Effects of Light, Nutrients, and Salts on Microbial Biofilm Productivity and Detrital Processing in Aquatic Mesocosms

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Effects of Light, Nutrients, and Salts on Microbial Biofilm Productivity and Detrital Processing in Aquatic Mesocosms

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology

by

Bethanie Brooke Howard-Parker
Southern Arkansas University-Magnolia
Bachelor of Science in Biology, and Agricultural Sciences, 2013

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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Anthropogenic activities associated with urbanization, agriculture, and resource extraction continue to increase to support increasing needs of the growing population. These activities increase the amounts of pollutants entering freshwater streams and put aquatic ecosystems at structural and functional risk. Aquatic microbes play an important role in detrital processing in streams as a key linkage in moving carbon from detrital stocks into aquatic food webs. My research investigates the effects of light, nutrients, and salts on detrital microbes and decomposition in freshwaters using a mesocosm approach. In chapter one, I modified a current priming effect (PE) hypothesis model to include light level, proposed a method for calculating quantitative values for potential PEs, and tested the PE hypothesis according to my model and proposed calculation methodology. I manipulated light level [ambient (full light available) or 19% of ambient] and phosphorus [P; added 10, 100, or 500μg/L dissolved inorganic P (DIP)], and measured the effects on autotrophic (algae) and heterotrophic (fungi) detrital microbial communities and decomposition of Quercus stellata (Post Oak) leaf litter in a 150-day experiment. The results illustrate the importance of considering light levels, nutrient ratios (rather than individual nutrients), and detrital recalcitrant organic matter (ROM) components in further PE model development and provide evidence that priming effects occur in aquatic systems. In chapter two, I compare two similar experiments investigating potential subsidy-stress responses of autotrophic (algae) and heterotrophic (fungi and bacteria) detrital microbial communities and decomposition of Liquidambar styraciflua (Sweet Gum) leaf litter to sub-lethal increases in two common sodium (Na) salts (i.e. NaCl and NaHCO₃). Treatments included ambient streamwater (SW; ~3mg/L Na) and NaCl and NaHCO₃ amended SW with target concentrations of 16, 32, and 64 mg/L Na salts. Experiment A was subjected to day/night light cycles and temperatures according to the season and ran for 134 days. Experiment B was subjected to 12hr light/dark cycles at a constant 4.5°C for 135 days. The data suggest low-level Na salt increases likely affect detrital biofilms and decomposition before toxicity occurs, may be
ion- and species-specific, and temperature plays a significant role in the magnitude of these effects.
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Dedication

I dedicate this work to my beloved friends and family who have traveled this road with me because I could not have gotten here without them.

To my friends: Joshua Taylor and Jenna Smith, thank you for being there and encouraging me along the way. You are both dear to me. Jean Adams, I think of you often. I wish you were here for me to be able to share this achievement with you today.

To my family: Mom (Beverly Moseley), thank you for being behind me 100% since day one and never giving up on me. Be it ‘dazzling’ or ‘baffling’, I have done my best. Memaw (Mary Bass), you have been my cheerleader my whole life, and it has meant the world to me. Nanny (Omer Renfro), I am eternally grateful that I got to spend so much time with you while you were on this earth. Thank you for pushing me to always ‘get my lessons’. Papaw (Alvin Bass), not a soul in this world inspired me more to continue my education than you. I wish you could be here for this. Dad (David Howard), I miss you terribly! You always pushed me so much, but you knew better than I what I could do. I would give anything to share this with you. Allan, you have been unbelievably patient and supportive. I could not ask for a better partner in life than you. Sarah, I ultimately did all of this for you. Every decision since the day you were born was made with you in mind, including going back to school. It has been a rough road at times, but to hear you say that what I have done has been an inspiration to you and that you are proud makes every struggle along the way worth it.
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Thesis Introduction

Effects of Light, Nutrients, and Salts on Microbial Biofilm Productivity and Detrital Processing in Aquatic Mesocosms
Introduction

Allochthonous inputs (originating from outside the stream) of detritus (dead organic matter subject to decomposition) into fresh headwaters is determined by the watershed and are the primary carbon (C) source for biota in these systems (Fisher and Likens, 1973; Hynes, 1975; Vannote et al., 1980). Detritus usually enters the stream with a high C:nutrient ratio (relatively low quality as determined by detritivores) because vegetation resorbs most nutrients prior to leaf abscission in the fall (Aerts 1996). Autochthonous inputs (originating from inside the stream) in the form of primary production can contribute to C standing stocks in headwaters, though to a much lesser degree than terrestrially derived detritus (Fisher and Likens, 1973). Detritus then is the basis of aquatic food webs (brown food webs) and sets the stage for C and nutrient cycling and energy transfer through successively higher trophic levels (Moore et al., 2004; Hagen et al, 2012).

Once detritus enters the stream, leaching of labile organic compounds begins and up to ~one-quarter of detrital biomass may be depleted within ~24hrs depending on the leaf litter species (Bärlocher, 1985; Webster and Benfield, 1986; Allan and Castillo, 2007). As leaching continues to a variable degree, heterotrophic aquatic microbes, the key linkage in moving C from detritus into the food web, rapidly colonize detritus (Bärlocher and Kendrick, 1975; Bärlocher, 1985; Gulis et al., 2006; Sanderman and Amundson, 2013). Detrital microbial communities forming biofilms on detritus are involved in a complex web of interaction known as the microbial loop, wherein detrital processing and relatively efficient nutrient and organic matter recycling occurs (Pomeroy et al., 2007). Fungi are the first heterotrophic microbes on the scene to begin the decomposition process and are quickly joined by a host of bacteria (Bärlocher and Kendrick, 1975). The rate of detrital processing by these microbes is determined in part by the ease with which complex molecules in the leaf litter detritus can be broken down (recalcitrance) (Danger et al, 2013; Pastor et al., 2014) and the development the microbial communities that colonize the leaf litter detritus (Strickland et al., 2009).
Increased microbial community biomass (productivity) leads to changes in the quantity of leaf litter as decomposition increases and changes in quality of leaf litter as C: nutrient ratios decrease (Romaní et al., 2006; Webster et al., 2009). Heterotrophic microbial detrital processing involves litter quantity decreases by enzymatic liberation of organic C (Turley, 1994; Munster and De Haan, 1998; Rier et al., 2007). This is known as decomposition through mineralization of organic matter and is primarily carried out by aquatic hyphomycetes, or fungi (Gessner and Chauvet, 1994; Munster and De Haan, 1998; Sanderman and Amundson, 2013). Heterotrophic bacteria also play a role in decomposition, though not as much as fungi (Baldy et al., 1995).

Another component of detrital processing is litter quality increases (decreasing C: nutrient ratios). Fungi and bacteria, which form part of the biofilm on C-rich detritus, are capable of assimilating nutrients from the water column (Suberkropp and Chauvet, 1995; Gulis et al., 2006; Suberkropp et al., 2010; Danger et al., 2013). In this way the detritus becomes a more nutritious food source (decreasing C: nutrient ratios) for detritivores (Cummins, 1973) as aquatic microbes are consumed along with their detrital substrates. Growth of detritivores depends heavily upon the quantity and quality of their food resource (Wallace et al., 1999; Chung and Suberkropp, 2009), which is why understanding microbial communities in terms of their effect on food resource quality and quantity is so important.

Nutrients are the major growth-limiting factor for heterotrophic aquatic microbes (fungi and bacteria) in unaltered headwaters (Elwood et al., 1981; Hecky and Kilham, 1988; Turley, 1994; Smith, 1998; Vitousek et al., 2008; Elser 2012). According to Liebig’s Law, growth is limited by the essential nutrient that is in the scarcest supply to the organism; the most limiting nutrient in freshwater streams is thought to be phosphorus (P) (Elwood et al., 1981). However nutrient loading into freshwater systems due to anthropogenic activities, such as expanding urbanization and agriculture, has become a well-known, global issue (Carpenter et al, 1998; Correll, 1998; Smith et al., 2003; Bernot et al., 2006; Jarvie et al., 2010) which can relieve nutrient constraints on growth (and therefore metabolic activity) of heterotrophic microbes.
There is also a growing awareness of problems with stream bank widening in relation to increasing land alterations for agricultural developments (Justus et al., 2010). These activities exacerbate nutrient loading and increase illuminance on headwater streams (Carpenter et al., 1998; Smith et al., 1999; Jarvie et al., 2010). Autotrophic microbes (algae) have been shown to have minimal response to nutrient increases alone (Greenwood and Rosemond, 2005), but increasing light from more open riparian canopies is a primary driver of increased algal growth (Triska et al., 1983; Smith, 1998) followed second by nutrient additions (Hill and Knight, 1988; Hill et al., 1995). Though autotrophic production usually contributes very little to the overall C standing stocks in freshwater systems (Fisher and Likens, 1973), increases in algal growth and metabolism translate to greater autochthonous C inputs into these freshwater streams in the form of dissolved organic C (DOC) (Danger et al., 2013). Part of algal metabolic activity is exudation of C into the surrounding water column as a byproduct of photosynthesis (Danger et al., 2013). This additional C source is then utilized by heterotrophic microbes, especially fungi, for growth and energy for processing detritus in the stream (Rier et al., 2007; Danger et al., 2013; Du et al., 2019). Depending on nutrient availability, detrital processing may be markedly increased or decreased (Guenet et al., 2010).

In addition to the aforementioned issues of nutrient loading and light level increases in low-order freshwater streams, data indicates the rise in freshwater salinity levels will continue to be a problem in the future (Hart et al., 1991; Ghassemi et al., 1995; Kaushal et al., 2005; Cañedo-Argüelles et al., 2016). As a result, studies involving salinity have seen a revival recently since early studies in the 70s. Expanding urbanization and agriculture as well as increased natural resource extraction, such as with fracking, and the application of deicer to paved roads in the winter are sources of salinity increases in freshwater streams (Postel, 1999; Blasius and Merritt, 2002; Kelly et al., 2008; Daley et al., 2009; Brantly et al., 2014). In most cases regulations for freshwater salinity levels are informed by research regarding toxic levels of salinity for aquatic macroinvertebrates, vertebrates, and macrophytes. However, sub-lethal salt
concentrations could impact detrital microbial communities before toxicity occurs which may have bottom up effects in brown food webs (Tyree et al., 2016; Entrekin et al., 2019). While small inputs of sodium (Na) may subsidize microbial growth (Odum et al., 1979), subsidy thresholds may differ between species. Likewise, anions associated with Na inputs have been understudied and may elicit differing responses in growth and metabolic activity depending on the microbial species (Cañedo-Argüelles et al. 2016 Zalizniak et al., 2006; Entrekin et al. 2019). Therefore, understanding how salinization of freshwaters impacts aquatic microbes is important given the close interaction of autotrophic and heterotrophic microbes (Pomeroy et al., 2007) comprising detrital biofilm and their role in detrital processing.

My research has primarily been concerned with how human activities impact freshwater systems from the bottom up. My overarching objective was to investigate the effects of additional stream inputs (i.e. increased light, nutrients, and salts) originating from anthropogenic activities on microbial communities and detrital processing. In my first chapter I modified a current model depicting the priming effect (PE) hypothesis (Guenet et al., 2010) to include light as a factor and proposed a method to calculate potential PEs. I tested this hypothesis and proposed calculation methods by investigating the effects of increasing light [ambient (full light available) or 19% of ambient] and nutrients [P as added 10, 100, or 500μg L\(^{-1}\) dissolved inorganic P (DIP)] on autotrophic and heterotrophic detrital microbial communities and decomposition of Quercus stellata (Post Oak) in a 150-day mesocosm experiment. In my second chapter I investigated potential subsidy-stress (Odum et al., 1979) effects of sub-lethal increases in two common Na salts (i.e. NaCl and NaHCO\(_3\)) on microbial communities and decomposition of Liquidambar styraciflua (Sweet Gum) leaf litter. Treatments included ambient streamwater (SW; ~3-4mg L NaCl/NaHCO\(_3\)) and NaCl and NaHCO\(_3\) amended SW with target concentrations of 16, 32, and 64mg L\(^{-1}\) Na salts. My 134-day experiment included natural day/night light cycles and temperatures according to the season (February-June). Within
chapter two, I compare my study results to a separate but similar study that used the same treatments but included 12hr light/dark cycles at a constant 4.5°C for 135 days.

References


Light and Dissolved Nutrients Mediate Recalcitrant Organic Matter Decomposition Via Microbial Priming in Experimental Streams

B Howard-Parker, B White, HM Halvorson, MA Evans-White
Abstract

Environmental factors such as nutrient and light availability may play important roles in determining the magnitude and direction of microbial priming and detrital decomposition and, therefore, the relative importance of microbial priming in carbon (C) dynamics in freshwater ecosystems. I integrated light availability with an existing conceptual model predicting the magnitude of the priming effect (PE) along a dissolved nutrient gradient (i.e. nutrient PE model). The modified light-nutrient PE model hypothesizes how light may mediate priming at any given nutrient concentration and provides a calculation method for quantitative PE values (i.e. light effect size at a given nutrient concentration). I used recirculating stream mesocosms with Quercus stellata (Post Oak) leaf litter as an organic matter (OM) substrate in a 150-day experiment to test the model predictions. I manipulated light levels [ambient (full light), shaded (~19% of ambient)] and phosphorus (P) concentration (10,100, 500 µg PO$_4$-P L$^{-1}$) in a fully factorial design. I also supplied all mesocosms with 500 µg L$^{-1}$ dissolved inorganic nitrogen (DIN). Microbial biomass, water column dissolved organic carbon (DOC), and leaf litter dry mass and recalcitrant OM [i.e. the fiber (cellulose + lignin) component of Post Oak substrate] were measured. Recalcitrant OM (ROM) k-rates (day$^{-1}$) were used to calculate the light effect size within P treatments as a log response ratio [LRR, ln(ambient k-rate/shade k-rate)] to ascertain PE magnitude and direction [positive (+) or negative (-)]. Light was an important driver of DOC, a potential source of additional labile organic matter (LOM) essential for priming heterotrophic microbes. There were weak PEs in total leaf litter dry mass remaining, but PEs were more pronounced in leaf litter ROM remaining. The strongest positive PEs (specific to litter ROM pools) occur in the highest P treatment, presumably due to a change in which nutrient, nitrogen (N) versus P, was a limiting factor for microbes based on nutrient ratios rather than P concentration alone. These results illustrate the importance of considering light levels, nutrient ratios (rather than individual nutrients), and detrital ROM components in further PE model development.
Introduction

The priming effect (PE) was first described by soil scientists and can be thought of as an alteration of the rate of carbon (C) turnover; specifically the PE is a change in the rate of recalcitrant organic matter (ROM) decomposition by heterotrophic microbes when labile organic matter (LOM) is added (Guenet et al., 2010; Jenkinson et al., 1985; Kuzyakov, 2010). Though evidence for the PE primarily comes from terrestrial ecosystems, it is thought to occur in aquatic systems as well (Guenet et al., 2010). The direction of PEs can either be positive (+) or negative (-), where a positive PE is an increase in the rate of ROM degradation in the presence of additional LOM and a negative PE is the decrease in the rate or lack of ROM degradation in the presence of additional LOM (Blagodatskaya & Kuzyakov, 2008; Guenet et al., 2010; Kuzyakov et al., 2000). Priming has implications for altering aquatic ecosystem C flux, however the factors determining the relative magnitude and direction of the PE in aquatic ecosystems are not well known and methods for calculating quantitative values for PEs remain elusive (Bengtsson et al., 2018).

As the environment is continually altered by anthropogenic activities, it is important to consider how these activities may affect conditions in forested headwaters and how various factors influence the aquatic PE hypothesis. Nutrients are among the major limiting factors for heterotrophic microbial (fungal and bacterial) growth and metabolism in unaltered, fresh headwater streams (Elser, 2012; Elwood et al., 1981; Strickland et al., 2009; Vitousek et al., 2008). Heterotrophic microbes are capable of assimilating nutrients from the water column, but they also liberate C and nutrients from leaf litter detritus through enzymatic degradation to help support their energetic and dietary needs (Cheever et al., 2013; Gauthier et al., 1990). Energy released from metabolizing LOM [i.e. dissolved organic C (DOC)] in the water, such as from algal exudates, may prime heterotrophic microbes toward greater decomposition (mining) of ROM to obtain limiting nutrients (a positive PE) (Danger et al., 2013; Lagrue et al., 2011; Rier et
al., 2007 & 2014), especially in low-nutrient environments (Blagodatskaya & Kuzyakov, 2008; Guenet et al., 2010; Jenkinson et al., 1985; Kuzyakov, 2010).

