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The Effects of Light on the Bacterial Biomass of Decomposing Leaf Litter in Freshwater

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by

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Abstract

Recent evidence has suggested that the presence of light (and in conjuction, periphytic algae) stimulates the growth and production of bacteria on decaying plant litter. However, the current method of measuring bacterial biomass, flow cytometry, drastically underestimates the bacterial biomass levels associated with litter samples. To determine and correct for this underestimation, the present study determined conversion factors using direct counting methods via epifluorescence microscopy. These conversion factors were then applied to flow cytometry counts for seven different studies. The studies analyzed the effects of light and other covarying factors on the bacterial biomass associated with various samples of leaf litter that were submerged in freshwater. In six out of the seven experiments I observed that the presence of light, and in tandem algae, positively affected litter-associated bacterial biomass. However, this relationship was likely complicated by a multitude of other factors, including time of litter decomposition, nutrient availability, temperature, and presence of other organisms, such as detrital consumers (shredders). These findings further advance the understanding of interactions between microbes associated with decaying leaf litter in freshwater environments, particularly between autotrophic periphytic algae and bacteria, and how algal stimulation of bacteria can vary with different environmental parameters.

Keywords: bacterial biomass, bacteria, algal priming, light effect, priming effect, detritus

Dedication

This senior thesis is dedicated to the friends, family, and mentors who supported me throughout the completion of this project.

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List of Abbreviations

DAPI	4′,6-diamidino-2-phenylindole
NaCl	sodium chloride
NaH_2PO_4	sodium dihydrogen phosphate
PBS	phosphate-buffered saline
SE	standard error

Chapter 1: Introduction

Decomposition of plant litter is an important process in many freshwater ecosystems, such as streams, the littoral zones of inland lakes, and wetlands (DeGasparro *et al.* 2019; Fennessy *et al.* 2019; Gessner 1997; Webster *et al.* 1999). The decomposition of plant litter in these environments plays a vital role in the recycling of nutrients, including but not limited to carbon and nitrogen (Fennessy *et al.* 2019; Howarth *et al.* 1988; Wyatt & Turetsky 2015). The organisms that drive the breakdown of leaf litter include detritivorous invertebrates referred to as "shredders" and heterotrophic microorganisms like bacteria and fungi (Findlay *et al.* 2002; Wallace & Webster, 1996). It has been suggested that shredders account for the majority of the mass loss in decaying leaf litter, followed by fungi and lastly bacteria; however, the contribution of the latter is still substantial enough not to be discounted (Findlay & Arsuffi 1989; Findlay *et al.* 2002). Moreover, though overall biomass of fungi is generally regarded as greater than that of bacteria, studies have shown that bacterial biomass tends to be less variable than fungal biomass, thus the former would serve as the more predictable component of microbial communities on leaf litter in freshwater communities (Findlay *et al.* 2002).

Heterotrophic microbes, such as fungi and bacteria, drive the decomposition of organic matter by assimilating and mineralizing carbon and other nutrients (Gessner *et al.* 2010). As such, microbes are limited by the availability of this carbon, especially in environments in which nutrients are scarce (Garcia-Pausas & Paterson 2011). Recent studies have illustrated that the addition of labile carbon to the environment by primary producers increases the amount of carbon available to these microbes and in turn positively stimulates their growth and production; this is referred to as the "priming effect" (Danger *et al.* 2013). The priming effect has been well-documented in terrestrial ecosystems (Cheng *et al.* 2013; Luo, Wang, & Sun 2016; Rousk, Hill,

& Jones 2014), benthic marine sediments (Gontikaki *et al.* 2015), wetlands (Wyatt & Turetsky 2015), and freshwater streams and lakes (Danger *et al.* 2013; Halvorson *et al.* 2019; Kuehn *et al.* 2014). In freshwater environments in particular, it is not only microbes that populate the leaf litter, but algae as well. Multiple studies that have manipulated algal photosynthesis have shown that algal activity is correlated strongly with both fungal and bacterial biomass and production; that is to say, in the presence of light, bacterial and fungal growth and activity are rapidly stimulated compared to in the dark (Danger *et al.* 2013; Halvorson *et al.* 2019; Kuehn *et al.* 2014). Some studies have even gone on to analyze whether the priming effect co-varies with other environmental properties, such as temperature or litter nutrient properties, and found that these characteristics can also influence microbial activities and growth (Francoeur *et al.* 2020; Pope *et al.* 2020).

A number of methods are used to quantify bacterial biomass; of these, two of the most favored methods are direct microscopy counts and flow cytometry. Direct counting methods employ the use of fluorescent dyes in order to manually count bacteria with the assistance of a microscope. Historically, dyes such as acridine orange and 4′,6-diamidino-2-phenylindole (DAPI) were favored (Kepner & Pratt, 1994); however, in recent years, a multitude of improved fluorescent dyes like SYBR Green have been produced that have enhanced the applications of this method (Buesing, 2005; Frossard, Hammes, & Gessner, 2016). Unfortunately, the direct counting method has two limitations: first and foremost, the method is exceptionally tedious and time-consuming, and secondly, there is a considerable measure of observer bias (Frossard, Hammes, & Gessner, 2016). As such, flow cytometry is an alternative to direct counts, and is faster, more cost-efficient, and has a wide variety of applications due to its adaptability (Frossard, Hammes, & Gessner, 2016; Hammes & Egli, 2010). However, flow cytometry has

one major pitfall - it drastically underestimates bacterial counts as compared to direct count methods, sometimes by 10 or 100 fold (Frossard, Hammes, & Gessner 2016).

The objective of the present study is two-fold. First, bacterial biomass samples were collected from a multitude of different studies conducted in the Kuehn laboratory and subjected to both flow cytometry and direct counting via epifluorescence microscopy. The results of the two methods were used to create conversion factors to account for flow cytometry underestimating bacterial biomass estimates. These conversion factors were then applied to the flow cytometry data obtained for seven studies to adjust the bacterial biomass estimates to measurements that would have been obtained with direct counts. These results were analyzed in terms of effects of light on the bacterial biomass of the samples.

Chapter 2: Methods

A total of seven studies are included in the bacterial biomass analyses of the present experiment. Two investigate only the effects of light on the bacterial biomass, one investigates the effects of light under covarying temperatures, two couple the effect of light availability with nutrient availability, and one investigates the effects of light coupled with invertebrate presence and absence.

