# Algal priming mediates the effects of light and nutrients on organic matter processing: insights from artificial and natural streams

by

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## Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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## Abstract

Environmental factors such as light and nutrients may play important roles in determining detrital decomposition through their effects on primary production. Thus, human activities that alter the availabilities of light and nutrients may have direct consequences on organic matter (OM) processing and nutrient cycling in freshwater ecosystems. I compared heterotrophic function (OM processing and respiration) between two P levels (5 µg P $L^{-1}$ and 51 µg P $L^{-1}$) and two shade levels (0% and 80% shade) in natural streams, as well as 3 P levels (10 µg P $L^{-1}$, 50 µg P $L^{-1}$ and 100 µg P $L^{-1}$) and 4 shade levels (0%, 50%, 80% and 100%) in artificial streams using the cotton-strip assay (CSA). Data from these experiments show a negative association between algal abundance (GPP and chlorophyll *a*) and recalcitrant OM (ROM) processing, implying a negative priming effect. Light was an important driver of negative priming which disproportionately affected ROM decomposition at lower P treatments over time. Overall, there were limited interactive effects between light and nutrient availability; rather, ROM processing was positively associated with P availability at all light levels while it was negatively associated with light availability at all P levels. There was no evidence to support positive priming (i.e., algal stimulation of ROM processing) in either experiment. Overall, results from this study illustrate the importance of considering light levels and nutrient availability when considering long term carbon and nutrient budgets in freshwater ecosystems.

## Summary for Lay Audience

Environmental factors such as light and nutrients may play important roles in determining detrital decomposition through their effects on primary production. Thus, human activities that alter the availabilities of light and nutrients may have direct consequences on organic matter (OM) processing and nutrient cycling in freshwater ecosystems. I used the cotton-strip assay (CSA), with artist's fabric as the designated OM substrate, to test an existing light-nutrient priming effect (PE) model in both natural and artificial streams. The light-nutrient PE model predicts how light availability mediates recalcitrant organic matter (ROM) processing at different levels of nutrient concentration. Two streams in southwestern Ontario, Canada were selected for a 21-day field study based on differing bioavailable phosphorus (P) concentrations to produce high (51 μg P $L^{-1}$) and low (5 μg P $L^{-1}$) P treatments. Light levels were manipulated through shade cloth to produce ambient and shaded (20% of existing light level) treatments. Recirculating sinuous flume mesocosms were used for a 28-day artificial stream experiment. Light (0%, 20%, 50% and 100% available light) and P (10, 50 and 100 μg P $L^{-1}$) availability were manipulated using a fully factorial design. Mesocosms were also saturated with 1500 μg $L^{-1}$ of dissolved nitrogen (N). For both experiments, I measured algal abundance (i.e., gross primary production and chlorophyll *a* concentration), ROM decomposition (i.e., tensile loss of cotton substrates) and community respiration. Data from these experiments show that algal presence inhibits decomposition through a negative priming effect. Light was an important driver of negative priming which disproportionately affected ROM decomposition at lower P treatments over time. Overall, there were limited interactive effects between light and nutrient availability; rather, they appeared to act mostly independent of one another. ROM processing was positively associated with P availability and negatively associated with light availability. There was no evidence of positive priming (i.e., algal stimulation of ROM processing) found in either experiment. Overall, these results illustrate the importance of considering light levels and nutrient availability when considering long term carbon and nutrient budgets in freshwater ecosystems.

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## 1. Introduction

Organic matter (OM) processing refers to the degradation of larger organic materials (such as dead plant and animal materials) into smaller organic and inorganic forms. In most streams, processing of OM is regarded as being largely biological (Ferreira et al., 2015) with microbial degradation being the key mechanism governing rates of OM break down (Rader et al., 1994; Chellaiah and Yule, 2018). According to a growing body of evidence, microbial OM processing is influenced by algal communities through a *priming effect* (Kuzyakov, 2010; Guenet et al., 2010; Benggston et al., 2018), which is a term used to describe the process through which heterotrophic decomposition of OM is modified by algae through several physical, chemical and biological processes in OM biofilms. Priming is well known in terrestrial soils and involves the release of labile dissolved organic matter into the rhizosphere by plants, which facilitates microbial decomposition of the more recalcitrant components of the soil OM pool (Kuzyakov 2010). Similarly, in aquatic environments, labile carbon (C) released by algae provides a source of C and energy for heterotrophic decomposers, which may subsequently stimulate the decomposition of more recalcitrant terrestrially derived organic matter (ROM; Guenet et al.2010, Kuehn et al. 2014). Accordingly, several researchers have reported enhanced ROM processing when algae and heterotrophs co-occur in benthic biofilms, with modifications ranging from 26-120% (Guenet, 2010). However, despite this evidence, numerous studies have also shown either no priming effect or negative priming whereby preferential use of labile C results in significant inhibition of microbial OM processing when algae are present (Halvorson et al., 2019; Soares et al., 2017).

Given these connections between autotrophic primary production and heterotrophic decomposition, it is thus likely that the response in OM processing is mediated by the factors that constrain algal growth. Because primary production in streams is often limited by the availabilities of light and nutrients, it is thus likely that heterotrophic function, too, is mediated by these resources. For instance, priming is expected to be positive in nutrient-stressed conditions when labile C produced through photosynthesis is used as a high-quality energy source to stimulate decomposition of recalcitrant OM by heterotrophic organisms. This effect, however, can turn negative in nutrient-rich environments where labile C, along with nitrogen (N) and phosphorus (P) from the water-column, are preferentially consumed (Kuzyakov, 2002) over the more recalcitrant, terrestrially derived materials. While numerous studies have attempted to validate this hypothesis

in aquatic ecosystems, the results thus far have been inconclusive. For instance, some studies have found evidence of positive priming effects under nutrient-limited conditions (Danger et al., 2013; Halvorson et al., 2016) while others have observed the most significant positive priming in nutrient-enriched settings (eg. Howard-Parker et al., 2019). Meanwhile, some studies have found no evidence of priming at all in aquatic ecosystems (Catalan et al., 2015; Soares et al., 2017; Elosegi et al., 2018). Due to this incongruent evidence, the goal of my study was therefore to further examine how light and nutrient availability interactively mediate heterotrophic OM processing in stream ecosystems.

## 1.1 Literature Review

### *1.1.1 Drivers of Organic Matter Processing*

OM is composed of organic compounds that have come from the feces and remains of organisms such as plants and animals. In streams, OM is critical as it provides a key energy source upon which stream food webs are based as well as many important ecosystem functions like nutrient retention and recycling. While some stream food webs can obtain energy from instream primary productivity (e.g., Bott et al. 1985), many stream food webs, especially those in heavily shaded headwater streams, are primarily driven by inputs of OM from the terrestrial environment (Tank et al. 2010). Of the terrestrial material entering the water column, leaf litter represents a major fraction, but other materials such as bark, wood and the feces or remains of dead or decaying organisms are also included. Once this material enters the lotic environment, it is immediately colonized by fauna and the breakdown process is initiated (Graca, 2001; Tank et al., 2010).

OM breakdown (or OM processing) refers to the process through which organic materials are broken down into smaller, inorganic forms (such as $CO_2$ or inorganic forms of nutrients) to be cycled back to the environment. In streams, there are several overlapping processes that contribute to OM processing. These include 1) leaching, 2) microbial conditioning, and 3) fragmentation by invertebrate shredding, as well as the physical abrasion of materials (Albelho, 2001). Leaching of soluble materials occurs mostly during the first days of immersion with rates of loss being dependent on the type of litter and its associated characteristics (Gessner, 1991). Microbial

conditioning is the result of microbial colonization in the water body, which causes mass loss when microbial decomposers, such as bacteria and fungi, assimilate C from the litter into their microbial biomass (Gulis et al., 1995). Lastly, physical fragmentation occurs when shredders or other materials (ie., sediment) break larger particles of OM into smaller pieces, which is important because it exposes more surface area to microbial action and facilitates consumption for other macroinvertebrates (Graca, 2001).

While all processes are critical, the processing of OM is regarded as being largely biological (Ferreira et al., 2015) with microbial degradation being the key mechanism governing rates of OM break down in most streams (Rader et al., 1994; Chellaiah and Yule, 2018). Microbial degradation of OM in streams is driven by heterotrophic organisms that use a mixture of hydrolytic and oxidative extracellular enzymes to mineralize C compounds (Luo et al., 2017). Generally, recalcitrant organic materials like plant litter are too large for direct absorption through cell membranes; thus, hydrolysis and oxidation by extracellular enzymes are required to break molecules down into smaller components to be moved across the cell membranes of heterotrophic organisms. These steps are considered integral components of nutrient cycling in aquatic ecosystems, and due to high energy demands, are often referred to as rate-limiting steps in OM processing (Boschker, 1997). Inputs of labile C from algae, however, can help overcome these rate-limiting steps by providing a high-quality energy source for increasing the expression of extracellular enzymes used by heterotrophic decomposers (Keuhn et al., 2014).

### *1.1.2 Benthic Biofilm Ecology and Algal Priming*

Microbial conditioning of OM occurs extensively in biofilms, making them 'hotspots' for mineralization and nutrient cycling in aquatic ecosystems (Guenet et al., 2010; Chellaiah and Yule, 2018). Biofilms are complex assemblages of surface-associated microbiota that develop on organic surfaces and other benthic substrata (eg. cobble). They are ubiquitous in streams and commonly include both autotrophic and heterotrophic organisms (Battin et al., 2003). Autotrophs in biofilms mostly include species of algae but can also include some phototrophic bacteria and protists (Allan & Castillo, 2007). When conditions promote the growth of phototrophic organisms, these communities serve as important primary producers and can thus contribute significantly to the net

primary production of an ecosystem (Biggs, 1996; Battin et al., 2003). In contrast, heterotrophic organisms in biofilms include certain types of bacteria and fungi who obtain their energy through the consumption of the dead or decaying OM (Allan & Castillo, 2007). By breaking down organic materials, heterotrophic organisms are responsible for much of the P, N, and C cycling that occurs in aquatic systems (Allan & Castillo 2007).

Biofilms, which are often only millimetres in thickness, are considered independent microbial landscapes (Battin et al., 2007). They are separated from the surrounding water column by an extracellular polysaccharide matrix (EPS) which acts as a barrier to trap extracellular enzymes, their lysis products, nutrients, and other by-products of algal and microbial respiration inside the biofilm (Freeman and Lock, 1995). Due to the confinement of these materials within the EPS matrix, interactions within periphytic communities are favoured. These interactions involve the sharing and exchanging of resources needed for growth and metabolism (Sinsabaugh et al., 2013), which may subsequently affect rates of OM processing in stream environments when heterotrophic organisms are involved. While there are many factors that influence microbial OM processing such as the size, chemical composition and elemental stoichiometry of the organic molecules (Chróst, 1992; Amon and Benner, 1996) as well as water temperature (Davidson & Janssens, 2006; Fereirra & Chauvet, 2011; Gholz et al, 2000), there is increasing evidence to suggest that microbial OM processing is in fact influenced by linkages with autotrophic production. According to a growing body of evidence, microbial OM processing is mediated by algal communities through a *priming effect* (Kuzyakov, 2010; Guenet et al., 2010; Benggston et al., 2018), which describes the process through which heterotrophic decomposition of OM is either inhibited or stimulated by algae through several physical, chemical and biological processes.

While priming has been shown to affect the processing of both dissolved (Hotchkiss et al., 2014; Morling et al. 2017; Wyatt & Rober 2020) and particulate (Rier et al. 2007, Guenet et al. 2010, Danger et al. 2013, Halvorson et al. 2016; Soares et al. 2017, Howard-Parker et al. 2020) OM, most studies tend to focus on the effects of priming on more recalcitrant, terrestrial derived OM (i.e., ROM). Compared to OM produced within the water body (i.e., autochthonous C produced through photosynthesis), ROM is relatively unavailable to heterotrophic organisms due to its complex molecular structure. Moreover, ROM has a lower C:N ratio making it less energetically favorable for heterotrophic organisms to obtain essential nutrients from (Tank et al.,

2010). Algae, however, can facilitate heterotrophic processing of ROM by providing high-quality energy sources for heterotrophic decomposers and by altering the physical and chemical conditions within biofilms.

Algae have been shown to alter several environmental conditions within biofilms including the pH, dissolved oxygen (DO) availability, and labile organic matter (LOM) availability (Wetzel, 2001) during photosynthesis (**Fig. 1.1**). These environmental changes may subsequently alter the behavior of the microbial communities within biofilms, thus influencing rates of ROM processing. For example, labile C released by algae such as photosynthate (a dissolved carbon compound primarily composed of simple carbohydrates and nitrogen and phosphorus rich amino acids (Jones & Canon, 1986; Biddanda & Benner, 1997)) provide heterotrophic bacteria and fungi with a high-quality source of C and energy, which may subsequently stimulate the decomposition of the recalcitrant, terrestrially derived OM (Kuehn et al. 2014; Wyatt & Turetsky, 2015). Algae also increase the pH and DO concentration within biofilms through photosynthesis which may enhance ROM decomposition by producing chemically favourable conditions for both oxidative and hydrolytic enzyme function of the heterotrophic organisms (Rier et al., 2007; Rier et al., 2014). Lastly, algae increase the surface area available for bacterial and fungal colonization (Rier and Stevenson, 2001), also potentially leading to enhanced rates of OM processing in stream environments.

Accordingly, several researchers have reported enhanced ROM processing (ie., a positive priming effect) in response to labile C exudates (Danger et al., 2013; Hotchkiss et al., 2014; Bianchi et al., 2015). However, numerous studies have also reported either no priming or significant inhibition of microbial ROM processing (ie., a negative priming effect) where preferential use of algal-derived labile C by heterotrophic organisms results in reduced decomposition of ROM (Lutz et al. 2012, Halvorson et al. 2016, Elosegi, 2018; Halvorson et al., 2019; Soares et al., 2017). To explain these divergent results, Guenet et al. (2010) suggested that the magnitude and direction of priming is largely dependent on environmental factors such as light and nutrient availability in aquatic ecosystems.

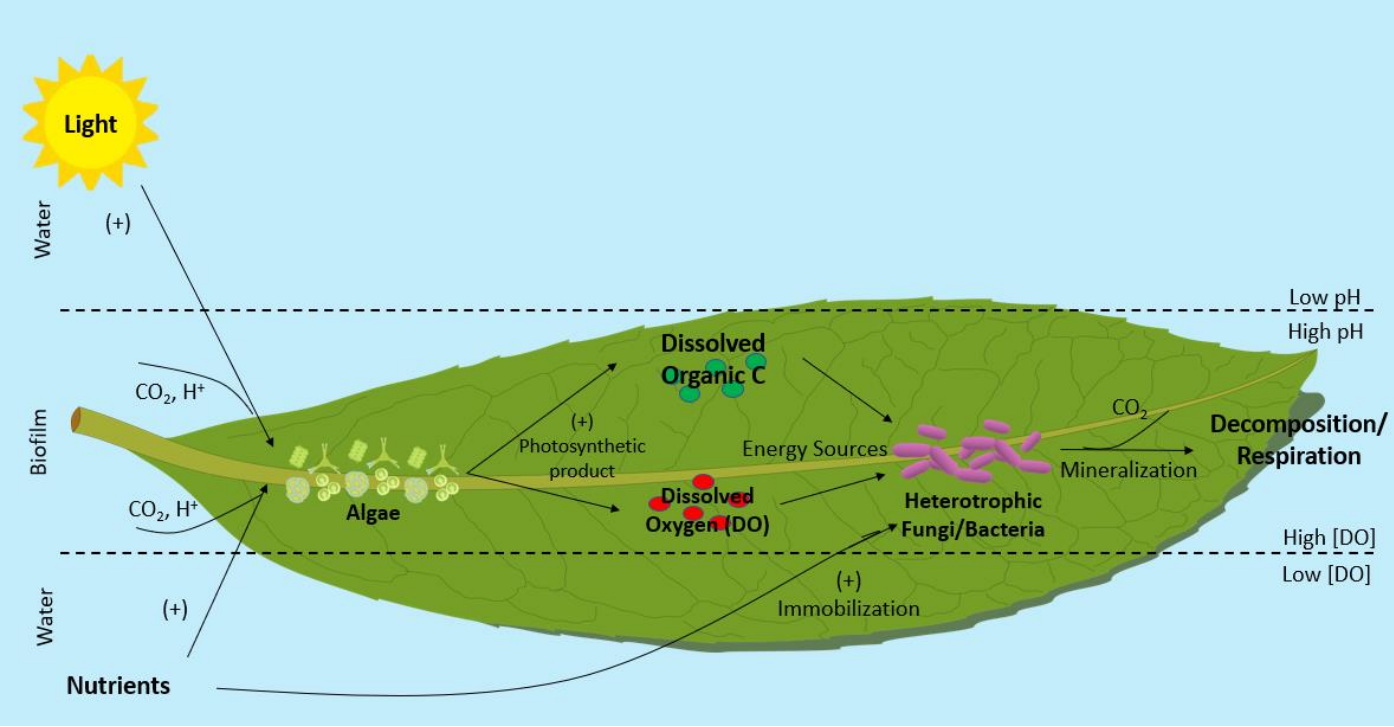


**Figure 1.1.** Schematic diagram of how light and nutrients mediate heterotrophic function through algal priming in organic matter (OM) biofilms. Arrows represent matter/energy transfers. Plus (+) signs represent a positive or stimulatory response.

### *1.1.3 Light and Nutrients as Controls of Algal Priming*

Given the linkages between autotrophic and heterotrophic organisms within stream biofilms, OM processing may be interactively mediated by the factors that constrain algal growth. Nutrients, particularly the availabilities of N and P, are among the major limiting factors for algal growth in many streams (Taulbee et al., 2005). Thus, it is likely that N and P availability play a strong role in controlling the direction and magnitude of algal priming in stream environments (Danger et al., 2013; Halvorson et al., 2016; Howard-Parker et al., 2020). Based on a series of priming studies from the terrestrial environment, Guenet et al., (2010) proposed a model that predicts a how algal priming intensity behaves at different nutrient concentrations in aquatic ecosystems. The model proposes a positive priming effect under nutrient limited (i.e., oligotrophic) conditions which then shifts to either a neutral or negative priming effect as nutrient concentration increases.

