AFTER THE FALL: HOW CHANGES IN TEMPERATE FORESTS ALTER WETLAND COMMUNITIES

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The composition of species is continually shifting due to natural succession, disturbance, and human influences. Predicting effects of these changes requires understanding species interactions, phenotypic traits responsible these interaction, and general relationships between diversity and ecological processes. In this thesis, I explore how changes in temperate forest tree composition alter processes within forest wetland ecosystems, the chemical traits of litter responsible for these effects, and general relationships between litter diversity and ecological processes.

Forest wetlands often receive massive amounts of tree leaf litter, and contain diverse food webs that recycle energy and nutrients within litter into myriad inorganic and organic forms. In the first study, I hypothesized that the abiotic and biological components of forest wetlands respond to changes in the input of tree leaf litter species. I provided different litter species to wetland communities in outdoor mesocosms, using ten common deciduous tree litter species. Effects were dramatic, including variation in the biomass, density, and survival of consumers. In this study, I also demonstrate that several traits of litter explain much of the variation in these effects. In the second study, I hypothesized that variation in litter inputs also induce phenotypic

changes in consumer development and morphology. Using wood frogs as a model species, I found that variation in litter species alters development rate and several morphological features, such as tail length, mouth size, and gut length. In the third study, I hypothesized that variation in litter inputs alters predator-prey interactions by changing the chemical and physical structure of wetland ecosystems. The results of this study suggest that interactions between litter resources and top-down interactions should be considered to accurately predict the consequences of shifting litter species composition. In the final study, I hypothesized that a general, positive relationship exists between litter chemical trait diversity and wetland consumer biomass. I found strong effects of trait diversity on decomposition rate, but no effects across a diverse array of consumer species. This suggests that wetland communities – although responsive to changes in single litter species chemistry – respond positively to increased litter species richness and may be resistant to fluctuations of litter chemical diversity.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

Superficially, graduate school is a timely string of classes, exams, papers, conferences, and seminars. Graduate students busy themselves with the day-to-day stresses of conducting successful research and pleasing their committee. However, such facets only exist to provide evidence of a journey fundamentally focused on adopting the qualities of an academic personality. Along this journey, we learn to listen and question; to accept while remaining skeptical; to realize we know nothing relative to all that can be known; and to heed advice whenever it is given. Some are gifted with these qualities from birth, but most must learn them through apprenticeship. This is the underlying nature of the bond between student and advisor. The feelings of frustration and annoyance often felt by graduate students have nothing to do with papers, failed experiments, or harsh criticism. Instead, these feelings are reflections of the mind's unwillingness to change, while external factors continue to push it along an accelerated path to intellectual maturity. Although this path has no end, the completion of a dissertation reflects the achievement of a mental state that will accompany a student for the rest of his or her life.

By these opinions, I do not mean to discredit or devalue the work of this text. The following chapters are my best effort to significantly advance our scientific knowledge and ecological understanding of this world. Many have assisted with experiments and contributed to their written description. Heated discussions with colleagues and advisors have contributed to every written word, and this dissertation could not be achieved without such input. However, I

personally value this assistance, contribution, and discussion as a means for self-reflection and self-improvement. For this reason, I consider my dissertation to reflect all the events which have transformed my personality over the past six years. Whether those events were small or large, short or long, inconsequential or of lasting importance, they still contributed to the person I am.

Hence, to thank all the people who have changed my life over the past six years is impossible, but I certainly want to express my gratitude towards those who have been with me all along. Of course, I need to thank my committee and particularly my advisor, Rick Relyea, who guided me along the path at every moment. Through morning coffee conversations, laughing over mud-coated waders after intense fieldwork, and evening chats on his front porch, I have learned more than I can possibly comprehend. His relentless effort to improve my academic prowess probably borders on the insane, given how much I pushed back over the years. Of course, he was aided by the Walt Carson, whose constant verbal battering over friendly drinks at the local bar have entirely transformed my perspective of academics and science. At the same time, Brian Traw's persistent smile was enough to clear up any fog of frustration while providing novel insight that shed perceptive light on any concept. Similarly, Michael Grabe provided cutting insight into any discussion, and always offered a unique perspective on ecological questions that generated thoughtful conversation an each meeting. Finally, Michael Vanni's academic dexterity in conducting research across multiple subfields of ecology have consistently guided and improved my approach to experimental design and interpretation of results.

I must also thank the postdocs and graduate students within my lab for their valuable assistance and conversations. In particular, I must thank Rickey Cothran, who has been a roommate, friend, advisor, and colleague. I have looked up to him from the first day we met and I feel truly privileged to learn and laugh with him. Similarly, John Hammond was always willing

to sit down for extended periods of time to discuss the details of any project or concept. I must also thank Jessica Hua, who has one of the most exuberant and enlightening personalities of any student whom I have had the privilege of working with. Her jovial outlook could probably cheer up a brick wall, and it was certainly appreciated on days when I felt like giving up. In addition, my conversations with Will Brogan have always provided insight into the values of applied ecology and have substantially altered the course of my conversations with other ecologists, conservationists, and non-academics. Devin Jones has been with me since my first day in Rick's lab, and has always provided the perfect mix of amusement and serious conversation for any day of work. Marnin Wolfe has been a true friend over the years, and we have had many engaging discussions that always seemed to result in mutual understanding and enlightenment. Other graduate students who have been extremely influential include Jason Hoverman, Josh Auld, RJ Bendis, Maya Groner, Heather Shaffery, Alison Hale, and Hao Ji. Their assistance in the field, advice, and conversations were always appreciated and I am truly grateful for my interactions with them.

In addition to the graduate students, a small army of undergraduates assisted with my research. Although I will not list every name (they are found in the acknowledgements section of each chapter), I would like to single out the assistance of Kate Henderson, Lindsay Skovira, Chris Hensley, and Erika Yates. Hours of Kate's life were spent over a microscope measuring preserved specimens, and I am not sure how the work would have been finished without her. When Lindsay assisted me with my work, I felt as though she assumed personal responsibility for the outcome of the study, which is more than anyone expects of any undergraduate. Chris served as an incredible field assistant who was generally more prepared than I was (and from that, one learns that the value of a researcher is not in intelligence, but in proper selection of

assistants). Erika assisted with several of my studies, and was extraordinarily patient with me, despite having to re-measure specimens on multiple occasions. Finally, I would like to extend my sincere gratitude to the many other undergraduate assistants that have helped me over the years, and I wish them the best of luck in their future endeavors.

In addition, I owe an enduring gratitude to my family, who has been there through every step. Undoubtedly, my work ethic comes from my father, who taught me the value of sweat and persistence. From him, I have learned that a patient attitude towards the resistant forces of life will always win in the end. No matter the time of day, my mother was always on the other end of the phone line to hear my complaints and rants. Our philosophical discussions served to calm me down, consider life in new ways, and provide life lessons that persistently reverberate through all of my actions. I saw a reflection of myself in my brother, who has grown up by my side on an alternative pathway to academic enlightenment and I am continually grateful for the opportunity to learn from him.

Finally, I dedicate this thesis to my grandfather, Melvin Aiken, who continues to be the most influential person in my life even after his death nearly a year ago. Early in his life, he was chemically blinded by the rage of a disgruntled customer. Despite a dozen eye surgeries, his sight was never restored and he remained blind for the rest of his life. During this time, he married my grandmother, had two amazing daughters, achieved wealth and success as a life insurance salesman, and has been nationally recognized for his achievements on numerous occasions. His intelligence and willpower were bewildering to the point of unexplainable, and his friendliness to others was staggering despite first-hand knowledge of the rage that exists in this world. If he could achieve such success in his lifetime with such a debilitating handicap as blindness, certainly I can obtain a measly doctorate degree. Of course, he would never describe

my work in such a manner. In fact, he always asked about my research, compelling me to find ways of describing my work without the use of scientific jargon. I have taken this skill, along with his relentless sense of humor, to academic and non-academic presentations, classrooms, and everyday interactions. His personality courses through my veins with every step I take, exists between every line of this dissertation, and will persist throughout my career and personal life.

From my grandfather, I have also gained an appreciation for human progress. Being blind for many years before our modern-day technological revolution, there was no way for him to envision the form of today's fashionable gadgetry. The concept of a laptop was already foreign to him; the idea of a smartphone must have been akin to walking down the yellow brick road in the Land of Oz. My brother and I often placed such gadgets in his hands, and watched him run his fingers over the smooth glass screens and miniaturized buttons. We would tell him of the latest innovations in nanotechnology and the cutting-edge science developing in the world. He often laughed at all of this, out of amazement and awe, and always wanted to learn more. To imagine the images of past and present floating in his mind necessarily impresses in one an appreciation for the rapid progress of human civilization. This is increasingly important, as the value of a scientist often rests in an ability to understand and integrate the past with the present. Because of him, I often shut my eyes travel backwards through the whirlwind of our modern progress, in an effort to see – and learn from – the origins of the storm.

To him, I raise a glass of beer and toast to a bright and brilliant future!

1.0 INTRODUCTION

In all ecosystems, the composition of species is continually shifting due to natural succession, disturbance, and human influence. A central goal of ecology is to predict the consequences of these shifts on the processes essential to ecological function (Hooper et al. 2005). Decomposition, which is the conversion of organic chemicals into inorganic resources necessary for plant growth, is one of the most vital processes (Gessner et al. 2010). Since this is primarily a metabolic process (Fierer et al. 2005), the rate of decomposition is largely determined by the chemistry of decomposing material (Wardle et al. 2006). Globally, primary production provides the largest source of decomposing material, and because litter chemistry differs among plant species (Webster and Benfield 1986), the plant species composition of a community strongly influences the decomposition process (Lecerf and Richardson 2009). This is particularly important to consider in temperate forests, where 70-90% of all vegetation senesces annually, and fuels the growth of diverse, multi-trophic communities (Facelli and Pickett 1991). These communities are vital to the decomposition process and comprise an important framework for energy and nutrient cycling (Moore et al. 2004). Despite this, we have a remarkably poor understanding of how litter chemistry – and changes in litter species composition – affects community structure and function.

This thesis explores how tree litter species composition in temperate forests can influence the communities arising from litter resources, specifically in forest wetlands. These systems are

crucial to the functioning of forests, as they receive and process enormous amounts of plant litter annually (Wetzel 2001, Williams 2005). In contrast to other environments in which litter is processed and broken down (e.g., soils, streams), energy and nutrient release in wetlands is relatively rapid and much of the released material from litter is retained within the system. These ecosystem characteristics allow for long and reticulated food webs with high biomass accumulation (Shurin et al. 2002). As such, these systems are metabolic hotspots within forests, outputting substantial amounts of atmospheric CO_2 and biomass to the surrounding forest (Williams et al. 2005, Mitsch and Gosselink 2007). Despite their ecological importance, forest wetlands remain remarkably understudied with regard to the effects of litter input on community-level processes.

In the second chapter, I hypothesize that the abiotic and biological components of forest wetland communities will respond to changes in leaf litter species inputs. I base specific predictions on prior knowledge of relationships between litter decomposition rate and the nutritional content, recalcitrance, and toxicity of the litter (e.g., Aerts 1997). In outdoor mesocosms, I provided diverse communities with inputs of 10 litter species in monoculture and mixture. Over the course of four months – a period comparable to the typical inundation period of temperate forest temporary wetlands – I assessed survival and biomass of microbes, algae, zooplankton, benthic arthropods, snails, and tadpoles. There were many dramatic effects of litter species composition, such as 80% mortality of American toad tadpoles (*Anaxyrus americanus*) in treatments with rapidly decomposing litter species. Many responses were correlated with components of litter chemistry, particularly soluble carbon which increased light attenuation, resulting in decreased dissolved oxygen and high consumer mortality. Importantly, many of the

litter species used in this study are the focus of current conservation efforts, and I discuss implications of results for forest management. This paper is co-authored by Rick Relyea and will be submitted to *Ecology Letters*.

Although this study only included measurements of consumer survival and biomass in response to litter inputs, chapter three considers alternative consumer responses. Possible responses of consumers to changing litter resources may consist of changes in survival, in addition to more subtle phenotypic changes that can potentially improve fitness under otherwise detrimental conditions. Previous work has shown that some tadpole species, particularly wood frogs (*Lithobates sylvaticus*), are able to alter phenotypes when faced with low per-capita resources or the threat of predation, and that these plastic changes are adaptive (Relyea 2002, Relyea and Auld 2004, 2005). In the third chapter, I hypothesize that tadpoles also exhibit phenotypic plasticity in response to changes in litter resource quality and per-capita litter availability. I reared wood frog tadpoles at two densities on one of six litter species that varied in nutrient content, toxicity, and recalcitrance. Once tadpoles reached the later stages of larval development, I measured several phenotypically plastic components of wood frog phenotypes: development rate, intestinal length, and several morphology features of the body, tail, and oral disc. Morphological changes often correlated with litter nutrient content, and responses to both lower tadpole density and increased litter nutrient content were often similar in direction and magnitude. However, for some morphological features, response to nutrients and density contrasted in direction and magnitude, which suggests a fundamental difference between the role of resource quality and quantity to wetland consumers. Rick Relyea is a co-author on this study, which is in press at *Ecology*.

The effect of resource quality is likely mediated by – and may mediate – both competitive and top-down interactions. Chapter four considers how litter inputs affect prey survival and performance when predators are present. In this chapter, I question how litter inputs that alter the physical and chemical complexity of the environment will change the outcome of predator-prey interactions. Specifically, I predicted that litter inputs which increase benthic surface area or decrease visibility of prey to predators (i.e. through litter leachates) will lead to reduced predation rates. By exposing gray tree frog tadpoles (*Hyla versicolor*) to newt predators that were either caged or free-swimming, I found that benthic surface area had little effect on tadpole growth or predation rates, and in contrast to predictions, I found that increasing litter leachates led to higher predation rates. Experimental results suggest that this effect was likely due to a concurrent reduction in tadpole growth caused by the leachates, which suggests an interaction between top-down and bottom-up forces on prey performance. Rick Relyea is a co-author on this study, which is in press at *Oecologia*.

Chapter five considers how bottom-up forces may interact with each other. Often, combinations of species of litter in mixture results are associated with non-additive rates of decomposition (Gartner and Cardon 2004, Lecerf and Richardson 2009, Gessner et al. 2010). However, the mechanisms underlying this phenomenon or the effects of litter mixing on other higher trophic levels, remains largely unknown. In this chapter, I question how mixing litter species in wetland mesocosms alters litter decomposition rate, as well as the composition and biomass of higher trophic levels. Although litter species cannot directly interact, several mechanisms have been hypothesized which point to ways that consumers may mediate, and subsequently be affected by litter mixing (Gessner et al. 2010). Exploring these mechanisms, I first hypothesize that the dissimilarity of litter chemistry provides more opportunities (niches) for

consumers of litter thus increasing overall resource use in the community (i.e. resource complementarity mechanism). I also explore whether average chemical traits of litter can predict these same responses (i.e. mass-ratio mechanism), or if responses may generally be predicted by the presence of individual litter species (i.e. selection effects mechanism). Exploring the effect of litter diversity on microbes, algae, zooplankton, snails, benthic detritivores, and amphibians, I found evidence in support of all three mechanisms. However, each mechanism affected a different component of the community. For example, litter chemical dissimilarity positively related to leaf litter decomposition rates, but had no effect on biomass of consumers. This study represents the first attempt to examine how the mixing of litter species in forests influences the structure and function of decomposer communities, and presents broader insight regarding the relationship between diversity and ecological function. This paper is co-authored by Rick Relyea and will be submitted to *Ecology*.

In the final chapter, I discuss the implications of this research for ecological theory and conservation biology, and suggest areas for further research. In particular, I discuss the larger role of wetlands in landscape-level ecosystem functioning and suggest how further work at the ecosystem level may explore this role in detail. Moreover, since the work contained in this thesis directly ties with the larger questions of global biodiversity loss, I suggest studies that may link this phenomenon with ongoing and predicted changes in the global environment.

2.0 RELATING LEAF LITTER SPECIES, DIVERSITY, AND CHEMISTRY TO PRODUCTION IN FOREST PONDS

2.1 INTRODUCTION

A central goal in ecology is to elucidate the effects of individual species on ecological function and to predict the consequences of species composition shifts on community and ecological function. This is often a daunting challenge, given the complexity of interactions that can exist within a single ecosystem (Simberloff 2004). Primary producers are frequently at the center of this complexity, providing live tissue for herbivores and their predators (Price et al. 1980) as well as dead organic material (i.e. litter) for a variety of consumers (Facelli and Pickett 1991, Moore et al. 2004). It is well-known that individual plant species, through interspecific differences in plant chemistry, physiology, and life history can have unique effects on rates of herbivory and decomposition, and subsequently on rates of nutrient cycling (Webster and Benfield 1986, Facelli and Pickett 1991, Scott and Binkley 1997, Cadotte et al. 2009). However, far less is known regarding how such differences among plant species specifically influence the structure and function of the biological communities underlying these processes, particularly among litter-based communities (Moore et al. 2004).

Understanding this connection is important for several reasons. Most importantly, the metabolism, behavior and life history of organisms that are involved in the decomposition

process determine the chemical and physical pathways of resources in any environment (Wardle et al. 2004). For example, if a community is dominated entirely by microbial organisms, this may translate into relatively slow releases of carbon and nutrients, whereas the presence of microbial grazers can promote microbial growth and activity (Cuffney et al. 1990). Mobile grazers may also transport energy and nutrients away from the place of litterfall (Polis et al. 1997). This can be quite extensive for some species, particularly emergent aquatic organisms such as mosquito larvae, midges, and tadpoles, which can move great distances away from their larval home (Beard et al. 2002, Dreyer et al. 2011). Hence, the composition of litter-based food webs can have important feedbacks to primary production by determining the pathway that energy and nutrients are recycled. It is also important to recognize that many species involved in the decomposition process are the focus of conservation concern (e.g., amphibians; Ficetola et al. 2011) and disease control (e.g., snails, mosquitoes). Hence, understanding how changes in plant composition influences litter-based communities can provide great insight into the role of plants in the movement of energy and nutrients through an ecosystem, and offer significant contributions to the field of conservation ecology.

Decomposition of litterfall and the activity of organisms in litter-based communities are critical features of ecological function in temperate forests. In these systems, up to 90% of all plant material eventually senesces, often in a single seasonal pulse, and is processed by complex food webs in both aquatic and terrestrial systems (Facelli and Pickett 1991, Wallace et al. 1997). After falling, litter is immediately colonized by bacteria and fungi that nutritionally enrich the litter. Fragments of litter and colonies of microbes are subsequently consumed by grazers and their predators. Through respiration, excretion, and egestion, energy and nutrients are released from the litter as inorganic compounds that are readily absorbed by primary producers (Gessner

et al. 1999). The rate of this process is generally accelerated in freshwater environments (e.g., streams, ponds) where physical abrasion and leaching from water hasten the decomposition process, leading to relatively high biological activity and longer food webs (Kaushik and Hynes 1971, Wallace et al. 1997, Wetzel 2001, Lecerf et al. 2007). A multitude of factors, including hydroperiod, canopy cover, and resource quantity and quality are thought to structure communities in these environments (Vannote et al. 1980).

Understanding the effects of litter on temperate forest aquatic communities and predicting the response of these communities to the chemistry of individual litter species has proven challenging. Although it is generally true that biomass production on or around nutrient-rich and labile litter species is greater than production around nutrient-poor litter (Yanoviak 1999, Motomori et al. 2001, Swan and Palmer 2006), single litter species consist of both beneficial and harmful chemical compounds that can influence biotic growth and fitness in contrasting ways (Wardle et al. 1997, Epps et al. 2007). In particular, acidic and structural compounds (e.g., phenolics and lignins, respectively) often remain in the litter after senescence and can inhibit microbial growth and activity, regardless of nutrient content (Webster and Benfield 1986, Hoorens et al. 2002, Ardón and Pringle 2008). On the other hand, litter with high amounts of structural compounds may also provide rigid and persistent substrate for microbial growth in addition to consumer refugia (Dudgeon and Wu 1999). Such effects of litter are further complicated by non-additive effects within litter mixtures that remain poorly understood (Kominoski et al. 2007, Gessner et al. 2010), but are likely generated by intricate interactions between decomposer fauna and litter chemistry.

Moreover, our understanding regarding the effects of litter inputs on aquatic communities is hindered by a severe bias of research towards lotic (i.e. flowing) habitats (e.g., streams, rivers),

whereas lentic (i.e. non-flowing) habitats, such as shallow ponds have received little attention. This is surprising, as these systems are common features of most forests and are centers of high biological activity (Wetzel 2001, Williams 2005). The effects of litter in these systems may be unique, as low outflow and high litter retention is coupled with high rates of leaching (Hodkinson 1975). Such retention can elevate available nutrients and promote primary and secondary production (Briand and Cohen 1989). However, retention of secondary compounds (e.g., phenolic acids) can reduce consumer survival and growth (Horne and Dunson 1995, Maerz et al. 2005), and potentially shade the benthos to the extent it constrains *in situ* primary production and reduces herbivore growth (Wetzel 2001). Also unlike streams, the effects of litter on biological communities can persist much longer in lentic systems as a result of longer litter retention. Whereas stream ecosystems experience a rapid surge of production following pulses of litter inputs (Wallace et al. 1997, Yang et al. 2010), lentic environments typically experience consumer biomass production over an extended period of time due to multiple breeding cycles. During this period, litter and water chemistry can change substantially, leaving late-breeding organisms with drastically different resource chemistry than their early-breeding counterparts (Fegraus and Marsh 2000). In addition, degradation, consumption, and dilution of soluble leachates will increase over time (Williamson et al. 1999, Wetzel 2001), likely resulting in a greater effect of more recalcitrant chemical factors (e.g., initial C:N, lignin).

The purpose of this study was to elucidate the variation of effects arising from inputs of natural, chemically distinct litter species on temperate forest pond communities. Recent work indicates that many common pond-breeding organisms are strongly impacted by variation in leaf litter chemistry (Maerz et al. 2005, Williams et al. 2008, Reiskind et al. 2009, Stoler and Relyea 2011, Cohen et al. 2012). However, these studies have only examined the effects of litter species

on individual consumers, while the holistic effects of litter on realistic pond communities has not been investigated. Moreover, past studies have also only examined the effects of litter over short experimental durations, leaving us with little information regarding how the effects of litter change throughout a growing season.

To determine the effects of leaf litter on aquatic communities, one can either take a phylogeny- or trait-based approach. The phylogeny-based approach assumes that taxonomically related species share similar chemical compounds and consequently have similar functional effects (Cadotte et al. 2009, Chiarucci et al. 2011). Although closely related tree species often have similar leaf chemistry, chemistry can differ among populations and among years, which results in significantly different decomposition rates (Aerts and de Caluwe 1997). This makes it difficult to generalize the effects of leaf litter on communities from a taxonomic perspective (McGill et al. 2006). Additionally, subtle differences in chemistry between related species can result in disproportionate changes in community responses (Stoler and Relyea 2011). In contrast, the trait-based approach has the advantage of providing better generality by positing that ecological function is quantitatively linked to the traits of the litter (McGill et al. 2006). Over the past decade, trait-based approaches have been widely used for linking the traits of plant species with primary production in old fields (McGill et al. 2006), and have more recently been used to link the chemical traits of plant litter to the process of decomposition (Meier and Bowman 2008).

Using outdoor mesocosms, we manipulated leaf litter in communities that representative of natural forest ponds. We generated 12 treatments, including a no-litter treatment, 10 monoculture treatments of litter from different tree species that varied in soluble carbon, the carbon to nitrogen ratio (C:N), lignin content, and phenolic content, and a substitutive mixed-litter treatment to investigate possible interactions among leaf litter species. We made several

predictions: 1) the presence of leaf litter, regardless of species, will elevate resource supply and subsequently increase the biomass and survivorship of community members, 2) as a consequence of species-specific leaf chemistry, individual litter species will have significantly different impacts on food web responses 3) as is frequently observed in streams, litter mixtures will have non-additive effects on pond community responses, 4) food web responses will be negatively impacted by soluble carbon, phenolics, lignin, and C:N, but positively influence by the overall decomposition rate of litter, and 5) the effects of more soluble chemical components will reduce over time, resulting in a stronger relationship of C:N and lignin with later responses.

2.2 METHODS

2.2.1 Experimental Design

The experiment was conducted at the University of Pittsburgh's Pymatuning Laboratory of Ecology in northwest Pennsylvania. We used a completely randomized design with 12 treatments, each replicated 4 times for a total of 48 experimental units. Based on prior experiments (Stoler and Relyea 2011), this level of replication was expected to provide sufficient power to differentiate responses among treatments. The 12 treatments consisted of 10 litter monocultures, a control treatment containing no leaves, and a substitutive mixture of all 10 litter species to test the existence of non-additive litter mixing effects (Table 2.1). For each litter species, we assessed four components of litter chemistry: soluble carbon, lignin, total phenolics, the ratio of carbon to nitrogen (C:N), and decomposition rate. Details of the methods used to assess litter chemical traits are available in Appendix A.

The experimental units were 800-L, black, polyethylene, cylindrical tanks that served as pond mesocosms. Each mesocosm was covered with a 60% shade cloth lid that prevented escape or entry of organisms and simulated moderate canopy cover (Schiesari 2006) while providing sufficient light for algal growth. On 16 April 2008, we added approximately 20 L of homogenized loamy soil to each mesocosm, which was then fully dried in the sun to desiccate and kill any soil macroinvertebrates. We then filled the mesocosms with well water between 18 and 21 April and allowed the soil to settle for 3 d. On 25 April, we collected 20-L buckets of water from five ephemeral forest ponds as sources of microbes and algae. From these same ponds, we collected zooplankton using a 250-μm zooplankton which was sufficiently small to collect many of the large-bodied zooplankton common of forest ponds. As is common to mesocosm experiments of this nature (e.g. Relyea 2005), we removed all predators to eliminate top-down pressure on zooplankton, mixed all zooplankton and water from the five ponds, and introduced equal aliquots of the slurry to all mesocosms.

We added leaf litter to the mesocosms on 27 and 28 April. Therefore, 28 April was defined as day 1 of the experiment. We collected the litter from forests in western PA within 1 wk after senescence during autumn 2007. We air dried litter for 1 wk after collection and stored it in a dry area through the winter. Previous work demonstrates that litter in the benthos of ponds does not significantly decay during the winter (A. Stoler, *unpublished data*). Although leached chemicals can photo-degrade or biologically decompose before the spring thaw, temperate forest ponds are typically covered in ice during the winter and metabolism in this environment substantially slows. Hence, it is likely that chemicals are retained in the water through the winter, so our use of non-decomposed autumn-shed litter is not likely to detract significantly from the realism of our experiment.

We added a total of 250 g of leaf litter to each mesocosm: 235 g of loose leaf litter and five mesh bags that each contained 3 g of litter (mesh size = 5 mm). Mesocosms assigned to the mixture treatment received equal proportions of all litter species, both as loose litter and in the mesh bags. The no-leaf treatment received empty mesh bags. Next, we added 25 g of rabbit chow to each mesocosm as an initial nutrient source. This pulse of nutrients, which is common of large mesocosm experiments (Morin 1983), contains phosphorus, nitrogen, and micronutrients. On day 11, we placed four ceramic tiles, oriented vertically on top of the litter and soil on the north side of the mesocosms, to serve as periphyton samplers.

We introduced several species of macroinvertebrates and anuran larvae into all mesocosms, including some of the most common consumers in our region. Between days 11 and 16, we added two species of benthic detritivores: isopods (*Asellus communis*) and amphipods (*Crangonyx psuedogracilis*). Using adults dipnetted from a local forest pond, we introduced 26 amphipods and 40 isopods to each mesocosm. To equalize early production of both species, we added the same number of gravid females to each mesocosm (8 amphipods; 6 isopods).

At the same time, we introduced two species of snails to each mesocosm: the pouch snail (*Physa acuta*) and ram's horn snail (*Helisoma trivolvis*). Both species are generalist feeders, although they are commonly considered to be grazers of periphyton. The snails were introduced as eggs to eliminate the risk of introducing nematode parasites that frequently live in adult snails and subsequently parasitize tadpoles. To obtain snail eggs, we collected approximately 500 adult pouch snails and 300 adult ram's horn snails from natural ponds and held them in the laboratory to reproduce. After reproduction, we removed, mixed, and introduced 10 egg masses of each species to the experiment by sinking a small cup containing the egg masses into the mesocosms.

We added five species of tadpoles to each mesocosm as they became available based on breeding phenology, including three spring-breeding species: wood frogs (*Rana sylvatica*); leopard frogs (*Rana pipiens*), and American toads (*Bufo americanus*), and two summer-breeding species: spring peepers (*Pseudacris crucifer*) and gray tree frogs (*Hyla versicolor*). Each mesocosm received 20 individuals of each species. Similar to the snails, tadpoles are generalist grazers and may even filter phytoplankton, although they are commonly considered as periphyton grazers. We collected amphibians as newly oviposited eggs from nearby ponds (8-29 egg masses per species), allowed them to hatch in pools containing aged well water, and fed them rabbit chow pellets *ad libitum*. Tadpoles of each species were early in development (Gosner stage 25; Gosner 1960) when added to the experiment. Initial masses (± 1 SE) of the five species were as follows: wood frogs = 52 ± 19 mg, leopard frogs = 36 ± 5 mg, toads = 25 ± 4 mg, spring peepers = 21 ± 23 mg, and gray treefrogs = 26 ± 11 mg. We added wood frogs and leopard frogs on day 16, American toads on day 31, spring peepers on day 38, and gray treefrogs on day 57.

Table 2.1. Treatments used in the experiment, including common names, abbreviations, and family. For all single litter species treatments, traits used in the redundancy analysis are given. Values for soluble carbon, lignin, and total phenolics are means of a analyses performed in triplicate; values in parentheses represent 95% confidence intervals (CI). Values for C:N represent single samples analyzed in a CHN analyzer with 4% measured accuracy. Values for decomposition rate are means of decay coefficients calculated for each treatment by sampling litter at monthly intervals during the experiment (*sensu* Petersen and Cummins 1974); values in parentheses represent 95% CI.

Treatment	Abbre-viation	Family	Species	Soluble carbon (%)	Lignin (%)	Phenolics (%)	C:N (g/g)	Decay Coefficient (k)
Red maple	RM	*Aceraceae*	*Acer rubrum*	40.0 (±10.5)	30.0 (±14.5)	8.04 (±1.56)	57.7	0.088 (±0.013)
Hybrid Chestnut	CH	*Fagaceae*	*Castanea dentata x C. mollissima*	39.2 (±7.7)	40.8 (±9.4)	5.11 (±0.57)	73.2	0.092 (±0.011)
Black oak	OAK	*Fagaceae*	*Quercus velutina*	29.0 (±3.5)	40.0 (±7.0)	4.55 (±0.28)	34.4	0.041 (±0.007)
American Beech	BCH	*Fagaceae*	*Fagus grandifolia*	23.4 (±4.9)	39.0 (±3.9)	4.21 (±0.74)	65.2	0.039 (±0.015)
Tulip poplar	TP	*Magnoliaceae*	*Liriodendron tulipifera*	43.8 (±2.5)	38.8 (±5.0)	0.63 (±0.06)	55.7	0.093 (±0.008)
Green ash	ASH	*Oleaceae*	*Fraxinus pennsylvanica*	26.3 (±10.5)	52.4 (±14.5)	3.76 (±1.15)	36.1	0.099 (±0.004)
Black cherry	CHER	*Rosaceae*	*Prunus serotina*	36.4 (±6.8)	29.0 (±12.0)	1.74 (±0.49)	46.5	0.097 (±0.011)
Black willow	BW	*Salicaceae*	*Salix nigra*	20.8 (±1.3)	36.4 (±2.3)	1.10 (±0.06)	32.2	0.081 (±0.007)
Bigtooth Aspen	ASP	*Salicaceae*	*Populus grandidentata*	22.8 (±3.1)	29.2 (±14.9)	1.63 (±0.05)	70.6	0.063 (±0.015)
American Elm	ELM	*Ulmaceae*	*Ulmus americana*	20.5 (±3.1)	36.3 (±11.7)	1.53 (±0.10)	47.6	0.095 (±0.007)
Mixture	MIX							
No Leaf	NL							

2.2.2 Abiotic measurements

To document how the leaf litter treatments affected abiotic conditions of the mesocosms, we quantified light attenuation, dissolved oxygen, temperature, temperature stratification, and pH at four sample dates (i.e. every 4 wks) using a calibrated electronic water meter (Multiline P4 Universal Meter, WTW). Details of these measurements can be found in Appendix A.

2.2.3 Biotic measurements

At multiple times during the experiment, we measured several biotic response variables. Details regarding the sampling methods are provided in Appendix A.

To quantify litter decay rate, we recorded the mass loss of litter in mesh litterbags. From each mesocosm, one litter bag was sampled after the second week, the fourth week, and once a month for the last three months (i.e. five sample dates). These values were used to calculate a decay rate coefficient for each mesocosm (*sensu* Petersen and Cummins 1974), which was used as a leaf litter trait in the trait based analysis described below.

To quantify algal and microbial production, we measured phytoplankton and periphyton monthly (phytoplankton: days 26, 48, 81, and 108; periphyton: days 33, 59, 82, and 111). Phytoplankton density was estimated using the concentration of chlorophyll *a* (chl *a*) in the water. Periphyton abundance was estimated from the biomass of periphyton scraped from half of a single ceramic tile.

We began estimating the abundance of the invertebrates after 2 months. Although earlier samples were taken, population sizes were very small and it was evident that most species had

not reached carrying capacity. Hence, these earlier samples are not reported. We quantified the density of zooplankton on two sample dates (days 81 and 109). *Daphnia pulex*, and *Scapholeberis mucronata* constituted all cladoceran species on both sample dates, and *Skistodiaptomus oregonensis* constituted between 95% and 92% of all copepod species on the first and second sample date, respectively. Other species were excluded from the analysis due to their low relative abundance. We measured abundance and biomass of both amphipods and isopods on days 62 and 90 and of both snail species on days 66 and 94. At the same time, we also quantified the number of snail egg masses of each species on the walls of the mesocosm.

For amphibians, we collected individuals as they metamorphosed and recorded each individual's time to metamorphosis. We held captured metamorphs in the lab containers containing wet sphagnum moss until their tails were fully resorbed (i.e. Gosner stage 45). We then preserved individuals in 10% formalin and quantified the mean mass of all metamorphs in each mesocosm. When the experiment was terminated on day 145, we collected all remaining tadpoles. For each amphibian species our response variables were total survival in a mesocosm (i.e. survival of tadpoles + metamorphs), total biomass in a mesocosm (i.e. biomass of tadpoles + metamorphs), mean individual mass of metamorphs from a mesocosm (i.e. for those species that completed metamorphosis), and mean time to metamorphosis from a mesocosm (i.e. for those species that completed metamorphosis).

2.2.4 Statistical analysis

2.2.4.1 Litter species-based analysis: To test for differences among leaf litter treatments, analysis of variance (ANOVA) techniques were employed. In all cases, transformation of

variables to meet assumptions of ANOVA and handling of missing values are described in Appendix A. All species-level analyses were conducted using IBM SPSS Version 19.

We tested for differences in decay rate constants among litter species with a univariate ANOVA. Average decay constants for each treatment are provided in Table 2.1.Test results and mass loss curves are provided in Appendix B.

To assess the effects of litter on the abiotic responses (pH, DO, temperature, temperature stratification, and light attenuation) we used a repeated-measures multivariate analysis of variance (rm-MANOVA). Upon finding a significant multivariate effect, we conducted univariate analyses to explore treatment differences, followed by Tukey's post-hoc treatment comparisons. A full description of our analysis is described in Appendix A. To test if the absence of litter (i.e. NL treatment) was associated with responses different from the mean of the other litter species treatments (excluding the mixture treatment), we conducted weighted planned comparisons that resulted in the comparison of no litter treatment responses with the average of all litter species treatment responses. In a similar manner, we conducted weighted planned comparisons to determine if mixture treatment responses were non-additive (i.e. comparing the expected and observed responses of the mixture treatment where expected responses were calculated as the mean treatment response of all monoculture litter species).

To assess the effect of litter treatment on the biotic responses that were measured at more than one time point, we again used rm-MANOVAs. We conducted one rm-MANOVA for response variables that were measured at two time points (snails, detritivores, and zooplankton) and another rm-MANOVA for response variables that were measured at four time points (phytoplankton and periphyton). Subsequent univariate analyses and Tukey's post-hoc comparisons were conducted as described for the abiotic analyses.

Because the amphibian response variables were only measured at a single time point, we separately analyzed these data using a combination of a MANOVA and several ANOVAs. The MANOVA included each amphibian species' survival and total biomass. Because mass at metamorphosis and time to metamorphosis had missing values due to complete mortality or incomplete development to metamorphosis for several species, these responses were analyzed separately using univariate ANOVAs. After finding significant univariate effects, we used Tukey's post-hoc test to determine which litter treatments differed and we conducted planned comparisons identical to those in the abiotic and biotic analyses.

2.2.4.2 Trait-based analysis: To explore the relationships between litter chemistry and the abiotic and biotic responses, we employed redundancy analyses (RDA). RDA is a constrained, linear, multivariate analysis that combines regression and ordination to explore how variation in the structure of an independent dataset (e.g., litter chemistry; litter decay coefficients) explains variation of a dependent dataset (e.g., abiotic and biotic response variables). Canonical axes (i.e. ecological gradients) for each data set are derived such that the first ecological gradient derived from the independent dataset explains the maximum variation within the dependent dataset. Because we wanted to explore how trait-response relationships changed over time, we conducted a separate analysis at each time point (i.e. four total analyses). We conducted a fifth analysis to explore trait-amphibian response relationships. Time to metamorphosis and mass at metamorphosis for leopard frogs, gray tree frogs, and spring peepers were excluded from the RDA due to substantial amounts of missing data. To interpret the importance of traits in determining ecological gradients and to interpret the strength by which responses were associated with these gradients, we followed the recommendation of Tabachnik and Fidell (1989), and considered loadings of ± 0.45 as fair, ± 0.55 as good, and ± 0.63 as excellent. All

data were centered and standardized prior to analysis. Multivariate normality of data for each analysis was verified by examining the scatterplot of Chi-squared values with squared Mahalanobis Distances, and assuming normality if the line was reasonably straight (Burdenski 2000). All ordination analyses were conducted using CANOCO, version 4.0.

