DO INHIBITORY INTERACTIONS BETWEEN DETRITIVORES INFLUENCE
LEAF BREAKDOWN?

A Thesis
by
MOIRA ANN HUTCHINGS

Submitted to the Graduate School
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

December 2014
Department of Biology

DO INHIBITORY INTERACTIONS BETWEEN DETRITIVORES INFLUENCE
LEAF BREAKDOWN?

A Thesis
by
MOIRA ANN HUTCHINGS
December 2014

APPROVED BY:

_____
Robert P. Creed, Ph.D.
Chairperson, Thesis Committee

_____
Michael M. Gangloff, Ph.D.
Member, Thesis Committee

_____
Michael D. Madritch, Ph.D.
Member, Thesis Committee

_____
Susan L. Edwards, Ph.D.
Chairperson, Department of Biology

_____
Max C. Poole, Ph.D.
Dean, Cratis Williams Graduate School

Abstract

DO INHIBITORY INTERACTIONS BETWEEN DETRITIVORES INFLUENCE
LEAF BREAKDOWN?

Moira Ann Hutchings
B.S., University of North Carolina at Asheville
M.S., Appalachian State University

Chairperson: Robert P. Creed

Recent research suggests that inhibitory interactions among detritivore

taxa may influence the effect of one or both taxa on detrital processing. Larvae of

the detritivorous stonefly *Tallaperla maria* were found to inhibit leaf consumption

by larvae of the cranefly *Tipula abdominalis* in a previous study. I conducted a

field experiment to determine whether *Tipula* consumption declined linearly with

increasing *Tallaperla* density or if there was a threshold density of *Tallaperla*

above which *Tipula* would cease to consume leaves. Two field experiments were

conducted in the winters of 2013 and 2014. In 2013, I added a single *Tipula* larva

to enclosures containing conditioned yellow birch leaves along with 0, 1, 2, 3, 4,

or 5 *Tallaperla* larvae. Enclosures with no insect larvae served as controls in

which only leaching and microbial breakdown occurred. The presence of *Tipula*

did not increase decomposition rates relative to microbial controls. Treatments of

Tallaperla in enclosures at 2, 4, and 5 densities resulted in a significant increase

in leaf breakdown relative to microbial controls. However, there were no differences among treatments containing insect larvae. In 2014, I added a single *Tipula* larva to enclosures containing 0, 1, 3, or 5 *Tallaperla*. Neither the presence of *Tipula* nor the addition of *Tallaperla* increased leaf breakdown rates relative to microbial controls. In 2013, there was evidence that leaf consumption by *Tipula* in the center of leaf packs declined in the presence of *Tallaperla*; evidence was less pronounced in 2014. In both years, leaf decomposition function was negatively correlated with increasing detritivore diversity. Leaf breakdown was not correlated with detritivore biomass in either year. My results indicate that *Tallaperla* may have inhibited *Tipula* leaf consumption.

Acknowledgements

I would like to thank the Department of Biology at Appalachian State University and my Thesis Committee, Dr. Michael Gangloff and Dr. Michael Madritch. To my advisor and Thesis Chair, Dr. Robert P. Creed, I give tremendous thanks and praise for helping me in all aspects of this project. I thank Michael Thomas, lab partner extraordinaire, for all his willing help on snowy and icy field work days. I could not have finished this without you. Thanks to Brett Davis and Connor Rice for assistance. Finally, I would like to thank my family: Ben, Brenna, Aidan, and Clara, for all their support and patience with me as I finished school while juggling work, home, and all your activities. We all made it.

Dedication

I would like to dedicate this work to my husband, Ben, and three children,

Brenna, Aidan, and Clara. You were there for me, and I did this all for you.

Table of Contents

List of Figures

Figure 8. Leaf mass lost (g) as a function of detritivore biomass (mg) for the Environmental Study Area stream in 2014.

Foreword

This Thesis was formatted according to guidelines for authors for the journal *Freshwater Science*, as per 2014 current updates.

INTRODUCTION

Many studies have examined the effects of biodiversity on the overall function or productivity of ecosystems (Tilman et al. 1996, McGrady-Steed et al. 1997, Jonsson and Malmqvist 2000, 2003, Jonsson et al. 2001, Wardle and Jonsson 2010). Most studies have reported positive influences of increasing species richness on ecosystem function (Naeem et al.1994, Tilman et al. 1996, Naeem and Li 1997, Jonsson and Malmqvist 2000, 2003, Cardinale et al. 2002, Paine 2002, Covich et al. 2004, Hooper et al. 2005). However, more recent work has shown that ecosystem function is not always influenced by species richness (Boyero et al. 2007, Creed et al. 2009, McKie et al. 2009, Rollins 2010).

Interference competition, in which one species directly alters resource attainment of another, as well as exploitative competition, in which species indirectly interact while using the same resources, can affect the function or productivity of a system (Lang and Benbow 2013). Interspecific competition may decrease ecosystem function if one organism is unable to effectively perform a function due to the behavior of another organism (Berendse 1979, Gulmon et al. 1983, Hooper 1998). An exception to this occurs when one taxon is both competitively and functionally dominant, in which case the dominant species performs at high enough levels that overall ecosystem function increases despite decreases in performance by the subordinate taxa (Creed et al. 2009, Rollins 2010). Inhibition of one subordinate species by another may also prevent increases in ecosystem function despite increases in species richness. This mechanism

has the opposite effect of mechanism such as complementarity or facilitation where increases in species richness can lead to increases in particular ecosystem functions. Such inhibition would result in low levels of function in a system, unless a functionally dominant species is present that is not affected by inhibition. In Southern Appalachian stream systems, such a competitively and functionally dominant detritivore is the caddisfly *Pycnopsyche gentilis* (Eggert and Wallace 2007, Creed et al. 2009, Rollins 2010, Tornwall 2011).

Lotic detritivores, including insect larvae, bacteria and fungi, and crustaceans, perform an important ecosystem function by converting course allochthonous detritus into forms readily usable by other species such as filter feeders and deposit feeders (Kaushik and Hynes 1969, Petersen and Cummins 1974, Wallace et al. 1982, Herbst 1982, Creed and Reed 2004). Fine particulate organic matter resulting from detritivore feeding afford increased surface area available for colonization by bacteria and fungi, which convert nitrates and nitrites not already leached from leaves into elemental nitrogen which then reenters the atmosphere, thus making detritivores important in nitrogen cycling (Skinner et al. 1999, Yee et al. 2007).

Three detritivores routinely dominate the biomass in Southern Appalachian headwater streams: *Pycnopsyche gentilis* (Trichoptera: Limnephilidae), *Tipula abdominalis* (Diptera: Tipulidae), and *Tallaperla maria* (Plecoptera: Peltoperlidae) (Stout et al. 1993, Eggert and Wallace 2007, Creed et al. 2009). These three taxa (hereafter referred to as *Pycnopsyche, Tipula,* and *Tallaperla*) may comprise about 95% of total detritivore biomass (Stout et al. 1993) and are also responsible for 54-

77% of annual detritivore production in headwater streams in the Southern Appalachians (Wallace et al. 1999).

Tallaperla and *Tipula* appear to be functionally subordinate to the caddisfly *Pycnopsyche gentilis* with respect to leaf breakdown (Eggert and Wallace 2007, Creed et al. 2009, Rollins 2010). Eggert and Wallace (2007) found that *P. gentilis* consumed leaves at a rate of 0.47g AFDM/ 1g insect AFDM/ day, which was significantly greater than *Tipula* (0.13g AFDM/ 1g insect AFDM/ day) and *Tallaperla* (0.09g AFDM/ 1g insect dry mass/ day). *Tipula* and *Tallaperla* actually consumed and assimilated greater quantities of epixylon (wood biofilms) than leaf matter when given the choice (Eggert and Wallace 2007). Rollins (2010) found no significant difference in leaf mass lost (LML) between treatments of *Pycnopsyche* alone and *Pycnopsyche* with either *Tipula* or *Tallaperla*. Further, there was no significant difference between LML in the *Tallaperla* alone treatment and the microbial controls which suggested that this species had little effect on leaf breakdown. Finally, Rollins'(2010) data suggested that *Tallaperla* may inhibit leaf consumption by *Tipula*. Rollins (2010) proposed two possible mechanisms that might explain this inhibition. First, Lieske and Zwick (2007) reported that the stonefly *Nemurella* preferentially consumed algal and microbial biofilms from stone surfaces over conditioned leaves. If *Tallaperla*, another stonefly, was removing microbial biofilms from leaf surfaces this could make these leaves less palatable to *Tipula* (Rollins 2010). Second, Rollins (2010) observed that *Tipula* is easily agitated and suggested that *Tipula* may abandon leaf packs in which *Tallaperla* is active or may not feed if unable to emigrate.