Algal production is usually low in forested headwaters, due firstly to low light levels (Mosisch et al., 2001; Hill et al., 1995) and secondly to nutrient limitation (Hill et al., 1988). Anthropogenic activities that alter riparian zones and open canopy cover remove light constraints on algal growth (Justus et al., 2010; Mosisch et al., 2001; Von Schiller et al., 2007) and may lead to microbial priming under low-nutrient conditions. Agriculture and urbanization can contribute to nutrient loading of aquatic environments via nutrient-enriched runoff from increased use of impervious surfaces, inefficient municipal waste handling, use of fertilizers, etc. (Carpenter et al., 1998; Elser, 2012; Smith, 1998). Studies show that as microbial growth is relieved from nutrient limitation, an increase in decomposition occurs (Scott et al., 2013). However, under high light conditions (i.e. unshaded conditions), nutrient enrichment may result in decreased ROM decomposition and potentially no or negative PE (Halvorson et al., 2016). The recent study by Halvorson et al. (2016) found nutrients (specifically P) and light interacted to influence detrital conditioning and decomposition by microbial communities, where light was observed to magnify effects of P on decomposition. This was especially true at lower nutrient concentrations, but at higher concentrations, high light suppressed decomposition slightly compared to lower light treatments at the same nutrient concentration, suggesting a PE. Under nutrient and light enrichment, heterotrophic microbes may use excess nutrients and LOM additions (DOC, potentially from algal exudates) from the surrounding environment rather than mining ROM for these resources (Blagodatskaya & Kuzyakov, 2008; Guenet et al., 2010; Jenkinson et al., 1985; Kuzyakov, 2010). Consequently, factors such as dissolved nutrients and light that control heterotrophic microbial activity and algal abundance may determine the magnitude and direction of PEs (Halvorson et al., 2016; Rier et al., 2014).
Calculating quantitative values for PEs has been difficult up to this point. It is common for decomposition studies to measure decomposition rates ($k$-rates) however, these measures often do not differentiate between LOM and ROM components of leaf litter and instead focus only on changes in measures such as total dry mass (TDM) or ash free dry mass (AFDM). At the heart of the PE hypothesis is the idea of changes in the rate of ROM degradation, so separating out ROM decomposition patterns in leaf litter is vital and may offer a way to calculate quantitative PE values.

The objectives of this work were 1) to improve the current conceptual model of the PE hypothesis by integrating a light level component, 2) to provide a potential method of deriving quantitative values for PEs, and 3) to directly test the PE hypothesis according to the model modifications and suggested calculations. I postulate a modified conceptual model (i.e. *light-nutrient PE* model, Figure 1) of the effects of light and nutrient availability on the potential presence and magnitude of aquatic PEs, which provides a means of calculating quantitative values for potential PEs, and I then used a manipulative experiment to test the PE hypothesis based on the conceptual model.

I used the existing conceptual model (i.e. *nutrient PE* model) presented by Guenet et al. (2010) as a beginning frame of reference, but the *light-nutrient PE* model (Figure 1) differs from Guenet et al.’s (2010) *nutrient PE* model in a few key ways. Guenet et al.’s (2010) *nutrient PE* model shows the response variable (algae density) to be a sigmoidal function of the independent variable (nutrient concentration) where algae density increases with increases in nutrient concentration. PE intensity is hypothesized to be a mirrored sigmoidal function of those two conditions, where positive PE intensity decreases with increases in nutrient concentration and algal density. The *light-nutrient PE* model (Figure 1) expresses ROM decomposition $k$-rates (rather than algal density) as a function of nutrient concentration and I present separate, superimposed regression lines illustrating how differing light levels within a given nutrient
concentration may affect ROM decomposition $k$-rates. While methods for calculating quantitative values for PEs were obscured in Guenet et al.'s (2010) model, the light regression lines in the new model suggest an effect size for light as a method for deriving quantitative values of potential PEs for specific concentrations of nutrients and light levels (Figure 1).

The model modifications were based on the following rationale: PEs are characterized by changes in ROM decomposition rates specifically after LOM additions (Jenkinson et al., 1985), algal exudates may contribute to the LOM pool to prime heterotrophs (Danger et al., 2013), especially in nutrient-limited environments (Guenet et al., 2010), and increases in the amount of algal exudates would depend on greater photosynthetic activity (Rier et al., 2007, 2014). It should be noted that I do not intend to imply that the shapes of the light-nutrient PE model (Figure 1) regression lines are a steadfast rule; rather, the model conveys a conceptual idea of light and nutrient mediated PEs.

The experimental approach to testing the light-nutrient PE model (Figure 1) included measurement and testing of algal biomass, water column DOC concentrations, and fungal biomass responses to light levels and nutrient amendments, for which I formulated the following sub-hypotheses: 1) increasing light will increase algal biomass within a given nutrient concentration due to relief of light-related growth limitations (except perhaps in the lowest P concentration where nutrient concentrations may limit algal growth), 2) as P concentrations increase, algal biomass and fungal biomass will increase due to relaxed P constraints on growth, and 3) water column DOC will increase with increasing algal biomass due to increased photosynthetic activity. I measured leaf litter ROM [i.e. fiber (cellulose + lignin) mass] decomposition over time and calculated $k$-rates (the model's primary response variable) which I predicted would increase with increasing P concentrations in shade and decrease with increasing P concentrations under increased light (Figure 1). Finally, I calculated light effect size as a log response ratio [LRR, $ln(ambient\ k-rate/shade\ k-rate)$] to ascertain PE magnitude and
direction [positive (+) or negative (-)] and predicted the greatest positive PE in the lowest P concentrations.

Methods

A manipulative experiment was carried out in a temperature-controlled, greenhouse environment where recirculating stream mesocosms were exposed to normal day/night cycles from July 2015 through December 2015 for the duration of 150 days. Average daytime greenhouse temperatures ranged from 17.7-25.4°C and nighttime temperatures ranged from 12.4-21.0°C over the course of the experiment (See Appendix A). Mesocosms were constructed from seven-liter oil pans, each maintained at a volume of four liters of dechlorinated water for the duration of the study and each supplied with ~15 g (dry mass) of leached Post Oak (Quercus stellata) leaf litter (hereafter, litter), on day 1. Post Oak was chosen because it is known to be a recalcitrant species and commonly used in litter studies (Scott et al., 2013; Halvorson et al., 2015). Mesocosms were also given miniature fountain pumps that were regularly maintained by clearing any algae or debris to encourage optimal flow. On day 1, mesocosms were inoculated with a small amount (5 ml) of slurry made from mixed litter and stream water collected from a local, second-order stream known to be low in nutrient concentrations to provide a naturally occurring consortium of microbes (Halvorson et al., 2015). Average mesocosm water temperatures ranged from 14.9-29.9°C over the course of the experiment as shown in Appendix B. Peak mesocosm water temperatures occurred during the month of July and, though they were higher than peak air temperatures, these temperatures are within the range of warmer water temperatures occurring in surface waters and isolated pools in Arkansas during the summer.

A full-factorial cross of two factors [light and phosphorus (P)] was used in a randomized layout. There were two levels of light [ambient (full light as determined by the time of year) and shade (~19% of full light)] and three levels of P (10, 100, 500 µg PO₄-P L⁻¹). Light treatments
were continuous for the duration of the experiment where shade treatments were achieved by triple-layering 1-mm mesh on top of the appropriate mesocosms and ambient treatments were left uncovered. Dissolved inorganic phosphorus (DIP) was added directly to the water column weekly to maintain target P concentrations. I provided the same amount of dissolved inorganic nitrogen (DIN) across treatments by direct addition to each mesocosm weekly to maintain a nitrogen (N) concentration of 500 µg L\(^{-1}\). There were 10 replicates per unique treatment combination resulting in 60 total experimental mesocosm units. Additionally, water was completely changed monthly for all mesocosms to prevent filamentous algal overgrowth, which may have resulted in self-shading and confound light treatment responses had it been allowed to remain.

A monthly sample plan was implemented where litter and suspended (i.e. planktonic) algae chlorophyll a (Chl a) were estimated as measures of algal biomass, water column samples were taken for DOC measures, litter ergosterol was estimated as a measure of fungal biomass, and litter was sampled for dry mass. Discs were cut with a cork borer (area=1.496 cm\(^2\)) from randomly selected litter from each mesocosm and algal biomass, fungal biomass, and litter dry mass were estimated. Suspended algae sampling was accomplished by pressure filtering (0.45 µm membrane filter pore size) a known volume from the water column of each mesocosm and DOC samples were taken as grab samples of the water column and were filtered prior to processing. Suspended algae and DOC samples were always taken prior to the monthly water change in mesocosms.

Suspended and litter algal biomass were estimated using 95% ethanol extraction of Chl a and standard spectrophotometric methods (APHA, 2005). Fungal biomass on litter was estimated by solid phase extraction of ergosterol and HPLC analysis (Gessner & Schmitt, 1996). Water column samples were analyzed for DOC by standard combustion catalytic oxidation methods (Eaton et al., 2005).
Total dry mass (TDM) was measured initially and at termination for each mesocosm by placing leaf litter in a drying oven at 48°C for at least 2 hours and bringing to room temperature in a desiccator for at least 30 minutes before weighing. A subset of litter was taken initially, dried (48°C, ≥2h), and sent for processing to the University of Arkansas Altheimer laboratory where the Ankom method (Ankom, 2013) was used to estimate the recalcitrant organic matter (ROM), or fiber (cellulose + lignin), portion of the leaf litter being supplied as substrate for the mesocosms. The analysis of the initial subset of litter showed the beginning fiber (cellulose + lignin) content to be ~43.5% of the initial total dry mass (TDM). This value was used along with the initial TDM values to calculate an initial fiber mass (g) of the litter substrate provided to each mesocosm. These same methods were used at termination to determine the remaining fiber content for each mesocosm. The final litter fiber content for each mesocosm was also given as a percentage (%), which I used along with final TDM values to derive final fiber mass (g) remaining of the original input.

Litter TDM and fiber mass values were used to calculate decomposition $k$-rates (day$^{-1}$) for each mesocosm. The exponential decay equation (Eq1) used for these calculations is as follows:

$$ k = \frac{\ln \left( \frac{M_f}{M_i} \right)}{t} $$

(Eq1)

In Eq1 above, $M_f =$ final mass, $M_i =$ initial mass, and $t =$ time in days (in this case, 150 days). Next, $k$-rates were used to calculate a light effect size as the log response ratio (LRR) within each P concentration as indicators of PE magnitude and direction [positive (+) or negative (-)] (Halvorson et al., 2019; Rosenberg et al., 2013). The LRR equation (Eq2) used for these calculations is as follows:
\[ LRR = \ln \left( \frac{k_a}{k_s} \right) \]  \hspace{1cm} (Eq2)

In Eq2 above, \( k_a \) = ambient \( k \)-rate and \( k_s \) = shade \( k \)-rate. Variance (\( V \)) around each LRR point (Halvorson et al., 2019; Rosenberg et al., 2013) was calculated as follows:

\[ V = \left[ \frac{s_{k_a}^2}{n_a(x_{k_a}^2)} \right] + \left[ \frac{s_{k_s}^2}{n_s(x_{k_s}^2)} \right] \]  \hspace{1cm} (Eq3)

In Eq3 above, \( s \) = standard deviation, \( x \) = mean, \( n_a \) = ambient sample size, \( n_s \) = shade sample size, and \( k \)-rates are denoted the same as in Eq2. The square root of VAR was taken to calculate standard error (SE) around each LRR point and used to estimate the 95%CI.

Repeated measures two-way analysis of variance (\( \alpha = 0.05 \)) was used to analyze data for suspended and litter algal biomass, water column DOC, and litter fungal biomass. Two-way analysis of variance (\( \alpha = 0.05 \)) was used to analyze data for litter TDM remaining, fiber mass remaining, and decomposition \( k \)-rates data. Data were log-transformed where necessary to meet test assumptions and Tukey’s HSD post hoc analysis was used when appropriate to discern significant pairwise comparisons. All data analyses were carried out using R statistical software, version 3.4.2.

**Results**

Light and time interacted to drive differences in suspended algal biomass (Table 1). Suspended algal biomass was similar across all sampling months for shaded treatments and these were similar to ambient treatments for the month of November (Figure 2). Suspended algal biomass was similar for ambient light treatments during August, September, October, and December (Figure 2). During August, suspended algal biomass was significantly lower in ambient treatments as compared to shaded treatments and was significantly higher in ambient treatments during September, October, and December as compared to shaded treatments.
Variation was similarly small from August to November for shaded and ambient light treatments, but much greater variation was observed in December for both light treatments (Figure 2). In general, suspended algal biomass tended to increase with increasing light (Figure 2), where time-pooled values showed suspended algal biomass concentrations under ambient conditions to be 0.062 mg L$^{-1}$ and 0.037 mg L$^{-1}$ under shaded conditions. Plot data are given in Appendix C.

Detrital algal biomass was statistically similar across low, moderate, and high P/shaded treatments and these were similar to low and moderate P/ambient treatments (Figure 3). Detrital algal biomass in the high P/ambient treatment was significantly greater than all other treatments (Figure 3) due to an interaction between light and P (Table 1). Variation tended to increase with increasing P/shaded conditions but was similar across all P/ambient conditions (Figure 3). There was a general trend of increased algal biomass with increased light, except in the lowest P concentration (Figure 3). Plot data are given in Appendix D.

Light and time interacted to yield significant differences between treatments over time for DOC (Table 1). No significant differences between DOC concentrations were found when comparing ambient and shaded treatments within any given sampling months except October where ambient treatments were significantly greater than shaded treatments (Figure 4). Ambient treatments differed from each other across all sampling months (Figure 4). Shaded treatments were only statistically similar during the months of October and November and these were similar to the ambient treatments during November (Figure 4). There was a trend of increasing DOC in the water column under both light treatments across time, but DOC was generally greater under ambient light as compared to shaded treatments (Figure 4). In fact, over the full duration of the study, DOC concentrations were significantly greater in the ambient treatments as compared to the shade treatments (Table 1), where time-pooled values showed DOC concentrations under ambient conditions to be 60.34 mg L$^{-1}$ and 45.23 mg L$^{-1}$ under shaded
conditions. Variation decreased over time but was small for all treatments (Figure 4). Plot data are given in Appendix E.

There were no significant interactions driving fungal biomass changes and light treatment effects were weak, but time and P treatments had independent main effects (Table 1). The P main effect was a significant increase in fungal biomass with increasing P, where moderate and high P treatments were similar but greater than low P treatments (Figure 5A). Fungal biomass fluctuated across time, but was statistically similar during August, November, and December (Figure 5B). Fungal biomass was greatest during October (Figure 5B). Fungal biomass in September was similar to fungal biomass in all other sampling months (Figure 5B). Variation was similarly small for all P treatments and across time (Figure 5A & 5B). Plot data for Figures 5A and 5B are given in Appendices F and G, respectively.

Total dry mass (TDM) remaining did not differ across treatments and variation tended to decrease with increasing P and increasing light (Table 2, Figure 6A). About 46.57-50.99% of initial TDM remained at termination (See Appendix H). However, light and P interacted to drive differences in litter fiber (cellulose + lignin) mass remaining (Table 2). Litter fiber mass remaining was similar across all P/shaded treatments and these were similar to low and moderate P/ambient light treatments (Figure 6B). High P/ambient light had less fiber mass remaining than all other treatments (Figure 6B). There was a weak trend of more fiber mass remaining with increasing P/shaded conditions (Figure 6B). Though low and moderate P/ambient treatment fiber mass remaining were similar, there was a trend of increase before significantly dropping below all other values in the highest P concentration (Figure 6B). Fiber mass remaining was usually less under ambient light as compared to shaded conditions, except in the low P treatments where the opposite was true (Figure 6B). Variation was similarly small across all treatments for fiber mass remaining (Figure 6B). Overall, ~66.7-79.7% of initial fiber mass remained at termination (See Appendix H). Plot data for Figures 6A and 6B are given in Appendices H.
Total dry mass decomposition $k$-rates did not differ, and variation was similar across treatments, but there was a significant interaction between light and P for fiber mass decomposition $k$-rates (Table 2, Figures 7A & 7B). For fiber $k$-rates, low, moderate, and high P/shade light treatments and low and moderate P/ambient light treatments were similar (Figure 7B). High P/ambient light had significantly faster fiber decomposition than other treatments (Figure 7B). In general, fiber decomposition slowed with increasing P/shaded conditions and fiber decomposition sped up with increasing P/ambient light (Figure 7B). There tended to be faster fiber decomposition with increased light, except in the low P where the opposite was true (Figure 7B). Variation was similar across treatments for litter dry mass $k$-rates (Figure 7A) and litter fiber mass $k$-rates (Figure 7B). Plot data for Figures 7A and 7B are given in Appendices I.