Priming is expected to be positive under nutrient limitation due to competition between decomposers and primary producers within the biofilm. When competition for limiting nutrients is maximized, algae are said to increase the proportion of labile C exudates released through photosynthesis (Wyatt et al., 2014). Energy released through metabolizing the labile C from algal exudates may prime heterotrophic organisms towards greater decomposition of ROM so that limiting nutrients can be obtained to support their energetic and dietary needs (Kuzyakov, 2002). In contrast, when nutrients are readily available in the water column, competition for limiting nutrients between autotrophic and heterotrophic organisms subsides. As a result, heterotrophs may preferentially use excess nutrients and algal exudates as an energy source for growth and reproduction (Kuzyakov, 2002) rather than investing energy in extracellular enzymes to free C, N and P from ROM (Halvorson et al., 2016). As a result, the effect of priming on ROM decomposition will weaken or become negative at these higher nutrient concentrations. Halvorson et al. (2019) confirmed this prediction in a meta-analysis using a small dataset. This study found that algal priming of ROM was generally positive at lower water-column dissolved N and P concentrations and negative at higher water-column concentrations.

In addition to nutrients, algal production is also limited by light availability, particularly in forested headwater streams (Hill et al., 1995; Mosisch et al., 2001). For example, algal biomass has been shown to be positively correlated with light availability during the accrual period,

resulting in rapid biomass formation when light is readily available (Singh and Singh, 2015). Thus, by increasing algal presence within the biofilm, human activities that increase light availability (i.e., removal of riparian canopy cover) may prime heterotrophic organisms towards positive priming due to greater bulk labile C availability within the biofilm (Halvorson et al., 2016). However, if labile C exudates, along with the available water-column N and P are sufficient to fulfill the dietary needs of heterotrophic organisms, preferential substrate use may result under these conditions, thus leading to a negative priming effect. Moreover, given that high light availability is also associated with nuisance algal growth and high proportions of filamentous green algae in upper biofilm layers of the biofilm, it may also lead to a phenomenon known as 'smothering'. By limiting the diffusion of essential resources like light, nutrients, and oxygen (Stevenson & Glover, 1993), smothering may negatively affect ROM processing, resulting in a negative priming effect.

In response to these conflicting ideas, Howard-Parker et al., (2020) created a conceptual model to help explain how light and nutrients interact with one another to influence ROM decomposition in aquatic ecosystems (**Fig. 1.2**). This model shows that as microbial growth (including both autotrophic and heterotrophic organisms) is released from nutrient limitation, an increase in ROM decomposition occurs, but only when light availability is limited. The authors expected a positive relationship to occur under these conditions because increased nutrient availability can both directly benefit heterotrophic function (i.e., they can directly acquire it from the water-column) as well as indirectly through stimulation of algal growth and hence labile C availability. However, under high light conditions (i.e., unshaded conditions), nutrient enrichment may result in decreased ROM decomposition. Here, the relationship between ROM decomposition and nutrient availability is expected to be negative because when neither light or nutrients are limiting to algal growth, accumulation of algae within the biofilm may result in preferential substrate use or potential smothering of the biofilm. Priming effects (PEs) in this model are calculated as the difference in ROM decomposition rates between biofilms grown in the presence of algae (i.e., high light) and biofilms grown without algae (i.e., low light). Like in the Guenet, (2010) model, a positive PE is expected under low nutrient conditions whereas a negative PE is expected under nutrient enriched conditions.

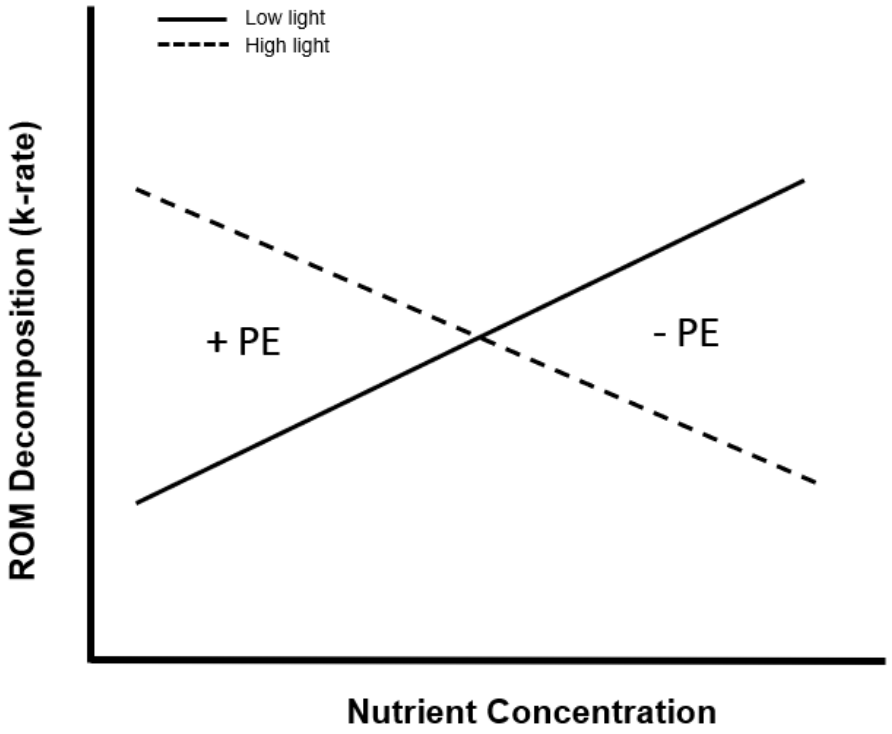


**Figure 1.2.** Light-Nutrient Priming Effect (PE) model modified from Howard-Parker et al. (2020). Recalcitrant organic matter (ROM) decomposition k-rates are presented on the y-axis and nutrient concentrations on the x-axis. Light availability is represented through solid (low light) and dotted (high light) lines. Priming effect (PE) are calculated as the difference in ROM decomposition between high-light and low-light incubated biofilms. The model assumes that the PE positive under low nutrient conditions which will then shift to a negative PE at higher nutrient concentrations.

Based on recent studies, there is evidence to suggest that light and nutrients do in fact interact with one another to influence ROM decomposition in aquatic ecosystems. Notably, studies by Howard-Parker et al., (2020) and Ashberry et al., (2021) found that nutrients (specifically P) and light interacted to influence algal biomass and labile C dynamics, which in turn affected detrital conditioning by microbial communities. Results of these studies differed, however, in that the greatest positive priming was observed in ambient treatments at the highest examined P concentration (500 µg P $L^{-1}$) in Howard-Parker et al., (2019), whereas Ashberry et al., (2021) found that decomposition was significantly decreased by ambient light at similar P concentrations, implying a negative priming effect of algae on OM processing. Conversely, at low nutrient concentrations (10 µg P $L^{-1}$), priming was shown to be positive in Howard-Parker et al., (2020) whereas Ashberry et al., (2021) found no evidence of positive priming at nutrient concentrations below 16 µg P $L^{-1}$. A third study by Elosegi et al., (2018), which examined the interactive effects of light and nutrients in real streams, found no evidence to support that algal priming is of any importance in the natural environment. Despite these differences, it is evident that both light and nutrients play a crucial role in controlling the magnitude and direction of priming, and that further studies are needed to understand their roles more clearly.

## 1.2 Knowledge Gaps

Given the linkages between autotrophic and heterotrophic communities, it is likely that the response in heterotrophic OM processing is interactively mediated by the availabilities of light and nutrients through modifications in primary production. Although several studies have quantified the consequences of algal presence on rates of heterotrophic function, there remains a notable gap in our understanding of how fluctuations in light and nutrient levels impact OM processing through their effects on primary production. To date, most studies examining the interactive effects of light and nutrients have produced inconclusive results as to which way priming behaves under different light and nutrient conditions, with outcomes ranging from strongly positive to strongly negative under similar environmental conditions. Moreover, these studies have been limited in the scope of light levels considered, often encompassing only "light" and "dark" conditions. Thus, to gain a more comprehensive understanding of this relationship, it is crucial to further investigate linkages

between light, nutrients and heterotrophic function, particularly across a broader spectrum of light levels.

Further, it has not yet been established whether there is a relationship between measures of algal assemblage and rates of OM processing in streams. That is, it is not yet known whether modifications to algal abundance for example, are associated with changes in heterotrophic function. Given that algal communities globally are being impacted by increasing anthropogenic nutrient inputs and losses of riparian vegetation, understanding these linkages would offer valuable insight into knowing how heterotrophic function may respond to human-driven environmental changes on stream ecosystems.

Lastly, because the bulk of existing research has predominantly been conducted within tightly controlled laboratory environments, there remains a significant knowledge gap in the understanding of the algal priming within the natural environment. At present, only a singular field investigation has been done to examine the effects of priming in natural streams. This study, however, took place in the fall season when algal accrual was at a minimum. Thus, understanding the implications of algal priming in the summer months is imperative in order to better inform management decisions during times when algal accrual is more substantial.

## 2.0 Research Objectives

The purpose of this study was to determine how light and nutrient availability (specifically P) interactively mediate heterotrophic function (measured as decomposition and respiration) through their effects on algal assemblages in streams. This goal was achieved by conducting parallel experiments using both natural and artificial streams. In the natural stream experiment, I aimed to assess the validity of the light-nutrient priming effect model presented by Howard-Parker et al., (2020) which concerns the mediation of OM processing in aquatic ecosystems by light and nutrient availability. My primary objective for this experiment was therefore to compare microbial function among streams exposed to varying light and nutrient availabilities.

Given the inherent limitations of controlling factors in a natural stream environment, I also conducted an artificial stream experiment to examine this relationship in a more controlled setting. Although I used a more nuanced experimental design, I maintained the same primary objective for this experiment which was to compare microbial function among streams exposed to varying light and nutrient availabilities. In addition, I included a further research objective which was to determine if the relative effects of light and nutrients on microbial function can be explained by algal assemblages (i.e., changes in algal abundance).

## 2.1 Hypotheses

**Objective 1:**

I predicted that the interactive effects of light and nutrients on OM processing in the field experiment would reflect the light-nutrient PE model presented by Howard Parker et al. (2020). Given the inherent connection between OM processing and heterotrophic respiration, I expected the relationship between light, nutrients, and respiration would be the same as described in this model. Thus, I expected P availability to increase both OM processing and respiration under low light conditions and suppress them under high light conditions. Like in Howard-Parker et al., (2020), priming was expected to be positive under P limitation and negative under P enriched conditions (**Fig. 2.1**). In contrast to the initial model, however, I chose to represent these relationships with curvilinear response curves to highlight how this relationship is not expected to be linear in nature.

My predictions for the artificial stream experiment were similar to those described for the field experiment such that positive priming was expected at low P concentrations and negative priming was expected at high P concentrations. However, since this experiment was done in a laboratory setting, more light and P levels were examined (**Fig. 2.2**). My predictions for each light level investigated were as follows:

- *No light (0% light availability)* – Given limited light availability, algae were expected to be absent or nearly absent under these conditions. Thus, only a limited stimulatory effect of P enrichment on heterotrophic activity was expected.
- *Low light (20% light availability)* – I expected P enrichment to increase heterotrophic activity under low light conditions due to the alleviation of P limitation. However, beyond moderate P levels, I expected this relationship to plateau given that saturating concentrations for heterotrophic activity would be reached.

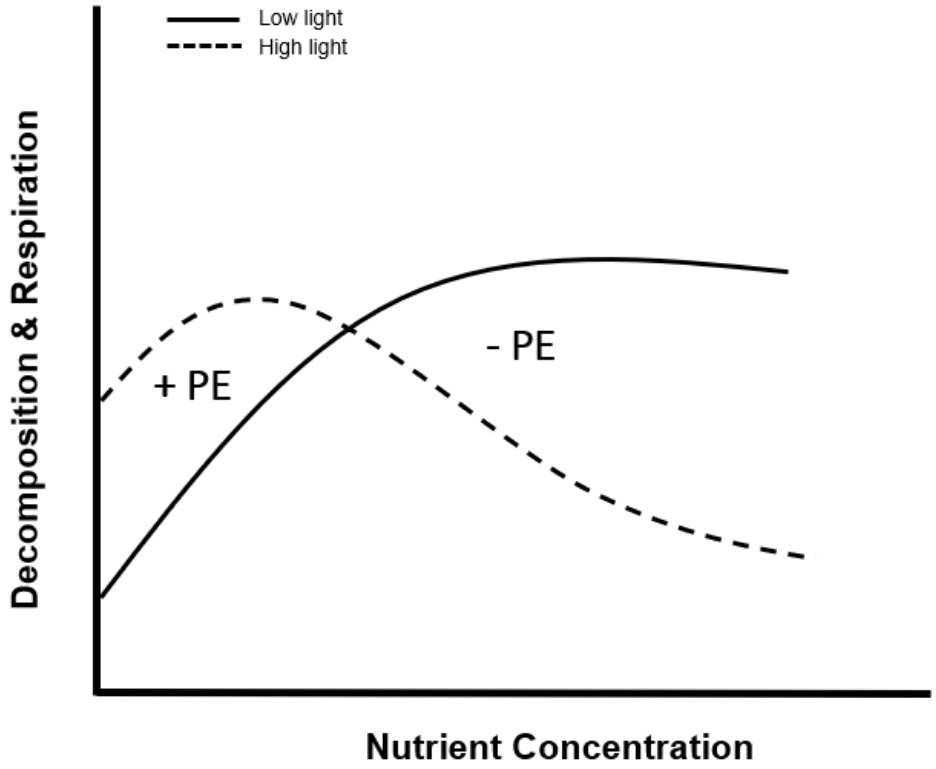


**Figure 2.1.** Prediction of how light and nutrients will interactively mediate heterotrophic function (decomposition and respiration) in natural streams. Rates of heterotrophic activity (ROM decomposition and respiration) are presented on the y-axis with nutrient availability on the x-axis. Light availability is represented through either solid or dotted lines. Note that the trend lines are drawn linearly here to represent a trend and not because the relationship is expected to be linear in nature.

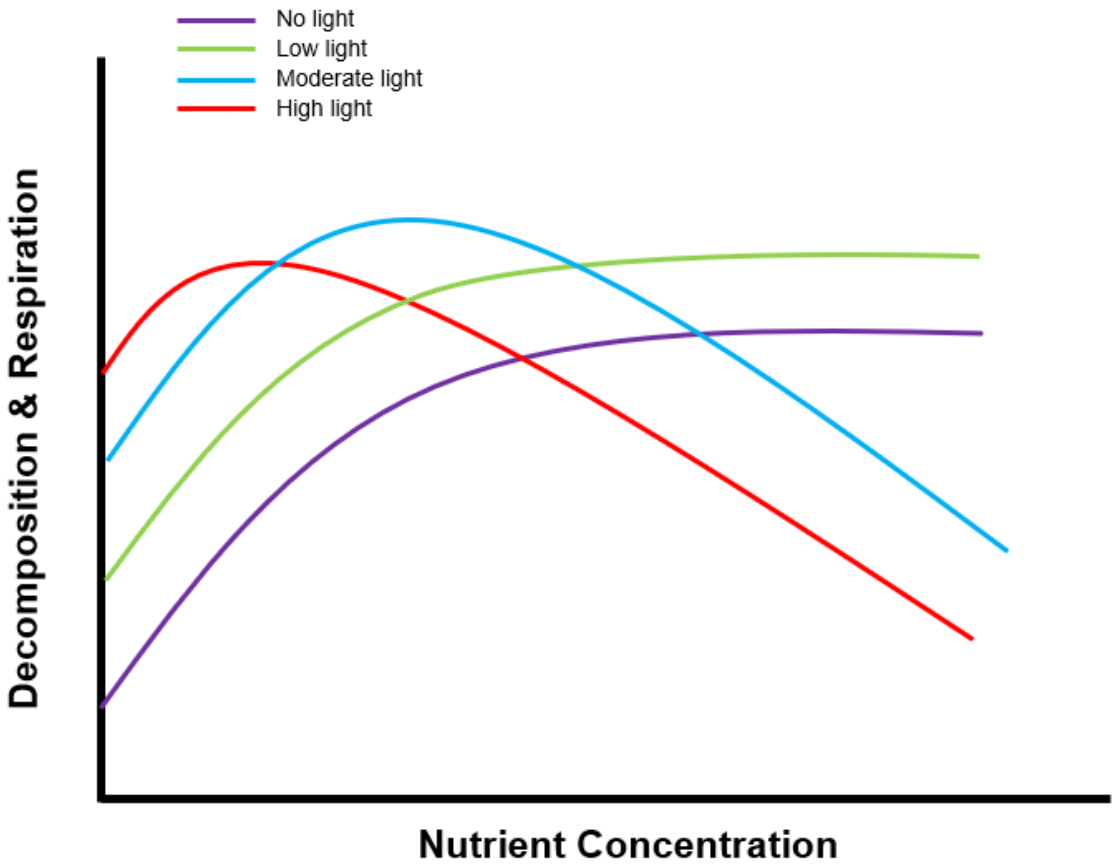


**Figure 2.2.** Prediction of how light and nutrients will mediate heterotrophic function (decomposition and respiration) in artificial streams. Rates of heterotrophic activity (ROM decomposition and respiration) are presented on the y-axis with nutrient availability on the x-axis. Light availability is indicated by the line colour.

- *Moderate light (50% light availability)* – Like the low light conditions, I expected ROM decomposition to increase with increasing P availability. Compared to low light levels, I anticipated that ROM decomposition at moderate light levels would be slightly elevated due to increased algal presence providing greater surface area for bacterial and fungal colonization along with greater bulk labile C availability. Beyond moderate P availability, I expected the relationship to become negative due to prolific algal growth and possible 'smothering' of the biofilm.

- *High light (100% light availability)* – Like moderate light levels, I expected the relationship between P availability and ROM processing to become negative. Due to the absence of any light constraints on algal growth, it was expected that this relationship would become negative before the moderate light availability treatment.

**Objective 2:**

With regards to the second objective, I predicted that both ROM decomposition and respiration would exhibit a subsidy-stress response to increasing algal abundance. Thus, maximum rates of OM processing and respiration were expected to occur at moderate levels of algal abundance (**Fig 2.3**).

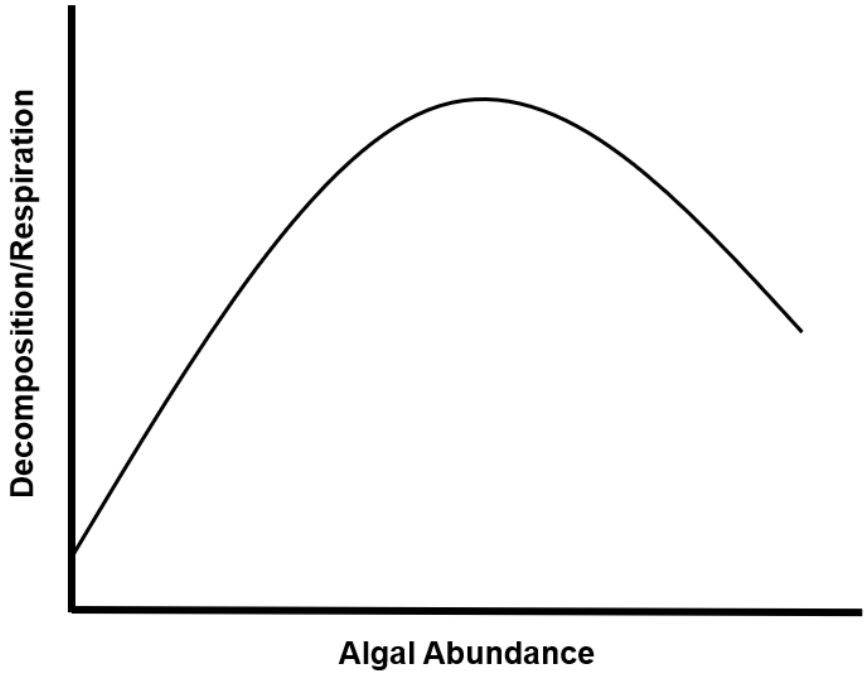


**Figure 2.4.** Predicted association of algal abundance and heterotrophic activity in artificial streams. It is expected that heterotrophic activity will be maximized at moderate levels of algal abundance before decreasing due to nuisance algal growth.

