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Non-Native Leaf Litter Modifies Algal Resources with Effects on Tadpole Growth

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NON-NATIVE LEAF LITTER MODIFIES ALGAL RESOURCES WITH EFFECTS ON

TADPOLE GROWTH

By

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ABSTRACT

Leaf litter species play a key role in determining the quality and quantity of algal resources in aquatic ecosystems as a cross-boundary subsidy. The invasion of non-native plant species into forests can alter aquatic resources. I investigated the effects of non-native leaf litter species on algal resources and green frog, *Lithobates clamitans*, development using a randomized complete block experiment that lasted 288 days. In experimental ponds, I added two non-native and two native leaf litter species in the presence and absence of tadpoles. Early in the experiment, Japanese honeysuckle and Chinese privet decreased periphyton N:P ratios and stimulated tadpole growth compared to native leaf litters. However, tadpole mortality in Japanese honeysuckle was high over winter compared to other leaf litters. Different litter species affected periphytic algal quality and quantity that modified tadpole growth suggesting that invasion of non-native terrestrial plants influence population dynamics of aquatic organisms.

INDEX WORDS: Leaf litter, amphibian larvae growth, mesocosm, periphyton

DEDICATION

This research is dedicated to all the people and institutions in my life who have helped me get to where I am in my academic career. I thank my parents, Julie and Luis Cruz, for their full support throughout my undergraduate and graduate career. I wouldn't have made it this far without their support and motivation. I would also like to thank my undergraduate advisor Dr. Natalie Hyslop for encouraging me to advance my career to graduate school and for laying the foundation of my research knowledge. I thank my graduate advisor and mentor Dr. Clifton Ruehl for his support and guidance throughout graduate school in both my thesis research and classes in general. He has made a great contribution to my understanding of how science works and helped give me the resources necessary to complete the research that I wanted to conduct. He never once gave up on me. I would also like to thank my committee members Dr. Troy Keller and Dr. Alan Wilson for their guidance throughout out my thesis research. Lastly, I would like to thank all my friends, family, past professors, the University of North Georgia, Columbus State University, and wonderful girlfriend Jessica who have all supported me and motivated me to keep on pushing regardless of my late nights grading papers and editing my thesis. My whole academic career would not be possible without these people in my life.

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Introduction

Identifying the mechanisms driving population dynamics is necessary for understanding changes in population structure and community dynamics. Cross-boundary subsidies, such as leaf litter from over-story vegetation, is an important resource for aquatic ecosystems and can influence population dynamics by being the dominant source of carbon, phosphorus, and nitrogen for the ecosystem (Nelson and Scott 1962; Fisher and Likens 1973; Webster and Benfield 1986; Bonner et al. 1997; Rubbo and Kiesecker 2004; Dodd 2010; Stoler and Relyea 2011, 2013). Nutrient cycling is also dependent on the quality and quantity of leaf litter that falls into the system. Changes in plant species composition surrounding aquatic systems can alter chemical concentrations, nutrient dynamics, and alter trophic structure (Facelli and Pickett 1991; Brown et al. 2006; Stoler and Relyea 2011, 2013). Leaf litter can vary in nutrient composition and decomposition rate depending on the species (Webster and Benfield 1986; Swan and Palmer 2006). For example, willow oak (*Quercus phellos)* is a deciduous tree found around aquatic ecosystems that produce leaf litter that have a slow and steady decomposition rate of high-quality nutrients. Comparatively, long leaf pine (*Pinus palustris*) occurs in a variety of habitats including those near aquatic systems that produce needles with a slow decomposition rate and low nutrients. Both species have been dominant in southeastern forests historically and both drop leaf litter throughout the year, but especially in the fall.

Non-native plants that colonize forests can introduce novel cross-boundary subsidies from the leaf litter that falls into nearby aquatic systems. Chinese privet (*Ligustrum sinense)* and Japanese honeysuckle (*Lonicera japonica)* are highly invasive flowering plants that are currently expanding into lowland and wetland habitats throughout the eastern United States (Bradley et al. 2010; Hanula and Horn 2011). Both species can dominate the understory of riparian habitats. For

