Data are mean values for each