2.3 RESULTS

Because the purpose of our experiment was to explore how manipulations of leaf litter influence pond community structure, we chose to highlight some of the most dramatic species-level responses from the analyses of variance and then provide a larger overall analysis of the population, community, and ecosystem responses based on the redundancy analyses. A complete analysis of treatment differences for each response variable is available in Appendix C. All multivariate and univariate test statistics can be found in the supplemental tables of Appendix D. All litter treatments are abbreviated as listed in Table 2.1.

2.3.1 Litter species-based analysis: abiotic response variables

There was a significant multivariate effect of treatment, time, and their interaction on abiotic response variables (Table D.1). Hence, we explored each abiotic response with univariate analyses.

2.3.1.1 Light attenuation: There was an effect of treatment, time, and their interaction on light attenuation (Table D.1; Figure 2.1a). Subsequent ANOVAs detected treatment effects on every sample date (Table D.2). Mesocosms without litter had lower light attenuation than the average

of all mesocosms containing litter on days 25, 80, and 112, but not on day 55 (Table D.8). Among the 10 litter species treatments, the most striking result was that RM and TP litter caused high rates of light attenuation early in the experiment. Indeed, the water in these treatments was black due to large amounts of soluble carbon. Over time, however, light attenuation in these treatments declined to be similar to the other litter species.

2.3.1.2 Dissolved oxygen: There was an effect of treatment, time, and their interaction on dissolved oxygen (Table D.1; Figure 2.1b). We found significant univariate effects of treatment on each sample date (days 19, 46, 76, 105; Table D.2). Mesocosms with no leaves had consistently higher concentrations of dissolved oxygen than the average of all mesocosms containing litter (Table D.8). Among the 10 litter species treatments, there was a general pattern of an initial decline in DO over time followed by a large increase by the final sample date. The most extreme decline in oxygen occurred in the TP treatment in which the concentration approached 1 mg/L. Dissolved oxygen was generally higher early in the experiment in mesocosms containing the more recalcitrant litter species (e.g., OAK, BCH, and ASP).

2.3.1.3 Temperature: There was no effect of litter treatment on temperature, but there were effects of time and their interaction (Table D.1; Figure 2.1c). Overall, temperature followed the trends in seasonal temperature, warming initially and cooling slightly toward the end of summer. Univariate analyses within each sample date only detected treatment effects on the first and second sample date (Table D.2). Early in the experiment, mesocosms lacking litter had lower temperatures than the average of all mesocosms containing litter (Table D.8). Among the 10 litter species treatments, those with darker, less transparent water early in the experiment (i.e. RM, CH, and TP) had higher water temperatures early in the experiment.

2.3.1.4 Temperature stratification: There were significant effects of treatment, time, and their interaction on temperature stratification (Table D.1; Figure 2.1d). We found univariate treatment effects on the first three sample dates (days 19, 47, and 75; Table D.2). There was no difference between mesocosms without litter and the average of all mesocosms containing litter (Table D.8). Among the 10 litter species treatments, stratification was generally greatest early in the experiment in TP and RM, which were the two litter treatments that had the greatest light attenuation due to their dark waters.

2.3.1.5 pH: There was an effect of treatment, time, and their interaction on pH (Table D.1; Figure 2.1e). Subsequent ANOVAs detected univariate treatment effects on all sample dates (days 19, 47, 75, and 105; Table D.2). Over time, pH generally increased in all of the treatments. Mesocosms without litter had consistently higher pH than the average of all mesocosms containing litter (Table D.8). Among the 10 litter species treatments, differences in pH were not substantial; the maximum range among treatments was 0.5 pH units, as observed on the first sample date.

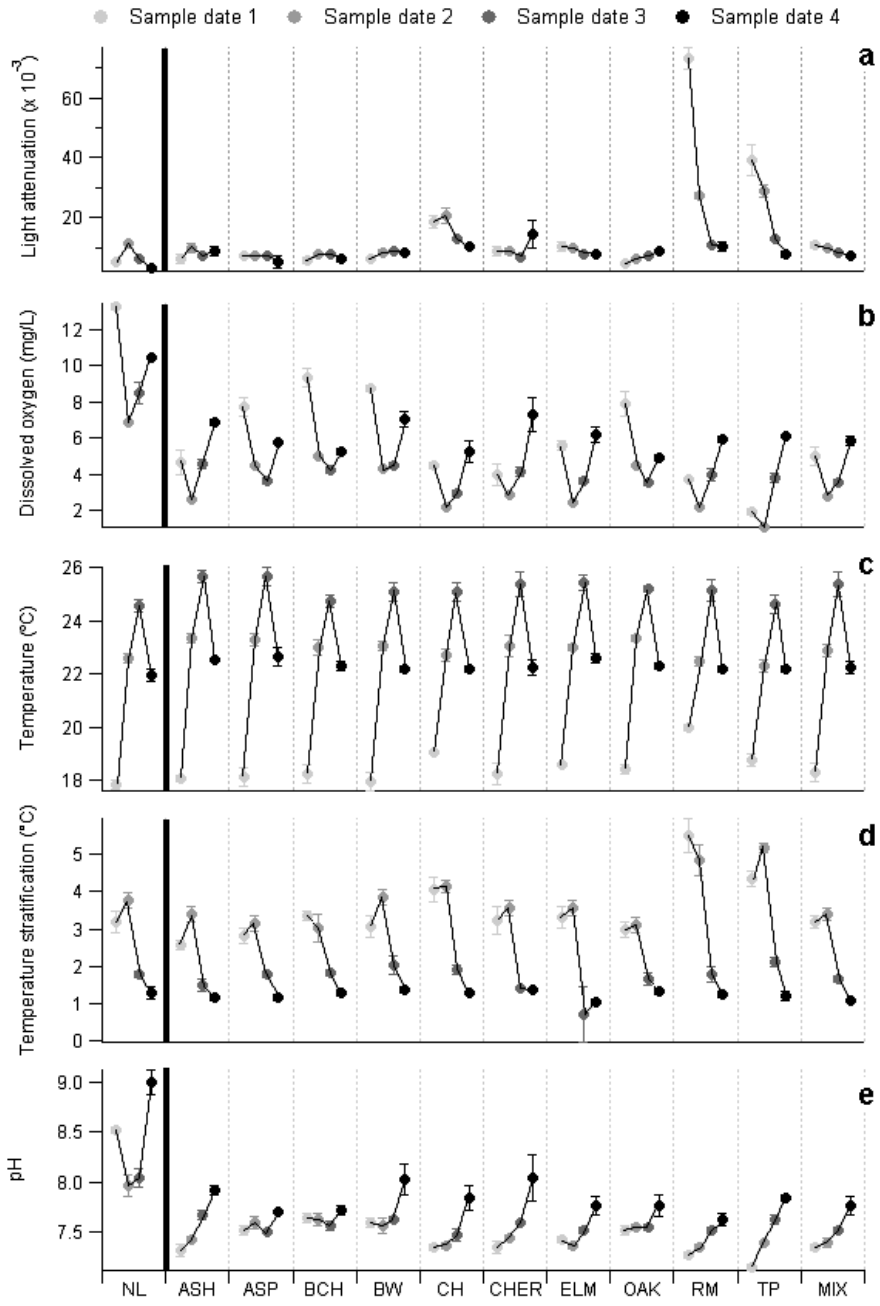


Figure 2.1. Abiotic responses for 10 litter monocultures, a mixed litter treatment, and a no-litter treatment: light attenuation (days 25, 55, 80, 112; panel a), dissolved oxygen near the benthos (days 19, 46, 75, 112; panel b), surface temperature (days 19, 47, 75, 105); panel c), temperature stratification between the top and bottom (days 19, 47, 75, 105; panel d), and pH (days 19, 47, 75, 105; panel e). Means ± 1 SE are presented.

2.3.2 Litter species-based analysis: biotic response variables

2.3.2.1 Phytoplankton and periphyton: There was a significant multivariate effect of treatment, time, and their interaction on chl *a* and periphyton biomass (Table D.3). Both responses exhibited univariate effects of treatment, time, and their interaction (Table D.3)

There were significant univariate effects of treatment on chl *a* concentration on the first, second and fourth sample day (days 26, 48, and 108; Table D.4; Figure 2.2a). Mesocosms without litter had higher chl *a* concentrations than the average of all mesocosms containing litter on the second sample date 48, but lower than the average on the third sample date (Table D.8). Among the 10 litter species treatments, there was a great deal of variation in chl *a* concentration on the first sample date that spanned nearly an order of magnitude. TP contained the lowest concentration of chl *a* while RM and CH contained the highest. Following the first sample date, the total range of phytoplankton densities decreased by an order of magnitude, yet still contained substantial variation. Later in the study, concentrations in TP remained the lowest among treatments whereas BW had the highest concentration.

There were significant univariate effects of treatment on periphyton biomass on the first and fourth sample dates (days 33 and 111; Table D.4; Figure 2.2b). On the first sample date, mesocosms without litter had more periphyton than the average of all mesocosms containing litter species treatments (Table D.8). Among the 10 litter species treatments, the more recalcitrant (i.e. lignified) litter species (e.g., BCH, OAK, ASP) had less periphyton than the more labile species (e.g., RM, TP, BW) on the first sample date. On the fourth sample date, biomass was generally greater than on the first date by an order of magnitude or more, yet there was substantial variation and no treatment differences were detected.

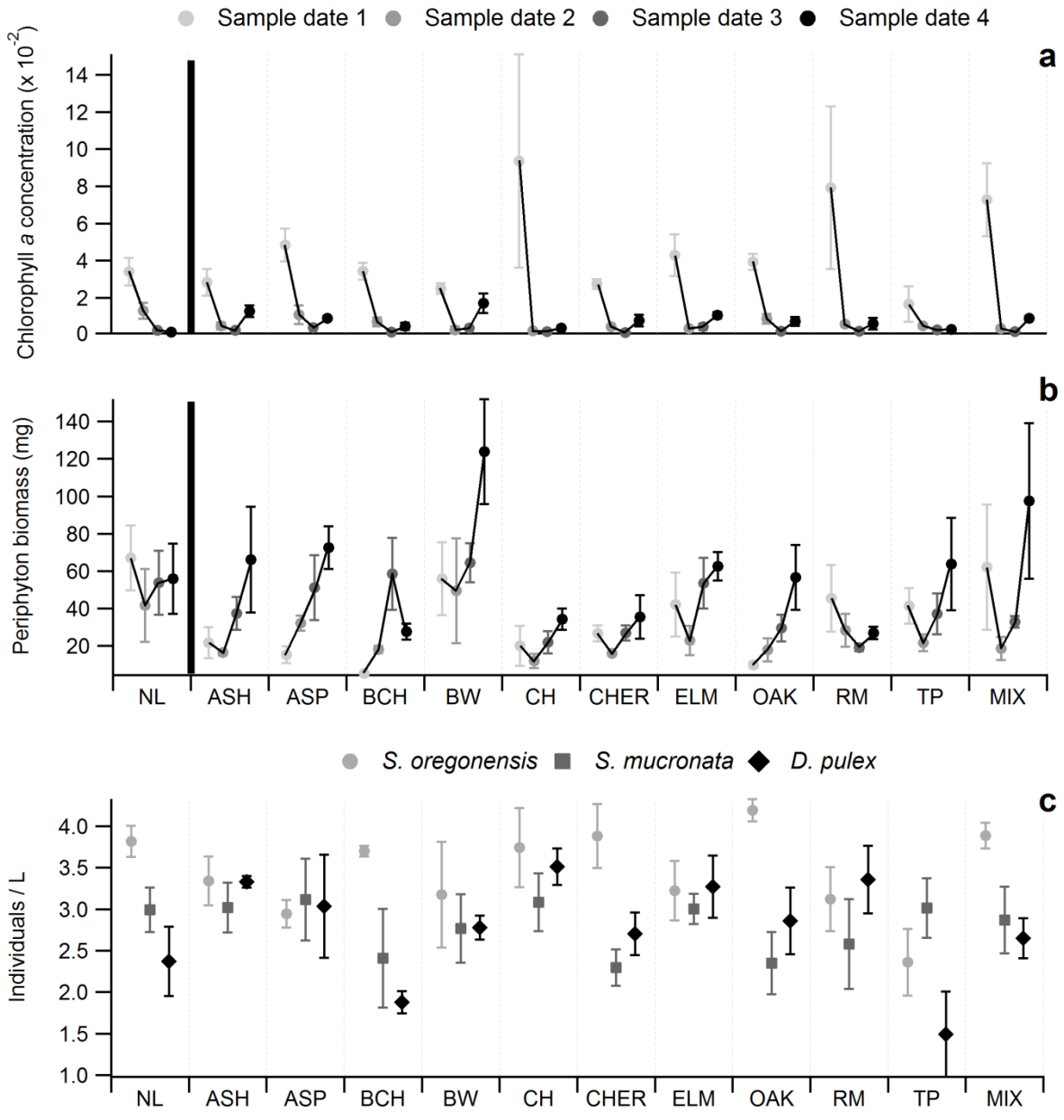


Figure 2.2. Phytoplankton concentration (measured as chlorophyll *a*; days 26, 48, 81, 108; panel a), periphyton biomass (days 33, 59, 82, 107; panel b), and average densities for the three dominant zooplankton species across both sample dates (days 81 and 109) for 10 litter monocultures, a mixed litter treatment, and a no-litter treatment. Values for zooplankton were log transformed. Means ± 1 SE are presented.

2.3.2.2 Zooplankton, snails, and detritivores: There was a significant multivariate effect of treatment and time on the abundance of zooplankton, snails, and arthropod detritivores. There was also a marginally significant time-by-treatment interaction (Table D.5).

For *D. pulex*, there was an effect of treatment and time on *D. pulex*, but no interaction (Table D.5; Figure 2.2c). Over time, there was an increase in the density of *D. pulex*. Despite a significant effect of treatment, post-hoc analyses did not reveal any significant differences among treatments. Mesocosms without litter did not differ from the average of all mesocosms containing litter (Table D.8).

For *S. oregonensis,* there was an effect of treatment and time but no interaction (Table D.5; Figure 2.2c). Over time, there was an increase in density. Despite a significant effect of treatment, post-hoc analyses did not reveal any significant differences among treatments. Once again, mesocosms lacking litter did not differ from the average of all mesocosms containing litter (Table D.8).

S. mucronata densities were affected by time, but there was no treatment effect or time-by-treatment interaction (Table D.5; Figure 2.2c). The density of these copepods increased over time.

Pouch snail density was affected by treatment and time, but there was no interaction (Table D.5; Figure 2.3a). Mesocosms without leaves had greater densities than the average of all mesocosms containing litter. Among the 10 litter species, there was a complete absence of pouch snails in TP mesocosm; relatively low densities also occurred in RM and CH.

Pouch snail biomass was affected by treatment and time and marginally by their interaction (Table D.5; Figure 2.3b). Significant treatment effects were found on both sample dates (days 66 and 94; Table D.6). We found no difference between mesocosms lacking leaves

and the average of all mesocosms containing leaves (Table D.8). Among the 10 litter species treatments, the lowest biomass values occurred with occurred with TP, RM, and CH. Indeed, pouch snail biomass in the TP treatment was zero on the first sample date. On the second sample date, the TP treatment continued to have the lowest total biomass of pouch snails, which reflected the low number of eggs laid prior to the adult snails dying.

Pouch snail egg production was affected by treatment, time, and their interaction (Table D.5; Figure 2.3c). There were significant univariate effects of treatment on both sample dates (days 65 and 93; Table D.6). Mesocosms without leaves had fewer egg masses than the average of all mesocosms containing litter species on the first sample date, but not on the second (Table D.8). Among the 10 litter species treatments, the number of egg masses varied widely throughout the experiment (6 to 98 and 4 to 158 on the first and second sample dates, respectively) with the lowest number in the TP and BCH litter. Specifically, on the first sample date fewer eggs were found in TP and BCH. The highest number of egg masses was found in CH.

The results for the ram's horn snails were quite different from the pouch snails. Ram's horn snail density exhibited no effects of treatment, time, or their interaction (Table D.5). Ram's horn biomass exhibited no effect of treatment, but there was an effect of time due to an increase in growth of all individuals between the first and second sample date (days 66 and 94; Table D.5).

Ram's horn egg production was affected by treatment and a time-by-treatment interaction, but not time (Table D.5; Figure 2.3d). A significant treatment effect was found only for the second sample date (day 93; Table D.6). Mesocosms without leaf litter did not differ from the average of all mesocosms containing litter species treatments (Table D.8). Among the 10 litter species treatments, the most striking response was within TP, as the number of egg masses

showed a sharp increase on the second sample date that did not occur in any other treatments. For amphipod density and total biomass, there were significant effects of treatment and time, but no interaction (Table D.5; Figure 2.4a,b). Both responses increased over time. Mesocosms without litter had lower amphipod biomass than the average of all mesocosms containing litter species, but there was no difference in amphipod density (Table D.8). Among the 10 litter species treatments, density and biomass were the lowest in the TP litter.

For isopod density and biomass, there was an effect of time but no effects of treatment or their interaction (Table D.5). Both responses increased over time.

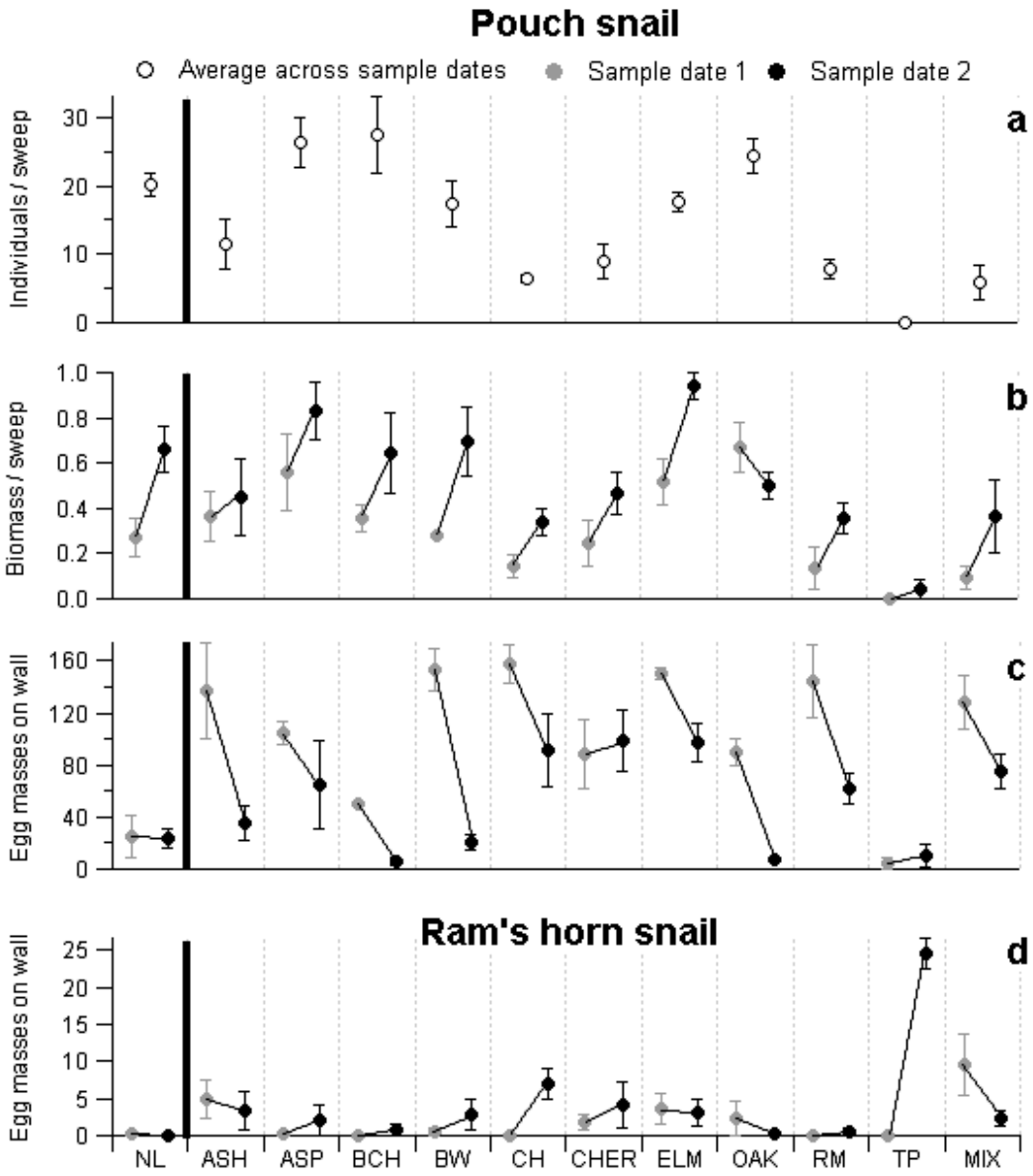


Figure 2.3. Average density of pouch snails across both sample dates (days 66 and 94; panel a); total biomass and egg density the pouch snail on both sample dates (panels b and c, respectively); and density of ram's horn snail egg density on both sample dates (panel d) for 10 litter monocultures, a mixed litter treatment, and a no-litter treatment. For ram's horn snails, biomass data were square-root transformed and egg density was rank transformed. Means ± 1 SE are presented.

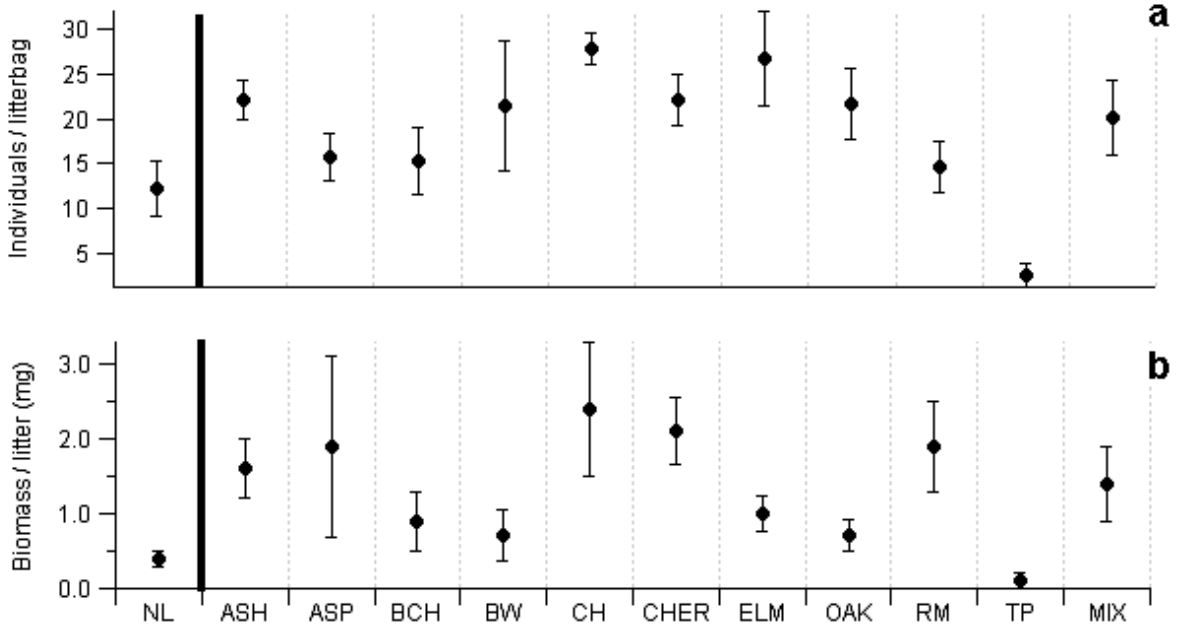


Figure 2.4. Average density and biomass of *C. psuedogracilis* (panels a and b, respectively) across both sample dates (days 62 and 90). Means ± 1 SE are presented.

2.3.2.3 Amphibians: There was a significant multivariate effect of litter treatment on all amphibian responses (Table D.7). Hence, we explored each response with univariate analyses.

For American toads, the litter treatments affected survival to metamorphosis, total biomass, and mass at metamorphosis mass, but not time to metamorphosis (Table D.7; Figure 2.5). Mesocosms without leaves had higher survival and larger mass at metamorphosis than the average of all mesocosms containing litter species (Table D.8). Among the 10 litter species treatments, toad survival to metamorphosis ranged from 20 to 96 %; the most striking pattern was that only 20% of toads survived with TP litter. Total toad biomass ranged from 0.6 to 1.8 g, and was lowest in TP largely due to their low survival. In contrast, average individual metamorph mass, which ranged from 0.08 to 0.14 g, was highest in the TP litter.

For wood frogs, all responses were affected by treatment (survival to metamorphosis was marginally significant; Table D.7; Figure 2.5). Mesocosms without leaves had smaller metamorphs than the average of all mesocosms containing litter species (Table D.8). Among the 10 litter species, wood frog survival to metamorphosis ranged from 51 to 95%; survival was lowest in RM and TP although no comparisons were significant ($P \geq 0.107$). Wood frog total biomass ranged from 5.4 to 10.2 g and was lowest in RM and TP treatments, but only significantly lower than biomass in ELM ($P \leq 0.028$). In contrast, average individual metamorph mass ranged from 0.36 to 0.63 g and was highest in TP; significantly higher than CHER, ASP, OAK, CH, BCH, and RM treatments ($P \leq 0.037$). Time to metamorphosis ranged from 41 to 51 d and was significantly greater in TP relative to all other treatments ($P < 0.001$).

For leopard frogs, the litter treatments affected survival to metamorphosis and individual mass at metamorphosis but not total biomass or time to metamorphosis (Table D.7; Figure 2.5). Mesocosms without litter had shorter time to metamorphosis and larger total biomass relative to the average of all litter species treatments (Table D.8). Among the 10 litter species treatments, leopard frog survival to metamorphosis ranged from 28 to 64% and was lowest in OAK and RM. Individual mass at metamorphosis ranged from 0.58 to 2.04 g was higher in TP than in all other litter species treatments ($P \leq 0.011$).

For spring peepers, the litter treatments affected survival to metamorphosis and total biomass (marginally significant), but not individual mass at metamorphosis or time to metamorphosis (Table D.7; Figure 2.5). Planned comparisons of mesocosms without leaves and the average of other litter species treatments were not conducted due to complete mortality among mesocosms without litter (Table D.8). Among the 10 litter species treatments, peeper survival to metamorphosis ranged from 0 to 26%. Survival was greatest in TP; significantly

greater than all treatments except ASH ($P \leq 0.038$). Total biomass ranged from 0 to 1.0 g and was greatest in TP, but only significantly greater than biomass in CHER and BCH treatments ($P = 0.039$).

For gray tree frogs, litter treatments affected survival and time to metamorphosis, but not total biomass or individual mass at metamorphosis (Table D.7; Figure 2.5). Mesocosms without litter had shorter time to metamorphosis than the average of all litter species treatments (Table D.8). Among the 10 litter species treatments, tree frog survival to metamorphosis ranged from 5 to 40%. Survival was highest in TP and was significantly greater than survival in all treatments except ASH, BW, and ELM ($P \leq 0.019$). Time to metamorphosis ranged from 40 to 83 d and was significantly shorter in RM and TP treatments relative to ASH ($P \leq 0.030$).

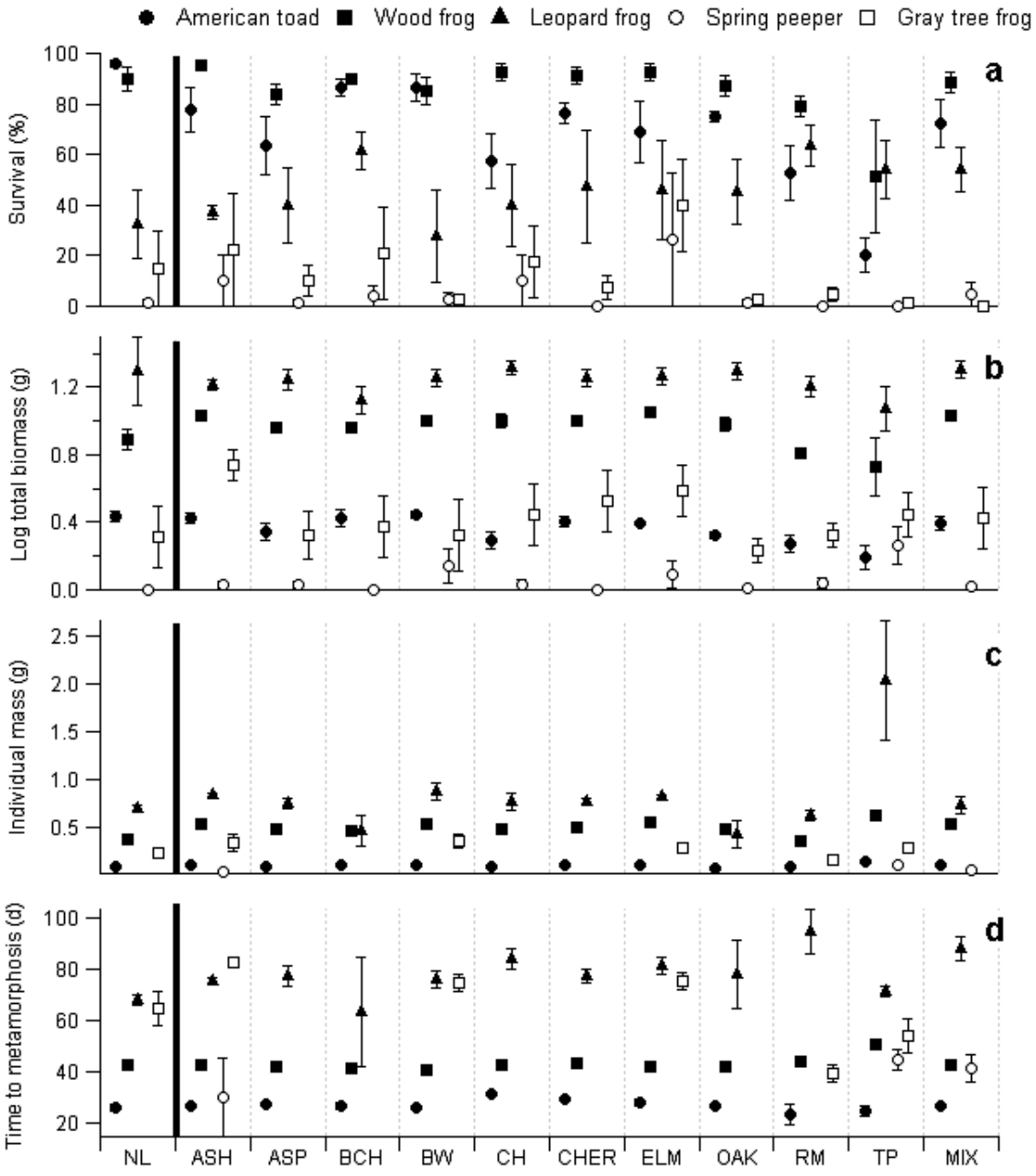


Figure 2.5. Survival (panel a), total biomass (i.e. tadpoles and metamorphs; panel b), individual metamorph biomass (panel c), and time to metamorphosis (panel d) of the five amphibian species for 10 litter monocultures, a mixed litter treatment, and a no-litter treatment. Spring-breeding species are indicated by filled symbols; summer-breeding species are indicated by open symbols. Note that total biomass is presented on a log scale. Means ± 1 SE are presented.

2.3.3 Litter species-based analysis: mixture effects:

Results of all planned comparisons can be found in Table D.9. Planned comparisons of mixture treatment responses to the average of all litter species treatments revealed only additive effects of mixing litter for our measured abiotic variables. Most biotic responses were additive as well, except for an antagonistic effect of mixing litter on pouch snail density and total pouch snail biomass on both sample dates (Figure 4a-b). Additionally, the litter mixture was associated with smaller spring peeper individual metamorph mass than the average of all litter species treatments. In contrast, mixing litter had a synergistic effect on ram's horn snails. There was a synergistic effect on egg production, but only on the first sample day (Figure 5f).

2.3.4 Trait-based analysis

To provide a general picture of how leaf litter affects population, community and ecosystem responses, we examined relationships between litter traits and these responses. To accomplish this, we conducted separate redundancy analyses on all responses variables measured within each sampling period and a fifth redundancy analysis on all amphibian response variables.

For all analyses, correlations of litter traits with measured responses were significant; the results of Monte Carlo permutation tests, amount response variation explained by each gradient, and percentage of trait-response relation variance are provided in Appendix E. In all cases, the first two gradients explained the most amount of variation among responses and among trait-response relations; additional gradients only explained minor amounts of additional variation.

2.3.4.1 First sample date: Among the litter traits, we found that both soluble carbon and decay rate loaded positively on the first ecological gradient, and phenolics loaded positively on the second gradient (Figure 2.6A). Following the recommended cut-off significance value of 0.55 or greater for loading values (Tabachnik and Fidell 1989), we did not consider lignin as strongly loaded onto either gradient. When we examined how the system responded to these gradients, we found that light attenuation, temperature, and temperature stratification were positively associated with the first gradient whereas DO and pH were negatively associated. Among all responses, phytoplankton was the only response that exhibited a positive association with the second gradient, although it did not meet the requirement for significant loading (score = 0.513).

2.3.4.2 Second sample date: Similar to the first date, the traits of soluble carbon and decay rate loaded positively onto the first ecological gradient. Decay rate also loaded positively onto the second ecological gradient whereas C:N loaded negatively (Figure 2.6B). When we examined how the system responded to these gradients, we found that light attenuation and temperature stratification were positively associated with the first gradient, whereas dissolved oxygen, pH, and temperature were negatively associated with this gradient, although the latter did not meet the requirement for significant loading (score = -0.516). In contrast, no response exhibited significant loading on the second ecological gradient.

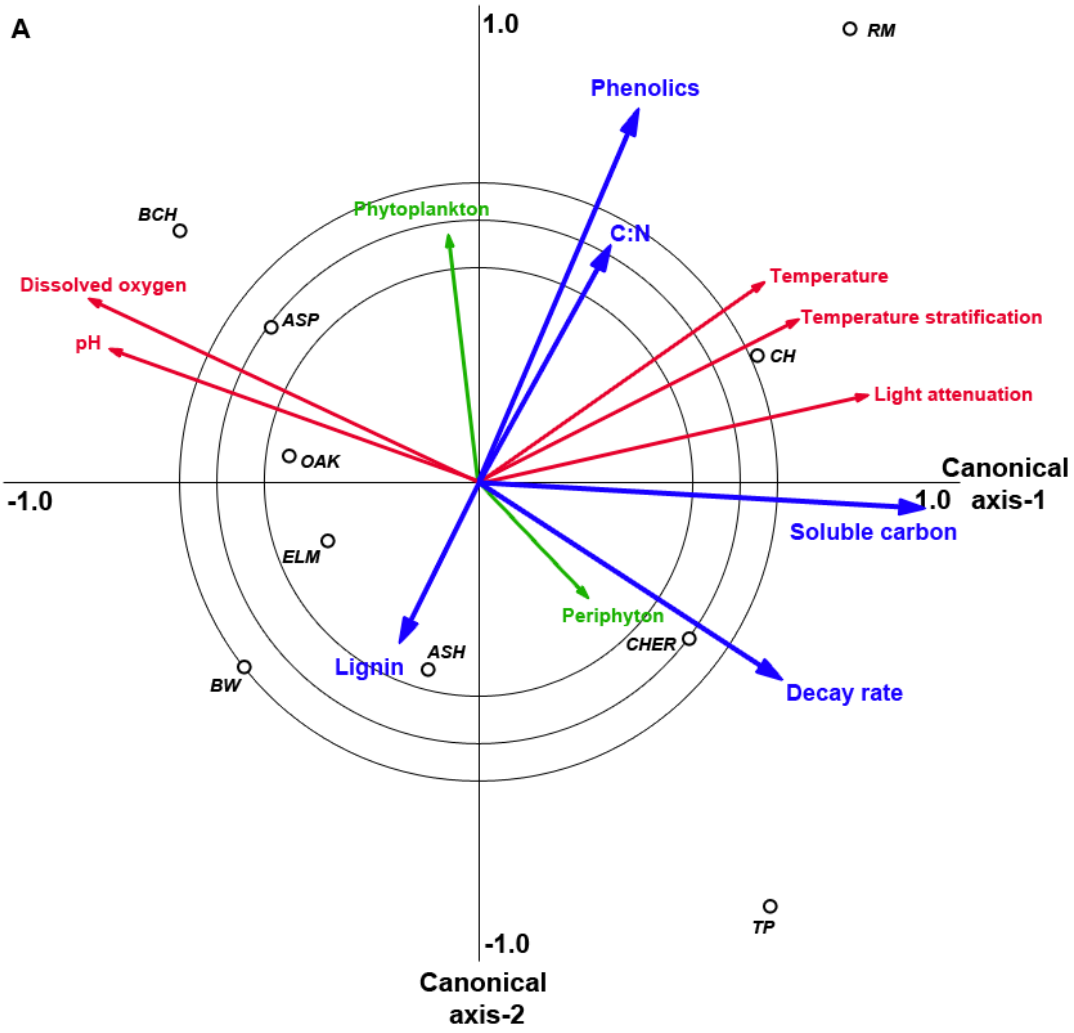
2.3.4.3 Third sample date: Among the litter traits, soluble carbon and C:N loaded positively onto the first ecological gradient, and decay rate and phenolics loaded positively onto the second gradient (Figure 2.6C). When we examined how the system responded to these gradients, it was clear that the majority of responses were negatively associated with the first gradient. Among was positively associated with the first gradient, whereas pouch snail biomass and density were

those responses that met the recommended cut-off value for significant loading, light attenuation negatively associated with this gradient. Pouch snail egg production was the only response positively associated with the second gradient.