example, forests heavily infested with privet have lower tree diversity and less native shrub cover resulting in an increase in the proportion of privet leaf litter (Hanula and Horn 2011). Previous experiments show that Chinese privet negatively impacts forest regeneration by outcompeting native shrubs and decreasing native tree seedling diversity (Kittell 2001). Privet has high quality leaf litter with lower lignin, cellulose, and low C:N ratios relative to the native flora of Georgia (Mitchell et al. 2011; Lobe et al. 2014). Privet also decomposes faster and has a five times greater increase in soil N mineralization rate compared to the native leaf litters in the floodplains of western Georgia (Mitchell et al. 2011). Strategies to remove privet and rehabilitate native plants and shrubs have proven successful (Hanula et al. 2009; Hanula and Horn 2011) but restoration can use strategies that damage the whole ecosystem. Privet has become so common throughout the southeast its leaf litter now subsidizes many ponds and wetlands and has potentially altered nutrient dynamics in these ecosystems. Both Japanese honeysuckle and Chinese privet have a broad range throughout the eastern United States that overlaps the ranges of many native amphibian species. However, little research has been done on how cross-boundary subsidies might affect amphibian larval development. Research on other honeysuckle species reveal that they decrease native floral species richness and abundance (Collier et al. 2002), decompose rapidly compared to native leaf litters (Brent and Stowasser 2009; Lewis and Brown 2010), and they alter aquatic ecosystem processes like nutrient cycling and decomposition (Mcneish et al. 2012). Some honeysuckle species have a higher nitrogen content than most Georgia native species (Blair and Stowasser 2009). Many studies have addressed honeysuckle species effects on terrestrial communities, but little research has considered how it might affect aquatic ecosystems (McNeish et al. 2012; Walting et al. 2012). Japanese honeysuckle creates large overarching canopies along streams that serves as a nutrient subsidy

(Mcneish et al. 2012). Walting et al. (2011) showed that leaf litter extracts from *Lonicera maackii* affected amphibian survivorship, behavior and increase their susceptibility to predation. With our current understanding of honeysuckle species, it is important to investigate the potential effects that Japanese honeysuckle may have on southeastern aquatic systems and amphibian development.

Amphibian densities and diversity are threatened by multiple factors including habitat loss, climate change, and disease. Habitat loss and change by the invasion of non-native plants likely impact breeding sites and the developmental rates of amphibians. Understanding these effects is important in wetland and riparian habitats because of human disturbances and the introduction of non-native species. For example, the invasion of non-native plant species into riparian habitats can alter environmental resources in breeding sites which can affect fitness (Stoler and Relyea 2013). These changes will likely scale up to alter nutrient cycling, food web dynamics, and pond community structure. Temporary ponds are used by a wide variety of invertebrates and amphibians as breeding sites (Wilbur 1997; Rubbo and Kiesecker 2004). Amphibian larvae are usually near the top of the food web (Stephens et al. 2013) and are often the most numerically dominant group in these ponds consuming mainly periphyton, seston, and zooplankton. Amphibian larvae often occur at high densities resulting in variation in development rate that is also influenced by the dominant substrate, the water chemistry, and the food supply. These factors are important to investigate because they affect tadpole larval mass and timing of metamorphosis which are traits closely linked to individual fitness (Berven 1990). The green frog (*Lithobates clamitans*), serves as a great model species for amphibians of the southern United States because it breeds throughout the southeast in many aquatic systems

(Jensen 2008), breeds throughout the spring, summer and fall, and co-occurs with all of the plant species in this study.

I evaluated the influence of non-native plant leaf litter on amphibian growth and development using experimental temporary pond ecosystems. Privet and Japanese honeysuckle served as the non-native litters, while willow oak and long leaf pine served as the two native litters. Nutrient releases from the decomposition of these leaf litters stimulated growth of primary producers that were consumed by green frog tadpoles. I hypothesize that the non-native plant leaf litter will produce higher quality algal resources that will promote greater tadpole mass and faster development.

Materials and methods

Experimental design and setup

The experiment was a randomized complete block design using mesocosms with eight treatments (four leaf litter types crossed with tadpole presence or absence) that were replicated five times to test for the effects of leaf litter type on periphyton and seston growth that influenced green frog growth and development. The experiment was conducted from late summer through early spring (31 July 2018 to 15 May 2019; 288 days) to replicate the larval period of many green frogs in the southeast United States. On 10 July 2018, 40 416-L plastic tanks (mesocosms) were filled with 265L of well water at the Columbus State University's Lynnhaven land preserve. Lynnhaven is a 28.3 ha hardwood forest located in Harris County, Georgia. All tanks were assembled in a 5x8 rectangular array to allow for a randomized complete block design with five blocks having eight treatments per block. The mesocosms were covered with shade cloth to prevent colonization or escape of animals. Each mesocosm had a standpipe placed at the 265L

mark to regulate water depth during rain events. Standpipes were equipped with a mesh covering to prevent the loss of tadpoles and algae. Four strips of flagging tape, suspended through the water column, were added to each tank to collect periphyton. On 17 July 2018, two weeks prior to the introduction of tadpoles, each tank received a plankton inoculate. The plankton inoculate was created by collecting 75.6L of plankton net-filtered water from a nearby pond and diluting the sample with another 75.6L of well water. The solution incubated outside in a large tank for 24 hours before adding 3L to each tank. Each tank also received 220g of leaf litter. The litter was added on 17 July 2018, which allowed the leaves time to settle to the bottom of the tank and release nutrients to stimulate the production of diverse microbial and producer guilds before tadpole additions.