# 3. Methods

## 3.1 Field Experiment

### *3.1.1 Study Design and Site Selection*

A field experiment was conducted using two streams in Southwestern Ontario, Canada (**Fig. 3.1**) during the late summer (August 8 – September 1 and August 9 – September 2) of 2021. The two study streams were Moorefield Creek and the South Thames River which are located in the Grand River watershed (Conestogo River subbasin) and Thames River watershed (Pittock Reservoir subbasin), respectively. Generally, southwestern Ontario experiences a warm, humid climate during the late summer with average temperature highs of 25°C in the month of August (Environment Canada). The region's geology consists of varying surface deposits overlying predominately calcareous bedrock from the Paleozoic age. Land use in the study area is characterized by patches of deciduous forests in an otherwise agriculturally dominated landscape. Agricultural activities include a mixture of rowcrops such as corn and soybean, as well as high-density livestock farms including beef, dairy, and poultry.

Moorefield Creek and the South Thames River were selected using GIS software and available water chemistry data. These streams were chosen such that they were similar in physical, chemical and hydrological properties. However, the streams differed in bioavailable P concentrations to enable the creation of nutrient treatment groups. Prior to the beginning of the experiment, water samples were collected to confirm concentrations of bioavailable P. Based on results, Moorefield Creek was selected as the low nutrient treatment stream (5 µg/L of SRP at the time of sampling) and the South Thames River (35 µg/L SRP) was selected as the high nutrient stream. Temperature, specific conductivity (µs/cm) and pH were also collected at this time to confirm that they were similar among the two study streams. In Moorefield Creek the average temperature, specific conductivity and pH were 22.2°C, 546 µs/cm and 7.88 respectively, and in the South Thames River, they were 23.2°C, 733 µs/cm and 8.19.

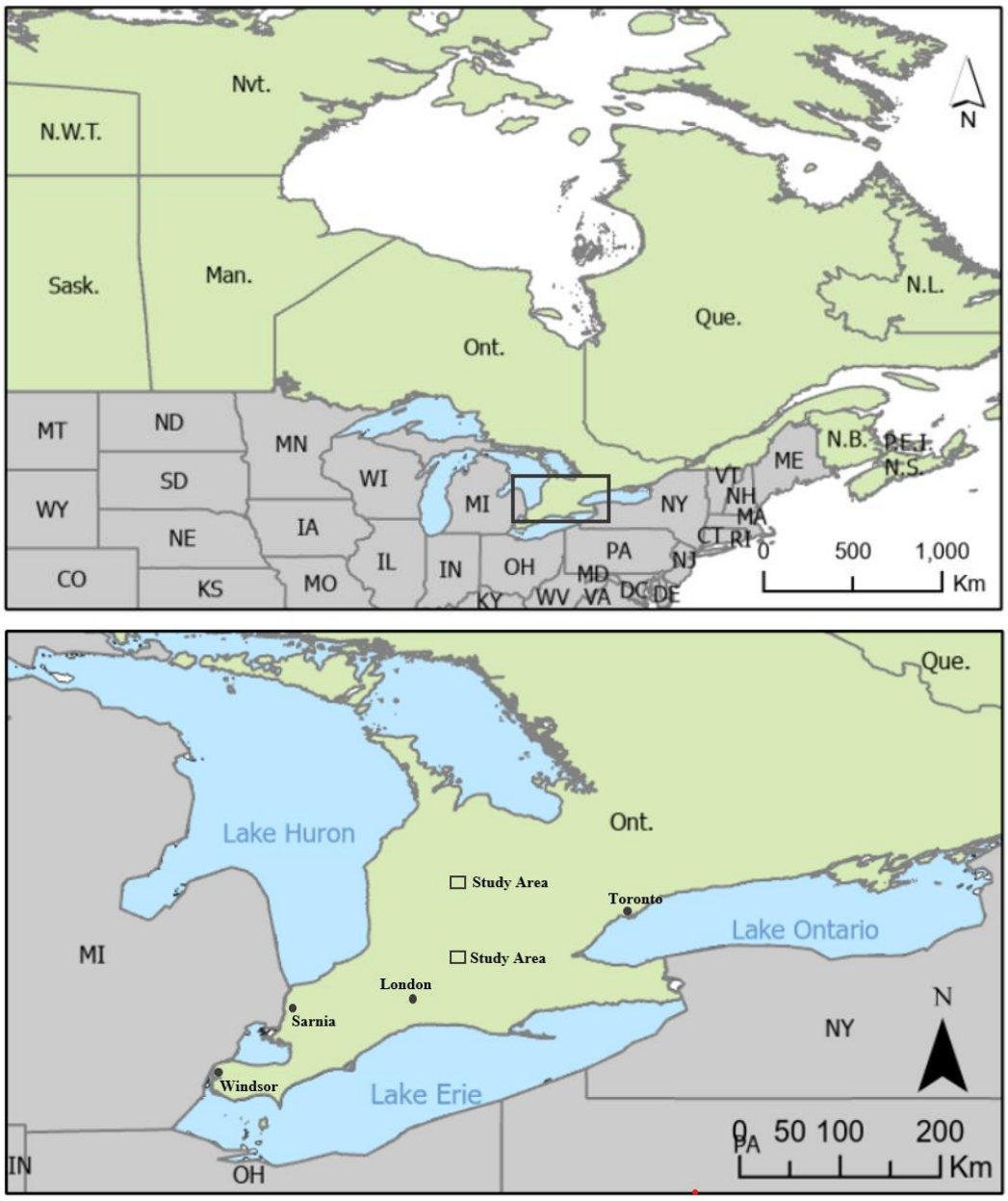


**Figure 3.1.** Maps displaying the location of study region within Canada (top) and the location of study area within the study region of southwestern Ontario (bottom).

A hierarchical study design was used to assess the interactive effects of light and nutrients across two phosphorus and two light treatment levels. Based on bioavailable P concentrations, there was one unenriched P treatment (Moorefield Creek) and one enriched treatment (South Thames River). Light treatments were implemented in each stream through the use of 80% shade cloth, which gave two light availability treatments of 20% and 100% (i.e., no shade cloth) light availability. By replicating light treatments 3 times within each stream, a total of 12 (N=12) sampling units were examined.

### *3.1.2 Experimental Set-up*

Prior to the beginning of the experiment, 3 sampling reaches were selected within each study stream, resulting in 6 total sampling locations (MC01, MC02 and MC03 in Moorefield Creek and ST01, ST02 and ST03 in the South Thames River; **Fig. 3.2**). Possible sampling reaches were narrowed down by finding areas in close spatial proximity to one another (within a few hundred metres) that exhibited no riparian canopy. Open canopy was a requirement to eliminate possible shading of strips from vegetation. Sampling reaches were also selected based on being within an operational sampling depth. An operational depth was defined as a sampling depth between approximately 20 to 50 cm so that it was neither too shallow (i.e., could dry up if water levels dropped) nor too deep (i.e., the stream bed could not be accessed with out being fully submerged in the water). Once the 3 reaches were finalized, one replicate of each light treatment was placed next to one another in the selected reach with a minimum of 1 m spacing between treatments (**Fig. 3.3**).

Light treatments were implemented in streams using shade structures (**Fig 3.4**). Shade structures were constructed by fastening rectangular sections of 80% shade cloth onto 6 pieces of rebar driven into the stream bed. The area of the stream bed covered by each shade structure was approximately 1.76 $m^2$ (given dimensions 1.6 m x 1.1 m for each structure). Additionally, the sides of shade structures, extending to about 10 cm above the stream surface, were screened using shade cloth to prevent angled sunrays from reaching the samples.

### *3.1.3 Stream Characterization*

Measurements of water physicochemical properties were collected to characterize water chemistry for each study reach. On 3 occasions, specific conductivity (lS/cm) and pH were determined using a hand-held multi-meter sonde (YSI, Professional Plus). Grab samples were also collected on 3 occasions from a well-mixed, flowing area at 60% depth, upstream of all sampling reaches. On the first and third sampling events, samples were analyzed for nitrate-nitrite N ($NO_2$ + $NO_3$) and SRP. On the second sampling event, samples were analyzed for total nitrogen (TN), total ammonia nitrogen ($NH_3$–N), total phosphorus (TP) and dissolved organic carbon (DOC) in addition to nitrate-nitrite N and SRP. Water samples were kept in a cooler while in the field and were frozen before being sent to an external lab for analysis.

Light availability was measured as photosynthetically active radiation (PAR) which was done at one sampling reach per stream; one logger was placed outside of the shade structure to capture light availability for the 100% light treatment, and one was placed underneath for the 20% light treatment. After the first 5 days of the experiment, however, the light logger for the shaded treatment at Moorefield Creek malfunctioned and therefore did not give accurate readings for the remainder of the experiment. Temperature was logged continuously through out the sampling period using hobo temperature loggers (TidbiT v2) anchored to the streambed at each sampling location within the stream and set to record water temperature at 15 min intervals.


**Figure 3.2.** Map displaying the locations of the 3 sampling reaches selected for Moorefield Creek (top) and the South Thames River (bottom).

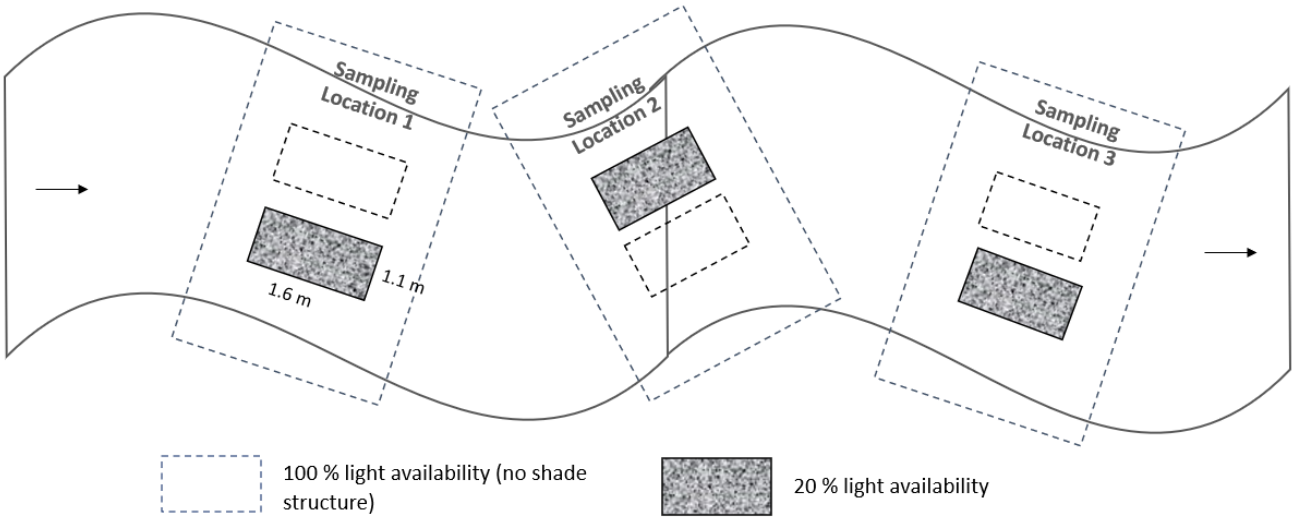


**Figure 3.3.** Experimental study design for placement of shaded and non-shaded structures in Moorefield Creek and South Thames River, the two study streams used for my field experiment in southwestern Ontario, Canada. Shaded and non-shaded structures were replicated 3 times in each stream, for a total of 12 (N=12) treatment groups.

**Figure 3.4.** Experimental set-up of shaded (left) and non-shaded structures (right; located underwater) used in the field experiment conducted in southwestern Ontario, Canada. Shaded and non-shaded structures were replicated 3 times in each Moorefield Creek and South Thames River, for a total of 12 (N=12) treatment groups.

Channel characteristics were measured as the average depth and flow velocity of the channel, which were measured for each individual light and phosphorus combination. Average velocity was measured by taking instantaneous velocity measurements at 20 locations above individual strips using a stream velocity meter (Swoffer Instruments, E-230-Model 2100). Sedimentation measurements were determined by collecting grab samples from 60% depth upstream of all 3 sampling reaches on 3 occasions and processing them for both TSS and turbidity.

In the lab, TSS was analyzed by filtering 1 L of distilled water through glass fiber filter paper (Whatman, 934-AH) using a vacuum filtration apparatus, and dried in the oven at 105°C over night. The blank filter papers were then ignited in a muffle furnace at 510°C for 15 minutes to get the filter dry weight. After measuring for dry filter weight, 750 mL of sample water was filtered with the vacuum apparatus. The oven dried mass provided the value of total suspended solid (TSS; mg/L). Turbidity (NTU) was measured using a Turner Designs Trilogy Fluorometer on turbidity mode. The fluorometer was set to continuous sampling, with 3 readings being taken 1/3 of a second apart per sample.

### *3.1.4 Sampling Procedures*

OM processing was assessed using the Cotton-Strip Assay (CSA; Tiegs et al., 2013). The CSA has been used in several studies in the place of the traditional leaf litter assay as a successful and effective way to measure OM decomposition in streams. A key advantage of using a standardized substrate such as cotton is that it eliminates the variation that stems from intrinsic substrate characteristics. Moreover, cotton breaks down faster than traditional leaf litter, therefore making the CSA a more timely method for measuring OM breakdown. The CSA is also unique in that, unlike most decomposition assays that rely on the determinations of mass loss for calculating decomposition rates, it uses the loss of tensile strength. By not having a mass component, the confounding variable of biofilm biomass is removed, making decomposition rates more meaningful and accurate. Lastly, because cotton strips are novel substrates with no organisms living on them prior to the experiment, we can be sure that the communities present at the time of sampling were colonized during the study period.

The preferred cotton substrate used in these studies is artist's fabric as it is composed primarily of cellulose (>95%), a C-based compound found naturally in detrital OM. Preparation, deployment, retrieval and processing of decomposition strips followed Tiegs et al. (2013) and used Fredrix-brand unprimed 12-oz. heavyweight cotton fabric, Style #548 (Fredrix, Lawrenceville, GA, USA). Prior to deployment, Fredrix brand fabric was cut into strips of 2.5 x 8 cm rectangles, with 3 mm of frayed "fuzz" left along the length of each strip. For field deployment, groups of 5 decomposition strips were attached, evenly spaced (around 20 cm), to metal chains using cable binders. Both ends of each chain was fastened to metal stakes and driven into the streambed parallel to the direction of flow. A total of 4 metal chains were deployed within each light treatment replicate, with chains being spaced at least 20 cm apart. Additionally, a minimum of 20 cm remained between decomposition strips and the edges of shade structures in the 20% light treatment.

Incubation of strips lasted for 21 days (August 8 – September 1 in Moorefield Creek and August 9 – September 2 in South Thames River). This amount of time was selected to yield approximately 50% tensile strength loss in cotton strips, a time when strips are thought to be the most biologically sensitive (Tiegs et al., 2013). Upon retrieval, strips were soaked in 70% ethanol (for 30 seconds to 1 minute) to sterilize strips and prevent further decomposition. After soaking, large pieces of sediment and debris were gently brushed from the strip. Strips were then transported to the lab and dried in an oven at 40°C for a minimum of 24 hours.

Tensile strength was measured using a tensiometer and motorized test stand (Force Gauge, Model M3-100). Approximately 1 cm portions of the ends of the cotton strips were placed in the grips of a tensiometer and pulled at a rate of 2 cm/min. The maximum tensile strength was recorded for each strip. Tensile strength loss was then expressed as the percent of the initial tensile strength lost per degree day (**Eq. 1**). Degree days was simply calculated by summing the mean daily temperatures in each stream for the entire incubation period.

*Tensile loss (%)*

$$T_{OM} = \frac{\left(\frac{\text{Tensile Strength}_{\text{REF}} - \text{Tensile Strength}_{\text{TRT}}}{\text{Tensile Strength}_{\text{REF}}}\right) \text{x } 100}{\text{Degree Days}} \quad (1)$$

Sampling of biofilm metabolism (gross primary production; GPP and community respiration; CR) was done using the light and dark bottle method. For each light *x* nutrient treatment, 1 light and 1 dark bottle of identical volumes (200 ml) were filled with water from the stream reach being sampled. Using an Ultrapen (Model PT5, Myron L Company), the initial temperature and DO concentration **(mg $L^{-1}$)** within each bottle were measured. Immediately after being measured, 1 strip from each light and nutrient treatment was placed into each of the light and dark bottles. Bottles were then capped (with no head space) and put back into the stream under the respective structure to incubate. After 1-2 hours, final temperatures and DO concentrations were recorded by placing the probe directly into bottles after removing decomposition strips. Upon removal from the chambers, strips were sterilized for 30 seconds in ethanol and then taken to the lab to be dried at 40°C for a minimum of 24 hours.

Differences between pre- and post-incubation DO concentrations were used to estimate CR and GPP from strips. In dark bottles, CR (i.e., DO consumption) was measured as the decrease in oxygen over the time incubated which was calculated using **Eq. 2**, modified from Hauer and Lamberti (2017). NEP, or the amount of carbon assimilation that occurred in excess of respiration, was measured in light bottles as the increase in oxygen over the time incubated (**Eq. 2**). By summing NEP and CR, GPP was determined.

$Respiration$:

$$R_{OM} = \frac{DO_{OM\ START} - DO_{OM\ END}}{t_{OM}}\ x\ Volume_{H2O}Chamber$$

Chlorophyll *a* concentration was also used to estimate algal abundance. Upon retrieval, strips were placed into 50 mL falcon (centrifuge) tubes then stored and frozen in the lab. The chlorophyll-a concentration from thawed strips was determined using fluorometric analysis in the lab. Chlorophyll *a* was extracted from the strips via a hot ethanol (containing 90% ethanol) non-acidification extraction. A Turner Designs Trilogy Fluorometer (Model: 7200-000) was then used to determine the concentration of chlorophyll *a* on each strip.