The goal of my research was to determine if *Tallaperla* does indeed inhibit leaf consumption by *Tipula* and at what densities the inhibition might occur. My null hypothesis was that the presence of *Tallaperla* would not inhibit the amount of leaf litter consumed by *Tipula*. Alternatively, *Tallaperla* may inhibit *Tipula*. I conducted enclosure experiments to determine if increasing *Tallaperla* abundance led to a linear decline in leaf consumption or to a step function response. A step function response would indicate that *Tipula* consumption is not affected by *Tallaperla* at low densities and that consumption would decrease considerably above a critical density.

METHODS

Study species

Larvae of the crane fly *Tipula abdominalis* are common detritivores in Southern Appalachian headwater streams (Stout et al. 1993, Wallace et al. 1999). Larvae typically measure 10-45 mm, depending on instar (McCafferty 1983). Tipulids are semi-voltine with most individuals spending the second year in the 4th larval instar stage (McCord et al. 2006). Only 4 larval instar stages are present. Larvae are reported to feed upon and live in leafy debris in streams and rivers (Cummins et al. 1973, Vannote and Sweeney 1985, Cook and Doran-Peterson 2010, but see Martin et al. 1980, Suberkropp 1992, Eggert and Wallace 2007). Martin et al. (1980) and Suberkropp (1992) suggested that *Tipula* larvae feed primarily on the fungal layer on the surface of conditioned leaves, based on the presence of gut bacteria specifically for fungal breakdown. Eggert and Wallace (2007) suggested that both *Tipula* and *Tallaperla* larvae have higher assimilation rates for wood biofilms (epixylon) despite the fact that they consume a larger quantity of leafy debris than woody debris.

Larvae of the stonefly *Tallaperla maria* are also common detritivores in Southern Appalachian headwater streams (Stout et al. 1993, Wallace et al. 1999). Larvae average 7-10 mm in length. This species is semi-voltine with an 18 month larval period following a 6 month egg diapause; diapause likely occurs to avoid hatching during summer when streams lack leaf material (O'Hop et al. 1984, Yokum et al. 1995). The

first instars appear in streams from November to January, with a total of 14 instars

occurring (Huryn and Wallace 1987).

Study sites

    Greene Creek site. -- Greene Creek, a headwater stream of the South Fork of the

New River, is located near the Blue Ridge Parkway north of Blowing Rock, in Watauga

County, North Carolina, USA. The site is divided into 3 areas. The first is a forested area

with a mix of birch, maple, oak, and hemlock trees, with the dominant riparian species

being Yellow Birch (*Betula alleghaniensis*) (Creed et al. 2009). The understory is

predominantly *Rhododendron maximum.* The stream flows out of the forested zone into a

transitional zone that has a canopy layer but contains less *Rhododendron* due to previous

cattle grazing damage (Creed et al. 2009). The third area flows through what was

formerly cattle pasture that is no longer grazed. Greene Creek averages about 8-9 cm in

depth with small pools up to 15 cm in depth. On average, the creek is 0.5-1.0 m wide.

The substrate is primarily bedrock, gravel, and cobble with some areas of sand and silt.

The section of creek used in the experiment was approximately 50 m in length and

located in the transitional zone.

    Environmental studies area site. -- I ran a second experiment in a small headwater

stream in the Environmental Study Area (ESA) on the campus of Appalachian State

University, Boone, Watauga County, North Carolina, USA from 24 January–21 March

2014. The unnamed stream flows through a forested area with a canopy of Yellow Birch,

Tulip Poplar, and oak with other hardwoods. Understory riparian zone vegetation is predominately *Rhododendron maximum* and mosses. The upper section of the stream flows into a small pond. The pond separates the upper and lower sections of the stream. The experimental reach was upstream of the pond and ~40 m long. Mean stream width in this section was 0.8-1.5 m wide and mean depth was 9-12 cm with some small pools averaging 18-25 cm deep. Substrate consisted mainly of cobbles, silt, and sand, with some smaller pebbles.

Tallaperla-Tipula inhibition experiment – Greene Creek

The effect of *Tallaperla* on *Tipula* was tested using in-stream enclosures. Enclosures were constructed from Rubber Maid ™ containers measuring 21 cm x 13.5 cm x 9 cm. The ends were removed and replaced with 243 μm screen sandwiched between 2 layers of 3 mm wire screen to prevent macroinvertebrates from entering the boxes. Boxes were held in place in the creek using two bricks attached with cable ties to either side.

Yellow Birch leaves were collected on site ~3 mo prior to the experiment. Leaves were picked from trees after the abscission layer formed but before they fell. Leaves were air dried in wire mesh boxes for 3 mo. Five gram (air dried mass) leaf packs were created and then soaked in deionized water for an hour to make them pliable. The leaf packs were constructed by securing the petiole ends of the leaves with binder clips. The leaf packs were attached to the upstream ends of the enclosures and conditioned in Greene Creek for 2 weeks, from 18 January–1 February 2013, to allow for microbial colonization.

Tallaperla and *Tipula* were collected from Greene Creek on 1 February 2013, the same day they were placed in the enclosures. Individuals of both taxa were sorted to create two groups of similar-sized individuals for use in the experiment. A single *Tipula* larva and 0, 1, 2, 3, 4, or 5 *Tallaperla* larvae were randomly assigned to the enclosures. Additional leaf packs were placed in boxes with no invertebrates added to determine the amount of leaf mass lost from leaching and microbial breakdown. The experimental design was a completely randomized design. The assignment of treatments to enclosures and treatment placement in the stream were also randomized. Five replicates of each treatment were used, for a total of 35 enclosures. The enclosures remained in the creek for 43 d from 1 February–15 March 2013. Daily water temperature was recorded using Onset HOBO logger temperature probes. Heavy rains during the week of March 1st led to flash-flooding in the creek, the result of which was a downstream displacement of the enclosures. Enclosures remained submerged during this period, but most were shifted from their original positions. They were replaced as close to their former positions as possible.

Upon termination of the experiment the leaf packs were carefully removed from the enclosures and placed into sealable plastic bags. Sediments from enclosures were also washed through a 240 µm screen to be sure that all *Tipula* and *Tallaperla* were recovered. A single *Tallaperla* was lost from two of the enclosures; box 16 (5 treatment) and box 22 (4 treatment). These two boxes were left with their original treatments for analysis since I did not know when the specimens were lost or died. In the lab, all sediment was rinsed from the leaves. Qualitative assessment was made of the amount of leaf damage that was present in each leaf pack. *Tipula* feed preferentially in the center of

leaf packs, thus variation in the amount of damage in the center of packs indicated changes in *Tipula* feeding rate. Leaves were then dried at 60° C for 4 d. Leaves were then weighed to calculate a dry mass. Leaves were combusted at 550° C for 3 hr to determine ash-free dry mass (AFDM). *Tipula* and *Tallaperla* recovered from the enclosures were preserved in 70% ethanol. Measurements of total body length were taken from all individual larvae used in the experiment after being preserved upon termination of the project. Mean length of *Tipula* was 30.78±3.4 mm. Mean length of *Tallaperla* was 5.98±2.8 mm. Dry mass of recovered insect larvae was estimated using length-mass formulas presented in Benke et al. (1999), allowing the determination of final, total detritivore biomass in each treatment.

Tallaperla-Tipula inhibition experiment – Environmental study area stream

The same enclosures were used in this experiment and were held in place in the creek via 2 bricks cable-tied to either side. Yellow Birch leaves used to construct leaf packs for the Environmental Study Area (ESA) site were also collected at Greene Creek approximately 3 mo prior to the experiment. Leaves were not collected in situ at ESA due to a necessary change in location from Greene Creek only days before the experiment was to be placed in the creek. Five gram (air dried mass) leaf packs were created and then soaked in deionized water for an hour to make them pliable. The leaf packs were constructed by securing the petiole ends of the leaves with binder clips.

The leaf packs were attached to the upstream ends of the enclosures and conditioned in

the ESA creek for two weeks, from 10-24 January 2014, to allow for microbial

colonization.