Sample Collection and Preservation

On each sampling event, two 13.5 mm disks or 1.7 cm long replicate sections of litter were collected from each sample. The two pieces of litter were placed into the appropriate labelled tubes. Ten mL of 2% formalin buffered with 0.1% sodium pyrophosphate were then added to each tube. The prepared bacterial biomass samples were placed on ice and sonicated for 1 minute (three intervals of 20 seconds) with a Branson 150 Sonifier in order to detach the bacterial cells from the suspended litter. Samples were then stored tightly-capped in darkness and refrigerated (4°C) until analysis via epifluorescence counts and flow cytometry.

Epifluorescence Counts

Samples were then chosen for epifluorescence microscopy. An equal number of samples from the light and dark conditions of Experiments 1 (specifically the *Liriodendron tulipifera* litter), 2 (*Liriodendron tulipifera* and *Quercus nigra*), 6 (*Typha latifolia*), and 7 (*Quercus alba*) were chosen create a flow cytometry conversion factor for each litter type. The samples represent conditions over the course of the sampling days and all four litter types used in the various experiments. Direct counting of bacterial cells via epifluorescence microscopy was performed using the method outlined by Buesing (2005) for each sample. A filtration system was assembled and a 25 mm nitrocellulose backing filter mounted onto a Millipore filtration unit and rinsed with

100 mL of Nanopure water. A 25 mm diameter, 0.2 µm pore size Anodisc supported Whatman filter (Whatman plc, Maidstone, Kent, UK) was then placed on top of the moistened backing filter and the filtration unit connected. One mL of Nanopure water was then added to the filtration unit. Samples were vortexed and allowed to settle for approximately ten seconds, then a 100 µL aliquot was removed from the sample and added to the Nanopure water contained within the filtration unit. Another 1 mL of Nanopure water was added to the filtration unit to ensure good suspension of the sample prior to filtration. The sample was then slowly filtered through the Anodisc filter by applying a vacuum of approximately 20 kPa. The filter was then removed and excess moisture wicked away by placing on a Kimwipe.

A concentrated staining solution was prepared by creating a 1:10 dilution of SYBR Green II solution in Nanopure water. From this stock solution, a working staining solution was freshly prepared for each set of samples by creating a 2.5% dilution from this concentrated staining solution. Following filtration, 100 µL of the working stain solution was pipetted onto a clean Petri dish and the dry Anodisc filter was placed on top. These dishes were incubated in the dark for 15 minutes to allow for thorough staining. The filters were then dried again via placement on a Kimwipe and mounted on a clean microscope slide. An antifading solution was prepared by combing 50% glycerol, 50% phosphate-buffered saline (PBS: 120 mM NaCl, 10 mM NaH_2PO_4, pH 7.5) and 0.1% *p*-phenylenediamine (Buesing 2005). A 30 µL aliquot of antifade solution was added to the filter, and a cover slip placed on top such that the antifading solution was evenly distributed. Prepared slides were placed on a tray, wrapped in aluminum foil to prevent fading, and frozen in darkness at -20°C until analyzed.

Slides were thawed and cells were counted using a Nikon Eclipse 80i epifluorescence microscope under oil immersion (1000× magnification). Bacterial cells were assigned into

categories according to size and shape, and cells were enumerated in a minimum of 10 fields (100×100 µm viewing window) until at least 250-300 cells had been counted. Video capture photographs were taken for biovolume estimates. Here, the areas and perimeters of these cells were recorded using digital imaging analysis software Nikon NIS Elements, version 4.60. These measurements were used in order to obtain biovolume estimates as outlined by Bjornsen (1986) and Krößbacher (1998). The biovolume was obtained with the following equation: Biovolume (μm^3) = 8.5 × (area in µm)$^{2.5}$ × (perimeter in µm)$^{-2}$ (Bjornsen 1986). The biomass of bacteria was then obtained by applying the following equation: Biomass (fg C) = 435 × (Biovolume in μm^3)$^{0.86}$ × 0.5 (Krößbacher 1998).

Flow Cytometry

After sonication, bacterial biomass samples were placed on ice and vortexed for 4 seconds and allowed to settle for 30 seconds. 15-mL centrifuge tubes were prepared each with a 40 µm sieve (MACS Smartstrainers, Miltenyi Biotec, Auburn, CA). Half a milliliter of each subsample was pipetted through the sieve into each appropriate tube. The sieve was rinsed with 4.5 mL of 1X concentration of PBS solution. The 15-mL tubes were then vortexed again for five seconds to mix the subsamples. 1 mL of the diluted samples were then transferred into appropriately labeled 1.5-mL conical centrifuge tubes.

Invitrogen bacterial counting kits for flow cytometry (Thermo Fisher, Waltham, MA) were used according to the manufacturer's protocols. Briefly, 1 µL of SYTO BC stain was added to each centrifuge tube and mixed by inversion. A 10 µL microsphere standard suspension was then added into each centrifuge tube. Samples were then counted and analyzed based on the method outlined by Halvorson *et al.* (2019): using a BD LSRFortessa Cell Analyzer (flow rate = 400 events/second), fluorescence was measured using a fluorescein [FITC] channel with a 530-

nm bandpass filter. Dyed controls containing only microbeads were included to establish a standard for comparion; bacterial cells were counted as particles with fluorescence above the microbeads (FITC 2 × 102). Cells/mL was converted from cells/g detrital C based on measured average leaf disc dry mass and C content.

Conversion Factor and Statistical Analysis

Once bacterial cell counts were obtained for samples from several different experiments using both epifluorescence counts and flow cytometry, the total sample cell counts obtained from each method were plotted against one another using linear regression to examine the relationship between total cell counts derived from flow cytometry and epifluorescence direct count methods. These regression equations were used as conversion factors for estimating bacterial cell counts among each of the leaf litter types examined (*Typha domingensis, Typha latifolia, Quercus alba,* and *Liriodendron tulipifera)*. These conversion factors were applied to all seven experiments to obtain corrected bacterial cell counts. Then, corrected cell counts were multiplied by average cell biomass (fg C/cell) from the appropriate leaf litter species to convert to bacterial biomass (mg C/g detrital C). The effects of light treatment and time (where appropriate) on bacterial biomass were then analyzed for each experiment using repeated-measures ANOVA in the statistical program R version 3.3.1.

