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Freshwater Invertebrate Assimilation Nutrients from Detrital Biofilms Under Light and Phosphorus Manipulation

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FRESHWATER INVERTEBRATE ASSIMILATION OF NUTRIENTS FROM DETRITAL BIOFILMS UNDER LIGHT AND PHOSPHORUS MANIPULATION

by

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ABSTRACT

Anthropogenic influences are increasing algal biomass and activity within aquatic ecosystems, stimulating debate regarding interactions between “green” and “brown” food webs. Specifically, in forested streams, the removal of forest canopy or riparian zones increases light exposure and nutrient fluxes to streams affecting primary productivity and carbon (C) flows across aquatic food webs. Further understanding the synergistic effects of light and nutrients on detrital biofilms may explain how aquatic detritivores respond to anthropogenic change. The objective of this study was to employ C and phosphorus (P) isotopic radiolabels to understand how microbial biofilm constituents (i.e heterotroph and autotroph) contribute C and P to detritivore nutrition under raised light and nutrient regimes. I conducted a feeding study using detritivorous caddisflies (i.e. *Pycnopsyche* sp.) and conditioned leaf litter across a fully-factorial experimental set up and fed radiolabeled litter in the lab. Additionally, I used dual labels of ^{14}C and ^{33}P to trace radiolabeled C and P into caddisfly tissues. I quantified microbial biomass and production rates, caddisfly consumption and egestion rates, and radiolabel assimilation, and incorporation efficiency. The results of my study report that 94-97% of detritivore C was incorporated from fungi and 1-3% from bacteria and algae. My study shows light and nutrients stimulate heterotrophic activity and determine the microbial quality of C and P available to detritivores. Future studies should assess physiological performance (i.e. growth rates) of caddisflies in response to light and nutrient exposed litter to understand how caddisflies are responding to potential elemental ratio imbalance

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CHAPTER I – BACKGROUND AND RESEARCH

Classic approaches to trophic ecology have focused on delineating relative contributions of ecosystem energy flow between "brown" and "green" food webs (Lindeman 1942; Brett et al. 2017). "Brown" food webs, or detritus-based systems, are driven by inputs of detrital subsidies and subsequent colonization by microbial decomposers (i.e. fungi and bacteria) that are critically important in providing nutrition to detritivorous consumers (Pollierer et al. 2012). In turn, "green" food webs are directly influenced by sunlight availability and nutrients that regulate autotroph biomass and nutrient contents, and therefore influence trophic level abundance (Oksanen et al. 1981; Sterner et al. 1997). While green and brown food webs differ in their energy basis, they are both limited by essential elements (e.g. nitrogen and phosphorus (P)) and dually play major roles in ecosystem-level energy flow and nutrient cycling (Evans-White & Halvorson 2017). In freshwater systems, detritivorous consumers derive nutrition from detrital periphyton comprised of microbial biomass (Bärlocher & Kendrick 1975; Mayer & Likens 1987). This periphyton consists of slow- and fast-turnover heterotrophic pools (i.e. fungi and bacteria) that may differ in nutritional quality for invertebrate assimilation (Dodds et al. 2014; Anderson et al. 2016). Research indicates that detritivorous macroinvertebrates assimilate most of their nutrition from microbial biomass compared to the leaf matrix (Petersen & Cummins 1974; Arsuffi & Suberkropp 1988). The high nutritional value of detrital microbes can be attributed to the higher accessibility of elements incorporated into heterotrophic microbial biomass relative to the litter matrix (Bärlocher 1985). When aquatic fungi colonize the leaf substrate, they assimilate carbon (C) and P within their biomass to a form more palatable for detritivores to ingest (Danger

et al. 2013). While the litter substrate is one major source of periphyton P accrual, heterotrophic microbes can also assimilate dissolved P from the water column (Kirchman 1994), enriching overall detrital quality to consumers with increasing dissolved nutrient availability (Danger et al. 2013; Manning et al. 2015).

Currently, most research on detrital microbes and detritivore nutrition has examined the importance of heterotrophic microbes in providing essential elements to detritivores (e.g., Chung and Suberkropp 2009; Halvorson et al. 2016a); however, the presence and activity of detrital-associated autotrophic microbes (i.e. algae) remain largely unexplored. This is due to the assumption that autotrophs play negligible roles in detrital-based food webs (Fisher and Likens 1973), especially in ecosystems of reduced light availability and low algal biomass, such as headwater streams (Roberts et al. 2007). Increasing research suggests that algae can play direct roles in "brown" food webs by stimulating heterotrophic activity on decomposing leaf litter and other organic substrates (Danger et al. 2013; Kuehn et al. 2014; Hotchkiss et al. 2016). Moreover, recent research indicates detritivorous animals can feed in "green" energy pathways, suggesting autotrophs play greater roles in detrital food webs than is classically assumed (Wolkovich et al. 2014). Algae play major roles supporting animal growth across a diversity of aquatic systems, likely due to the high nutritional quality of algal amino and fatty acids (Guo et al. 2016; Brett et al. 2017; Crenier et al. 2017). In recent years, anthropogenic deforestation, riparian removal, and fertilizer application have increased light availability and nutrient fluxes to aquatic systems (Neill et al. 2001; Kaylor et al. 2016; Carpenter et al. 1998), magnifying the potential role of algae in detrital food webs (Bumpers et al. 2017).

In the presence of light, algae can assimilate dissolved P into detrital periphyton, increasing periphyton P content (Persson et al. 2010, Halvorson et al. 2016b) and simultaneously increasing concentrations of C-rich polyunsaturated fatty-acids (Guo et al. 2016). This increase in detrital C quality and P content is likely greater under high P concentrations through increased algal biomass in detrital periphyton, leading to altered physiological processes (e.g. assimilation and growth) of aquatic detritivores (Gonzalez et al. 2014; Guo et al. 2016). One recent study indicates increased detrital P content reduces P assimilation efficiencies of detritivorous caddisflies (Halvorson et al. 2016a), which may be due to a switch in the major limiting element required for insect growth and nutrition (Frost et al. 2006). However, this study did not address autotrophic components of detrital periphyton, and algae may dampen the negative assimilatory response to P by increasing bioavailable C that becomes limiting under P enrichment (Boersma and Elser 2006). Interactions between light and P availability are important to understand the process of eutrophication (Correll 1996), which remains understudied in brown food webs (Rosemond et al. 2015).