2.3.4.4 Fourth sample date: Among the litter traits, soluble carbon loaded positively onto the first ecological gradient and decay rate loaded negatively onto the second gradient (Figure 2.6D). No other trait was found to significantly contribute to either the first or second gradient. When we examined the how the system responded to these gradients, it was clear that the majority of responses were negatively associated with either the first or second gradient. Among those variables that met the recommended cut-off value for significant loading, pouch snail density, and pouch snail biomass were negatively associated with the first ecological gradient. Dissolved oxygen exhibited a negative association with the second gradient. No other responses exhibited substantial associations with any gradient.

2.3.4.5 Amphibians: Among the litter traits, decay rate loaded positively on the first ecological gradient whereas phenolics loaded negatively on this gradient (Figure 2.7). Soluble carbon loaded positively on the second gradient. Neither lignin nor C:N significantly loaded onto either gradient. When we examined amphibian responses to these gradients, it was clear that responses were either positively associated with the first gradient or negatively associated with the second, with the exception of wood frog time to metamorphosis which was positively associated with the latter. Among those responses that exhibited a significant, positive association with the first ecological gradient were mass at metamorphosis of wood frogs and American toads, and the survival to metamorphosis of leopard frogs, spring peepers, and gray tree frogs. Among those

responses that exhibited a significant, negative association with the second ecological gradient were survival to metamorphosis of American toads and leopard frogs, and total biomass of American toads.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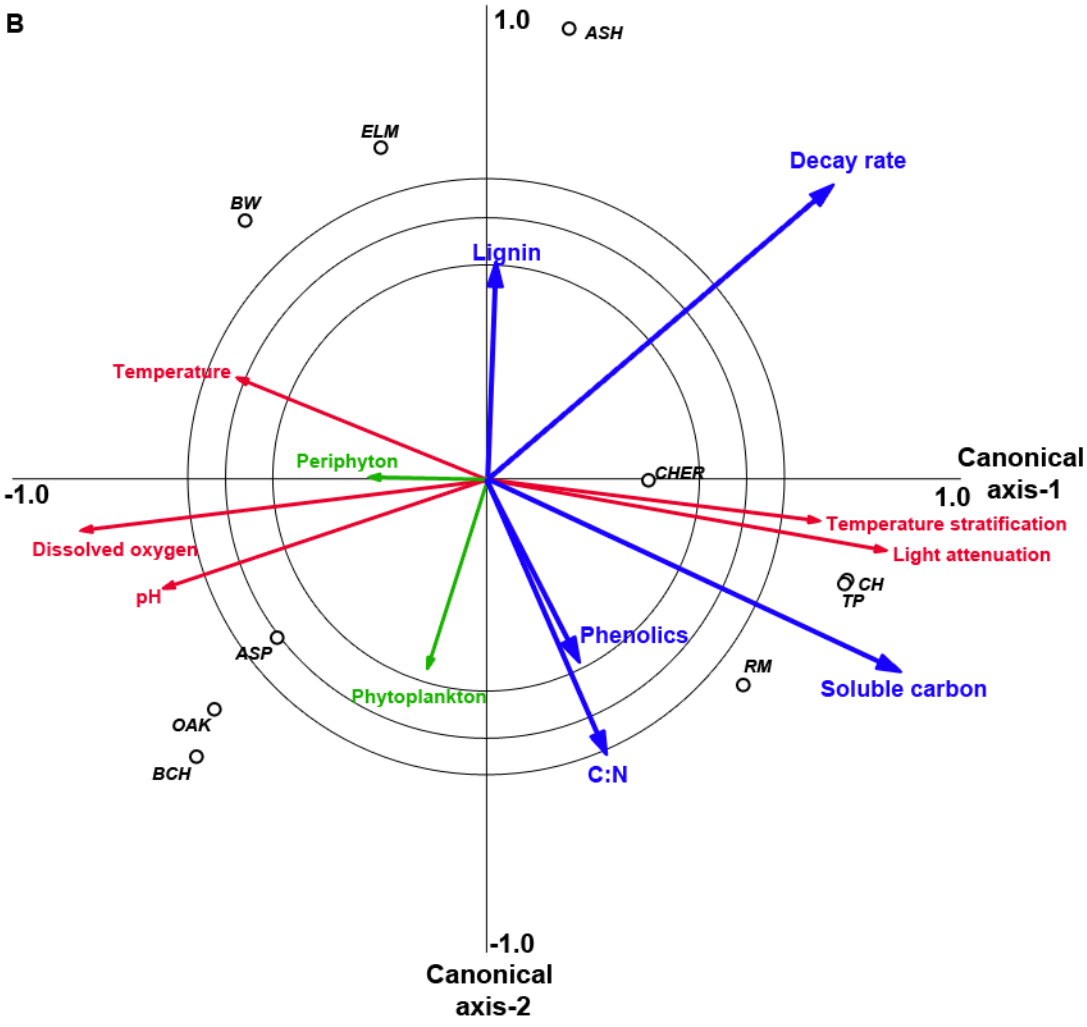
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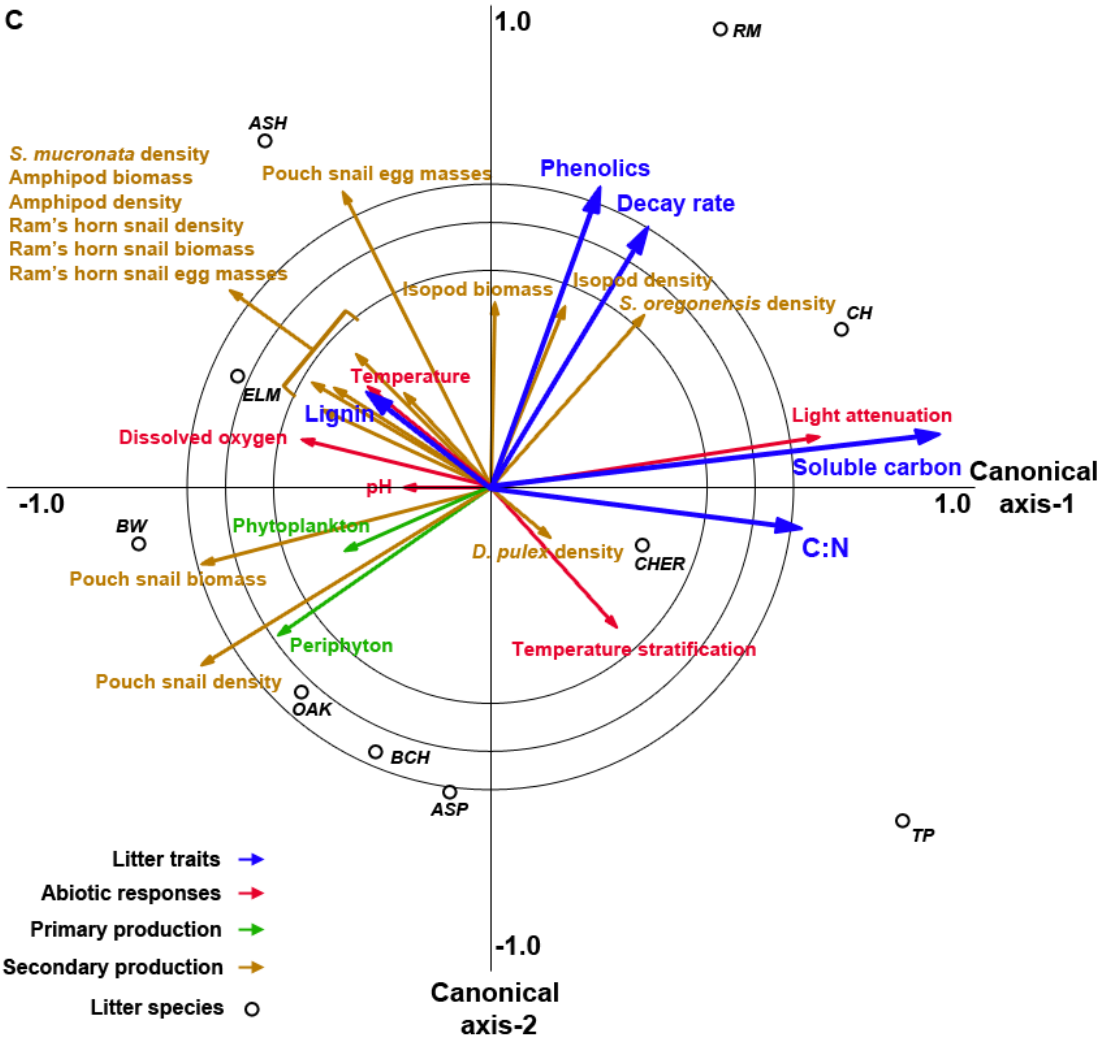
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Amphipod biomass
Amphipod density
Ram's horn snail density
Ram's horn snail biomass
Ram's horn snail egg masses
Pouch snail egg masses
Phenolics
Decay rate
Isopod biomass
Isopod density
S. oregonensis density
CH
ELM
Temperature
Lignin
Light attenuation
Dissolved oxygen
Soluble carbon
Canonical
axis-1
pH
-1.0
1.0
BW
Phytoplankton
D. pulex density
CHER
C:N
Pouch snail biomass
Periphyton
Temperature stratification
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Primary production
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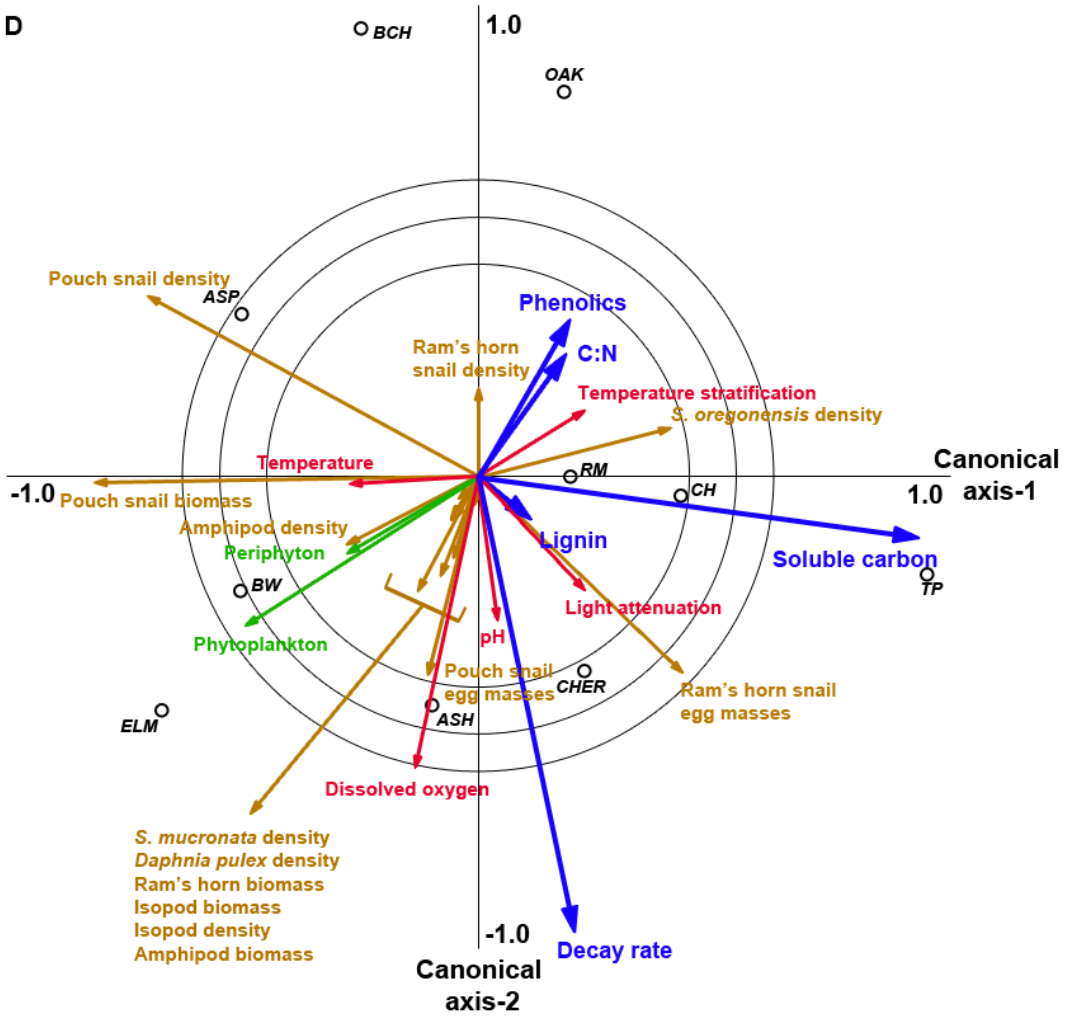

Figure 2.6. Biplot of redundancy analysis for responses taken during the first sample period (panel a), second sample period (panel b), third sample period (panel c), and fourth sample period (panel d). Independent values are litter attributes, and dependent values are responses among the 10 litter species treatments. The locations of species along ecological gradients are indicated by open circles and are abbreviated as in Table 1. Lengths of arrows indicate the importance of an independent variable to the gradients (i.e. loading on axes) whereas directions of arrows indicate the direction of change along that gradient. The three rings indicate cutoff points for fair (± 0.45), good (± 0.55), and excellent (± 0.63) loadings as recommended by Tabachnik and Fidell (1989).

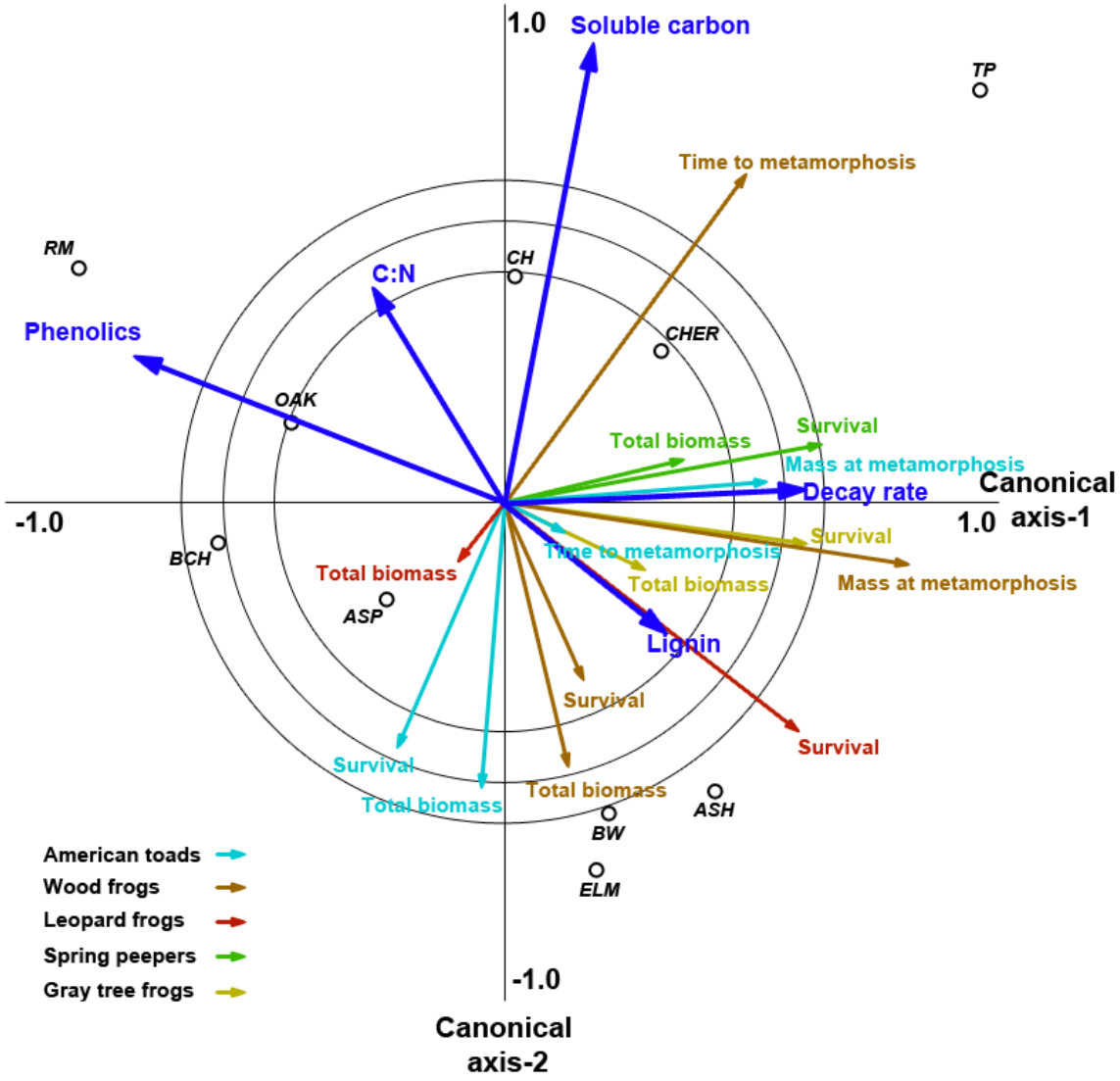

Figure 2.7. Biplot of redundancy analysis with independent values as litter attributes, and with dependent values amphibian responses among the 10 litter species treatments. Individual size at metamorphosis and time to metamorphosis for leopard frogs, gray tree frogs, and spring peepers were omitted due to missing values (see text for further explanation). Interpretation as in Figure 2.6.

2.4 DISCUSSION

These findings represent one of the most comprehensive studies exploring the relationship between qualitative differences in leaf litter chemistry and ecological function of any litter-based ecosystem. By incorporating a large number of litter species and exploring over 30 population, community, and ecosystem responses across multiple trophic levels and time points, we are able to consider the impacts of realistic changes in forest composition. At the same time, we were able to generalize the effects of litter both in terms of litter taxonomy as well as litter chemistry, which makes our results applicable to ecosystems containing a wide range of litter species. Overall, the results of this study illustrate the complex impact of litter species variation, composition, and chemistry on abiotic and biological variables in ponds and other wetlands across multiple trophic levels.

In several ways, our findings advance knowledge regarding the relationship between litter inputs and ecological function, particularly in lentic systems. First, although previous studies contrasting the presence and absence of one or two litter species have suggested that inputs of litter are generally associated with greater amounts of respiration relative to primary production (Fisher and Likens 1972, Wallace et al. 1997, Rubbo et al. 2006), our results indicate that this trend is not so simple; phytoplankton, periphyton and consumer responses varied widely among litter species, often producing average results that were indistinguishable from mesocosms without litter. Furthermore, although interpreting the effects of individual litter species with diverse chemical profiles can be difficult, our analyses reveal that a single chemical factor,

soluble carbon, often accounts for much of this variation. In addition, our results demonstrate that trends can dramatically change in both magnitude and direction over time, making the role of litter inputs both species- and time-dependent.

2.4.1 Effect of litter absence

We found variable effects of litter absence that provided equivocal support for the idea that litter inputs to wetlands promote secondary production leading to increased heterotrophy (i.e. respiration in excess of primary production; Rubbo et al. 2006). The absence of litter led to low light attenuation values similar to the most recalcitrant litter species (e.g., beech, oak). Lower attenuation likely contributed to the increase in observed algal production. In turn, pH and dissolved oxygen increased in the absence of litter, likely due to reduced microbial respiration and increased algal production. These differences were maintained throughout the experiment, indicating that the effect of litter on environmental attributes is both strong and persistent through time. Although these abiotic responses suggest lower levels of secondary production and heterotrophy in the absence of litter, biotic responses to the absence of litter were far less common and did not strongly indicate any change in these factors. Indeed, both periphyton and phytoplankton increased without litter, but these effects were short-lived and phytoplankton actually decreased below the average of all litter species by the end of the experiment. Responses related to consumers did not show any clear trend towards decreased secondary production or heterotrophy.

Responses resulting from the no-litter treatment contrast with findings from previous studies of litter removal or exclusion. For example, Rubbo et al. (2006) found relatively small

increases in dissolved oxygen and no changes in pH following litter removal whereas we found extreme effects on both responses. In the same study, they attributed the rise in dissolved oxygen to loss of secondary production, not a rise in gross primary production. In contrast, we found few shifts in consumer biomass and density and significant shifts in phytoplankton and periphyton production, indicating a reversed trend from their study. Lack of a significant change in consumer responses is particularly surprising, as past studies of litter removal or exclusion generally find substantial reductions in litter-based fauna (Sayer 2006). However, it is worth noting that while the literature is replete with examples of litter removal, the focus is generally on the removal of litter biomass without regard to litter species or litter quality, and the species of litter removed is rarely specified (e.g., Wallace et al. 1997, Rubbo et al. 2006). This is important, since our study demonstrates that the effects of litter species inputs are highly litter species-specific. For example, as compared with the average of all litter species treatments, phytoplankton density was higher without litter. However, pairwise comparisons reveal that this effect was largely a result of the relatively high density of phytoplankton in BW; statistically, the concentration of chlorophyll *a* without leaves was not different from all other litter species treatments. Similarly, as compared with the average of all litter species treatments, pouch snail density was higher without litter. However, pairwise comparisons revealed that pouch snail density without leaves was similar to all treatments except CH and TP. Hence, a major implication of our study is that the overall role of litter inputs on wetlands cannot be generalized as consistently influencing ecological function in one way; the actual influence is highly reliant on the litter species present.

2.4.2 Responses of the community to different species of litter

In agreement with our second hypothesis, most components of our communities were sensitive to the species of litter that was in the community. This resulted in unique response sets associated with each litter species. No litter species was consistently associated with high or low biological biomass, densities, or survival; all litter species appeared to benefit some aquatic species and harm others. For example, American beech litter promoted pouch snail biomass and density throughout the study yet it was also associated with smaller wood frog and leopard frog metamorphs. Despite such varied effects, elm litter was often ranked highest among treatment comparisons, causing relatively high pouch snail biomass, densities, and egg production, as well as high amphipod densities, high toad biomass, high survival to metamorphosis of leopard and tree frogs, and large wood frog metamorphs. In contrast, tulip poplar and red maple litter were often ranked lowest with regard to consumer biomass, density, and survival.

Our results differed from those found in prior work contrasting the effects of litter species in wetlands. For example, Rubbo and Kiesecker (2004) assessed the effects of red maple and oak litter on a simple community including wood frogs, Jefferson's salamanders (*Ambystoma jeffersonianum*), spotted salamanders (*A. maculatum*), bacteria, algae, and zooplankton. They found strong differences in almost every response measured, including chlorophyll *a*, bacterial production, cladoceran densities, and survival of two amphibian species. In contrast, we detected few differences between oak and maple litter; the only differences detected in our study were in pouch snail biomass and wood frog mass at metamorphosis. One explanation for this disparity may be the higher level of species diversity in our study. Stability is often associated with increased community diversity (Ives and Carpenter 2007) and may be greater when generalist

consumers are present (Morin 1999). In particular, generalist grazers such as the pouch snail, may buffer the process of carbon exchange against disturbances in input quality (Kondoh 2003). Although gastropods are frequently found in systems with high amounts of litter input (Mason 1970; Brady and Turner 2010), little is known of their response to differing litter quality. However, concentration of nutrients and phenolics can positively and negatively influence (respectively) the feeding rates of some species (e.g., *Pomacea canaliculata*; Qiu and Kwong 2009). Hence, it is possible that generalist feeders such as the pouch snail may buffer pond communities from changing resources, and further work should address this hypothesis. It is worth noting that if such buffering by generalists does occur, it is likely previous studies without such generalists overestimated the effects of litter on pond-dwelling organisms.

2.4.3 Effects of mixing litter

Despite the numerous ways in which litter chemistry affected abiotic and biotic response variables, the responses associated with the mixed litter treatment were surprisingly additive, which refutes our third hypothesis. Among the 44 tests for non-additivity that we conducted in our analysis, only pouch snail biomass, density, and egg production exhibited positive results. Given their low densities and biomass in such treatments as TP and RM, these results suggest pouch snails are particularly sensitive to the chemistry of those leaf species, which both include high levels of soluble carbon. In addition, these results may be a further consequence of a generalist buffering the community from changes in input quality, and removal of pouch snails from our communities may result in greater antagonism among responses.

The lack of non-additivity among other responses is surprising given the abundance of antagonistic and synergistic effects arising from mixed litter assemblages in lotic ecosystems (reviewed in Lecerf et al. 2007, Lecerf and Richardson 2009, Kominoski et al. 2009, but see Srivastava et al. 2009). Although this may be a consequence of differences between lotic and lentic ecosystems, our lack of non-additivity may also be due to the high diversity of our mixed litter assemblage. Most non-additive responses are observed with low species richness relative to our mixture treatment, allowing a higher representation of individual litter species. Indeed, our findings were similar to those found in pond mesocosms when mixtures were composed of highly diverse assemblages of litter species (Stoler and Relyea 2011). In reality, many temperate and boreal forests are relatively low-diversity assemblages, with several rare species often present (Braun 1950). Non-additivity may occur in such scenarios, particularly if individual litter species have a relatively strong influence on ecological function (Wardle et al. 1997).

2.4.4 Insights from a trait-based approach

Although much can be learned by comparing litter species, we were able to gain additional insight by taking a trait-based approach to the effects of leaf litter on aquatic communities. The association between litter traits and responses in our study illustrates the complex influence of litter chemistry on ecological function in a multi-trophic system. Litter traits are differentially associated with individual responses and those associations change over time. As indicated by results of Monte Carlo analyses, the overall significance of litter trait-response relationships decreased as our study progressed. This suggests that pulses of litter exert variable and diminishing effects on pond communities over time. Such results have appeared before in the

literature (e.g., Moran and Hodson 1989) and are likely due to an increasing percentage of relatively non-influential, recalcitrant compounds in later stages of litter decay (Melillo et al. 1982). An alternative explanation is that unmeasured traits may exert some control during later stages. Although multiple other components of litter chemistry have occasionally been published as important drivers of ecological function (Epps et al. 2007), the broad traits that we used are the most often cited determinants of litter palatability and quality (Taylor et al. 1989; Ardón and Pringle 2008). Our study demonstrates the usefulness of these traits in determining multi-trophic community composition and productivity.

Among all litter traits, soluble carbon substantially contributed to the primary ecological gradient on all sample dates and to the secondary gradient for amphibian responses. Furthermore, many responses had large loadings on the same axis as soluble carbon, suggesting that this single factor exerted substantial control over community attributes throughout the experiment. Gradients of DOC, which generally arise from inputs with variable amounts of soluble carbon, are known to regulate ecosystem function in larger lentic systems by reducing ultraviolet radiation, binding to contaminants, and providing valuable sources of organic energy through microbial mineralization (Williamson et al. 1999; Wetzel 2001). Under low to moderate levels of DOC, phytoplankton benefit from this source of energy whereas benthic primary production is generally limited by increased light attenuation (Klug 2002; Karlsson et al. 2009). At sufficiently high levels, soluble carbon may pose a direct toxicity risk to some species (Horne and Dunson 1995) and attenuate enough light to shade out phytoplankton. Under these same conditions, increased microbial biomass and lower photosynthetic rates are likely associated with reduced dissolved oxygen (Klug 2002). Oxygen levels found among some treatments (e.g.; TP, RM) were likely to cause substantial fitness costs for many species, particularly amphibians (McIntyre

and McCollum 1999). However, many species may also exhibit adaptations to such severe conditions and changes in bottom-up resource supply (McIntyre and McCollum 1999, Horne and Dunson 1995, Schiesari et al. 2009) and some tadpoles were observed to bob to the surface for air when oxygen was particularly low. However, such conditions surely impose a strong selective filter on most animals.

Total phenolic content of litter, which may act as a similar filter (Ardón and Pringle 2008), substantially contributed to the secondary gradient for the first and third sample dates, and to the primary gradient for amphibian responses. Generally, responses were negatively correlated with this gradient, although phytoplankton may be an exception. Phenolic acids are often implicated as deterrents to microbial growth in aquatic systems (Ardón and Pringle 2008) and thereby reduce resources for microbivores. Phytoplankton populations are also negatively influenced by inputs of phenolic acids, although low levels may be beneficial (Herrera-Silveira and Ramirez-Ramirez 1996). Zooplankton sensitivity to phenolics is largely unknown, and our study suggests that zooplankton are not affected. Anecdotal evidence also suggests that zooplankton species are among the least sensitive aquatic animals to phenolics (DeGraeve et al. 1980). Among the few, higher-level organisms that have been experimentally challenged with phenolics, amphibians appear to be among the most sensitive (Maerz et al. 2005). Our study supports this trend; while phenolics were only of secondary importance for most species in our communities, they constituted the primary ecological gradient in the analysis of amphibian responses.

The ratio of carbon to nitrogen in litter was less important in associations with amphibian responses, but it did load strongly onto at least one axis for the first three sample dates. For the first two sample dates, its relevance to the ecological gradients and to community responses was

nearly identical to that of phenolics. Increases in either chemical trait are likely to result in inhibitory responses, either due to increased toxicity or decreased nutritional quality. Elevated C:N is regularly implicated in reducing litter quality, slowing litter decomposition, and reducing nutrient cycling (Melillo et al. 1982; Scott and Binkley 1997). This trend was further supported on the third sample date when C:N was negatively associated with the majority of biological responses. In addition, although the associations were relatively weak, it is worth noting that similar negative associations between C:N and biological responses were found on the fourth sample date and for nearly all amphibian responses. Hence, our study find support that C:N does play a significant role in aquatic production (Moran and Hodson 1989), yet our study also suggests that other chemical traits may be more important to community dynamics.

Of those additional traits, decomposition rate was also a substantial component in our analyses. As an independent gradient, it was most strongly associated with amphibian responses and constituted the primary ecological gradient. Interestingly, the majority of responses associated with spring-breeding species were associated with the gradient of soluble carbon while summer-breeding species responses were associated with decomposition rate. These associations were likely the result of the strong filter that soluble carbon appeared to exert on communities early in the experiment that dissipated as the season progressed. Decomposition rate, which comprehensively describes the quality of litter throughout a season, was more closely associated with responses after the effects of soluble carbon dissipated. In agreement with this finding, Prescott (2005) suggested that decomposition rate may not be the most important factor in determining forest production and individual chemical attributes may actually provide more useful predictive power.

A correlation of decomposition rate with consumer production is a novel and important finding based on past literature. Certainly, the presence of litter can promote secondary production (Vitousek 1982; Wallace et al. 1997) and thousands of studies have elucidated the complex factors that regulate decomposition rate. However, few studies have linked these two research foci to examine how differences in litter quality and decomposition rate alter production in litter-based food webs (Marcarelli et al. 2011). The few studies that have examined the influence of individual litter species on consumers have found that consumer growth and density on or around litter either lacks strong correlation with decomposition rate or is best correlated with complex combinations of chemical components (e.g., Sayer 2006). Moreover, as our study suggests, some types of compounds released from litter are not necessarily beneficial for consumer growth and survival. Perhaps it is only when those compounds dissipate, does litter decomposition rate become a useful predictor of system productivity.

2.4.5 Consequences to ecological function and forest change

Our study suggests that shifts in the composition of North American temperate from past and ongoing human-driven disturbances are likely to influence pond community composition and function. For example, American chestnut has been forced into rarity and local extinction throughout much of its original range by an invasive fungal disease, and replaced by oaks (Moser et al. 2009). Our study suggests that this turnover was associated with increased pond clarity and dissolved oxygen with simultaneous changes in species composition and biomass production, particularly among snail species. Similarly, Dutch elm disease and emerald ash borers threaten most northeastern populations of elm and ash and many populations have already experienced

substantial mortality (Moser et al. 2009). Our study suggests that the loss of either species may be associated with the loss of amphibian and snail biomass, yet the actual effects will depend on which tree species replaces them. Similar effects may occur as oak and beech stands are taken over by red maple and cherry due to overbrowsing by deer (Abrams 1998, 2003).

Concurrent with these widespread changes are local losses of tree species resulting from selective and non-selective logging for timber harvesting, agriculture, and other industries (Abrams 2003). Although conservation and management practices are continually improving, this activity is often conducted with little regard for the fate of the pond ecosystems that exist in the forests. Like previous studies, our current experiment indicates that the loss of litter subsequent to logging is likely to dramatically shift the function of ponds. However, unlike previous studies our data suggests that the direction and magnitude of this shift is highly dependent on what species of tree is lost. This finding suggests that the influence of canopy cover, which is often regarded as a critical gradient in structuring aquatic communities (Werner and Glennemeier; Skelly et al. 2002), may be mediated by the species generating that cover. Indeed, manipulations of resource availability conducted alongside manipulations of canopy cover rarely consider the nutritional quality of material lost following reductions in canopy cover. For the sake of improved conservation and management practices, future work should elucidate the interaction between canopy cover and changes in quality of resources inputs to wetlands relative to the quality of inputs lost.

Anthropogenic influences are certainly not the only mechanism by which forest composition changes, and shifts in tree species also occur as a result of natural succession towards climax assemblages (Braun 1950). Our study suggests how pond community dynamics likely change with forest succession. For example, species including bigtooth aspen, black

willow, and tulip poplar are early-succession species that are ultimately replaced by climax assemblages. Although these species may be an ephemeral stage in forest succession, the duration of their presence is extends well beyond the generation times of the species living in the ponds. Of those tree species in our study typically associated with primary succession, tulip poplar has the most pronounced influence on pond dynamics, with extreme losses of water clarity, dissolved oxygen, and consumer production. In contrast, the nutrient-rich chemistry of black willow litter was associated with high primary production and substantially greater survival of spring breeding amphibians.

Although it is useful to consider the impacts of individual litter species, diverse assemblages of species are typically the rule. Responses from our mixture treatment serve to indicate that the combined effects of multiple litter species on pond systems may be simply additive, in contrast to the non-additivity predicted by prior studies in streams and terrestrial systems (Lecerf et al. 2007, Lecerf and Richardson 2009, Kominoski et al. 2009). However, we did find important exceptions to this additivity, indicating that the diversity of tree species can have important consequence for some pond inhabitants, particularly the highly generalist grazers such as pouch snails. Future work should concentrate on three goals: first, we need to understand what components of food webs are responsive to litter mixtures; why these components are sensitive; and how their presence influences the responses of other species to litter mixtures. Second, realistic mixtures are frequently comprised of rare and dominant species, and recent studies suggest that species evenness is as important, if not more important, to ecological function than species richness (Hillebrand et al. 2008, Swan et al. 2009). In the context of litter inputs, it is possible that the biomass of any single litter species in mixture is not linearly related to its biological effect. Finally, the effect of single species in relation to the chemistry of the

other species in mixture deserves attention. For example, chemical dissimilarity among litter species is hypothesized to generate non-additive effects (Kominoski et al. 2009). Recent innovations in statistical methodology may offer logistically feasible mechanisms for exploring these complex questions, including the use of multivariate trait-space to illustrate the characteristics of species mixtures with simple indices (Schleuter et al. 2010).

2.4.6 Conclusions

Despite decades of literature emphasizing the importance of litter inputs to aquatic systems, our study is among the first to trace the impacts of litter through multiple trophic levels in pond ecosystems. While the assumed role of litter inputs is to increase heterotrophy (Rubbo et al. 2006), our results suggest that the actual effect of litter on system production is species-dependent. This result is important given the growing acceptance that litter quality can influence function as much as or more than litter quantity. Measuring quality is not straightforward, as litter species are complex combinations of multiple chemical factors (Epps et al. 2007). Advanced statistical techniques, such as the ordination analyses demonstrated in our study, will be needed to fully interpret the effects of litter chemistry on ecological function.

Taken together, our study provides a unique perspective of how inputs of leaf litter alters phytoplankton, periphyton, and consumer production in ponds. Future work should aim to elucidate the specific changes in nutrient and energy flow resulting from qualitative changes in litter inputs. This will require tracing elemental compounds through the environment (Scott and Binckley 1997). Stoichiometric changes among primary and secondary producers, which are increasingly found in a variety of systems, may also be provide deeper and valuable insight into

the effects of litter quality on nutrient cycling in detritus-based systems (Sterner and Elser 2002). In addition, tracing elemental compounds within the environment may be just as important as tracing them out of the environment. On a regular basis, lentic ecosystems transform litter inputs into massive pulses of CO_2 and outflow of inorganic compounds (Lennon 2004). Changes in litter quality, as mediated by the responses demonstrated in our present study, are likely to substantially alter the magnitude and duration of those pulses. Similarly, emergent organisms can have a substantial impact on the fertility of riparian habitat through multiple pathways (Dreyer et al. 2011). In particular, amphibians process an enormous amount of nutrients on land and are an important prey item for many terrestrial predators (Beard et al. 2002). Our study offers substantial insight into what may occur along these important functional pathways and future work should explore these pathways.

2.5 ACKNOWLEDGEMENTS

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3.0 LEAF LITTER QUALITY INDUCES MORPHOLOGICAL AND DEVELOPMENTAL CHANGES IN LARVAL AMPHIBIANS

3.1 INTRODUCTION

Variation in environmental resources can have profound effects on the fitness of an individual. Resource limitation can promote competition while hindering development, growth, and other physiological processes (Price 1992). As a means of improving fitness, organisms frequently exhibit resource-induced phenotypic changes (i.e. phenotypic plasticity; Agrawal 2001, Weiner et al. 2004, Pigliucci 2005). For example, to improve resource use efficiency, many plant species growing in resource-limited environments alter growth rates and resource allocation strategies, including changes in allocation to root versus shoot growth (Weiner 2004). Similarly, many animal species can alter behavior, morphology, development, and life history traits; examples include insects (Bernays 1986, Greene 1989, Thompson 1992, Reiskind et al. 2009), fish (Day et al. 1994), and amphibians (Walls et al. 1993, Relyea 2002). These phenotypic changes are likely adaptive responses that improve individual performance, affect ecological interactions, and may lead to species diversification (Agrawal 2001, Miner et al. 2005).