Leaf litter from four species, long leaf pine, willow oak, Japanese honeysuckle, and Chinese privet were collected from May-June 2018 then air dried in the sun for 2 weeks in vented plastic bags. The leaves collected were a combination of both senesced and fresh leaves to replicate the litter seen in wetlands during the summer to spring season. Both long leaf pine and willow oak typically occur throughout the southeast in wetlands and served as native leaf litter treatments. Chinese privet and Japanese honeysuckle served as the non-native leaf litter treatments. All leaf litter was collected from various wetland and hardwood forests throughout north and west Georgia. Each tank received a monoculture of leaf litter to investigate the effects of each specific leaf litter individually.

I collected a large clutch of green frog eggs on 22 July 2018 from a nearby pond and transferred the eggs to pans filled with aerated tap water at the Columbus State University aquatic lab. This species was selected because it is common in various ecotones, wetlands, and temporary ponds throughout the southeast and breeds throughout the spring, summer and fall

(Jensen 2008). On 30 July 2018, when tadpoles were free swimming (7-days old), a 100 were randomly added to 20 of the 40 tanks (2000 total) to grow and develop until metamorphosis.

Primary Producers

Seston and periphyton serve as the main food resource for tadpoles. Samples were collected on 15 September 2018 (day 46) and 30 April 2019 (day 273) to assess primary production early in the experiment and at the end of the study. Seston was assessed by estimating biomass, chlorophyll-*a* concentrations, total nitrogen (TN), and total phosphorus (TP). Four samples of the water column were collected with a 200ml tube sampler and combined for a total sample of 800mls of water from each tank and were transferred to the lab. All samples were filtered through a 150μm filter to separate zooplankton and larger debris. The 800ml sample was divided into a 200ml subsample that was vacuum filtered through a GF/F filter (47 mm filter dia., 1.0 μm pore dia.; Wyvern Scientific Inc), dried, and weighed to assess seston biomass. Another 200ml subsample was vacuum filtered through a GF/F filter (47 mm filter dia., 1.0 μm pore dia.; Wyvern Scientific Inc., Cambridge, Ontario, Canada) and chlorophyll was extracted from the filter using 90% acetone and a magnesium buffer at -4°C for 48 hours. A final 20ml subsample was stored at -4°C and assayed for TN and TP using the Boyd method of extraction within 30 days.

To determine chlorophyll-*a* concentration, I used a modified version of the EPA method 150.1 (EPA 1991) which incorporates spectrophotometric analysis (Hach DR2700, Loveland, CO, USA). Absorbances were measured before and after acidification with 0.1N hydrochloric acid to calculated chlorophyll-*a* corrected (i.e., corrected for phaeophytin). Periphyton samples were collected from the periphyton strips, i.e., orange flagging tape, suspended though the water column on the same dates. The strips were scrubbed and rinsed into a homogenized slurry using

a hand blender. Area specific biomass, chlorophyll a, TN and TP were quantified using the same procedures as seston.

Tadpole growth and development

During weeks 7, 20, and 41 (September 9, 2018, December 8, 2018, and May 15, 2019), fifteen tadpoles were haphazardly collected using a hand net from each tank to assess tadpole mass at the beginning of the experiment, during the middle of winter, and just prior to metamorphosis in the spring. Tadpoles were weighed after being patted with a paper towel, and immediately placed back into their respective tanks. During the last sampling week (41), all tadpoles were collected and weighed along with any metamorphosed individuals and released back at the site of egg collection. Tadpoles were considered to have metamorphosed when their tail had completely resorbed (Gosner stage 46; Gosner, 1960). Survivorship was assessed as the percent survival calculated as the number of final tadpoles divided by the number of tadpoles initially added. To account for tanks with no survivors, one was added to all the survivor proportions then the -log was taken to estimate the instantaneous mortality rate. Total tadpole mass was analyzed with mortality as a covariate to account statistically for losses throughout the study.

Water chemistry

Temperature (°C), and sunlight intensity (lux) were measured throughout the first half of the experiment to account for environmental variation among tanks prior to the overwintering period. These data were not collected during the winter to avoid disturbing the tadpoles during this stressful period. Data was collected using eight data loggers placed at the bottom center of each mesocosm that collected data every hour (HOBO Pendant® Temperature/Light 64K Data

Logger, Onset Computer Corporation, Bourne, MA, USA). The HOBO data loggers collected data for 3 days at a time and were rotated among blocks during the experiment.

Statistical Analysis

To test if the response variables, primary producer biomass, TN, TP, and chlorophyll-*a*, varied between treatments, repeated measures analysis of variance was used that tested for leaf litter-treatment effects, time effects, tadpole presence/absence effects, block effect, and the interaction of all the independent variables. Since block only had a significant effect on tadpole mass, it was removed from all other models. For all significant univariate tests, *post hoc* comparisons between means was conducted using Tukey's HSD. To test if individual tadpole mass, total tadpole mass, and instantaneous mortality rate varied among treatments, a one-way analysis of variance was used to test for leaf litter-treatment effects followed by a Tukey's HSD. Periphyton biomass and ash free dry mass data were log10 transformed to meet assumptions of normality.