The objective of this study was to determine the effects of light and nutrient availability on "brown" and "green" energy pathways in detrital biofilms and the relative importance of algal, bacterial, and fungal C and P to detritivore nutrition. I used ^{14}C- and ^{33}P as tracers of detrital microbial C and P in order to quantify their impact on the growth of the aquatic caddisfly larvae *Pycnopsyche* spp., a common aquatic detritivore that responds strongly to detrital P enrichment (Halvorson et al. 2015). The overarching hypotheses were: (1) given the high quality of algal nutrients (Guo et al. 2016), larvae fed light-exposed detritus will exhibit greater incorporation efficiency of autotrophic C

compared to heterotrophic (i.e. bacterial and fungal) C. (2) Larvae will also increase P incorporation efficiency on low-nutrient detritus due to stronger P limitation of growth (Halvorson et al. 2016a). Finally, (3) larvae will incorporate heterotrophic C more efficiently on low light-incubated litter. In this way, light and nutrient manipulations will alter the magnitude of heterotrophic versus autotrophic microbial nutrients contributing to larval growth.

CHAPTER II – METHODS AND EXPERIMENTAL APPROACH

Leaf litter conditioning and enrichment:

A field detrital conditioning period occurred at the University of Southern Mississippi's Lake Thoreau Environmental Center with *Pycnopsyche* feeding trials conducted in the laboratory. Tulip poplar, *Liriodendron tulipifera*, leaves were collected during October 2017 at Lake Thoreau, air dried for 24 hours, and cut into 14 mm standardized leaf discs.

Leaf discs were mounted on Plexiglas plates and conditioned under either High light exposure or Low light conditions in eight flume mesocosms containing stream water from Big Creek, a 2nd-order low-nutrient forested stream in De Soto National Forest, Mississippi. For 7 weeks, leaf discs occupied the flumes with 1/3 of water replaced by fresh water from Big Creek every 5 days. Four low-nutrient flumes received no nutrient amendments (Low nutrient treatment) while four high-nutrient flumes received nutrient amendments of $NaNO_3$ and Na_2HPO_4 to raise concentrations of the water by a targeted 400 µg L^{-1} $N\text{-}NO_3$ and 60 µg L^{-1} $P\text{-}PO_4$, with new amendments during each water change (High nutrient treatment). All flumes were shaded by light canopy to reduce UV exposure and solar heating; each of the 8 flumes was divided into one half receiving ambient light (Light treatment; light canopy transmitted 51% and 23% of ambient photosynthetically active radiation (PAR) and UV transmission, respectively) and one half fully shaded with solar shade cloth (Low light treatment; PAR and UV transmission below detection). In two flumes I placed HOBO Onset Loggers to monitor temperatures, and after each nutrient amendment, I collected, froze, and subsequently thawed and filtered water

samples to measure concentrations of P-PO_4, N-NH_4, and N-[NO_3+NO_2] using a SEAL Autoanalyzer 3.

Litter biofilm characterization:

After conditioning, leaves were removed from Plexiglass plates and brought back to the laboratory. A subsample of leaf discs from each diet treatment were collected to characterize initial microbial production, biomass, and elemental composition before dual-labeling with ^{14}C and ^{33}P. Litter fungal biomass and production were estimated using the concentration of the fungal-specific sterol, ergosterol (Gessner 2005) and incorporation of ^{14}C-acetate into ergosterol (Suberkropp and Gessner 2005), respectively. Two disks from each of the 4 flumes per treatment (n=16) were placed into 4 mL sterile filtered (0.22 µm) Big Creek water and given 5.0 mM ^{14}C-acetate (specific activity = 16.0 µCi/mg). Samples were incubated at 15°C under 300 µmol photons m^{-2} s^{-1} PAR for 5 hrs, filtered, and frozen until ergosterol saponification and extraction in pentanes. Ergosterol was concentrated in methanol and measured by high-performance liquid chromatography; fractions were collected and mixed with 10 mL EcoLume to measure ^{14}C disintegrations per minute (DPMs) using a Beckman LS6500 Scintillation Counter. I calculated fungal biomass using a conversion of 5 µg ergosterol mg^{-1} fungal biomass and 43% C in fungal biomass. Fungal production rates were calculated using a conversion of 12.6 µg fungal biomass $nmol^{-1}$ acetate incorporated. For each treatment, one kill control containing ^{14}C-acetate and 3% formalin was incubated and extracted to subtract background DPMs due to non-biological uptake.

Algal biomass and production rates were estimated using chlorophyll-a and incorporation of ^{14}C-sodium bicarbonate into carbohydrates, respectively. Chlorophyll-a

was quantified after extraction in hot 90% ethanol, steeping samples at 4°C in darkness overnight, and measuring absorbance on a spectrophotometer at 665 and 750 nm before and after acidification with HCl. Algal production rates were measured using algal incorporation of ^{14}C-bicarbonate (specific activity = 8.4 mCi mmol^{-1}). (Francoeur et al. 2006). Leaf discs were placed into a scintillation vial with 5 mL of water and incubated with 0.5 µCi of $NaH^{14}CO_3$. After incubating, samples were administered formalin to end incorporation of the radioisotope and frozen. After, a conversion factor of 11.1 Chl-a mg^{-1} algal C from unpublished data was applied to convert chlorophyll-a values to algal specific C. Daily specific rates of C assimilation was then calculated utilizing gains of algal specific C g^{-1} (Kuehn et al. 2014).

Bacterial biomass was analyzed by extracting two leaf discs from each treatment, placed into a solution of 2% buffered formalin, and sonicated at setting 4 for 20 second intervals with a Branson 150 sonifier to dislodge bacteria from leaf material. After, flow cytometry was used to quantify bacterial biomass with the application of bacterial stain and plastic microbeads from an Invitrogen bacteria counting kit for flow cytometry (Thermo Fisher, Waltham, MA). These samples were then processed on a LSRFortessa Cell Analyzer by quantifying number of cell events captured via flow cytometry. Cell abundances were later converted to bacterial biomass based on a subset of samples which provided a conversion to epifluorescence counts (Buesing 2005) corrected for the low efficiency of flow cytometry cell counting (Frossard et al. 2016, epifluorescence count=7.40 x flow cytometry cell count + 6.19 x 10^7 cells), assuming cells were 50% C and using an empirically-derived cell biomass of 95 fg cell^{-1} . Bacterial incorporation of ^{3}H-Leucine into proteins was used to quantify bacterial production rates. Production

samples were placed into 20 mL scintillation vials and initiated with the addition of 2.5 µM {4,5-^{3}H]- leucine and 4 mL of filtered water. Scintillation vials were placed sideways with in an incubator to submerge the leaf disc and allow for thorough incubation. Bacterial uptake of leucine was terminated via the addition of 5% TCA and, after, heated at 80°C for 30 minutes. Samples were centrifuged for protein extraction and production rates were quantified as total uptake as µg bacterial C g^{-1} detrital C hr^{-1} using the conversion factor of 1.44 kg C mole $^{-1}$ leucine incorporated (Gillies et al. 2006; Buesing & Marxen, 2005).