Chapter 3: Results

Epifluorescence – Flow Cytometry Conversions

Four conversion factors were generated from the linear regression models calculated for each of the litter types examined (*Typha domingensis, Typha latifolia, Quercus alba,* and *Liriodendron tulipifera)* (**Figure 1**), where *y* is the epifluorescence direct cell count and *x* is the flow cytometry cell count. For *T. domingensis* the equation produced was $y = 4.05x + (2.44 \times 10^7)$ (R^2=0.919). For *T. latifolia*, the equation generated was $y = 4.0163x + (1 \times 10^8)$ (R^2=0.0215). For *Q. alba*, the resulting equation was $y = 7.12x + (5.87 \times 10^7)$ (R^2=0.272) and for *L. tulipifera*, the equation produced was $y = 7.40x + (6.19 \times 10^7)$ (R^2=0.110). Overall, it was found that flow cytometry counts generally did underestimate bacterial cell counts compared to epifluorescence direct counts by approximately 5-15 times, depending on the leaf litter type (**Figure 1**). The conversion factor observed for *T. domingensis* was by far the strongest relationship, with an R^2 of 0.919, which exhibited 5-6 orders of magnitude difference between the two counting methods. The other three litter types had much greater variation in the relationships between the two types of counts, ranging anywhere from 5 to 15 orders of magnitude depending on the sample.

Lotic Experiment

This experiment examined the effects of light on two types of leaf litter, tulip poplar (*Liriodendron tulipifera*) and water oak (*Quercus nigra*) in experimental streams. There was a significantly greater bacterial biomass in the light treatment for the water oak litter (P=0.023) with a 30% difference between light and dark, but this was not the case for the tulip poplar litter, where the bacterial biomass was actually slightly greater in the dark (16% difference) (**Table 1, Figure 2**).

Light Mesocosm Experiment

The light mesocosm experiment examined the effects of light, time, and both light and time together on bacterial biomass of *Typha domingensis* leaf litter in the littoral zone of Lake Thoreau. Both light (P=0.029) and time (P <0.001) had a significant effect on bacterial biomass, but there was no statistically significant interaction between light and time (P=0.374) (**Table 2**). Consistent with the lotic field experiment, the bacterial biomass was much greater in the light than in the dark, with a 26% difference between the two conditions (**Figure 3**).

Invertebrate Mesocosm Experiment

This mesocosm experiment examined the effects of light on the bacterial biomass of tulip poplar leaf litter in the presence or absence of two different invertebrate species - *Pycnopsyche* caddisfly (shredder) or *Macrobrachium* shrimp (omnivorous) in experimental streams. In the caddisfly study, a statistically significant effect of time (P <0.001), but not light (P=0.185), on bacterial biomass was observed in the presence of the caddisfly (**Table 3**). Similarly, in the absence of the caddisfly, there was a statistically significant effect of time on the bacterial biomass, but not light (P <0.001 and P=0.319, respectively) (**Table 3**), with the bacterial biomass greater in the dark than in the light at a 17.6% difference (**Figure 4**). In contrast, in the presence of the caddisfly, the bacterial biomass was greater in the light (10% difference). Additionally, there was an interaction between light and time that also affected the bacterial biomass (P<0.001) (**Table 3**). On the other hand, in the presence of the shrimp, there was a significant effect of both light (P=0.0411) and time (0.0455) on the bacterial biomass, but in the absence of the shrimp, only time (P<0.001) had an effect on the bacterial biomass (**Table 3**). In both the absence and presence of the shrimp, bacterial biomass was greater in the light (14% difference and 26% difference, respectively) (**Figure 4**).

Nutrient Mesocosm Experiment (1)

This experiment analyzed the effect of light on the bacterial biomass of tulip poplar litter in both high-nutrient and low-nutrient conditions in experimental stream mesocosms. There were no significant effects noted (**Table 4**). In both high- and low-nutrient conditions, the bacterial biomass was only slightly greater in the dark (5% difference and 2% difference, respectively) (**Figure 5**).

Temperature Mesocosm Experiment

This experiment analyzed the effects of light on the bacterial biomass of *Typha domingensis* litter at four different temperatures - 7, 14, 21, and 28°C - in greenhouse mesocosms at the University of Alabama. Results indicated that overall there was no statistically significant effect of light or time on bacterial biomass, although at 7°C there was a significant interactive effect of light and time on bacterial biomass (P=0.049) (**Table 5**). In all cases, the average bacterial biomass was greater in the light, most notably so at 21°C and 28°C (**Figure 6**). In order of increasing temperatures, the percent differences were 3%, 41%, 45%, and 56%, respectively.

Nutrient Mesocosm Experiment (2)

This experiment investigated the effects of light on the bacterial biomass of *Typha latifolia* leaf litter in high-nutrient and low-nutrient conditions in greenhouse mesocosms at the University of Alabama. There were no statistically significant effects of light, time, or light and time on the bacterial biomass (**Table 6**), but in both high- and low-nutrient conditions, the bacterial biomass was higher in the light (14.5% difference and 18% difference, respectively) (**Figure 7**).

Wood Veneer Mesocosm Experiment

This experiment analyzed the effect of light, time, and both light and time on the bacterial biomass of *Quercus alba* wood veneers in both high-nutrient and low-nutrient conditions in greenhouse mesocosms at the University of Alabama. In both cases, there was a statistically significant effect of light and time (P=0.028 and P=0.025, respectively) (**Table 7**). In the low-nutrient condition, there was an effect of time as well (P=0.002), but not interactions of light and time, while in the high-nutrient condition, there was no effect of time but an interactive effect of light and time (P=0.014) (**Table 7**). Under both nutrient conditions, bacterial biomass was consistently higher in the light than in the dark (33.7% difference and 40.2% difference, respectively) (**Figure 8**).

Chapter 4: Discussion

The purpose of the first phase of this study was to generate conversion factors that could correct for underestimations of bacterial cell counts performed by flow cytometry for each of four litter types - *Typha domingensis, Liriodendron tulipifera, Quercus alba,* and *Typha latifolia*. Although four conversion factors were generated and applied to the seven experiments discussed, only the *T. domingensis* conversion factor provided a robust linear relationship between epifluorescence counts and flow cytometry counts. This is likely a result of experimental design. Compared to the litter from the other experiments, the *T. domingensis* litter pieces were more uniform. For example, in the tulip poplar experiments, leaf discs were derived from many different leaves, which would lead to varying nutrient compositions, thickness, and other characteristics, while the *T. latifolia* litter pieces differed in thickness compared to the *T. domingensis* litter pieces. Furthermore, the *T. domingensis* litter experiment was the longest of all the performed studies at eight months; the others were of comparably shorter duration. Because of this difference in methodology, the *T. domingensis* litter exhibited the highest range of bacterial biomass as measured by flow cytometry, while the other litter types exhibited much smaller ranges in bacterial abundance (see range along the X-axis in **Figure 1**). In order to refine the conversion factors produced in this study, it may be necessary to conduct longer studies with each of the representative litter types to arrive at a better understanding of the relationship between epifluorescence and flow cytometry bacterial biomass estimations.