Results

Experimental conditions

During the summer and fall (3 August 2018 to 16 November 2018; Days 3 to 111), sunlight intensity decreased over time ($F_{4,112} = 378.91$; $P < 0.001$; Fig. 1) and this effect depended on leaf litter type ($F_{12,112} = 9.56$; $P < 0.001$). Willow oak and longleaf pine treatments had 40-65% higher sunlight intensity than the Japanese honeysuckle and Chinese privet treatments in the beginning of the experiment (3-16 August 2018) and at the end of the summer (7-19 September 2018; Tukey, $P < 0.05$). From 20 August 2018 to 4 September 2019, the willow oak treatments had 71% more sunlight intensity than the Japanese honeysuckle treatments (Tukey, $P < 0.05$) and the longleaf pine and Chinese privet treatments had no difference from one another. At the end of the fall season, all treatments had the same sunlight intensity. Sunlight intensity also varied

between leaf litter type regardless of season with willow oak and long leaf pine having 30-60% higher sun light intensity than Chinese privet and Japanese honeysuckle up until the winter season ($F_{3,28} = 92.50$; $P < 0.001$). The average highest and lowest temperatures per instrument rotation in each treatment decreased over time but did not vary between treatments (Fig. 2).

Primary Producers

Seston biomass did not differ among leaf litter types ($F_{1,27} = 2.08$; $P = 0.126$) or because of tadpole presence ($F_{1,27} = 0.029$; $P = 0.865$), but did decrease on average 99% over time ($F_{1,27} = 25.53$; $P < 0.001$, Fig. 3). Seston N:P ratios depended on leaf litter type ($F_{3,18} = 8.08$; $P = 0.001$). Seston N:P ratios also decreased on average 20% over time ($F_{1,18} = 7.04$; $P = 0.016$, Fig. 4). Long leaf pine produced lower N:P ratios than all other treatments (Tukey, $P < 0.05$). Pine had an average N:P ratio of 7.44 while privet produced the highest ratios with an average of 16.22 (Tukey, $P < 0.05$). Sestonic chlorophyll-a concentrations did not differ among treatments ($P > 0.05$).

Periphyton biomass changed over time ($F_{1,32} = 10.90$; $P = 0.002$) and the effect depended on tadpole presence ($F_{1,32} = 6.03$; $P = 0.020$) and the leaf litter type ($F_{3,32} = 6.00$; $P = 0.002$; Table 1 and 2; Fig. 5). The amount of periphyton biomass depended on the presence or absence of tadpoles ($F_{1,32} = 4.67$; $P = 0.038$) and the effect of tadpoles on biomass depended on the leaf litter type ($F_{3,32} = 8.68$; $P < 0.001$). Finally, there was a strong interaction between time, tadpole presence, and leaf litter type ($F_{3,32} = 4.26$; $P = 0.012$).

On the first sampling date (15 September 2018), when tadpoles were present, Chinese privet had the most periphyton biomass, willow oak and long leaf pine had similar and intermediate values, and Japanese honeysuckle had the least amount of periphyton biomass

(Tukey, P < 0.05; Table 1 and 2; Fig 5). When compared to to treatments with no tadpoles, the presence of tadpoles reduced periphyton biomass in Japanese honeysuckle by 99.9%, while Chinese privet had an increase of 100% in biomass when (Tukey, P < 0.05). When tadpoles were absent, Chinese privet treatments had more periphyton biomass than both willow oak and longleaf pine by 228% and 531% respectively while Japanese honeysuckle had more biomass than Willow oak and longleaf pine by 2,121% and 4,178% respectively (Tukey, P < 0.05).

On the second sampling date (30 April 2019), when tadpoles were present, periphyton biomass in willow oak treatments was 82.9% greater compared to when tadpoles were absent (Tukey, P < 0.05; Table 1 and 2; Fig 5). The Japanese honeysuckle and Chinese privet treatments had 99% and 90% lower periphyton biomass respectively when tadpoles were absent relative to the first sampling date when tadpoles were absent (Tukey, P < 0.05). When tadpoles were present, there was a 96% reduction in the amount of periphyton biomass in the Chinese privet treatment, and a 12.5% increase in biomass in the willow oak treatment when compared to the first sampling date (Tukey, P < 0.05). Periphyton ash free dry mass (AFDM) saw the same results as the periphyton biomass except there was no overall effect of tadpole presence on AFDM (Table 1).

The percent organic content in periphyton did not differ between-treatments (Fig. 6). There was an effect of time on periphyton organic content that was affected by tadpole presence and leaf litter type ($F_{3,32} = 3.25$; $P = 0.035$). On the first sampling date (15 September 2018), when tadpoles were present, Japanese honeysuckle showed lower percent organic content (30%) compared to the treatment without tadpoles. Longleaf pine and Chinese privet saw a 22.1% and 5.2% increase respectively in percent organic content when tadpoles were present. On the second sampling date (30 April 2019), when tadpoles were present, only longleaf pine saw a decrease

(7.6%) in percent organic content compared to when tadpoles were absent. The other leaf litter treatments had no difference in percent organic content compared to when tadpoles were absent. When tadpoles were absent, Japanese honeysuckle had a 22% decrease, longleaf pine had a 15% increase, and Chinese privet had a 18% decrease in percent organic content over time. When tadpoles were present, the Chinese privet treatment had a 19.5% decrease in percent organic content overtime.