Caddisfly sampling and acclimation:

In February 2018, 80 individual 4th and 5th-instar larval caddisflies (*Pycnopsyche* sp.) were collected from Chamber Springs, Arkansas and brought back to the University of Southern Mississippi. Head capsule widths were measured for each individual to classify instar stage. A subset of 20 caddisflies was randomly selected, removed from their larval cases, blotted and weighed, and frozen to analyze initial elemental composition and dry mass. The remaining 60 caddisflies were then randomly assigned among the 4 respective leaf litter diets (incubated under Low Light or High Light and Low or High nutrients), and placed into plastic cup feeding chambers in an environmental chamber at 15°C and a 12:12 hr light cycle. Feeding chambers were filled with 100 mL Chamber Springs water with constant aeration. A 2 mm screen mesh was used in feeding cups to allow for the accumulation of egesta. Caddisflies were given 5 unlabeled leaf discs for a 5-day acclimation period prior to feeding on radiolabeled litter.

Two days into the acclimation period, I measured larval consumption and egestion rates. Consumption rates were calculated using the pre- and post-weights of leaf

discs after feeding to larvae. Pre-consumption leaf discs were lightly blotted, weighed, and immediately re-wetted before feeding to caddisflies. After the 3-day feeding period, the remaining leaf discs were collected from chambers, oven dried at 50°C, and measured for post-consumption dry weights. A subset of leaf discs was also blotted, weighed, and oven dried to establish a blotted-dry mass regression and calculate the dry weight of pre-consumption litter fed to caddisflies. Total consumption was calculated as the difference in dry weight between pre- and post-consumption litter. Egestion rates were determined by placing caddisflies in fresh filtered stream water. After 2 days, accumulated egesta were filtered onto pre-ashed and oven dried, pre-weighed glass fiber 25 mm filters (Pall Inc.; Port Washington, NY) with 1 micron sized pores. Egesta were oven dried and re-weighed to determine total egesta dry weight before elemental analysis.

After the acclimation period, all 60 feeding chambers were transferred to a fume hood to commence feeding on radiolabeled leaf discs. Feeding chambers remained under constant aeration and were placed in a water bath at 15°C maintained by a Fisher Scientific Aquatemp 1006 equipped with a heat exchanger.

Dual radiolabeling:

Remaining conditioned leaf disks from each of the four diets were used for ^{14}C labeling of different pools of microbial C, each accompanied by dual ^{33}P labeling of microbial P, prior to feeding to caddisflies. To label primarily slow-turnover eukaryotic (e.g. fungal) microbial C, 50 leaf discs from each diet were sorted into specimen cups each containing 50 mL of sterile filtered (0.22-µm) Big Creek water and 0.33 µCi mL^{-1} ^{14}C-acetate (specific activity = 16.0 µCi/mg; 0.25acetate). Samples were incubated under 300 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) with constant

aeration at 15°C for 3 days to allow thorough incorporation of the radiolabel (Chung and Suberkropp 2009). To label faster-turnover, primarily prokaryotic (e.g. bacterial) microbial C, leaf discs were incubated in a thousand-fold diluted ^{14}C-acetate solution under a shorter labeling duration compared to slow-turnover C labeling. Five sets of 8 discs from each diet were assigned sterile scintillation vials containing 5 mL sterile-filtered stream water and 0.33 nCi mL^{-1} ^{14}C-acetate (specific activity = 16.0 µCi/mg; 0.25 µM acetate). Samples incubated at 15°C under 300 µmol photons m^{-2} s^{-1} for 1 hr. To label autotrophic (algal) C, 5 sets of 8 discs from each diet were allocated among sterile scintillation vials containing 8 mL sterile-filtered stream water and 0.05 µCi mL^{-1} ^{14}C-sodium bicarbonate (specific activity = 56 mCi $mmol^{-1}$). Samples incubated at 15°C under 300 µmol photons m^{-2} s^{-1} for 4 hr.

All radiolabel solutions were simultaneously amended with ^{33}P-orthophosphate to achieve 0.01 µCi mL^{-1} (specific activity = 155.8 Ci mg^{-1}; 12.36 µg P-PO_4 L^{-1} at 0.0002% ^{33}P), permitting dual labeling of microbial P during the associated label period (Halvorson et al. 2016a). Given contrasting dual-label timeframes (3 d, 4 hr, or 1 hr), this resulted in respective labeling of slow, mid-, or fast-turnover pools of microbial P prior to feeding. During all three dual labeling trials, one vial per diet containing 2-4 discs in 5 mL of the appropriate radiolabel solution and 4.8% formalin served as controls for abiotic uptake of radiolabel.

At the end of each labeling trial, all discs were gently rinsed four times with unlabeled stream water. A subset of radiolabeled discs (five per diet from each radiolabel incubation) were immediately frozen to measure total microbial ^{14}C and ^{33}P pre-consumption. Remaining radiolabeled discs were transferred into caddisfly feeding

chambers of the appropriate diet assignment (5 discs/chamber) and randomly assigned one of the 3 radiolabel categories (slow-turnover heterotrophic, fast-turnover heterotrophic, or autotrophic C).

Radiolabel consumption, egestion, and incorporation:

Caddisflies were allowed to feed on radiolabeled discs in the fume hood for a period of 36-48 hrs. Residual (i.e. uneaten) leaf discs were collected and frozen to determine post-consumption radiolabel remaining. I also filtered accrued egesta onto 25 mm glass fiber filters and froze the filters to determine egested ^{14}C and ^{33}P.

Concluding the feeding study, I provided caddisflies with 100 mL fresh stream water and 5 unlabeled leaf discs for a 24-hour gut clearance period to assimilate labeled C and P within their guts (Chung and Suberkropp 2009) and egest excess radiolabel. Accumulated egesta were filtered through a 25 mm filter and frozen for later analyses. Caddisflies were pulled from feeding chambers, removed from their cases, rinsed 3 times with unlabeled stream water, lightly blotted, weighed, and frozen.