Tallaperla and *Tipula* were collected from the ESA creek both upstream and

downstream of the pond on 24 January 2014, the same day they were placed in the

enclosures. Specimens of both taxa were sorted to create 2 groups of similar-sized

individuals for use in the experiment. A single *Tipula* larva and 0, 1, 3, or 5 *Tallaperla*

larvae were randomly assigned to the enclosures. Additional leaf packs were placed in

enclosures with no invertebrates added to determine leaf mass lost due to leaching and

microbial breakdown. The experimental design was a completely randomized design. The

assignment of treatments to enclosures and treatment placement in the stream was also

randomized. Five replicates of each treatment were used, for a total of 25 enclosures.

The enclosures remained in the creek for 56 d from 24 January–21 March 2014. Daily

water temperature was recorded using Onset HOBO logger temperature probes.

Upon termination of the experiment the leaf packs were carefully removed from

the enclosures and placed into sealable plastic bags. Sediments from enclosures were also

washed through a 240 µm screen to be sure that all *Tipula* and *Tallaperla* were

recovered. A single *Tallaperla* was lost from 3 of the enclosures; box 1 (5 treatment), box

4 (5 treatment), and box 6 (3 treatment). These boxes were kept with their original

treatments for analysis since I did not know when the specimens were lost or died. In the

lab, all sediment was rinsed from the leaves. A similar qualitative assessment of *Tipula*

damage in the center of leaf packs as described above was made. Leaves were then dried

at 60° C for 4 d. Leaves were then weighed to calculate a dry mass. Leaves were

combusted at 550° C for 3 hr to determine ash-free dry mass (AFDM). *Tipula* and *Tallaperla* recovered from the enclosures were preserved in 70% ethanol. Measurements of total body length were taken from all individual larvae used in the experiment after being preserved upon termination of the project. Mean length of *Tipula* was 31.35±5.0 mm. Mean length of *Tallaperla* specimens used was 5.99±2.4 mm. Dry mass was calculated for preserved insect larvae using length-mass formulas presented in Benke et al. (1999) in order to determine final, total detritivore biomass for each treatment.

Statistical analyses

For both study years, Leaf Mass Lost (LML) data were analyzed using one-way analysis of variance (ANOVA). Tukey's Test was used to determine significant differences among treatment means. Linear regression analysis was performed to determine the relationship between treatment Shannon-Wiener diversity (H') and LML. A qualitative assessment was made to determine the amount of leaf damage indicative of *Tipula* feeding that was present in the middle of leaf packs with different densities of *Tallaperla.* Chi-Square Analysis was performed on the qualitative data. Regression analysis was performed to determine whether LML was driven by detritivore biomass.

RESULTS

Greene Creek site

From 1 February–15 March 2013 the mean water temperature at the Greene Creek site was 5.68° C with a maximum temperature of 8.80° C and a minimum of 1.83° C.

Mean leaf mass lost (LML) for the microbial control was 0.818 g (SE± 0.021). Mean LML for treatments 0, 1 and 3 did not significantly differ from LML observed in the microbial control (Fig. 1). Mean LML for treatments 2, 4 and 5 were significantly greater than that observed for the microbial control ($F_{6, 25}$=3.51, p=0.012)(Fig. 1). However, there was no significant difference between any of the treatments containing *Tallaperla* and *Tipula*. Qualitative examination of leaf packs for areas of greatest damage showed that *Tipula* consumption in the middle of leaf packs significantly declined with increasing numbers of *Tallaperla* (χ^2=4.5, 0.05<p<0.025)(Fig. 2). There was a negative relationship between Shannon-Wiener diversity and LML ($F_{1,22}$=6.42, p=0.039, r^2=0.680)(Fig. 3).

Total detritivore biomass increased with increasing *Tallaperla* number ($F_{1, 26}$= 0.14.005, p<0.001, r^2=0.35)(Fig. 4A). However, there was no relationship between LML and total detritivore biomass ($F_{1,27}$= 0.258, p= 0.616, r^2= 0.000)(Fig. 4B).

Environmental study area site

Water temperature at the ESA site from 24 January–21 March 2014 averaged 3.68° C, with a maximum of 9.60° C and a minimum of 0.02° C.

Mean leaf mass lost (LML) for the microbial control was 0.682 g (SE± 0.156). Mean LML for treatments containing insects did not differ significantly from the microbial control ($F_{4,25}$=0.55, p=0.698)(Fig. 5). Qualitative examination of leaf packs showed that there was no significant effect of *Tallaperla* density on *Tipula* consumption in the middle of leaf packs (χ^2=3.6, 0.10<p<0.05)(Fig. 6). There was a negative relationship between increasing Shannon-Weiner diversity and LML function ($F_{1,13}$=4.75, p=0.041, r^2=0.184)(Fig. 7).

A positive relationship was found between total detritivore biomass and treatment ($F_{1,18}$=5.27, p= 0.034, r^2=0.227)(Fig. 8A). However, there was no relationship between LML and total detritivore biomass at the end of the experiment ($F_{1,19}$ = 0.003, p = 0.960, r^2 = 0.000)(Fig. 8B).

DISCUSSION

Competition between species may affect the function or productivity of stream ecosystems (Lang and Benbow 2013). Interspecific competition can take the form of inhibition, in which one species indirectly impedes the function of another species. Rollins (2010) observed inhibition of *Tipula* by *Tallaperla*. In contrast, I did not observe any significant inhibition of *Tipula* mediated leaf decomposition (LML) by *Tallaperla* in my study. However, I did observe a behavioral change in the amount of leaf consumption by *Tipula* in the center of leaf packs in the Greene Creek experiment. Leaf packs containing *Tipula* alone did not differ significantly in leaf mass lost (LML) from microbial control. Leaf packs containing increasing numbers of *Tallaperla* did in some treatments exhibit significantly greater LML than microbial control, although the amount of LML was quite small. The lack of significant LML in detritivore packs relative to microbial packs confirms similar results from Rollins (2010) who found *Tallaperla* and *Tipula-Tallaperla* combinations play minor roles in leaf pack breakdown in Southern Appalachian streams.

Tipula feeding damage, which is characterized by feeding on the leaves in the middle of leaf packs, decreased with added *Tallaperla* in the Greene Creek experiment. In packs containing 3+ *Tallaperla*, *Tipula* was often recovered from the heart of the pack surrounded by wholly intact leaves. While this pattern could denote some amount of *Tipula* feeding inhibition, the LML data does not suggest less overall feeding by all of the detritivores. I suggest a possible reason for the lack of a decrease in LML even with less

Tipula damage; my personal observation during visual leaf analysis was that packs with 3 or more *Tallaperla* had more damage typical of *Tallaperla* feeding. The increase in *Tallaperla* feeding might have offset the lack of *Tipula* feeding in those packs with higher *Tallaperla* densities. Detritivore biomass in leaf packs containing 5 *Tallaperla* and 1 *Tipula* was about 67% higher than that in a single *Tipula* pack, and total detritivore biomass significantly increased with increasing *Tallaperla* density in the treatments. However, neither experiment showed a significant relationship between LML and overall detritivore biomass which supports the idea that declining leaf consumption by *Tipula* was compensated for by increased consumption by *Tallaperla*.

Previous researchers have suggested that *Tallaperla* plays an important role in leaf breakdown in Appalachian streams (Wallace et al. 1970, Hutchens and Wallace 2002, but see Eggert and Wallace 2007, Rollins 2010). Eggert and Wallace (2007) found that *Tipula* and *Tallaperla* consumed significantly less leaf mass than did *Pycnopsyche gentilis*, and that these two species consumed wood biofilms preferentially over leaves. Rollins (2010) and others (Cummins et al. 1973, Herbst 1982) found that *Tipula* monocultures had significant impact on LML compared with leaching and microbial controls, but I did not observe significant effects of *Tipula* on leaf breakdown. Regardless of year or site, I found no major effect of either detritivore on leaf pack breakdown. A re-evaluation of the relative importance of these detritivore species in headwater stream systems seems in order in light of my findings along with those of Eggert and Wallace (2007) and Rollins (2010).