The experiments to which these conversion factors were applied investigated the relationship of light and algae to bacteria established on decaying leaf litter in a variety of freshwater environments. Some of these studies also manipulated other factors of the environment (e.g., temperature and nutrients). A multitude of findings suggest that periphytic

algae can positively affect bacterial growth and accrual on decaying leaf litter in freshwater environments. For example, some studies have suggested that periphytic algae increase the surface area, which in turn leads to more space for bacteria to colonize, as there is a positive correlation between algal biomass and bacterial biomass (Carr *et al.* 2005; Rier & Stevenson, 2001). Additionally, the dissolved organic carbon that is released into the environment by photosynthesizing algae can be taken up and utilized by resident bacteria (Arvola & Tulcnen, 1998). Therefore, bacterial growth on leaf litter in freshwater environments may be be positively associated with light, as this means more significant periphytic algae are present.

In this study, six out of the seven experiments indicated that there was an overall average higher biomass in the light treatments, and by extension, the presence of algae. However, in only four of these experiments was the effect of light on bacterial biomass statistically significant. This overall trend is consistent with my hypothesis and previous studies showing algae stimulate heterotrophic activity, particularly bacterial growth (Kuehn *et. al* 2014) and bacterial biomass accrual (Gu & Wyatt 2016); however, the results also indicate that several other factors can either negatively or positively affect the bacterial biomass in addition to light, such as the elapsed time of litter decomposition, nutrient availability, and the presence of invertebrates.

Leaf litter type can also be important in the dynamics of microbial colonization and litter decomposition; for example, in two of the three experiments that used fast-decomposing tulip poplar litter, bacterial biomass was actually greater in the dark than in the light. In other, relatively more slowly-decomposing or recalcitrant litter types such as water oak and *Typha*, there was often greater bacterial biomass in the light, athough the degree varied across experiments. The trend that light/algae stimulate bacteria on more slowly-decomposing substrates, such as *Typha* and wood, may be consistent with algal stimulation of bacteria by

provisioning labile C that otherwise constrains bacterial activity and biomass accrual on recalcitrant substrates (Kuehn *et al.* 2014; Wyatt *et al.* 2019). Similar to my studies using submerged leaf litter, terrestrial studies have indicated that tree species have an effect on aspects of bacterial microbial communities such as nutrient uptake and enzyme activity (Amin *et al.* 2013; Weard *et al.* 2010). This observation may also extend to freshwater bacterial communities; leaf litter type may influence multiple aspects of the bacterial interactions with the leaf litter and algae.

Across my experiments, temperature effects could also influence bacterial responses to light and algae. This effect has been observed in other studies (Gu & Wyatt 2016; Pope *et al.* 2020). In the temperature mesocosm experiment, differences in bacterial biomass were the greatest between dark and light at the two highest temperatures of 21°C and 28°C. Studies indicate that algal photosynthesis reaches its peak between 20°C and 25°C (Yokohama *et al.* 1973), so the rate at which the algae are photosynthesizing may also affect bacterial growth rates and biomass accrual, indicating possibly greater effects when algal photosynthetic activity is greater.

It is possible that interactions with other microbes such as fungi may also be at play in these experiments. Competition with fungi may cause bacterial biomass to be lower due to competition for nutrients such as nitrogen and phosphorus. Indeed, it has been found that fungal biomass usually exceeds bacterial biomass, especially on larger organic substrates such as leaves and wood (Findlay *et al.* 2002). It has been observed that with the introduction of nutrients such as nitrogen and phosphorus, elevated algal biomass levels only stimulate heterotrophic fungi and not heteretrophic bacteria, implying there may be some antagonistic relationship between fungi and bacteria when it comes to nutrient competition (Wyatt *et al.* 2019). The bacterial growth in

these experiments may have been overshadowed by the responses of fungal biomass, particularly those that manipulated nutrient availability. This phenomenon could explain why many of the observed effects on bacterial biomass were not statistically significant.

It is also possible that the activity of larger invertebrates may cause disruptions in the microbial communities that are established on decaying litter. For example, studies have shown that the presence of some shredder species causes nutrient enrichment that specifically enhances fungal activity (Chung & Suberkropp 2008; Villanueva, Albariño, & Canhoto 2012). As previously stated, fungi can outcompete the bacterial communities. Bacteria are important food sources for detritivores, thus it is possible that in experiments that involve litter decay in streams and lakes, bacterial biomass may be affected by these organisms feeding on the bacterial colonies (Perlmutter & Meyer, 1991). Additionally, detritivores are selective of leaf litter type and even preferentially seek out litter that has established microbial communities, which may also account for variations in bacterial biomass (Graça 2001).

In conclusion, my study shows that light and presence of periphytic algae often positively stimulate bacterial biomass on decaying leaf litter in freshwater, but this relationship can be complicated by other aspects of the environment, sometimes even multiple aspects working together. Bacteria are important members of microbial communities, serving as decomposers as well as sources of nutrition for organisms that feed on biofilms. In the presence of algae, the species of bacteria may shift in these biofilms in response to the labile carbon being introduced, which could affect energy flow processes at the microorganism level. In addition, bacteria are responsible for many vital nutrient transformations, such as nitrogen and phosphorus. If there is greater microbial biomass on leaf litter in the light, that means more nutrients are being immobilized from the water column, which may have implications for the environment as a

whole. The relationship between heterotrophic bacterial communities and periphytic algae on leaf litter in freshwater environments is important and has the potential to substantially impact the environment. In particular it is worth investigating how the increased bacterial biomass in the light affects the nutrient supplies, and in turn, other organisms, in these environments.