Radioactive pre- and post-consumption leaf discs, egesta, and caddisfly samples were thawed and digested at 60°C in 1 mL (leaf discs) or 2 mL (caddisflies, egestion filters) Soluene-350 tissue solubilizer. Caddisflies were macerated beforehand to enhance tissue digestion. Afterward, I transferred 0.1 mL digested sample into new scintillation vials to achieve detectable ^{33}P decay relative to background radioactivity (see below). All samples were decolorized with 0.1 mL 30% hydrogen peroxide overnight. Samples were then suspended in 10 mL EcoLume scintillation fluid (MP Biomedicals) for determination of ^{14}C and ^{33}P contents.

Sample analysis:

After sample suspension in scintillation fluid, all samples were allowed to settle for 3 days, then placed into a Beckman Coulter LS 6500 liquid scintillation counter to quantify radioactive ^{33}P and ^{14}C counts per minute (CPMs) on four separate dates over a minimum of three half-lives of ^{33}P (75 days), using a wide counting window (0-250 keV) to measure both ^{14}C and ^{33}P activity. CPMs were converted to ^{14}C- and ^{33}P-specific disintegrations per minute (DPMs) extrapolated to the time of freezing each sample, using the decline in measured CPMs due to ^{33}P decay over time, and employing separate ^{14}C and ^{33}P quench curves (Duhamel et al. 2006; Halvorson et al. 2016a). To account for non-biological uptake of radiolabel, from ^{14}C and ^{33}P DPMs of each sample, I subtracted the proportion of leaf disc ^{14}C and ^{33}P measured in killed control leaf discs, relative to living discs, from each diet and radiolabel category.

Total ^{14}C and ^{33}P DPMs consumed by each caddisfly was calculated from the total of each radioisotope fed to caddisflies (average DPMs disc^{-1} of pre-consumption discs from each diet and label category, multiplied by 5 disks), minus ^{14}C and ^{33}P measured post-consumption (total DPMs of residual discs). Total DPMs egested and incorporated were determined as calculated ^{14}C and ^{33}P DPMs in egesta and caddisfly samples, respectively. I calculated ^{14}C and ^{33}P-specific assimilation efficiencies using the equation

$$Assimilation\ efficiency\ = \frac{(DPMs\ consumed - DPMs\ egested)}{DPMs\ consumed}$$

And I determined ^{14}C and ^{33}P-specific incorporation efficiencies using the equation

$$Incorporation\ efficiency\ = \frac{DPMs\ incorporated}{DPMs\ consumed}$$

Initial (non-radioactive) leaf litter diets and larvae were dried, and larvae were weighed to infer dry mass from blotted weights of radiolabeled individuals of each instar (4^{th} instar: y = 0.216 (Blotted weight) - 2.932; 5^{th} instar: y = 0.276 (Blotted weight) - 8.996). Consumption and egestion rates were calculated as total dry mass consumed or egested, divided by trial duration and larval dry mass. Litter and larvae were ground and analyzed to determine %P contents by combustion, hot HCl extraction, and subsequent dilution to determine PO_4 concentrations using a SEAL Autoanalyzer 3 (Mequon, WI). Sample %C and %N contents were determined using the Costech Elemental Combustion System. Acclimation egesta were similarly processed, with the exception that filters were halved, each half re-weighed, and then analyzed separately for either P or C/N contents.

Statistical analyses:

Effects of light and nutrient treatments on initial leaf litter microbial biomass and production rates were analyzed using a full factorial two-way analysis of variance (ANOVA). Similarly, larval dry mass consumption and egestion rates during the acclimation period were compared using two-way ANOVA. Dietary effects on larval ^{33}P- and ^{14}C-specific assimilation and incorporation efficiencies were also assessed using two-way ANOVA, with separate ANOVAs for each of the 3 radiolabel categories for each isotope. Response variables were log-transformed where necessary to improve homogeneity of variances. All statistical analysis were conducted using the statistical program R, version 3.4.3.

CHAPTER III - RESULTS

Microbial biomass and production across leaf litter diets:

Fungal biomass was not significantly different across light or nutrient treatments (Fig. 1A; Table 1); however, fungi exhibited higher average biomass values (range = 190-250 mg C g^{-2} detrital C) across treatments compared to autotroph (range = 3.2-7.6 mg autotroph mg C g^{-2} detrital C) and bacterial biomass (range = 1.0-1.4 mg bacterial mg C g^{-2} detrital C) (Fig. 1). Autotroph biomass was significantly higher in the High Light compared to the Low light treatment ($p < 0.05$) and, bacterial biomass was significantly higher within High nutrient treatments ($p = 0.001$; mean = 1.31) (Fig. 1C; Table.1).

Fungal production rates were stimulated by light ($p = 0.041$) and increased nutrients ($p = 0.043$; Fig.1; Table 2) with the highest fungal production rates occurring under High nutrient and High Light exposed treatments (mean = 1386 ug C / g detrital C / hour). Autotrophic production rates reported significant interactions of light and nutrients ($p < 0.001$) (Fig.1; Table. 2). A Tukey's HSD analysis reported significantly higher autotrophic production rates in High Light-exposed High nutrient treatments compared to all other treatments ($p < 0.05$; mean = 214.61 ug C / g detritus / hour). Bacterial production rates reported significant ($p < 0.001$) interactive effects of light and nutrients. A Tukey's HSD reported significantly higher bacterial production rates in Low-Light High nutrient conditions ($p < 0.001$; mean = 171.2 ug C / g detrital C / hour) compared to High Light High nutrient conditions, Low Light Low nutrient conditions, and High Light Low nutrient treatments (Fig.1; Table. 2).

Consumption and egestion rates:

Average caddisfly consumption rates ranged from 0.16- 0.37 mg /mg /day and a two-factor ANOVA reported significantly greater consumption on high-nutrient litter ($p = 0.02$; mean = 0.39 mg / mg / day) (Fig. 2; Table 3). Average egestion rates ranged from (0.16 – 0.39 mg / mg/ day) and, similar to consumption rates, a two-factor ANOVA reported significantly greater egestion rates on high-nutrient litter ($p = 0.02$; mean = 0.37 mg / mg / day) (Fig. 2; Table 3).