Two previous mechanisms have been suggested as to why *Tipula* might be inhibited by *Tallaperla*. First, Rollins (2010) observed that *Tipula* larva are easily

agitated; thrashing and swimming away when disturbed or touched. I also observed this behavior when collecting *Tipula*. Second, some stoneflies scrape biofilms from leaf and stone surfaces, possibly making leaves less palatable to other detritivore species if they reduce the abundance of the microbial layer covering the leaves (Lieske and Zwick 2007). *Tallaperla* may remove biofilm from leaf surfaces, making them less palatable to *Tipula*. Consistent with this idea is the fact that LML data were not significantly different with increased *Tallaperla* densities which suggests *Tallaperla* is not feeding on the actual leaf tissue. Given that qualitative evidence suggested that *Tipula* feeding damage decreased with increased *Tallaperla* density, especially in the Greene Creek experiment, I feel that Rollins' theory regarding *Tipula* anti-predator behavior may better explain the low rates of *Tipula* feeding in these headwater stream systems. However, I cannot reject the second mechanism until there is a quantitative assessment of microbial biofilm abundance when exposed to various densities of *Tallaperla*.

I examined the relationship between the Shannon-Weiner diversity index and ecosystem function, as represented by leaf breakdown, because recent experiments have reported negative relationships between function and detritivore diversity (Boyero et al. 2007, Creed et al. 2009, McKie et al. 2009, Encalada et al. 2010, Rollins 2010). I also found negative relationships between detritivore species diversity and leaf breakdown in both experiments. My experiments were not traditional BEF experiments; rather they were an examination of inhibitory interactions between two detritivore taxa. However, as inhibition is an alternative mechanism to other frequently suggested explanations for positive BEF relationships (i.e., facilitation and complementarity), an evaluation of the relationship between diversity and function was relevant even if I was only examining the

effects of 2 taxa. Both sites show that with increasing *Tallaperla* density (and reduced detritivore diversity) there was a small increase in LML. This suggests that although *Tallaperla* may inhibit *Tipula* feeding, the increase in stonefly numbers and biomass may have offset the reduced leaf consumption by *Tipula*. The magnitude of this effect, however, was small which suggests that neither of these taxa are dominant detritivores in Southern Appalachian streams.

There was an interesting difference in the results between the 2 experiments. In 2013, some *Tipula-Tallaperla* treatments had significantly less leaf mass remaining than did the microbial controls. Further, 80% of leaf packs in the single *Tipula* treatment showed feeding damage in the center of the leaf pack that is typical of this species. In 2014, there were no significant differences in leaf mass remaining among any of the treatments. Further, in the single *Tipula* treatment, the number of leaf packs with obvious *Tipula* damage in the center dropped to 20%. Differences were also seen in the 3 and 5 *Tallaperla* treatments. Some of the differences between the 2 experiments over the 2 years were likely due to differences in water temperature. In Greene Creek, mean water temperature was $5.68°$ C. Examination of temperature data showed that during the first month of the experiment only 6 days had water temperatures below $4°$ C. However, in 2014, mean water temperature was $3.68°$ C, and during the first month 21 days had water temperatures below $4°$ C. Six days had water temperatures at or just above $0°$ C. This is unusually cold for these and other headwater streams (Argue et al. 2011). More snow and ice were observed around the enclosures during the 2014 experiment. Differences in temperature and weather patterns may have influenced detritivore feeding rates which could explain the lack of differences among treatments in 2014. Further, cooler water

temperatures may have altered *Tipula's* response to *Tallaperla* which may have caused the different results in the qualitative data between seasons.

Many researchers studying biodiversity and ecosystem function relationships over the past two decades have reported increased function with increased diversity (Naeem et al. 1994, Tilman et al. 1996, Naeem and Li 1997, Jonsson and Malmqvist 2000, 2003, Cardinale et al. 2002, Paine 2002, Covich et al. 2004, Hooper et al. 2005). Two mechanisms have been suggested to explain why function may increase with increasing diversity. Complementarity occurs when the function of one organism allows another organism to utilize a resource more efficiently (Fridley 2001). Complementarity is related to niche partitioning because organisms are using the same resource in different ways at the same time (Fridley 2001). Facilitative interactions, often called 'positive interactions', occur when one organism benefits from another with no harm coming to either participant (Bruno et al. 2003). Facilitation often involves one organism improving the local environment in a way favorable to another organism (Stachowicz 2001). Facilitation becomes a mutualism if both participants benefit from the interaction. The differences between complementarity and facilitation are very subtle. Researchers doing BEF studies often use the terms almost interchangeably; Flombaum et al. (2014) and Turnbull et al. (2013) suggest that the effects of these mechanisms are difficult to quantify and need to be further understood and developed before positive diversity results can be attributed to either interaction.

Notably lacking in discussions of the relationship between diversity and function are mechanisms that can produce negative relationships between diversity and function. Competitively dominant taxa can reduce diversity; their effect on function appears to be

the same regardless of whether the competitively dominant species is a functionally dominant species or not (Cardinale et al. 2002, Creed et al. 2009, Rollins 2010). *Pycnopsyche gentilis*, the dominant insect detritivore in Appalachian headwaters, may overwhelm litter diversity effects which have been important in other studies because litter diversity typically influences detritivore feeding efficacy (Swan 2011).

Interspecific competition has been well-studied for the last 50 years and competitive hierarchies have been described for species in a number of systems (Paine 1966, Lubchenco 1978, Gurevitch et al. 1990, Keddy et al. 2000, Segre et al. 2014). How are so many positive relationships between diversity and function being reported when competition is undoubtedly occurring among the species that are present in the system under study? As a type of competition, inhibition is poorly represented in the literature, perhaps because inhibitory interactions can be more subtle than are direct competitions (Hargrave et al. 2011). My research suggests that inhibition can lead to reduced function even with increasing detritivore diversity. Research in other systems examining possible inhibitory interactions is needed in order to determine if inhibition regularly impacts ecosystem function.

In conclusion, my results show mixed support for the hypothesis that *Tallaperla* inhibits feeding by *Tipula*. Given that LML did not differ across larval treatments, but behavioral changes in feeding patterns by *Tipula* were noted, it is difficult to state that inhibition definitively occurred. However, neither species appears to strongly infuence leaf breakdown, which confirms earlier work by Eggert and Wallace (2007) and Rollins (2010) in regards to low leaf mass breakdown attributable to *Tipula* and *Tallaperla* larvae. These species have long been considered functionally important taxa (Wallace et

al. 1970, Cummins et al. 1973, Herbst 1980, 1982, Hutchens and Wallace 2002), but recent work (e.g., Rollins 2010) and my study seem to indicate that this is not the case. Function may be more dependent upon detritivore biomass or species identity rather than diversity. Negative relationships were found between function and diversity in both study years. Future studies need to consider the impact of mechanisms such as inhibition and competition on ecosystem function in order to more accurately determine the importance of species diversity on ecosystem processes.

LITERATURE CITED

Argue, D. M., J. P. Pope, and F. Dieffenbach. 2011. Characterization of major-ion chemistry and nutrients in headwater streams along the Appalachian National Scenic Trail and within adjacent watersheds, Maine to Georgia. NPS Scientific Investigative Report 2011-5151.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the Southeastern United States. Journal of the North American Benthological Society 18:308-343.

Berendse, F. 1979. Competition between plant populations with different rooting depths: theoretical considerations. Oecologia 43:19-26.

Boyero, L., R. G. Pearson, and M. Bastian. 2007. How biological diversity influences ecosystem function: a test with a tropical stream detritivore guild. Ecological Research 22:551-558.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution 18:119-125.

Cardinale, B. J., and M. A. Palmer. 2002. Disturbance moderates biodiversity-ecosystem function relationships: experimental evidence from caddisflies in stream mesocosms. Ecology 83:1915-1927.

Cardinale, B. J., M. A. Palmer, and S. L. Collins. 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415:426-429.

Cook, D. M., and J. Doran-Peterson. 2010. Mining diversity of the natural biorefinery housed within *Tipula abdominalis* larvae for use in an industrial biorefinery for production of lignocellulose ethanol. Insect Science 17:303-312.

Covich, A. P., M. C. Austen, F. Barlocher, E. Chauvet, B. J. Cardinale, C. L. Biles, P. Inchausti, et al. 2004. The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. BioScience 54:767-775.

Creed, R. P., R. P Cherry, J. R. Pflaum, and C. J. Wood. 2009. Dominant species can produce a negative relationship between species diversity and ecosystem function. Oikos 118:723-732.

Creed, R. P., and J. M. Reed. 2004. Ecosystem engineering by crayfish in a headwater stream community. Journal of the North American Benthological Society 23:224-236.

Cummins, K. W., R. C. Petersen, F. U. Howard, J. C. Wuycheck, and C. S. Holt. 1973. The utilization of leaf litter by stream detritivores. Ecology 54:336-345.

Eggert, S. L., and J. B. Wallace. 2007. Wood biofilm as a food resource for stream detritivores. Limnological Oceanography 52:1239-1245.