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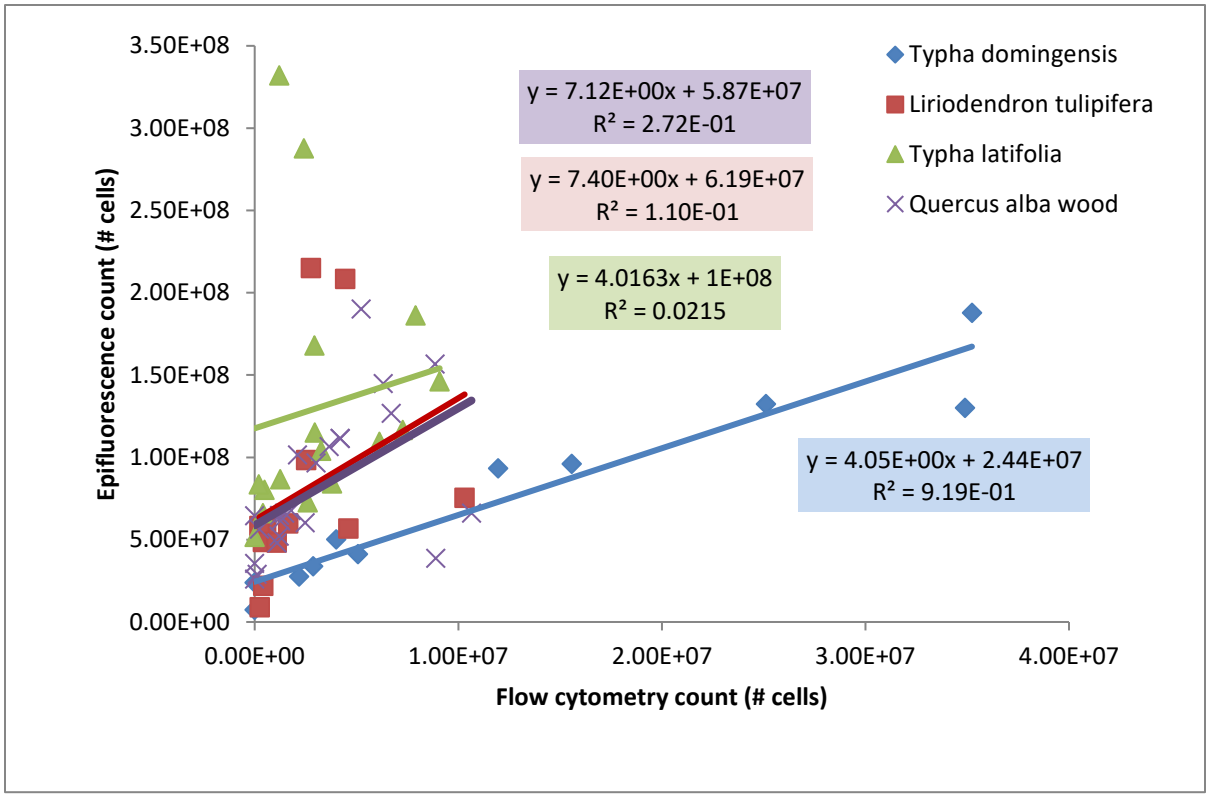

Figure 1. Line graph plot of conversion factors generated by comparison of epifluorescence microscopy bacterial cell counts and flow cytometry counts. The solid lines represent the linear equations that were generated for each litter species, which were used as conversion factors to correct flow cytometry counts.

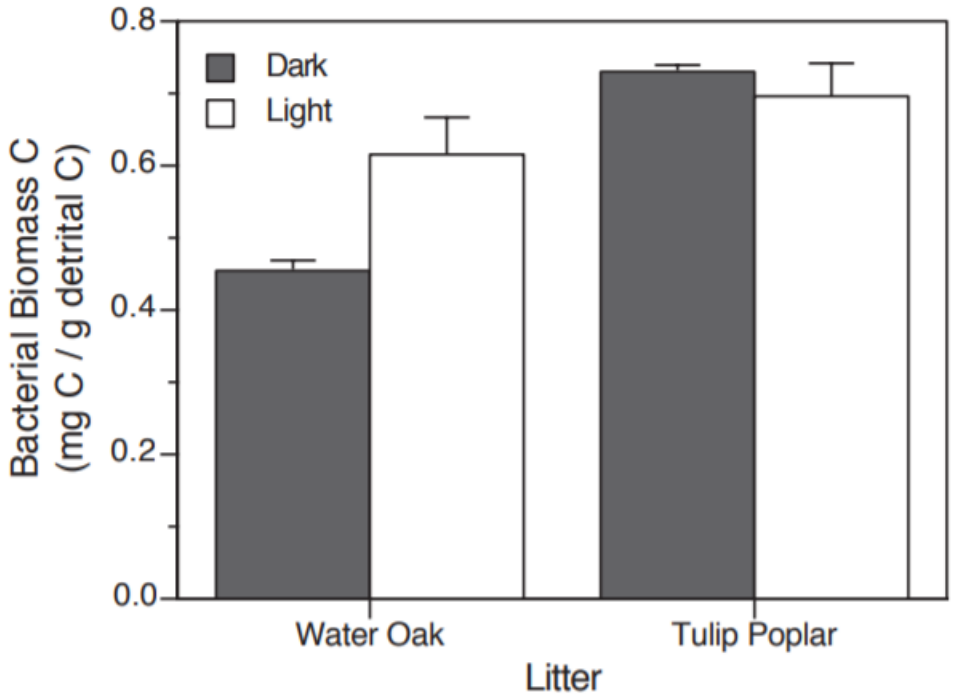

Figure 2. *Lotic Experiment*- Mean + SE bacterial biomass counts of water oak and tulip poplar leaf litter subjected to dark and light treatments. See **Table 1** for associated statistics.

Litter	Factor	F-value	P-value
Water Oak	Light	$9.12_{1,6}$	0.023**
Tulip Poplar	Light	$0.55_{1,6}$	0.487

Table 1. *Lotic Experiment*- Repeated-measures ANOVA results testing the effects of light on bacterial biomass. Data presented are only for the fourth sampling date, for which there was a full sample set available. ** denotes effects that are statistically significant.