Assimilation and incorporation of microbial ^{14}C

On average, caddisflies assimilated slow-turnover heterotrophic ^{14}C with the greatest efficiency (range = 0.81-0.88) compared to autotrophic ^{14}C or fast-turnover heterotrophic ^{14}C (0.50-0.76 and 0.42-0.72, respectively). In turn, caddisflies incorporated slow-turnover heterotrophic ^{14}C with greatest efficiency (range = 0.12-0.16) compared to autotrophic ^{14}C (range = 0.05-0.10) or fast-turnover heterotrophic ^{14}C (range = 0.09-0.37).

Assimilation efficiency of slow-turnover heterotrophic ^{14}C displayed no significant differences across treatments. Results from a two-way ANOVA showed caddisfly assimilation of autotrophic-derived ^{14}C significantly differed ($p = 0.02$) between nutrient treatments, with Low nutrient conditions reporting higher average assimilation efficiency (mean = 0.67; Fig. 3; Table 4). Assimilation efficiency of fast-turnover heterotrophic ^{14}C significantly differed between Low and High Light treatments ($p =0.001$; Fig. 3; Table 4) with higher average assimilation efficiencies on Low Light conditioned litter (mean = 0.69; Fig. 4; Table. 5).

Much like assimilation, incorporation of slow-turnover heterotrophic-derived ^{14}C exhibited no significant differences across treatments; however, caddisfly incorporation efficiency of autotrophic ^{14}C was significantly higher on Low Light compared to High Light treatment litter *(p* = 0.008; Fig. 3; Table. 4). Incorporation efficiency of fast-turnover heterotrophic ^{14}C exhibited a significant difference (*p* = 0.031) in response to nutrient treatments with higher average incorporation efficiencies occurring under Low nutrients (mean = 0.33; Fig. 3; Table. 4).

Assimilation and incorporation of microbial ^{33}P:

On average, caddisflies assimilated fast-turnover ^{33}P with the highest efficiency (range = 0.75- 0.85) compared to mid-turnover (0.67-0.81) and slow-turnover (0.39 – 0.91) pools of ^{33}P. Additionally, incorporation efficiency of mid-turnover pools of ^{33}P was highest (0.36-0.40) compared to slow (0.14-0.75) and fast-turnover (range = 0.33-0.42).

Light and nutrient treatments significantly interacted (p = 0.038) to affect caddisfly assimilation efficiencies of slow-turnover ^{33}P (Fig. 4; Table. 5). ^{33}P assimilation efficiencies significantly increased under Low nutrient and High Light exposed conditions (p = 0.01; mean = 0.912). A Tukey's Honestly Significant Difference (HSD) indicated assimilation efficiency on Low Light Low nutrient litter was significantly lower than all other diets ($p < 0.05$). Assimilation efficiency of mid-turnover ^{33}P exhibited no significant difference across treatments. Similarly, assimilation of fast-turnover ^{33}P did not respond to treatments (Table 4, Fig. 3).

Light and nutrient treatments also interacted significantly (p = 0.002) to affect caddisfly incorporation efficiency of slow-turnover pools of ^{33}P (Table. 5), with the

highest ^{33}P incorporation efficiency on Low-nutrient dark-incubated litter compared to all other diets (Tukey HSD, $P<0.05$; Fig. 4). Similar to patterns in assimilation, incorporation efficiency of both fast- and mid-turnover ^{33}P did not significantly differ across treatments (Table 5).

CHAPTER IV – DISCUSSION

By employing dual tracers to label contrasting pools of microbial C and P, my study helps resolve the nutritional value of detrital microbial nutrients and shows bottom-up effects of light and dissolved nutrients on acquisition of microbial biomass by detritivorous consumers. On average, caddisflies incorporated heterotrophic microbial C at higher efficiencies than autotrophic microbial C, refuting my first hypothesis that autotrophic C would be of greater value compared to heterotrophic C. While incorporation of fast- and mid-turnover microbial P did not show dietary effects, incorporation of slow-turnover microbial P expressed higher efficiencies under low nutrient conditions, supporting hypothesis 2. Finally, detritivore incorporation efficiency of both slow- and fast-turnover heterotrophic C reported higher values on High Light exposed litter, rejecting hypothesis 3.

My study affirms others' showing light and dissolved nutrients can determine the biomass and activity of detrital microbial C pools available to detritivorous consumers (Danger et al. 2013; Halvorson et al. 2016a). Detrital algal biomass and production increased strongly with light exposure, as did fungal production rates, suggesting autotrophs may have stimulated heterotrophic activity on litter (Danger et al. 2013; Kuehn et al., 2014). In addition, high algal activity in high light exposed conditions may increase algal-derived C flow into fungal pools as fungi mineralize dead algal biomass for C and P. Conversely, bacterial production rates were significantly higher under dark and high nutrient conditions, suggesting bacterial activity was reduced by algal photosynthesis, perhaps due to competition with algae for dissolved nutrients (Scott et al. 2008). Together, these findings suggest that in the presence of light, algal stimulation of

heterotrophs within the biofilm may govern the bioavailability of microbial pools of C and P for consumption.

Consumption and egestion rates determine the amount of energy and nutrients available to support animal growth and therefore indicate flexibility in response to dietary changes (Frost et al. 2005; Halvorson et al. 2018). In the present study, detritivore consumption rates increased on high nutrient litter compared low nutrient litter, consistent with others' findings (Halvorson et al. 2016b). A potential explanation to these results is a release of nutrient limitation and increased feeding on high-quality litter biofilm. Interestingly, caddisflies increased consumption and egestion rates despite similar fungal and algal biomass (within a given light treatment) and strictly higher bacterial biomass on high-nutrient litter. This suggests that bacterial biomass is a strong positive indicator of litter quality, or perhaps caddisflies increased consumption because microbes were more nitrogen- and P-rich on high-nutrient litter, given these microbial groups exhibit substantial stoichiometric flexibility (Persson et al. 2010; Gulis et al. 2017). By affecting consumption and egestion rates, variation in microbial biomass and stoichiometry may govern the magnitude of microbial support of detritivore assimilation and growth.