Periphyton N:P ratios varied between leaf litter type ($F_{3,30} = 4.48$; P = 0.010; Fig. 7; Table 3). The N:P ratios also decreased over time for all eight treatments ($F_{1,30} = 103.01$; P < 0.001) and the degree of decline depended on leaf litter type ($F_{3,30} = 6.61$; P = 0.001; Fig. 7). On the first sampling date (15 September 2018), Japanese honeysuckle had the lowest N:P ratio with an average ratio of 6.4, Chinese privet had an intermediate ratio of 11.5, and willow oak and longleaf pine had the highest ratios of 16.2 and 19.5 respectively (Tukey, P < 0.05). On the second sampling date (30 April 2019), there was no difference in N:P ratios, but willow oak did average over a 50% lower ratio than Chinese privet and Japanese honeysuckle.

Primary Consumer

Tadpole mass increased over time ($F_{3,39} = 563.10$; P < 0.001; Fig. 8) and varied by leaf litter type ($F_{3,13} = 5.69$; P = 0.010). The amount of biomass accumulation over time also depended on leaf litter type ($F_{9,39} = 2.16$; P = 0.047). Over the last 3 sampling dates (September 9, 2018, December 8, 2018, and May 15, 2019) Japanese honeysuckle litter produced on average between 30-100% larger tadpoles than the other leaf litter treatments. On the second sampling date (September 9, 2018), the long leaf pine treatment had the smallest individuals with an average of 0.153g, the Japanese honeysuckle treatment had the largest individuals with an average of 0.610g tadpoles, and the Chinese privet and willow oak treatments were not different from each other averaging

between 0.293g and 0.228g. On the last two sampling dates (December 8, 2018 and May 15, 2019), there was no difference between tadpole mass in the long leaf pine, willow oak, and Chinese privet treatments. Japanese honeysuckle treatments had the largest tadpoles (Tukey, $P < 0.05$). A one-way ANOVA, with mortality as a covariate, of the total tadpole mass at the end of the experiment (15 May 2019) revealed that Chinese privet was different from the other treatments ($F_{3,19} = 6.35$; $P = 0.005$; Fig. 9). Chinese privet total mass was 121.5% larger than the three other treatments. Japanese honeysuckle, willow oak, longleaf pine, and Chinese privet had a total tadpole mass of 6.26g, 9.98g, 10.74g and 19.9g respectively.

Analysis of the tadpole mortality in each leaf litter type revealed a significant effect of leaf litter type on survivorship ($F_{3,16} = 5.87$; $P = 0.007$). Japanese honeysuckle had a two-fold increase in mortality compared to the three other treatments (Fig. 10).

Discussion

Leaf litter species play a key role in determining the quality and quantity of algal resources in temporary ponds by providing allochthonous and cross-boundary subsidies that would not normally be present (Bonner et al. 1997; Rubbo and Kiesecker 2004; Brown et al. 2006; Rubbo et al. 2008; Stoler and Relyea 2011, 2013). Leaf litter can vary in nutrient composition and rate of decomposition depending on the plant species (Webster and Benfield 1986; Swan and Palmer 2006). The results of this study indicate that non-native plants like Japanese honeysuckle and Chinese privet can have important impacts on primary producers and amphibian larval growth. Nutrient cycling in these systems was dependent on the presence of consumers and the species of leaf litter that entered the system. The response of periphytic algae to these different litter species has important implications on how aquatic ecosystems can function.

Algal mass and stoichiometry

Nutrient additions can increase periphyton and tadpole biomass (Leibold and Wilbur 1992). Tadpole grazing by some ranid species increase periphyton biomass (Kupferberg 1997), cause no effect (Morin et al. 1988), or reduce periphyton biomass (Dickman 1968; Brönmark et al. 1991; Leibold and Wilbur 1992). I found that differences in periphyton biomass depended on both the leaf litter species and the presence of tadpoles. Chinese privet and Japanese honeysuckle stimulated the accumulation of the largest amount of periphyton biomass initially but over time the biomass decreased. This suggests that these non-native leaf litters decompose faster and provide a large amount of nutrients to fertilize periphyton compared to native leaf litters (Brent and Stowasser 2009; Lewis and Brown 2010; Mitchell et al. 2011; Lobe et al. 2014). Tadpole grazing reduced periphyton biomass in Japanese honeysuckle, had no effect on longleaf pine or willow oak, and stimulated periphyton growth in Chinese privet. After seven months of development, tadpole grazing increased periphyton biomass in willow oak. Japanese honeysuckle had a five-fold decrease in periphyton biomass while the other treatments saw no reduction or increase in biomass. The slower decomposition rate of the willow oak leaves provided fresh nutrients for periphyton growth over time, while the non-native treatments released nutrients early in the study and tadpoles depended on a feedback loop between grazing and excretion of inorganic nutrients to complete development (Fig. 11). The feedback loop was strong in privet as evidenced by the greater periphyton biomass in tanks with tadpoles relative to the tanks without tadpoles during the second part of the study. I suspect that as tadpoles develop, competition for nutrients between tadpoles and periphyton increases as tadpoles store more nutrients and excrete less, causing a reduction in nutrient cycling. Nitrogen decreased in all treatments over time as the tadpoles continued to grow, leaving less nitrogen in the system to