The light effect sizes within a given P concentration, which were calculated as log response ratios (LRR, Eq2), serve as quantitative values for PEs where positive (+) LRR = positive (+) PE and negative (-) LRR = negative (-) PE. The magnitude and direction of LRRs/PEs for litter TDM and fiber mass and are visualized in Figures 8 along with the 95% confidence interval (95%CI) around each point. Litter TDM LRRs/PEs were negative under the low and moderate P conditions and became positive in the high P concentration (Figure 8). Litter fiber mass LRRs/PEs were negative under low P conditions and became increasingly positive in moderate to high P concentrations (Figure 8). The 95%CI widened with increasing P concentrations for litter TDM and fiber mass and 95%CIs were generally wider for fiber mass as compared to TDM (Figure 8). Trends in litter fiber mass LRRs were more pronounced than in TDM LRRs, resulting in decreasing amounts of overlap and eventually distinct separation in 95%CIs with increasing P (Figure 8). Plot data are given in Appendix J.

Discussion

By mediating heterotrophic activity, environmental factors such as nutrient and light availability may influence the magnitude and sign of priming (Guenet et al., 2010; Evans-White
& Halvorson, 2017), and therefore determine the role of priming in C fluxes within freshwater systems (Webster et al., 1999; Benstead et al., 2009). Light and nutrient manipulations resulted in changes in aquatic microbial communities (algal and fungal biomass), the size of the water column labile organic matter (LOM) pool measured as dissolved organic C (DOC), and ultimately litter recalcitrant organic matter (ROM) decomposition \( k \)-rates. Light was shown to be a statistically important driver of suspended algal biomass and DOC over time. Light also interacted with phosphorus (P) to significantly drive changes in litter algal biomass. This reinforces the importance of including light along with nutrients as factors in further priming effect (PE) model development such as in the light-nutrient PE model. The study also illustrates the importance of separating out ROM decomposition \( k \)-rates for use as a response variable in PE model development, as PEs specific to ROM pools were clearly more pronounced than in total dry mass (TDM) data in this work.

One noteworthy caveat is that the light-nutrient PE model may lead to the assumption that the PE patterns postulated are in response to changes in the concentration of only a single nutrient (as was the original perception) because the x-axis is non-specific regarding nutrient identity. However, the effects of increased nutrient levels may depend on the availability of other nutrients in the system [i.e. the ratio of nutrients such as nitrogen (N):P; Halvorson et al., 2019]. It is important to note that the experimental nutrient manipulations, which included increasing concentrations of dissolved inorganic P (DIP) together with a consistent concentration of dissolved inorganic N (DIN), led to decreasing N:P ratios across treatments. While the sub-hypotheses regarding the model parameters were met reasonably well, N:P ratios could explain some of the patterns seen in the data and why the calculated PEs were counter to the predictions.

I predicted an increase in algal biomass with increasing light due to relief of light-related limitation, but that there would be little to no difference between light treatments regarding algal biomass at lower P concentrations due to nutrient limitations. The data for algal biomass
support this hypothesis. I also predicted an increase in algal biomass with increasing P concentrations within a given light level due to relief of P constraints. The detrital algal biomass data for higher light levels support the hypothesis, but algal biomass at lower light levels remained statistically similar across P treatments. Algal cellular nutrient content was likely altered by interactions of changing light and nutrient availability in the study and therefore could have altered which nutrient would limit algal growth (Sterner & Elser, 2002; Verhoeven et al., 1996). The demand for P could have become greater under higher light levels, but lower light levels could result in a higher N demand for algae to produce N-rich pigments like chlorophyll (Elliott & White, 1994; Healey, 1985; Sterner & Elser, 2002). This may explain why litter algal biomass did not respond as expected with increasing P (decreasing N:P)/shade conditions; N was becoming increasingly limiting to algal growth in the shade.

I predicted and then observed that fungal biomass increased with increasing P concentrations as found in many previous studies, but I also saw shaded ROM k-rates were significantly slower than ambient light ROM k-rates in the highest P concentration (opposite of the model prediction). The ROM k-rates data resulted in the greatest positive (+) PE where P concentrations were highest which was counter to the predictions. This could have been due to decreasing N:P ratios, competition between algae and fungi for nutrients in the water column, and insufficient DOC to stimulate fungi towards mining ROM for limiting nutrients under shaded conditions. Fungal acquisition of N from the water column may have further constrained algal productivity due to increased N demand by algae as N:P ratios decreased under shaded conditions. These constraints on algae may also have been partially responsible for decreased DOC exudation which therefore became energetically limiting to fungi. Limitation of algal-derived carbon energy (DOC) would have reduced the ability of fungi to mine ROM for N in shaded conditions as N:P ratios decreased. Under ambient light conditions, the switch in algal demand for more P instead of N may have provided competitive relief for algae and, along with
relieved light constraints, algae may have exuded more DOC that could then be used by fungi as an energy source for mining ROM.

The PE predictions assumed that additions of DOC to the water column (presumably from algal exudates) provide energy for priming heterotrophic microbes, especially fungi. Evidence that fungi take up algal-derived DOC in aqueous environments has recently been provided by a study showing mutual exchange of C and nutrients between algae and fungi clearly showing that fungi do receive C derived specifically from living algae (Du et al., 2019). The data show water column concentrations of DOC increase as algal biomass increase. DOC levels did not differ as strongly as algal biomass between the two light treatments, likely indicating high efficiency of heterotrophic turnover of algal-derived DOC within the water column in ambient light. Moreover, the data are not entirely conclusive as to the origins of the additional DOC, only that DOC did increase under ambient light conditions over time and, therefore, could be a potential source of energy for heterotrophic microbes. Firstly, suspended algae might have had an advantage over detrital algae in intercepting more light for photosynthetic activity leading to more exudation of DOC. Detrital algae may have become crowded and slightly more shaded within the detrital biofilm, reducing photosynthesis and exuding less DOC. Secondly, trends in DOC mimicked trends in suspended algae over time across both light treatments. However, amounts of additional DOC were relatively large so it is reasonable to suggest that algal exudates were not the only source of DOC. Photodegradation of litter detritus could have been another potential source of additional water column DOC (Wetzel et al., 1995) since litter decomposition increased with increasing light. Increasing DOC could have also been due to increased fungal processing of litter in ambient light conditions which would result in increased leaching of the litter.

Radioisotopes could be used to trace proportional usage of DOC from different sources by heterotrophic microbes to confirm whether fungi are using a substantial amount of algal-
derived DOC as an energy source for mining litter ROM in low nutrient environments. Additionally, radioisotope tracers could improve understanding of the part played by heterotrophic bacteria in the cycling of algal- versus detrital-derived DOC in stream systems. I did not measure bacterial responses, but studies have shown heterotrophic bacteria can assimilate algal-derived DOC to enhance growth rates (Kuehn et al., 2014). However, many recent priming studies that quantified bacterial activity or biomass found relatively weak or negligible responses for bacterial growth/production (Halvorson et al., 2019; Soares et al., 2017). Heterotrophic bacteria can preferentially use DOC from different sources and the preference is likely specific to the type of bacteria present. Terrestrial literature indicates gram-negative bacteria have the most affinity for DOC originating from autotrophic exudation (Hotchkiss et al., 2014). Using radioisotopes to link DOC (and its origin) to fungi and heterotrophic bacteria (especially according to species), could assist understanding of the level of competition and perhaps mutualism that may occur between biofilm microbes as it relates to PEs and may reveal mechanisms of PEs.

I chose the log response ratio (LRR) as a measure of effect size based on ROM k-rates, which is common in ecological studies (Halvorson et al., 2019; Rosenberg et al., 2013), to quantify PEs as suggested by the light-nutrient PE model. I saw weak PEs within the litter TDM data, and this could be because algae in the study did not differ enough between light regimes to provide sufficient contrast for testing PEs, which may be a problem in other studies as well. However, I saw a strong positive PE in the highest P treatment (lowest N:P) specific to litter ROM, which may have been obscured had I not separated out litter OM pools. As discussed previously, I presume the PE results are due to a change in which nutrient, N or P, was a limiting factor for microbial productivity and the different ways in which microbes handled nutrient availability depending on light. The trend in ROM decomposition in ambient light is still indicative of positive (+) PEs occurring in low nutrient environments, but in this case, it is due to increasing N limitation as indicated by the decreasing N:P ratio. This suggests that predicting
PEs reliably will require consideration of changing nutrient ratios rather than changing concentrations of a single nutrient.

**Conclusions**

Overall, the data provide further evidence that human activities affecting nutrient and light levels in headwaters can result in altered detrital decomposition and therefore C and nutrient cycling at the ecosystem level through the mediation of microbial priming. These results provide yet another reason why shading by the riparian canopy is an important part of managing human impacts on streams. The modifications of the PE model, though imperfect, are a step in the right direction to improve understanding of the occurrence of PEs in aquatic environments. The use of isotope tracers to link LOM to microbial biomass, photosynthetic efficiencies of algae, and competition for or exchange of resources between algae and heterotrophic microbes are all seemingly important parts of the PE puzzle that deserve further consideration in hypothetical model development. Additionally, improvements to the PE model could be made by incorporating a wider range of light levels and nutrient concentrations (with special consideration for nutrient ratios) as well as a variety of leaf litter species. More data points would help to discern the actual shape of the resulting PE curves and help to identify how positive/negative PE change points differ at varying light levels under similar nutrient concentrations.

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dedication of 2015 ecoREU student Scott Hamby. Funding for this project was jointly provided by National Science Foundation Biology REU (NSF DBI 1359188) and University of Arkansas.

References


Tables

Table 1 Repeated measures two-way analysis of variance ($\alpha = 0.05$) output for suspended (i.e. planktonic) and litter algal biomass estimated as chlorophyll a (Chl a; mg L$^{-1}$ and mg cm$^{-2}$, respectively), dissolved organic carbon (DOC; mg L$^{-1}$) in the water column, and detrital fungal biomass estimated as ergosterol (mg cm$^{-2}$). All data were log-transformed to meet the assumption of normality. Significant $p$ values are shown in bold. Tukey’s HSD was conducted post hoc as necessary to discern significant comparisons. Factor abbreviations are as follows: L = light treatment, P = phosphorus treatment, T = time/repeated measures increments. Column header key: df=degrees freedom, F=ANOVA test statistic, $p$=probability value.

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Table 2 Two-way analysis of variance (α = 0.05) output for litter mass remaining (g) and $k$-rates (day$^{-1}$) for total dry mass (TDM) and fiber (cellulose + lignin). Laboratory analysis results for litter components were provided as percentages (%) and converted to grams (g) for statistical analyses. Significant $p$ values are shown in bold. Tukey’s HSD was conducted post hoc as necessary to discern significant comparisons. Factor abbreviations are as follows: L = light treatment and P = phosphorus treatment. Column header key: df=degrees freedom, F=ANOVA test statistic, $p$=probability value

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Figure 1 Aquatic light-nutrient priming effect (PE) model modified from Guenet et al.’s (2010) nutrient PE model. Recalcitrant organic matter (ROM) decomposition k-rates (day^{-1}) are presented on the y-axis. Nutrient concentrations presented on the x-axis increase from left to right. The model assumes additional labile organic matter (LOM) inputs in the form of dissolved organic carbon (DOC), presumably from algal exudates. The filled boxes connected by a solid line show k-rates along an increasing nutrient gradient under low light conditions and the open boxes connected by a dashed line show k-rates along an increasing nutrient gradient under high light conditions. (Note: The purpose of the trend lines is to help conceptually illustrate PEs, not to imply that ROM decomposition trends are linear in nature.) As with Guenet et al.’s (2010) nutrient PE model, the light-nutrient PE model predicts the greatest positive (+) PE when nutrients are most limited. The shaded wedges between trend lines indicate a light effect size (ES) as a method for quantifying the magnitude and direction of PE within a given nutrient concentration. Under low light, increased nutrients stimulate ROM decomposition by permitting greater heterotrophic growth and investment in nutrient-rich ROM degradative enzymes. Under high light, algae shift from positively priming decomposition (insufficient algae to allow preferential use of labile algal C), to negative priming decomposition (heterotrophs switch to reliance on labile algal C instead of ROM for growth).
Figure 2 Mean ± 1 SE suspended (i.e. planktonic) algal biomass estimated as chlorophyll a (Chl a; mg L⁻¹) across repeated measures increments in months abbreviated as follows: Aug = August, Sep = September, Oct = October, Nov = November, Dec = December. Shaded treatments = black line and ambient light treatments = gray line. Data were log-transformed for statistical analyses. Significant differences due to a light x time interaction as per repeated measures two-way analysis of variance (α=0.05) and Tukey’s HSD post hoc analyses are indicated by letters.
Figure 3 Mean ± 1 SE detrital-associated (litter) algal biomass estimated as chlorophyll a (Chl a; mg cm\(^{-2}\)) across low, moderate, and high (10, 100, and 500 µg L\(^{-1}\)) phosphorus (P) treatments. Shaded treatments = black bars and ambient light treatments = white bars. Data were log-transformed for statistical analyses. Significant differences due to a light x P treatment interaction as per repeated measures two-way analysis of variance (α=0.05) and Tukey’s HSD post hoc analyses are indicated by letters.
Figure 4 Mean ± 1 SE water column dissolved organic carbon (DOC, mg L⁻¹) across repeated measures increments in months abbreviated as follows: Sep = September, Oct = October, Nov = November, Dec = December. Shaded treatments = black line and ambient light treatments = gray line. Data were log-transformed for statistical analyses. Significant differences due to a light x time interaction as per repeated measures two-way analysis of variance (α=0.05) and Tukey's HSD post hoc analyses are indicated by letters.
Figure 5  (A) Mean ± 1 SE detritus-associated (litter) fungal biomass estimated as ergosterol (mg cm\(^{-2}\)) across low, moderate, and high (10, 100, and 500 µg L\(^{-1}\)) phosphorus (P) treatments.  
(B) Mean ± 1 SE litter detritus fungal biomass estimated as ergosterol (mg cm\(^{-2}\)) across repeated measures increments in months abbreviated as follows: Aug = August, Sep = September, Oct = October, Nov = November, Dec = December. Data were log-transformed for statistical analyses. Significant differences due to P main effects (A) and time main effects (B) as per repeated measures two-way analysis of variance (\(\alpha=0.05\)) and Tukey’s HSD post hoc analyses are indicated by letters.
Figure 6 Mean ± 1 SE total dry mass (TDM, g) remaining (A) and fiber (cellulose + lignin) mass (g) remaining across low, moderate, and high (10, 100, and 500 µg L⁻¹) phosphorus (P) treatments. Shaded treatments = black bars and ambient light treatments = white bars. Significant differences in fiber mass remaining (B) due to a light x P interaction as per repeated measures two-way analysis of variance (α=0.05) and Tukey’s HSD post hoc analyses are indicated by letters. Horizontal dashed lines are provided for comparison and indicate initial TDM (A) and fiber mass (B) values (g)
Figure 7 Mean ± 1 SE total dry mass (TDM) and fiber (cellulose + lignin) decomposition k-rates (day⁻¹) across low, moderate, and high (10, 100, and 500 µg L⁻¹) phosphorus (P) treatments (A and B, respectively). Shaded treatments = black lines and ambient light treatments = gray lines. Significant differences in fiber decomposition k-rates (B) due to a light x P interaction as per repeated measures two-way analysis of variance (α=0.05) and Tukey’s HSD post hoc analyses are indicated by letters.
Figure 8 Log response ratio [LRR, calculated as \( \ln(\text{ambient} \ k \text{-rate day}^{-1}/\text{shade} \ k \text{-rate day}^{-1}) \) ± 95% confident interval (CI) as quantification of priming effect (PE) within a given phosphorus (P) concentration across low, moderate, and high (10, 100, and 500 µg L\(^{-1}\)) P treatments. Positive (+) LRR = positive (+) PE and negative (-) LRR = negative (-) PE. Total dry mass (TDM) LRR/PE = black line and fiber (cellulose + lignin) mass LRR/PE = gray line.
Appendices

Appendix A

Mean monthly greenhouse temperatures (°C) where the mesocosm experiment was carried out. Monthly abbreviations are as follows: Jul=July, Aug=August, Sep=September, Oct=October, Nov=November, Dec=December.