## 3.2 Artificial Stream Experiment

### *3.2.1 Study Design and Experimental Setup*

An artificial stream experiment was conducted over a 27-day period in the summer (June 29 – July 26) of 2020. Nine artificial streams were randomly assigned to one of three phosphorus (P) treatment levels: 1) an unenriched P treatment (10 µg P $L^{-1}$), 2) an enriched P treatment (50 µg P $L^{-1}$), and 3) an enriched P treatment (100 µg P $L^{-1}$). Within each of the nine artificial streams, four shade levels (0%, 50%, 80% and 100%) were established. Shade levels were chosen to reflect different amounts of riparian shading while experimental P concentrations were chosen based on regional nutrient criteria (Chambers et al., 2012) and were reflective of P concentrations observed in human-influenced streams. P concentrations below 10 µg $L^{-1}$ P were not considered given previous studies which found very little biological activity under this level (e.g., Ashberry et al., 2021).

Shade treatments were implemented by dividing individual artificial streams into four treatment levels by covering sections of channel with shade cloth. Three different shade cloths were used to give 100% (no shade cloth), 80%, 50% and 0% shade, resulting in 12 unique treatment combinations, each with 3 replicates (N=36). Shade treatments were arranged such that channels receiving a particular light treatment were alternated between P treatments (i.e., each light treatment was installed once in the left, middle, and right channels among the three artificial streams within each P treatment) and that channels receiving 80% and 1000% shade were paired together (**Fig. 3.6).**


**Figure 3.6.** Schematic diagram (left) and experimental set-up (right) for the artificial stream experiment at the Thames River Experimental Stream Science (TRESS) centre in London, ON Canada.

The artificial stream experiment took place at the Thames River Experimental Streams Sciences (TRESS) Centre in London, Ontario, Canada. Artificial streams consisted of sinuous flumes (0.15 m deep by 0.20 m wide by 7.0 m long) that received a continuous supply of water from the Lake Huron Water Supply System through individual diaphragm pumps. P treatments (10, 50 and 100 $\mu g\ L^{-1}$ P) were achieved by adding concentrated P ($KH_2PO_4$) to the inflow of each artificial stream via chemical dosing pumps. Total nitrogen (N) concentration was kept constant among all streams at a continuous concentration of 1500 $\mu g\ L^{-1}$ N. N was delivered to streams by adding concentrated N ($NH_4NO_3$) to the common water supply of the facility. Flow rates of chemical dosing and diaphragm pumps were calculated and calibrated daily to ensure target nutrient concentrations were maintained throughout the study period. To confirm target nutrient concentrations, water samples were collected on four occasions and analyzed for Nitrate-Nitrite and SRP concentrations.

Total light availability (measured as photosynthetically active radiation; PAR) above shade treatments, water temperature, flow velocity and substrate were equal among artificial streams. Light availability and water temperatures in artificial streams fluctuated daily and reflected diurnal cycles and day-to-day variability in ambient weather conditions. Flow velocity for all streams was set to 0.4 $m\ s^{-1}$ and was measured daily to ensure the selected velocity was maintained throughout the experiment. Substratum in all experimental flumes was cobble (D50 – 46 mm) which was placed to a depth of ~ 10cm.

Prior to sampling, artificial streams were inoculated with algae from 5 local streams with ambient nutrient concentrations that reflected the experimental P concentrations used in the study design. Inoculum was collected by scraping algae from several (10-20) cobble substrates from randomly selected reaches in the selected streams and rinsing it into two 1 L Nalgene bottles. Bottles were then filled with water and mixed thoroughly. An equal volume (~110 mL) from each bottle was then poured into each artificial stream after the clean cobble and sampling materials were put into the streams. Streams were inspected before sampling and all large grazers (ie., snails and crayfish) were removed.

### *3.2.3 Sampling Procedures*

Preparation of cotton strips in the artificial stream experiment was the same as described for the field experiment. For deployment in artificial streams, strips were anchored to the stream bed using steel washers. Incubation of the strips (N=540) lasted for up to 27 days.

Tensile loss (N = 396) was sampled on 9 occasions. Sampling of strips fell on every third day following deployment. At each sampling event, 1 strip from each unique treatment combination was retrieved. On the seventh sampling event (day 21 of the experiment), 2 additional strips (for a total of 3) were collected. Retrieval and processing of strips was the same as described for the field experiment. Tensile loss was then calculated using **Eq. 1**, with incubation time (days) being used in place of degree days.

Sampling of biofilm metabolism (GPP and CR) occurred on the seventh sampling event (day 21) of the experiment. This duration was selected to align with approximately 50% tensile strength loss in decomposition strips. Processing of strips for GPP (N = 36) and CR (N = 36) was done using the same light and dark bottle method that was described for the field experiment.

Cotton strips were also sampled for algal abundance (N = 72), measured as chlorophyll *a* concentration, on the 21$^{st}$ day of the experiment. At each sampling event, 2 strips were collected from each unique treatment combination. Retrieval and processing of strips was the same as described for the field experiment.

## 3.3 Data analysis

### *3.3.1 Field Experiment*

Prior to analyses, data were tested for normality by checking residual q-q plots and were found to be suitable for applying parametric tests. A nested hierarchical Generalized Linear Mixed Model (GLMM) using Type III sums of squares was used to partition the variance associated with tensile loss per degree day, respiration, GPP and chlorophyll *a*. The fixed effects were phosphorus, shade as well as their interaction term (phosphorus * shade). Structure, which represented the 6 random locations where light treatments were placed within the stream, was nested within stream and included as a random factor in the model. The GLMM analysis was followed by Tukey's post-

hoc tests (alpha = 0.05) to determine pairwise comparisons. Both the GLMM and post-hoc analyses were done using Tibco Statistica software (Version 13.5).

### *3.3.2 Artificial Stream Experiment*

Like the field experiment, data were tested for normality using residual q-q plots and were found suitable for parametric analysis. For each sampling event, individual nested hierarchical GLMM's using Type III sums of squares were used. The fixed effects were phosphorus, shade as well as their interaction term (phosphorus x shade). Table, which represented the 9 different artificial stream channels used in the experiment, was included as a random variable in the model which was nested within phosphorus. On days where an interaction was found between shade and phosphorus, individual GLMM's were run to compare the effects at phosphorus at each given shade level. This model only had one fixed effect of phosphorus, with table being including as a nested random effect.

GLMMs with same model structure as described above were also run for day 21 of the experiment to partition the variance associated with tensile loss, respiration, chlorophyll *a* and GPP. GLMM analysis for day 21 was followed with individual GLMMs for individual shade levels (where an interaction term was found) and Tukey post-hoc analysis. All statistical analyses were done using Tibco Statistica software (Version 13.5).

# 4. Results

## 4.1 Field Experiment

### *4.1.1 Experimental Conditions*

SRP concentrations in the study reaches were generally consistent throughout the 3-week study period (**Table 4.1**). Based on the 3 sampling events, mean SRP concentration in the South Thames River (51 ± 15.0 µg P $L^{-1}$) was approximately 10 times higher than that in Moorefield Creek (5 ± 0.3 µg P $L^{-1}$). In contrast, dissolved N ($NO_2$ + $NO_3$) concentrations varied substantively over the study period. In both streams, $NO_2$ + $NO_3$ concentrations were higher at the beginning of the experiment compared to the end. In the South Thames River, the concentrations ranged from 313 to <2 µg N $L^{-1}$ (i.e., below detectable limit) whereas in Moorefield Creek, $NO_2$ + $NO_3$ concentrations ranged from 1320 to 138 µg N $L^{-1}$. Ammonia concentrations were low at both sites, with the concentration being below the detectable limit (i.e., <3 µg N $L^{-1}$) in the South Thames River and 12 µg N $L^{-1}$ in Moorefield Creek. Consequently, the stoichiometric ratios of dissolved inorganic nitrogen to SRP ($NO_2$ + $NO_3$ + $NH_4$ : SRP) differed between the study streams, which were calculated as 2:1 in the South Thames River and 193:1 in Moorefield Creek.

Water temperatures were marginally warmer (23.39 ± 3.28 °C) in the South Thames River compared to Moorefield Creek (21.86 ± 2.50 °C). Similarly, the degree days in the South Thames River (546.54 ± 4.57 °C) were higher than those in Moorefield Creek (510.48 ± 3.20 °C). Both streams exhibited alkaline pH values, with mean pH values differing by less than 0.2 between the South Thames River (8.27 ± 0.20) and Moorefield Creek (8.02 ± 0.14). The specific conductivity of the South Thames River (750.33 ± 35.65 µs/cm), however, was notably higher than that of Moorefield Creek (535.53 ± 7.88 µs/cm).

Turbidity was about 4 times higher in the South Thames River (53.10 ± 34.23 NTU) compared to Moorefield Creek (12.48 ± 5.69 NTU). Although the concentrations of TSS were found to be low in both streams, the South Thames River (0.024 ± 0.008 mg/L) showed slightly elevated levels compared to Moorefield Creek (0.004 ± 0.003 mg/L). Lastly, DOC concentration was more than 6 times greater in the South Thames River (26.3 mg/L) compared to Moorefield Creek (3.9 mg/L) which was likely due to the elevated P concentrations contributing to higher rates of algal growth.

Mean water velocity differed between the streams by 33%, with the average velocity being slightly elevated in Moorefield Creek (0.063 ± 0.023 m/s) compared to the South Thames River (0.042 ± 0.019 m/s). Stream depth varied significantly between the two study streams, with Moorefield Creek (45.2 ± 3.6 cm) being substantially deeper than the South Thames River (20.1 ± 4.20 cm).

For the first 5 days of the experiment (i.e., before the PAR logger malfunction in Moorefield Creek), total photosynthetically active radiation (PAR) was between 80-85% (81.9% in Moorefield Creek and 83.6% in the South Thames River) lower in the shade structures compared to the full light treatments at each site. Total PAR for each light treatment was determined by summing all recorded PAR values (**µmol/cm$^2$/s**) up until the equipment malfunction.

To demonstrate that strips were exposed to similar amounts of light availability over the entire study period, total PAR for each stream is provided in **Fig. 4.1**. Total PAR was determined by summing all recorded PAR values (**µmol/cm$^2$/s**) throughout the 21-day sampling period from loggers exposed to full light conditions. Total PAR for each stream was 3.39 mol at Moorefield Creek and 3.19 at the South Thames River (**Fig. 4.2**). Assuming that shade structures continued to provide the same level of shade that they did in the first 5 days of the experiment (i.e., before malfunctioning), light availability in these structures was estimated to be between 0.54 and 0.64 mol/cm$^2$/s, given an 80-85% reduction in light availability.

**Table 4.1.** Mean physical and chemical characteristics over the 21-day study period for Moorefield Creek and South Thames River between August 8th and September 1st, 2021. Nutrients, specific conductivity, TSS, turbidity and pH were measured on 3 occasions; velocity and depth were measured on 1 occasion; and temperature was logged continuously throughout the experiment.

| Parameter | Moorefield Creek | South Thames River |
|---|---|---|
| **Temperature (°C)** | 21.86 (± 2.50) | 23.39 (± 3.28) |
| **Degree Days (°C)** | 510.48 (± 3.20) | 546.54 (± 4.57) |
| **TN (µg N/L)** | 1650 | 666 |
| **$NO_2$ + $NO_3$ (µg N/L)** | 953 (± 408) | 108 (± 102) |
| **$NH_4^+$ (µg N/L)** | 12 | <3* |
| **TP (µg P/L)** | 17 | 155 |
| **SRP (µg P/L)** | 5 (± 0.3) | 51 (± 15.0) |
| **Specific Conductivity (µm/cm)** | 535.53 (± 7.88) | 750.33 (± 35.65) |
| **DOC (mg C/L)** | 3.9 | 26.3 |
| **pH** | 8.02 (± 0.14) | 8.27 (± 0.20) |
| **Turbidity (NTU)** | 12.48 (± 5.69) | 53.10 (± 34.23) |
| **TSS (mg/L)** | 0.004 (± 0.003) | 0.024 (± 0.008) |
| **Velocity (m/s)** | 0.063 (± 0.023) | 0.042 (± 0.019) |
| **Depth (cm)** | 45.2 (± 3.6) | 20.1 (± 4.20) |

* *Below detectable limit*

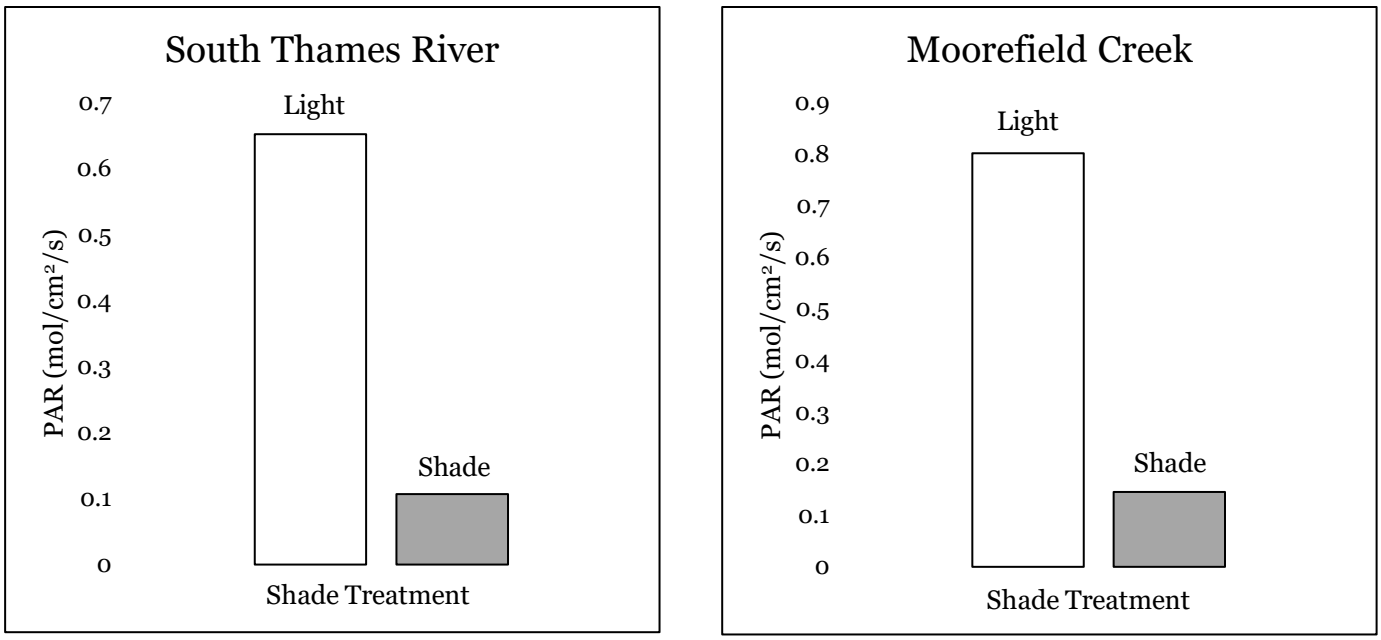


**Figure 4.1.** Total light availability, measured as photosynthetically active radiation (mol/cm$^2$/s), for light and shaded treatments in the South Thames River (bottom) and Moorefield Creek (top). Data shown here is from the first 5 days of the field experiment in southwestern Ontario prior to malfunctioning of PAR loggers. PAR was continuously logged at 5-minute intervals throughout the experiment.

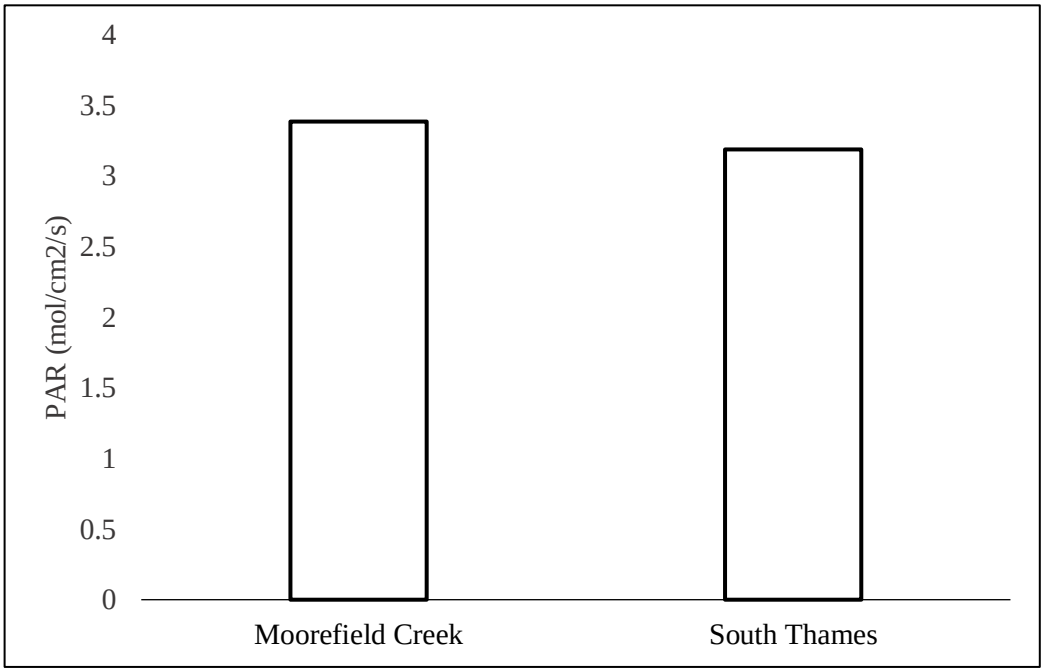


**Figure 4.2**. Total light availability, measured as photosynthetically active radiation (mol/cm$^2$/s), in light treatments over the 21-day study period in southwestern Ontario, Canada. PAR was continuously logged through the experiment at 5-minute intervals.

### *4.1.2 Organic Matter Processing*

Mean tensile loss after 21 days of incubation among all 154 cotton strips was 0.13% per degree day (**Fig. 4.3**). A hierarchical generalized linear mixed model (GLMM) found no interactive effects of shade and phosphorus ($F=1.9$, $p=0.168$) on tensile loss per degree day. Percent tensile loss per degree day did differ, however, between P levels ($F=161.9$, $p < 0.001$), with tensile loss being 33% faster in the high P stream, the South Thames River, compared to the low phosphorus stream, Moorefield Creek. No main effect of shade was found ($F=0.4$, $p=0.532$).

Mean respiration was $0.18 \pm 0.08$ µg $O_2$ $hr^{-1}$ for all 38 strips collected from the field (**Fig. 4.4**). The hierarchical GLMM found no significant interaction ($F=0.15$, $p=0.705$) nor main effects of phosphorus ($F=3.50$, $p=0.135$) or shade ($F=0.08$, $p=0.76$) on respiration.