fertilize the periphyton. Tadpoles store nutrients for development and excrete less nutrients over time, resulting in fewer nutrients available to periphyton for growth. Periphyton nutrient quality is important because quality determines tadpole growth and developmental rates.

Nitrogen and phosphorus primarily determine nutrient quality of resources, often colimit periphyton growth, and subsequently limit growth of other organisms in aquatic systems (Dodds et al. 2002; Rier and Stevenson 2006; Elser et al. 2007). Periphyton N:P ratios decreased over time for all the treatments but varied among leaf litter species. When leaf litter was first added to the system, Japanese honeysuckle and Chinese privet produced periphyton with a low N:P ratio compared to willow oak and longleaf pine. This is likely because these non-natives decomposed rapidly and released large amounts of nitrogen and phosphorus (Brent and Stowasser 2009; Lewis and Brown 2010; Mitchell et al. 2011; Lobe et al. 2014). This pattern implies that these non-native species only introduce this cross-boundary subsidy of increased nutrients for a short time period. High litter N stimulates periphyton growth on leaf surfaces (Webster & Benfield, 1986), which will in turn increase periphyton abundance. However, over time willow oak produced the highest quality periphyton (lowest N:P ratio) and the highest periphyton biomass compared to the other treatments. Therefore, the slow release of nutrients by willow oak provides a small but consistent supply of nutrients for periphyton growth. Although Japanese honeysuckle and Chinese privet had similar trends in terms of their influence on periphyton quality and quantity, by winter, all the Japanese honeysuckle leaves had fully decomposed, while the other treatments had large numbers of leaves remaining including Chinese privet. This result suggests that although Chinese privet releases many nutrients early, leaves also contain recalcitrant components that continue to release nutrients for a long period relative to Japanese honeysuckle.

Green frog larvae

Tadpoles are generalist grazers and consume both periphyton and seston as a resource (Wassersug 1972; Seale and Bekvar 1980), but I focus on periphyton consumption as the primary resource since there was no evidence that tadpoles consumed seston. Primary resources with high nitrogen and phosphorus content produce larger tadpoles (Schiesari 2006; Stoler and Relyea 2013; Stephens et al. 2017). Stephens et al. (2017) found that high amounts of N in the early stages of tadpole development and high P during bone developmental stages positively influenced tadpole growth and development. Japanese honeysuckle leaf litter produced the highest quality and quantity of periphyton early in the experiment and tadpoles from tanks with Japanese honeysuckle litter were larger than tadpoles from other litter treatments. These results suggest that high quality periphyton may be more beneficial to tadpole growth during the early stages of development.

The total tadpole mass in each tank was greatest in Chinese privet leaf litter treatments, contrary to individual mass results. The total mass in Chinese privet treatments was 85-218% greater than all the other treatments. Mortality in Japanese honeysuckle treatments likely explains the discrepancy between individual mass and total mass as mortality was two times higher in the Japanese honeysuckle than the three other treatments. Walting et al. (2011) found that honeysuckle species extracts negatively affected amphibian survivorship by interfering with respiratory physiology which may have contributed to this mortality. Additionally, most tadpoles died during the winter after all the Japanese honeysuckle leaf litter had fully decomposed. The lack of leaf litter complexity and refuge for tadpoles likely contributed to the higher mortality rates in this treatment compared to the others. The large sizes of individuals in the Japanese honeysuckle treatments may have been due to cannibalism but no research has shown evidence

of this behavior in green frog tadpoles. The largest growth seen in the tadpoles happened in the Japanese honeysuckle shortly after the overwintering die off which suggests that a decrease in competition and an increase in primary production in response to the regeneration of nutrients from decaying tadpoles likely contributed to this growth although it's not statistically supported. Japanese honeysuckle and Chinese privet indirectly produced larger tadpole individuals, but Japanese honeysuckle had a higher mortality rate. This difference in mortality suggests that Chinese privet leaf litter provides a better resource for producing larger and more tadpoles than the other litters considered in this experiment.