Comparisons of assimilation and incorporation efficiencies across major microbial biomass pools may help resolve microbial contributions to detritivore energy and nutrient demands (Sinsabaugh et al. 1985). Here, assimilation efficiency is defined as the efficiency of digesting and passing ingested material through the gut membrane into the available metabolic pool. In turn, incorporation efficiency describes how ingested material is respired or excreted versus subsequently incorporated to synthesize new

biomass (growth) (Sperfeld et al. 2017). Across all diets, I found that slow-turnover heterotrophic C was the highest quality for assimilation, but fast-turnover heterotrophic C was the highest quality for incorporation. Autotrophic C was assimilated and incorporated with lower efficiency. This suggests that slow-turnover fungal C may be easily digested, but supports a greater proportion of post-assimilatory loss, most likely respiration, compared to algal or fast-turnover bacterial C. In turn, bacterial C may not be assimilated very efficiently because of the small size of bacterial cells, which easily become entrained within the ingested matrix and are therefore less susceptible to enzymatic activity in the digestive tract. Alternatively, bacterial C assimilation by caddisflies may be low because bacteria were more easily sloughed off litter during feeding, leading to measurement of non-ingested bacteria within egesta (Herbst 1982). While my methods did not permit resolution of heterotrophic versus autotrophic P, I found that acquisition of microbial P depended on labeling turnover time, with general support that microbial P is assimilated highly efficiently, and fast- and mid-turnover microbial P is of greater value for incorporation compared to slow-turnover microbial P. This may reflect greater recalcitrance of slow-turnover P pools such as polyphosphate P (Rier et al. 2016). In contrast, fast- turnover pools (i.e. bacterial P) may be associated with higher microbial surface area to volume ratios, faster growth rates, and low C:P ratios, providing a greater proportion of high-quality P supporting P-rich detritivore growth (Elser et al. 2003).

My data suggest that light and dissolved nutrients can further affect the efficiency of detritivore assimilation and incorporation of microbial nutrients. Incorporation efficiencies suggest that nutrient-rich, fast-turnover heterotrophic C is higher quality and

efficiently incorporated by caddisflies compared to lower-nutrient, fast-turnover heterotrophic C; however, these trends were not apparent for slow-turnover heterotrophic C, which was assimilated and incorporated with similar efficiency regardless of diet. Given that fast-turnover heterotrophic C was also assimilated more efficiently on dark-incubated litter, my data suggest light and nutrient limitation may cumulatively decrease the nutritional quality of fast-turnover heterotroph C (namely bacteria) for detritivores. Autotrophic C was assimilated more efficiently on low-nutrient diets and incorporated more efficiently on dark-incubated litter, suggesting that caddisflies acquired algal C more efficiently when feeding on litter of lower algal biomass. My findings suggest that not all pools of detrital microbial C are equally valuable to detritivore nutrition; indeed, even within pools of similar microbial C (e.g., autotrophic or heterotrophic C), environmental factors can affect C quality for detritivore assimilation and growth. This may partly explain responses of detritivore growth to factors including leaf type and dissolved nutrient availability (Halvorson et al. 2018). While algal biomass reported highest values under light-exposed conditions, C within algal biomass was no higher-quality than heterotrophic microbial C. One potential mechanism linking increased algal biomass, production rates, and nutrient availability to detritivore nutrition are autotrophic flows of carbon (i.e. algal exudates) into heterotrophic C pools as forms more bioavailable for detritivore utilization. This possibility aligns with the reported increases in light exposed algal and fungal production rates suggesting importance of 'priming effects' in detrital-based food webs. In contrast, caddisflies assimilated fast-turnover heterotrophic C more efficiently under dark treatments; however, caddisflies did not

show elevated incorporation efficiency suggesting bacterial carbon could be proportionally respired instead of incorporated in the dark.

My dual-labeling approach permits comparison of C relative to P incorporation efficiencies toward detritivore assimilation and growth. Across experimental treatments, incorporation efficiencies of microbial P were on average higher than C incorporation efficiencies. This may reflect high C losses to respiration and comparative retention of P as a limiting element essential in the production of RNA (and thus protein synthesis) and phospholipids (Sterner and Elser 2002). Notably, incorporation efficiencies of slow-heterotrophic pools of P were highest under dark and low nutrient treatments. This may be explained by a greater degree of P-limitation on these diets (Halvorson et al. 2016a). Further work from my study may determine the C:P stoichiometry of different microbial contributions to detritivore growth under contrasting light and nutrient availability.

Overall, my study highlights the importance of environmental conditions on the availability of aquatic microbial nutrients to consumers. I show that fast-turnover heterotrophic C is high-quality for detritivore incorporation; however, 94-97% of total microbial C was incorporated from fungi with 1-3% C from bacteria and algae. These results are consistent with prior studies that found nutrition required for growth of *Pycnopsyche gentilis* was mainly derived from fungal biomass (Chung and Suberkropp 2009). Additionally, detrital stoichiometry and biofilm composition has been shown to exert a strong control on detritivore growth rates and assimilatory processes (Fuller et al. 2015). Holistically, these findings suggest that biofilm elemental composition and identity regulate the magnitude of C and P detritivore incorporation compared to detrital C (Chung & Suberkropp 2009; Cummins 1974).

As anthropogenic modification of the landscape reduces canopy cover and increases nutrient flux into streams interactions between “green” and “brown” food webs may be amplified (Bumpers et al. 2017). The synergistic effects of light and nutrients on microbial biofilms and detritivore nutrition, presented in this study, should be taken into account when assessing consumer responses to resource changes. Additionally, as atmospheric CO_2 levels increase, it may stimulate aquatic autotrophic activity through alterations in allochthanous input stoichiometry (e.g. Tulip poplar leaf stoichiometry) that intensifies the role of autotrophic-heterotrophic interactions in stream trophic food webs (Van de Waal et al. 2010; Roberts & Howarth 2006). Future studies should address physiological performance (e.g. growth rates) of detritivores fed conditioned leaf discs to analyze levels of tolerance for P limitation and performance. Understanding this may elucidate how invertebrate species will physiologically respond under light and dissolved nutrient effects on biofilms, shifts in stream invertebrate community composition, and complexity of aquatic trophic webs.

TABLES

Table 1. Results from a two factor ANOVA for fungal, algal, and bacterial biomass in response to light (L) and nutrient (N) treatments. Significance ($P < 0.05$) is indicated by bolded values.

Variable	Factor	F-ratio	P-value
Fungal	L	0.57	0.46
	N	0.12	0.73
	N x L	0.14	0.72
Algal	L	8.66	**0.01**
	N	3.03	0.10
	N x L	3.15	0.10
Bacterial	L	2.3	0.15
	N	17.0	**< 0.01**
	N x L	0.05	0.83

Table 2. Results from a two factor ANOVA for fungal, algal, and bacterial production in response to light (L) and nutrient (N) treatments. Significance ($p<0.05$) is represented by bolded text.