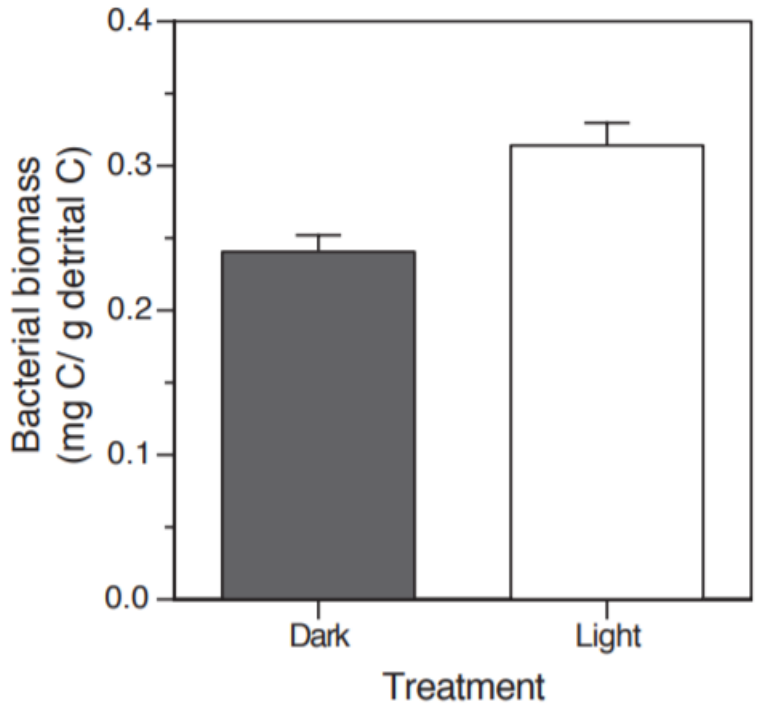

Figure 3. *Light Mesocosm Experiment*- Mean + SE bacterial biomass counts of *Typha domingensis* litter subjected to dark and light treatments. See **Table 2** for associated statistics.

Litter	Factor	F-value	P-value
Typha domingensis	Light	$6.51_{1,10}$[a]	0.029**
	Time	$31.81_{7,70}$[a]	<0.001**
	Light x Time	$1.10_{7,70}$[a]	0.374

Table 2. *Light Mesocosm Experiment*- Repeated-measures ANOVA results testing the effects of light, time, and both light and time on bacterial biomass. [a] denotes data that was log10-transformed before analysis. ** denotes effects that were statistically significant.

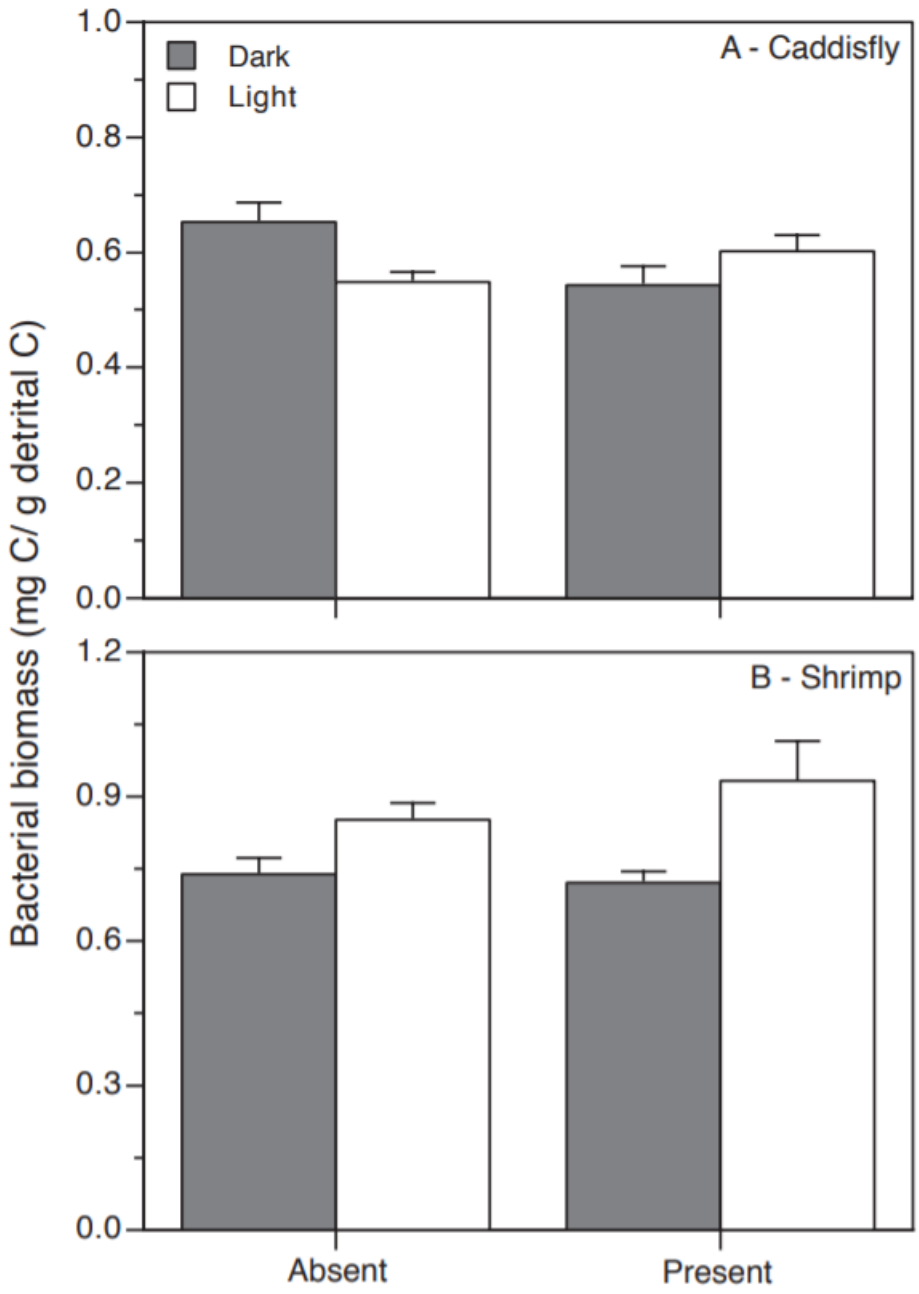

Figure 4. *Invertebrate Mesocosm Experiment*- Mean + SE bacterial biomass counts of tulip poplar leaf litter subjected to both light and dark treatments as well as presence or absence of a shredder species- A. *Pycnopsyche* caddisfly or B. *Macrobrachium* shrimp. See **Table 3** for associated statistics.

Litter	Consumer	Factor	F-value	P-value
Tulip Poplar	Caddisfly- P	Light	$2.24_{1,6}$[a]	0.185
		Time	$12.39_{3,18}$[a]	<0.001**
		Light x Time	0.70	0.567
	Caddisfly- A	Light	$7.75_{1,6}$	0.319
		Time	$25.66_{3,18}$	<0.001**
		Light x Time	$8.58_{3,18}$	<0.001**
Tulip Poplar	Shrimp- P	Light	$6.72_{1,6}$[a]	0.041**
		Time	$3.27_{3,18}$[a]	0.046**
		Light x Time	$1.22_{3,18}$[a]	0.332
	Shrimp- A	Light	$5.69_{1,6}$[a]	0.544
		Time	$10.03_{3,18}$[a]	<0.001**
		Light x Time	$0.53_{3,18}$[a]	0.670

Table 3. *Invertebrate Mesocosm Experiment*- Repeated-measures ANOVA results testing the effects of light, time, and both light and time on tulip poplar litter either in the presence (P) or absence (A) of shredder species *Pycnopsyche* caddisfly and *Macrobrachium* shrimp. [a] denotes data that was log10-transformed before analysis. ** denotes effects that were statistically significant.