Phenotypic responses to resource fluctuations are often studied in the context of variation in resource quantity (i.e. changes in competition), but resource fluctuations can occur due to changes in resource quality (Thompson 1992, Marcarelli et al. 2011). In many systems, resources

are derived from both inorganic and organic sources whose quality is a function of their chemical composition. *In situ* changes in production or changes in resource inputs from surrounding ecosystems (i.e. resource subsidies; Polis et al. 1997) can lead to both quantitative and qualitative resource variation. Resource chemistry is determined by numerous factors, including biological causes (e.g., changes in resource stoichiometry) and abiotic causes (e.g., rainfall, temperature) and it can change independently of resource quantity (Marcarelli et al. 2011). Several studies have found that the effects of different resource chemistry on individual phenotypes can be substantial, particularly for morphological traits (Greene 1989, Thompson 1992, Day et al. 1994), and may have significant implications for ecological interactions (Greene 1989). Hence, discerning how chemical variation in resources alters phenotypes can greatly improve our understanding of how organisms respond to environmental variation.

Plant litter represents a resource in terrestrial and aquatic ecosystems that can vary in both quantity and quality. Whereas litter quantity is simply a function of how much litter is produced, litter quality varies due to interspecific and intraspecific variation in tissue chemistry that remains after senescence (Ostrofsky 1993, Webster and Benfield 1986). Such variation can have important effects on litter-based food webs, which often contain diverse communities of microbes and larger consumers that mineralize and process the nutrients of litter (Facelli and Pickett 1991). For example, elevated nutrient content in litter can promote microbial growth, whereas increased concentrations of structural (e.g., lignin, cellulose) or toxic compounds (e.g., phenolics) can slow or inhibit such growth. Although the effects of litter quality on ecosystem-level processes (e.g., decomposition, nutrient cycling) are well studied (Marcarelli et al. 2011), less attention has been given to the effect on individuals within such food webs.

Moreover, when the effects of litter quality on individuals are considered, the focus is commonly on the survival and growth of individuals. However, changes in litter quality might also alter many other traits of consumers—such as morphological traits—and do so in ways that could represent adaptive responses, similar to how changes in living plant chemistry are known to influence herbivore morphology (Bernays 1986). Despite the potential importance of such changes, there appears to have only been one study that has ever examined how senesced leaf litter alters the morphological traits of consumers. In that study, Reiskind et al. (2009) found that adult mosquitoes developed different wingspans when larvae were fed different litter types.

Recently, there has been growing interest in examining how differences in leaf litter species and chemistry affect the survival and growth of wetland organisms. Much interest has surrounded larval amphibians, which feed off microbial and algal communities growing on litter surfaces (i.e. periphyton; Altig et al. 2007, Schiesari 2006). To date, the focus of this work has been on the survival and growth of consumers in the system (Rubbo and Kiesecker 2004, Maerz et al. 2005, Williams et al. 2008, Stoler and Relyea 2011, Cohen et al. 2012). For example, Cohen et al. (2012) found that tadpole growth was positively related to litter nitrogen (N) content whereas Maerz et al. (2005) found that increased concentrations of polyphenols in litter can have severely adverse effects on tadpole survival. Such effects may be due to changes in the nutritional quality of litter resources (Cohen et al. 2012), or more direct effects of changing aquatic chemistry (e.g., from leached soluble carbon and phenolics; Horne and Dunson 1995, Maerz et al. 2005). However, there has never been an investigation of whether manipulations of litter species or chemistry can induce morphological changes in tadpoles.

Although there has been no examination of litter-induced changes in tadpole morphology, there has been a great deal of work examining how tadpole morphology changes in response to

resource quantity, predation risk, and pesticides (Relyea 2000, 2002, Relyea and Auld 2004, 2005, Relyea 2012). Wood frog tadpoles (*Lithobates sylvaticus* [*Rana sylvatica*]) are particularly well studied for their response to reductions in per-capita resource quantity; lower resources induce slower growth and development, and higher foraging activity. Morphologically, lower resource quantity induces relatively smaller tails, larger bodies, longer intestines, and wider mouths, although the magnitude of response depends on the presence of predation risk (Relyea 2002, Relyea and Auld 2004, 2005). These morphological changes appear adaptive, as they improve the growth performance of tadpoles (Relyea 2002) likely due to increased assimilation and growth efficiency (Sibly 1981, Wassersug and Yamashita 2001). Given the variety of morphological responses to variation in resource quantity, it is reasonable to ask if tadpoles also have the ability to alter their morphology in response to variation in resource quality.

In this study, we investigated whether tadpole consumers can respond to changes in leaf litter quality by altering their internal and external morphology. Using six litter species that varied in nutrient content, recalcitrance, and toxin content, we analyzed the species-specific effects of each litter species and the effects of individual litter chemical components. To investigate how responses to litter chemistry interact with resource quantity, we also manipulated litter species at two densities of tadpoles. We predicted that tadpoles given litter with high N will exhibit morphological responses similar to tadpoles experiencing low competition (e.g., shorter intestines, smaller bodies, and larger tails). In contrast, we predicted that tadpoles given litter with elevated phenolic content or lignin (i.e. structural compounds) will exhibit morphological responses similar to tadpoles experiencing high competition. Regarding effects of density, we predicted that decreasing per-capita resource supply would increase the magnitude of phenotypic responses to litter species.

3.2 METHODS

3.2.1 Experimental design

Our experiment was conducted at the Pymatuning Laboratory of Ecology in northwest Pennsylvania. The experiment used a completely randomized design with six leaf litter species treatments crossed with two tadpole densities. To increase the applicability of our work with regard to realistic changes in resource chemistry, we used litter species that are dominant in eastern North America and common to areas where wood frogs breed in northeastern temperate forests: American sycamore (SYC), bigtooth aspen (ASP), black willow (BW), sugar maple (SM), red pine (RP), and white pine (WP; Table 3.1). All species vary substantially in multiple aspects of litter chemistry, including total N, total phenolic content, and total lignin, thereby allowing us to determine the specific components of litter chemistry that are responsible for morphological changes. Each of the 12 treatment combinations was replicated four times, for a total of 48 experimental units.

The experimental units were 100-L outdoor, plastic mesocosms covered by a 60% shade mesh cloth to simulate a moderate amount of canopy cover and prevent entrance of unwanted organisms. Mesocosms were filled with well water on 6 May. We then introduced microbes, algae, and zooplankton to each mesocosm by providing an aliquot of water taken from five nearby wetlands. A small amount of rabbit chow was provided to each mesocosm as a form of nutrients to accelerate growth of microfauna.

We added leaf litter to the mesocosms on 7 May. We collected litter immediately after senescence during the autumn prior to the experiment and allowed it to dry indoors during the

winter in an unheated facility. We placed 100 g of litter into each mesocosm. This provided a litter density within the natural range for the northeastern United States (Rubbo et al. 2008) and a similar density relative to previous experiments (Stoler and Relyea 2011). After adding litter, we allowed periphyton, phytoplankton, and algae to develop for 2 wks before tadpoles were added.

We collected the wood frogs as 10 egg masses from a local wetland and placed all masses in wading pools containing aged well water where they hatched and were then fed rabbit chow *ad libitum*. After reaching stage 25 (Gosner 1960) and a safe handling mass (66.8 mg; 1 SE = ±3.4), we added tadpoles to mesocosms on 23 May (hereafter, day 0). We mixed tadpoles from all egg masses and placed 20 and 40 individuals in low and high density treatments, respectively. This established natural densities of tadpoles and replicated the two lower experimental densities of Relyea and Auld (2004, 2005). Twenty additional tadpoles were selected haphazardly to assess 24-hr survival, which was 100%.

Tadpoles developed in mesocosms until day 23, at which time we collected and euthanized all surviving individuals and preserved them in 10% formalin. We stopped development of tadpoles at this time because several individuals were at Gosner stage 41. At this stage, tadpole body mass reaches a peak and is soon followed by metamorphosis.

We digitally imaged all preserved tadpoles from the low-density treatments, and 20 randomly selected individuals from the high-density treatments. Because survival was high across all treatments, we were able to image at least 15 individuals per mesocosm. We took separate pictures of the right lateral side, oral disc, and uncurled intestines. For images of the lateral side, we ensured that the tail was on the same focal plane as the body in the image by propping the tail on top of a glass slide to so that the center line of the individual was parallel with the focal plane of the camera.

From these images, we made morphological measurements using ImageJ (Version 1.6.0_20, NIH). We chose to conduct linear measurements instead of landmark-based geometric measurements (e.g., Van Buskirk 2011) because linear dimensions are often easier to visually interpret and both methods often provide the same general illustration of body shape. We began by measuring several dimensions on the right side of the body. We made five measurements identical to those made in Relyea (2001): body length, body depth, tail length, tail depth, and tail muscle depth. Next, we measured several dimensions of the oral disc. We imaged the oral disc after forcing the mouth open by pinning down the lower labium. For mouthparts, we traced the length of each denticle row excluding any gaps in keratinization and denticle structure. As is common for wood frogs, particularly among individuals under high competition (Relyea and Auld 2004), the fourth denticle row was frequently missing or lacked keratinization. When this occurred, the length of this denticle row was given a measurement of zero. The total keratinized length for each denticle row was summed into a single measure. We also measured the width of the beak and traced the length of the lower beak edge. Finally, we dissected the intestines, and measured intestine length by tracing the entire length of the intestines from the end of the lower stomach to the beginning of the colon.

To elucidate potential chemical mechanisms underlying changes in tadpole growth, development, and morphology, we assessed three key components of litter chemistry: total N, percentage of total phenolics, and percentage of total lignin. We also analyzed total phosphorous, but this was highly correlated with total N, so we dropped it from our analysis. Details regarding the chemical analyses can be found in Appendix A.

Table 3.1. The leaf litter species used in the experiment, including common names, abbreviations, and family. Values for total lignin, total phenolics, and total nitrogen are mean values based on analyses that were performed in triplicate.

Treatment	Abbreviation	Family	Species	Lignin (%)	Phenolics (%)	Nitrogen (%)
American sycamore	SYC	Platanaceae	*Platanus occidentalis*	24.0	0.5	1.0
Bigtooth aspen	ASP	Salicaceae	*Populus grandidentata*	23.9	0.2	0.9
Black willow	BW	Salicaceae	*Salix nigra*	14.9	1.0	1.0
Red pine	RP	Pinaceae	*Pinus resinosa*	7.7	1.0	0.4
Sugar maple	SM	Aceraceae	*Acer saccharum*	7.3	2.1	0.7
White pine	WP	Pinaceae	*Pinus strobus*	20.5	0.2	0.6

3.2.2 Statistical analysis

Mass and all morphological dimensions were log-transformed to fit a normal distribution prior to all analyses, and morphological dimensions were mass-adjusted (see Appendix B). During digital analysis, all images from one high-density replicate of red pine were lost, and it was not possible to re-image them because the tadpoles had already been dissected. This replicate was removed from all analyses. Preliminary analysis revealed no significant differences in survival among the 12 treatment means, which ranged from 92 to 100%.

Prior work has demonstrated that the numerous dimensions of the oral disc are typically correlated and can therefore be simplified with ordination analysis without significant loss of information (Relyea and Auld 2005). Following mass-adjustments, we included all mouth dimensions in a principal components analysis (PCA). The first axis explained 71% of the variation, so we used the scores associated with axis as a single response variable (hereafter, "mouth size") in place of all mouthpart dimensions.

As a result of these analyses, our dataset included individual mass, developmental stage, and seven mass-adjusted morphological measurements: intestine length, mouth size, body length, body depth, tail length, tail depth, and muscle width. We also attempted to reduce external body dimensions using PCA, but the resulting axes did not produce interpretable gradients. Consequently, we retained all external body dimensions as separate response variables in our analysis. In all cases, we used the mean responses from a mesocosm as our response variables. Preliminary analyses revealed that mass-adjustment of linear dimensions also removed any correlations between developmental stage and linear dimensions, and that adding development stage as a covariate in our analyses did not change the interpretation of our results.

We analyzed the effects of density and litter species on the nine response variables using a multivariate analysis of variance (MANOVA) with litter species and density as fixed effects in a full-factorial model. Upon finding a significant multivariate effect, we conducted univariate analyses. For significant univariate effects of litter, we conducted Tukey's post-hoc pairwise comparisons to determine treatment differences.

To assess the effect of litter chemistry on growth, development, and morphological dimensions at different density levels, we conducted a multivariate multiple regression analysis on mesocosm means of phenotypic responses. Preliminary analysis revealed that all regressions were best fit by a linear model. Thus, we employed the general linear model (GLM) procedure in SPSS, using a model that included density as a fixed factor, total N, total lignin, and total phenolics as covariates, and the nine response variables as dependent variables. The model included all main effects and all three possible interactions of density with the covariates. Upon finding a significant multivariate effect, we conducted separate univariate Pearson correlation analyses to determine correlation coefficients.

3.3 RESULTS

3.3.1 Effects of litter species and tadpole density on tadpole morphology

We found a significant multivariate effect of litter species, tadpole density, and their interaction on mass, development, and relative morphology of tadpoles (Table 3.2). As a result, we conducted univariate ANOVAs on each response. When we detected a litter species-by-density interaction, we conducted separate univariate ANOVAs within each density treatment.

The mass of individual tadpoles was marginally affected by litter species, and significantly affected by density, and their interaction (Table 3.2, Figure 3.1A). At low density, litter species affected mass ($F_{5,18} = 5.442$, $P = 0.003$). Mean comparisons indicated that tadpoles raised with SYC ha 19 to 25% more mass than any other treatment ($P \leq 0.042$). At high density, litter species had a marginal effect on mass ($F_{5,17} = 2.722$, $P = 0.055$); mass in BW tended to be greater than in RP, yet there were no significant differences among the pairwise comparisons ($P \geq 0.068$). Relative to low density treatments, individuals at high density were an average of 30% less massive across all litter treatments.

The developmental stage of the tadpoles was affected by litter species, density, and their interaction (Table 2; Figure 3.1B). Litter species affected developmental stage at low density ($F_{5,18} = 6.585$, $P = 0.001$), but not at high density ($F_{5,17} = 1.865$, $P = 0.154$). At low density, tadpoles in WP were one to two developmental stages behind individuals in SM, BW, and SYC ($P \leq 0.022$). Additionally, tadpoles in RP were about one stage behind individuals in SYC ($P = 0.017$). Relative to low density treatments, developmental stage decreased at high densities among SM, ASP, and SYC treatments (1.1 to 3.5%), whereas stage increased slightly (1.3%) in WP.

Tadpole mouth size was affected by litter species and density but not their interaction (Table 2; Figure 3.2A). Averaged across both density treatments, tadpoles in the SYC treatment developed larger mouths than individuals in all other treatments ($P \leq 0.048$). In addition, tadpoles in the BW treatment developed larger mouths than in the WP treatment ($P = 0.003$). Averaged across all litter treatments, mouth size was larger at high density than at low density. Intestine length was affected by litter species, density, and their interaction (Table 1; Figure 3.2B). At low density, litter species affected intestine length ($F_{5,18} = 3.686$, $P = 0.018$); tadpole

intestines in the BW treatment were 13 to 14% shorter than in the RP and WP treatments, respectively ($P \leq 0.038$) and 12% shorter than in the SYC treatment ($P = 0.068$). At high density, litter species had a marginal effect ($F_{5,17} = 2.754$, $P = 0.053$); intestines were 12% shorter in the ASP treatment than in the SYC treatment ($P = 0.038$). Relative to low density treatments, intestines increased in length among all treatments, yet this increase was subtle ($\leq 3.5\%$) among ASP, SP, and WP treatments while intestinal length increased by 12, 13, and 20% among SYC, SM, and BW treatments, respectively.

Body length and depth were affected by litter species and density, but not their interaction (Table 2; Figure 3.3A,B). Averaged across the density treatments, tadpole bodies in the SYC treatment were 2.7 to 2.9% longer than in the RP or SM treatments ($P \leq 0.054$). In the WP, RP, and SYC treatments, individuals had 3.4 to 5.3% deeper bodies than in the ASP or BW treatments ($P \leq 0.026$). Additionally, bodies in SM were 3.9% deeper than in BW treatments ($P = 0.002$). Averaged across all litter treatments, bodies were 4.2% longer and 2.2% deeper at high density than at low density.

Tail length, tail depth, and tail muscle width were affected by litter species and density, and there was a marginal litter-by-density interaction on tail length (Table 2; Figure 3.3C-E). Regarding tail length, litter species had an effect at both densities (low density: $F_{5,18} = 10.039$, $P < 0.001$; high density: $F_{5,17} = 2.876$, $P = 0.046$); At low density, tails in the BW treatment were 8.0 to 10.9% longer than in the WP and RP treatments ($P \leq 0.004$). Tails in ASP were 8.6% longer than in RP ($P = 0.002$) and 5.7% longer than in WP ($P = 0.059$). At high density, mean comparisons failed to reveal any significant differences among treatments ($P \geq 0.086$). Relative to low density treatments, tail length of individuals decreased 1.2 to 4.2% among SM, ASP, SYC, and BW treatments while tail length increased 2.9% with RP.

Regarding tail depth, tadpoles in the SM, ASP, and BW treatments had 4.3 to 6.3% deeper tails than in RP and WP ($P \leq 0.040$) when averaged across both density treatments. Averaged across all litter treatments, tails were 2.2% deeper at low density than at high density.

Regarding tail muscle depth, tail muscles were 5.6% wider in the BW treatment than in the SYC treatment ($P = 0.046$) and slightly deeper than in the SM treatment ($P = 0.073$) when averaged across both density treatments. Averaged across all litter treatments, tail muscles were 5.0% deeper at low density than at high density.

Table 3.2. Results of a MANOVA and subsequent ANOVAs on mass, development stage, and seven mass-adjusted morphological dimensions of wood frog tadpoles. All measurements were performed on preserved tadpoles that were raised in mesocosms for 23 days. The term "mouth size" represents the first axis of a PCA conducted on 10 dimensions of the oral disc.

	Litter species			Density			Litter species x density		
	F	df	P	F	df	P	F	df	P
MANOVA	5.221	45,124	<0.001	15.903	9,27	<0.001	1.963	45,124	0.002
Univariate effects									
Mass	2.364	5,35	0.060	1.499	1,35	<0.001	5.421	5,35	0.001
Development stage	5.412	5,35	0.001	4.155	1,35	0.049	2.220	5,35	0.074
Mouth size	11.876	5,35	<0.001	42.195	1,35	<0.001	1.255	5,35	0.305
Intestines	3.564	5,35	0.010	26.190	1,35	<0.001	2.917	5,35	0.026
Body length	2.543	5,35	0.046	61.029	1,35	<0.001	1.033	5,35	0.414
Body depth	11.307	5,35	<0.001	16.612	1,35	<0.001	0.893	5,35	0.496
Tail length	11.515	5,35	<0.001	4.791	1,35	<0.001	2.301	5,35	0.066
Tail depth	8.029	5,35	<0.001	8.277	1,35	0.007	0.339	5,35	0.886
Tail muscle depth	2.813	5,35	0.031	19.782	1,35	<0.001	0.865	5,35	0.514

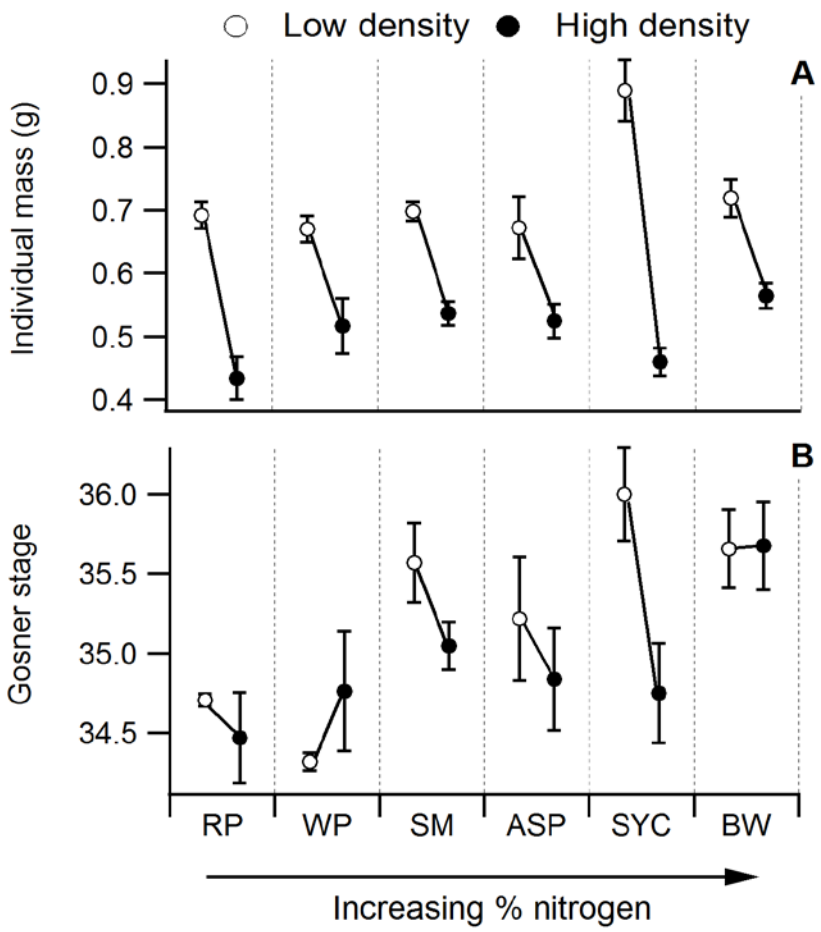

Figure 3.1. Individual mass (a) and Gosner stage (b) of wood frog tadpoles in six different litter treatments at two density levels. Responses were measured on tadpoles preserved on day 23 of the experiment. Litter treatments and abbreviations are found in Table 1. Data are means ± 1 SE.

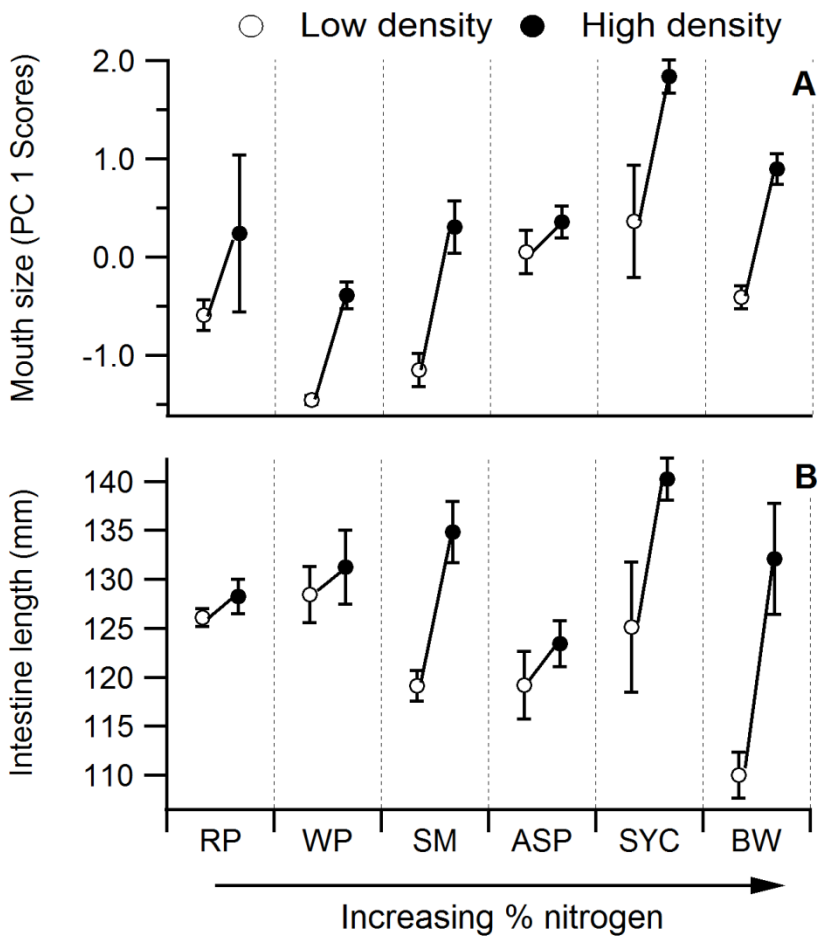

Figure 3.2. Mass-independent mouth size (a) and intestine length (b) of wood frog tadpoles in six different litter treatments at two density levels. Responses were measured on tadpoles preserved on day 23 of the experiment. Mouth size data represent principal component scores of a single axis that explain the majority of variation among 10 mass-independent measurements of the oral disc. Litter treatment abbreviations are found in Table 1. Data are back-transformed means ± 1 SE.

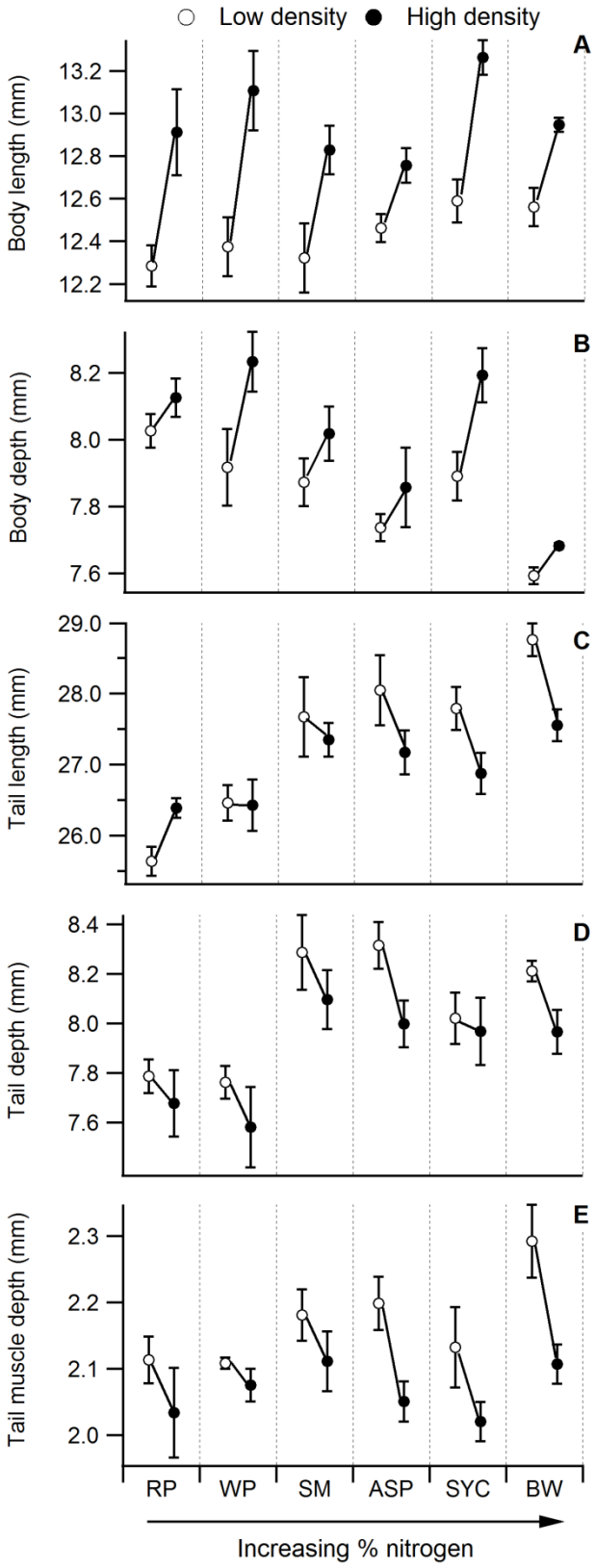
Low density
High density
A
Body length (mm)
13.2
13.0
12.8
12.6
12.4
12.2
B
Body depth (mm)
8.2
8.0
7.8
7.6
C
Tail length (mm)
29.0
28.0
27.0
26.0
D
Tail depth (mm)
8.4
8.2
8.0
7.8
7.6
E
Tail muscle depth (mm)
2.3
2.2
2.1
2.0
RP
WP
SM
ASP
SYC
BW
Increasing % nitrogen

Figure 3.3. Mass-independent body length (a), body depth (b), tail length (c), tail depth (d), and tail muscle width of wood frog tadpoles in six different litter treatments at two density levels. Responses were measured on day 23 of the experiment. Litter treatment abbreviations are found in Table 1. Data are back-transformed means ± 1 SE.

3.3.2 Relationships between tadpole phenotypes and litter chemistry

When we tested for relationships between tadpole phenotypes and the chemical traits of the six litter species, we found significant multivariate effects of density ($F_{9,31} = 11.601$, $P < 0.001$), total N ($F_{9,31} = 12.892$, $P < 0.001$), lignin ($F_{9,31} = 2.699$, $P < 0.019$), a marginally significant effect of phenolics ($F_{9,31} = 2.087$, $P = 0.062$), and a significant density-by-N interaction ($F_{9,31} = 2.268$, $P = 0.044$). We did not find significant density-by-lignin or density-by-phenolic interactions ($P \geq 0.717$).

We then examined the univariate regression coefficients (Table 3). Because of the density-by-N interaction, we conducted univariate regression analyses on the effects of N within each density level. At low density, there were significant negative relationships of N with intestine length and body depth; there were significant positive relationships of N with development stage, mouth size, tail depth, tail length, body length, and tail muscle depth. At high density, N was positively related to mouth size, tail length, and tail depth, and negatively related to body depth. For the percentage of total lignin, there were no significant univariate relationships with any response variable. For the percentage of total phenolics, there was a negative relationship with tail depth across both densities.

Table 3.3. Univariate regression coefficients of the correlation between three litter chemical components (total nitrogen, total lignin, total phenolics) with nine developmental and morphological responses of wood frog tadpoles. Because there was a significant interaction of density with nitrogen, coefficients at both density levels are provided.

	Nitrogen			
Measurement	Low Density	High Density	Lignin	Phenolics
Mass	0.378	-0.288	0.047	0.037
Development stage	**0.664**	0.368	0.037	0.216
Mouth size	**0.531**	**0.590**	-0.114	-0.122
Intestines	**-0.487**	0.144	0.045	-0.025
Tail depth	**0.592**	**0.483**	0.155	**0.290**
Tail length	**0.815**	**0.515**	-0.037	0.139
Body depth	**-0.608**	**-0.492**	0.226	-0.048
Body length	**0.453**	0.048	0.009	-0.151
Tail muscle depth	**0.448**	0.030	-0.007	0.176

Note: Coefficients in boldface are significant ($P < 0.05$).

3.4 DISCUSSION

While previous studies have demonstrated the effects of resource quantity on tadpole morphology (Relyea 2000, 2002, Relyea and Auld 2004, 2005), our study is the first to demonstrate that variation in resource quality can induce dramatic effects on tadpole phenotypes. All measured developmental and morphological responses exhibited at least marginally significant changes in response to the leaf litter treatments. In many cases, the magnitudes of changes caused by resource quality were equal to or greater than those induced by changes in resource quantity (i.e. competition).

3.4.1 Effects of litter quality on phenotypes

The primary question posed by this study is how litter quality influences tadpole phenotypes. Many responses could be generalized through correlations with litter chemistry, and particularly nutrient content. Litter species with greater N content (e.g. sycamore, black willow), which was positively correlated with litter P content, were associated with shorter intestines, larger mouths, longer and shallower bodies, longer and deeper tails, and deeper tail muscles. These correlations indicate wood frogs are capable of ingesting the nutrients in litter, either by direct litter consumption or grazing of microbial communities. Since the litter was generally un-fragmented by the end of the study, it is also likely that the majority of resources were microbial-derived. Interestingly, there was a positive correlation of litter nutrients with development rate, yet no correlation of mass with nutrients. This suggests that wood frog tadpoles use nutrients towards development instead of growth. Schiesari (2006) also found evidence of this trend, noting that

leopard frogs (*L.* [*R.*] *pipiens*) gained more mass than wood frogs when provided high N resources, while wood frogs developed faster than leopard frogs in the same conditions. Similarly strong effects of litter nutrients have also been noted in mosquitoes; Walker et al. (1997) noted that mosquito larvae (*Aedes triseriatus*) increased both development rate and body size with increasing litter N content.

Surprisingly, there were few correlations of total lignin or total phenolics with tadpole responses. This is interesting because past studies have demonstrated strong negative association between lignin and litter decomposition rate, which is largely regulated by the grazing of consumers (e.g., tadpoles) on the litter surface (Melillo et al. 1982, Aerts 1997, Swan and Palmer 2006). Moreover, studies have revealed negative effects of phenolic leachates on tadpole survival (Maerz et al. 2005). There are at least three potential explanations for non-significant effects of phenolics and lignins on tadpole phenotypes. First, wood frogs may be adapted to moderate amounts of phenolic leachates and generally poor-quality substrate; they are one of the few anuran species that consistently inhabits closed-canopy wetlands, which have high inputs of leaf litter and low primary production due to a high amount of shading from the overhead canopy (Werner et al. 2007). This hypothesis appears unlikely, as wood frogs are negatively impacted by dissolved organic carbon and low pH (Horne and Dunson 1995), which are both associated with high phenolic leachates. An alternative explanation is that lignin and phenolic content are inversely related to each other and subsequently counterbalanced their effects. However, there is no evidence for such a relationship in our study and such a relationship has not been reported in the literature. A more likely explanation is that the concentration of secondary compounds in the litter was not sufficiently high to elicit a response from the tadpoles. Previous studies demonstrating an effect of litter phenolic chemistry on tadpoles used litter of an invasive species

(*Lythrum salicaria*) with a dry weight consisting of over 20% phenolic content (Maerz et al. 2005, Brown et al. 2006). In contrast, the highest concentration of phenolic content among our native litter species was 2.1%. Given that litter phenolic content of most native, temperate deciduous tree species is generally between 0-2% (Ostrofsky 1993), our results suggest that the effects of phenolics in native litter species may be largely overshadowed by nutrient content.

3.4.2 Interaction of litter quality and density

Another central question of this study is how the effects of litter chemistry on tadpole phenotypes compare to the effects of per-capita resource quantity. One prediction is that the effects of increasing N content, decreasing lignin content, or decreasing phenolic content would parallel the effects of decreasing density on phenotypes. Although we found no correlation of phenotypic traits with lignin or phenolics, correlations with N provided mixed support for this prediction. For several phenotypic traits in our study, including developmental stage, intestinal length, tail depth, tail length, body depth, and tail muscle depth, responses to increasing litter N were in the same direction as decreasing density. For other phenotypic traits, including mouth size and body length, responses to increasing litter N were in the opposite direction as decreasing density. Moreover, most phenotypic responses exhibited a weaker response to litter N at high density. In addition, increasing density decreased tadpole mass while increasing litter N had no significant effect on tadpole mass at either density level. These results indicate that increased litter nutrient content generate many of the same phenotypic responses as decreasing density, however the relationship is not perfect. Reasons for this are unclear and warrant further research, such as an investigation of how tadpoles allocate nutrients at different densities.

It is worth noting that the interaction effects observed for several phenotypic responses were not merely due to changes in response magnitude, but also to changes in response direction. This suggests that tadpole development strategies depend on relative litter nutrient content and competitor density, in addition to the unique chemical composition of each litter species. For example, intestinal length and mouth size generally increased at higher densities, yet this was not the case for individuals in bigtooth aspen treatments. One explanation may be the relatively low phenolic and high N content of aspen leaves, which likely promoted microbial growth and efficient tadpole grazing, even at high tadpole densities. In contrast, the relative lack and nutrients and high recalcitrance of the two conifer litters (i.e. red and white pine) may explain the consistently long intestinal length, large body size, and short tail lengths in these treatments. As another example, the relatively high tadpole mass in sycamore may have been generated by distinctively large surface area of the litter species. Large surface area, combined with high N content, can promote microbial growth (Gunnarsson et al. 1988), may have reduced the energetic demands of tadpole foraging, and allowed energy to be used in other aspects of the phenotype.

3.4.3 Implications of results for changes in forest composition

Our study suggests that changes or heterogeneity in forest composition will have cascading effects on consumer phenotypes and potentially on consumer fitness. This is important, considering the numerous impacts that humans are currently exerting on forest structure and function. For example, sugar maple is undergoing a dramatic decline in abundance due to climate change, deer browsing, and other factors (Lovett and Mitchell 2004). Multiple species are likely to replace this, including red maple (*A. rubrum*), which differs substantially in chemistry and

may induce changes in wetland food webs (Stoler and Relyea *in review*). Natural succession in forests may also shift tree composition, through the replacement of fast growing tree species (e.g., pines and poplars) with more shade tolerant and slow growing species (e.g., maples, oaks; Abrams 1998). Our results indicate that wood frogs may cope with such changes through phenotypic plasticity, yet future research should elucidate whether such plasticity will influence ecosystem processes within wetlands (e.g., rate of litter decomposition) and across aquatic-terrestrial boundaries (e.g., organic subsidies to land). Such effects may provide a novel link between forest diversity and ecological function.

The importance of phenotypic changes will also depend on whether they are adaptive within and among ecological contexts. Although Relyea (2002) suggests that the phenotypic changes observed in our study may be adaptive, explicit tests of this with regard to litter-induced changes should be considered in the future. Moreover, many phenotypic changes were in the opposite direction to changes that wood frogs exhibit when challenged with predators (Relyea 2002), indicating potential maladaptation in the context of predator presence. Additionally, litter-induced phenotypic changes may not occur among amphibian populations or species less adapted to the litter-based conditions of closed-canopy wetlands. Further studies on the combined effects of litter chemistry and predation for wood frogs and other amphibian species should be conducted to fully elucidate the effects of changing forest composition on amphibian fitness.