### *4.1.3 Algal Abundance*

Mean chlorophyll *a* concentration for the 36 collected strips was $3.27 \pm 0.61$ µg/$cm^2$ (**Fig. 4.5**). A GLMM found a significant interaction ($F = 3.73$, $p < 0.001$) between the main effects of P and shade on chlorophyll *a* concentration from the collected strips. Post-hoc analysis revealed that chlorophyll *a* concentrations only differed between P levels when there was no shading ($p=0.012$) whereas there was no significant effect of P in shaded conditions ($p=0.731$). In full light conditions, mean chlorophyll *a* concentration was approximately 45% higher in the high P stream compared to the low P stream. In low light conditions, however, chlorophyll *a* was only 28% higher in the high P stream compared to the low P stream.

Mean GPP for all 36 strips was 0.52 µg $O_2$ $hr^{-1}$ $\pm$ 0.06 µg $O_2$ $hr^{-1}$ (**Fig. 4.6**). The GLMM indicated that there was a significant interaction between shade and P ($F=7.53$, $p=0.01$) on GPP. Like chlorophyll *a,* post-hoc analysis revealed that P only had a significant main effect on GPP in high light treatments ($p=0.021$) and its effect was not significant in shaded treatments ($p=0.090$). In full light conditions, GPP differed by approximately 55% between the high P stream and the low P stream, whereas GPP in low light conditions differed by only 6% between high and low P treatments.

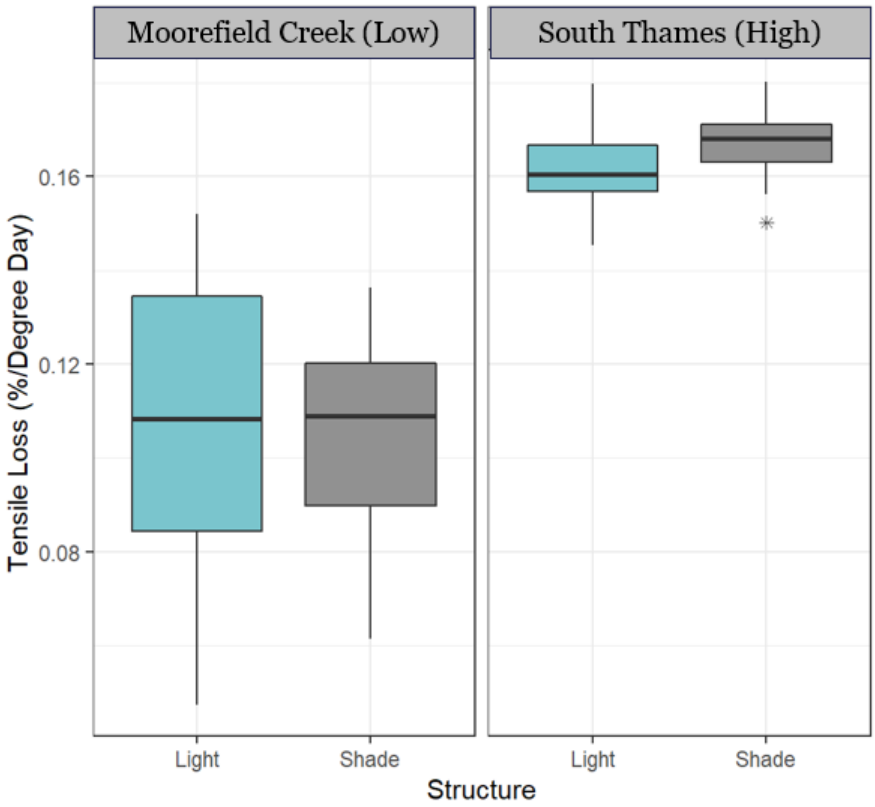


**Figure 4.3.** Boxplot summarizing tensile loss of cotton substrates (N=154) incubated for 21 days in the field experiment conducted in southwestern Ontario, Canada. Boxplots show the mean, median, interquartile range, and the whiskers denote the 5th and 95th percentiles for tensile loss per degree day. The Asterisk (*) represents an outlying data point.

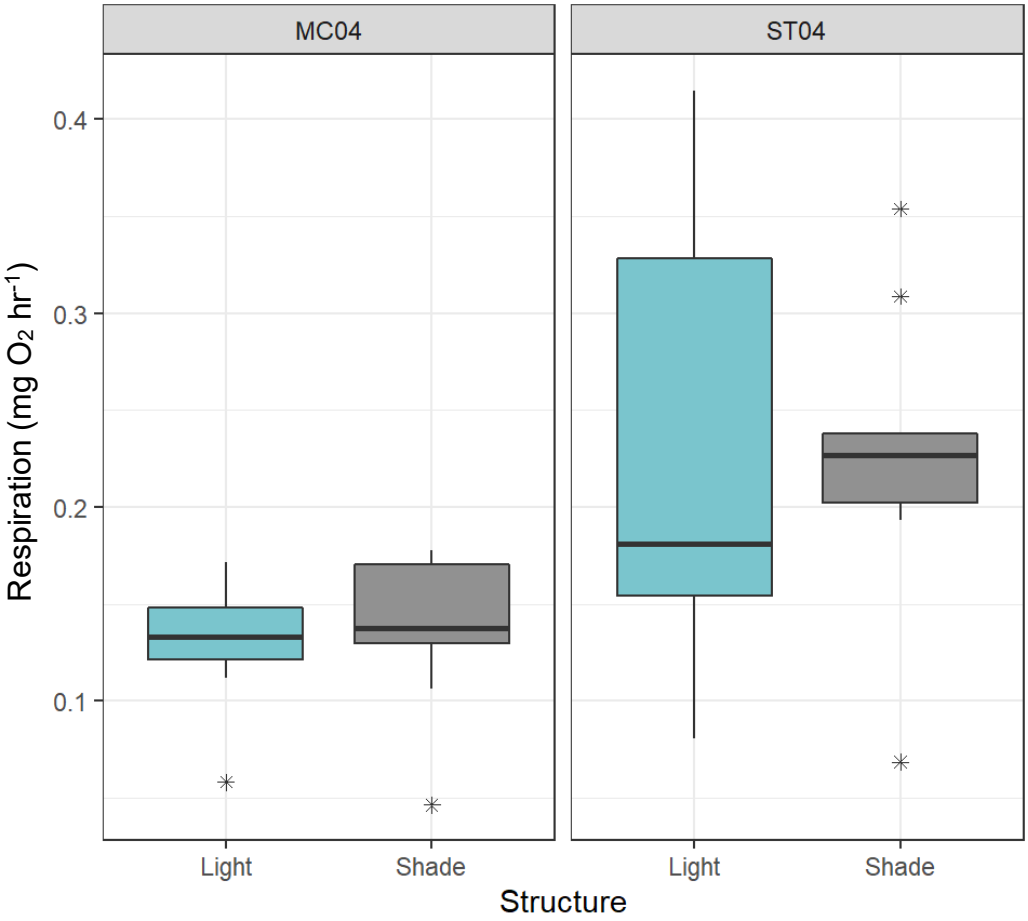


**Figure 4.4.** Boxplot summarizing respiration from cotton substrates (N=36) after 21 days of incubation in the field experiment conducted in southwestern Ontario, Canada. Boxplots show the median and interquartile range, and whiskers denote the 5th and 95th percentiles for respiration. Asterisks (*) represent outlying data points.

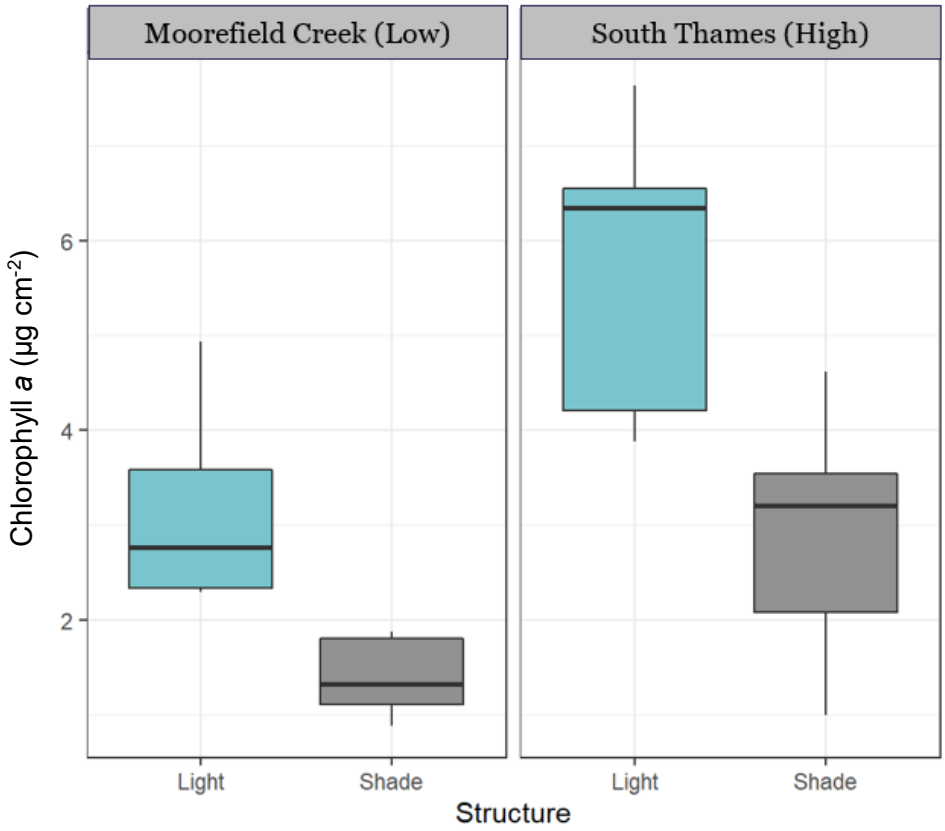


**Figure 4.5.** Boxplot summarizing chlorophyll *a* concentration (N=36) from cotton substrates incubated for 21 days in the field experiment conducted in southwestern Ontario, Canada. Boxplots show the median and interquartile range and the whiskers denote the 5th and 95th percentiles for chlorophyll *a* concentration.

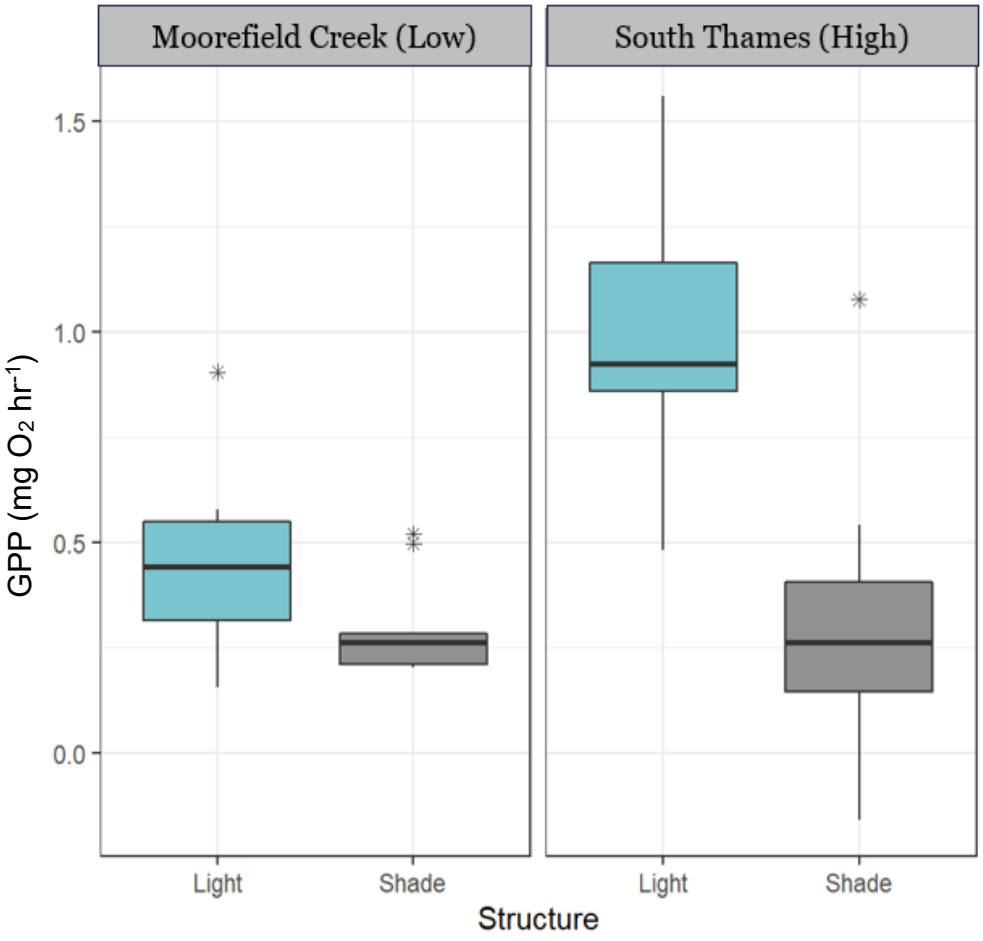


**Figure 4.6.** Boxplot summarizing GPP (N=36) from cotton substrates incubated for 21 days in the field experiment conducted in southwestern Ontario, Canada. Boxplots show the median and interquartile range, and the whiskers denote the 5th and 95th percentiles for GPP. Asterisks (*) represent outlying data points.

## 4.2 Artificial Stream Experiment

### *4.2.1 Time Series*

Results of the GLMM analysis for each sampling day of the experiment are shown in **Appendix A1.** All streams exhibited similar rates of tensile loss for approximately the first 3 days (and up to 5 days) of the experiment. Differences in rates of tensile loss emerged among shade treatment groups after 6 days of incubation. Post-hoc analysis found that rates of tensile loss differed most consistently between ambient light treatments (0% shade) and heavily shaded (80% and 100% shade) treatments on sampling days where shade had a main effect, as well as between moderately shaded treatments (50% shade) and fully shaded treatments (**Appendix A2**). Generally, tensile loss per day was greatest in fully shaded treatments, followed by moderately shaded treatments, and full light treatments (**Fig. 4.7**).

Differences among P treatment groups began on day 12 of the experiment. Pairwise comparisons for sampling events beyond day 12 revealed that tensile loss differed consistently between the unenriched P treatment group (10 μg P $L^{-1}$) and the two enriched P treatment groups (50 μg P $L^{-1}$ and 100 μg P $L^{-1}$). Differences between the enriched treatment groups, however, were not significant ($p<0.05$) on any day (**Appendix A3**). Mean tensile loss was generally greater in the enriched P treatments compared to the unenriched P treatment over the entire duration of the experiment (**Fig. 4.7**).

Shade and P showed significant interactive effects on rates of tensile loss per day on day 21, day 24, and day 27 of the experiment (**Appendix A4**). Post-hoc GLMs comparing the effects of P on tensile loss at each shade level found that P had a significant main effect at all examined shade levels in the experiment on days 21, 24 and 27 (**Appendix A5**). Tukey HSD post-hoc tests revealed that on all 3 sampling events, the unenriched P treatment differed from both enriched P treatments at each shade level (**Appendix A6**). There were two occasions where all three P treatments differed from one another which occurred on day 21 (80% shade) and day 27 (0% shade).

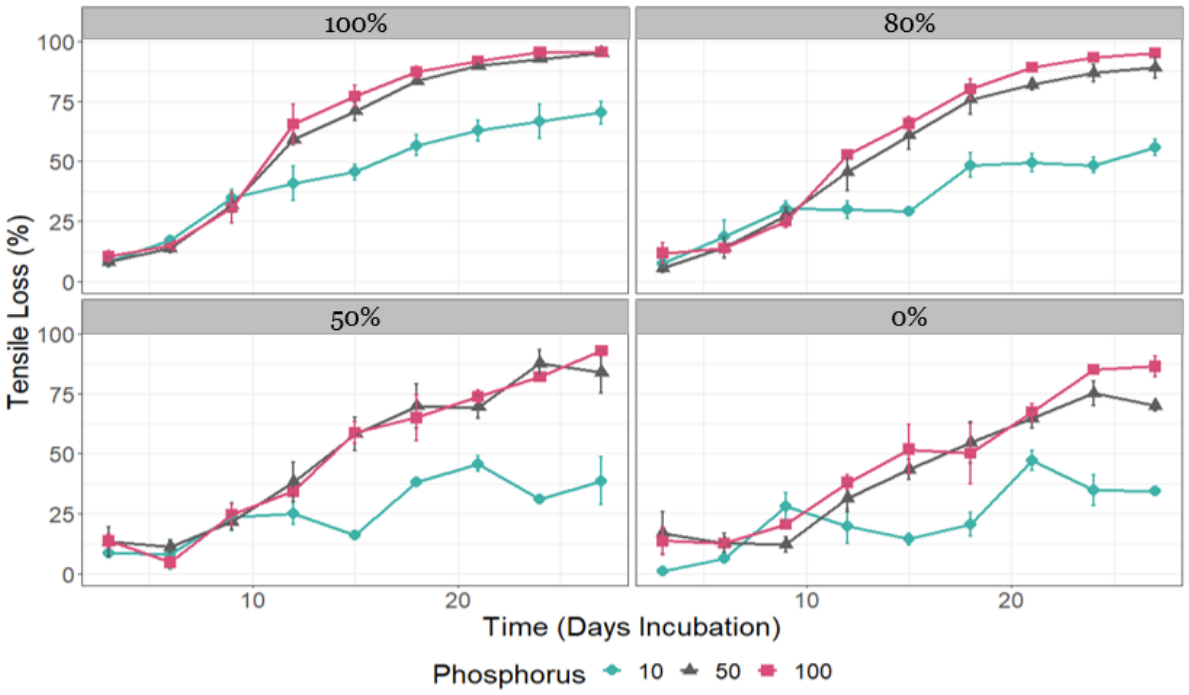


**Figure 4.7.** Mean tensile loss of all cotton substrates (N=372) incubated in the 27-day artificial stream experiment conducted at the Thames River Experimental Steam Sciences (TRESS) Centre in London, Ontario, Canada. Percent tensile loss is given on the y-axis with time on the x-axis. Coloured lines represent different phosphorus treatment groups while panels represent different shade treatments.

### *4.2.2 Day 21*

Following the 21-day incubation period, the mean tensile loss observed in the 163 cotton strips sampled was 69.39% ± 1.75%, corresponding to a mean daily loss of 3.30% $day^{-1}$ ± 0.10% $day^{-1}$. GLMM analysis found a significant interaction term (F=2.4, p=0.034) between P and shade on tensile loss per day (**Fig. 4.8**). Subsequent individual GLMM's for each shade treatment revealed that P had a significant main effect at each examined shade level given p-values of p=0.052 at 0% shade, p=0.018 at 50% shade, p=0.001 at 80% shade and p=0.004 at 100% shade.