Encalada, A. C., J. Calles, V. Ferreira, C. M. Canhoto, and M. A. S. Graca. 2010. Riparian land use and the relationship between the benthos and litter decomposition in tropical montane streams. Freshwater Biology 55:1719-1733.

Flombaum, P., O. E. Sala, and E. B. Rastetter. 2014. Interactions among resource partitioning, sampling effect, and facilitation on the biodiversity effect: a modeling approach. Oecologia 174:559-566.

Fridley, J. D. 2001. The influence of species diversity on ecosystem productivity: how, where, why? Oikos 93:514-526.

Gulman, S. L., N. R. Chiariello, H. A. Mooney, and C. C. Chen. 1983. Phenology and resource use in three co-occurring grassland annuals. Oecologia 58:301-310.

Gurevitch, J., P. Wilson, J. L. Stone, P. Teese, and R. J. Stoutenburgh. 1990. Competition among old-field perennials at different levels of soil fertility and available space. Journal of Ecology 78:727-744.

Hargrave, C. W., K. D. Hambright, and L. J. Weider. 2011. Variation in resource competition across a gradient of increasing intra- and interspecific richness. Ecology 92:1226-1235.

Herbst, G. N. 1980. Effects of burial on food value and consumption of leaf detritus by aquatic invertebrates in a lowland forest stream. Oikos 35:411-424.

Herbst, G. N. 1982. Effects of leaf type on the consumption rates of aquatic detritivores. Hydrobiologia 89:77-87.

Hooper, D. U. 1998. The role of complementarity and competition in ecosystem responses to variation in plant diversity. Ecology 79:704-719.

Hooper, D. U., F. S. Chapin, J. J. Ewell, A. Hector, P. Inchausti, and S. Lavorel. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75:3-35.

Hooper, D. U., and P. Vitousek. 1997. The effect of plant composition and diversity on ecosystem processes. Science 277:1302-1305.

Huryn, A. D., and J. B. Wallace. 1987. The exopterygote insect community of a mountain stream in North Carolina, USA: life histories, production, and functional structure. Aquatic Insects 9:229-251.

Hutchens, J. J., and J. B. Wallace. 2002. Ecosystem linkages between southern Appalachian headwater streams and their banks: leaf litter breakdown and invertebrate assemblages. Ecosystems 5:80-91.

Jonsson, M., and B. Malmqvist. 2000. Ecosystem process rate increases with animal species richness: evidence from leaf eating, aquatic insects. Oikos 89:519- 523.

Jonsson, M., and B. Malmqvist. 2003. Mechanisms behind positive diversity effects on ecosystem functioning: testing the facilitation and interference hypotheses. Oecologia 134:554-559.

Jonsson, M., B. Malmqvist, and P. Hoffsten. 2001. Leaf litter breakdown rates in boreal streams: does shredder species richness matter? Freshwater Biology 46:161-171.

Kaushik, N. K., and H. B. N. Hynes. 1969. Experimental study on the role of autumn-shed leaves in aquatic environments. Journal of Ecology 56:229-245.

Keddy, P., C. Gaudet, and L. H. Fraser. 2000. Effects of low and high nutrients on the competitive hierarchy of 26 shoreline plants. Journal of Ecology 88:413-423.

Lang, J. M., and M. E. Benbow. 2013. Species interactions and competition. Nature Education Knowledge 4:8.

Lieske, R., and P. Zwick. 2007. Food preference, growth and maturation of *Nemurella pictetii* (Plecoptera: Nemouridae). Freshwater Biology 52:1187-1197.

Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. American Naturalist 112:23-29.

Martin, M. M., J. S. Martin, J. J. Kukor, and R. W. Merritt. 1980. The digestion of protein and carbohydrate by the stream detritivore *Tipula abdominalis*. Oecologia 46:360-364.

McCafferty, W. P. 1983. Aquatic entomology: the fishermen's and ecologist's illustrated guide to insects and their relatives. Jones and Bartlett Publishers, Inc., Sudbury, Massachusetts.

McCord, S. B., A. D. Christian, and R. S. Grippo. 2006. Biomass dynamics of *Tipula* (Insecta:Diptera) in forested streams of the interior highlands, Arkansas. Journal of the Arkansas Academy of Science 60:74-79.

McGrady-Steed, J., P. M. Harris, and P. J. Morin. 1997. Biodiversity regulates ecosystem predictability. Nature 390:162-165.

McKie, B. G., M. Schindler, M. O. Gessner, and B. Malmqvist. 2009. Placing biodiversity and ecosystem functioning in context: environmental perturbations and the effects of species richness in a stream field experiment. Oecologia 160:757-770.

Naeem, S., L. J. Thompson, S. P. Lawler, and J. H. Lawton. 1994. Declining biodiversity can alter the performance of ecosystems. Nature 368:734-737.

Naeem, S. and S. Li. 1997. Biodiversity enhances ecosystem reliability. Nature 390:507-509.

O'Hop, J., J. B. Wallace, and J. D. Haefner. 1984. Production of a stream shredder, *Peltoperla maria* (Plectoptera: Petroperlidae) in disturbed and undisturbed hardwood catchments. Freshwater Biology 14:13-21.

Paine, R.T . 1966. Food web complexity and species diversity. American Naturalist 100:65-67.

Paine, R. T. 2002. Trophic control of production in a rocky intertidal community. Science 296:736-739.

Petersen, R. C. and K. W. Cummins. 1974. Leaf processing in a woodland stream. Freshwater Biology 4:343-368.

Rollins, M. 2010. MS Thesis, Appalachian State University. Pp. 51.

Segre, H., R. Ron, N. De Malach, Z. Henkin, M. Mendel, and R. Kadmon. 2014. Competitive exclusion, beta diversity, and deterministic vs stochastic drivers of community assembly. Ecology Letters Early View, doi: 10.1111/ele.12343.

Skinner, B. J., S. C. Porter, and D. B. Botkin. 1999. The blue planet: an introduction to earth system science. 2nd edition. John Wiley and Sons, New York, New York.

Stachowicz, J. J. 2001. Mutualism, facilitation, and the structure of ecological communities. Bioscience 51:235-246.

Stout, B. M., III, E. F. Benfield, and J. B. Webster. 1993. Effects of a forest disturbance on shredder production in southern Appalachian headwater streams. Freshwater Biology 29:59-69.

Suberkropp, K. 1992. Interactions with invertebrates. *in* The Ecology of Aquatic Hyphomycetes. Barlocher, F. Ecological Studies 94:118-134.

Swan, C. M. 2011. Consumer presence and resource diversity independently induce

    stability of ecosystem function in a piedmont stream. Ecosphere 2: art136.

    http://dx.doi.org/10.1890/ES11-00274.1

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by

    biodiversity in grassland ecosystems. Nature 379:718-720.

Tornwall, B. M. 2011. Effects of a lowhead dam on a dominant detritivore and detrital

    processing in a headwater stream. MS Thesis. University of North Carolina at

    Greensboro. Pp. 49.

Turnbull, L. A., J. M. Levine, M. Loreau, and A. Hector. 2013. Coexistence, niches, and

    biodiversity effects on ecosystem functioning. Ecology Letters 16:116-127.

Vannote, R. L., and B. W. Sweeney. 1985. Larval feeding and growth rate of the stream

    cranefly *Tipula abdominalis* in gradients of temperature and nutrition.

    Proceedings of the Academy of Natural Sciences of Philadelphia 137:119-128.

Wallace, J. B., J. R. Webster, and T. F. Cuffney. 1982. Stream detritus dynamics:

    regulation by invertebrate consumers. Oecologia 53:197-200.

Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1999. Effects of resource

    limitation on a detrital-based ecosystem. Ecological Monographs 69:409-442.

Wallace, J. B., W. R. Woodall, and F. F. Sherberger. 1970. Breakdown of leaves by

    feeding of *Peltoperla maria* nymphs (Plecoptera: Pletoperlidae). Annals of the

    Entomological Society of America 63:562-567.

Wardle, D. A., and M. Jonsson. 2010. Biodiversity effects in real ecosystems –a response

    to Duffy. Frontiers in Ecology and the Environment 8:10-11.

Yee, D., M. G. Kaufman, and S. A. Juliano. 2007. The significance of ratios of detritus types and microorganism productivity to competitive interactions between aquatic insect detritivores. Journal of Animal Ecology 76:1105-1115.