Indirect effects on environmental conditions

Sunlight intensity varied between the native and non-native treatments. Sunlight was only measured through the summer and fall seasons and all treatments experienced a steady decrease in sunlight intensity with seasonal changes. The amount of sunlight that reached the leaf litter was 50-65% higher in the native leaf litter treatments. Periphyton accumulation in the non-native treatments on the surface of the water contributed to the shading effect. This increased sunlight intensity in the native treatments could have contributed to periphyton growing directly on the leaf litter that would not have been measured with my methods. The shading effect in the non-native treatments has serious implications in preventing periphyton from growing on the surface of the leaf litter and on ground surfaces. Temperature steadily decreased in all treatments in the fall season. There was no variation or treatment effect on the temperature in the tanks despite decreased sunlight, this could have been due to the systems being above ground tanks. More research should be done in deeper, concealed ponds to understand the effects of increased algal shading on temperature.

Invasive plants

Prior research has established that variation in leaf litter quality can also cause different responses depending on what type of native trees dominate the forest. For example, studies have found that various mixtures of leaf litters can either decrease or increase consumer responses to introduced leaf litters (Rubbo and Kiesecker 2004; Stoler and Relyea 2011, 2013). Increasingly, humans are exerting large direct impacts on forests that often cascade though many compartments with unknown influences on community and population structure. Human disturbance of forests often corresponds with the introduction of non-native species resulting in successional changes in forest composition that are unique relative to the evolutionary histories of the species living in those forests. The establishment of non-native species can prevent native leaf litters from entering aquatic systems by excluding them from riparian areas and the transitional zones around wetlands and ponds. For example, McNeish (2016) found that dense canopies of honeysuckle can act as a barrier preventing other leaf litters from entering aquatic systems, creating a monoculture of litter type subsidizing these aquatic systems. As forest composition shifts, new leaf litter types have profound effects on aquatic communities through cross-boundary exchange. Newly established tree species subsidize aquatic systems with novel leaf litter that can alter the quality and quantity of primary producers resulting in cascading effects on upper trophic levels. Plant species vary in the timing and quantity of leaf litter they drop. The presence of consumers along with new leaf litter types also can induce different producer responses. For example, this research showed how the response of the producer to each leaf litter type was also dependent on the presence of a consumer in both a positive and negative way. The interaction between time and leaf litter type on algal resources has serious implications on how communities can be affected. The results of this study show the pathway through which

leaf litter type can alter characteristics of a community by changing resource quality and quantity that affects the growth and survival of individuals.

This research is the first to examine the temporal effects of leaf litter on primary producer and tadpole growth. Research in the past has primarily focused on amphibian species with relatively short larval periods, potentially missing out on the effects leaf litter decomposition can have on slower developing species through seasonal succession. With these results, I have shown how leaf litter type can have different affects depending on how long the litter has been in the system. This has implications for understanding how species with different developmental rates can be affected by novel leaf litters. Further research on the effects of non-native leaf litter on consumer function should be conducted to understand how ecosystems can be affected by these novel cross-boundary subsidies.

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Table 1: Results of repeated measures ANOVA that tested how treatments affected (A) periphyton dry mass and (B) periphyton ash-free dry mass. Significance is noted with an asterisk.

(A) Periphyton dry mass

Treatment	DF	F	P
Leaf litter	3	0.46	0.124
Tadpole presence	1	1.03	0.038*
Leaf litter x tadpole presence	3	8.68	<0.001*
Time	1	10.90	0.002*
Time x leaf litter	3	6.00	0.002*
Time x tadpole presence	1	6.03	0.02*
Time x leaf litter x tadpole presence	3	4.26	0.012*

(B) Periphyton ash-free dry mass

Treatment	DF	F	P
Leaf litter	3	1.72	0.183
Tadpole presence	1	3.39	0.075
Leaf litter x tadpole presence	3	8.05	<0.001*
Time	1	9.44	0.004*
Time x leaf litter	3	5.66	0.003*
Time x tadpole presence	1	5.16	0.03*
Time x leaf litter x tadpole presence	3	4.42	0.01*

Table 2: The mean (± 1 SE) periphyton dry mass in μg/cm^2 in four different leaf litter types on day 46 and day 273 with the presence and absence of tadpoles.

Species	Tadpole presence	Day 46	Day 273
Japanese honeysuckle	Absent	2.31 (0.60)	0.077 (0.41)
	Present	0.02 (0.01)	0.051 (0.03)
Chinese privet	Absent	0.341 (0.22)	0.032 (0.11)
	Present	0.682 (0.19)	0.022 (0.01)
Willow oak	Absent	0.104 (0.05)	0.117 (0.08)
	Present	0.076 (0.04)	0.214 (0.09)
Longleaf pine	Absent	0.054 (0.02)	0.037 (0.01)
	Present	0.057 (0.02)	0.211 (0.19)

Table 3: The absolute mean values (± 1 SE) of periphyton total nitrogen and total phosphorus in four different leaf litter types on day 46 and day 273 with the presence and absence of tadpoles in μg/cm².