Post-hoc pairwise comparisons showed that only the two enriched P treatments differed from the unenriched P treatment in terms of tensile loss (**Appendix A7**) within the majority of the examined shade levels (0%, 50% and 100%). Tensile loss was between 40% and 50% higher in the enriched P treatments compared to unenriched treatment at these shade levels. Only in the 80% shade level, did all 3 P treatment groups differ significantly, with losses in the most enriched treatment exceeding the moderately enriched treatment by approximately 15%.

Mean respiration for all 36 cotton strips on day 21 was 0.168 ± 0.003 μg $O_2$ $hr^{-1}$ (**Fig. 4.9**). The GLMM found no interactive effects (F=1.89, p=0.14) of shade and P on respiration, nor main effect of P (F=1.28, p=0.35). Respiration did differ between shade treatments, particularly if p-values above 0.05 are considered (F=2.92, p=0.06), which is reasonable given the small sample size per treatment combination (n=3). Post-hoc analysis, however, did not indicate any significant pairwise comparisons (p>0.1) for any shade levels, likely due to limited statistical power.

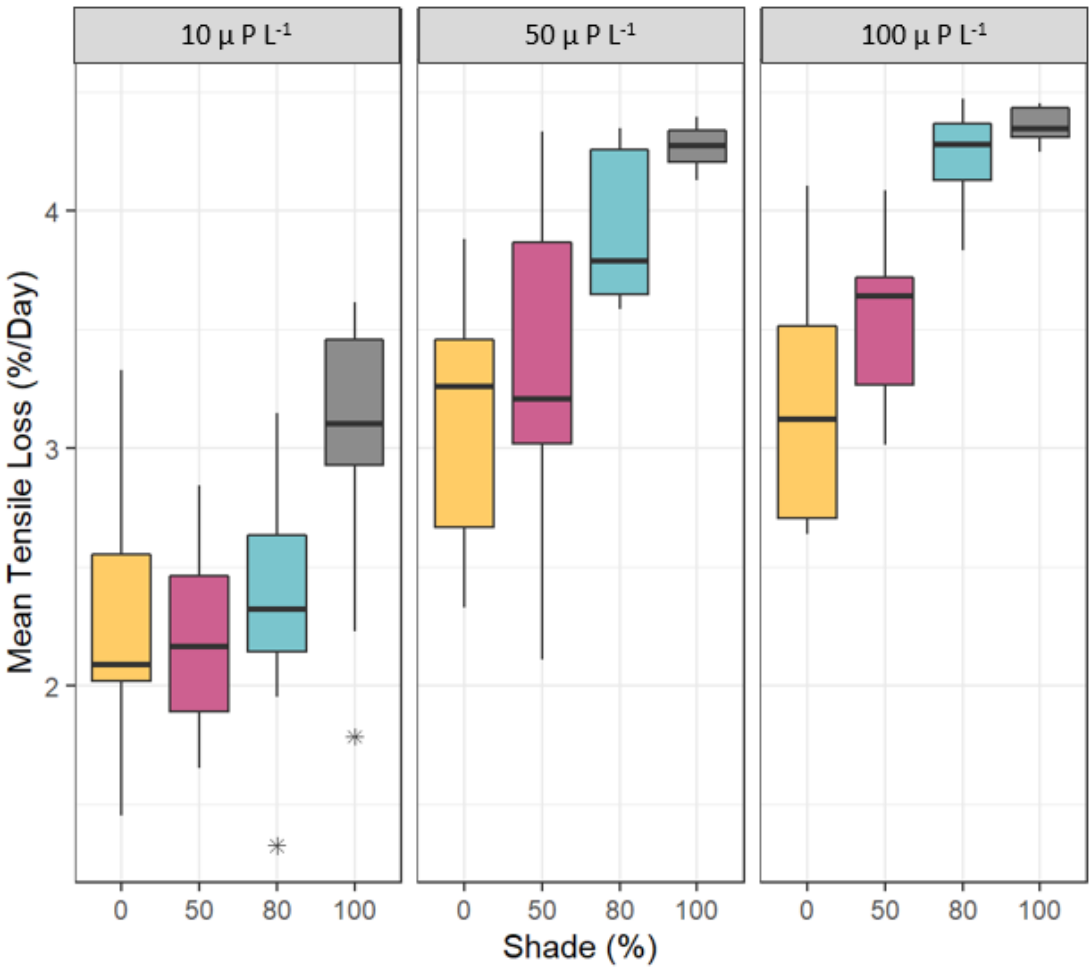


**Figure 4.8.** Boxplot summarizing tensile loss per day of cotton substrates (N=163) retrieved from artificial streams following 21 days of incubation. Boxplots show the median and interquartile range, and whiskers denote the 5th and 95th percentiles for percent tensile loss per day.

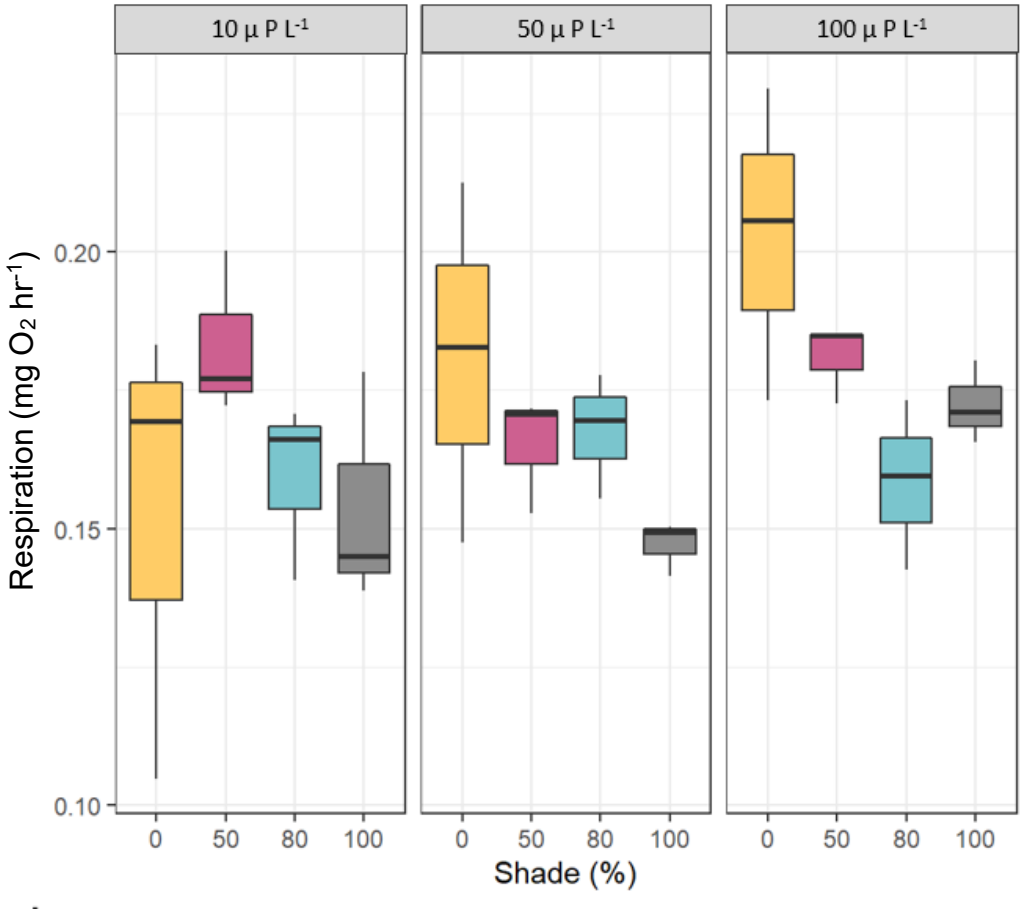


**Figure 4.9.** Boxplot summarizing mean respiration (mg $O_2$ $hr^{-1}$) on cotton substrates (N=36) retrieved from artificial streams following 21 days of incubation. Boxplots show the median and interquartile range, and whiskers denote the 5th and 95th percentiles for respiration.

### *4.2.3 Algal Abundance*

Mean chlorophyll *a* concentration from all 36 collected strips was 15.73 ± 2.23 μg/cm$^2$ (**Fig. 4.10**). A GLMM showed significant interaction between P and shade (F=2.28, p=0.049). Post-hoc analyses revealed that phosphorus did not show a significant main effect (p>0.05) at any shade level, likely due to limited statistical power. To increase power, Tukey pairwise comparisons were done. Results demonstrated there were in fact differences in chlorophyll *a* concentration between P treatment groups at some shade levels (0% and 80% shade). In unshaded treatments, all 3 P treatment groups differed, with chlorophyll *a* concentrations being significantly greater in the enriched (p=0.061) and moderately enriched P group (p=0.043) compared to the unenriched P group. At 80% shade, the enriched P treatment group was only greater than the moderately enriched P treatment (p=0.019).

GPP was, on average, 0.574 ± 0.096 μg $O_2$ hr$^{-1}$ for all 36 collected strips (**Fig 4.11**). The GLMM did not find a significant interaction of phosphorus and shade (F=1.09, p=0.404), nor main effect of P (F=1.55, p=0.286). GPP did, however, differ between shade treatments (F=70.77, p<0.001). Pairwise comparisons revealed that GPP differed between all shade treatment groups (p<0.05), with productivity being higher in unshaded treatments compared to shaded treatments. The only exception was that the unshaded and moderately shaded (50%) treatment groups did not differ (p=0.302) from one another.

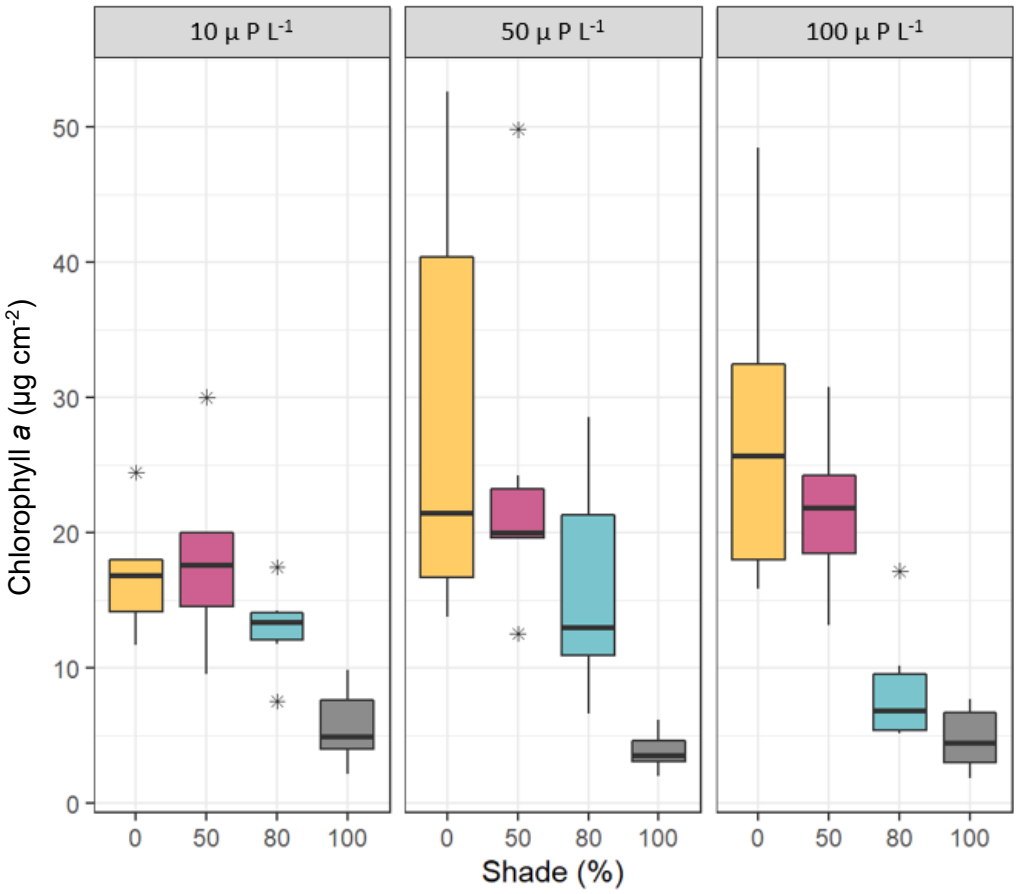


**Figure 4.10.** Boxplot summarizing mean chlorophyll *a* concentration (µg $cm^{-2}$) on cotton substrates (N=36) after 21 days of incubation in artificial streams. Boxplots show the median and interquartile range, and whiskers denote the 5th and 95th percentiles for chlorophyll a concentration.

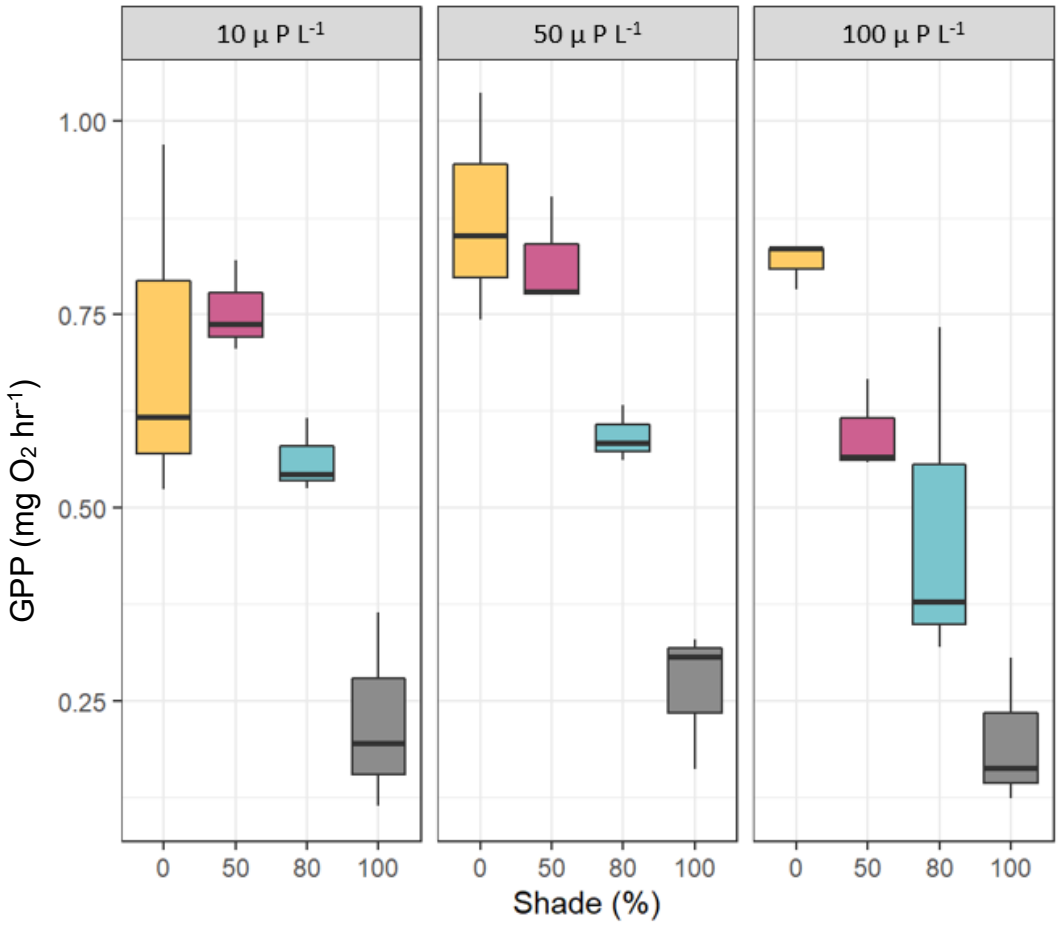


**Figure 4.11.** Boxplot summarizing mean gross primary production (GPP; mg $O_2$ $hr^{-1}$) on cotton substrates (N=36) after 21 days of incubation in artificial streams. Boxplots show the median and interquartile range, and whiskers denote the 5th and 95th percentiles for GPP.

### *4.2.4 Relationship Between Algal Abundance and Tensile Loss*

A negative linear relationship with a slope of -0.12 was found between GPP and tensile loss per day ($R^2 = 0.18$; **Fig. 4.12**) for all samples from all treatment levels. Tensile loss per day also decreased with increasing GPP within the three tested P levels. Slopes of the resultant regression lines decreased with P concentration from -0.15 ($R^2 = 0.58$) at 100 µg P $L^{-1}$ to -0.13 ($R^2 = 0.02$) at 50 µg P $L^{-1}$ to -0.09 ($R^2 = 0.35$) at 10 µg P $L^{-1}$. Within P treatment groups, shade treatments generally transitioned from the highest shade intensity to the lowest as GPP increased, specifically in the two enriched P treatment groups. There was no discernible pattern of shade treatments in the unenriched P treatment group, aside from fully shaded treatments being associated with lower GPP values and greater tensile loss.

Like GPP, a negative linear relationship with a slope of -0.13 was also found between chlorophyll *a* concentration and tensile loss per day ($R^2 = 0.03$; **Fig. 4.13**) for all samples from all treatment levels. Percent tensile loss per day also decreased with increasing chlorophyll *a* concentration within the 3 tested phosphorus levels, but there was, however, no clear pattern regarding the steepness of slopes within individual P treatment groups (**Fig. 4.13**). The steepest slope (-0.49, $R^2 = 0.35$) in this case belonged to the unenriched P treatment group, followed by the most enriched (-0.35, $R^2 = 0.58$) and moderately enriched (-0.07, $R^2 = 0.024$) P groups. Regarding the arrangement of shade levels, there was again a clear progression from the highest shade intensity to the lowest within the two enriched P groups as chlorophyll *a* concentration increased.