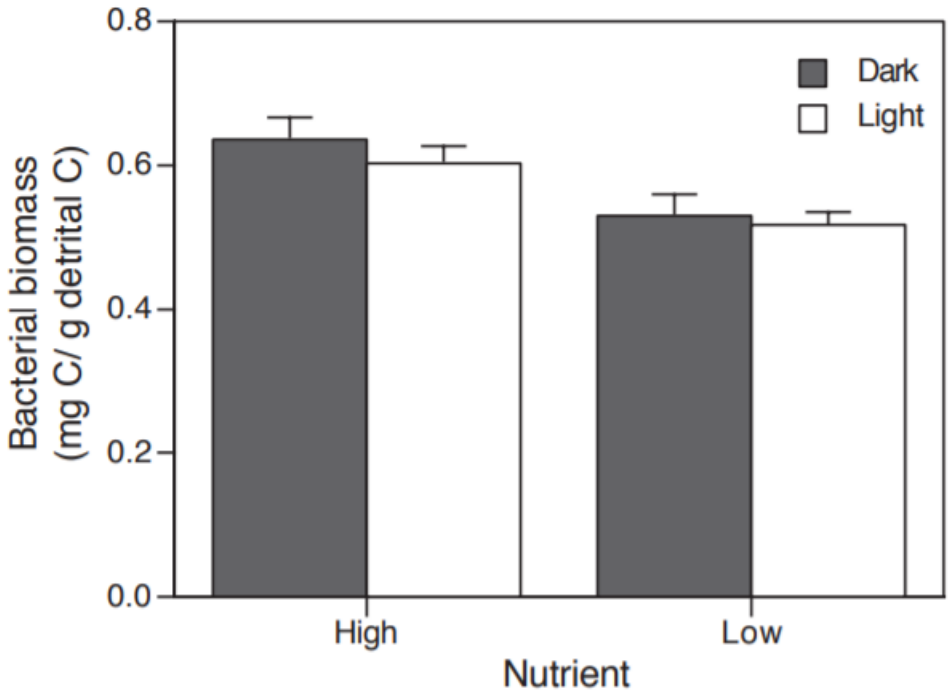

Figure 5. *Nutrient Mesocosm Experiment (1)*- Mean + SE bacterial biomass counts of tulip poplar leaf litter subjected to both light and dark treatments and high and low nutrient treatments. See **Table 4** for associated statistics.

Nurtient	Factor	F-value	P-value
Low Nutrient	Light	$0.14_{1,6}$	0.725
High Nutrient	Light	$0.74_{1,6}$	0.424

Table 4. *Nutrient Mesocosm Experiment (1)*- Repeated-measures ANOVA results testing the effects of light on high-nutrient and low-nutrient tulip poplar samples subjected to light and dark treatments.

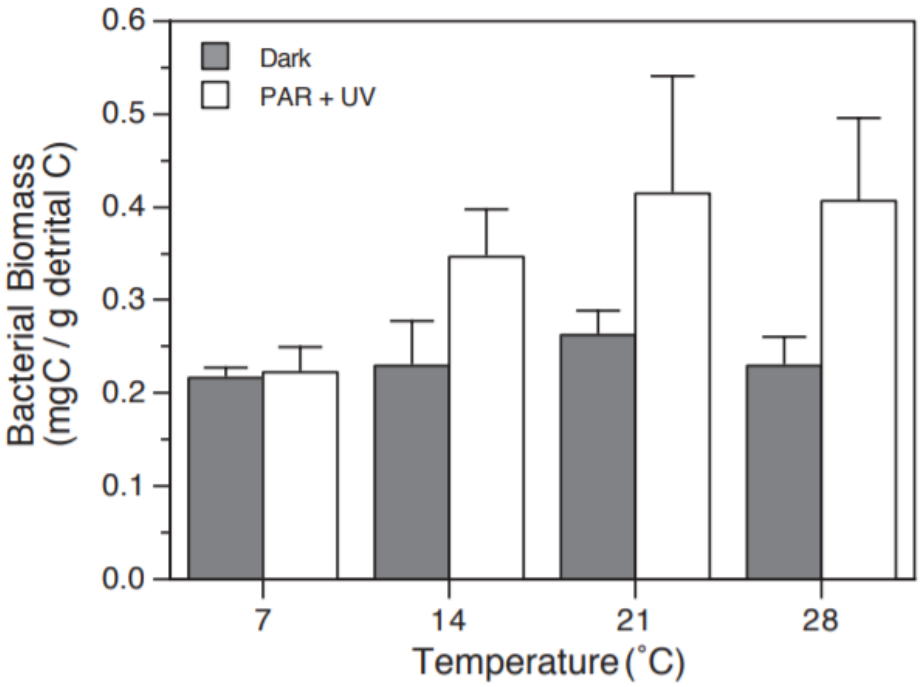

Figure 6. *Temperature Mesocosm Experiment*- Mean + SE of bacterial biomass counts of *Typha domingensis* leaf litter subjected to light and dark treatments at four different temperatures - 7, 14, 21, and 28 degrees Celsius. See *Table 5* for associated statistics. PAR + UV refers to photosynthetically active radiation plus ultraviolet light.

Temperature	Factor	F-value	P-value
7°C	Light	$<0.01_{,4}$[a]	0.623
	Time	$0.09_{1,4}$[a]	0.059
	Light x Time	$6.82_{1,4}$[a]	0.04**
14°C	Light	$2.80_{1,4}$	0.17
	Time	$<0.01_{1,4}$	0.98
	Light x Time	$0.26_{1,4}$	0.64
21°C	Light	$1.21_{1,4}$	0.333
	Time	$0.70_{1,4}$	0.451
	Light x Time	$0.04_{1,4}$	0.862
28°C	Light	$4.56_{1,4}$	0.100
	Time	$0.57_{1,4}$	0.492
	Light x Time	$0.03_{1,4}$	0.864

Table 5. *Temperature Mesocosm Experiment*- Repeated-measure ANOVA results testing both the effects of light, time, and both light and time on *Typha domingesis* litter subjected to light and dark treatments at four different temperatures. [a] denotes data that was log10-transformed prior to analysis. ** denotes effects that were statistically significant.