Variable	Factor[a]	F-ratio	*P*-value[b]
Fungal	L	5.2	**0.04**
	N	5.1	**0.04**
	N x L	0.3	0.58
Algal	L	27.1	**< 0.01**
	N	16.2	**< 0.01**
	N x L	10.3	**< 0.01**
Bacterial	L	21.4	**< 0.01**
	N	41.0	**<0.001**
	N x L	24.0	**<0.001**

Table 3. Results from a two factor ANOVA for consumption and egestion rates across light (L) and nutrient (N) treatments. Significance ($p<0.05$) is represented by bolded text.

Variable	Factor	F-ratio	*P*-value
Consumption	L	0.63	0.43
	N	5.41	**0.02**
	N x L	1.15	0.29
Egestion	L	2.01	0.16
	N	5.30	**0.03**
	N x L	0.13	0.72

Table 4. Results from a two-factor analysis of variance (ANOVA) for caddisfly radioisotope C^{14} assimilation or incorporation efficiencies in response to light and nutrient treatments. Significance is indicated by bolded values ($p < 0.05$). Specific constituents of the biofilm are indicated by autotrophic, slow-turnover heterotrophic pools of C^{14}, and fast-turnover heterotrophic pools of C^{14}. Terms [A] = assimilation efficiency and [I] incorporation efficiency.

Variable	Factor[a]	F-ratio	*P*-value[b]
Slow-turnover ^{14}C [A]:	L	0.94	0.35
	N	1.21	0.29
	N x L	0.40	0.53
Autotrophic ^{14}C [A]:	L	2.64	0.13
	N	5.96	**0.03**
	N x L	0.47	0.50
Fast-turnover ^{14}C [A]:	L	15.9	**< 0.01**
	N	0.16	0.69
	N x L	1.80	0.2
Slow-turnover ^{14}C [I]:	L	1.12	0.31
	N	< 0.01	0.93
	N x L	0.11	0.74
Autotrophic ^{14}C [I]:	L	9.30	**< 0.01**
	N	3.40	0.085
	N x L	0.38	0.055
Fast-turnover ^{14}C [I]:	L	2.80	0.11
	N	5.55	**0.03**
	N x L	0.18	0.67

Table 5. The results from a two-factor analysis of variance (ANOVA) portraying ^{33}P radioisotope assimilation and incorporation by caddisflies in response to light (L) and nutrient (N) treatments. Significance is indicated by bolded values ($p < 0.05$). Specific constituents of the biofilm are indicated by slow, mid, and fast heterotrophic pools of P^{33}. Terms [A] = assimilation efficiency and [I] incorporation efficiency.

Variable	Factor[a]	F-ratio	*P*-value[b]
Slow-turnover ^{33}P [A]:	L	7.33	**0.02**
	N	5.03	**0.04**
	N x L	5.15	**0.04**
Mid-turnover ^{33}P [A]:	L	< 0.01	0.97
	N	< 0.01	0.97
	N x L	1.21	0.29
Fast-turnover ^{33}P [A]:	L	2.81	0.11
	N	0.41	0.53
	N x L	3.75	**0.07**
Slow-turnover ^{33}P [I]:	L	14.4	**< 0.01**
	N	17.4	**< 0.01**
	N x L	13.1	**< 0.01**
Mid-turnover 33P [I]:	L	0.12	0.74
	N	0.10	0.75
	N x L	0.11	0.74
Fast-turnover 33P [I]:	L	0.67	0.42
	N	0.75	0.40
	N x L	0.07	0.79

Table.6: Average temperature and dissolved nutrients across experimental conditions with standard errors.

Parameter	Low- nutrient	High- nutrient
Temperature (C)	10.2	9.5
[$N\text{-}NO_3$+NO_2] (ug/L)	61.5 (17.3)	256.3 (54.5)
[$P\text{-}PO_4$] (ug/L)	12.4 (0.8)	33.9 (4.4)
[$N\text{-}NH_4$](ug/L)	12.4 (6.6)	17.4 (7.2)

FIGURES

Figure.1: Mean ± SE biofilm constituent measurements for biomass and production in response to light and nutrient treatments.

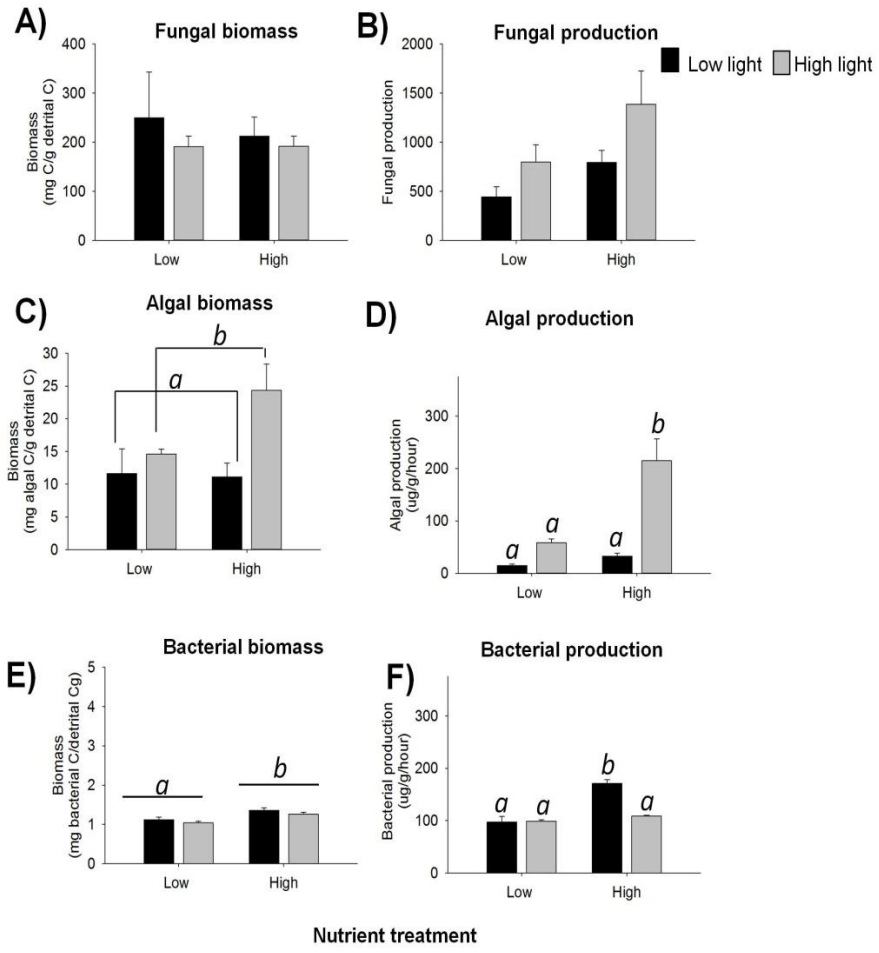


Figure 2. Mean ± SE caddisfly consumption and egestion rates in response to light and nutrient treatments.