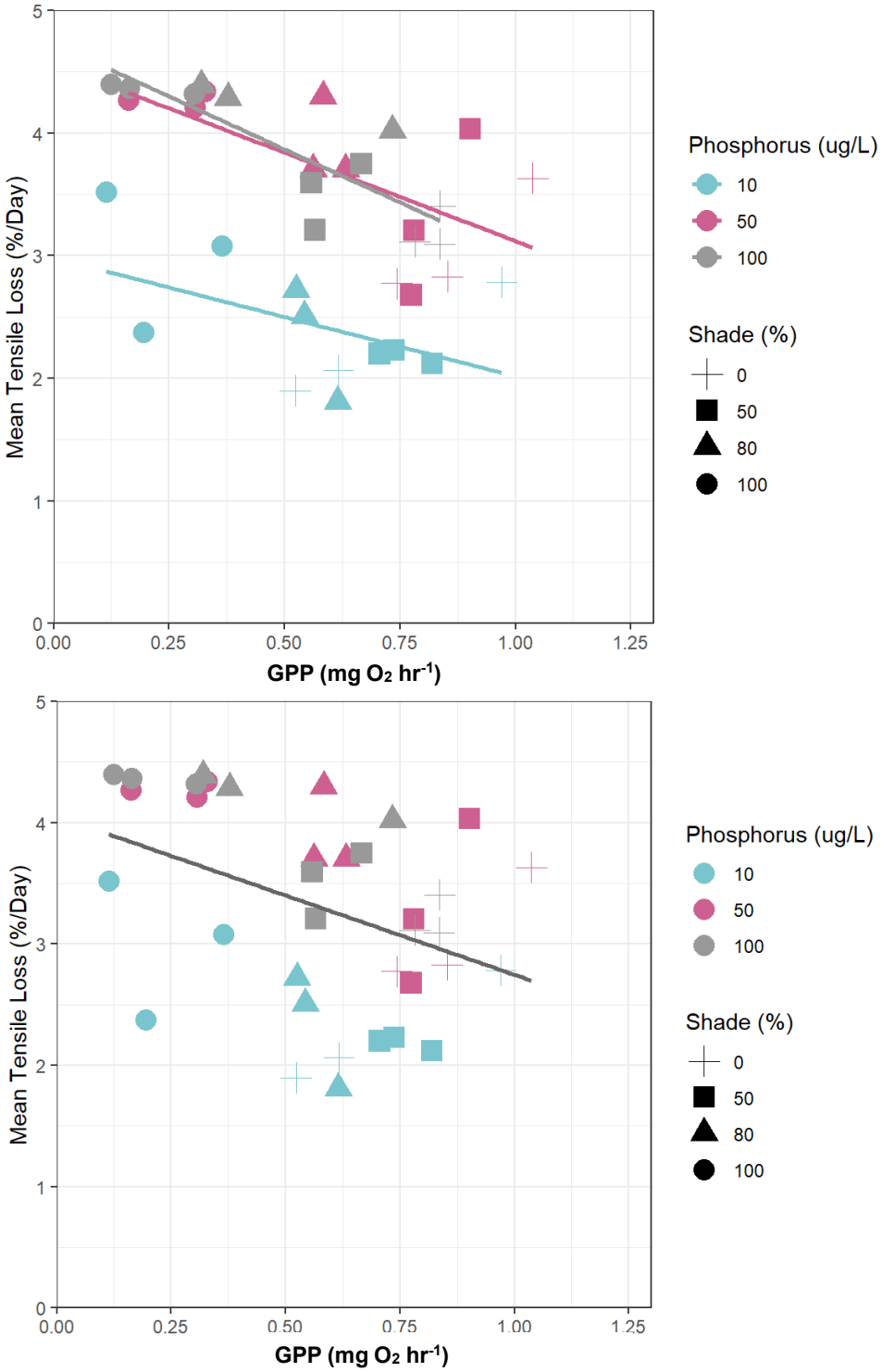


**Figure 4.12.** Linear regression of tensile loss (% per day) and GPP from cotton substrates (N=36) in artificial streams after 21 days of incubation. Tensile loss values are given as the mean tensile loss of the 3 strips collected from each treatment combination in artificial streams. Colours represent P treatments while shapes represent light treatments. The top panel shows the relationship for all samples from all treatments and the bottom panel shows the relationship within P treatment groups.

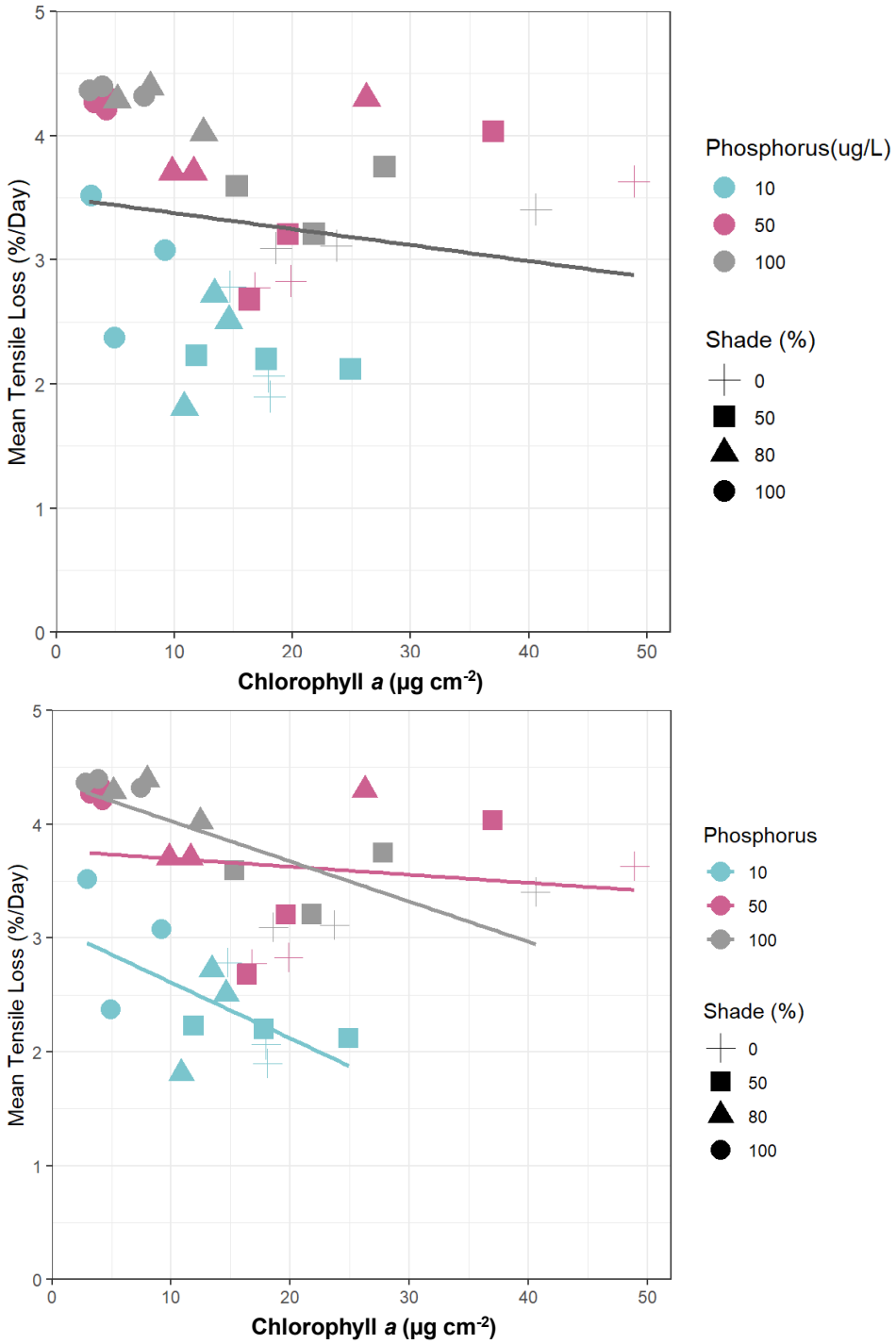


**Figure 4.13.** Linear regression of tensile loss (% per day) and chlorophyll *a* concentrations from cotton substrates (N=36) in artificial streams after 21 days of incubation. Tensile loss values are given as the mean tensile loss from the 3 strips collected per treatment combination in artificial streams. Colours represent P treatments while shapes represent light treatments. The top panel shows the relationship for all samples from all treatments and the bottom panel shows the relationship within P treatment groups.

# 5.0 Discussion

## 5.1 Evidence of Priming

Through experimental manipulation of light and nutrients, I found that algal accrual on coarse particulate OM can hinder ROM decomposition through a negative priming effect. In contrast to my initial prediction, there was no discernable evidence of positive priming observed in my study. Rather, negative priming was observed at all examined P concentrations whereby cellulose substrates incubated in high light conditions experienced higher rates of algal accrual compared to those substrates incubated in low light conditions, which translated to slower rates of cellulose decomposition specifically in artificial streams. While these results may appear to contradict the initial predictions, mine is not the first study to report only evidence of algal inhibited priming in response to changes in light and nutrient availability. For example, in the study by Ashberry et al., (2021), which examined priming intensity across a gradient of P enrichment (1 to 1024 μg P $L^{-1}$), only evidence supporting negative priming of cellulose decomposition was found. In this study, negative priming was observed above P concentrations of 16 μg P $L^{-1}$, with no evidence of priming potential below this concentration. Results of the current study align with findings in Ashberry et al. (2021) such that only evidence of negative priming was found, however, in my study, evidence of negative priming was found at concentrations below 16 μg P $L^{-1}$ (i.e., 10 μg P $L^{-1}$) despite being absent in Ashberry et al. (2021).

The negative priming of cellulose decomposition in my study was likely the result of the preferential use of algal exudates by heterotrophic organisms. Although algae have been shown to release a smaller fraction of photosynthate under nutrient-replete conditions compared with those that experience nutrient stress (Wyatt et al., 2014, Wyatt and Turetsky, 2015), higher algal biomass associated with increasing P and light availability could have led to higher bulk labile C release. Having a greater pool of labile C available to heterotrophic organisms as a direct energy source may have reduced the need for bacteria and fungi to invest in energy-intensive processes to obtain organically bound cellulose from within cotton substrates, thus leading to the lower rates of decomposition observed in my experiment. This pattern is consistent with my prediction based on Howard-Parker et al. (2020) whereby priming would be either negative or greatly diminished due to preferential substrate use when there is greater algal presence in OM biofilms.

It is also possible that enriched P conditions affected the nature of the labile C itself which further contributed to the negative priming observed in the experiment. Indeed, Wyatt et al. (2014) demonstrated that labile C sourced from green algae, such as Cladophora, could potentially become more labile (i.e., degradable) in response to elevated N and P concentrations. While direct measurement of algal communities was not conducted in my study, it is conceivable that the high light and P conditions impacted the nature of labile C sourced from green algae, leading to an even greater preference towards algal-exuded labile C as an energy source in place of ROM. Additionally, the microbial community may have been affected such that microbes targeting cellulose were outcompeted or not able to colonize under these high light and nutrient conditions, further inhibiting cellulose processing from cotton substrates. Data from my study support this, as decreases in tensile loss were found to be directly associated with increasing levels of both GPP and chlorophyll a*,* suggesting a negative relationship between biofilm thickness and tensile loss. Because phototrophic organisms like algae tend to dominate the upper layers of biofilms where light availability is optimal (Proia et al., 2017), steep diffusion gradients may have arisen that hindered essential resource access for heterotrophic bacteria and fungi that are prevalent in the lower layers of the biofilm. This smothering effect could have led to challenges for heterotrophic microorganisms in acquiring the resources they needed for growth and metabolism (such as light, dissolved oxygen, and the nutrients N and P), consequently resulting in reduced cellulose processing.

While negative priming was observed in artificial stream settings, there was no evidence to support this in my field experiment. Microbial degradation in my study streams, measured as the temperature corrected breakdown rate of cellulose, did not differ between ambient and shaded conditions in either study stream. Although there was a trend for unshaded treatments to have higher chlorophyll *a* and GPP than shaded ones, the differences were not consistent and they did not translate to slower rates of cellulose breakdown. This lack of association between algal abundance and heterotrophic activity may indicate that priming (either positive or negative) is of minor importance in a field setting. Elosegi et al., (2018) reached a similar conclusion about priming in the field environment while studying OM breakdown in temperate streams. Although the study by Elosegi et al. (2018) took place during early autumn when algae are not as abundant as they are in late summer, our results were similar in that no priming effect was observed. To my knowledge, there are no other field experiments that have taken place in similar stream

environments that my study can be compared to. However, according to a meta-analysis conducted by Bengssten et al. (2018) concerning the significance of priming across a diverse range of freshwater systems, such as lakes and rivers, the authors similarly arrived at the conclusion that the priming effect might be minimal or even absent within natural environments.

Overall, findings from my field experiment, along with other studies in freshwater ecosystems, suggest that other environmental factors may outweigh the influence of algal growth on OM decomposition dynamics in the natural stream environment. While my experimental design aimed to reduce the potentially confounding effects of other environmental variables by placing shaded and unshaded reaches very close to one another other, there are some environmental variables at the microhabitat scale that can influence decomposition rates in a field setting. Indeed, litter decomposition is sensitive to small differences in microhabitat scale factors such as flow velocity, sediment deposition and differences in biological communities (Albelho, 2001). Notably in my study, the presence of large grazers (particularly crayfish found near the cotton strips) may have played a crucial role in decomposition in the field experiment that was not accounted for in the artificial stream experiment as they were removed prior to incubation.

Despite my initial predictions, there was no evidence to support positive priming in either natural or artificial streams. Based on the conceptual model developed by Howard-Parker et al. (2020), positive priming would have been expected to occur in both unenriched experimental P treatments used in my study. Instead, I observed negative priming in artificial streams at low P concentrations while priming was completely absent in natural streams. An absence of positive priming could potentially be attributed to the fact that the experimental P concentrations used in the study were not low enough to establish a competitive environment within the biofilm. Indeed, studies have shown that dissolved SRP concentrations required to saturate specific cellular growth rates in periphyton can be as low as 0.3 – 0.6 µg P $L^{-1}$ (Bothwell, 1988) for some periphytic diatoms. Other microbial species such as green algae, however, require greater concentrations of bioavailable P (between 25 – 50 µg P $L^{-1}$) to saturate cellular growth rates (Bothwell, 1989). Depending on what microbial species are present, cellular growth may have been saturated at the concentrations of 5 µg P $L^{-1}$ and 10 µg P $L^{-1}$ that were used in my study. If cellular growth was in fact saturated, competition for the available P resources may have been limited, negating the need for heterotrophic investment into degradative enzymes.

A lack of N limitation may have also contributed to the absence of positive priming in my study. Although the P treatments covered a range of N:P ratios, with the most enriched P treatment theoretically being N-limited (N:P = 15:1), maintaining a water-column N concentration at a constant 1500 µg dissolved inorganic N $L^{-1}$ in artificial streams likely minimized the possibility of N limitation in this experiment. Similarly, my study streams in the field experiment were also exposed to high dissolved inorganic N concentrations, despite the high P study stream being N-limited (N:P = 2:1). There have been several studies in both soils (eg. Moorhead and Sinsabaugh, 2006; Chen et al., 2014) and aquatic ecosystems (eg. Soares et al., 2017) that have documented that positive priming is associated with N-mining by heterotrophic organisms. In aquatic systems, N is the nutrient that most closely regulates the development of fungi on leaf litter, with P being secondary (Jabiol et al., 2018). Therefore, positive priming of coarse particulate OM in aquatic ecosystems might be driven more by N limitation than by P limitation. Thus, N limitation in streams may be a prerequisite for positive priming.

Lack of positive priming may also be attributed to negligible nutrient concentrations in my substrates (as indicated by C:N and C:P ratios). This was listed as a potential reason that Ashberry et al. (2021) did not find positive priming, as they found limited N and P bound within cotton strips. Although this was not directly measured in my study, given that Ashberry et al. (2021) also used artist's fabric as the cotton substrate, it is reasonable to assume that my substrates also had negligible N and P. Lack of organically bound N and P may have further contributed to preferential substrate use in low P conditions as it would have been more energetically favourable to use labile C sourced from algae as an energy source for growth and reproduction rather than investing it in extracellular enzymes to obtain what was organically bound within cotton strips. Moreover, if priming is in fact primarily associated with N-mining, then the availability of organically bound N with substates would be an especially important factor to consider for positive priming. Thus, a lack of N associated with cotton substrates, coupled with high-water column dissolved N, would discourage fungi from investing such resources in N-mining.

## 5.2 Effect of Light and Nutrients on OM Processing

In terms of the environmental controls on priming intensity, I initially predicted that priming intensity may be interactively mediated by light and P availability. However, I instead observed that they acted largely independent of one another to influence OM decomposition in both natural and artificial streams. Although a significant interaction term between light and nutrients was found between days 21 and 27 for the artificial stream experiment, there was no clear pattern of light-dependent or P-dependent effects on OM processing. That is, there was no consistent pattern over these 3 sampling events which demonstrated that light was only important at certain nutrient concentrations or conversely that P was only important at certain light levels. Instead, data collected in my study showed that both light and P had consistent effects on organic matter processing, regardless of the availability of the other.

Regarding nutrient availability, my primary finding was that P enrichment increased rates of tensile loss from cotton substrates, irrespective of light level. Elevated P concentrations in both natural and artificial streams were associated with higher rates of tensile loss, with P availability increasing decomposition by a factor of 40-50% in artificial streams and 33% in natural streams. An increase in microbial OM processing in response to P amendment aligns with other studies which have shown that as microbial growth is released from nutrient limitation, an increase in decomposition occurs (eg. Scott et al., 2013). P availability is thought to increase microbially driven OM processing by facilitating the growth and reproduction of the heterotrophic organisms, thus leading to a larger pool of heterotrophic organisms being present within the biofilm. Indeed, heterotrophic abundance has been shown to be positively correlated with P availability in stream biofilms, including both bacterial (Rier and Stevenson, 2001) and fungal species (Gulis 2003, Fereira, 2015; Howard-Parker et al., 2020). Fungal biomass in particular has been shown to respond positively to P enrichment, which may imply that the stimulatory effect of P on OM processing could be driven by fungal, rather than bacterial action (Gulis, 2003). Moreover, P enrichment, by stimulating algal growth may have increased the surface area available for bacterial and fungal colonization, further increasing the pool of heterotrophic organisms involved in C processing. Forthcoming community analyses from my experiment will help confirm whether there was a larger heterotrophic community contributing to higher C processing rates and, too, whether increases in C processing were bacterially or fungally driven.

Stimulation of heterotrophic activity through P amendment is also seen over the entire 27-day study period in artificial streams. Despite having similar rates of tensile loss for the first 9 days (i.e., first 3 sampling events) of the experiment, increased P availability after day 9 resulted in 30-40% increases in tensile loss from cotton substrates, which the persisted until the final day of the experiment. During this study period, P stimulation of ROM decomposition was slightly greater in unshaded and moderately shaded treatments compared to completely shaded treatments. For instance, in completely shaded conditions on day 15 of the experiment, enriched P treatments exhibited approximately 30% higher tensile loss than the unenriched P treatment. When shade was absent or more moderate, the distinctions between the unenriched and enriched P treatments were closer to 40%, suggesting P enrichment may have a greater effect light grown conditions compared to shaded conditions.