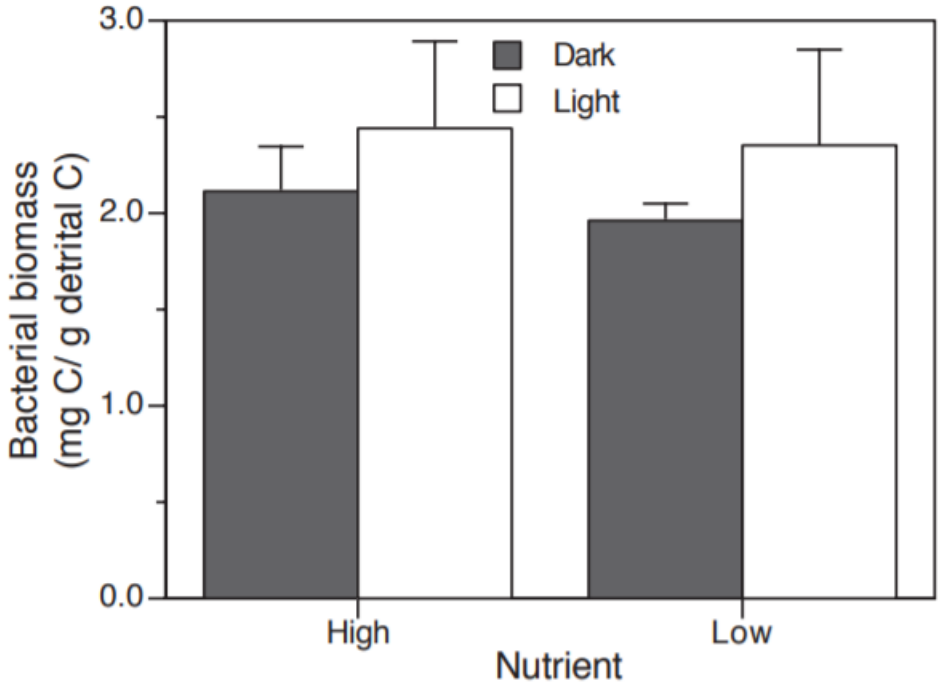

Figure 7. *Nutrient Mesocosm Experiment (2)*- Mean + SE bacterial biomass counts of *Typha latifolia* litter subjected to light and dark treatments at both high and low nutrient concentrations. See **Table 6** for associated statistics.

Nutrient	Factor	F-value	P-value
Low Nutrient	Light	$0.503_{1,4}$[a]	0.517
	Time	$0.183_{1,4}$[a]	0.691
	Light x Time	$0.544_{1,4}$[a]	0.502
High Nutrient	Light	$0.414_{1,4}$	0.555
	Time	$2.119_{1,4}$	0.219
	Light x Time	$0.085_{1,4}$	0.785

Table 6. *Nutrient Mesocosm Experiment (2)*- Repeated-measures ANOVA results testing the effects of light, time, and light and time on *Typha domingensis* litter subjected to light and dark treatments at both high and low nutrient concentrations. [a] denotes data that was log10-transformed before analysis.

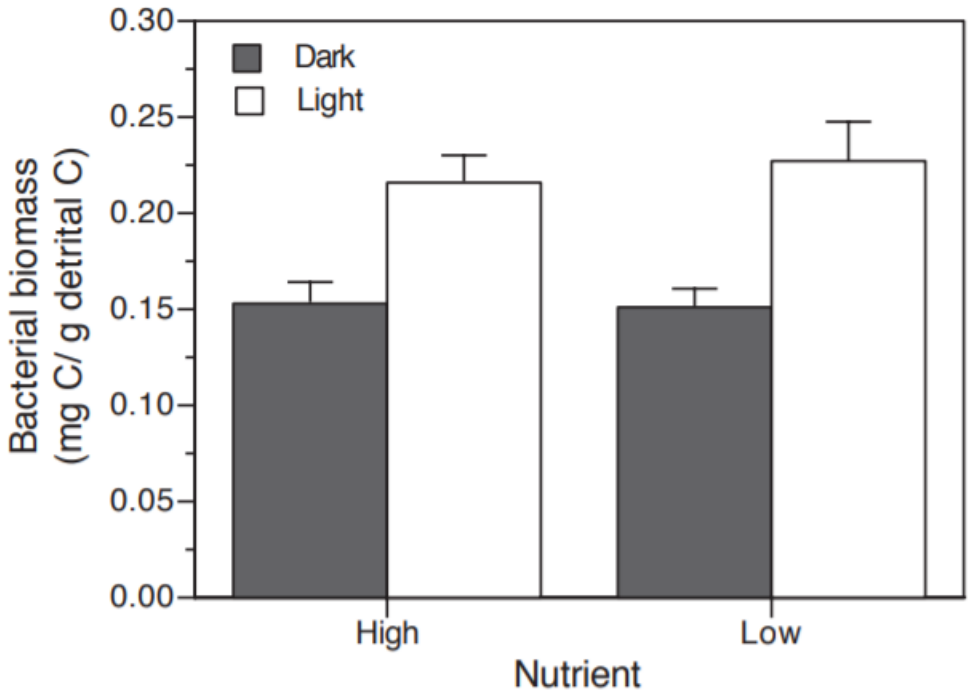

Figure 8. *Wood Veneer Mesocosm Experiment*- Mean + SE bacterial biomass counts of *Quercus alba* litter (wood veneers) subjected to light and dark treatments at both high and low nutrient concentrations. See **Table 7** for associated statistics.

Nutrient	Factor	F-value	P-value
Low Nutrient	Light	$11.33_{1,4}$	0.0281**
	Time	$50.950_{1,4}$	0.00204**
	Light x Time	$0.007_{1,4}$	0.93664
High Nutrient	Light	$12.11_{1,4}$	0.0253**
	Time	$0.854_{1,4}$	0.4076
	Light x Time	$17.148_{1,4}$	0.0144**

Table 7. *Wood Veneer Mesocosm Experiment*- Repeated-measures ANOVA results testing the effects of light, time, and light and time on *Quercus alba* litter subjected to light and dark treatments at both high and low nutrient concentrations. ** denotes effects that were statistically significant.