3.4.4 Conclusions

Discussions of resource subsidies in ecosystems have focused on either quality or quantity, but rarely consider the impacts of both simultaneously (Marcarelli et al. 2011). This disconnect has

resulted in the use of separate analyses to uncover the effects of resource chemistry and quantity, and has led to little comparison of their effects. This is particularly the case with leaf litter; except for a few notable studies (e.g., Maerz et al. 2005) the majority of community-level studies have ignored the impacts of litter species variation even though ecosystem ecologists continually stress the importance of this variation for whole-ecosystem function (Scott and Binkley 1997, Aerts 1997). Our study is among the first to examine how litter quality alters consumer morphology, and the first study to examine the effects of litter quality on tadpole morphology. In doing so, we have shown that variation in litter chemistry can have an equal, if not greater, impact on individual-level processes than resource quantity. Future work should escalate this research to the community level, and attempt to understand how resource variation impacts food web structure and function.

3.5 ACKNOWLEDGEMENTS

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4.0 BOTTOM-UP MEETS TOP-DOWN: LEAF LITTER INPUTS INFLUENCE PREDATOR-PREY INTERACTIONS IN WETLANDS

4.1 INTRODUCTION

Ecological function is reliant on the flow of resource subsidies between food webs and on the biological processes that assimilate and process these inputs (Polis et al. 1997; Marcarelli et al. 2011). In food webs, the most common conceptual role of subsidies is the bottom-up supply of energy and nutrients that permit greater *in situ* production than internal resources would allow (Polis et al. 1997). However, resource subsidies also alter the structural complexity of an environment by generating microhabitat and altering other abiotic factors (e.g., water chemistry; Dobson et al. 1992; Richardson 1992; Moore et al. 2004). Both bottom-up forces and environmental changes can alter food web dynamics, particularly predator-prey interactions (Crowder and Cooper 1982). For example, the response of prey to the threat of predators is often mediated by bottom-up energy availability, and prey may be able to utilize changes to the environment as chemical or physical refugia (Flecker and Allan 1984; Carpenter et al. 2010; Evans et al. 2011). Consideration of subsidies as mediating predator-prey dynamics is rare, yet is critical to assessing the full impact of subsidies on ecological function.

Senescent plant tissue (i.e. litter) is one of the largest sources of coarse particulate subsidies (Polis et al. 1997) and ecological function is often reliant on the decomposition of this resource pool. This is particularly true in temperate forests where 70 to 90% of all terrestrial leaf tissue and substantial amounts of senescent leaf tissue and woody debris fall to the ground each year (Facelli and Pickett 1991). Much of this litter gravitates towards streams and wetlands containing heterotrophic food webs that rapidly decompose the litter (Marcarelli et al. 2011). These inputs, which frequently exhibit interspecific variation in both chemical and physical structure (Webster and Benfield 1986), can have positive bottom-up effects on prey growth through the provision of nutrients and energy (Wallace et al. 1997; Motomori et al. 2001; Leroy and Marks 2006; Stoler and Relyea 2011). In contrast, leachate from litter can contain detrimental levels of dissolved organic carbon (DOC) and other compounds such as phenolic acids that interfere with growth and development of prey and their predators (Horne and Dunson 1995, Maerz et al. 2005, Canhoto and Laranjeira 2007). Changes in growth can have substantial consequences for predator-prey dynamics. Higher growth can result in prey reaching size-refugia from gape-limited predators and possessing greater evasion speeds (Wilbur et al. 1983), whereas lower growth can make prey easier to catch.

Leaf litter inputs can also alter the physical and chemical environment in multiple ways that may directly interfere with predator-prey dynamics (Richardson 1992; Yee and Juliano 2006). First, litter inputs to wetlands persist as relatively stationary sources of microhabitat that can provide shelter and visual protection from predators (Richardson 1992; Dudgeon and Wu 1999). Indeed, in artificial wetland mesocosms a greater percentage of prey are often found hiding in litter when predators are present (e.g., Hoverman and Relyea 2008). Second, leachate from litter inputs can darken the water (Karlsson et al. 2009), which may increase prey survival

by making it harder for visual predators to find and catch prey. Acid leachates from litter (e.g. phenolics) may interfere with prey responses to predators by reducing pH, which may reduce the effectiveness of chemical cues emitted by predators (i.e. kairomones) that prey use to detect and respond to predators (Brown et al. 2002; Leduc et al. 2004). Finally, changes in predation rates resulting from such direct effects of litter may have further effects on prey by changing per-capita resource availability for prey. Although these effects may not be of great importance in lotic (i.e. flowing) systems such as streams and rivers where litter and leachates rapidly flow downstream (Dobson et al. 1992; Richardson 1992; Dudgeon and Wu 1999), they are likely important in lentic (i.e. non-flowing) systems where material is retained for much longer periods of time.

Our goal was to investigate how predator-prey interactions respond to changes in benthic surface cover and water clarity generated by inputs of leaf litter of equal biomass. We made three predictions 1) increased benthic surface cover would increase prey survival due to increased refuge availability; 2) decreased water clarity from litter leachate would increase prey survival due to reduced visual detection by predators; and 3) the combination of increased benthic surface cover and decreased water clarity would increase prey survival more than either factor alone. These predictions assume that the influence of litter on pH or prey growth rate is negligible. If increased structure or decreased water clarity cause decreases in prey growth, which would make the prey more susceptible to gape-limited predators, then the predicted increases in prey survival could be weakened or even reversed.

To test these predictions, we altered surface cover and water clarity in the benthos of outdoor, artificial wetland mesocosms by manipulating the species of litter inputs. Using wetland mesocosms, we examined how these manipulations affected the growth and survival of gray tree

frog tadpoles (*Hyla versicolor*) when in the presence of adult eastern red-spotted newts (*Notophthalmus viridescens*) as predators. Although these predictions could be tested using artificial structure and coloring agents, this would preclude any bottom-up effects of litter chemistry on prey.

4.2 METHODS

4.2.1 System background

Our experiment was conducted at the University of Pittsburgh's Pymatuning Laboratory of Ecology in northwest Pennsylvania. Senesced tree litter constitutes a dominant source of nutrient and energy subsidies to the ponds that these newts and tadpoles cohabit. In the region where this study was conducted and where organisms were collected, red maple (*Acer rubrum*), red pine (*Pinus resinosa*), and oak (*Quercus* spp.) constitute three of the dominant tree species. In addition, these trees are associated with contemporary changes in forest diversity. Currently, maples are increasing in abundance throughout eastern temperate forests through such forces as selective browsing by mammals (e.g. white tailed deer, *Odocoileus virginianus*) and human-driven fire suppression (Abrams 1998, 2003). At the same time, the ranges of many maple species are predicted to shift northwards following current models of climate change (Hansen et al. 2001; Iverson and Prasad 2001).

The ranges of gray tree frogs and newts overlap in most areas where these tree species are dominant. Both species can be found in a wide variety of habitats, from large lakes to small wetlands, open- to moderate-canopy systems, and from deciduous to coniferous forests (Lannoo

2005, Werner et al. 2007). Many of these habitats receive substantial inputs of litter from surrounding trees, either from overhead litterfall or wind-blown inputs. Often, these inputs are concentrated in shallow littoral zones where amphibians spend much of their time foraging and seeking refuge (Porej and Hetherington 2005). Gray tree frogs are a summer-breeding species that typically lays its eggs during June and July (Kiesecker and Skelly 2000). They are active foragers, typically metamorphosing in 3 to 6 wks (Werner et al. 2007). In many systems, they constitute an important prey item for eastern red-spotted newts, which are predominantly visual predators that forage during both day and night (Martin et al. 1973) and are often keystone predators in vernal ponds (Wilbur et al. 1983). Gray tree frogs and other prey items of eastern red-spotted newts, including larval newt conspecifics, are alerted to the threat of predation through chemical cues (i.e. kairomones; Dodson et al. 1994, Relyea 2001, Mathis 2003). Upon eating and digesting tadpole prey, newts release kairomones that induce phenotypic responses in gray tree frog tadpoles that make the tadpoles less susceptible to predation (Lawler 1989; Relyea 2001). These include relatively immediate reductions in movement and activity to diminish visual detection, and more gradual changes in body shape that increase chances of escape.

4.2.2 Experimental design

The experiment used a completely randomized design with six treatments in which we crossed two benthic surface cover treatments (oak versus pine litter) and three water clarity treatments. Low, medium, and high water clarity treatments were generated using variable amounts of red maple litter that was removed prior to the experiment to avoid altering the total biomass of litter in treatment, which might confound results. Due to expected variability in predator feeding

behavior, we replicated each treatment eight times, resulting in 48 experimental units. Although it was unlikely that litter inputs would have any effect on tadpole survival or mass over the short duration of our study (Stoler and Relyea 2011), we assessed these direct, bottom-up effects by including two control replicates for each of the six treatments that included a caged predator. This design produced a total of 60 experimental units.

Our experimental units were 100-L plastic wading pools. The pools were 1 m in diameter and approximately 0.2 m in height. Each pool was covered with a 60% shade-cloth lid to prevent escape or entry of any organisms and to simulate a medium level of canopy cover relative to the range of canopy cover in ephemeral wetlands (Werner and Glennemeier 1999).

Pine and oak litter were placed into mesocosms on 8 June 2011. Litter used for this experiment was collected immediately after senescence during the autumn prior to the experiment. While the chemistry of this litter is substantially different from older litter to which summer-breeding amphibians would be naturally exposed, prior work has demonstrated that the physical structure of oak and pine does not deteriorate much from the time of senescence and the time of the experiment. Furthermore, stained water due to red maple leachate remains dark throughout the spring and summer in many ponds (A. Stoler, unpubl. data). To manipulate low and high benthic surface cover, we added 100 g of red pine needles or 100 g of oak leaves to the mesocosms, respectively. This biomass is within the range of observed litter inputs to forest wetlands (Rubbo et al. 2008) and is similar to the biomass of inputs used in past experiments (e.g., Stoler and Relyea 2011). These two species were used to manipulate structure due to their common co-occurrence, conservation concern, and similarity in lignin content and breakdown

rate (Webster and Benfield 1986), which indicates a similarity in physical rigidity. Biomass was used to standardize inputs in accordance with the methods of nearly all other litter manipulation studies and mesocosm experiments (e.g., Rubbo et al. 2008).

Maple litter was added to mesocosms on 10 June 2011. To generate high, medium, or low water clarity, we added 15, 50, or 85 g of maple litter to the mesocosms. These amounts span the range of observed red maple inputs to forest wetlands as observed in field surveys (A. Stoler, unpubl. data); the highest biomass leached sufficient DOC into the water so that the benthos was no longer visible. Because we wanted an equal biomass of benthic leaf litter in all treatments, we placed the maple litter into 5 mm mesh bags that were later removed. Soluble carbon began leaching from leaves almost immediately and clarity ceased to change after 2 d. Bags were left in mesocosms for 9 d and were removed prior to tadpole introduction, while oak and pine litter were kept in the mesocosms for the duration of the study. Although red maple served as the primary source of leachate, both oak and pine do leach some carbon into the water. However, this amount is nominal relative to the leachate of maple, primarily due to the slow decomposition rate of oak and pine species (Webster and Benfield 1986).

Two days after maple litter introduction, in accordance with common protocol for setting up mesocosms, we collected and mixed water from six nearby ponds to serve as a source of periphyton, phytoplankton, zooplankton, bacteria and fungi. We inoculated each mesocosm by placing 1.5-L aliquots of the water into all mesocosms. We chose ponds for water collection based on their proximity to tree species whose litter was represented in this experiment. We allowed the mesocosms to sit for 7 d prior to the introduction of tadpoles. Given this short time period, there was no substantial increase in zooplankton that could serve as an alternative food

source for the newts. Hence, we made no attempt to quantify zooplankton. Growth of periphyton biomass was quantified in a previous study (Stoler and Relyea 2011), that found greater growth among conifer litter treatments relative to broadleaf litter treatments, and relatively low growth of periphyton with red maple litter relative to oak litter. Hence, we did not quantify periphyton biomass in the current study.

In accordance with accepted IUCAC protocol, the gray tree frogs were collected as 24 amplecting pairs that were allowed to oviposit into laboratory containers. After oviposition, we transferred eggs to outdoor wading pools. Tadpoles were fed rabbit chow *ad libitum* until introduced into the experiment when they reached a safe handling mass (initial mean mass ± 1 SE = 25 ± 18 mg). On 19-June-2011 (defined as day 0 of the experiment), individuals from all 24 clutches were mixed and 30 tadpoles were placed into each mesocosm. This resulted in a density of 38 tadpoles / m^2, which is well within the natural range of densities for *H. versicolor* (Relyea and Hoverman 2003). Thirty additional tadpoles were chosen at random to assess 24-hr survival post-handling, which was 100%.

We collected the newts from a local wetland and held them in laboratory tubs containing filtered water and refugia for 7 d. While in the lab, we kept four individuals in each container and fed each tub 15 to 20 gray tree frog tadpoles daily (at a size that was similar to the tadpoles they would experience during the experiment). To ensure that newts used in the experiment had similar propensities to consume tadpoles, we attempted to feed all individuals two tadpoles prior to introduction into mesocosms. We only used individuals that readily ate both tadpoles.

On day 1 of the experiment (20 June 2011), one newt was introduced into each mesocosm. This resulted in a density of approximately 1 individual / m^{-2}, which is comparable to densities observe in natural ponds (Gill 1978). Since predator-prey interactions can be altered by

phenotypic changes that tadpoles undergo when sensing predatory risk, we caged all newts for the first 2 d to provide tadpoles with predator cues. Cages were made of corrugated drain pipe, capped on both sides by 1-mm mesh and held in place along the edge of each mesocosm with binder clips. Immediately after placing newts in cages, each newt was fed 300 mg of gray tree frogs to cause the production of kairomones by the newts. This biomass of prey is sufficient to elicit a response by tadpoles (Schoeppner and Relyea 2005). The newts were not fed for the next 2 d, which is a sufficient time to ensure they are hungry when released (Lefcort and Blaustein 1995). On day 3, all newts in the uncaged treatments were released from their cages; all cages were left in the mesocosms. Newts in mesocosms assigned to the caged-predator treatment were not released. Instead, they were fed 300 mg of tadpoles on day 1, 3, and 5 so that the tadpoles were continually exposed to the kairomones. To equalize disturbance caused by feeding the caged newts, all empty cages were also lifted out of the water and placed back after each feeding.

To monitor tadpole survival over time, we randomly selected a single replicate from each treatment on each morning of the experiment, removed all litter, and netted and counted all tadpoles. We stirred the litter in all other mesocosms to equalize the disturbance generated by this activity. Mortality was $\leq 30\%$ by the third day, so we attempted to increase the rate of predation by increasing visibility in the water. To do this, we replaced the 60% shade cloth lids with 10% shade cloth lids (made of nylon window screen).

The experiment ended on day 7. By that time, newts had foraged for 4 d. In addition, the tadpoles in some treatments had grown nearly 10-fold, indicating a potential size refuge from newts. Upon termination, we collected all newts and placed them into individual containers. Because tadpole survival may be influenced by the body size of a predator, we measured the snout vent length (SVL) of each newt using digital calipers. While treatments may have

influenced newt body mass, SVL was unlikely to change significantly over the short duration of the experiment (average growth rate of adult newts is ~ 5 mm SVL per yr; Caetano and Leclair Jr. 1996).

After measuring the newts, all tadpoles were removed from each mesocosm and counted to determine percent mortality. The tadpoles from each mesocosm were weighed and we used the mean individual mass as our response variable. To verify that leaf litter did not exert a bottom-up influence on tadpole over the short duration of our study, tadpoles in caged-predator replicates were also weighed. We did not attempt to assess tadpole behavior among treatments, as the dark water of high-leachate treatments made it difficult to see individuals and posed a sampling bias.

4.2.3 Water chemistry

Using the method of Collier (1987), we quantified the concentration of DOC in the water column via spectrophotometric absorbance, which has been shown to be accurate across large ranges and types of DOC. We took samples on day 3, after the newts were released, and kept samples at 4 °C for 2 d until they were processed. We filtered samples through a 0.42 µm cellulose membrane and allowed samples to reach room temperature before assaying in a spectrophotometer (Perkin Elmer UV/Vis Lambda 20 Spectrophotometer). Absorbance values were transformed to g m^{-3} of DOC via the equation:

$$DOC\ (g\ m^{-3}) = 59.6a + 1.9$$

where *a* is equal to the absorbance of the sample at 360 nm with a path length of 1 cm in acrylic cuvettes. We also quantified pH in all mesocosms on day 7 with a handheld meter (P4 Multiline

Meter, WTW Instruments). Sub-sampling of treatments for dissolved oxygen and temperature revealed no difference among treatments, which was expected due to the high surface area to volume ratio of our mesocosms that allowed rapid surface air and heat exchange.

4.2.4 Statistical analysis

We used multivariate analyses of variance (MANOVA) to test for effects of surface cover and leachate on tadpole mortality, tadpole mass, pH, and the mass of DOC in the water column. This effectively controlled for type I error when conducting subsequent univariate analyses. We employed type III sums of squares based on unweighted marginal means to account for our unbalanced experimental design due to missing replicates. We used a full-factorial model including benthic surface cover treatments and red maple-leachate treatments as independent, fixed factors. Preliminary tests revealed that inclusion of newt SVL as a covariate in the model had no effect on the biological interpretation of results, so this covariate was dropped from the multivariate model. For leachate treatments, we used Tukey's test to conduct mean comparisons between treatments after finding significant univariate effects. Caged-predator treatments were not included in this analysis as they were used only to confirm a lack of any direct, bottom-up effect of litter; however, values and ranges of these treatments are reported in Table 4.1. All variables were assessed for normality using probability plots. Percent mortality was log-transformed to fit a normal distribution. One newt escaped from an uncaged-predator replicate containing oak and a low maple leachate, so we discarded all data from this replicate. Using

Dixon's Q-test (confidence level = 95%; Sokal and Rohlf 1995) we detected one outlier among mortality responses in the high maple leachate and oak litter treatment, and so discarded all data from this replicate.

4.3 RESULTS

Caged-predator treatments confirmed that tadpole mortality and individual mass did not differ among caged-predator controls, thus indicating no bottom-up effect of litter over the short duration of our study. Across all caged-predator treatments, mortality was never higher than 6%. Means and ranges of tadpole mortality and individual mass are provided in Table 4.1. For all uncaged-predator treatments, our analysis revealed a significant multivariate effect of benthic surface cover species and maple leachate level on response variables. There was no interaction between surface cover species and red maple leachate (Table 4.2A).

Table 4.1. Means ± one standard error of tadpole mortality and individual mass among caged-predator treatments. Values are divided among the three levels of maple leachate treatments (low, medium, and high) within the two treatments of benthic surface cover (pine and oak).

	Mortality (%)			Individual mass (mg)		
	Low	Medium	High	Low	Medium	High
Pine litter	1.5 ± 1.1	3.1 ± 2.2	0.0 ± 0.0	231.9 ± 21.4	275.3 ± 11.8	219.9 ± 43.2
Oak litter	1.5 ± 1.1	0.0 ± 0.0	1.5 ± 1.1	263.3 ± 4.5	206..5 ± 28.6	198.9 ± 11.4

4.3.1 Effect of surface cover species

We did not detect any univariate effects of benthic surface cover species on tadpole mortality, but there was a marginal effect on individual tadpole mass (Figure 4.1; Table 4.2B). Tadpoles in oak litter treatments were approximately 10% smaller relative to individuals in pine litter treatments.

We detected a significant effect of benthic surface cover species on DOC concentration in the water column, as measured by absorbance, and on pH (Table 4.2B, Figure 4.2). DOC concentration was approximately 21% higher in oak litter treatments relative to pine litter treatments. Mean comparisons revealed that pH was approximately 0.3 pH units less in oak litter treatments relative to pine litter treatments.

4.3.2 Effect of red maple leachate

Red maple leachate affected tadpole mortality (Table 4.2B, Figure 4.1A). Mean comparisons found that mortality in high- and medium-leachate treatments was at least 13% greater than in low-leachate treatments ($P \leq 0.007$). Mortality among high- and medium-leachate treatments did not differ ($P = 0.78$).

Red maple leachate also affected individual tadpole mass (Table 4.2B, Figure 4.1B). Tadpoles in high-leachate treatment were 50 mg (26%) smaller than tadpoles in medium-leachate treatment ($P < 0.001$), and tadpoles in the medium-leachate treatment were 36 mg (16%) smaller than tadpoles in low -leachate treatments ($P = 0.011$).

The mass of DOC in the water column was also affected by maple leachate (Table 4.2B; Figure 4.2A). Mean comparisons revealed that mass of DOC was significantly different between all three levels of maple leachate ($P < 0.001$). Mass of DOC was 30% greater in medium-leachate treatments relative to low-leachate treatments, and 48% higher in high-leachate treatments relative to medium-leachate treatments.

Red maple leachate also influenced pH (Table 4.2B, Figure 4.2B). Mean comparisons revealed that pH differed between all leachate levels ($P < 0.001$). Water in low-leachate treatments was 0.4 pH units greater than medium-leachate treatments, which were approximately 0.5 pH units greater than high-leachate treatments.

Table 4.2. A) Multivariate and B) univariate results of the MANOVA for benthic surface cover and leachate treatments on mass of DOC, mortality, tadpole mass, and pH in mesocosms containing uncaged predators. Univariate results for the interaction term are not provided, as the multivariate effect was not significant. In the table of univariate results, degrees of freedom are written as subscripts with F values.

A. Multivariate	F	*P*
Benthic surface cover	$12.354_{4,37}$	<0.001
Maple leachate	$49.254_{8,74}$	<0.001
Benthic cover x maple leachate	$1.389_{8,74}$	0.22

B. Univariate	Tadpole mortality		Tadpole mass		DOC		pH	
	F	*P*	F	*P*	F	*P*	F	*P*
Benthic surface cover	$0.098_{1,40}$	0.76	$3.899_{1,40}$	0.055	$26.260_{1,40}$	<0.001	$49.865_{1,40}$	<0.001
Maple leachate	$8.781_{2,40}$	0.001	$26.042_{2,40}$	<0.001	$567.456_{2,40}$	<0.001	$145.345_{2,40}$	<0.001

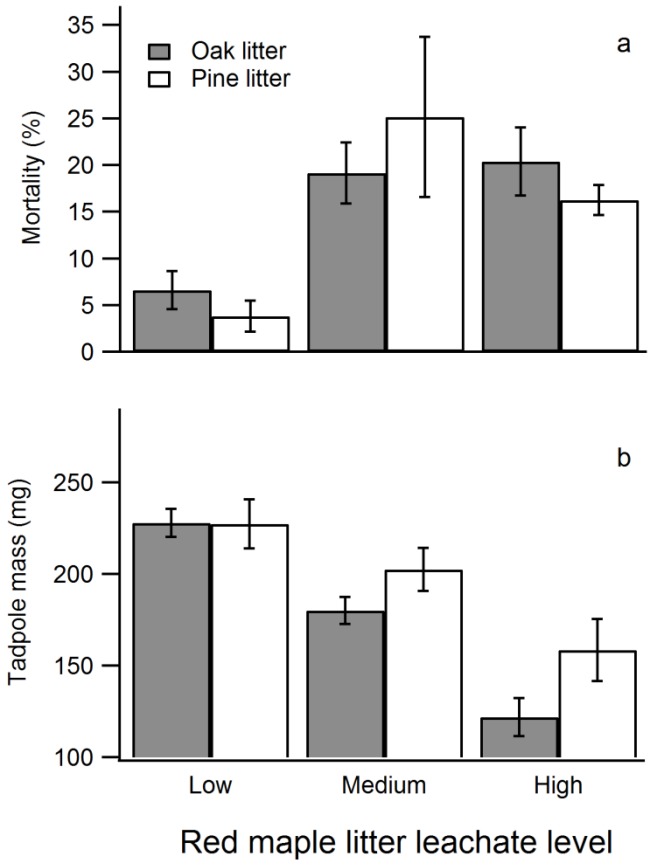

Figure 4.1. Effect of leachate level from red maple litter and benthic surface cover (oak versus pine litter) on individual tadpole a) mortality and b) mass (means ± 1 SE). Results displayed are for uncaged-predator treatments only.

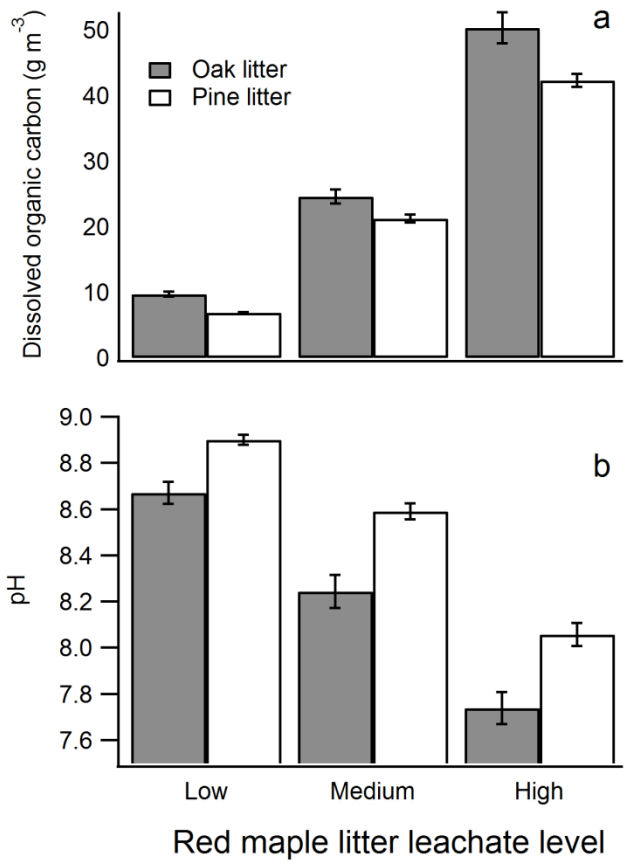

Figure 4.2. Effect of leachate level from red maple litter and benthic surface cover (oak versus pine litter) on a) mass of DOC and b) pH (means ± 1 SE). Results displayed are for uncaged-predator treatments only.

4.4 DISCUSSION

Our study suggests that leaf litter inputs alter the physical and chemical environment of wetlands in a manner that influences prey growth and subsequent interactions between predators and prey. While tadpole mortality was relatively low (0 to 6%) when newts were caged, tadpole mortality ranged from 5% to more than 20% with lethal predators as the amount of red maple leachate increased. However, mortality was unaffected by our manipulation of benthic surface cover using oak or pine litter. In addition, tadpole mass increased as maple leachate decreased and individuals reached a potential size refuge from predation by the conclusion of the study.

These results refuted our three predictions, which were based on the assumption that visibility was the dominant factor influencing newt-tadpole interactions (Martin et al. 1973). It is possible that this assumption was incorrect; indeed, studies examining other newt-prey and newt-predator interactions indicate that newts are responsive to chemical cues (Dodson et al. 1994, Mathis 2003). However, there is no strong indication that newts use chemical cues when detecting heterospecific prey items (Martin et al. 1973). Moreover, this suggests that the increased mortality of tadpoles recorded in the high-leachate treatments of our study was due to increased perception of tadpole cues in these treatments. This is unlikely, as experimental manipulations of prey cues in increasingly high- and low-light environments registered no change in newt predation activity (Martin et al. 1973). Hence, it is unlikely that changes in predator perception of prey chemical cues were a major mechanism underlying our results.

One possible explanation for our results is that treatments with elevated DOC (i.e. high maple leachate) decreased light availability in the visible spectrum more for the tadpoles than the newts, thereby affording newts a visual advantage. Such differences in spectral sensitivities have

been documented for other aquatic organisms and their predators, particularly among fish (Endler 1992). While newts are visual predators, their spectral sensitivity is unknown, so we cannot determine if elevated DOC altered their visual acuity in our study. However, many species of aquatic prey, including tadpoles, use chemical cues to detect their predators (Dodson et al. 1994; Brönmark and Hannsson 2000), so it is unlikely that differences in visual acuity had a strong influence on tadpole predation. It is more likely that high amounts of maple leachate interfered with the chemical cues of newts or reduced prey size, thereby making it easier for the newts to consume more tadpoles.

Chemical cues such as kairomones are common in pond environments (Dodson et al. 1994), and previous work demonstrates that gray tree frogs reduce their movement when kairomones are present (Schoeppner and Relyea 2005). However, the effectiveness of chemical alarm cues can be pH dependent. In streams, Brown et al. (2002) found that a reduction of pH by one unit (i.e. from 7 to 6) reduced predator avoidance behavior of a minnow and dace species, likely due to a permanent deformation of the alarm cue's molecular structure. Such changes in pH can be caused by litter inputs, particularly when litter species rich in phenolic acids (e.g., red maple) are introduced into the system. Although our observed pH values (pH 7.8 to 8.9) were not below neutral and were not within a range that would directly harm tadpoles (Grant and Licht 1993), the decline in pH of 0.9 units with increased maple leachate may have been sufficient to alter the detection of kairomones by the prey. In turn, this would have prevented tadpoles from activating their normal suite of anti-predator strategies, such as hiding or reducing movement, which would result in increased predation rates and decreased tadpole survival. This hypothesized mechanism certainly requires further investigation.

Differences in predation may have also been affected by differences in tadpole size. Body size is a critical factor in determining prey survival, particularly when the predator is gape-limited (Wilbur et al. 1983). Unlike many predators that pierce or chew their prey (e.g., dragonfly larvae), newts consume tadpoles by engulfing the body (Wilbur and Fauth 1990). Larger prey are both faster and more difficult to engulf, making successful predation attempts harder (Relyea 2004). In our study, tadpole mass increased as leachate decreased; by the end of the experiment tadpoles in low-leachate treatments were twice the mass of tadpoles in high-leachate treatments and were likely closer to a size refuge from newt predation. Hence, larger body size likely contributed to increased tadpole survival under low-leachate treatments.

Differences in body size among treatments may have been caused by variation in litter inputs that differed in the quality and availability of resources (Brinson et al. 1981; Webster and Benfield 1986; Marcarelli et al. 2011). Low tadpole survival has been associated with red maple litter in wetland mesocosms, likely due to large inputs of DOC and phenolic acids that can inhibit periphyton production through shading and chemical inhibition, and can also interfere with gill functioning (Rubbo and Kiesecker 2004, Maerz et al. 2005). High levels of red maple may increase aerobic microbial respiration, leading to reduced dissolved oxygen and suffocation of tadpoles (Wassersug and Feder 1983; A. Stoler, unpubl. data). In addition, DOC leached from maple litter darkens the water column and reduces algal growth, which is a nutrient-rich food source for gray tree frog tadpoles (Kupferberg 1997). In contrast, pine litter possesses relatively little soluble carbon (Berg and McClaugherty 2008), resulting in clearer water that promotes greater algal productivity (Karlsson et al. 2009). Indeed, in mesocosms of similar size and with similar litter species to those used in this experiment, Stoler and Relyea (2011) found greater biomass of algal dominated periphyton in litter treatments with relatively clear water.

Given the nominal mortality among caged-predator treatments, tadpole mortality was not likely a direct result of litter chemistry or leachates in our study. Moreover, we did not find any dead tadpoles and did not note any individuals that appeared sickly or weak in either caged- or uncaged-predator treatments. Considering the detrimental effects of leachates when tadpoles are exposed for longer durations, it is possible that more time is needed for leachates to have pronounced bottom-up effects on tadpole fitness (Rubbo and Kiesecker 2004; Stoler and Relyea 2011). Yet even over short durations, the presence of sublethal stressors can have important consequences on tadpole fitness when combined with other stressors (Relyea 2003), such as elevated kairomone levels and the sight of a free-swimming predator. Hence, an important implication of our study that deserves further investigation is that bottom-up stresses caused by the effects litter inputs on the chemical and physical environment may exacerbate the effects of stress from top-down forces.

Further work should aim to understand how increasing environmental and ecological complexity mediate the effect of litter inputs on predator-prey dynamics. It is worth noting that natural water chemistry may substantially differ from that of our mesocosms and will depend on many environmental variables (e.g., timing of litterfall, hydroperiod, soil composition, temperature). Understanding how such climactic factors influence the effects of leaf litter and predators on prey fitness is necessary to fully elucidate how our experimental results translate to natural phenomena. Further work should also aim to understand how increasing food web complexity mediates these effects. For example, the presence of litter grazers with functionally different feeding habits can facilitate consumer growth (Iwai and Kagaya 2007), which may have further impacts on predation rates. Incorporation of such complexity may offer detailed and important insight into the effects of litter in natural food webs.

4.4.1 Implications for future shifts in forest composition

By manipulating leaf litter species, the results of our study suggest that predicted changes in eastern temperate forests of the United States have the potential to dramatically change the dynamics of forested wetlands. While red maple naturally colonizes forests through succession—replacing trees such as pines and poplars (*Populus* spp.)—it is rapidly increasing in abundance throughout the northeastern United States due to fire suppression and selective mammalian browsing (Abrams 1998, 2003). At a local scale, many forests are becoming near-monocultures of red maple. Our study indicates that such shifts in forest tree composition will influence predator-prey dynamics in wetlands. This is important for both wetlands and the surrounding forest since many predator and prey species, particularly amphibians, significantly contribute to nutrient cycling in large regions surrounding wetlands (Beard et al. 2002). Thus, our study suggests a biological consequence of changing forest composition that should be considered to fully estimate future changes in the ecological functioning of forests.

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5.0 RESOURCE COMPLEMENTARITY AND LITTER CHEMISTRY DRIVES ECOSYSTEM PROCESSES IN FOREST WETLANDS

5.1 INTRODUCTION

Recent, unprecedented losses of global biodiversity have led researchers to question how the loss of species and shifts in species composition will influence ecosystem processes (Hooper et al. 2005, de Bello et al. 2010). The decomposition of organic material is among the most important processes on Earth, with up to 90% of all primary production entering the pool of dead organic material annually (Facelli and Pickett 1991, Moore et al. 2004, Gessner et al. 2010). The diversity (i.e. identity, composition, and abundance) of plant litter species is thought to predict the rate of litter decomposition within an ecosystem (Gartner and Cardon 2004, Hättenschwiler et al. 2005). This is evidenced by past manipulations of plant litter richness, which have resulted in non-additive – and often synergistic – functional responses to increased richness (e.g., decomposition rate, detritivore growth; Gartner and Cardon 2004, Hättenschwiler et al. 2005). Although this general finding indicates that plant diversity loss may have negative impacts on decomposition, the mechanisms underlying this relationship remain unclear and largely untested.

The decomposition rate of plant litter is determined by an interaction between litter chemistry and the consumers that use litter as an energy and nutrient resource. Consumers generally exhibit an affinity for nutrient-rich and labile material which subsequently decomposes

relatively quickly and promotes secondary growth (Swan and Palmer 2006). In contrast, nutrient-poor, recalcitrant, or toxic material generally inhibits consumer grazing, decomposes slowly, and inhibits secondary growth (Ardón et al. 2009). Based on these relationships, one hypothesis relating litter diversity to the decomposition process suggests that the rate of the decomposition is determined by the mean chemical trait values of all species in mixture (i.e., mass-ratio hypothesis; Grime 1998, Díaz et al. 2007). In contrast, the selection hypothesis poses that non-additive effects occur because individual litter species facilitate or inhibit litter mixture decomposition, owing to their unique chemistry and effect on biotic interactions among microbes and consumers (Hättenschwiler et al. 2005). Non-additive effects may also occur as a result of resource complementarity, when two or more litter species in mixture provide a more complete diet for consumers, thus leading to elevated consumer biomass and faster decomposition rates (Schindler and Gessner 2009, Gessner et al. 2010, Vos et al. 2013). More specifically, this hypothesis posits that increasing chemical dissimilarity of litter will be positively related to litter decomposition rate and consumer activity (Epps et al. 2007, Gessner et al. 2010). Given that complementarity has been demonstrated to generate synergistic effects in other systems (Hooper et al. 2005), there has been much interest in determining to what extent this mechanism influences litter decomposition. Although current evidence finds equivocal support for all three hypotheses (Gessner et al. 2010), this is likely due to inappropriate experimental designs (Dias et al. 2013). In this study, we address and correct these design flaws to explicitly test the mechanism of resource complementarity.

The most fundamental challenge to testing this mechanism is the need to define the traits that determine the dissimilarity between litter species. Several studies have used single traits in their definition (Wardle et al. 1997, Epps et al. 2007, Schindler and Gessner 2009, Vos et al.

2013). However, different parts of the decomposition process are likely regulated by different litter traits and manipulation of single trait dissimilarity cannot account for the effects of all other relevant traits, particularly as many are uncorrelated within litter (e.g., phenolics and nitrogen, Epps et al. 2007). Several multivariate indices have been proposed that offer superior methods of measuring qualitative differences among litter species (Schleuter et al. 2010). These indices essentially describe the volume encompassed by a group of species in n-dimensional trait space, and subsequently describe chemical dissimilarity as a single, continuous measure defined by several multivariate axes. The values of such trait indices remain largely unexplored with regard to the decomposition process (Gessner et al. 2010).

A second major challenge to testing the relationship between chemical dissimilarity and function is that chemical dissimilarity is often confounded with other variables, particularly species presence / absence, and average trait chemistry. Certain litter species, by virtue of their unique chemistry, are likely to be represented more among mixtures with low chemical dissimilarity (Dias et al. 2013), which increases the opportunity for selection effects. Additionally, mixtures with low dissimilarity are more likely to have extreme values of trait means (Dias et al. 2013). These relationships have likely confounded past manipulations of litter dissimilarity with mass-ratio effects (Dias et al. 2013). It is possible to remove these confounds by incorporating a sufficient number of chemically distinct and different litter species into an experimental design, while maintaining relatively small maximum levels of trait dissimilarity, and forcing equal representation of species across all levels of trait dissimilarity. Although no study has employed this design, the wide variation in leaf litter chemistry frequently found within ecosystems (Ostrofsky 1997) certainly offers the opportunity to overcome this challenge, in order to isolate the effects of litter chemical dissimilarity on the decomposition process.