Moreover, in addition to finding a positive association between P amendment and heterotrophic function, data from artificial streams also provided evidence of a possible biological threshold for OM processing rates. Given that cellulose processing rates did not differ between the two enriched P treatments at any of the examined shade levels, it may imply that heterotrophic organisms were functioning at or near their biological capacity at the lesser enriched P concentration of 50 µg P $L^{-1}$. A similar observation was made in a meta-analysis by Woodward et al. (2012) which focused on the impact of nutrient pollution on the breakdown rates of litter (specifically Oak and Alder) across northern European streams. Their findings revealed that OM processing peaked at approximately 50 µg P $L^{-1}$, and then subsequently declined as P concentrations surpassed 100 µg P $L^{-1}$ (up to concentrations of 1000 µg P $L^{-1}$). The decline in OM processing was likely the result of deteriorating environmental conditions, along with increased biofilm thickness that suppressed invertebrate and microbially mediated breakdown of OM. Although, I did not observe a decline in OM processing as my experimental P concentrations did not go beyond 100 µg P $L^{-1}$, my study does support the notion of having heterotrophic C processing rates peak at concentrations of approximately 50 µg P $L^{-1}$ of SRP.

Overall, P amendment had a greater effect on OM processing rates in artificial streams than it did in natural streams, despite experimental P concentrations being similar. In artificial streams, P increased decomposition by a factor of 40-50% in artificial streams compared to only 33% in natural streams. This outcome aligns with findings from a meta-analysis by Fereira et al., (2015)

examining the effects of nutrient enrichment on decomposition rates in streams. In this study, responses to nutrient enrichment were found to be much stronger in laboratory studies than what was observed in field studies. This finding is not surprising given that growth rates of benthic species are typically higher in laboratory settings compared to field settings due to the absence of uncontrolled environmental variables as well the presence of shredders and large grazers. Although GPP did not vary substantively between artificial and natural streams in my study, chlorophyll *a* concentrations were much greater in artificial streams (range of 2.16 – 49.82 $\mu g/cm^2$) compared to natural streams (range of 0.88 – 7.63 $\mu g/cm^2$). Given connections between autotrophic and heterotrophic organisms within OM biofilms, a larger heterotrophic community may have also been present in artificial streams, partially explaining why tensile loss was greater in this environment compared to natural streams.

In contrast to the effect of P enrichment, light availability was found to reduce rates of decomposition by heterotrophic organisms through its effects on algal abundance. Specifically, in the artificial stream experiment, I observed a positive association between light availability and algal abundance (measured as GPP and chlorophyll *a*), which resulted in slower rates of tensile loss across all examined P levels. On day 21, for example, light availability increased both GPP and chlorophyll *a* concentration by a magnitude of between 3 and 4, which reduced percent tensile loss day per day by approximately 35% to 40% across all P levels. This finding agrees with the study by Ashbery et al., (2021) where strips incubated in light conditions were found to have higher rates of algal accrual than strips incubated in dark conditions, which resulted in lower losses in tensile strength, lower fungal biomass, and lower extracellular enzyme activity. Similarly, Halvorson et al., (2016) found light-incubated leaf litter to have slower decomposition and lower fungal biomass accrual compared to dark-incubated controls due to higher levels of algal accrual.

Light availability, by increasing algal biomass and consequently biofilm thickness, may have resulted in lower ROM decomposition by microbial organisms by increasing competition for essential resources within the biofilm. As biofilms become thicker, the diffusion of dissolved oxygen, light and nutrients from the water column becomes increasingly impaired, potentially leading to the competitive exclusion of heterotrophic organisms which are dominant in the lower layers of the biofilm. Preferential substrate use may have also played a role in decreasing decomposition rates from the cellulose substrates in high light conditions. Studies have shown a

reduction in degradative enzyme activity (Ashberry et al., 2021) in light-grown conditions as well as an accompanied increase in the activity of labile-C associated enzymes (Wagner et al., 2015). These observed shifts in enzyme activity support the theory that fungi grown in the presence of algae do allocate metabolic resources away from degradative enzyme production, thus decreasing recalcitrant C acquisition from the cotton substrate.

Like with P enrichment, the negative effect of light availability on cellulose processing can also be seen over the entire 27-day study period. As illustrated in **Fig. 4.7,** increasing light availability was found to be associated with a reduction in the steepness of decomposition curves over time, thereby affecting the time required to attain specific amounts of tensile loss. For example, by examining the highest phosphorus level (100 µg P $L^{-1}$), there is a 5-day discrepancy in achieving 50% tensile loss between the highest and lowest shade treatment levels. Under minimal light availability, it took roughly 10 days to achieve a 50% reduction in tensile loss. In contrast, within higher light conditions, the progression took approximately 12 to 14 days under moderate shade and extended to 15 days under the lowest shade level. Similar results were observed by Brady et al. (2021) whereby light incubation of leaf litter (*Macrobrachium* and *Pycnopsyche*) slowed decomposition rates, prolonging the time taken to reach 30% mass loss by upwards of 15 days. Data from this study also showed a light-induced decoupling of fungal growth rates from biomass accrual during experiment, indicating faster fungal turnover possibly because fungi relied on algal-derived C exudates to support growth and sporulation instead of investing in degradative enzymes to breakdown endogenous litter C (Brady et al., 2021). This observation may help explain why heterotrophic decomposition of OM in streams became suppressed in light-grown conditions in my study.

Although my analysis failed to identify any P-dependent effects of light on tensile processing (i.e., an interaction between light and P availability), it is interesting to note that the magnitude of the light effect changed depending on the particular P level examined. At higher P concentrations, light appeared to have a lesser impact than it did at lower P concentrations with regards to the maximum amount of tensile loss observed at the end of the study. In both enriched P treatments, light was found to have a significant effect on OM processing rates, although it only resulted in a 10% (from 95% to 85%) reduction in tensile loss between the highest and lowest shade levels. At low P levels, the effect of light was found to have a more significant impairment

on OM processing rates, with reductions in OM processing amounting to approximately 30% between shaded and unshaded treatments. In fully shaded treatments, peak losses attained by the end of the experiment were approximately 70-75%, whereas maximum losses reached for both of the high light treatments were only around 40-50%.

Having a disproportionate impact of increased light availability on ROM processing in low P treatments is contrary to what I would have expected given the varying demands for N and P by microbial organisms under different light conditions. For example, it has been observed that microbial N uptake tends to be higher in low light conditions (eg. Rees & Syrett 1979), while P uptake is generally greater in ambient light conditions (eg. Wagner et al., 2015). A shift towards greater P preference would thereby intensify the competition for limited P resources among microbial organisms under high light conditions. Based on the theory that the strongest positive priming effect occurs under nutrient limitation, one would expect that ROM decomposition would be highest in the unenriched P treatment where competition for limiting P is maximized. However, I instead observed the strongest negative priming effect under low P conditions. This, along with previous observations, may further suggest that algal priming is less tied to P-mining in aquatic ecosystems and other factors such as N-mining may be more important.

Lastly, I initially predicted that community respiration would mirror the patterns found for decomposition. However, no association was found between respiration and either light or nutrients. Generally, respiration rates were greater in high phosphorus conditions compared to low phosphorus conditions (particularly in the field experiment), however, the observed differences were not found to be significant. My ability to detect differences in respiration between treatment groups may have been limited by confounding effects of algal photosynthesis and respiration using the light and dark bottle method. Both over- and under-estimations of microbial respiration are possible through this method due to algal communities continuing to respire (consuming oxygen) or photosynthesize (producing oxygen) in dark bottles. In addition, this method does not account for the heterogeneity in microbial communities. Different microbial communities can exhibit varying rates of photosynthesis and respiration, thus, if the composition of microbial communities changes across sampling locations, it can introduce variability into the measurements and make it challenging to attribute oxygen changes solely to microbial respiration from light and nutrients.

## 6.0 Conclusions and Future Directions

My study, which examined the effects of light and nutrients (particularly bioavailable P) on heterotrophic function through their effects on primary production, provides further evidence of algal inhibited decomposition of OM in streams. A reduction in detrital decomposition signifies an impairment to ecosystem function as it can negatively impact C cycling at an ecosystem level. Moreover, because OM is also considered a long-term source of nutrients (Mulholland, 2004), reduced detrital processing can also have repercussions on N and P cycling in streams. Indeed, research by Halvorson et al., (2019) has established a connection between algal priming intensity and N and P loss from plant litter, reinforcing the link between macronutrient cycling and algal priming in aquatic ecosystems. Algal-induced suppression of decomposition may result in a build-up of OM and the subsequent emergence of hypoxic conditions in both the local stream environment as well as further downstream locations. Accumulation of OM can create hypoxic conditions as dissolved oxygen (DO) is consumed from the water column when it is eventually broken down by microbes (Halvorson et l., 2019). Such consequences could have profound impacts on local food webs which are crucial for both human and wildlife survival.

Not only did my study demonstrate adverse impacts of algal presence on OM decomposition, it also highlights the varying significance of priming under different environmental contexts. Priming appears to be of particular importance under low nutrient conditions, whereby increases in light availability were shown to have more negative effects on organic matter processing than they did at high P concentrations. This finding may be of particular importance for low-order forested streams which are typically characterized by low background nutrient availability (Roberts et al., 2007). Due to severe constraints on algal growth, these streams may be particularly susceptible to increases in light availability from anthropogenic activities, possibly leading to long-term effects on C and nutrient budgets. This finding provides yet another reason to focus stream restoration efforts on human activities that modify riparian shading, such as canopy removal, to counteract human-induced impacts on streams.

When considering long-term C and nutrient budgets, it is equally important to take into account rising anthropogenic inputs of nutrients, specifically N and P. Although elevated P concentrations did not appear to increase priming intensity in my study, it did increase rates of ROM decomposition in both natural and artificial streams. In addition, based on my discovery of

a nutrient threshold, increases in detrital decomposition may be more pronounced when the initial P concentration is low. For example, streams with background nutrient concentrations below 50 μg P $L^{-1}$ may be more stimulated by increasing water-column P availability from human activities than streams with background nutrient concentrations above this concentration. As a consequence, low-order forested streams with low nutrient availability may again be disproportionately impacted by changes human activities that affect nutrient inputs to streams.

Given that global temperatures continue to rise due to climate change, it is increasingly important to understand how temperature interacts with light and nutrient availability to influence heterotrophic function and ecosystem productivity. Warming can significantly shift microbial metabolism and community dynamics, potentially reshaping OM breakdown rates and nutrient cycling patterns at an ecosystem level. Because the combined effects of temperature, light, and nutrient availability are not likely linear; complex interactions or thresholds likely exist beyond which ecosystem functions are disproportionately affected. Thus, future studies on algal priming would benefit from assessing the interactive effects of all three factors to determine which stream types might be most affected by such changes.

Future studies should also consider whether biofilm assemblage composition is related to observed rates of C and nutrient processing in aquatic ecosystems. While an association between algal abundance and heterotrophic function was found in my study, a significant knowledge gap remains regarding potential shifts in biofilm assemblage composition that could be driving alterations in C decomposition by microbial organisms. The forthcoming data from this study will shed light on whether the anticipated transition from diatom-dominated algae to green algae indeed contributed to the decline in carbon processing, as I initially hypothesized. Too, determining whether a relationship exists between the composition of heterotrophic assemblages and decomposition rates would also be beneficial. Again, forthcoming taxonomic data from this study will be able to answer whether associations can be drawn between bacterial and fungal communities and the decomposition process. Understanding these population level dynamics is important for the development of bioindicator and bioassessment tools for water resources management, whereby the types of species present in a water column may be used to assess and predict rates of decomposition.

Lastly, additional tests of algal-mediated priming effects in field settings are certainly necessary. To date, results of priming in natural stream environments remains controversial and it is unclear whether if it is of any real significance outside of laboratory settings. Particular importance should be placed on conducting these field studies during the summer months when algal accumulation is at a maximum. Future priming studies (in both natural and laboratory environments) should be done across a greater number of P levels, specifically at low P levels to increase the potential of finding a positive priming effect. My study failed to find algal enhanced priming at P concentrations of 5 µg P $L^{-1}$ (field) and 10 µg P $L^{-1}$ (artificial streams), thus future studies might want to consider P concentrations below this. Such experiments will improve our understanding of how P availability drives C storage and nutrient cycling in stream ecosystems.

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**Appendix A1**

GLMM results for each sampling event of the 27-day artificial stream experiment at the Thames River Experimental Stream Sciences (TRESS) Centre in London, Ontario, Canada. Table shows F- and p-values for the main effects of Phosphorus, Shade and their interaction term. Bolded results indicate that a significant effect was found ($p<0.05$).

| Time (Days) | Shade | Phosphorus | Shade*Phosphorus |
|---|---|---|---|
| **3** | F=0.29; p=0.831 | F=0.78; p=0.500 | F=0.38; p=0.877 |
| **6** | **F=5.95; p=0.005** | F=0.08; p=0.921 | F=1.58; p=0.210 |
| **9** | **F=7.40; p=0.002** | F=1.13; p=0.385 | F=1.17; p=0.366 |
| **12** | **F=12.27; p=0.000** | **F=12.78; p=0.007** | F=0.44; p=0.841 |
| **15** | **F=23.60; p=0.000** | **F=38.92; p=0.000** | F=0.76; p=0.612 |
| **18** | **F=18.30; p=0.000** | **F=16.58; p=0.004** | F=0.20; p=0.972 |
| **21** | **F=38.39; p=0.000** | **F=20.44; p=0.002** | **F=2.40; p=0.034** |
| **24** | **F=17.90; p=0.000** | **F=145.60; p=0.000** | **F=3.70; p=0.014** |
| **27** | **F=20.34; p=0.000** | **F=50.54; p=0.000** | **F=3.20; p = 0.026** |

**Appendix A2**

Tukey HSD post-hoc results for the effect of shade on days 6 to 18 of the artificial stream experiment conducted at the Thames River Experimental Stream Science Centre in London, Ontario, Canada. Bolded values indicate a significant result ($p<0.05$). Only days where shade was found to have a significant main effect are shown. Days where there was no effect of shade (day 3) or days where an interaction between shade and phosphorus was found (days 21-27) are omitted.

| | **Pairwise Test** | | | | | |
|---|---|---|---|---|---|---|
| **Day** | 0%:50% | 0%:80% | 0%:100% | 50%:80% | 50%:100% | 80%:100% |
| **6** | 0.6050 | 0.1390 | 0.1701 | 0.0116 | **0.0149** | 0.9995 |
| **9** | 0.6793 | **0.0678** | **0.0018** | 0.4322 | **0.0189** | 0.3251 |
| **12** | 0.9289 | **0.0558** | **0.0003** | 0.1710 | **0.0008** | **0.0642** |
| **15** | 0.1471 | **0.0018** | **0.0002** | 0.1693 | **0.0003** | **0.0009** |
| **18** | **0.0197** | **0.0004** | **0.0002** | 0.1811 | **0.0081** | 0.4156 |

## Appendix A3

Tukey HSD post-hoc results for the effect of phosphorus on days 12 to 18 of the artificial stream experiment conducted at the Thames River Experimental Stream Science Centre in London, Ontario, Canada. Bolded values indicate a significant result ($p<0.05$). Only days where phosphorus was found to have a significant main effect are shown. Days where there was no effect of phosphorus (days 3 to 9) or days where an interaction between shade and phosphorus was found (days 21-27) are omitted.

| | **Pairwise Test** | | |
|---|---|---|---|
| **Day** | 10:50 µg P $L^{-1}$ | 10:100 µg P $L^{-1}$ | 50:100 µg P $L^{-1}$ |
| **12** | **0.0053** | **0.0007** | 0.5842 |
| **15** | **0.0001** | **0.0001** | 0.2407 |
| **18** | **0.0001** | **0.0001** | 0.9971 |

**Appendix A4**

Results of individual GLMM's comparing phosphorus treatments for each examined shade level on day 21, day 24 and day 27 of the artificial stream experiment conducted at the Thames River Experimental Stream Science Centre in London, Ontario, Canada. Values are given as p-values, with bolded values indicating a significant result ($p<0.05$).

| Day | 0% Shade | 50% Shade | 80% Shade | 100% Shade |
|---|---|---|---|---|
| **21** | **0.052** | **0.018** | **0.001** | **0.004** |
| **24** | **0.001** | **0.000** | **0.000** | **0.005** |
| **27** | **0.000** | **0.005** | **0.000** | **0.001** |

**Appendix A5**

Tukey HSD post-hoc results comparing phosphorus treatment groups on day 21, day 24 and day 27 of the artificial stream experiment conducted at the Thames River Experimental Stream Science Centre in London, Ontario, Canada. Bolded values indicate a significant result ($p<0.05$).

| Pairwise Test | 0% Shade | 50% Shade | 80% Shade | 100% Shade |
|---|---|---|---|---|
| **10:50 µg P $L^{-1}$** | Day 21: **p= 0.0055** | Day 21**: p=0.0002** | Day 21: **p=0.0001** | Day 21: **p=0.0001** |
| | Day 24: **p=0.0023** | Day 24: **p=0.0003** | Day 24: **p=0.0004** | Day 24: **p=0.0110** |
| | Day 27: **p=0.0005** | Day 27: **p=0.0144** | Day 27: **p=0.0011** | Day 27: **p=0.0018** |
| **10:100 µg P $L^{-1}$** | Day 21: **p=0.0018** | Day 21: **p=0.0002** | Day 21: **p=0.0001** | Day 21: **0.0001** |
| | Day 24: **p=0.0009** | Day 24: **p=0.0003** | Day 24: **p=0.0003** | Day 24: **p=0.0070** |
| | Day 27: **p=0.0002** | Day 27: **p=0.0063** | Day 27: **p=0.0005** | Day 27: **p=0.0017** |
| **50:100 µg P $L^{-1}$** | Day 21: p= 0.8520 | Day 21: p=0.5308 | Day 21: **p=0.0317** | Day 21: p=0.7459 |
| | Day 24: p=0.3604 | Day 24: p=0.5086 | Day 24: p=0.2851 | Day 24: p=0.8917 |
| | Day 27: **p=0.0165** | Day 27: p=0.7145 | Day 27: p=0.4296 | Day 27: p=0.9943 |

**Appendix A6**

Tukey HSD post-hoc results for day 21 of the artificial stream experiment conducted at the Thames River Experimental Stream Science Centre in London, Ontario, Canada. Bolded values indicate a significant result ($p<0.05$).

| Pairwise Test | 0% Shade | 50% Shade | 80% Shade | 100% Shade |
|---|---|---|---|---|
| **10:50 µg P $L^{-1}$** | **p=0.0055** | **p=0.0002** | **p=0.0001** | **0.0001** |
| **10:100 µg P $L^{-1}$** | **p=0.0018** | **p=0.0002** | **p=0.0001** | **0.0001** |
| **50:100 µg P $L^{-1}$** | p=0.852 | p=0.5308 | **p=0.0317** | 0.7459 |