<table>
<thead>
<tr>
<th></th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>26.2</td>
<td>25.4</td>
<td>23.4</td>
<td>19.9</td>
<td>17.7</td>
<td>20.6</td>
</tr>
<tr>
<td>Night</td>
<td>22.7</td>
<td>21.0</td>
<td>19.7</td>
<td>14.3</td>
<td>12.4</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Appendix B

Mean mesocosm water column temperatures (°C) according to unique treatment over time. Unique (full factorial cross) treatment column abbreviations are as follows: Light factor with two levels (ambient and shade = Amb and Shd, respectively) and phosphorus factor with three levels (10, 100 and 500 µg L\(^{-1}\) = 10, 100, and 500, respectively). Monthly abbreviations are as follows: Jul=July, Aug=August, Sep=September, Oct=October, Nov=November, Dec=December.

<table>
<thead>
<tr>
<th>Unique Treatment</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amb-10</td>
<td>29.7</td>
<td>25.6</td>
<td>26.5</td>
<td>22.4</td>
<td>15.5</td>
<td>22.1</td>
</tr>
<tr>
<td>Amb-100</td>
<td>29.9</td>
<td>25.3</td>
<td>26.2</td>
<td>22.1</td>
<td>15.4</td>
<td>22.3</td>
</tr>
<tr>
<td>Amb-500</td>
<td>27.9</td>
<td>25.2</td>
<td>26.0</td>
<td>22.1</td>
<td>15.3</td>
<td>22.0</td>
</tr>
<tr>
<td>Shd-10</td>
<td>29.8</td>
<td>25.5</td>
<td>25.9</td>
<td>22.2</td>
<td>15.1</td>
<td>21.9</td>
</tr>
<tr>
<td>Shd-100</td>
<td>29.6</td>
<td>25.0</td>
<td>25.5</td>
<td>21.7</td>
<td>14.9</td>
<td>21.9</td>
</tr>
<tr>
<td>Shd-500</td>
<td>29.8</td>
<td>25.0</td>
<td>25.5</td>
<td>21.8</td>
<td>14.9</td>
<td>21.6</td>
</tr>
</tbody>
</table>

Appendix C

Mean suspended (i.e., planktonic) algal biomass as chlorophyll a (mg L\(^{-1}\)) across time and according to light treatment. SE = standard error. Monthly abbreviations are as follows: Aug=August, Sep=September, Oct=October, Nov=November, Dec=December.

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Time-pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Ambient</td>
<td>0.017</td>
<td>0.004</td>
<td>0.039</td>
<td>0.006</td>
<td>0.050</td>
<td>0.006</td>
</tr>
<tr>
<td>Shade</td>
<td>0.027</td>
<td>0.007</td>
<td>0.030</td>
<td>0.002</td>
<td>0.029</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Appendix D

Mean detrital-associated (litter) algal biomass as chlorophyll a (mg cm$^{-2}$) across time and according to light treatment. SE = standard error. The table reflects six unique treatments from the full factorial cross of light and phosphorus (P) manipulations as follows: Light factor with two levels (ambient and shade) and P factor with three levels (10, 100 and 500 µg L$^{-1}$ = 10, 100, and 500, respectively).

<table>
<thead>
<tr>
<th>P Treatment</th>
<th>Ambient</th>
<th>Shade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>10</td>
<td>5.2E-04</td>
<td>8.9E-05</td>
</tr>
<tr>
<td>100</td>
<td>7.2E-04</td>
<td>1.5E-04</td>
</tr>
<tr>
<td>500</td>
<td>1.3E-03</td>
<td>2.4E-04</td>
</tr>
</tbody>
</table>

Appendix E

Mean water column dissolved organic carbon (DOC, mg L$^{-1}$) across time and time-pooled according to light treatment. SE = standard error. Monthly abbreviations are as follows: Sep=September, Oct=October, Nov=November, Dec=December.

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Time-pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Ambient</td>
<td>9.516</td>
<td>2.026</td>
<td>44.851</td>
<td>4.921</td>
<td>33.391</td>
</tr>
</tbody>
</table>

Appendix F

Mean detrital-associated (litter) fungal biomass estimated as ergosterol (mg cm$^{-2}$) according to phosphorus (P) treatment. P treatment with three levels were as follows: (10, 100 and 500 µg L$^{-1}$ = 10, 100, and 500, respectively). SE = standard error.

<table>
<thead>
<tr>
<th>P Treatment</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.036</td>
<td>0.003</td>
</tr>
<tr>
<td>100</td>
<td>0.047</td>
<td>0.004</td>
</tr>
<tr>
<td>500</td>
<td>0.051</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Appendix G

Mean detrital-associated (litter) fungal biomass estimated as ergosterol (mg cm\(^{-2}\)) over time. SE = standard error. Abbreviations for sampling months are as follows: Aug=August, Sep=September, Oct=October, Nov=November, Dec=December.

<table>
<thead>
<tr>
<th>Sampling Month</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug</td>
<td>0.043</td>
<td>0.004</td>
</tr>
<tr>
<td>Sep</td>
<td>0.050</td>
<td>0.005</td>
</tr>
<tr>
<td>Oct</td>
<td>0.060</td>
<td>0.004</td>
</tr>
<tr>
<td>Nov</td>
<td>0.036</td>
<td>0.004</td>
</tr>
<tr>
<td>Dec</td>
<td>0.036</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Appendix H

Mean detrital (litter) total dry mass (TDM) remaining and fiber mass remaining as a percentage (%) and in grams (g) according to unique treatment. SE=standard error. Unique (full factorial cross) treatment column abbreviations are as follows: Light factor with two levels (ambient and shade = Amb and Shd, respectively) and phosphorus factor with three levels (10, 100 and 500 \(\mu\)g L\(^{-1}\) = 10, 100, and 500, respectively).

<table>
<thead>
<tr>
<th>Unique Treatment</th>
<th>TDM Remaining Mean (%)</th>
<th>TDM Remaining Mean (g)</th>
<th>SE</th>
<th>Fiber Mass Remaining Mean (%)</th>
<th>Fiber Mass Remaining Mean (g)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amb-10</td>
<td>49.07</td>
<td>7.441</td>
<td>0.234</td>
<td>73.76</td>
<td>4.867</td>
<td>0.129</td>
</tr>
<tr>
<td>Amb-100</td>
<td>50.14</td>
<td>7.633</td>
<td>0.249</td>
<td>74.76</td>
<td>4.953</td>
<td>0.173</td>
</tr>
<tr>
<td>Amb-500</td>
<td>48.01</td>
<td>7.282</td>
<td>0.203</td>
<td>66.69</td>
<td>4.402</td>
<td>0.130</td>
</tr>
<tr>
<td>Shd-10</td>
<td>46.57</td>
<td>7.075</td>
<td>0.248</td>
<td>73.01</td>
<td>4.826</td>
<td>0.178</td>
</tr>
<tr>
<td>Shd-100</td>
<td>49.81</td>
<td>7.559</td>
<td>0.263</td>
<td>77.99</td>
<td>5.150</td>
<td>0.165</td>
</tr>
<tr>
<td>Shd-500</td>
<td>50.99</td>
<td>7.756</td>
<td>0.350</td>
<td>79.72</td>
<td>5.277</td>
<td>0.232</td>
</tr>
</tbody>
</table>
Appendix I

Mean calculated decomposition $k$-rates (day$^{-1}$) for detrital (litter) total dry mass (TDM) and fiber (cellulose + lignin) mass. Litter TDM was directly measured initially and at termination in grams (g) and these data were used to calculate TDM decomposition $k$-rates. Laboratory analysis results for litter fiber content of an initial subsample of litter and for each mesocosm at termination were provided as percentages (%) and converted to grams (g) to be used in the calculation of fiber mass decomposition $k$-rates. SE=standard error. Unique (full factorial cross) treatment column abbreviations are as follows: Light factor with two levels (ambient and shade = Amb and Shd, respectively) and phosphorus factor with three levels (10, 100 and 500 µg L$^{-1}$ = 10, 100, and 500, respectively).

<table>
<thead>
<tr>
<th>Unique Treatment</th>
<th>TDM $k$-rate</th>
<th>TDM SE</th>
<th>Fiber Mass $k$-rate</th>
<th>Fiber Mass SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amb-10</td>
<td>-0.0048</td>
<td>0.0002</td>
<td>-0.0020</td>
<td>0.0002</td>
</tr>
<tr>
<td>Amb-100</td>
<td>-0.0046</td>
<td>0.0002</td>
<td>-0.0019</td>
<td>0.0002</td>
</tr>
<tr>
<td>Amb-500</td>
<td>-0.0049</td>
<td>0.0002</td>
<td>-0.0027</td>
<td>0.0002</td>
</tr>
<tr>
<td>Shd-10</td>
<td>-0.0051</td>
<td>0.0002</td>
<td>-0.0021</td>
<td>0.0002</td>
</tr>
<tr>
<td>Shd-100</td>
<td>-0.0047</td>
<td>0.0002</td>
<td>-0.0017</td>
<td>0.0002</td>
</tr>
<tr>
<td>Shd-500</td>
<td>-0.0045</td>
<td>0.0003</td>
<td>-0.0015</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Appendix J

Mean calculated log response ratio (LRR) values within a given phosphorus (P) concentration. LRR is a light effect size that serves to estimate the direction and magnitude of the priming effect (PE) for detrital (litter) total dry mass (TDM) and fiber (cellulose + lignin) mass across three levels of P treatments (10, 100 and 500 µg L$^{-1}$ = 10, 100, and 500, respectively). SE=standard error of LRR. 95% CI=LRR 95% confidence interval based on LRR SE.

<table>
<thead>
<tr>
<th>P Treatment</th>
<th>TDM LRR/PE</th>
<th>TDM SE</th>
<th>TDM 95% CI</th>
<th>Fiber Mass LRR/PE</th>
<th>Fiber Mass SE</th>
<th>Fiber Mass 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-0.0709</td>
<td>0.0145</td>
<td>0.0284</td>
<td>-0.0331</td>
<td>0.0328</td>
<td>0.0644</td>
</tr>
<tr>
<td>100</td>
<td>-0.0094</td>
<td>0.0134</td>
<td>0.0263</td>
<td>0.1574</td>
<td>0.0364</td>
<td>0.0714</td>
</tr>
<tr>
<td>500</td>
<td>0.0855</td>
<td>0.0198</td>
<td>0.0387</td>
<td>0.5809</td>
<td>0.0517</td>
<td>0.1013</td>
</tr>
</tbody>
</table>
Chapter 2

Cooler Temperatures Amplify Microbial Biofilm Subsidy-Stress Responses to Low-Level Sodium Salt Increases in Freshwater

B Howard-Parker, A Mogilevski, T Williams, S Entrekin, MA Evans-White
Abstract

Freshwater salinization is a global issue due to anthropogenic activities, yet sub-lethal and ion-specific salinization effects on freshwater microbes remain understudied. My objective was to investigate effects of two common sodium (Na) salts (NaHCO₃ and NaCl) on heterotrophic (fungi and bacteria) and autotrophic (algae) microbes and detrital processing across a sub-lethal concentration gradient (16, 32, 64mg L⁻¹) relative to unamended streamwater (SW, ~3-4mg L⁻¹ NaCl/NaHCO₃). Microbes may follow subsidy-stress patterns when exposed to low-level salt inputs because salts are a physiological requirement. I used the common Liquidambar styraciflua (sweet gum) as leaf litter substrate in a 134-day microcosm experiment in a greenhouse with temperatures (17.3°C-23.4°C daytime; 12.6°C-20.4°C nighttime) and light cycles typical of the season (February to June). A second 135-day experiment was performed using an environmental chamber set at 4.5°C with 12-hour dark/light cycles. Species- and ion-specific subsidy-stress responses to Na salt increases were observed where NaHCO₃ was a better subsidy for fungi and NaCl was a better subsidy for algae. Responses were strongest in cooler temperatures (4.5°C). Detrital biofilm subsidy-stress responses have broad implications in carbon flux, nutrient cycling, and biodiversity in aquatic food webs, but these results are particularly informative for at-risk areas with low freshwater salinity.

Introduction

Salinization of freshwater ecosystems is an increasing global issue (Hart et al., 1991; Ghassemi et al., 1995; Kaushal et al., 2005; Cañedo-Argüelles et al., 2016). Though the types and relative amounts of the main cations [Magnesium (Mg²⁺), Calcium (Ca²⁺), Potassium (K⁺), and Sodium (Na⁺)] and anions [Bicarbonate (HCO₃⁻), Carbonate (CO₃²⁻), Chloride (Cl⁻), and Sulfate(SO₄²⁻)] occur naturally in freshwaters (Griffith, 2014), varying with geology and precipitation (Allen and Castillo, 2007; Gibbs, 1970), human inputs can increase ionic
concentrations in freshwaters above region-specific normal levels (Jackson and Jobbágy, 2005; Qian and Mecham, 2005). Anthropogenic activities in urban (Blasius and Merritt 2002; Kelly et al. 2008) and agricultural lands (Postel, 1999) as well as resource extraction can lead to increased salt concentrations in freshwaters (Daley et al., 2009; Brantley et al., 2014). Increasing salinization may lead to water quality degradation (Vengosh, 2013), loss of utility for humans and habitat for biota (Jin et al., 2011), general toxicity (Kaushal et al., 2005), loss of density and biodiversity for macroinvertebrates (Cañedo-Argüelles et al., 2013), and altered rates of detrital decomposition (Kaspari et al., 2009).

There is a paucity of studies investigating salinity effects on aquatic biota and ecosystems from an ion-specific perspective (Cañedo-Argüelles et al. 2016) because composite measures such as total dissolved solids (TDS) and electrical conductance (EC), or conductivity, levels have historically been used to describe “salinity” of aquatic environments. Further, water quality standards are often based on these types of total salinity measurements. However, ionic regime can differ among streams of similar TDS or EC (Kunz et al., 2013; Griffith, 2014) which may cause variation in biological responses because they likely vary with ion type and relative amount (Entrekin et al., 2018). Therefore, additional studies are needed that examine biological responses while manipulating ion types and amounts.

Many studies have investigated the negative and potentially lethal effects of large salt increases (up to 1000mg L-1), but fewer studies have investigated the effects of low-level salinization (Hart et al., 1991). Biotic metabolic responses to sub-lethal salt increases may take place before acute toxicity (i.e., mortality) occurs (Zalizniak et al., 2006; Tyree et al., 2016) and long-term responses are likely ion- and species-specific (Zalizniak et al., 2006; Entrekin et al. 2018). Experiments using sub-lethal salt manipulations are needed to examine the potential for subsidy-stress responses (Odum et al., 1979), especially since evidence shows low level salt increases are occurring in freshwaters (Olson, 2019). The subsidy-stress hypothesis (Odum et al., 1974) indicates small inputs may initially subsidize or increase productivity for biota, cause a
productivity plateau at some moderate input level, and finally stress or decrease productivity with relatively higher inputs. The precise characteristics of this hump-shaped curve would likely differ in shape or magnitude according to the species of interest and type and amount of inputs.

Despite their important role in ecosystem processes such as in detrital processing and nutrient cycling (Cummins, 1974; Hieber & Gessner, 2002), microbial species responses to salinization have garnered less attention than vertebrate, macroinvertebrate, and macrophyte responses. Detrital microbial biofilms are comprised of a wide variety of heterotrophic (fungal and bacterial) and autotrophic (algal) species and this variability in community composition lends to variability in salt tolerances and potential metabolic responses to salinization, even at low levels (Hart et al., 1991; Nielson et al., 2003). Microbial populations within detrital biofilms may exhibit subsidy-stress patterns in response to sub-lethal salinization that are ion- and species-specific because salts are a physiological requirement (Kaspari et al., 2009; Kaspari et al., 2014).