We employed this strategy with a common multivariate trait index to explicitly test the mechanism of resource complementarity in vernal, temperate forest ponds. These systems typically receive substantial inputs of leaf litter (Rubbo et al. 2006, Earl et al. 2012), and give rise to a decomposition process that often involves diverse and massive food webs (Williams 2005). Recent studies have shown that differences litter chemistry can exert strong impacts on organisms across trophic levels (Maerz et al. 2005, Williams et al. 2008, Brady and Turner 2010, Stoler and Relyea 2011, Earl et al. 2012, Cohen et al. 2012). The few studies demonstrating effects of litter mixing exhibit results that vary in magnitude from almost completely additive (Stoler and Relyea 2011, *in review*) to highly non-additive (Rubbo and Kiesecker 2004), yet no study has explicitly tested a particular mechanism underlying the effects of litter mixing. In this study, we primarily tested the hypothesis that litter chemical dissimilarity positively relates to the rate of litter decomposition and with the biomass of higher trophic level components. However, by removing confounding relationships between litter chemical dissimilarity, litter species composition, and trait means, we were also able to explore the effects of the latter two litter diversity attributes, which correspond to selection and mass-ratio effects, respectively.

5.2 METHODS

The experiment was conducted at the University of Pittsburgh's Pymatuning Laboratory of Ecology in northwestern Pennsylvania. There were three treatments, including low, medium, and high leaf litter chemical dissimilarity. Each treatment was replicated 20 times for a total of 60 experimental units. Experimental units consisted of 500-L polyethylene mesocosms covered by a 60% mesh cloth to simulate moderate levels of canopy cover and to prevent unwanted escape or

entry of organisms. Prior to filling mesocosms with water and litter, 20-L of loamy soil was spread on the bottom of each mesocosm. Soil was allowed to fully dry in the sun prior to filling mesocosms in order to desiccate and kill any soil organisms. We filled mesocosms with well water between 3 and 7 May and allowed soil to settle for 1 wk before introducing litter.

5.2.1 Collection of leaf litter and analysis of litter chemistry

In autumn 2009, we collected 20 species of broadleaf and coniferous tree litter from western Pennsylvania within 1 wk of senescence (Table 5.1) and analyzed several chemical components of each species. We collected litter from various locations throughout western Pennsylvania. Each species was collected from a single location to reduce intraspecific chemical variation among tree species. Litter was air-dried in an unheated garage through the winter. After drying, we used a Wiley mill (Thomas Scientific, New Jersey) to grind samples of leaf tissue to < 0.5 mm. We used these samples to assess litter carbon (C), nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), total phenolics, tannin, lignin, and soluble carbon (methods for chemical analyses may be found in Appendix H). These chemical components were chosen to describe litter chemistry because they are the most widely published of forestry studies and many of them have known correlations with litter decomposition rate (Epps et al. 2007).

Table 5.1. The litter species used in the study, including family and species names, and their decomposition rates, measured as the coefficient of decay (k; Petersen and Cummins 1974). Numbers in parentheses represent statistically similar groups and order of k (1 = slowest decomposition; 6 = fastest decomposition). Decomposition rates were not measured for conifers.

Common name	Family	Species	Abbreviation	k
Red maple	Aceraceae	Acer rubrum	RM	0.056 (6,7)
Sugar maple	Aceraceae	Acer saccharum	SM	0.057 (7)
American sweetgum	Altingiaceae	Liquidambar styraciflua	SGUM	0.044 (4,5)
Yellow birch	Betulaceae	Betula alleghaniensis	BIR	0.046 (5,6)
American beech	Fagaceae	Fagus grandifolia	BCH	0.022 (1)
American sycamore	Fagaceae	Plantanus occidentalis	SYC	0.023 (1)
Chinese chestnut	Fagaceae	Castanea mollissima	CHCH	0.035 (3,4)
Hybridized chestnut	Fagaceae	Castanea mollissima x Castanea dentata	HYCH	0.056 (6,7)
Black oak	Fagaceae	Quercus velutina	BOAK	0.027 (1,2)
White oak	Fagaceae	Quercus alba	WOAK	0.042 (3,4,5)
Sassafras	Lauraceae	Sassafras albidum	SASS	0.035 (3,4)
Tulip poplar	Magnoliaceae	Liriodendrum tulipfera	TP	0.073 (8)
Green ash	Oleaceae	Fraxinus pennsylvanica	GASH	0.066 (8)
Northern tamarack	Pinaceae	Larix laricina	TAM	--
Red pine	Pinaceae	Pinus resinosa	RP	--
Eastern white pine	Pinaceae	Pinus strobus	WP	--
Bigtooth aspen	Salicaceae	Populus grandidentata	BASP	0.033 (2,3)
Quaking aspen	Salicaceae	Populus tremuloides	QASP	0.035 (3,4)
Black cherry	Rosaceae	Prunus serotina	CHER	0.070 (8)
Black willow	Salicaceae	Salix nigra	BW	0.042 (3,4,5)

5.2.2 Calculation and manipulation of litter chemical dissimilarity

We used values of chemical traits to calculate and manipulate litter chemical dissimilarity. To manipulate litter mixture diversity while maintaining constant litter species richness, we first calculated chemical diversity of all possible four-species litter combinations, given the pool of 20 litter species. The choice of four species as the mixture richness value provided sufficient spread among potential values of chemical dissimilarity to delineate distinct ranges of chemical dissimilarity while keeping richness logistically feasible and realistic. Chemical dissimilarity was calculated as Rao's quadratic entropy (i.e. RaoQ; Botta-Dukat 2005, Epps et al. 2007, Laliberte and Legendre 2010) after reducing trait dimensionality via principal components analysis. Details on the calculation of RaoQ may be found in Appendix I.

After calculating RaoQ values for all possible four-species mixtures, we used the resulting distribution of RaoQ values to delineate ranges of low, medium, and high chemical dissimilarity, corresponding to the three dissimilarity treatments. We selected 20 mixtures from each of these ranges which corresponded to the 20 replicates within each dissimilarity treatment. No two mixtures (i.e. dissimilarity treatment replicates) were identical in species composition. To avoid bias from an increased presence of a particular litter species, we limited the appearance of individual litter species within dissimilarity treatments to between three and five instances. Details on the method of delineating these ranges and selecting mixtures may be found in Appendix I.

On 14 May (i.e. day 0), we placed litter into mesocosms. A total of 200 g was placed into each mesocosm, consisting of 50 g of each of the four component species. To ensure that litter was homogenously mixed across the benthos of each mesocosm, we thoroughly mixed all

component species before they were placed into mesocosms. To measure decomposition rate of litter and provide a means of sampling benthic grazers, we also added three mesh bags to each mesocosm containing pre-weighed amounts of litter. The mesh size of bags was 5 mm, which permitted the consumers contained in our mesocosms to graze off of contained litter. Each bag contained a mixture of litter species, including 1.5 g of each litter species present in the tank. Since coniferous needles could not be contained within this mesh size, they were excluded from the bags, yet still placed in the water to ensure that all mesocosms had an equal total biomass of litter.

5.2.3 Constructing the aquatic community

On 16 May, we collected 15 L of water from 10 ephemeral ponds to serve as sources of microbes and algae. From five of these ponds, we collected zooplankton using a 250-μm plankton tow net, which was sufficient to capture the larger-bodied zooplankton typical of forested ephemeral ponds. All species feed on phytoplankton and other organic material from the water column. Following removal of all predators to eliminate top-down pressure on zooplankton, we mixed the pond water samples and the zooplankton samples and introduced equal amounts (2.5 L) of the slurry into each mesocosm. On 23 May, we added 10 $\mu g\ L^{-1}$ of phosphorus (as Na_2HPO_4) and 72 $\mu g\ L^{-1}$ of nitrogen (as $NaNO_3$) to each mesocosm as a pulse of nutrients at the Redfield ratio. This nutrient addition accelerates growth of phytoplankton and periphyton and adjusts mesocosm nutrient levels to those commonly found in mesotrophic systems (Downing and McCauley 1992). At this time, we also placed three clay tiles to serve as periphyton samplers; the tiles were oriented vertically on top of the litter on the north side of each mesocosm,

We added 15 individuals of each of three species of spring-breeding larval anurans to mesocosms: wood frogs (*Lithobates sylvaticus*), American toads (*Anaxyrus americanus*), and spring peepers (*Psuedacris crucifer*). Larval anurans are commonly considered periphyton grazers, although they may also filter phytoplankton from the water column (Altig et al. 2007). We collected amphibians as newly oviposited eggs from nearly wetlands (9 to 18 egg masses per species), allowed them to hatch in aged well water, and fed them rabbit chow *ad libitum*. Wood frogs and toads were Gosner stage 25 (Gosner 1960) when introduced to the mesocosms and spring peepers were stage 27. Tadpole mean masses (± SE) were as follows: wood frogs = 65 (3.67 mg), American toads = 29 (1.05 mg), and spring peepers = 50 (2.99 mg). Wood frogs were introduced on 27 May whereas toads and spring peepers were introduced on 28 May. To test for effects of handling on tadpole survival, we assessed 24-hr survival in the lab for all three species, which was 100%.

We introduced several species of macroinvertebrates into each mesocosm, including some of the most common consumers in our region. All species were generalist grazers that consume both algae and microbes from substrates. Two species of snails, the pouch snail (*Physa acuta*) and the ram's horn snail (*Helisoma trivolvis*), were introduced on 23 May. Both species were introduced as eggs to avoid potential introduction of parasites common to adult snails in the area. To obtain egg masses, we collected 100 adult snails of each species from local ponds on 10 May and allowed them to lay egg masses in 14-L plastic bins. Ten egg masses of each species were introduced into each mesocosm by haphazardly selecting egg masses from all bins, and gently scraping them into a cup which was then placed on the benthos of the mesocosm. On 2 June, we added ~40 individuals of one amphipod species, *Crangonyx psuedogracilis*, and ~40 individuals of one isopod species, *Asselus communis*. Using dipnets, we collected amphipods and

isopods from two ponds where they occurred at high densities, removed all other organisms from collections and placed equal aliquots (0.4 L) into each mesocosm. The date of amphipod and isopod additions marked the last day of additions to the community and day 0 of the experiment.

5.2.4 Abiotic measurements

To assess how litter chemical dissimilarity affected the abiotic conditions of the mesocosms, we measured light attenuation, dissolved oxygen, pH, and temperature on monthly (i.e. every 4 wks) with calibrated meters. We measured light attenuation on days 35, 74, and 105; dissolved oxygen and temperature on days 37, 65, and 97; pH on days 35, 74, and 97. Details of these measurements may be found in Appendix C.

5.2.5 Biotic measurements

We measured several biotic response variables at multiple times during the experiment. Further details regarding the sampling methods are provided in Appendix C.

We quantified leaf litter decomposition for each species in each mesocosm monthly (i.e. three sample dates; days 47, 74, and 108). To measure leaf litter decomposition rate, we recorded the mass loss of litter in mesh bags. Decomposition rate of conifer litter species was not measured since these species were not included in mesh bags.

We quantified algal and microbial biomass monthly (i.e. three sample dates; phytoplankton on days 42, 75, and 107; periphyton on days 33, 72, and 103). We estimated

phytoplankton as the biomass of chlorophyll *a* (chl *a*) in the water using pipe samples and fluorometric analysis. We estimated periphyton biomass as the mass of material scraped from half of a clay tile.

We quantified the abundance of zooplankton during the second and third month of the experiment (days 75 and 107). Although samples were taken during the first month (day 42), these samples were not enumerated, as zooplankton were not very abundant and it was clear that populations were still growing to carrying capacity. Among the collected samples, all zooplankton communities were comprised of no more than five species. The most dominant species were the copepod *Microcyclops rubellus*, and the two cladocerans *Schapholeberis mucronata* and *Daphnia pulex*. A single ostracod species (Order: Podocopida) and the cladoceran *Chydorus sphericus* were less common, but were found in substantial numbers within many mesocosms.

We quantified the biomass of amphipods and isopods monthly (days 47, 74, and 108). We estimated biomass by collecting individuals grazing off the leaves contained in mesh bags used for sampling litter decomposition rate. Since an unequal biomass of litter was in each bag, we corrected all biomass measurements by dividing recorded values by the total amount of litter in each bag.

We quantified the biomass of pouch snails and ram's horn snails monthly (days 56, 88, and 109). We estimated biomass as the mass of snails collected in a net swept along the bottom and up the wall of a mesocosm. While sorting, we also found substantial numbers of a third snail species, the two-ridge ram's horn snail (*H. anceps*), which were likely introduced with the zooplankton, microbes, and algae. Thus, we also estimated biomass for this species.

For the three species of amphibians, we recorded their survival to metamorphosis and mass at metamorphosis. We did not include time to metamorphosis as a response, as many toads and spring peepers had not begun the process of metamorphosis by the conclusion of the study. Metamorphosis of larval anurans began on 14 June. After this date, we checked mesocosms daily for metamorphosing individuals, and continued checking until 18 July. On this date, the last metamorph emerged and we verified the absence of remaining tadpoles by observing each tank for at least 10 min while gently disturbing the benthos. We ended the experiment on 31 August, which was the date of the last snail sample and established an experimental duration well-within the hydroperiod range of typical vernal ponds common to the area.

5.2.6 Statistical analyses

We analyzed for the effects of litter chemical dissimilarity, chemical trait means, and litter species presence / absence on litter decomposition rate, abiotic responses, and biotic responses. To compare response measurements conducted throughout the community, we standardized the data to a mean of zero and unit variance prior to all analyses. For each analysis, multivariate normality of data was verified by examining the scatterplot of Chi-squared values with squared Mahalanobis Distances, and assuming normality if the line was reasonably straight (Burdenski 2000). A single high chemical dissimilarity replicate was removed from the study due to the presence of a periphytic algal bloom that dominated the system, generated outlying abiotic responses, and led to high mortality among several community components.

5.2.6.1 Effects of litter chemistry and dissimilarity on litter decomposition rate: Unlike all other responses, litter decomposition rates were measured for each species of litter within each

mesocosm (except for conifer litter species, whose absence from mesh bags precluded measurement of decomposition rate). Hence, litter species decomposition rates were analyzed separately from the other response variables.

To assess the influence of litter chemical dissimilarity on decomposition rate of individual litter species in mesh bags, we employed analysis of variance (ANOVA) using a linear mixed model. Since natural variation in decomposition rates among litter species is likely to account for a large portion of total variance in decomposition rate, we included both litter chemical dissimilarity and identity of litter species in mesh bags in the model as fixed factors, as well as their interaction. Since more than one litter species was associated with each mesocosm, we included mesocosm in the model as a random factor. Preliminary analysis revealed a non-significant litter chemical dissimilarity by litter identity interaction, so this term was dropped from the model. Post-hoc treatment comparisons were conducted using the Bonferroni confidence interval adjustment method.

5.2.6.2 Effect of litter chemical dissimilarity and trait means on abiotic and biotic responses: To assess the effect of litter chemical dissimilarity on responses, we employed multivariate ANOVA (MANOVA). For responses measured at multiple time points, we employed repeated-measures MANOVA (rm-MANOVA). Multivariate analyses were followed by univariate ANOVAs after detecting a significant multivariate effect. For rm-MANOVAs, we conducted univariate ANOVAs on responses within sample dates if a significant time-by-treatment interaction was detected. When a significant univariate effect was detected, we conducted treatment comparisons using Tukey's HSD tests. Due to differences in response types and number of times each response was measured, we conducted four separate analyses to fully assess the influence of litter chemical dissimilarity on community responses. First, we conducted

a rm-MANOVA on the four abiotic measures, which were sampled three times during the study. Second, we conducted a rm-MANOVA on the following biotic responses that were also sampled three times: phytoplankton biomass, periphyton biomass, biomass of each snail species, and biomass of each benthic detritivore species. Third, we conducted a rm-MANOVA on zooplankton species densities, which were measured twice. Finally, we conducted a MANOVA on amphibian responses, as these were assessed only once during the study.

To assess the influence of mass-ratio effects, we followed each multivariate analysis with a multivariate multiple linear regression (MMLR) analysis that examined the effects of litter chemical trait means on abiotic and biotic responses. Chemical trait means were calculated as community-weighted trait means (details in Appendix D) and standardized to a mean of zero and unit variance prior to all analyses. As intended by our experimental design, preliminary analysis via multivariate analysis of variance (MANOVA) verified the lack of difference in trait means across litter chemical dissimilarity treatments ($P = 0.809$). Due to the large number of traits used in this study, and the large number of associated trait means, we first reduced the number of trait means by conducting a PCA. This resulted in two PCs that explained 65% of total variation in trait means. The first PC had positive loadings of nutrient trait means (percent N, P, Mg, Ca, and K in mixtures) and a negative loading of the mean percent carbon in mixtures. The second PC had positive loadings of mean percent carbon and phenolic in mixtures and a negative loading of the mean percent lignin in mixture. Hence, the first PC (herein "nutrient means") describes the nutrients available in different litter mixtures and the second PC (herein "structural means") describes their solubility, recalcitrance, and toxicity.

We used these two PCs in MMLR analyses with a model that included nutrient means, structural means, and their interaction. For repeated measures, we also included time and all

possible two- and three-way interactions in the model. When a significant multivariate multiple regression was found, multiple regressions were conducted on individual responses. When an interaction between time and another factor was significant, multiple regressions were conducted within sample dates. Similar to our analyses on litter chemical dissimilarity effects, we conducted separate MMLR analyses on abiotic responses, non-zooplankton and non-amphibian biotic responses, zooplankton responses, and amphibian responses.

5.2.6.3 Effect of species presence on abiotic and biotic responses: To assess whether responses could be attributed to the presence or absence of individual litter species, we employed redundancy analyses (RDA). RDA is a constrained, linear, multivariate analysis that combines regression and ordination to explore how variation in the structure of an independent dataset (e.g., species presence and absence) explains variation of a dependent dataset (e.g., abiotic and biotic response variables). Canonical axes (i.e. ecological gradients) for each data set are derived such that the ecological gradients derived from the independent dataset explain the maximum variation within the dependent dataset. Because we wanted to explore how litter species-response relationships changed over time, we conducted a separate analysis at each time point (i.e. three total analyses). We conducted a fourth analysis to explore trait-amphibian response relationships. To interpret the importance of species in determining response gradients and to interpret the strength by which species were associated with these gradients, we followed the recommendation of Tabachnik and Fidell (1989), and considered loadings of ± 0.45 as fair, ± 0.55 as good, and ± 0.63 as excellent. To assess whether response gradients significantly explained the variability among response variables, we conducted permutation tests (ter Braak and Verdonschot 1995). All ordination analyses were conducted using CANOCO, version 4.0.

5.3 RESULTS

5.3.1 Effects of litter chemistry and dissimilarity on litter decomposition rate

Our analysis of litter decomposition rates detected a significant effect of litter species ($F_{16,155}$ = 77.763, $P < 0.001$) and litter chemical dissimilarity ($F_{2,54} = 3.281$, $P = 0.045$). Decomposition rates varied tremendously among litter species. American beech, American sycamore, and black oak were among the slowest decomposing species (Table 5.1). They decomposed 59% to 71% slower than the fastest decomposing species, which were green ash, black cherry, and tulip poplar. Among the three levels of litter chemical dissimilarity, pairwise comparisons revealed that decomposition rates in the high-dissimilarity treatment were 15% faster than in the low-dissimilarity treatment ($P = 0.040$; Figure 5.1). There were no other significant differences between dissimilarity treatments ($P \geq 0.523$).

5.3.2 Effect of litter chemical dissimilarity and trait means on abiotic and biotic responses

5.3.2.1 Abiotic responses: Our analysis of abiotic responses to litter chemical dissimilarity did not detect a multivariate effect of litter chemical dissimilarity, time, or their interaction (Table 5.2). On further analysis of abiotic responses to litter trait means, we detected a marginal multivariate effect of structural means and a significant interaction of structural means with time. We did not detect multivariate effects of nutrient means or any other interaction (Table 5.3).

pH was affected by the interaction between structural means and time, so we conducted regressions within sample dates. The regression was significantly negative on the first sample date (β = -0.426, t = -3.554, P = 0.001; Figure 5.2), but not significant on the second and third sample dates (P $\geq$ 0.246).

Dissolved oxygen was affected by structural means, but not time or any interaction with time. Across all sample dates, there was a negative regression between dissolved oxygen and structural means (β = -0.206, t = -2.786, P = 0.006; Figure 5.3).

Light attenuation was affected by the interaction between structural means and time, so we conducted regressions within sample dates. There was a positive regression between light attenuation and structural traits on the first sample date (β = 0.663, t = 6.694, P < 0.001; Figure 5.2), but no significant regression on second or third sample date (P $\geq$ 0.282).

Temperature was not affected by structural means or an interaction between structural traits and time.

5.3.2.2 Phytoplankton, periphyton, snails, and benthic detritivores: Our analysis of phytoplankton, periphyton, ram's horn snail, two-ridge ram's horn snail, pouch snail, amphipod, and isopod biomass did not detect a multivariate effect of litter chemical dissimilarity, time, or their interaction (Table 5.2). On further analysis of these responses to litter trait means, we detected a multivariate effect of structural means, but no effect of nutrient means or time. In addition, we detected a marginally significant interaction of nutrient means and time, but no effect of any other two- or three-way interaction (Table 5.3).

Both phytoplankton and periphyton biomass were affected by structural means but not the interaction between nutrient means and time. Across sample dates, there was a negative

regression between structural means and phytoplankton biomass (β = -0.262, t = -3.598, P < 0.001; Figure 5.3), and a positive regression between structural means and periphyton biomass (β = 0.273, t = 3.758, P < 0.001; Figure 5.3).

Regarding biomass of snails, we detected a significant effect of structural means on ram's horn snail biomass, but not on pouch snail or two-ridge ram's horn snail biomass. We also detected an effect of the interaction between nutrient means and time on two-ridge ram's horn snail biomass, but not on ram's horn or pouch snail biomass. Across sample dates, there was a negative regression between structural means and ram's horn snail biomass (β = -0.201, t = -2.716, P = 0.007; Figure 5.3). Within sample dates, there was a positive regression between two-ridge ram's horn snail biomass and nutrient means on the third sample date (β = 0.271, t = 2.129, P = 0.038; Figure 5.2), but no significant regression on the first or second sample date (P ≥ 0.588).

Regarding biomass of benthic detritivores, we detected a marginally significant interaction between nutrient means and time, yet analyses within sample dates did not reveal any significant regressions (P ≥ 0.109).

5.3.2.3 Zooplankton: Our analysis of zooplankton abundances did not detect multivariate effects of litter chemical dissimilarity or time, but did detect an effect of their interaction (Table 5.2). On further analysis of zooplankton responses to litter trait means, we detected a multivariate effect of nutrient means and structural means, but no effect of time or any two- or three-way interaction (Table 5.3).

D. pulex abundance was affected by the interaction between litter chemical dissimilarity and time, but not by chemical trait means (Table 5.3). Within sample dates, there was no effect of litter chemical dissimilarity on the abundance of *D. pulex* during the second sample (P =

0.194), but there was an effect on the third sample date ($F_{2,56} = 3.591$, $P = 0.034$). Treatment comparisons of *D. pulex* abundances during the third sample date revealed that abundance in the medium diversity treatment was 59% to 69% higher than in the high and low diversity treatments, respectively, although the effects were only marginally significant ($P \leq 0.063$).

M. rubellus abundance was not affected by the interaction between litter chemical dissimilarity and time, but was affected by nutrient means, and marginally affected by structural means. Across sample dates, there was a positive regression between abundance and nutrient means ($\beta = 0.305$, $t = 3.452$, $P = 0.001$; Figure 5.3), but no significant regression of abundance with structural means ($P = 0.104$).

S. mucronata abundance was not affected by the interaction between litter chemical dissimilarity and time, and marginally affected by structural means. However, across sample dates, there the regression between abundance and structural means was not significant ($P = 0.254$).

C. sphericus abundance was not affected by the interaction between litter chemical dissimilarity and time, but was affected by nutrient means. Across sample dates, there was a negative regression between abundance and nutrient means ($\beta = 0.256$, $t = 2.854$, $P = 0.005$; Figure 5.3).

Ostracod abundance was not affected by the interaction between litter chemical dissimilarity and time, or chemical trait means ($P \geq 0.775$).

5.3.2.4 Amphibians: Our analysis of amphibian survival and mass at metamorphosis did not detect an effect of litter chemical dissimilarity (Table 5.2). On further analysis of these responses to chemical trait means, we detected effects of nutrient means, structural means and their interaction (Table 5.3).

Wood frog biomass was affected by both trait means, but not their interaction, and survival was not affected by either trait means or their interaction. There was a positive regression between wood frog biomass and nutrient means (β = 0.558, t = 5.071, P < 0.001), and a negative regression with structural means (β = -0.289, t = -2.281, P = 0.026; Figure 5.4).

American toad biomass was not affected by either trait means or their interaction, and survival was affected by structural means, but not nutrient means or the trait means interaction. There was a positive regression between American toad survival and structural means (β = 0.283, t = 2.224, P = 0.030; Figure 5.4).

Spring peeper biomass was affected by both trait means, and survival was marginally affected by nutrient means but not structural means the trait means interaction. There was a positive regression between nutrient means and spring peeper survival (β = 0.338, t = 2.710, P = 0.009). There was also a positive regression between nutrient means and spring peeper biomass (β = 0.398, t = 3.272, P = 0.002), and a negative regression with structural means (β = -0.544, t = -4.894, P < 0.001; Figure 5.4).

Table 5.2. ANOVA results on the influence of time and litter chemical dissimilarity on abiotic and biotic components of the mesocosm system.

	Time		Litter dissimilarity		Litter dissimilarity x time	
	F	P	F	P	F	P
Abiotic responses						
Multivariate	**$0.001_{8,49}$**	**>0.999**	**$0.320_{8,106}$**	**0.957**	**$1.134_{16,98}$**	**0.336**
pH	$0.001_{2,112}$	>0.999	$0.999_{2,56}$	0.375	$1.644_{4,112}$	0.168
Dissolved oxygen	$0.001_{2,112}$	>0.999	$0.290_{2,56}$	0.932	$0.782_{4,112}$	0.539
Light attenuation	$<0.001_{2,112}$	>0.999	$0.071_{2,56}$	0.932	$0.534_{4,112}$	0.711
Temperature	$<0.001_{2,112}$	>0.999	$0.182_{2,56}$	0.834	$1.332_{4,112}$	0.270
Phytoplankton, periphyton, snails, and benthic detritivores						
Multivariate	**$0.001_{14,43}$**	**>0.999**	**$0.350_{14,100}$**	**0.985**	**$0.933_{28,86}$**	**0.568**
Phytoplankton	$0.001_{2,112}$	>0.999	$0.047_{2,56}$	0.954	$1.403_{4,112}$	0.238
Periphyton	$<0.001_{2,112}$	>0.999	$0.688_{2,56}$	0.507	$0.952_{4,112}$	0.437
Ram's horn snails	$<0.001_{2,112}$	>0.999	$1.011_{2,56}$	0.370	$0.312_{4,112}$	0.870
Pouch snails	$<0.001_{2,112}$	>0.999	$0.475_{2,56}$	0.624	$0.461_{4,112}$	0.764
Two-ridge ram's horn snails	$0.001_{2,112}$	>0.999	$0.365_{2,56}$	0.696	$1.360_{4,112}$	0.252
Isopods	$<0.001_{2,112}$	>0.999	$0.201_{2,56}$	0.819	$0.176_{4,112}$	0.950
Amphipods	$0.001_{2,112}$	>0.999	$0.903_{2,56}$	0.411	$0.687_{4,112}$	0.602
Zooplankton densities						
Multivariate	**$0.002_{5,52}$**	**>0.999**	**$0.950_{10,104}$**	**0.483**	**$2.188_{10,104}$**	**0.024**
D. pulex	$0.001_{1,56}$	0.972	$0.170_{2,56}$	0.844	$4.791_{2,56}$	0.012
S. mucronata	$<0.001_{1,56}$	0.993	$3.334_{2,56}$	0.043	$0.800_{2,56}$	0.454
M. rubellus	$0.001_{1,56}$	0.974	$0.047_{2,56}$	0.954	$0.963_{2,56}$	0.388
Ostracod	$0.001_{1,56}$	0.982	$3.146_{2,56}$	0.051	$0.713_{2,56}$	0.495
C. sphericus	$0.002_{1,56}$	0.961	$0.144_{2,56}$	0.954	$2.248_{2,56}$	0.115

Table 5.2 (continued)

Amphibian survival and biomass						
Multivariate	--	--	**$0.623_{12,102}$**	**0.818**	--	--
Wood frog survival	--	--	$1.123_{2,54}$	0.333	--	--
Wood frog biomass	--	--	$0.029_{2,54}$	0.972	--	--
American toad survival	--	--	$1.337_{2,54}$	0.271	--	--
American toad biomass	--	--	$0.674_{2,54}$	0.514	--	--
Spring peeper survival	--	--	$0.019_{2,54}$	0.981	--	--
Spring peeper biomass	--	--	$0.149_{2,54}$	0.862	--	--

Table 5.3. ANOVA results of the influence of time, nutrient means, and structural trait means on abiotic and biotic components of the mesocosm system. Trait means represent two principal components that explained 80% of the total variation in trait means. See text for further explanation. There were no significant effects of time ($P \geq 0.999$) or the three-way interaction between time, nutrient traits, or structural traits ($P \geq 0.351$).

	Nutrient means		Structural means		Time x nutrients		Time x structure		Nutrients x structure	
	F	P	F	P	F	P	F	P	F	P
Abiotic responses										
Multivariate	**$0.386_{4,52}$**	**0.822**	**$2.271_{4,52}$**	**0.074**	**$0.303_{8,48}$**	**0.961**	**$7.603_{8,48}$**	**<0.001**	**$0.679_{4,52}$**	**0.610**
pH	--	--	$0.834_{1,55}$	0.365	--	--	$6.696_{2,110}$	0.002	--	--
Dissolved oxygen	--	--	$5.128_{1,55}$	0.028	--	--	$1.553_{2,110}$	0.216	--	--
Light attenuation	--	--	$2.866_{1,55}$	0.096	--	--	$15.024_{2,110}$	<0.001	--	--
Temperature	--	--	$0.812_{1,55}$	0.371	--	--	$0.015_{2,110}$	0.985	--	--
Phytoplankton density and biomass of periphyton, snails, and benthic detritivores										
Multivariate	**$0.524_{7,49}$**	**0.812**	**$3.955_{7,49}$**	**0.002**	**$1.809_{14,42}$**	**0.070**	**$0.719_{14,42}$**	**0.743**	**$1.144_{7,49}$**	**0.351**
Phytoplankton	--	--	$11.328_{1,55}$	0.001	$0.332_{2,110}$	0.718	--	--	--	--
Periphyton	--	--	$13.128_{1,55}$	0.001	$2.240_{2,110}$	0.111	--	--	--	--
Ram's horn snails	--	--	$4.042_{1,55}$	0.049	$2.016_{2,110}$	0.138	--	--	--	--
Pouch snails	--	--	$0.549_{1,55}$	0.462	$1.004_{2,110}$	0.370	--	--	--	--
Two-ridge ram's horn snails	--	--	$0.021_{1,55}$	0.886	$4.427_{2,110}$	0.014	--	--	--	--
Isopods	--	--	$1.223_{1,55}$	0.274	$2.786_{2,110}$	0.066	--	--	--	--
Amphipods	--	--	$0.041_{1,55}$	0.840	$1.496_{2,110}$	0.228	--	--	--	--

Table 5.3 (continued)

Zooplankton densities										
Multivariate	**$3.973_{5,51}$**	**0.001**	**$2.110_{5,51}$**	**0.079**	**$0.639_{5,51}$**	**0.671**	**$0.700_{5,51}$**	**0.626**	**$1.617_{5,51}$**	**0.172**
D. pulex	$2.014_{1,55}$	0.162	$0.054_{1,55}$	0.817	--	--	--	--	--	--
S. mucronata	$1.268_{1,55}$	0.265	$3.029_{1,55}$	0.087	--	--	--	--	--	--
M. rubellus	$8.659_{1,55}$	0.005	$3.321_{1,55}$	0.074	--	--	--	--	--	--
Ostracod	$0.051_{1,55}$	0.822	$0.083_{1,55}$	0.775	--	--	--	--	--	--
C. sphericus	$7.314_{1,55}$	0.009	$2.195_{1,55}$	0.144	--	--	--	--	--	--
Amphibian survival and biomass										
Multivariate	**$6.063_{6,50}$**	**<0.001**	**$10.542_{6,50}$**	**<0.001**	--	--	--	--	**$2.269_{6,50}$**	**0.052**
Wood frog survival	$0.062_{1,55}$	0.805	$1.832_{1,55}$	0.181	--	--	--	--	$1.757_{1,55}$	0.190
Wood frog biomass	$24.749_{1,55}$	<0.001	$6.854_{1,55}$	0.011	--	--	--	--	$0.226_{1,55}$	0.636
American toad survival	$0.732_{1,55}$	0.396	$4.010_{1,55}$	0.050	--	--	--	--	$0.680_{1,55}$	0.413
American toad biomass	$0.230_{1,55}$	0.634	$0.079_{1,55}$	0.780	--	--	--	--	$1.554_{1,55}$	0.218
Spring peeper survival	$3.708_{1,55}$	0.059	$0.100_{1,55}$	0.753	--	--	--	--	$1.150_{1,55}$	0.299
Spring peeper biomass	$8.456_{,55}$	0.005	$33.827_{1,55}$	<0.001	--	--	--	--	$2.818_{1,55}$	0.099

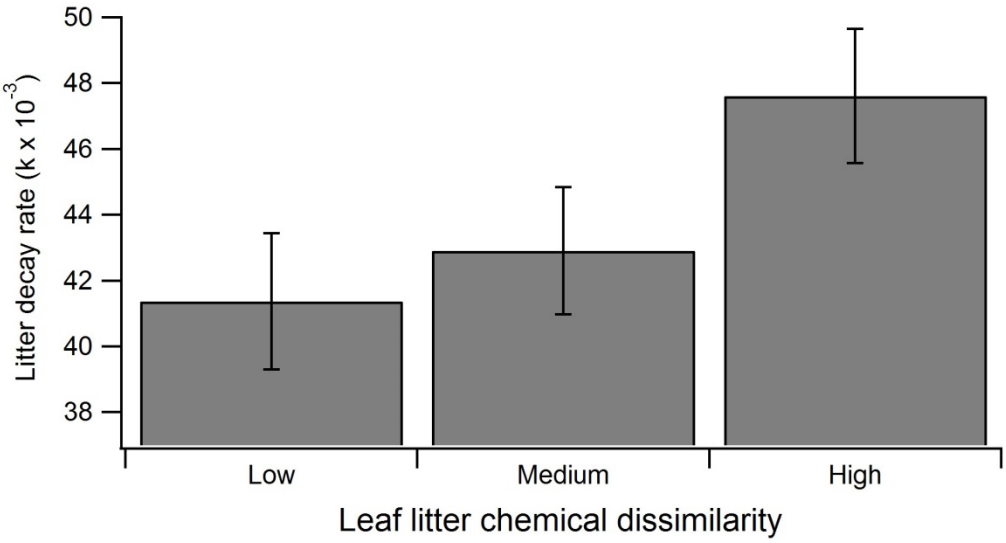

Figure 5.1. Effect of litter chemical dissimilarity on average decomposition rate (measured as the coefficient of decay [k] *sensu* Petersen and Cummins 1974) of individual species in mixture. Bars represent the average decomposition rate of all individual litter species found within all mesocosms of a single diversity treatment, over three months of decomposition. Bars are ± 1 SE.

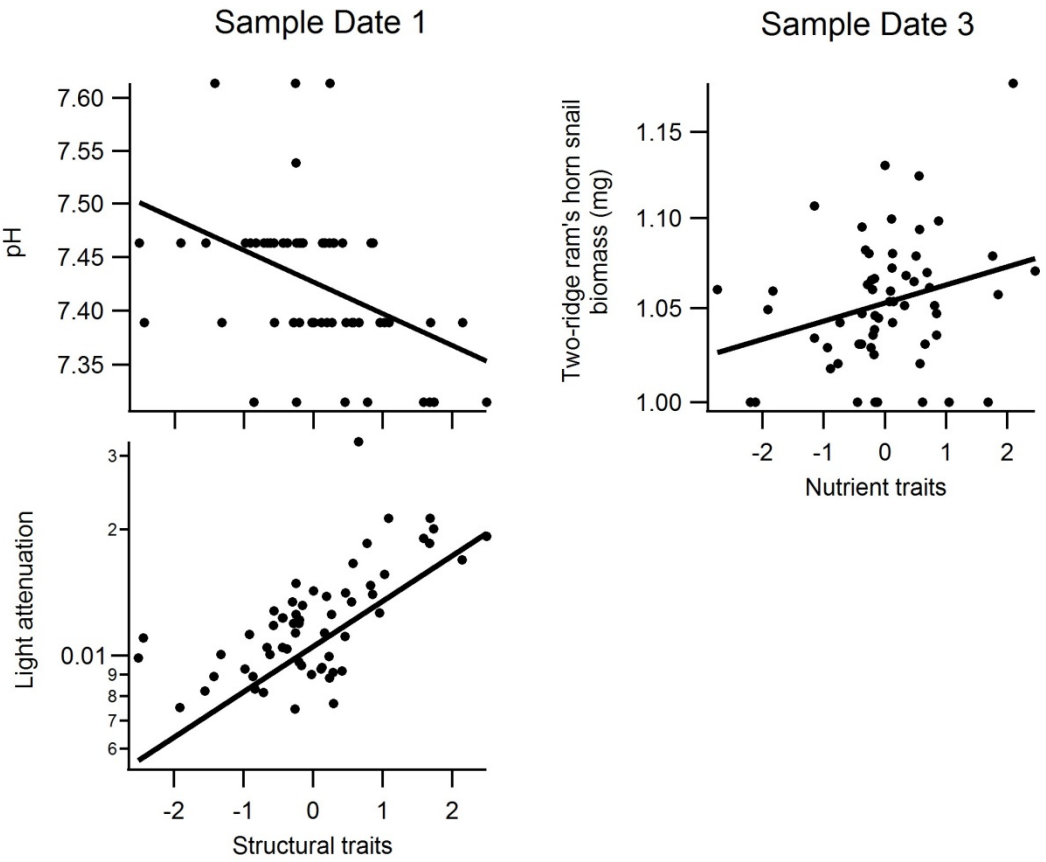

Figure 5.2. Regressions of community responses to nutrient and structural trait means within sample dates. Only significant correlations are shown; there were no significant correlations between trait means and responses on the second sample date.