Yokum, K. A., T. R. Angradi, and D. C. Tarter. 1995. Ecology of *Peltoperla arcuta* and *Tallaperla maria* at the Fernow experimental forest, Tucker County, WV. Psyche 102:151-168.

FIGURE LEGENDS

Figure 1. Mean (+ 1 SE) leaf mass lost (g) as a function of treatment in the 2013 Greene Creek stream. All treatments except the microbial control (MC) contained a single *Tipula*. The numbers for the treatments represent the number of *Tallaperla* enclosed with the *Tipula*. Letters above bars represent results of Tukey's Test. Bars with the same letters are not significantly different.

Figure 2. Qualitative analysis of percent (mean ± 1 SE) of leaf packs with damage to the center of the pack made by *Tipula*, as a function of treatment in the Greene Creek stream in 2013.

Figure 3. Regression analysis of leaf mass lost as a function of the Shannon-Weiner diversity index. Data are from the Greene Creek stream in 2013. Numbers above the data points refer to the particular treatments.

Figure 4. Leaf mass lost (g) as a function of detritivore biomass (mg) for the Greene Creek stream in 2013. **A.** Total Detritivore Biomass vs Treatment. **B**. Leaf mass lost vs Total Detritivore Biomass

Figure 5. Leaf mass lost (g) as a function of treatment in the 2014 Environmental Study Area stream. All treatments except the microbial control (MC) contained a single *Tipula*. The numbers for the treatments represent the number of *Tallaperla* enclosed with the *Tipula*. Letters above bars represent results of Tukey's Test. Bars with the same letters are not significantly different.

Figure 6. Qualitative analysis of percent (mean ± 1 SE) of leaf packs with damage to the center of the pack made by *Tipula,* as a function of treatment in the Environmental Study Area stream in 2014.

Figure 7. Regression analysis of leaf mass lost as a function of the Shannon-Weiner diversity index. Data are from the Environmental Study Area stream in 2014. Numbers above the data points refer to the particular treatments.