Fungal colonization, growth, extracellular cellulytic activity, sporulation, spore germination, and respiration have been shown to decrease with sodium chloride (NaCl) increases up to 5+ times greater than most freshwater salinity values, but still less than marine salinity values (Datta & Kulkarni, 2004; Franco et al., 2008; Canhoto et al., 2017; Chen et al., 2017; Table 1). Fungi tended to respond similarly even when exposed to relatively lower salinities (still up to 2-3 times greater than most freshwaters; Table 1) and when comparing different ions, Cl\(^{-}\) appears to be more stressful to fungi than other anions regardless of the associated cation (Georgieva et al., 2012; Bencherif et al., 2015; Scott et al., 2015). Additionally, heterotrophic bacteria have been shown to decrease in relative abundance when salinity increases from freshwater to seawater concentrations which included the ions Cl\(^{-}\), SO\(_4^{2-}\), Mg\(^{2+}\), and Ca\(^{2+}\) (Zhang et al., 2013; Table 1). On the other hand, bacteria have a hump-shaped curve across a gradient of calcium carbonate (CaCO\(_3\)) increases up to 2-3 times greater than freshwaters (Bencherif et al., 2015; Table 1). This indicates that, as with fungi, Cl\(^{-}\) may be the
more stressful anion for bacteria (as compared to CO$_3^{2-}$) and that the type and intensity of responses to various salts differ between fungi and bacteria.

Microbial algae have also been shown to have negative responses to salinity increases depending on ions present. Recent studies show D. salina biomass decreases with increasing NaCl, which is not surprising considering the salinity increases went well beyond average saltwater concentrations (Nemtseva et al., 2013; Table 1). Zuo et al (2014) showed C. reinhardtii cell density and cell growth decreased with NaCl and sodium carbonate (Na$_2$CO$_3$) concentrations increasing to brackish water ranges (Table 1). The Zuo et al. (2014) study also reported a decrease in pigment and photosynthetic ability with an overall decline in oxygen (O$_2$) production within algal cells as salinity increased. This is similar to terrestrial studies showing decreased photosynthetic ability and photochemical reactions with increasing NaCl (Chen et al., 2017; Table 1). In contrast to the effects of different salt anions on heterotrophs though, Zuo et al. (2014) indicates CO$_3^{2-}$ as being more toxic than Cl$^-$ to algae. Additionally, the previously mentioned study by Scott et al. (2015) resulted in increased relative and total abundance for microalgae along with an increase in chlorophyll a (Chla) in Cl$^-$ dominant wetlands. In that same study, however, autotrophic prokaryotic bacteria saw a decrease in diversity in the Cl$^-$ dominant wetlands, so they may be affected by anions more similarly to their heterotrophic bacterial cousins than to organisms that share similar energy acquisition functional traits.

In contrast to stress and toxicity seen at higher salinities as outlined above, streams with low salinity (i.e. $\leq$~200µS/cm; Griffith, 2014) experiencing small, additional salt inputs may result in heterotrophic microbial subsidy-stress responses prior to toxicity. In terrestrial ecosystems, detrital decomposition rates have been shown to increase with low salt inputs (Kaspari et al. 2009, Table 1), likely due to a relief of nutritional (i.e. Na) constraints on decomposers. Sodium concentrations are generally low in northwest Arkansas freshwaters, for example, and may be a limiting growth factor for microbes (APCEC, 2014). Small Na salt additions may act as a subsidy for heterotrophic microbial production (leading to increasing
detrital decomposition) up to some salt concentration threshold before declining thereafter. Additionally, changes in respiration rates often coincide with changes in heterotrophic microbial biomass and decomposition since $O_2$ is consumed as decomposition proceeds (Gessner & Chauvet, 1994). The specific anions associated with the Na salt may mediate the magnitude of these effects depending on the role of a specific anion in osmoregulatory function (Kaspari et al., 2009; Kaspari et al., 2014).

The effects of salt increases on autotrophic (algal) communities may also indirectly affect detrital decomposition. Along with heterotrophic microbes, algae are an integral part of detrital biofilms and interact with heterotrophic microbes to affect decomposition processes, where increased algal biomass is often shown to stimulate detrital processing performed by heterotrophic microbes (Danger et al., 2013; Lagrue et al., 2011; Rier et al., 2007 & 2014). This effect, known as a positive ‘priming effect’, is thought to occur in low-nutrient environments (Guenet et al., 2010), as is found in many unaltered freshwater ecosystems, presumably due to the additional carbon energy from algal exudates exploited by heterotrophs for the purpose of mining recalcitrant organic matter for more nutrients (Blagodatskaya & Kuzyakov, 2008; Guenet et al., 2010; Jenkinson et al., 1985; and Kuzyakov, 2010). If salt compounds were to act as a subsidy for algal production, heterotrophic microbial detrital processing may be increased, and the magnitude of this effect would be mediated by the specific anion involved. However, ions or overall salt concentrations that act as stressors to algae may limit growth or photosynthetic ability, and therefore algal exudates, so that no heterotrophic ‘priming’ would occur and decomposition would then be primarily mediated by salt effects on the heterotrophic microbes alone.

Our overarching objective was to evaluate the effects of two common Na salts [NaCl and sodium bicarbonate (NaHCO$_3$)] on detrital microbial community productivity (biomass) and function (detrital processing) across a presumed sub-lethal concentration gradient using two similar laboratory microcosm studies. I hypothesized the following:
1) Autotrophic (algal) and heterotrophic (fungal and bacterial) microbial biomass will increase with increasing salinity (i.e. a subsidy response), potentially plateauing at some salt concentration threshold, or even declining in the higher salt concentration treatments (i.e. a stress response) (Odum et al, 1974). 2) Detrital decomposition and respiration patterns will follow fungal biomass patterns (Gessner & Chauvet, 1994). 3) NaHCO₃ will be a better subsidy or relieve more osmoregulatory stress for heterotrophic microbes (fungi and bacteria) than NaCl given the evidence suggesting Cl⁻ is a more stressful anion to heterotrophs (Georgieva et al., 2012; Zuo et al., 2014; Bencherif et al., 2015; Scott et al., 2015). 4) NaCl will be a better subsidy or relieve more osmoregulatory stress for autotrophic microbes (algae) than NaHCO₃ given the evidence suggesting HCO₃⁻ is a more stressful anion to autotrophs (Zuo et al., 2014; Scott et al., 2015).

Methods

Experiment A

A manipulative microcosm study was carried out in a climate-controlled greenhouse from February to June 2016. Microcosms were exposed to natural day/night light cycles according to the season for the duration of the experiment. Greenhouse temperatures were taken every hour during the experiment by a digital temperature monitoring and control system. Daytime temperatures ranged from 17.3°C-23.4°C and nighttime temperatures ranged from 12.6°C-20.4°C (Appendix K). Microcosm daytime water temperatures ranged from 15.1°C-29.6°C (Appendix L).

Microcosms were constructed using 50-ml conical centrifuge tubes with 1mm vinyl-coated mesh squares placed inside to form a pouch for suspending leaf litter discs in treatment water. Discs (area = 1.496 cm²) were cut from Liquidambar styraciflua (sweetgum) leaf litter using a cork borer, leached for one week in dechlorinated water, and sets of 10 discs were placed inside the mesh pouch of each microcosm.
Stream water was collected from a local stream known to have low ambient Na salt concentrations (Musto, 2013; Tyree et al. 2016), which was verified with laboratory analysis (Arkansas Water Resource Center Water Quality Laboratory, Fayetteville, AR). Collected stream water was filtered with a 32-μm mesh to remove large particulate matter and then used to make bulk treatment solutions to be randomly assigned to microcosms. Treatment solutions included ambient SW with no salt amendments (Na+, Cl, HCO3− = ~2.68, ~2.17, ~3.48mg L−1, respectively; Table 2), NaHCO3 amendments to achieve a target concentration gradient with 3 levels (16, 32, and 64mg L−1 NaHCO3), and NaCl amendments to achieve a target concentration gradient with 3 levels (16, 32, and 64mg L−1 NaCl).

The salt types and concentration levels were chosen based on Arkansas Regulation 2 (APCEC, 2014) stating Cl− and HCO3− are dominant ions in northwest Arkansas streams and Cl− ions should not exceed a one-third increase beyond reference concentrations in the area (10-13mg L−1) or 15mg L−1, whichever is highest. Further, Arkansas Regulation 2 (APCEC, 2014) states that different ions may require different regulatory limits unless Cl−:HCO3− + SO42− > 1 or the effects of an ionic mixture is the same as when Cl−:HCO3− + SO42− > 1. Therefore, I aimed for a low-level NaCl concentration gradient with Cl− values falling below, at (approximately), and above this regulatory limit which are summarized in Table 2. The SW SO42− = 2.59mg L−1, so Cl−:HCO3− + SO42− was always > 1 across the NaCl treatment gradient. I used the same salt concentration gradient with the NaHCO3-amended treatments to achieve HCO3− values falling below, at (approximately), and above the regulatory limits as well, which are summarized in Table 2. The Cl−:HCO3− + SO42− was always < 1 in the NaHCO3-amended treatments. In this way, potential salt treatment effects can be compared, regardless of slight differences in individual ionic concentrations because, according to Arkansas Regulation 2, differing effects would mean that regulations different from those governing Cl− are needed for HCO3− due to the lower ratio values seen in NaHCO3− treatments (APCEC, 2014).
Microcosm treatments were randomly assigned, and each treatment had 10 replicates (n=10, N=70). Bulk treatment solutions were added to their respective microcosms as necessary to maintain a volume of 50-ml. Microcosms were completely refreshed weekly with new treatment solutions to provide necessary nutrients for continued microbial community development. Microcosms were aerated daily by bubbling each microcosm individually using aquarium-grade tubing and air pumps to prevent anoxic conditions. The leaf discs remained in incubations for a total of 134 days.

A Membrane Inlet Mass Spectrophotometer (MIMS) was used to detect O$_2$ change over time as a measure of microbial community respiration (CR) rates (Halvorson et al., 2016), which were taken on the day of termination. Three leaf discs from each treatment were placed in glass scintillation vials filled with dissolved oxygen (DO) saturated SW, sealed air-tight, and incubated in the dark for 2-3 hours at 22°C before measuring DO. The incubation temperature was chosen based on the average greenhouse temperatures the leaf discs had been exposed to during the experiment. Ambient SW was used for incubations to standardize across treatments. Leaf discs were removed from incubation water after respiration measurements, dried for at least 24 hours at ≥48°C, desiccated until they reached room temperature, and weighed to obtain final dry mass measurements. CR rates were corrected for water volume and final dry mass of leaf discs.

Detrital decomposition was estimated as percent (%) dry mass loss per day over the 134-day experimental period. A subsample of leaf discs used as detrital substrate for setting up the microcosms was taken and dried (48°C, ≥24 h), desiccated, and weighed to obtain an initial dry mass. The initial and final dry mass measurements were used to calculate % dry mass loss per day for each treatment.

On day 134, samples were taken from each microcosm to assess microbial biomass. Two discs were subsampled from each microcosm for chlorophyll a (Chla) analysis as an estimate of algal biomass, three discs were taken for ergosterol analysis as an estimate of fungal biomass, and two discs were used for bacterial enumeration to be used later on as part
of bacterial biomass calculations. Discs for Chla analysis were preserved in 95% ethanol, discs for ergosterol analysis were preserved in 100% methanol, and discs for bacterial enumeration were preserved in 37% formaldehyde. All preserved samples were kept in the dark at 4°C until processed.

Chla analysis was performed using ethanol extraction and standard spectrophotometric methods (Steinman et al. 2007). Ergosterol analysis was performed by alkaline-methanol lipid extraction and purification using a solid phase extraction (SPE) vacuum manifold and high-performance liquid chromatography (HPLC) (Gessner 2005); the only methodological difference was the samples were preserved in only methanol and later KOH was added to achieve a concentration of 8g/L for processing. Bacterial enumeration was performed using DAPI staining and light excitation (Porter and Feig 1980). Bacteria were counted based on morphology [i.e. cocci (round-shaped) and bacilli (rod-shaped)] because evidence suggests that shape may lend to functional advantages for bacteria depending on nutrient or ion availability in their environment (Young, 2006). It should be noted that while all processed samples included 10 replicates per treatment (n=10; N=70), bacteria samples only included 5 replicates per treatment (n=5; N=35) due to mechanical failure of the dark refrigeration unit used to store the samples. High temperature and high light exposure for an extended amount of time compromised the integrity of half of the bacteria samples after DAPI staining before enumeration occurred, therefore I choose not to use those samples.

All data were analyzed and graphically visualized using R version 3.4.2 (R Core Team, 2017). Data for ergosterol, % leaf litter dry mass loss per day, and CR were analyzed using one-way ANOVAs (α=0.05, aov function in the stats package). Tukey's HSD (TukeyHSD function in the stats package) post hoc testing was used to make pairwise comparisons where ANOVA revealed significant p values. Kruskal-Wallis test (α=0.05; kruskal.test function in the stats package) was used to analyze Chla, cocci, bacilli, and total bacteria data due to assumption violations for parametric testing. Dunn’s test (dunn.test function in the dunn.test package) was
used, post hoc, for pairwise comparisons as necessary. Graphs were produced using the ggplot function in the ggplot2 package.

Experiment B

A manipulative microcosm study was carried out in a climate-controlled environmental chamber (Conviron Chamber, Model E15; Fuller et al. 2015) for 135 days in 2018. The environmental chamber temperature was held at 4.5°C with 12-hour light/dark cycles (Fuller et al., 2015) to emulate average seasonal temperature conditions from January through March 2018 and light cycles provided standardization.

Microcosms were constructed by replacing the bottom of a 120-mL specimen cup with 1mm vinyl-coated mesh and nesting it inside a second 120-mL specimen cup to suspend leaf litter discs within treatment solutions. The same type and number of discs were cut, leached, and placed on top of the mesh inside each microcosm as described in Experiment A.

The same stream water from Experiment A was collected, filtered to remove large particulate matter, and then used to make bulk treatment solutions to be randomly assigned to microcosms. Treatment solutions included SW, NaHCO$_3$ additions, and NaCl additions in the same concentrations as in Experiment A (Table 2). Microcosm treatment solutions were maintained at a 120-mL volume throughout the 135-day experiment. Microcosms were replicated, aerated, and refreshed the same as in Experiment A.

A respirometer (PyroScience FireSting O$_2$ Meter) was used to detect O$_2$ change over time as a measure of CR at termination. One leaf disc from each microcosm was prepared and incubated in the dark as described for Experiment A to obtain CR rates. In addition, a second round of incubations were done on the same discs in the light with all other conditions held the same in order to obtain net community respiration (NCR) rates. Since CR values were negative, gross primary production (GPP; Bott, 2007) was calculated as NCR-CR. Leaf discs were removed from incubation water after these measurements and final dry mass was obtained as described for Experiment A. These measurements were corrected for water volume and dry disc
mass as before. Final dry mass measurements were used in conjunction with initial dry mass measurements from a subset of leaf discs to calculate % loss per day as described in Experiment A.

At termination on day 135, three discs were taken from each microcosm to assess microbial biomass. Two discs per microcosm were used for Chla analysis to estimate algal biomass and one disc from each microcosm was used for ergosterol analysis to estimate fungal biomass. Discs for Chla analysis were sonicated for 10 minutes to suspend algae in 10-mL of respective salt treatment solution and pressure filtered onto a glass fiber filter. Filters were added to centrifuge tubes and covered with 95% ethanol. Determination of Chla thereafter was done as described for Experiment A. Discs for ergosterol analysis were preserved and processed as described for Experiment A.

All data were analyzed and graphically visualized using the same functions and packages in R version 3.4.2 (R Core Team, 2017) as in Experiment A. Data for % litter dry mass loss per day and CR were analyzed using one-way ANOVA (α=0.05). Data for Chla, ergosterol, NCR, and GPP were analyzed using Kruskal-Wallis (α=0.05) because assumptions for parametric testing could not be reasonably met. Dunn’s test was used, post hoc, for pairwise comparisons as necessary.

Results

Experiment A

There were no differences across treatment medians for Chla (Table 3). Median values were ~16-40% lower than the SW median for all treatments except for 32mg L⁻¹ NaHCO₃ where the median was ~8% greater (Figure 1a; Appendix M). Medians were always slightly lower for NaCl treatments compared to NaHCO₃ treatments within each concentration level (Figure 1a; Appendix M). The interquartile range (IQR, a measure of variability), was greatest in and similar
across SW, 32mg L\(^{-1}\) NaHCO\(_3\), and 32mg L\(^{-1}\) NaCl (2.1e-04, 2.2e-04, and 2.1e-04, respectively) (Figure 1a; Appendix M).