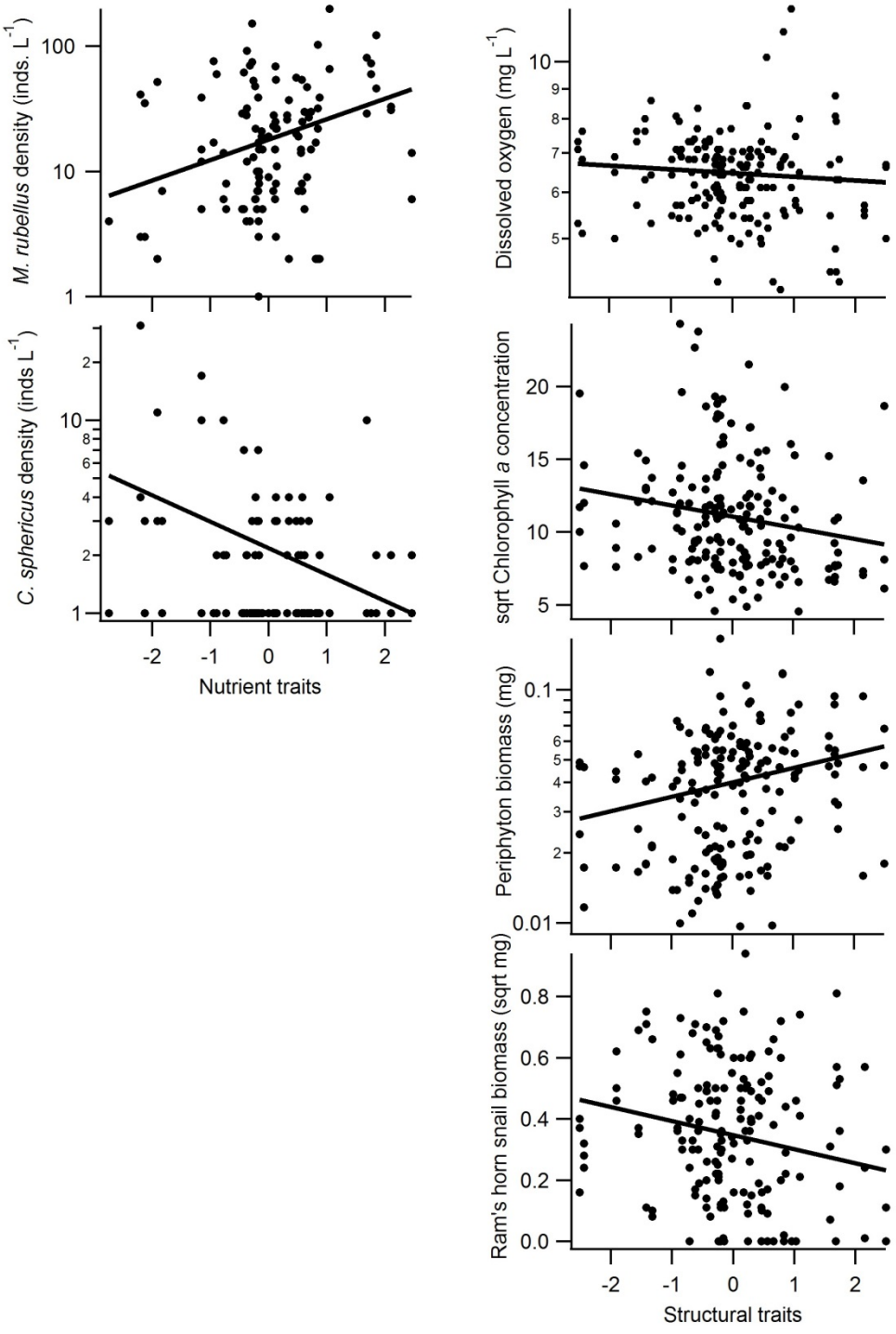

Figure 5.3. Regressions of community responses to nutrient and structural trait means across sample dates. Only significant correlations are shown. Note that chlorophyll *a* biomass and ram's horn snail biomass are square-root transformed.

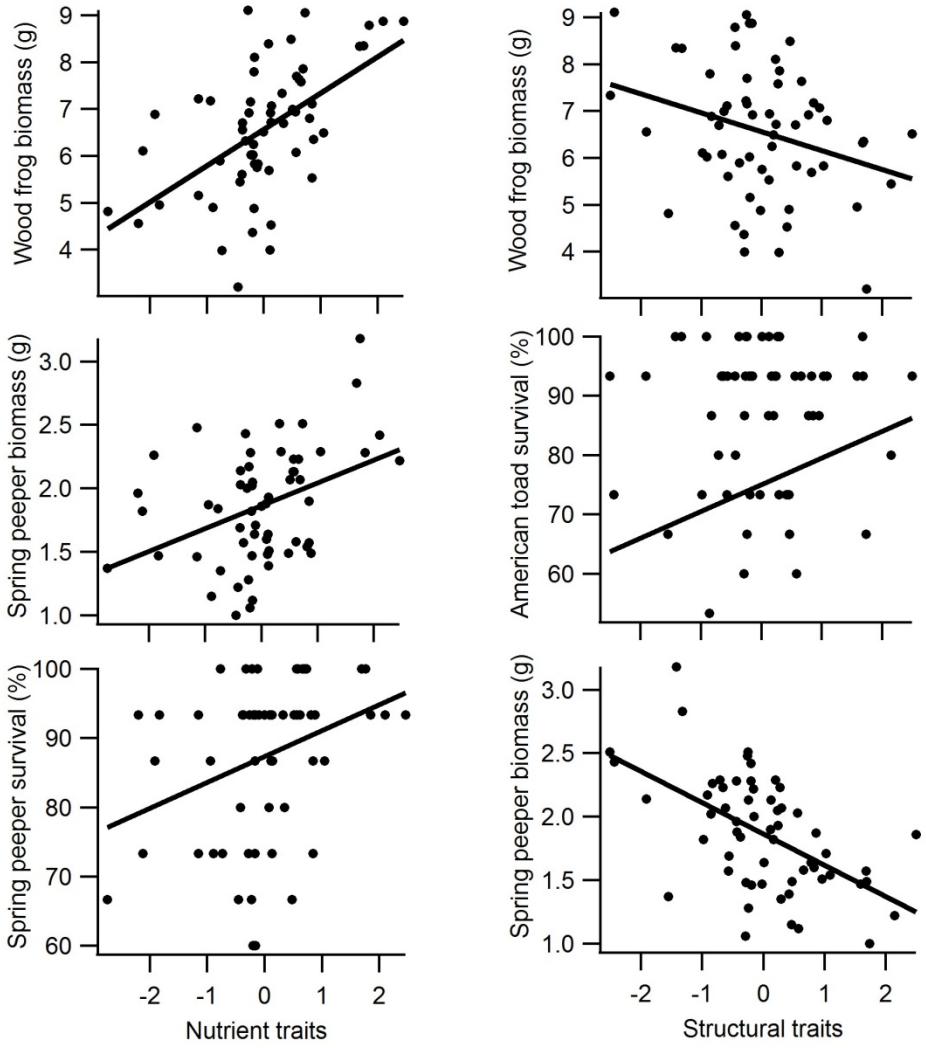

Figure 5.4. Regressions of amphibian responses to nutrient and structural trait means. Only significant correlations are shown.

5.3.3 Effect of litter species presence on abiotic and biotic responses

To assess the potential for selection effects in our study, we used redundancy analysis to determine if abiotic and biotic responses associated with the presence or absence of individual leaf litter species. Litter species abbreviations are found in Table 5.1.

5.3.3.1 First sample date: On the first sample date, litter species explained a significant amount of variability in the abiotic and biotic response variables (permutation test: F = 1.695; P = 0.002; Figure 5.5a). Presence of SM and HYCH litter positively associated with the first axis, whereas presence of CHCH litter negatively associated with the second axis. Regarding responses to these axes, we found that light attenuation was positively associated with the presence of SM and HYCH litter, while phytoplankton density, pH, and dissolved oxygen were negatively associated the presence of SM and HYCH litter. Pouch snail biomass positively associated with the presence of CHCH litter).

5.3.3.2 Second sample date: On the second sample date, litter species presence did not explain variation in the abiotic and biotic response variables (permutation test: F = 1.307; P = 0.182; Figure 5.5b). However, the presence of QUASP litter did exhibit a positive association with the second axis, and we found that *M. rubellus* abundance was also positively associated with this axis.

5.3.3.3 Third sample date: On the third sample date, litter species explained a marginally significant amount of variability in the abiotic and biotic response variables (permutation test: F = 1.211; P = 0.068; Figure 5.5c). The presence of BIRCH litter was positively associated with the first axis whereas the presence of CHCH litter was negatively associated with the second axis. Regarding responses to these axes, we found that biomass of ram's horn and pouch snails

was positively associated with the presence of BIRCH litter, and periphyton biomass was negatively associated the presence of BIRCH litter. The biomass of two-ridge ram's horn snails was negatively associated with the presence of CHCH litter.

5.3.3.4 Amphibians: We found that litter species explained a significant amount of variation in the amphibian responses (permutation test: F = 1.498; P = 0.0260; Figure 5.5d). The presence of QUASP litter negatively associated with the first axis, but no other litter species showed any association with either axis. Regarding amphibian responses to this axis, we found that biomass of wood frogs and spring peepers positively associated with the presence of QUASP litter.

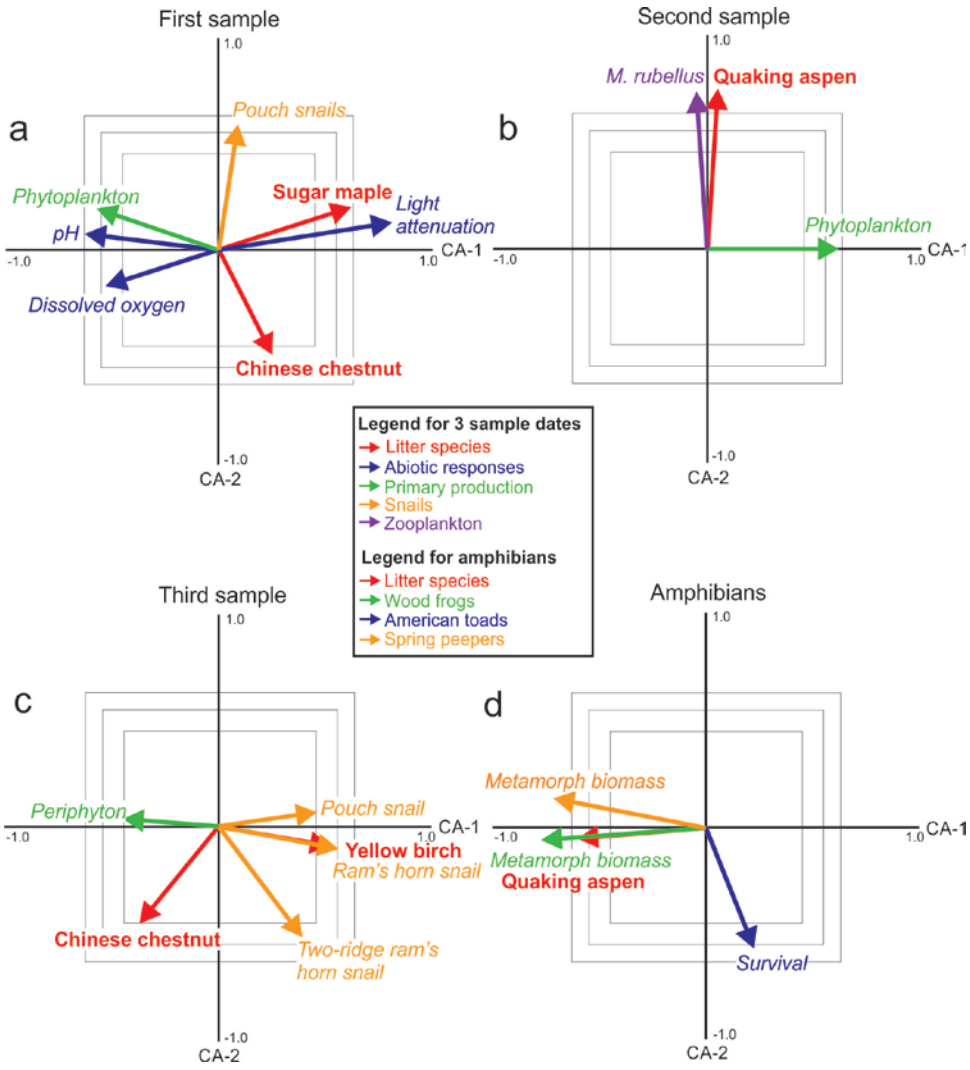

Figure 5.5. Biplots of redundancy analyses showing the relationship between litter species presence / absence and community responses for a) the first sample date, b) the second sample date, c) the third sample date, and d) amphibians. Independent values are species presence / absence and dependent values are responses within each mesocosm. Lengths of arrows indicate the importance of an independent variable to the axes (i.e. loading on axes) whereas directions of arrows indicate the direction of change along that axis. The three squares indicate cutoff points for fair (± 0.45), good (± 0.55), and excellent (± 0.63) loadings on each canonical axis (CA) as recommended by Tabachnik and Fidell (1989). Litter species or community responses whose loadings did not meet the lowest cutoff were excluded from biplots.

5.4 DISCUSSION

Our study found that three separate components of leaf litter diversity, including litter chemical dissimilarity, litter chemical trait means, and the presence of individual litter species had differential effects individual processes and components within an aquatic community. By removing confounding relationships between litter chemical dissimilarity, litter species presence and absence, and litter chemical trait means, we were able to examine the independent effects of each litter diversity component. In agreement with our first hypothesis, litter chemical dissimilarity was positively related to the decomposition rate of individual litter species. However, in contrast to our predictions, the processes among higher trophic levels did not respond to changes in litter chemical dissimilarity. Instead, many of these variables responded to litter chemical trait means and the presence of individual litter species. Hence, our study suggests that independent components of resource diversity differentially determine aquatic community processes across multiple trophic levels.

5.4.1 Effect of litter diversity on litter decomposition rate

We observed a 16% increase in decomposition rate between low and high chemical dissimilarity treatments. This is a substantial increase relative to the majority of past litter mixing studies, which have found between 1% to 65% synergistic increases in decomposition rate as a result of increasing litter diversity (Hättenschwiler et al. 2005). However, this range of synergy represents studies that manipulated litter species richness rather than functional dissimilarity of the litter

chemistry. Subsequently, this range also represents mixtures whose dissimilarity likely extends beyond the range of chemical diversity manipulation in our study, and also confounds diversity with mixture chemistry (Vos et al. 2013).

To our knowledge, there are only three experiments across both aquatic and terrestrial studies that have explicitly examined the relationship between litter trait dissimilarity and decomposition rates. Among two of these studies, responses to dissimilarity were either non-existent (Schindler and Gessner 2009) or relatively minor in comparison to the influence of other environmental factors, such as temperature (Lecerf et al. 2011). In contrast, Meier and Bowman (2008) manipulated chemical dissimilarity of litter mixtures in soil and found positive relationships of litter chemical dissimilarity with net N mineralization. However, none of these studies attempted to control the confounding relationships between litter chemical dissimilarity, trait means, or species presence, leading to somewhat ambiguous interpretation of results. In contrast, our experimental design explicitly attempted to remove these relationships, making it likely that litter trait dissimilarity was largely responsible for the change in decomposition rate.

Several other studies have found decomposition to be predicted by initial variation in single chemical traits (Wardle et al. 1997, Meier and Bowman 2008, Schindler and Gessner 2009, Lecerf et al. 2011, Vos et al. 2013). For example, Wardle et al. (1997) found that the mass of litter remaining in mixture was inversely related to the initial variation of leaf nitrogen content, resulting in up to a 65% synergistic increase of decomposition rate from expected values. Interestingly, several of these studies have also found interactions between litter trait means and litter species identity, which our study did not. One possible reason for this contrast may be our use of multiple traits to determine dissimilarity. Indeed, the decomposition of litter is controlled by multiple, and often uncorrelated traits (Epps et al. 2007), and the manipulation of

single trait variation may lead to undesired variation in another trait. For example, a mixture that has low dissimilarity in nitrogen content may have high dissimilarity in phenolics. In this scenario, the presence or absence of a leaf with high phenolics may lead to selection effects. Our use of a multivariate index likely removed this problem, and subsequently isolated the effect of resource complementarity on the decomposition process.

5.4.2 Effects of diversity on higher trophic levels.

Although litter chemical dissimilarity was positively related to litter decomposition rate, we found no effect of chemical dissimilarity on the biomass of any consumer species. This is surprising, as there is evidence that leaf decomposition positively relates to the quality of resources for consumers (Smock and MacGregor 1988, Sweeney 1993). One possible explanation for this result is that consumers exhibited compensatory feeding at lower levels of litter dissimilarity. Several studies of aquatic macroinvertebrates demonstrate compensatory feeding on litter substrates when litter quality is relatively low (Lindroth et al. 1993, Swan and Palmer 2006). This is a plausible explanation for our results, as litter mixtures of low dissimilarity likely provided incomplete resources for consumers, making it a relatively low quality diet. Microbial communities may have exhibited similar compensatory dynamics, and subsequently buffered higher trophic levels from experiencing the variation in litter resource dissimilarity. Indeed, studies of litter decomposition in soil have found that microbial activity and rates of nutrient mineralization increase with litter chemical diversity, yet microbial nutrient

biomass remained similar across all levels of diversity (Meier and Bowman 2008, 2010). This explanation may be explored further by measuring microbial and consumer metabolism through such measures as respiration, ingestion, excretion, and egestion rates.

Another possible explanation for the lack of consumer response to litter chemical dissimilarity is that plasticity in consumer stoichiometry reduced the apparent influence of litter chemical dissimilarity on the community. Indeed, there is increasing evidence that many aquatic consumers exhibit stoichiometric plasticity in response to changing resource quality without substantial changes in survival, growth, or fitness (Cross et al. 2005). This would lead to elevated rates of litter decomposition as observed in our study, as well as elevated levels of inorganic nutrients in the water column, but not increased resource quantity or quality for consumers. This explanation may be explored further by measuring microbial and consumer nutrient.

Although consumers showed little response to litter chemical dissimilarity, we did find that many species were highly sensitive to average litter chemistry, and particularly to levels of soluble carbon and phenolics. This result is in agreement with previous studies showing similar sensitivity of consumers to these compounds, particularly with respect to tadpoles (Horne and Dunson 1995, Rubbo and Kiesecker 2004, Maerz et al. 2005, Stoler and Relyea *in review*). The negative effects of phenolics are likely direct, due to the ability of these compounds to bind with active proteins (Maerz et al. 2005). In contrast, the negative effect of soluble carbon on consumers is likely indirect: elevated levels of soluble carbon decreases light attenuation, primary production, pH, and dissolved oxygen. Simultaneously, the increase in decomposition associated with higher levels of soluble carbon is associated with higher levels of aerobic respiration on the litter surface, further decreasing dissolved oxygen levels. Indeed, we observed a negative relationship between structural trait means, chl *a* in the water column, and dissolved

oxygen. We also observed a positive relationship of soluble carbon with light attenuation, pH, and periphyton biomass. These changes indicate a simultaneous decrease in algal production and increase in microbial biomass on the benthos.

Previous studies suggest that many of the species used in our study lack tolerance to such conditions. Wood frogs are particularly sensitivity to high levels of dissolved organic carbon (Horne and Dunson 1995, Stoler and Relyea *in review*), and spring peepers are sensitive to high levels of phenolics (Stoler and Relyea *in review*). Overall, tadpole performance appears to consistently improve as the amount of soluble leachates in litter decreases (Williams et al. 2008). Indeed, we also found decreased wood frog and spring peeper biomass with increasing litter concentrations of phenolics and soluble carbon. Interestingly, American toad survival was positively associated with increasing soluble carbon, despite a sharp reduction in survival found in Stoler and Relyea (*in review*). Additionally, our current study found no relationship between *P. acuta* biomass and litter chemistry although a negative relationship of *P. acuta* biomass with soluble carbon was one of the strongest effects in Stoler and Relyea (*in review*). One cause for these contrasts may be that moderate levels of soluble carbon can actually benefit aquatic organisms, since it serves as potential energy resource (Wetzel 2001, Williamson et al. 1999). Indeed, the maximum level of soluble carbon in our study was certainly below that of Stoler and Relyea (*in review*), potentially allowing less sensitive organisms such as *P. acuta* and American toads to persist and even flourish.

There were also several relationships between litter nutrient content and consumer responses. Biomass of wood frogs, survival of American toads, and biomass of spring peepers all increased with nutrient content. These results are not surprising; several studies note that the performance of tadpoles and other consumers is positively correlated with litter nutrient content

(Moran and Hodson 1989). For example, Kupferberg (1997) demonstrated that tadpole growth rate increases with protein content of algal resources. Similar to our study, Cohen et al (2012) found that American toad survival increased with relative nutrient content of litter resources. However, it is worth noting that the effects of litter nutrients in their study were relatively weak compared to factors such as dissolved oxygen and phenolics. Indeed, Stoler and Relyea (*in review*) also found that the effects of nutrients were relatively weak in comparison to the effects of elevated soluble carbon and phenolics. Thus, our study finds partial support for the notion that litter nutrient concentration is an important determinant of consumer biomass (Moran and Hodson 1989), yet we find support for the overriding effect of leached litter components in forest wetlands (Stephens and Berven *in review*, Stoler and Relyea *in review*).

5.4.3 Effects of individual litter species

Often, the litter species with extreme amounts of soluble carbon or phenolics were often associated with community processes, thus providing greater evidence for the strong effect of litter leachates and nutrients. For example sugar maple exhibited associations with pH, dissolved oxygen, light attenuation and phytoplankton biomass, and also had the highest levels of tannin and phenolics among all litter species in our study, relatively high soluble carbon, and low lignin. Chinese chestnut litter, which exhibited an association with *P. acuta* and *H. anceps* biomass, contained the second lowest lignin content and second highest tannin content of all species in our study. Quaking aspen litter, which showed an association with *M. rubellus* densities, wood frog biomass, and spring peeper biomass, contained the lowest phenolic and soluble carbon content,

relatively low tannin content, the highest amounts of lignin and nitrogen, and relatively high phosphorus levels. Yellow birch litter, which showed an association with *H. trivolvis* and periphyton biomass, had relatively high nitrogen content.

The association of these species with community responses suggests that the effects of average litter chemistry may be due to the presence of single litter species with extreme chemistry. It is possible that the presence of these species altered the influence of other species in mixture (i.e. selection mechanism). However, our study cannot verify this mechanism, as there were no monoculture litter species treatments. The more parsimonious explanation is that the chemical uniqueness of these litter species simply altered average mixture chemistry, and subsequently influenced community responses. Indeed, the responses which associated with each litter species also tend to associate with the chemical traits represented by those species. For example, analysis of trait mean regressions revealed that wood frog biomass was positively associated with litter nutrient content and negatively associated with secondary compound content, and was positively associated with the presence of quaking aspen litter which has both high nutrient content and low amounts of secondary compounds.

5.4.4 Implications for forest management

Such species-specific influences, in addition to the effects of average mixture chemistry and chemical diversity have strong implications for the functioning of forests. Over the past hundred years, temperate forests have undergone massive shifts in composition, such as the complete loss of American chestnut due to invasive fungal disease (Smock and MacGregor 1988). Ongoing changes include the loss of oaks due to over-browsing by mammals (Abrams 2003), decimation

of eastern hemlock (*Tsuga canandensis*) and ash due to invasive diseases and insects (Orwig and Foster 1998, Kovacs et al. 2010) massive changes in composition and succession from practices such as fire suppression and selective logging (Abrams 2003). In turn, a few opportunistic species such as black cherry and red maple are encroaching on novel territory (Abrams 1998). Often, such encroaching species are well defended through chemical traits (Cappuccino and Arnason 2006), are subsequently likely to drive mixture chemistries to extreme values. Simultaneously, our study suggests that the loss of diversity that will be associated with these changes is likely to reduce the rate of decomposition and nutrient cycling while increasing carbon storage in wetlands. Given the connectance of wetlands to surrounding riparian zones and to the rest of the forest (Wetzel 2001, Dreyer et al. 2011, Reinhardt et al. *in press*), our study provides a unique perspective on how changing compositions of forest vegetation are likely to alter the ecosystem ecology of temperate forests.

It is worth noting that several restoration and conservation processes are also changing forest composition, and may alter ecological processes. For example, there is currently substantial effort to reintroduce the American chestnut tree species into northeast forests with a hybridized and disease resistant American chestnut (Thompson 2012). We included an earlier generation of this hybrid species (i.e. HYCH) in our study. Interestingly, this species had the lowest lignin content of all species, highest phosphorus content, and nearly highest tannin, phenolic, and soluble carbon content. Given these extreme chemical characteristics, our study suggests that wetland consumer production may be increased by the reintroduction of a chestnut species into temperate forests, particularly if it reaches the dominance once assumed by American chestnut (i.e. up to 25% of species composition; Thompson 2012).

5.4.5 Conclusions

Our study isolates the effect of litter resource complementarity on wetland ecosystem processes, and reveals how various components of litter diversity, including trait dissimilarity, trait means, and the presence of individual litter species can alter a forested aquatic environment. We detected directionality with regard to the influence of these diversity components across the food web. In particular, litter chemical dissimilarity positively correlated with litter decomposition rates, indicating that consumers increasingly ingested litter resources as litter resource diversity increased. However, this was not reflected in consumer responses, which were largely determined by litter trait means. In addition, we found that the presence of chemically unique litter species strongly influences abiotic responses and consumer processes, which serves to bolster conclusions regarding the overriding effects influence of litter chemistry on wetland community components. The activity and stoichiometry of microbial communities may mediate this disconnect in the way litter diversity influences the various levels of a forest wetland food web, and this deserves further investigation. In addition, it is increasingly accepted that changes in diversity and species composition will not occur randomly or evenly (Walker 1992, Walker et al. 1999), and further research is needed to understand how expected changes in local tree composition and evenness will influence wetland processes. Our study provides the necessary first step to predict such responses and further research will likely continue to solidify our understanding of the relationship between diversity and ecological function.

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6.0. CONCLUSION

The ecology and conservation of wetlands is important at the landscape level, because wetlands connect with surrounding ecosystems in a variety of ways. Inputs to wetlands can be derived from large tracts of forests, since they are often at the lowest topographical points in an area and are gravitational attractors for mobile material (Wetzel 2001). In turn, material exported from wetlands may spread over an equally large spatial expanse: wetlands release gas (i.e. through inorganic chemical reactions, algal photosynthesis, or respiration; Wetzel 2001, Rubbo et al. 2006), mineral nutrient runoff, and living organic material that migrates throughout the forest (Beard et al. 2002, Dreyer et al. 2011, Reinhardt et al. *in press*). Consequently, reductions or increases in wetland functioning can have broad consequences for forest nutrient cycling. The studies in this thesis suggest that changes in leaf litter chemistry may lead to such functional shifts in wetlands.

However, further work is needed to directly link leaf litter inputs with changes at the ecosystem level. First, greater attention should be given to microbial community composition and function. Bacteria and fungi are ubiquitous components of aquatic systems (Mitsch and Gosselink 2007), are likely responsible for an enormous amount of respiration and nutrient mineralization (Hall and Meyer 1998), and evidence suggests that populations strongly respond to the chemistry of litter inputs (Rubbo and Kiesecker 2004). Second, although mesocosms are an excellent venue to manipulate conditions and test hypotheses, observations and manipulative

studies in natural wetlands are needed to discern natural phenomena. Third, to explicitly investigate how litter inputs alter ecosystem function, we need to conduct ecosystem-scale measurements of nutrient and energy fluxes such as gas release and nutrient export. These measurements should be conducted in both mesocosms and natural systems to test hypotheses and discern natural patterns.

Consideration should also be given for how leaf litter may influence biomass inputs to wetlands, particularly eggs from terrestrial organisms. Aside from litter, a major energy and nutrient resource in wetlands comes from breeding organisms that annually oviposit a substantial biomass of eggs (Regester et al. 2006, Reinhardt et al. *in press*). Importantly, many of these organisms have a choice regarding the placement of their eggs, and frequently choose locations that will benefit the fitness of their offspring (Jaenike 1978). For example, several studies have demonstrated that adult amphibians and mosquitoes choose their breeding location based on resources, predators and contaminants (Resetarits and Wilbur 1989, Vonesh and Buck 2007, Reiskind et al. 2009). My thesis demonstrates clear links between the chemistry of litter inputs and tadpole survival, so it is reasonable to expect breeding amphibians may also use the physical and chemical qualities of litter inputs as an oviposition cue. Testing of this hypothesis will enhance our understanding of landscape-level reproduction patterns, and serve to compare the role of litter inputs with other environmental factors already known to influence consumer fitness.

Such studies are needed to properly place the effects of litter chemistry in the context of other environmental factors and global patterns. For example, chemical contaminants (e.g., pesticides, nutrients from fertilizer), are frequently found in wetlands, and often have detrimental effects on ecosystem functioning, such as the generation of algal blooms that lead to consumer

mortality (Relyea and Diecks 2008). Given that the chemistry of some litter inputs can promote or inhibit algal blooms, it is reasonable to predict that litter inputs may interact with contaminants. Other environmental factors, such as temperature and precipitation, may also interact with leaf litter inputs. Decomposition is primarily a metabolic process and its rate is governed by both temperature and the chemistry of litter (Fierer et al. 2005). Consequently, the effect of temperature on the breakdown rates of litter species depends on their litter chemistry. For example, temperature causes a greater increase in breakdown rates of recalcitrant litter species (e.g., *Quercus* spp.) than labile species (e.g., *Acer* spp.; Fierer et al. 2005). As temperatures continue to rise, we need to understand how realistic shifts in forest composition will interact with temperature to affect wetland function.

There is ample ecological theory to generate specific hypotheses and predictions for these avenues of future research. For example, optimal oviposition theory (Jaenike 1978) can help to predict the effects of litter on consumer breeding patterns. Metabolic theory (Brown et al. 2004) may predict the interaction between litter inputs and temperature. Theories concerning the role of limiting nutrients on nutrient release (e.g., Tilman 1982) can generate predictions for the ecosystem-scale effects of litter inputs, and stoichiometric theory (Sterner and Elser 2002) may predict what types of microbial organisms will dominate a system. By integrating theory with empirical studies in a system of major conservation concern, this work can both inform conservation management and advance understanding of ecological phenomena.

APPENDIX A

CHAPTER TWO: SUPPLEMENTAL METHODS

A.1 LITTER CHEMICAL ANALYSIS

Prior to introduction, we assessed four components of litter chemistry to explore how abiotic and biotic responses change with litter chemistry, each a significant driver of decomposition rate and microbial colonization. We ground all leaf tissue to < 0.5 mm using a Wiley mill prior to analysis. We measured percent total phenolics with the Folin-Ciocalteu reagent after ethanol extraction. We assessed C:N content of the leaf litter with a CHN analyzer (University of George Stable Isotope Laboratory). Finally, we measured percent soluble carbon (SC) and lignin by carbon fractionation. The SC fraction was measured by three repeated extractions with 95% ethanol, three extractions with deionized water, followed by another single extraction with 95% ethanol. The sample was then dried for 24 hrs at 60 °C and reweighed. The difference between initial weight and final weight was noted as SC. Remaining sample was then digested with 72% sulfuric acid and autoclaved to extract cellulose and hemicellulose, and filtered onto pre-weighed

ash free filter paper. Filters with sample were weighed, ashed at 550 °C in aluminum tins and reweighed. Lignin content was the difference of sample mass post-sulfuric acid digestion and ashing.

A.2 ABIOTIC MEASUREMENTS

A.2.1 Dissolved oxygen and pH

We measured dissolved oxygen and pH just above the leaf litter layer in the benthos while we measured temperature just below the surface. However, since the litter significantly changed the color of the water in some treatments, which had the potential to cause temperature stratification, we also measured temperature just above the litter to assess temperature difference between the top and bottom of each mesocosm.

A.2.2 Light attenuation

We recorded photosynthetic active radiation (PAR) in the water column at depths of 2 cm and 22 cm below the water's surface using an underwater quantum sensor (LI-COR, Lincoln, Nebraska, USA). We used the differences in these values to measure light attenuation using the formula

$$K = \frac{\ln(L_2 / L_{22})}{d}$$

where L_2 is PAR at a depth of 2 cm, L_{22} is PAR at a depth of 22 cm, and d is the difference in depth between the two PAR measurements.

On day 114, we excluded two experimental units from the analysis due to the presence of algal mats on the water surface, which generated negative attenuation values. The excluded measures included one no-leaf treatment and one aspen litter treatment.

A.3 BIOTIC MEASUREMENTS

We measured decay rate of leaf litter by sampling a single mesh litter bag from each mesocosm each month, rinsed off the detritivores and snails, and recorded the mass of the litter after drying it for 24 hrs at 65 °C. We then used these values to determine the litter decay rate for each mesocosm with the following equation (Petersen and Cummins 1974).

To measure chl *a* concentration in the water column, we sampled 200 mL of water just below the surface at the four cardinal directions and in the center of the mesocosm. We did this by plunging a 200 mL plexiglass tube sampler in each location below the water surface and capping both ends with a rubber ball to seal the sample as it was brought above the water surface. We pooled and filtered all five samples through GF/C filters (Whatman, Kent, UK), and immediately froze the filters for chl *a* analysis by fluorometry after ethanol extraction (Arar and Collins 1997).

To measure periphyton biomass, we gently lifted one ceramic tile from each tank and vigorously scrubbed the surface of the top half of the tile onto a pre-weighed, oven-dried (65 °C, 24 hr) GF/C filter. We dried the filters again (65°C for 24 hr) and re-weighed to determine total dry mass of periphyton.

We sampled zooplankton via the tube sampling method used to measure chl *a* concentration. However, to capture zooplankton that might reside lower in the water column, we

collected the entire height of the water column in the north sample of the mesocosm by plunging a 1-m length of 5-cm diameter PVC pipe into the water column until it touched the benthos. We pooled and filtered water from all five samples through a 62-μm Nitex screen (Small Parts, Miami, Florida, USA), preserved all zooplankton in 30% ethanol. We enumerated cladoceran and copepod individuals to determine their density.

We sampled amphipods and isopods by collecting the individuals rinsed from the litter bags into a 500-μm sieve. Individuals were preserved in 70% ethanol and later counted to determine density. To measure total biomass of each species within a sample, we measured head length of individuals and converted values to individual mass by using established head length-mass relationships (Benke et al. 1999).

We sampled snails by sweeping an aquarium net along the soil layer from the center to the wall of each mesocosm, and then up along the wall of the mesocosm. This method excluded snails that were < 2 mm (i.e. the mesh size of aquarium net). We hand-sorted snails from the soil and litter, counted individuals by species, and determined total biomass of all individuals from each mesocosm after gently blotting the snails dry. We returned the entire content of the net sweeps, including the snails, to their respective mesocosms.

For each amphibian species, we recorded time to metamorphosis, total biomass at metamorphosis, total biomass of remaining tadpoles, and survival to metamorphosis. Once metamorphosis of larval amphibians began, we checked the mesocosms daily for metamorphosing individuals. We removed individuals with emergent forelimbs to the lab and held them in 1-L containers with moist sphagnum moss. Once tails had resorbed to ≤ 2 mm, metamorphosis was considered complete. For each individual, we recorded time to metamorphosis, euthanized them in 2% MS-222 (tricaine methanesulfonate), and preserved them

in 10% formalin. At the end of the experiment, all metamorphosed individuals were weighed to determine total species biomass and counted to determine survival to metamorphosis. By this time, several leopard frog, spring peeper, and gray treefrog tadpoles had not yet begun the process of metamorphosis; many had not grown past Gosner stage 36 (Gosner 1960). Thus, at the conclusion of the experiment (day 147), we collected any tadpoles remaining in the tanks, separated by species, preserved in 10% formalin, weighed them, and added this value to total metamorph biomass to determine total amphibian biomass. For these three species, we treated total metamorph biomass and total biomass (which included mass of remaining tadpoles) as two separate variables.

A.4 STATISTICAL ANALYSES

For all analyses of variance, upon finding a significant multivariate effect, we examined univariate effects to determine which response variables were significantly affected by litter treatments. Whenever a significant time-by-treatment interaction was found, we performed ANOVAs within each sample date. After finding significant univariate effects, we used Tukey's mean comparisons to determine which litter treatments differed.