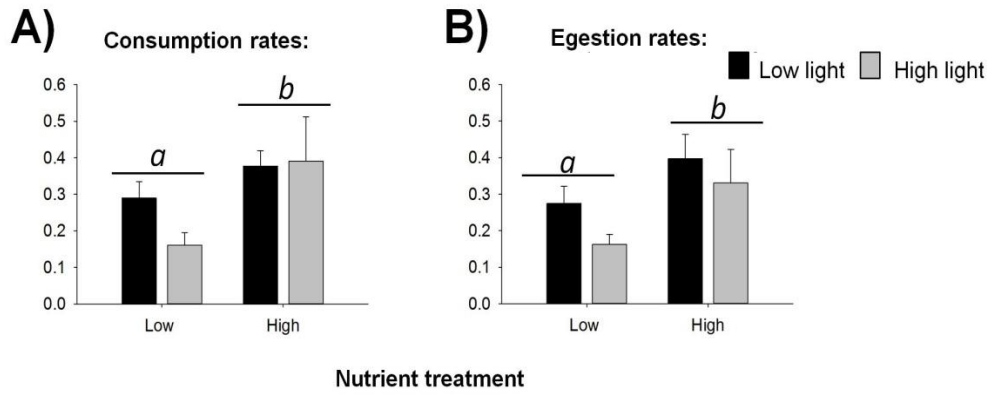


Figure 3: Mean ± SE caddisfly assimilation and incorporation efficiencies of slow-turnover heterotrophic (A,B), autotrophic (C,D), and fast-turnover heterotrophic (E,F) ^{14}C in leaf litter diets incubated under contrasting light and nutrient levels prior to feeding.

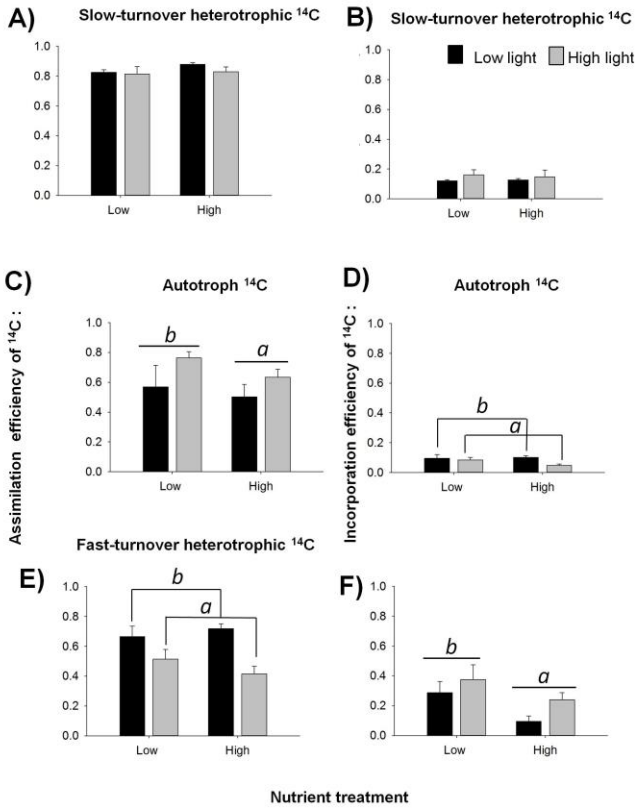
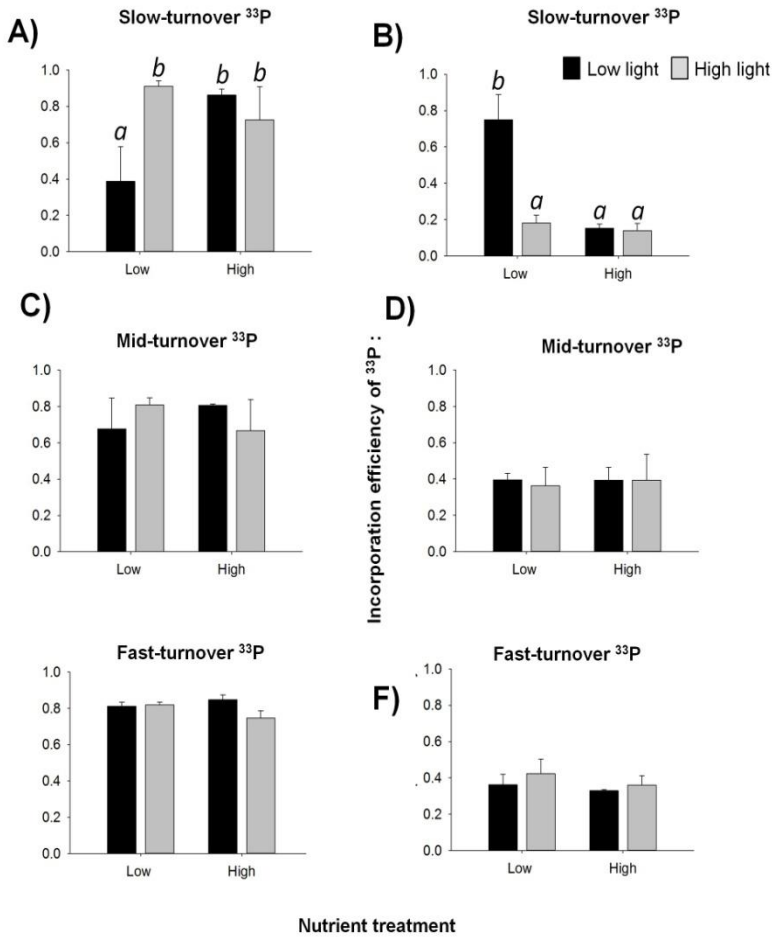


Figure 4. Mean ± SE assimilation and incorporation efficiencies of slow-turnover (A,B), mid-turnover (C,D), and fast-turnover (E,F) ^{33}P in leaf diets by caddisflies across light and nutrient treatments.



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