Mean fungal biomass (Table 3) did not differ across treatments but was \(~8.12-13.60\%\) greater in 16-32mg/L NaHCO\(_3\) and \(~7.21-11.30\%\) greater in 16-32mg L\(^{-1}\) NaCl than in SW treatments (Figure 2a; Appendix M). Mean fungal biomass was \(~8.63\%\) and \(~6.97\%\) less in 64mg/L NaCO\(_3\) and NaCl, respectively, than in SW (Figure 2a; Appendix M). There was a weak hump-shaped pattern in the data for both Na salt treatments (Figure 2a). The greatest variation occurred in the 16-32mg L\(^{-1}\) NaCl treatments with IQRs of \(~5.47\) and \(~6.60\), respectively (Figure 2a; Appendix M).

There was a significant salt treatment effect on cocci-type bacterial biomass medians across treatments (Table 3). The 16mg L\(^{-1}\) NaHCO\(_3\) treatment differed from 64mg L\(^{-1}\) NaHCO\(_3\) and NaCl treatments but was similar to all others (Figure 3a). The 64mg L\(^{-1}\) NaHCO\(_3\) and NaCl treatments were similar to each other and all others except 16mg L\(^{-1}\) NaHCO\(_3\) (Figure 3a). The 16-32mg L\(^{-1}\) NaHCO\(_3\) treatments were \(~97.04-94.23\%\), respectively, lower than SW and the 64mg L\(^{-1}\) NaHCO\(_3\) treatment was \(~23.08\%\) greater (Figure 3a; Appendix M). The 16-32mg L\(^{-1}\) NaCl treatments were \(~94.23-88.46\%\), respectively, lower than SW and the 64mg L\(^{-1}\) NaCl treatment was \(~38.46\%\) greater (Figure 3a; Appendix M). Variation was smallest in 16-32mg L\(^{-1}\) NaHCO\(_3\) (IQRs = 5.8e-08 and 7.6e-08, respectively) and 16-32mg L\(^{-1}\) NaCl (IQRs = 2.2e-06 and 3.0e-07, respectively), which were all smaller than SW (IQR = 2.6e-06) (Figure 3a; Appendix M). Variation was greatest in 64mg L\(^{-1}\) NaHCO\(_3\) (IQR = 4.5e-06) and NaCl (IQR = 1.3e-05) treatments, which were both greater than SW (IQR = 2.6e-06) (Figure 3a; Appendix M).

Median bacilli-type bacterial biomass did not differ across treatments (Table 3). The 16, 32, and 64mg L\(^{-1}\) NaHCO\(_3\) treatments medians were all lower than SW by \(~98.63, ~71.39, and \(~36.84\%\), respectively (Figure 3b; Appendix M). The 16 and 32mg L\(^{-1}\) NaCl treatments were \(~97.95\) and \(~92.63\%\) lower than SW, but the 64mg L\(^{-1}\) NaCl treatment was \(~57.89\%\) greater (Figure 3b; Appendix M). Variation was smaller than SW (IQR = 1.7e-06) in all NaHCO\(_3\)
treatments (16, 32, and 64mg L\(^{-1}\) NaHCO\(_3\) IQRs = 4.5e-08, 1.1e-07, and 6.1e-07, respectively) (Figure 3b; Appendix M). The 16-32mg L\(^{-1}\) NaCl treatments (IQR = 9.1e-07 and 1.0e-07, respectively) varied less than SW (IQR = 1.7e-06), but 64mg L\(^{-1}\) NaCl treatment (IQR = 3.7e-06) varied distinctly more (Figure 3b; Appendix M).

Median total bacterial biomass, consisting of cocci- and bacilli-type bacteria, differed across treatments in initial statistical testing (Table 3), but post hoc testing did not detect any significant comparisons. Medians for 16, 32, and 64mg L\(^{-1}\) NaHCO\(_3\) treatments were ~97.83, ~96.09, and ~10.87%, respectively, less than for SW (Figure 3c; Appendix M). The 16-32mg L\(^{-1}\) NaCl treatment medians were ~95.87 and ~90.87% less than SW, but the 64mg L\(^{-1}\) median was ~63.04% greater than SW (Figure 3c; Appendix M). Variability was smaller than SW (IQR = 4.2e-06) for 16-32mg L\(^{-1}\) NaHCO\(_3\) treatments (IQRs = 1.0e-07 and 1.9e-07, respectively) and 16-32mg L\(^{-1}\) NaCl treatments (IQRs = 3.1e-06 and 3.8e-07, respectively) (Figure 3c; Appendix M). The 64mg L\(^{-1}\) NaHCO\(_3\) treatment (IQR = 4.8e-06) varied more than SW (IQR = 4.2e-06), but 64mg L\(^{-1}\) NaCl had the greatest variation (IQR = 1.6e-05) (Figure 3c; Appendix M).

Salt treatments had a significant effect on mean microbial community respiration (CR; O\(_2\) consumption) rates (Table 3). Mean CR was more negative overall with NaHCO\(_3\) amendments compared to SW, where CR was ~11.94, ~5.40, and ~1.55 times more negative than SW in 16, 32, and 64 mg L\(^{-1}\) NaHCO\(_3\), respectively (Figure 4; Appendix M). Means for 16, 32, and 64 mg L\(^{-1}\) NaCl treatments were ~65.46%, ~1.44 times, and ~2.22 times, respectively, more positive than SW means (Figure 4; Appendix M). Notably, SW and all NaHCO\(_3\) treatments had negative values; all NaCl treatments had positive mean values (Figure 4; Appendix M). Variation was greater in 16, 32, and 64mg L\(^{-1}\) NaHCO\(_3\) treatments (IQRs = 0.0114, 0.0135, and 0.0053, respectively) (Figure 4; Appendix M). Variation was smaller in 16-32mg L\(^{-1}\) NaCl treatments (IQR = 0.0019 and 0.0018, respectively) than in SW (IQR = 0.0025) but varied about the same in 64mg L\(^{-1}\) (IQR = 0.0025) (Figure 4; Appendix M).
Mean % leaf litter dry mass loss did not differ across salt treatments (Table 3). Means for 16, 32, and 64mg L\(^{-1}\) NaHCO\(_3\) treatments were lower than SW mean by ~2.56, ~12.82, and ~2.56\% (Figure 5a; Appendix M). The 16 and 64mg L\(^{-1}\) NaCl means were ~5.13 and ~7.69\% greater than the SW mean, but the 32mg L\(^{-1}\) treatment was ~7.69\% lower than SW (Figure 5a; Appendix M). Variation was smallest for 16mg L\(^{-1}\) NaHCO\(_3\) (IQR = 0.03), which was smaller than in SW (IQR = 0.05), but there was greater variability in 32 and 64mg L\(^{-1}\) NaHCO\(_3\) treatments (IQRs = 0.10 and 0.11, respectively) (Figure 5a; Appendix M). The 16 and 32mg L\(^{-1}\) NaCl treatments (IQRs = 0.09 and 0.08, respectively) also varied more than SW (IQR = 0.05), but 64mg L\(^{-1}\) NaCl variation (IQR = 0.05) was about the same (Figure 5a; Appendix M).

**Experiment B**

Median Chla values significantly differed across treatments (Table 4). Medians for SW and 16-32mg L\(^{-1}\) NaCl were similar to each other as well as to 16mg L\(^{-1}\) NaHCO\(_3\) and 64mg L\(^{-1}\) NaCl (Figure 1b; Appendix N). Medians for 32-64mg L\(^{-1}\) NaHCO\(_3\) were similar to each other and 16mg L\(^{-1}\) NaHCO\(_3\) and 64mg L\(^{-1}\) NaCl (Figure 1b; Appendix N). Medians for all NaHCO\(_3\) treatments were lower than SW (Figure 1a; Appendix N). Medians for 16-32mg L\(^{-1}\) NaCl were ~1.147 times greater than SW, but 64mg L\(^{-1}\) NaCl was ~23.53\% less than SW which resulted in a hump-shaped curve (Figure 1b; Appendix N). Variation showed a steady decrease with increasing NaHCO\(_3\) (16, 32, 64mg L\(^{-1}\) IQRs = 0.0014, 0.0000, 0.0000, respectively) as compared to SW (IQR = 0.0022) (Figure 1b; Appendix N). Variation was greatest in 32-64mg/L NaCl treatments (IQRs = 0.0079 and 0.0039, respectively), which were both greater than SW (IQR = 0.0022), but 16mg L\(^{-1}\) NaCl was somewhat smaller (IQR = 0.0019)(Figure 1b; Appendix N).

Salt treatments had a significant effect on median ergosterol concentrations (Table 4). Median ergosterol concentration for 32mg L\(^{-1}\) NaHCO\(_3\) was statistically similar to 16 and 32mg
L⁻¹ NaHCO₃ (Figure 2b; Appendix N). Medians for SW and all NaCl treatments were similar to each other as well as to 16 and 32mg L⁻¹ NaHCO₃ treatments (Figure 2b; Appendix N). Medians were ∼2.48, ∼6.66, and ∼5.22 times greater for 16, 32, and 64mg L⁻¹ NaHCO₃ treatments, respectively, than SW, yielding a hump-shaped curve (Figure 2b; Appendix N). Medians were consistently lower than SW for 16, 32, and 64mg L⁻¹ NaCl treatments by ∼26.65, ∼22.05, and ∼41.57%, respectively (Figure 2b; Appendix N). Variation was greater than SW (IQR = 2.50) in all NaHCO₃ treatments (16, 32, 64mg L⁻¹ IQRs = ∼9.35, ∼4.49, ∼4.59, respectively), but especially in the lowest NaHCO₃ concentration (Figure 2b; Appendix N). Variation was smaller than SW (IQR = 2.50) in all NaCl treatments (16, 32, 64mg L⁻¹ IQRs = ∼2.37, ∼2.08, ∼1.17, respectively), but especially in the highest NaCl concentration (Figure 2b; Appendix N).

There was no difference across treatments for mean microbial CR (Table 4), which were all negative values (Figure 6a; Appendix N). The mean 16mg L⁻¹ NaHCO₃ value was ∼23.1% more negative than SW mean and 32-64mg L⁻¹ NaHCO₃ means were ∼2.1-14.6% less negative than SW (Figure 6A; Appendix N). Mean values for NaCl treatments were always more negative than SW by ∼0.6-17.4% (Figure 6A; Appendix N). Variation was smaller than SW (IQR = ∼0.0062) in 16mg L⁻¹ NaHCO₃ (IQR = ∼0.0031) and across all NaCl treatments (16, 32, 64mg L⁻¹ IQRs = ∼0.0040, ∼0.0016, ∼0.0032, respectively) (Figure 6a; Appendix N). Variation was greatest in 32-64mg L⁻¹ NaHCO₃ treatments (IQRs = 0.0090 and 0.0087, respectively) (Figure 6a; Appendix N).

Initial statistical testing detected significant differences between treatments for median microbial NCR values (Table 4) but post hoc testing did not discern any significant comparisons. Median values were negative for SW and across all treatments (Figure 6b; Appendix N). The 16, 32, and 64mg L⁻¹ NaHCO₃ medians were less negative than SW by ∼50.00, ∼51.32, and ∼9.21%, respectively (Figure 6b; Appendix N). The 16mg L⁻¹ NaCl treatment was ∼99.34% more negative than SW, but 32 and 64mg L⁻¹ NaCl were ∼59.21 and ∼71.05%, respectively, less
negative than SW (Figure 6b; Appendix N). Variation was smallest in NaHCO₃ treatments for 16-32mg L⁻¹ NaHCO₃ (IQRs = 0.0041 and 0.0043, respectively) and greatest for 64mg L⁻¹ NaCOH₃ (IQR = 0.0235), but all varied less than SW (IQR = 0.0338) (Figure 6b; Online Resources 4). Variation was greatest in NaCl treatments for 16mg L⁻¹ (IQR = 0.0307) and smallest for 32-64mg L⁻¹ (IQRs = 0.0082 and 0.0030, respectively), but all varied less than SW (IQR = 0.0338) (Figure 6b; Appendix N).

Similar to NCR results, initial statistical testing showed salt treatments had a significant effect on median GPP values (Table 4) but post hoc testing did not detect any significant comparisons. It is important to note that median values for 16-32mg L⁻¹ NaHCO₃, and 32-64mg L⁻¹ NaCl were positive while medians for SW, 64mg L⁻¹ NaHCO₃, and 16mg L⁻¹ NaCl were negative (Figure 6c; Appendix N). Medians NaHCO₃ for 16-32mg L⁻¹ were ~1.83 and ~1.02 times, respectively, less negative than SW and 64mg L⁻¹ NaHCO₃ was ~2.38% more negative than SW (Figure 6c; Appendix N). The 16mg L⁻¹ NaCl treatment was ~2.86 times more negative than SW and the 32-64mg L⁻¹ NaCl treatments were ~1.71 and ~2.05 times less negative than SW (Figure 6c; Appendix N). Variability trends were similar to trends seen in NCR (Figures 6b and 6c; Appendix N).

There was no difference between treatments for mean % litter dry mass loss per day (Table 4). The 16-32mg L⁻¹ NaHCO₃ treatments were ~31.58 and ~10.53% greater, respectively, than SW and the 64mg L⁻¹ NaHCO₃ treatment was ~10.53% less than for SW (Figure 5b; Appendix N). The 16mg L⁻¹ NaCl treatment was ~21.05% greater than SW and mass loss for the 32-64mg/L NaCl treatments were about the same as for SW (Figure 5b; Appendix N). Weak hump-shaped patterns were seen in the data for both Na salt amendments (Figure 5b; Appendix N). Variation in 16 and 64mg L⁻¹ NaHCO₃ were the same (IQRs = 0.09) and smaller than SW (IQR = 0.10), but 32mg L⁻¹ NaHCO₃ varied the most (IQR = 0.17) and was greater than SW (Figure 5b; Appendix N). Variation in 16mg L⁻¹ NaCl (IQR = 0.15) was greater
than SW (IQR = 0.10) and 32-64mg L\(^{-1}\) NaCl varied the least (IQRs = 0.08 and 0.05, respectively) which was less than for SW (IQR = 0.10) (Figure 5b; Appendix N). was wide-ranging for all treatments (Figure 5b).

Discussion

Experiment A

Algal biomass was overall unresponsive to Na salt treatments, appearing not to be subsidized by Na salts at these relatively low inputs in either NaHCO\(_3\) or NaCl treatments, at least at warmer temperatures as may be seen in spring and early summer (Appendix K). These results did not support the hypothesis regarding subsidy-stress for autotrophs. There were no patterns to suggest that one salt type was better than the other at supporting algal communities, which did not support the hypothesis that NaCl would be the better subsidy for autotrophs. The relatively low concentrations of Chla indicate there likely was not enough algae (and therefore photosynthetic by-product exuded) to effectively positively ‘prime’ fungi. Algal biomass data suggest that any changes in detrital processing at warmer temperatures may be mostly dependent on how low inputs of Na salts affect heterotrophic microbes with little influence from algae.

Fungal biomass data suggest that fungi may be weakly subsidized up to 32mg L\(^{-1}\) NaCl- and NaHCO\(_3\)-amended treatments, which was supportive of the subsidy-stress hypothesis for heterotrophic microbes. The increasing relative variability within a specific salt treatment gradient is further evidence that stress/toxicity thresholds are being approached beyond subsidization plateaus (Odum et al., 1979). The differences in how well the Na salts subsidize fungi appear to be influenced by the associated anion and, potentially, how that anion affects fungal osmoregulation. The variability in the data were also supportive of the hypothesis that
NaHCO₃ would be better at supporting fungal communities since the largest variability was seen in the NaCl-amended treatments.