Figure 8. Leaf mass lost (g) as a function of detritivore biomass (mg) for the Environmental Study Area stream in 2014. **A.** Total Detritivore Biomass vs Treatment. **B**. Leaf mass lost vs Total Detritivore Biomass
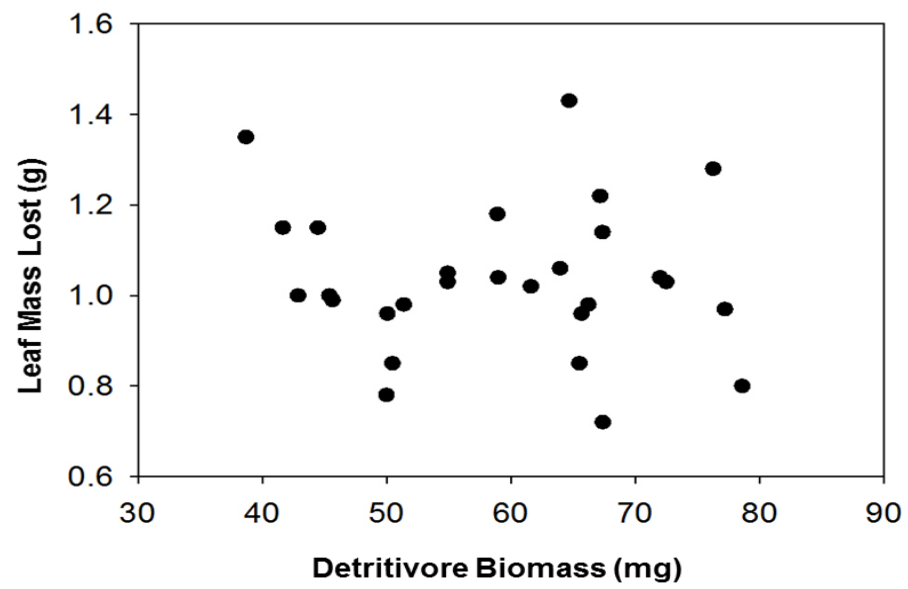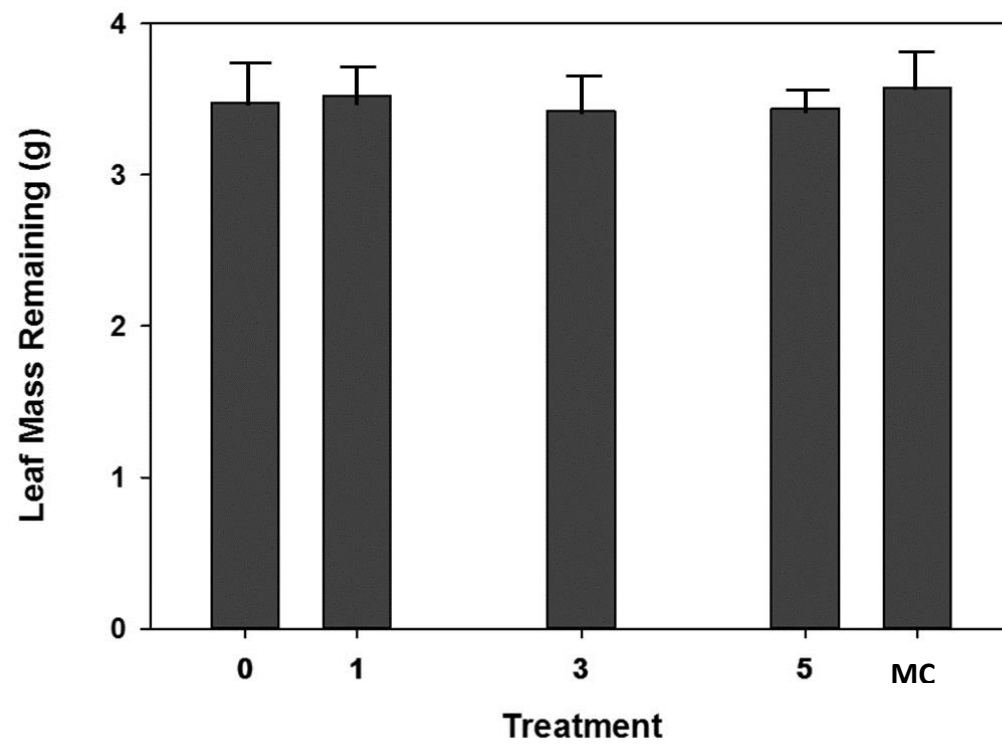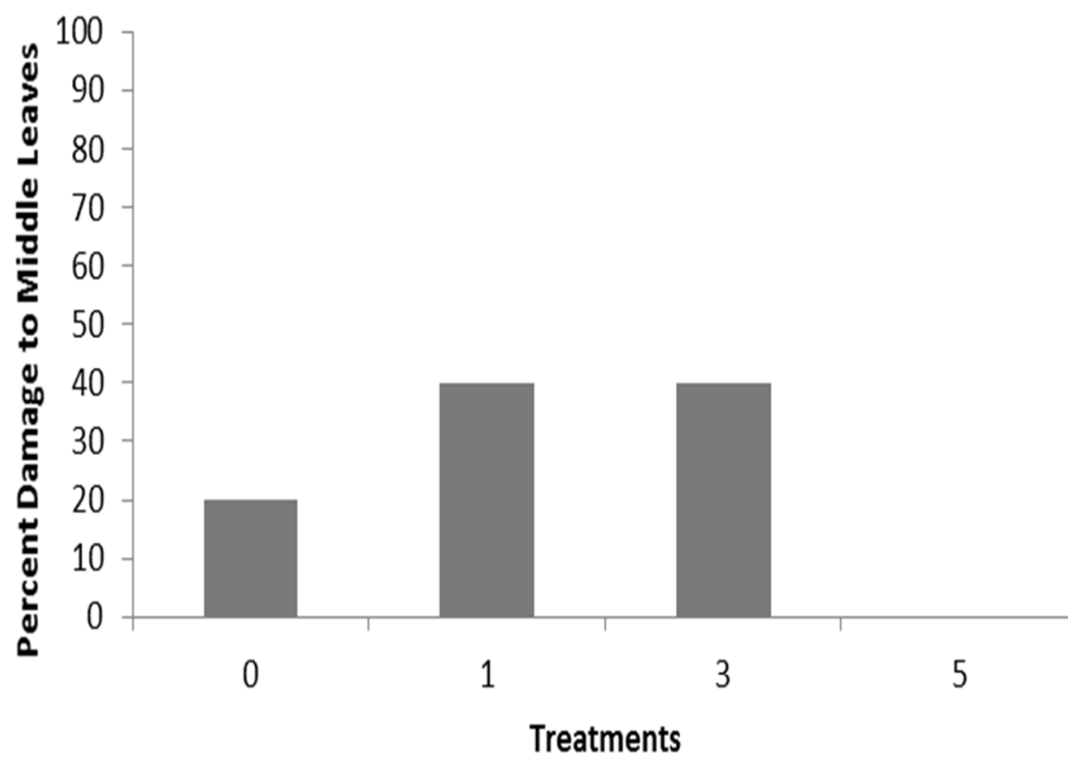
Figure 1.
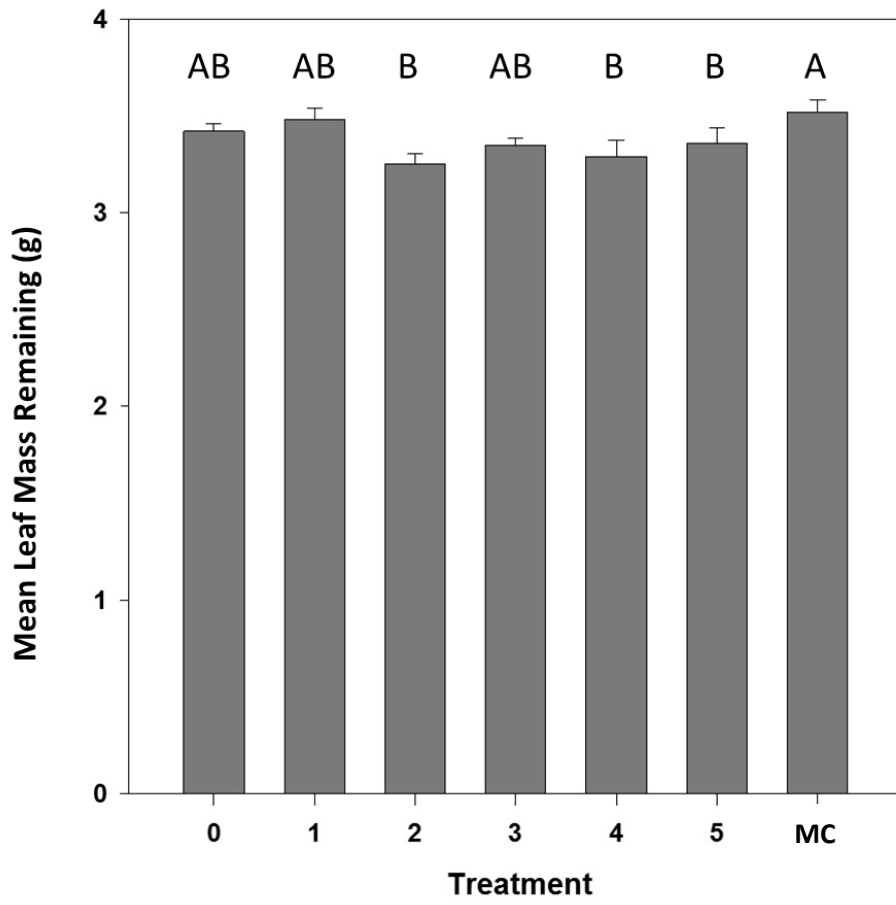
Figure 2.
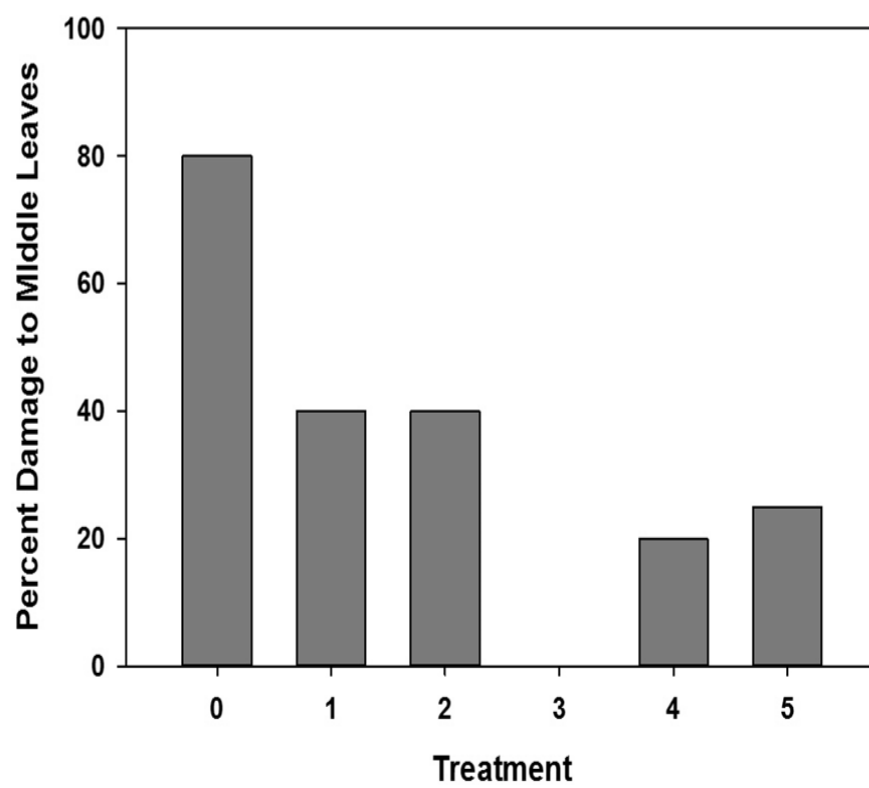
Figure 3.
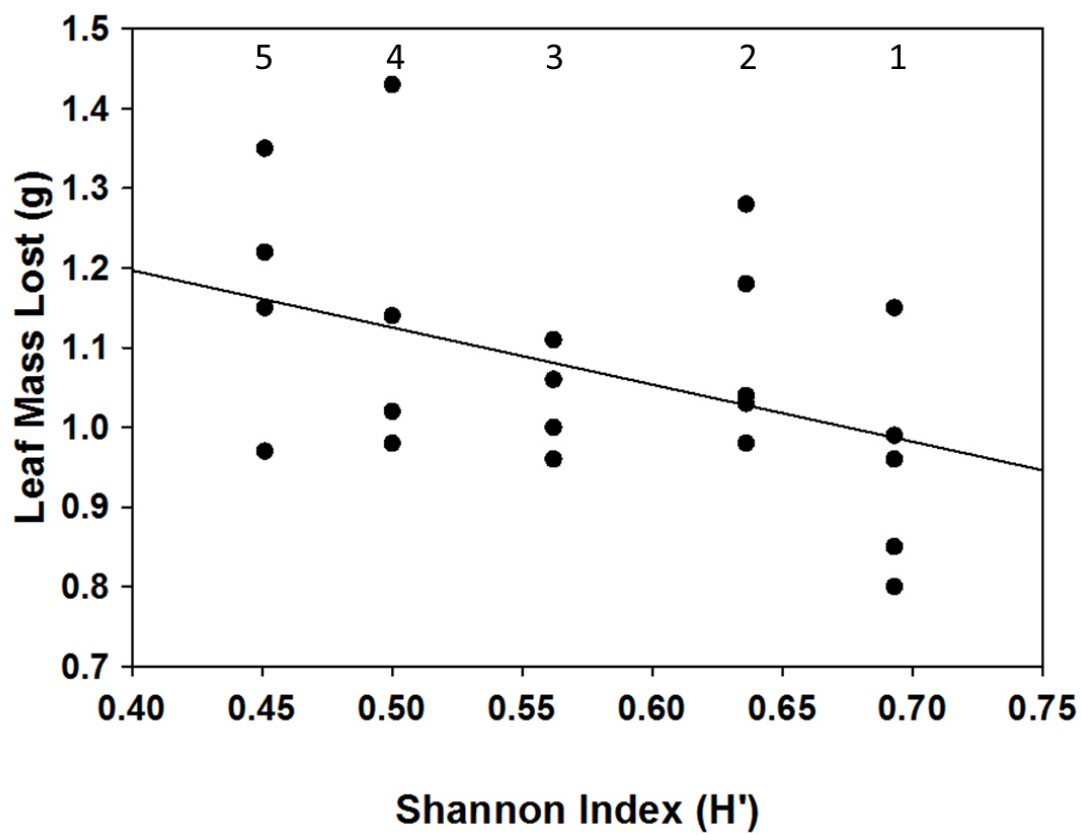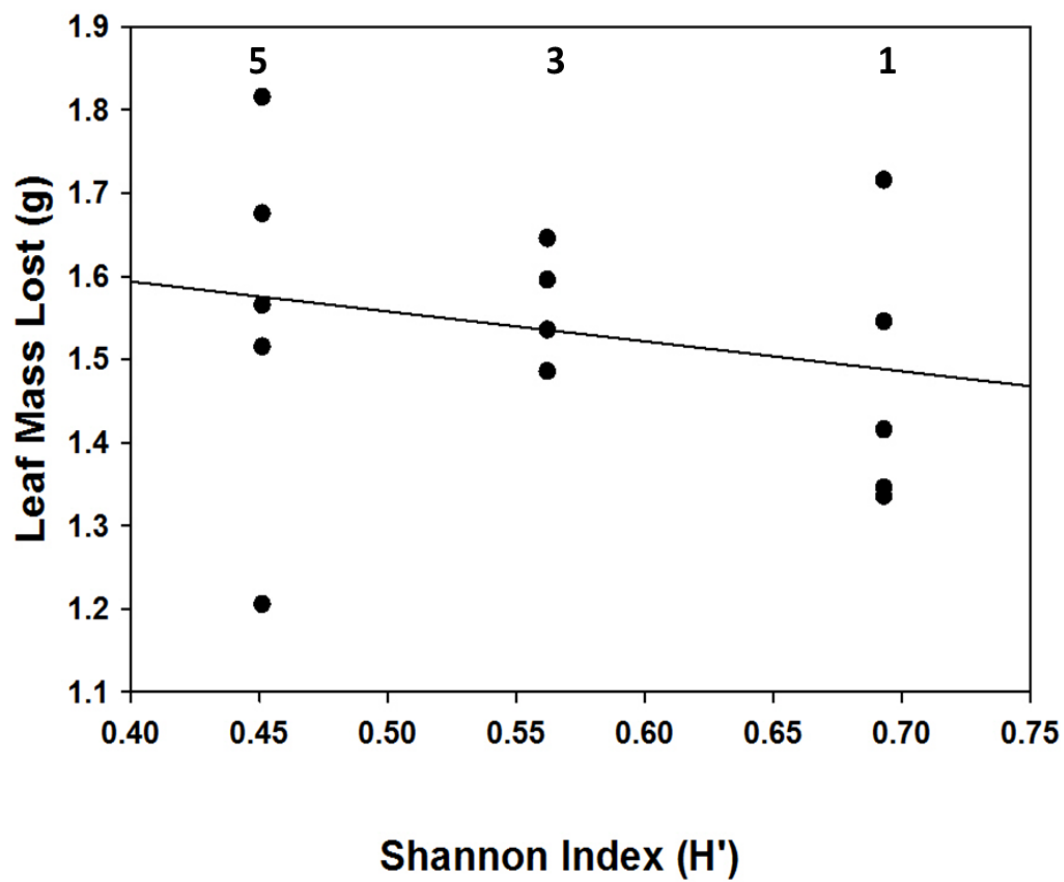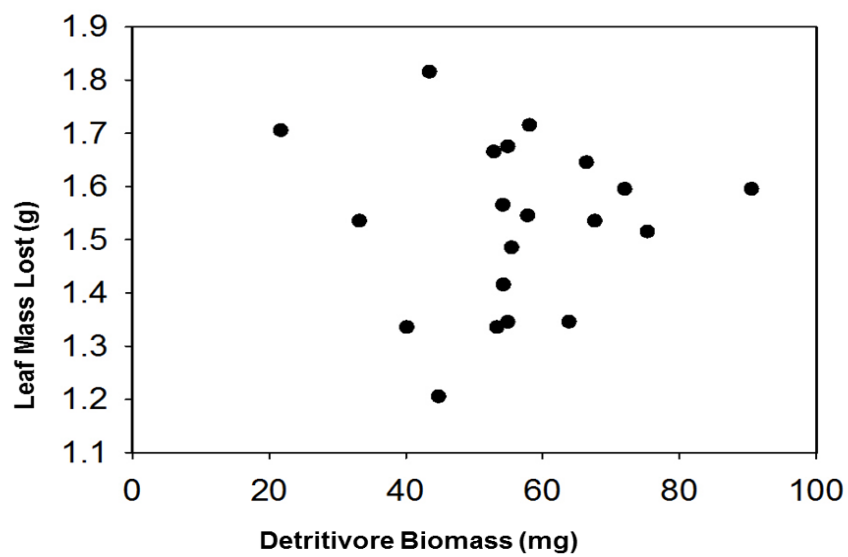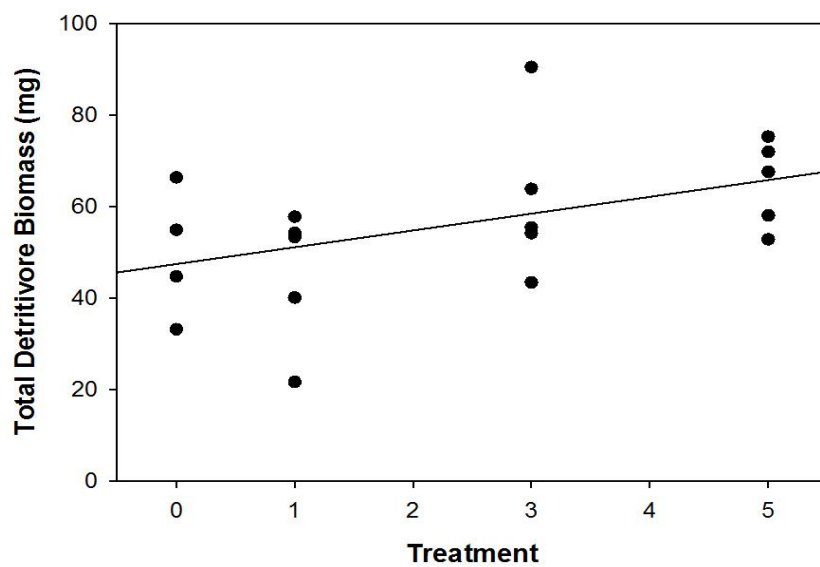
Figure 4.