For all analyses, we transformed all responses as necessary to meet assumptions of homoscedasticity and normality. Due to multiple low values or zeros in the dataset for ram's horn snail egg production and amphibian survival, these responses were rank-transformed. In the analysis of abiotic variables, two replicates had algal mats at the surface of the mesocosm which prevented us from measuring light attenuation. Likewise, there were two replicates with missing periphyton measurement on both the first and second sample set, and two replicates with missing

values for zooplankton on the third sample set. When these missing values occurred, we excluded corresponding replicates from corresponding analyses. There was never more than one replicate from any treatment removed and removal had no effect on the outcome of significance tests. Analyses were conducted using SPSS (Version 18.0).

In conducting the redundancy analyses (RDA), we included five leaf litter traits in the independent dataset: total phenolics, C:N content, percent lignin content, percent soluble carbon, and litter breakdown rate. For the dependent datasets, we conducted a separate RDA for each set of responses measured with the same frequency (i.e. four samples of abiotic conditions, phytoplankton and periphyton; 2 samples of zooplankton, snails, and detritivores; 1 sample of amphibians).

Biplots produced from RDA scores visualize the dominant ecological relationships, where axes are linear combinations of independent variables that explain the most possible variation among the dependent variables. Lengths of arrows indicate the importance of an independent variable to the gradients (i.e. loading on axes) while directions of arrows indicate the direction of change along that gradient. The cosine of an arrow with an axis or another arrow is the correlation coefficient of that variable with the axis and other variables, respectively. In all analyses, C:N, lignin, soluble carbon, total phenolics, decomposition rate, and time (coded as a dummy variable) were included as independent variables. All data were centered and standardized prior to analysis. Significance of canonical axes was determined using a Monte Carlo permutation test (number of permutations = 499). Analyses were conducted using CANOCO (Version 4.0)

APPENDIX B

CHAPTER TWO: LITTER DECOMPOSITION

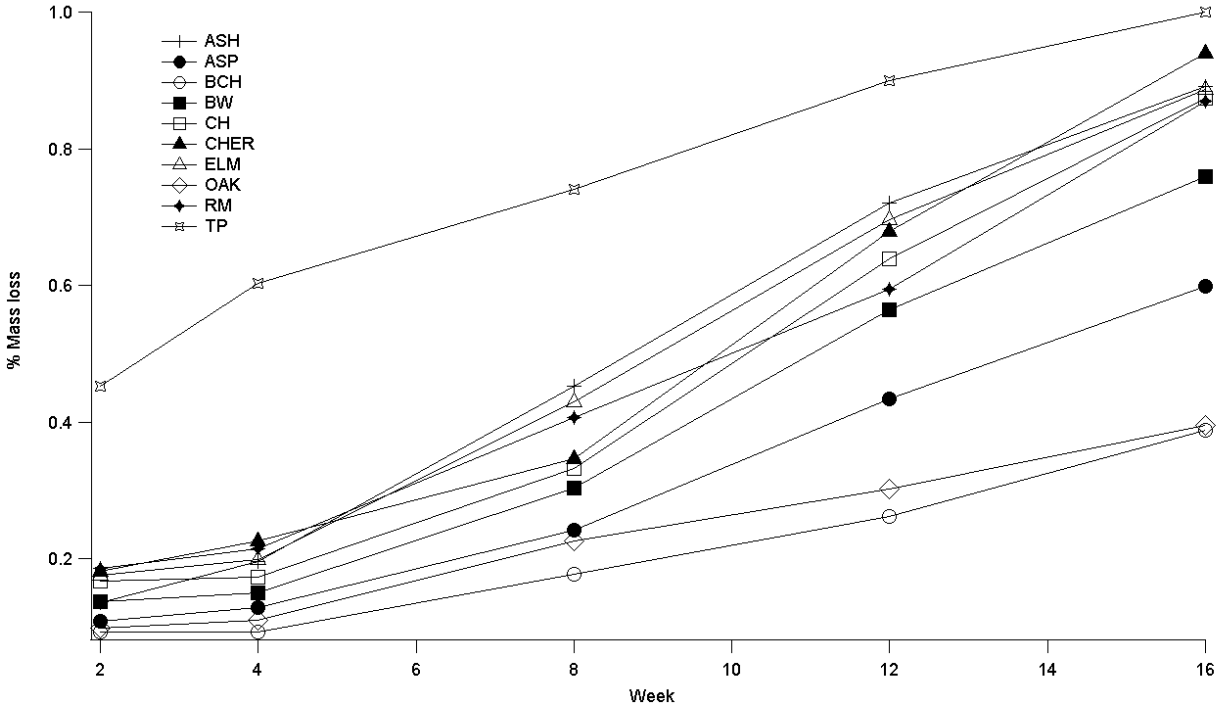

Figure B.1. The figure below depicts mass loss of each litter species over the duration of the experiment. ANOVA results: $F = 49.267$; $df = 9,30$; $P < 0.001$. Data points are means of the four mesocosms.

APPENDIX C

CHAPTER TWO: SUPPLMENTAL RESULTS

C.1 ABIOTIC RESPONSES

C.1.1 LIGHT ATTENUATION

Univariate effects of treatment were found on all four sample dates (days 25, 55, 80, and 112; Table C2). On day 25, OAK had lower attenuation than ELM, CH, TP, and RM ($P \leq 0.022$); BCH, ASH, BW, ASP, and CHER were less than CH, TP, and RM ($P \leq 0.014$); TP and RM was greater than all other treatments ($P \leq 0.022$). On day 55, TP, RM, and CH had greater attenuation than all other treatments ($P \leq 0.013$). On day 80, CHER, ASH, ASP, OAK, BCH, and ELM had greater attenuation than RM, CH, and TP ($P \leq 0.025$); and BW was greater than CH and TP. On day 112, no differences were detected among litter species treatments ($P \geq 0.118$).

C.1.2 Dissolved oxygen

Univariate effects of treatment were found on all four sample dates (days 19, 46, 76, 105, TableC2). On day 19, TP had lower dissolved oxygen than all treatments ($P < 0.001$); RM, CHER, CH, and ASH were less than ASP, OAK, BW, and BCH ($P \leq 0.009$); and ELM was less than BW and BCH ($P \leq 0.039$). On day 46, dissolved oxygen in TP remained lower than all treatments ($P < 0.001$); CH, RM, ELM and ASH was lower than BW, ASP, OAK, and BCH ($P \leq 0.047$); CHER was lower than BCH ($P = 0.024$). On day 76, CH was lower than RM, CHER, BCH, BW, and ASH ($P \leq 0.019$). On day 105, OAK had lower dissolved oxygen than ASH, BW, and CHER ($P \leq 0.014$); and CH and BCH was lower than BW and CHER ($P \leq 0.036$).

C.1.3 Temperature

Since there was a time by treatment interaction, we explored univariate effects of treatment at each sample date. Univariate effects of treatment were found only on the first two sample dates (days 19 and 47; Table C2). On day 19, temperature in RM was lower than all treatments except CH and TP ($P \leq 0.018$). On day 47, no differences were detected among litter species treatments ($P \geq 0.095$).

C.1.4 Temperature stratification

Univariate effects of treatment were found only on the first three sample dates (days 19, 47, and 75; Table C2). On day 19, stratification was lower in ASH than in CH, TP, and RM ($P \leq 0.016$);

ASP was lower than TP and RM ($P \leq 0.016$); and BW, CHER, ELM, BCH, and CH were lower than RM ($P \leq 0.027$). On day 47, stratification was lower in BCH, OAK, ASP, ASH, ELM, and CHER relative to RM and TP ($P \leq 0.028$); and BW was lower than TP ($P = 0.016$). On day 75, stratification in ELM was lower than BW and TP ($P \leq 0.028$).

C.1.5 pH

Univariate effects of treatment were found on all four sample dates (days 19, 47, 75, and 105; Table C2). On day 19, pH was lower in TP than ELM, ASP, OAK, BW, and BCH ($P \leq 0.002$); RM was lower than ASP, OAK, BW, and BCH ($P \leq 0.008$); ASH, CHER, and CH were lower than BW and BCH ($P \leq 0.008$); and ELM was lower than BCH ($P = 0.024$). On day 47, pH in RM was lower than in ASP and BCH ($P \leq 0.042$); and CH and ELM were lower than BCH ($P \leq 0.042$). On days 75 and 105, no differences were detected among litter species treatments ($P \geq 0.097$).

C.2 PHYTOPLANKTON AND PERIPHTYON RESPONSES

C.2.1 Phytoplankton

Univariate effects of treatment on chl *a* concentration were found on the first, second, and fourth sample dates (days 26, 48, and 108; Table C4). On day 26, concentration in TP was lower

relative to RM and CH ($P \leq 0.048$). On day 48, no significant differences were found among the litter species treatments ($P \geq 0.131$). On day 108, concentration in TP was lower relative to BW ($P = 0.024$).

C.2.2 Periphyton

Univariate effects of treatment on periphyton biomass were found on the first and fourth sample date (days 33 and 111; Table C4). On day 33, biomass in BCH was less relative to RM, TP, ELM, and BW ($P \leq 0.049$). On day 111, no significant differences were found among the litter species treatments ($P \geq 0.068$).

C.3 ZOOPLANKTON, SNAILS, AND ARTHROPOD DETRITRIVORE RESPONSES

C.3.1 *D. pulex*

Since there was no time by treatment interaction (Table C5), we conducted treatment comparisons on values averaged across sample dates (days 81 and 109). The only difference found among litter species treatments was lower densities of *D. pulex* densities in BCH relative to CH ($P = 0.055$).

C.3.2 *S. oregonensis*

Since there was no time by treatment interaction (Table C5), we conducted treatment comparisons on values averaged across sample dates (days 81 and 109). No differences were found among litter species treatments ($P \geq 0.303$).

C.3.3 *S. mucronata*

No effect of litter treatment was found (Table C5).

C.3.4 Pouch snails

There was no time by treatment interaction on pouch snail density (Table C5), so we conducted treatment comparisons on values averaged across sample dates (days 66 and 94). Densities in TP were less than BW, ELM, OAK, ASP, and BCH ($P \leq 0.002$); CH, RM, and CHER also had lower densities than OAK, ASP, and BCH ($P \leq 0.008$); and ASH had lower densities than ASP and BCH ($P \leq 0.026$).

There was a marginally significant time by treatment interaction on pouch snail biomass (Table C5), and we found univariate effects of treatment on both sample dates (days 66 and 94; Table C6). On day 66, there was lower biomass in TP relative to ASP, ELM, and OAK ($P \leq 0.009$); and lower biomass in RM and CH than OAK ($P \leq 0.004$). On day 94, there was lower biomass in TP relative to BCH, BW, ASP, and ELM ($P \leq 0.029$); and lower biomass in CH and RM relative to ELM ($P \leq 0.021$).

There was a significant time by treatment interaction on pouch snail egg production (Table C5), and we found univariate effects of treatment on both sample dates (days 65 and 93; Table C6). On day 65, the number of eggs found on mesocosm walls was less in TP relative to ASP, ASH, RM, ELM, BW, and CH ($P \leq 0.010$) and the number of eggs were less in BCH relative to RM, ELM, BW, and CH ($P \leq 0.028$). On day 93, the number of eggs found on mesocosm walls was less in BCH, OAK, and TP relative to CH, ELM, and CHER ($P \leq 0.030$); and the number of eggs in BW was less than the number in CHER ($P = 0.049$).

C.3.5 Ram's horn snail

There was no effect of treatment on either density or biomass, but there was an effect of treatment and a time by treatment on egg production (Table C5). We found significant univariate effects on both sample dates (days 65 and 93; Table C6). On the first sample date, there were no differences among litter species treatments ($P \geq 0.502$). On the second sample date, there more egg masses on mesocosm walls in TP relative to OAK, BCH, RM, and ASP.

C.3.6 *C. psuedogracilis*

There was an effect of treatment but no time by treatment interaction on both biomass and density (Table C5), so we conducted treatment comparisons on values averaged across sample dates (days 62 and 90). Densities of *C. psuedogracilis* was less in TP than in ASH, CHER, ELM, and CH ($P \leq 0.035$). Similarly, biomass was less in TP than in ASP, ASH, RM, CHER, and CH ($P \leq 0.050$).

C.3.7 *A. communis*

Neither density nor biomass were affected by litter treatment (Table C5).

C.4 AMPHIBIAN RESPONSES

C.4.1 American toads

Univariate effects of treatment were found for survival to metamorphosis, total biomass, and individual mass at metamorphosis (Table C7). Survival to metamorphosis in TP was lower relative to BW and BCH ($P \leq 0.030$). Total biomass was less in TP relative to ASH, BCH, and BW ($P \leq 0.031$). Individual mass at metamorphosis was greater in TP relative to CH, RM, and OAK ($P \leq 0.040$).

C.4.2 Wood frogs

Significant or marginally significant univariate effects of treatment were found for all responses (Table C7). There were no significant treatment differences for survival ($P \geq 0.107$). Total biomass in RM was lower relative to ASH and ELM ($P \leq 0.032$); and biomass in TP was lower relative to ELM ($P = 0.028$). Individual mass at metamorphosis was lower in RM relative to all treatments except BCH ($P \leq 0.028$); and TP had larger metamorphs than BCH, CH, OAK, ASP, and CHER ($P \leq 0.037$). Time to metamorphosis was longer in TP relative to all treatments ($P < 0.001$).

C.4.3 Leopard frogs

Univariate effects of treatment were found for survival to metamorphosis and individual mass at metamorphosis (Table C7). Survival to metamorphosis in OAK and RM were lower relative to CHER, ASH, BW, and ELM ($P \leq 0.042$); survival in BCH was lower relative to ASH, BW, and ELM ($P \leq 0.50$). Individual mass at metamorphosis was greater in TP relative to all other treatments ($P \leq 0.003$).

C.4.4 Spring peepers

There were significant or marginally significant univariate effects of treatment on survival to metamorphosis and total biomass (Table C7). Survival in TP was greater relative to all other treatments except ASH ($P \leq 0.038$). Total biomass in TP was greater relative to BCH and CHER ($P \leq 0.039$).

C.4.5 Gray tree frogs

There were significant or marginally significant univariate effects of treatment on survival to metamorphosis and time to metamorphosis (Table C7). Survival to metamorphosis in BCH, CH, and OAK was lower relative to ELM and TP ($P \leq 0.026$); and survival was lower in ASP, CHER, and RM relative to TP ($P \leq 0.019$). Time to metamorphosis was shorter in RM relative to BW, ELM, and ASH ($P \leq 0.012$); and TP was shorter relative to ASH ($P = 0.030$).

APPENDIX D

CHAPTER TWO: SUPPLEMENTAL TABLES

Table D.1. rm-ANOVA results of the 12 litter treatments on all abiotic responses.

	Treatment		Time		Time x treatment	
	F	P	F	P	F	P
Repeated Measure Multivariate Analysis						
	$9.758_{55,142}$	<0.001	$2697.147_{15,20}$	<0.001	$3.387_{165,200}$	<0.001
Univariate Tests of Between and Within Subject Effects						
pH	$29.800_{11,34}$	<0.001	$111.482_{3,102}$	<0.001	$4.523_{33,102}$	<0.001
Dissolved oxygen	$59.848_{11,34}$	<0.001	$186.475_{3,102}$	<0.001	$12.018_{33,102}$	<0.001
Temperature	$0.753_{11,34}$	0.682	$3582.633_{3,102}$	<0.001	$6.098_{33,102}$	<0.001
Temp. stratification	$11.531_{11,34}$	<0.001	$366.411_{3,102}$	<0.001	$4.146_{33,102}$	<0.001
Attenuation	$28.606_{11,34}$	<0.001	$22.722_{3,102}$	<0.001	$11.910_{33,102}$	<0.001

Table D.2. Univariate results of the 12 litter treatments on all abiotic results on each sample date

	First sample			Second sample			Third sample			Fourth sample		
	F	P	df	F	P	df	F	P	df	F	P	df
Light attenuation	38.244	<0.001	11,36	25.445	<0.001	11,36	17.770	<0.001	11,36	2.562	0.017	11,34
Dissolved oxygen	34.805	<0.001	11,36	25.256	<0.001	11,36	21.809	<0.001	11,36	11.890	<0.001	11,36
Temperature	5.281	<0.001	11,36	2.301	0.030	11,36	1.154	0.352	11,36	1.071	0.410	11,36
Temperature stratification	8.820	<0.001	11,36	7.991	<0.001	11,36	2.206	0.037	11,36	1.246	0.294	11,36
pH	69.952	<0.001	11,36	12.546	<0.001	11,36	11.915	<0.001	11,36	11.346	<0.001	11,36

Table D.3. rm-ANOVA results of the 12 litter treatments on phytoplankton and periphyton responses.

	Treatment		Time		Time x treatment	
	F	P	F	P	F	P
Repeated Measure Multivariate Analysis						
	$2.769_{22,66}$	0.001	$89.166_{6,29}$	<0.001	$2.007_{66,161}$	<0.001
Univariate Tests of Between and Within Subject Effects						
Phytoplankton	$2.576_{11,34}$	0.017	$127.418_{3,102}$	<0.001	$2.542_{33,102}$	<0.001
Periphyton	$3.072_{11,34}$	0.006	$23.929_{3,102}$	<0.001	$2.119_{33,102}$	0.002

Table D.4. Univariate results of the 12 litter treatments on phytoplankton and periphyton responses at each sample date.

	First sample			Second sample			Third sample			Fourth sample		
	F	P	df	F	P	df	F	P	df	F	P	df
Phytoplankton	2.629	0.014	11,36	2.180	0.039	11,36	1.492	0.177	11,36	4.739	<0.001	11,36
Periphyton	3.789	<0.001	11,35	1.132	0.367	11,35	1.905	0.072	11,36	2.087	0.048	11,36

Table D.5. rm-MANOVA results of the 12 litter treatments on zooplankton, snails, and arthropod detritivores responses.

	Treatment		Time		Time x treatment	
	F	P	F	P	F	P
Repeated Measure Multivariate Analysis						
	$2.312_{143,205}$	<0.001	$32.856_{13,22}$	<0.001	$1.269_{143,205}$	0.059
Univariate Tests of Between and Within Subject Effects						
S. oregonensis density	$2.156_{11,34}$	0.043	$62.787_{1,34}$	<0.001	$1.820_{11,34}$	0.089
S. mucronata density	$0.625_{11,34}$	0.794	$143.874_{1,34}$	<0.001	$1.185_{11,34}$	0.334
D. pulex density	$4.234_{11,34}$	0.001	$7.872_{1,34}$	0.008	$0.477_{11,34}$	0.905
Pouch snail density	$10.942_{11,34}$	<0.001	$12.466_{1,34}$	0.001	$1.668_{11,34}$	0.124
Pouch snail biomass	$6.898_{11,34}$	<0.001	$37.233_{1,34}$	<0.001	$1.932_{11,34}$	0.070
Pouch snail egg mass density	$9.237_{11,34}$	<0.001	$56.377_{1,34}$	<0.001	$3.277_{11,34}$	0.004
Ramshorn snail density	$1.279_{11,34}$	0.278	$0.039_{1,34}$	0.845	$0.647_{11,34}$	0.776
Ramshorn snail biomass	$0.742_{11,34}$	0.692	$6.823_{1,34}$	0.013	$0.436_{11,34}$	0.928
Ramshorn snail egg mass density	$2.345_{11,34}$	0.028	$0.471_{1,34}$	0.497	$3.360_{11,34}$	0.003
Amphipod density	$3.343_{11,34}$	0.003	$17.384_{1,34}$	<0.001	$1.449_{11,34}$	0.197
Amphipod biomass	$3.548_{11,34}$	0.002	$28.277_{1,34}$	<0.001	$1.816_{11,34}$	0.090
Isopod density	$1.046_{11,34}$	0.430	$40.765_{1,34}$	<0.001	$1.244_{11,34}$	0.297
Isopod biomass	$1.439_{11,34}$	0.201	$17.837_{1,34}$	<0.001	$0.797_{11,34}$	0.642

Table D.6. Univariate results of the 12 litter treatments on zooplankton, snails, and arthropod detritivores responses.

	First sample			Second sample		
	F	P	df	F	P	df
*S. oregonensis**	--	--	--	--	--	--
*S. mucronata**	--	--	--	--	--	--
*D. pulex**	--	--	--	--	--	--
Pouch snail density*	--	--	--	--	--	--
Pouch snail biomass	5.183	<0.001	11,36	4.959	<0.001	11,36
Pouch snail egg density	7.857	<0.001	11,36	5.258	<0.001	11,36
Ram's horn snail density**	--	--	--	--	--	--
Ram's horn snail biomass**	--	--	--	--	--	--
Ram's horn snail egg density	1.555	0.155	11,36	3.894	0.001	11,36
Amphipod density*	--	--	--	--	--	--
Amphipod biomass*	--	--	--	--	--	--
Isopod density**	--	--	--	--	--	--
Isopod biomass**	--	--	--	--	--	--

* no significant time by treatment interaction; analyses were not conducted on individual sample dates

**no significant treatment effect; analyses were not conducted on individual sample dates

Table D.7. MANOVA and univariate results of the 12 litter treatments on amphibian survival and total biomass (i.e. metamorphs and tadpoles) for all anuran species in the experiment. Due to several missing values resulting from complete mortality or incomplete development to metamorphosis for several species. individual mass at metamorphosis and time to metamorphosis were analyzed with separate ANOVAs.

	Treatment	
	F	P
MANOVA	$1.950_{110,215}$	<0.001

	Survival to metamorphosis		Total biomass (metamorphs & tadpoles)		Individual mass at metamorphosis		Time to metamorphosis	
Species	F	P	F	P	F	P	F	P
Wood frog	$1.937_{11,36}$	0.067	$3.368_{11,36}$	0.003	$10.332_{11,36}$	<0.001	$11.549_{11,36}$	<0.001
American toad	$3.637_{11,36}$	0.002	$3.430_{11,36}$	0.002	$2.536_{11,36}$	0.017	$1.823_{11,36}$	0.086
Leopard frog	$4.707_{11,36}$	<0.001	$0.823_{11,36}$	0.618	$5.278_{11,34}$	<0.001	$1.827_{11,34}$	0.088
Spring peepers	$4.622_{11,36}$	<0.001	$2.037_{11,36}$	0.053	$1.585_{2,5}$	0.293	$0.139_{2,5}$	0.873
Gray tree frogs	$5.269_{11,36}$	<0.001	$0.703_{11,36}$	0.727	$1.789_{5,12}$	0.190	$7.514_{5,12}$	0.002

Table D.8. Results of weighted planned comparisons of no litter (NL) treatment responses with average response of all litter species treatments. Comparisons were only conducted on responses that exhibited a significant univariate effect of treatment.

Responses with time by treatment interactions	Time 1			Time 2			Time 3			Time 4		
	T	df	P	t	df	P	t	df	P	t	df	P
pH	24.526	33	<0.001	-9.508	33	<0.001	10.126	33	<0.001	-10.213	33	<0.001
DO	10.338	33	<0.001	-8.210	33	<0.001	13.437	33	<0.001	-8.897	33	<0.001
Temperature	2.737	33	0.010	1.601	33	0.119	-	-	-	-	-	-
Temperature Stratification	1.129	33	0.267	0.118	33	0.907	-0.196	33	0.846	-	-	-
Light attenuation	5.303	33	<0.001	0.132	33	0.895	5.120	33	<0.001	3.458	31	<0.001
Phytoplankton	0.105	33	0.917	-2.359	33	0.024	-	-	-	4.653	33	<0.001
Periphyton	-3.063	33	0.004	-	-	-	-	-	-	0.251	33	0.804
Pouch snail egg density	-	-	-	-	-	-	4.218	33	<0.001	1.478	33	0.149
Pouch snail biomass	-	-	-	-	-	-	0.485	33	0.631	-1.327	33	0.194
Ram's horn snail egg density	-	-	-	-	-	-	-	-	-	2.635	33	0.013

Responses without time by treatment interactions	t	df	P
S. oregonensis density	-0.822	31	0.417
D. pulex density	1.134	31	0.265
Pouch snail density	-2.066	33	0.047
Amphipod density	1.714	33	0.096
Amphipod biomass	2.297	33	0.028

Table D.8. (continued)

Amphibian responses	Survival to metamorphosis			Total biomass (metamorphs & tadpoles)			Individual mass at metamorphosis			Time to metamorphosis		
	t	df	P	t	df	P	t	df	P	t	df	P
Wood frog	-	-	-	1.558	33	0.129	5.964	33	<0.001	0.920	33	0.364
American toad	4.033	33	0.460	-2.042	33	0.049	0.875	33	0.338	-	-	-
Leopard frog	0.302	33	0.765	-	-	-	0.990	31	0.330	-	-	-
Spring peepers	1.406	33	0.169	1.724	33	0.094	-	-	-	-	-	-
Gray tree frog	0.830	33	0.413	-	-	-	-	-	-	-6.006	12	<0.001

Table D.9. Results of weighted planned comparisons of mixture treatment responses with average response of all litter species treatments. Comparisons were only conducted on responses that exhibited a significant univariate effect of treatment.

Responses with time by treatment interactions	Time 1			Time 2			Time 3			Time 4		
	T	df	P	t	df	P	t	df	P	t	df	P
pH	1.485	33	0.147	1.665	33	0.105	1.130	33	0.267	0.495	33	0.624
DO	0.626	33	0.535	0.307	33	0.761	1.399	33	0.171	0.390	33	0.699
Temperature	0.896	33	0.377	0.208	33	0.837	-	-	-	-	-	-
Temperature Stratification	1.252	33	0.219	1.611	33	0.117	0.083	33	0.934	-	-	-
Light attenuation	0.032	33	0.975	1.264	33	0.215	1.328	33	0.193	0.860	32	0.396
Phytoplankton	-1.857	33	0.072	0.931	33	0.358	-	-	-	-0.923	33	0.362
Periphyton	-1.730	32	0.093	-	-	-	-	-	-	-1.409	33	0.168
Pouch snail egg density	-	-	-	-	-	-	-0.980	33	0.334	-1.616	33	0.116
Pouch snail biomass	-	-	-	-	-	-	2.529	33	0.187	1.348	33	0.187
Ram's horn snail egg density	-	-	-	-	-	-	-	-	-	-0.234	33	0.817

Responses without time by treatment interactions	t	df	P
S. oregonensis density	1.352	31	0.186
D. pulex density	1.722	31	0.095
Pouch snail density	3.023	33	0.005
Amphipod density	-0.236	33	0.815
Amphipod biomass	-0.588	33	0.561

Table D.9. (continued)

Amphibian responses	Survival to metamorphosis			Total biomass (metamorphs & tadpoles)			Individual mass at metamorphosis			Time to metamorphosis		
	t	df	P	T	df	P	t	df	P	t	df	P
Wood frog	-	-	-	-1.521	33	0.138	1.269	33	0.213	0.390	33	0.699
American toad	0.451	33	0.655	-0.869	33	0.391	0.139	33	0.890	-	-	-
Leopard frog	- 0.740	33	0.465	-	-	-	0.809	31	0.424	-	-	-
Spring peepers	1.318	33	0.197	0.150	33	0.881	-	-	-	-	-	-
Gray tree frog	0.752	33	0.457	-	-	-	-	-	-	27.989	10	<0.001

APPENDIX E

CHAPTER TWO: RESULTS OF THE TRAIT-BASED ANALYSIS

Table E.1. Monte Carlo permutation tests on the significance of the first two canonical axes for each RDA and amount of response and trait-response relation variation explained by each axis. The cumulative variation explained is simply the sum of the variation explained by the first two axes.

	Permutation test		Response variation explained (%)			Trait-response relation variation explained (%)		
Analysis	F	P	1st axis	2nd axis	Cumulative	1st axis	2nd axis	Cumulative
Sample 1	8.155	0.002	39.1	12.3	51.4	71.7	22.5	94.2
Sample 2	5.537	0.002	38.3	4.1	42.4	83.9	8.9	92.8
Sample 3	2.483	0.002	12.4	7.2	19.6	44.3	25.8	70.1
Sample 4	2.347	0.002	10.3	6.8	17.1	39.3	25.9	65.2
Amphibian	3.485	0.002	18.3	11.9	30.3	54.1	35.2	89.3

APPENDIX F

CHAPTER THREE: DETAILS ON LITTER CHEMICAL ANALYSES

All chemical analyses were performed in triplicate after grinding dried litter using a Wiley mill. To determine total N, samples were sent to the University of Georgia Isotope Laboratory and analyzed with a CHN analyzer. The percentage of total phenolics was determined by spectrophotometry using the Folin-Ciocalteu reagent after extraction in 70% acetone (Graça et al. 2008). The percentage of total lignin was determined via carbon fractionation using water and ethanol to remove soluble components followed by acid digestion to remove cellulose and hemicellulose (Moorhead and Reynolds 1993).

APPENDIX G

CHAPTER THREE: DETAILS ON MASS-ADJUSTMENT METHODOLOGY

Differences in morphology can be due to differences in both size and relative shape. To assess the impact of density and litter species on relative shape, we mass-adjusted all body, tail, oral disc, and intestine dimensions. To accomplish this, we began by conducting a multivariate analysis of covariance (MANCOVA) on all individuals to verify that there were no mass-by-treatment interactions (i.e. the covariation between mass and a given dimension were parallel among all treatments). We then conducted the analysis again without the interactions and saved residuals for every dimension of each individual. Estimated marginal means for each treatment were added to these residuals to calculate mass-adjusted dimensions. This approach has been used successfully in a large number of past studies (e.g., Relyea 2012).

APPENDIX H

CHAPTER FIVE: METHODS OF LITTER CHEMICAL ANALYSIS

We measured percent total phenolics via spectrophotometry following addition of Folin-Ciocalteau reagent to ethanol extractions of litter. We measured percent tannin with radial diffusion assays, using ethanol extractions of litter placed into agar media mixed with bovine serum albumin. We measured nitrogen with a CHN analyzer. In addition, we measured phosphorus, magnesium, calcium, and potassium via atomic absorption spectrophotometry. Nitrogen, phosphorus, magnesium, calcium, and potassium were measured by the University of George Stable Isotope Laboratory (Athens, Georgia, USA).

We measured percent soluble carbon (SC) and lignin by carbon fractionation. The SC fraction was measured by three repeated extractions with 95% ethanol, three extractions with deionized water, followed by another single extraction with 95% ethanol. The sample was then dried for 24 hrs at 60 °C and reweighed. The difference between initial weight and final weight was noted as SC. Remaining sample was then digested with 72% sulfuric acid and autoclaved to extract cellulose and hemicellulose, and filtered onto pre-weighed ash free filter paper. Filters with sample were weighed, ashed at 550 °C in aluminum tins and reweighed. Lignin content was the difference of sample mass post-sulfuric acid digestion and ashing.

APPENDIX I

CHAPTER FIVE: DETAILS ON THE MANIPULATION AND CALCULATION OF LITTER CHEMICAL DISSIMILARITY

Chemical dissimilarity was calculated as Rao's quadratic entropy (i.e. RaoQ; Botta-Dukat 2005, Epps et al. 2007, Laliberte and Legendre 2010) based on the 10 measured chemical traits after reducing trait dimensionality via principal components analysis. RaoQ is defined as the average dissimilarity d_{ij} between a set of R species from a pool of species without replacement, taking into account the abundance ρ of each species. RaoQ is then calculated as:

$$\mathrm{RaoQ} = \sum_{i=1}^{R}\sum_{j=1}^{R} \rho_i \rho_j d_{ij}$$

where d_{ij} is defined as the standardized Euclidean distance between species with regard to their respective trait k values, and is calculated for species with n trait values as

$$d_{ij} = \frac{1}{n}\sqrt{\sum_{k=1}^{n}\left(t_{ik} - t_{jk}\right)^2}$$

Hence, RaoQ can be conceptually thought of as an index of dissimilarity among species (Botta-Dukat 2005, Epps et al. 2007, Laliberte and Legendre 2010).

Values of RaoQ were normally distributed with a mean $\overline{RaoQ}$, so standard deviations were employed to determine ranges of low, medium, and high chemical diversity. The low range of RaoQ values was bounded by $\overline{RaoQ} - \sigma_{RaoQ}$ and $\overline{RaoQ} - 2\sigma_{RaoQ}$ whereas the high range of RaoQ values was bounded by $\overline{RaoQ} + \sigma_{RaoQ}$ and $\overline{RaoQ} + 2\sigma_{RaoQ}$. This generated ranges consisting of ~900 mixtures. We used this range size to delineate the medium range of RaoQ values by selecting the first 450 mixtures with RaoQ values below $\overline{RaoQ}$ and the first 450 mixtures above $\overline{RaoQ}$. Use of standard deviations produced ranges of RaoQ values were sufficiently similar to one another within ranges yet quantitatively separated from each other. These three RaoQ ranges were then used to designate replicates for each of the three experimental treatments of our study. At random and without replacement, we selected 20 litter mixtures from each of the three RaoQ ranges, and these served as individual replicates (60 total experimental units).

To avoid bias from an increased presence of a particular litter species, we assessed the frequency of each litter species among 20 mixtures within each treatment. If an individual litter species appeared more than five times among the 20 mixtures in a dissimilarity treatment, we randomly selected a mixture with that species from within the corresponding dissimilarity treatment and replaced it with another randomly selected mixture. This was repeated until all litter species appeared at least three times and no more than five times among all mixtures within treatments.

APPENDIX J

CHAPTER FIVE: DETAILS OF ABIOTIC AND BIOTIC MEASUREMENTS

J.1 ABIOTIC MEASUREMENTS

To measure light attenuation, we used a submersible quantum sensor (Li-Cor Instruments, Nebraska, USA) to measure the amount of photosynthetically active radiation (PAR) at 2 and 22 cm below the water surface (L_2 and L_{22}, respectively), and calculated the light attenuation (K) between these two depths via the formula

$$K = \frac{\ln(L_2/L_{22})}{20}$$

Because cloud cover can affect the amount of light entering mesocosms, we performed measurement on cloudless days. We measured dissolved oxygen and temperature using a microelectrode array probe (YSI, Ohio, USA) at sunset, just above the litter layer on the benthos. We measured pH just below the surface using a single-junction electrode (Oakton Instruments, Illinois, USA).

J.2 BIOTIC MEASUREMENTS

J.2.1 Leaf litter decomposition rate

To measure the decomposition rate of each litter species in each mesocosm, we gently lifted mesh bags containing preweighed litter mixtures to the surface, placed them in a plastic container, and transported them to the lab for analysis. In the lab, we rinsed the litter of all sediment and grazing organisms. We then sorted the mixture of litter in each mesh bag by species, placed individual litter species in pre-weighed aluminum tins, and dried each litter tin for 24 hours at 80 ºC. For each species within each mesocosm, we recorded the final mass of dried litter and subtracted this value from the initial dry mass of that species to determine amount of mass loss. Using the three values of mass loss obtained for each species from each sample date, we calculated a single litter decomposition rate for each species in each mesocosm using an exponential decay function (Petersen and Cummins 1974).

J.2.2 Phytoplankton and periphyton biomass

To measure phytoplankton, we took water samples from the north, south, east, west, and center of each mesocosm using a 0.25-m length and 5.08-cm diameter PVC pipe that was plunged vertically into the water and sealed at one end with a rubber ball. Each pipe sample collected a 450 ml of water. We mixed water from all samples taken from a mesocosm, and vacuum-filtered 1 L of this water through a 0.45-µm cellulose membrane (Millipore Corporation, Massachusetts, USA). We immediately placed filters into a -20 ºC freezer to lyse cells. After freezing, we

extracted chl *a* by placing the filters in 90% methanol and used a fluorometer (TD-700, Turner Designs) to determine the biomass of chl *a* in the sample (Arar and Collins 1997). To measure periphyton, we used a toothbrush to vigorously brush all material from half of single ceramic tile. We then vacuum filtered material on a pre-weighed 1.2 µm glass fiber filter (Whatman Inc). After allowing filters to oven-dry for 24 hrs at 60 ºC, we reweighed to determine total periphyton biomass.

J.2.3 Zooplankton

To sample zooplankton populations, we measured 1 L of water collected from tube samples used for estimating phytoplankton, and filtered this water through a 62-µm nylon mesh. We preserved filtered zooplankton in 30% ethanol. Numbers of each species were recorded to determine species density in each sample.

J.2.4 Amphipods and isopods

To sample amphipod and isopod populations, we collected all individuals rinsed from litter bags through and preserved them in 70% ethanol. We measured biomass by placing all individuals of a single species into pre-weighed aluminum tins, which were dried for 24 hrs at 60 ºC and reweighed to determine total biomass.

J.2.5 Snails

To sample populations, we swept a 0.5-mm mesh net across the sediment layer and up the wall of each mesocosm, starting in the center of the mesocosm and moving towards the same direction for each sample. We sorted snails from the litter and preserved all individuals in 70% ethanol. We placed individuals of a single species into pre-weighed aluminum tins, which were dried for 24 hrs at 60 ºC and reweighed the tins to determine biomass of snails.

J.2.6 Amphibians

To sample amphibian metamorph mass and survival, we checked mesocosms daily and removed any individuals with two emergent forelimbs. In the lab, we held individuals in 1-L containers with moist sphagnum moss until their tails resorbed to < 2 mm. At this stage, metamorphosis was considered complete. We euthanized individuals with MS-222 (tricaine methanosulfate) and preserved them in 10% formalin. Metamorph mass was measured as the average individual mass in a mesocosm.

APPENDIX K

CHAPTER FIVE: CALCULATION OF TRAIT MEANS

Chemical means of mixtures for each trait were calculated as community-weighted means (CWMs), which is the sum of trait values multiplied by the abundance of each species in mixture (Lavorel et al. 2008)

$$\mathrm{CWM} = \sum_{i=1}^{n} \rho_i \times \mathrm{trait}_i$$

Since biomass of all component litter species within a mixture was equal, all calculations were performed with equal abundance values for each component species. All trait values were standardized to a mean of zero and a standard deviation of one prior to calculation of RaoQ or CWM values. All calculations were performed using the package FD (Laliberte and Legendre 2010) in R (Version 2.15.1).

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