It is unclear whether bacterial biomass data align with the subsidy-stress predictions for heterotrophic microbes. Since the bacterial community likely consisted of mixed species, some bacterial species may express greater tolerance to higher salinities while still being growth-limited in lower salinity environments due to nutrient and/or osmoregulatory constraints. In this case, small Na salt increases up to 32mg L⁻¹ may not have offered enough Na subsidy or osmoregulatory relief to greatly increase biomass for salt-tolerant bacteria. However, these smaller Na salt additions could have been stressful and even toxic to salt-intolerant bacteria, driving down biomass in these otherwise competitively advantaged species. This may explain decreases in bacterial biomass in the study with up to 32mg L⁻¹ Na salt additions. Continued increases in Na salt concentration up to 64mg L⁻¹ may have been enough to cause replacement of most salt-intolerant bacteria with salt-tolerant species, and Na and/or osmoregulatory constraints on salt-tolerant species may have been relieved at these higher concentrations. Some of the wide variation in the data at the highest Na salt concentrations may have been due to such extreme species replacement shifts. Unfortunately, the study was limited in that I did not attempt genomic analysis to delineate bacterial species, which would be important to understand if species replacement had been taking place with low-level salinization. The hypothesis that NaHCO₃ may be better at supporting heterotrophic communities is reasonably supported given the greater variability and therefore stress seen in the NaCl treatments.

Decomposition appeared slightly suppressed even though the fungal data indicate weak subsidization by Na salts, which does not support the prediction that decomposition patterns would have followed fungal biomass patterns. This was especially true for NaHCO₃ treatments. This may be because HCO₃⁻ could be used as a C source by heterotrophs rather than relying
solely on C from the leaf litter substrate, which would result in somewhat suppressed detrital mass loss in those treatments while maintaining or even slightly increasing fungal biomass.

More negative CR values correspond to greater respiration or O₂ consumption and less negative values correspond to lowered respiration. The CR data trends in this experiment were strong and show that NaHCO₃ treatments resulted in increased CR while NaCl treatments resulted in suppressed respiration. These results did not seem to be explained by patterns in fungal biomass because the relationships seen in the fungal biomass data were weak and the patterns were similar for both salt treatments; the CR data showed strong and opposing trends between salts. Therefore, CR data did not support the hypothesis that respiration trends would follow fungal biomass trends. These and the decomposition results suggest that exposure to low-level salt inputs at warmer temperatures (Appendix K) may result in fungal biomass being decoupled from decomposition and respiration measurements. Bacterial biomass changes may help explain some of the CR patterns seen. If species replacement was occurring within bacterial communities, NaHCO₃ treatments may have led to a larger population of aerobic bacteria where NaCl treatments may have led to a larger population of aerobic bacteria.

Experiment B

Algal biomass had a strong stressful/toxic response to NaHCO₃ treatments while NaCl treatments elicited a strong subsidy-stress response. The findings fit the prediction for autotrophic subsidy-stress responses to small Na salt inputs. The data also supported the predictions that NaCl would be a better subsidy for autotrophs given the clear hump-shaped subsidy-stress curve and increased variability characteristic of these types of responses. These relationships were considerably stronger at cooler temperatures (4.5°C) than those seen in Experiment A.

Fungal biomass data showed a strong subsidy-stress response to NaHCO₃ and a weak subsidy-stress response to NaCl treatments, which was supportive of the subsidy-stress
predictions for heterotrophic microbes. These data also supported the predictions that NaHCO$_3$ would be a better subsidy for fungal biomass. As with the algal biomass data, these patterns were more pronounced in the cooler temperatures of this experiment as compared to Experiment A.

Decomposition data trends were weak, but reasonably aligned with the hypothesis that decomposition patterns would follow fungal biomass patterns considering the hump-shaped curves and variability. However, the weak decomposition relationships coupled with the strong responses of fungal biomass to NaHCO3 treatments further indicate that fungi may have used HCO$_3^-$ as an additional C source for growth. Additionally, Chla concentrations were somewhat greater in NaCl treatments than in NaHCO3 treatments or even algal biomass in Experiment A, so there may have been additional algal exudates available for fungal use in NaCl treatments. This may have provided enough C energy to fungi to continue detrital processing at rates comparable to the NaHCO3 treatments where much more fungal biomass was found. A limitation of both studies was that I did not include a means of tracking the movement of C from different potential sources. Using isotopic tracers could help determine whether fungi are prone to use HCO$_3^-$ as a significant C source for biomass increases or algal C as an energy source to support detrital processing activity.

The CR responses to Na salt treatments did not show any clear patterns of changes in respiration despite changes in fungal biomass so these data, like in Experiment A, do not support the hypothesis that respiration patterns would follow fungal biomass trends and suggest a decoupling of CR from fungal biomass and decomposition measurements. Additionally, negligible CR and decomposition changes with strong subsidization of fungi in NaHCO$_3$ treatments indicate the use of HCO$_3^-$ by fungi to increase biomass. The NCR data revealed net heterotrophy in all treatments evidenced by all negative NCR values, meaning O$_2$ consumption always overpowered any potential O$_2$ evolution. The GPP data patterns were similar to NCR.
patterns and though most values were positive as would be expected, some values were
negative. Negative GPP values indicate strong heterotrophic activity in those treatments that
overpowered autotrophic production.

Conclusion

Sodium (Na) salts may cause a subsidy-stress response in detrital biofilms, even at sub-
lethal levels and the data suggest effects may be ion- and species-specific, but temperature
also seems to play a significant role in the magnitude of these effects. Algae may be more likely
to experience stress with small HCO$_3^-$ inputs and be subsidized with small Cl- inputs in cooler
temperatures (4.5°C), but these effects may dissipate as temperatures increase (Appendices K
& L). One noteworthy caveat is that light may have affected these results. Experiment A would
have had shorter light periods according to the season than Experiment B and this could have
been an additional constraint on algal productivity in Experiment A, accentuating the differences
seen in algal responses between the two experiments. Fungi may be less vulnerable to shifts in
biomass with small Na salt inputs in warmer temperatures, but in cooler temperatures fungi may
experience significant biomass increases in the presence of HCO$_3^2$-. In fact, fungi may use
HCO$_3^2$- as a C subsidy in addition to C found in organic matter to increase biomass in cooler
temperatures. Bacteria may be significantly impacted by small Na salt increases such that
species replacement may occur, resulting in lower diversity at least in warmer temperatures.
Rates of detrital processing by heterotrophic microbes may not be prone to significant alteration
due to small Na salt inputs, but Na salts may influence the quality of detritus as a food source
for detritivores due to salt effects on microbes within the biofilm. Further experimentation is
needed to understand how microbial community changes after exposure to small Na salt
increases may affect secondary production. Measures of CR may become decoupled from
fungal biomass and decomposition with salt increases, which would render CR a less
appropriate measure for estimating fungal productivity. Concerning Arkansas-specific water quality regulations, these results support the development of separate criteria for specific ions. The study shows differing ion- and species-specific responses in the presents of inputs that are within regulatory limits, indicating these limits should be revisited and limitations for one ion may not be effectively extrapolated to other ions in an effort to protect freshwater ecosystems. Broadly, this study provides evidence that Na salt additions, even at low, sub-lethal levels, could have bottom up effects on aquatic food webs, especially during colder months.

Disclaimer

Some data from Chapter 2 Experiment B has been used as part of a Master of Science thesis by Anastasia Mogilevski completed at University of Central Arkansas.

Acknowledgements

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References


Keywords: Priming effect Microbial activity Hotspots Rhizosphere Detritusphere C and N modeling Soil organic matter turnover Enzyme activities. *Soil Biology and Biochemistry*, 42, 1363–1371.


Swan C (2007) Assessing the role of road salt runoff on the critical ecological interactions that regulate carbon processing in small, headwater streams in the Chesapeake Bay watershed. Final Report. MWRRC (Project #2007MD148B)


### Tables

*Table 1* Primary literature investigating effects of increasing salinity on heterotrophic and/or autotrophic microbes. Microbial group: H=heterotrophs, A=autotrophs, B=both. Salt concentration ranges have been standardized from values reported in the literature with salinity expressed here as Conductivity (µS/cm)

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Microbial Group</th>
<th>Salt Type or Ions Present</th>
<th>Conductivity</th>
<th>Decreases with Salt Increases</th>
<th>Increases with Salt Increases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Datta &amp; Kulkarni</td>
<td>2004</td>
<td>H</td>
<td>NaCl</td>
<td>10400-82600</td>
<td>Colonization</td>
<td></td>
</tr>
<tr>
<td>Chen et al.</td>
<td>2017</td>
<td>H</td>
<td>NaCl</td>
<td>11200-89500</td>
<td>Colonization (suppressed hyphal growth); sporulation; spore germination</td>
<td></td>
</tr>
<tr>
<td>Bencherif et al.</td>
<td>2015</td>
<td>H</td>
<td>CaCO₃</td>
<td>18200-49500</td>
<td>Colonization</td>
<td>Sporulation; ergosterol</td>
</tr>
<tr>
<td>Georgieva et al.</td>
<td>2012</td>
<td>H</td>
<td>CaSO₄ NaCl</td>
<td>778-2100788-2100</td>
<td>Abundance; diversity</td>
<td>Abundance; diversity</td>
</tr>
<tr>
<td>Kaspari et al.</td>
<td>2009</td>
<td>NaCl NaCl</td>
<td>NaCl NaCl Na₂PO₄</td>
<td>780-9116.64</td>
<td>Detrital decomposition</td>
<td>Detrital decomposition</td>
</tr>
<tr>
<td>Canhoto et al.</td>
<td>2017</td>
<td>H</td>
<td>NaCl NaCl NaCl NaCl</td>
<td>0-24.96</td>
<td>Sporulation; respiration; mass loss; growth</td>
<td></td>
</tr>
<tr>
<td>Scott et al.</td>
<td>2015</td>
<td>H</td>
<td>NaSO₃ CaCl</td>
<td>22158.2417312.88</td>
<td>Diversity</td>
<td>Diversity</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>2013</td>
<td>H</td>
<td>Ca²⁺, Mg²⁺, Cl⁻, SO₄⁻⁡</td>
<td>530.4-40840.8</td>
<td>Relative abundance</td>
<td>Species replacement</td>
</tr>
<tr>
<td>Franco et al.</td>
<td>2013</td>
<td>H</td>
<td>NaCl</td>
<td>7800-15600</td>
<td>Extracellular activity; growth</td>
<td></td>
</tr>
<tr>
<td>Nemtseva et al.</td>
<td>2013</td>
<td>A</td>
<td>NaCl Na₃CO₃</td>
<td>93.1-279.2g/L</td>
<td>Biomass; antioxidant production</td>
<td></td>
</tr>
<tr>
<td>Zuo et al.</td>
<td>2014</td>
<td>A</td>
<td>NaCl Na₃CO₃</td>
<td>1.8233-27.3499</td>
<td>Density; growth; photosynthetic ability; O₂ production</td>
<td></td>
</tr>
<tr>
<td>Tyree et al.</td>
<td>2016</td>
<td>NaCl</td>
<td></td>
<td>4.68-218.4</td>
<td>Respiration</td>
<td>Decomposition</td>
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Table 2 Total sodium (Na) salt amendments with specific anion target concentrations. Salt type: NaCl = sodium chloride amended; NaHCO₃ = sodium bicarbonate amended. All values expressed in mg L⁻¹

<table>
<thead>
<tr>
<th>Salt Type</th>
<th>Total Amendment</th>
<th>[Cl⁻]</th>
<th>[HCO₃⁻]</th>
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</thead>
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<tr>
<td>NaCl</td>
<td>16</td>
<td>10.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>19.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>39.18</td>
<td></td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>16</td>
<td></td>
<td>12.92</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td></td>
<td>24.54</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td></td>
<td>47.79</td>
</tr>
</tbody>
</table>

Table 3 Experiment A statistical analyses (α = 0.05) results. Response variable key: Chl a = chlorophyll a, a proxy for algal biomass; Ergo = ergosterol, a proxy for fungal biomass; Cocci = cocci-type bacterial biomass; Bacilli = bacilli-type bacterial biomass; Total Bact = total (cocci+bacilli) bacterial biomass; Dry Mass Loss = leaf litter detritus dry mass loss; CR = community respiration (O₂ consumption). Key to statistical output column headers: df = degrees freedom, F = ANOVA test statistic or X² = Kruskal-Wallis test statistic, p = probability value. Significant p values in bold. Post hoc testing specified as necessary

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Statistical Test</th>
<th>df</th>
<th>F or X²</th>
<th>p</th>
<th>Post Hoc Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a (mg mg⁻¹ dry mass)</td>
<td>Kruskal-Wallis</td>
<td>6</td>
<td>4.384</td>
<td>0.625</td>
<td>N/A</td>
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<tr>
<td>Ergo (µg mg⁻¹ dry mass)</td>
<td>1-way ANOVA</td>
<td>6</td>
<td>0.517</td>
<td>0.793</td>
<td>N/A</td>
</tr>
<tr>
<td>Cocci (mg C mg⁻¹ dry mass)</td>
<td>Kruskal-Wallis</td>
<td>6</td>
<td>16.613</td>
<td>0.011</td>
<td>Dunn’s</td>
</tr>
<tr>
<td>Bacilli (mg C mg⁻¹ dry mass)</td>
<td>Kruskal-Wallis</td>
<td>6</td>
<td>9.646</td>
<td>0.140</td>
<td>N/A</td>
</tr>
<tr>
<td>Total Bact (mg C mg⁻¹ dry mass)</td>
<td>Kruskal-Wallis</td>
<td>6</td>
<td>14.099</td>
<td>0.029</td>
<td>Dunn’s*</td>
</tr>
<tr>
<td>Dry Mass Loss (% day⁻¹)</td>
<td>1-way ANOVA</td>
<td>6</td>
<td>1.290</td>
<td>0.275</td>
<td>N/A</td>
</tr>
<tr>
<td>CR (mg O₂ mg⁻¹ dry mass hr⁻¹)</td>
<td>1-way ANOVA</td>
<td>6</td>
<td>153.600</td>
<td>&lt;0.001</td>
<td>Tukey’s HSD</td>
</tr>
</tbody>
</table>

* indicates post-hoc tests revealed no differences between medians though Kruskal-Wallis testing was significant
Table 4 Experiment B statistical analyses ($\alpha = 0.05$) results. Response variable key: Chl a = chlorophyll a, a proxy for algal biomass; Ergo = ergosterol, a proxy for fungal biomass; Dry Mass Loss = leaf litter detritus dry mass loss; CR = community respiration (O$_2$ consumption); NCR = net community respiration (O$_2$ consumption + O$_2$ evolution); GPP = gross primary production (NCR-CR). Key to statistical output column headers: df = degrees freedom, F = ANOVA test statistic or $X^2$ = Kruskal-Wallis test statistic, $p$ = probability value. Significant $p$ values in bold. Post hoc testing specified as necessary.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Statistical Test</th>
<th>df</th>
<th>F or $X^2$</th>
<th>$p$</th>
<th>Post Hoc Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a (mg mg$^{-1}$ dry mass)</td>
<td>Kruskal-Wallis</td>
<td>6</td>
<td>32.170</td>
<td>$&lt;0.001$</td>
<td>Dunn’s</td>
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<tr>
<td>Ergo (μg mg$^{-1}$ dry mass)</td>
<td>Kruskal-Wallis</td>
<td>6</td>
<td>23.500</td>
<td>$&lt;0.001$</td>
<td>Dunn’s</td>
</tr>
<tr>
<td>Dry Mass Loss (% day$^{-1}$)</td>
<td>1-way ANOVA</td>
<td>5</td>
<td>0.907</td>
<td>0.496</td>
<td>N/A</td>
</tr>
<tr>
<td>CR (mg O$_2$ mg$^{-1}$ dry mass hr$^{-1}$)</td>
<td>1-way ANOVA</td>
<td>6</td>
<td>0.887</td>
<td>0.510</td>
<td>N/A</td>
</tr>
<tr>
<td>NCR (mg O$_2$ mg$^{-1}$ dry mass hr$^{-1}$)</td>
<td>Kruskal-Wallis</td>
<td>6</td>
<td>13.837</td>
<td>$0.032$</td>
<td>Dunn’s*</td>
</tr>
<tr>
<td>GPP (mg O$_2$ mg$^{-1}$ dry mass hr$^{-1}$)</td>
<td>Kruskal-Wallis</td>
<td>6</td>
<td>15.212</td>
<td>$0.019$</td>
<td>Dunn’s*</td>
</tr>
</tbody>
</table>

* indicates post-hoc tests revealed no differences between medians though Kruskal-Wallis testing was significant.
Figures