A.
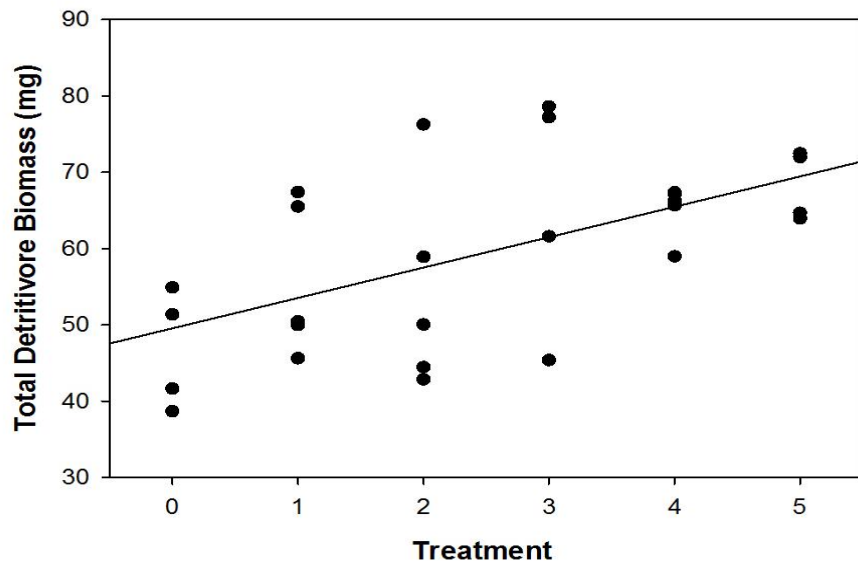
B.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

A.

B.

Vita

Moira Gougeon Hutchings was born in Michigan in 1978. She is the daughter of Rick and Teresa Gougeon. Moira graduated from the inaugural class of the International Baccalaureate Program at Independence High School in Charlotte, NC in 1997. In 2000, Moira married Benjamin Hutchings. They currently live in North Carolina with their three brilliant and wonderful children; Brenna Therese, Aidan Thomas, and Clara Eileen. Moira went to culinary school before turning her interests to the Biological Sciences. She received her B.S. in Biology, Magna Cum Laude, with departmental honors from the University of North Carolina at Asheville in 2007. She received her M.S. in Biology from Appalachian State University in December 2014.