Figure 1 (a) Experiment A chlorophyll a concentrations (mg mg\(^{-1}\) dry mass) by salt treatment. (b) Experiment B chlorophyll a concentrations (mg mg\(^{-1}\) dry mass) by salt treatment. Salt treatment key: SW = ambient streamwater (3mg L\(^{-1}\) Na); HCO3-16, -32, -64 = sodium bicarbonate (NaHCO\(_3\)) amended (16, 32, 64mg L\(^{-1}\) Na, respectively); CL-16, -32, -64 = sodium chloride (NaCl) amended (16, 32, 64mg L\(^{-1}\) Na, respectively). Diamonds = means. Horizontal lines = medians. Bars = standard error. Letters indicate differences between treatments according to post hoc testing.
Figure 2 (a) Experiment A ergosterol concentrations (μg mg⁻¹ dry mass) by salt treatment. (b) Experiment B ergosterol concentrations (μg mg⁻¹ dry mass) by salt treatment. Salt treatment key: SW = ambient streamwater (3mg L⁻¹ Na); HCO₃-16, -32, -64 = sodium bicarbonate (NaHCO₃) amended (16, 32, 64mg L⁻¹ Na, respectively); CL-16, -32, -64 = sodium chloride (NaCl) amended (16, 32, 64mg L⁻¹ Na, respectively). Diamonds = means. Horizontal lines = medians. Bars = standard error. Letters indicate differences between treatments according to post hoc testing.
Figure 3 (a) Experiment A cocci-type bacterial biomass [mg carbon (C) mg\(^{-1}\) dry mass] by salt treatment. (b) Experiment A bacilli-type bacterial biomass (mg C mg\(^{-1}\) dry mass) by salt treatment. (c) Experiment A total bacterial (cocci+bacilli) bacterial biomass (mg C mg\(^{-1}\) dry mass) by salt treatment. Salt treatment key: SW = ambient streamwater (3mg L\(^{-1}\) Na); HCO\(_3\)-16, -32, -64 = sodium bicarbonate (NaHCO\(_3\)) amended (16, 32, 64mg L\(^{-1}\) Na, respectively); CL-16, -32, -64 = sodium chloride (NaCl) amended (16, 32, 64mg L\(^{-1}\) Na, respectively). Diamonds = means. Horizontal lines = medians. Bars = standard error. Letters indicate differences between treatments according to post hoc testing.
Figure 4 Experiment A community respiration (CR, O₂ consumption) rate (mg O₂ hr⁻¹ mg⁻¹ dry mass) by salt treatment. Measured after 2-hr dark incubation. Salt treatment key: SW = ambient streamwater (3mg L⁻¹ Na); HCO₃-16, -32, -64 = sodium bicarbonate (NaHCO₃) amended (16, 32, 64mg L⁻¹ Na, respectively) ; CL-16, -32, -64 = sodium chloride (NaCl) amended (16, 32, 64mg L⁻¹ Na, respectively). Diamonds = means. Horizontal lines = medians. Bars = standard error. Letters indicate differences between treatments according to post hoc testing.
Figure 5 (a) Experiment A leaf litter dry mass loss (% day\(^{-1}\)) by salt treatment. (b) Experiment B leaf litter dry mass loss (% day\(^{-1}\)) by salt treatment. Salt treatment key: SW = ambient streamwater (3mg L\(^{-1}\) Na); HCO3-16, -32, -64 = sodium bicarbonate (NaHCO\(_3\)) amended (16, 32, 64mg L\(^{-1}\) Na, respectively); CL-16, -32, -64 = sodium chloride (NaCl) amended (16, 32, 64mg L\(^{-1}\) Na, respectively). Diamonds = means. Horizontal lines = medians. Bars = standard error.
Figure 6 (a) Experiment B community respiration (CR, $O_2$ consumption) rate (mg $O_2$ hr$^{-1}$ mg$^{-1}$ dry mass) by salt treatment. Measured after 2-hr dark incubation. (b) Experiment B net community respiration (NCR, $O_2$ consumption + $O_2$ evolution) rate (mg $O_2$ hr$^{-1}$ mg$^{-1}$ dry mass) by salt treatment. Measured after 2-hr light incubation. (c) Experiment B gross primary production (GPP) rate (mg $O_2$ hr$^{-1}$ mg$^{-1}$ dry mass) by salt treatment. GPP = NCR-CR. Salt treatment key: SW=ambient streamwater (3mg L$^{-1}$ Na); HCO$_3$-16, -32, -64 = sodium bicarbonate (NaHCO$_3$) amended (16, 32, 64mg L$^{-1}$ Na, respectively); CL-16, -32, -64 = sodium chloride (NaCl) amended (16, 32, 64mg L$^{-1}$ Na, respectively). Diamonds = means. Horizontal lines = medians. Bars = standard error.
Appendices

Appendix K

Experiment A greenhouse temperatures (°C) taken every hour during the experiment. Limits were set on a digital temperature monitoring and control systems at 7.2°C (lower limit) and 26.7°C (upper limit). Missing data points are the result of an equipment malfunction that specifically affected temperature data logging, but not temperature regulation.

\[
\begin{array}{|c|c|ccc|ccc|}
\hline
\text{Week} & \text{Date} & \text{Night} & & \text{Day} & & \\
& & \text{Minimum} & \text{Maximum} & \text{Mean} & \text{Minimum} & \text{Maximum} & \text{Mean} \\
\hline
1 & February 19 & 13.3 & 22.8 & 17.0 & 17.2 & 25.0 & 23.0 \\
4 & March 11 & 11.1 & 17.8 & 12.6 & 11.7 & 19.4 & 17.3 \\
7 & April 1 & 11.1 & 19.4 & 13.4 & 11.1 & 20.6 & 18.8 \\
10 & April 22 & 12.8 & 24.4 & 15.6 & 12.8 & 25.6 & 22.7 \\
13 & May 19 & - & - & - & - & - & - \\
17 & June 13 & 18.3 & 22.8 & 20.4 & 17.8 & 26.1 & 23.4 \\
\hline
\end{array}
\]

Appendix L

Experiment A microcosm mean water temperatures (°C). Salt treatment key: SW = ambient streamwater (3mg L\(^{-1}\) Na); HCO3-16, -32, -64 = sodium bicarbonate (NaHCO\(_3\)) amended (16, 32, 64mg L\(^{-1}\) Na, respectively) ; CL-16, -32, -64 = sodium chloride (NaCl) amended (16, 32, 64mg L\(^{-1}\) Na, respectively)

\[
\begin{array}{|c|c|c|c|c|c|c|c|}
\hline
\text{Salt Treatment} & \text{Week 1} & \text{Week 4} & \text{Week 7} & \text{Week 10} & \text{Week 13} & \text{Week 17} & \text{Week 19} \\
\hline
SW & 22.14 & 19.12 & 22.6 & 22.5 & 15.1 & 29.4 & 28.2 \\
HCO3-16 & 22.99 & 19.09 & 23.4 & 23.8 & 15.9 & 29.6 & 28.2 \\
HCO3-32 & 22.44 & 18.76 & 23.4 & 23.3 & 15.9 & 29.6 & 28.6 \\
HCO3-64 & 22.76 & 18.85 & 23.2 & 23.4 & 15.9 & 29.6 & 28.6 \\
CL-16 & 23.25 & 18.6 & 21.2 & 23.5 & 15.3 & 29.1 & 27.9 \\
CL-32 & 22.6 & 18.72 & 21.2 & 23.4 & 15.4 & 29.2 & 28.2 \\
CL-64 & 22.74 & 18.83 & 21.1 & 23.3 & 15.4 & 28.9 & 27.2 \\
\hline
\end{array}
\]
### Appendix M

Experiment A response variable medians, means, and interquartile range (IQR) by salt treatment. Salt treatment key: SW = ambient streamwater (3mg L\(^{-1}\) Na); HCO3-16, -32, -64 = sodium bicarbonate (NaHCO\(_3\)) amended (16, 32, 64mg L\(^{-1}\) Na, respectively); CL-16, -32, -64 = sodium chloride (NaCl) amended (16, 32, 64mg L\(^{-1}\) Na, respectively). Chla = chlorophyll \(a\), a proxy for algal biomass. Ergo=ergosterol, a proxy for fungal biomass. Cocci=cocci-type bacterial biomass. Bacilli=bacilli-type bacterial biomass. Total Bact=total (cocci+bacilli) bacterial biomass. CR=community respiration (O\(_2\) consumption). Dry Mass=leaf litter detritus dry mass.

| Salt Treatment | Chla (mg mg\(^{-1}\) dry mass) Median | Mean | IQR | Ergo (µg mg\(^{-1}\) dry mass) Median | Mean | IQR | Cocci (mg carbon mg\(^{-1}\) dry mass) Median | Mean | IQR | Bacilli (mg carbon mg\(^{-1}\) dry mass) Median | Mean | IQR | Total Bact (mg O\(_2\) hr\(^{-1}\) mg\(^{-1}\) dry mass) Median | Mean | IQR | CR (mg O\(_2\) hr\(^{-1}\) mg\(^{-1}\) dry mass) Median | Mean | IQR | Dry Mass (% loss day\(^{-1}\)) Median | Mean | IQR |
|---------------|------------------------------------|------|-----|--------------------------------------|------|-----|--------------------------------------|------|-----|--------------------------------------|------|-----|--------------------------------------|------|-----|--------------------------------------|------|-----|--------------------------------------|------|-----|--------------------------------------|------|-----|
| SW            | 2.5e-04                            | 2.8e-04 | 2.1e-04 | 6.3374                             | 6.1643 | 2.5098 | 2.6e-06                             | 2.1e-06 | 2.1e-06 | 1.9e-06                             | 1.1e-06 | 1.7e-06 | 4.6e-06                             | 3.2e-06 | 4.2e-06 | -0.0052                             | -0.0055 | 0.0025 | 0.39                               | 0.39 | 0.05 |
| HCO3-16       | 1.9e-04                            | 1.8e-04 | 6.1e-05 | 6.7393                             | 6.6648 | 2.9701 | 7.6e-08                             | 5.8e-08 | 7.5e-08 | 5.0e-07                             | 4.5e-08 | 9.7e-07 | 1.0e-07                             | 9.7e-07 | 1.0e-07 | -0.0649                             | -0.0712 | 0.0114 | 0.37                               | 0.38 | 0.03 |
| HCO3-32       | 2.7e-04                            | 3.9e-04 | 2.2e-04 | 6.8914                             | 7.0024 | 2.0719 | 1.5e-07                             | 5.0e-07 | 7.6e-08 | 4.4e-08                             | 3.7e-07 | 1.1e-07 | 1.8e-07                             | 8.7e-07 | 1.9e-07 | -0.0375                             | -0.0352 | 0.0135 | 0.35                               | 0.34 | 0.10 |
| HCO3-64       | 2.1e-04                            | 1.5e-03 | 1.1e-04 | 5.5417                             | 5.6322 | 1.7856 | 3.2e-06                             | 4.4e-06 | 4.5e-06 | 1.2e-06                             | 1.2e-06 | 1.2e-06 | 6.1e-07                             | 5.6e-06 | 5.8e-06 | -0.0129                             | -0.0140 | 0.0053 | 0.40                               | 0.38 | 0.11 |
| CL-16         | 1.5e-04                            | 1.7e-04 | 6.3e-05 | 7.4060                             | 6.6089 | 5.4699 | 1.5e-07                             | 1.3e-07 | 2.2e-06 | 3.9e-08                             | 5.4e-07 | 9.1e-07 | 1.9e-07                             | 1.8e-06 | 3.1e-06 | -0.0017                             | -0.0019 | 0.0019 | 0.43                               | 0.41 | 0.09 |
| CL-32         | 1.6e-04                            | 2.2e-04 | 2.1e-04 | 7.3005                             | 6.8607 | 6.5960 | 3.0e-07                             | 6.7e-07 | 3.0e-07 | 1.4e-07                             | 5.2e-07 | 1.0e-07 | 4.2e-07                             | 1.1e-06 | 3.8e-07 | 0.0023                              | 0.0024 | 0.0018 | 0.34                               | 0.36 | 0.08 |
| CL-64         | 2.0e-04                            | 2.2e-04 | 1.1e-04 | 5.4034                             | 5.7347 | 1.2472 | 3.6e-06                             | 7.6e-06 | 1.3e-05 | 3.0e-06                             | 2.3e-06 | 3.7e-06 | 7.5e-06                             | 1.0e-05 | 1.6e-05 | 0.0060                              | 0.0067 | 0.0025 | 0.43                               | 0.42 | 0.05 |
Experiment B response variable medians and means by salt treatment. Salt treatment key: SW = ambient streamwater (3mg L$^{-1}$ Na); HCO3-16, -32, -64 = sodium bicarbonate (NaHCO$_3$) amended (16, 32, 64mg L$^{-1}$ Na, respectively); CL-16, -32, -64 = sodium chloride (NaCl) amended (16, 32, 64mg L$^{-1}$ Na, respectively).

Chla = chlorophyll a, a proxy for algal biomass; Ergo=ergosterol, a proxy for fungal biomass; CR=community respiration (O$_2$ consumption); NCR=net community respiration (O$_2$ consumption + O$_2$ evolution); GPP=gross primary production (NCR-CR).

<table>
<thead>
<tr>
<th>Salt Treatment</th>
<th>Chla (mg mg$^{-1}$ dry mass)</th>
<th>Ergo (μg mg$^{-1}$ dry mass)</th>
<th>CR (mg O$_2$ hr$^{-1}$ mg$^{-1}$ dry mass)</th>
<th>NCR (mg O$_2$ hr$^{-1}$ mg$^{-1}$ dry mass)</th>
<th>GPP (mg O$_2$ hr$^{-1}$ mg$^{-1}$ dry mass)</th>
<th>Dry Mass (% loss day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW</td>
<td>0.0017</td>
<td>0.0029</td>
<td>1.8191</td>
<td>1.6918</td>
<td>2.5049</td>
<td>0.0091</td>
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<tr>
<td>HCO3-16</td>
<td>0.0000</td>
<td>0.0012</td>
<td>6.3294</td>
<td>7.9807</td>
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<td>-0.0106</td>
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<td>HCO3-32</td>
<td>0.0000</td>
<td>0.0001</td>
<td>13.9338</td>
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<td>HCO3-64</td>
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Thesis Conclusions

Effects of Light, Nutrients, and Salts on Microbial Biofilm Productivity and Detrital Processing in Aquatic Mesocosms
Conclusions

At least half of detrital carbon (C) goes on to support secondary production (growth of primary consumers) in freshwater streams, thus detritus is the basis of aquatic food webs (Allan & Castillo, 2007). Several factors contribute to how quickly detritus can be decomposed by heterotrophic microbes, so it is important to identify factors influencing heterotrophic microbial productivity and detrital processing because changes in detrital quality and quantity through decomposition can have ecosystem effects.

The supply of C and nutrients in the water column can influence the rate of detrital processing by mediating microbial productivity and therefore activity (Suberkropp and Chauvet, 1995; Gulis et al., 2006; Rier et al., 2007; Suberkropp et al., 2010; Danger et al., 2013). Low, sub-lethal inputs of salt ions may subsidize or stress detrital biofilm microbes, resulting in changes in detrital quality and potentially altering detrital quantity. Increased decomposition rates may result in the depletion of detrital standing stocks too quickly and result in trophic cascading from the bottom up. Nutrient-rich microbes making up the biofilm on detritus create a nutritious food source for detritivorous primary consumers in streams (Cummins, 1973; Sterner & Elser, 2002; Benstead et al., 2009) and though this can mean increases in secondary production in the short term, some primary consumers may have physiological advantages over others which could lead to decreasing biodiversity in freshwater ecosystems due to interspecific competition in the long run (Sterner & Elser, 2002).

It is important to protect biofilm structure in order to preserve biofilm function. Research involving anthropogenic stream inputs help to inform regulatory policies that allow us to maintain healthy freshwater ecosystems. My work provides more evidence that human activities can indirectly influence freshwater ecosystems from the bottom up. Specifically, my research shows increased in light, nutrients, and salts of anthropogenic origin can alter biofilm productivity and detrital processing. The implications of these added resources are manifold and are of sizable importance considering anthropogenic activities increasing nutrients, light, and salts in
Freshwater streams are likely to continue into the foreseeable future in an effort to sustain our growing population's demands. (Carpenter et al, 1998; Smith et al, 1999; Smith, 1998; Vitousek et al, 2008).

References