Species	Tadpole presence	TN Day 46	TN Day 273	TP Day 46	TP Day 273
Japanese honeysuckle	Absent	48.68 (7.86)	0.44 (0.10)	7.70 (1.08)	0.48 (0.17)
	Present	1.08 (0.34)	0.22 (0.05)	0.164 (0.05)	0.35 (0.16)
Chinese privet	Absent	7.46 (4.64)	0.19 (0.02)	0.78 (0.56)	0.24 (0.07)
	Present	19.27 (4.96)	0.27 (0.04)	1.56 (0.40)	0.21 (0.05)
Willow oak	Absent	1.44 (0.40)	0.15 (0.02)	0.13 (0.06)	0.26 (0.44)
	Present	1.81 (0.45)	0.15 (0.13)	0.11 (0.20)	0.34 (0.05)
Longleaf pine	Absent	0.76 (0.17)	0.08 (0.20)	0.05 (0.01)	0.10 (0.02)
	Present	0.99 (0.19)	0.11 (0.03)	0.06 (0.01)	0.29 (0.22)
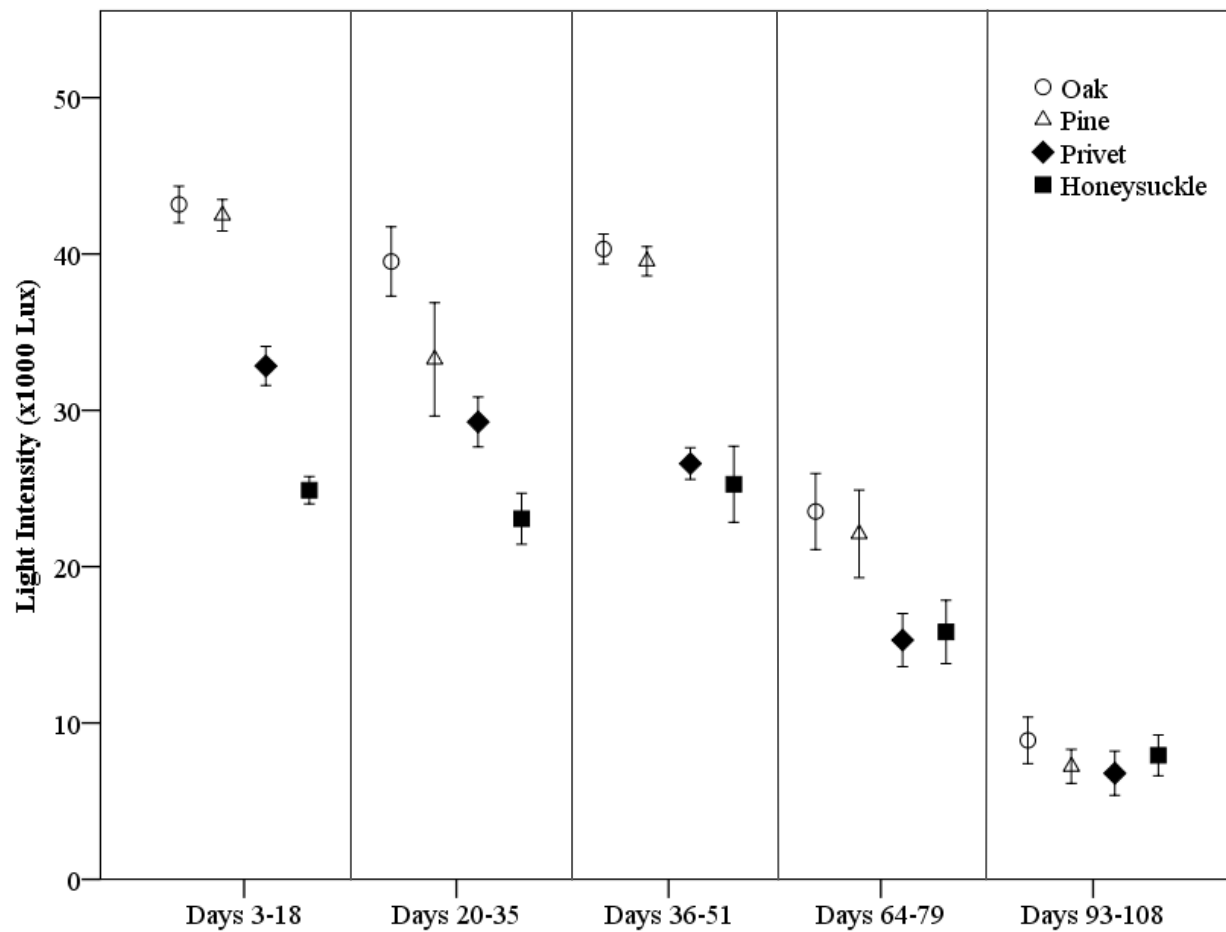
Figure 1: Sunlight intensity in each leaf litter treatment on five rotational periods throughout 2018. Filled in shapes represent the non-native treatments. Each point represents the untransformed treatment mean ± 1 SE.
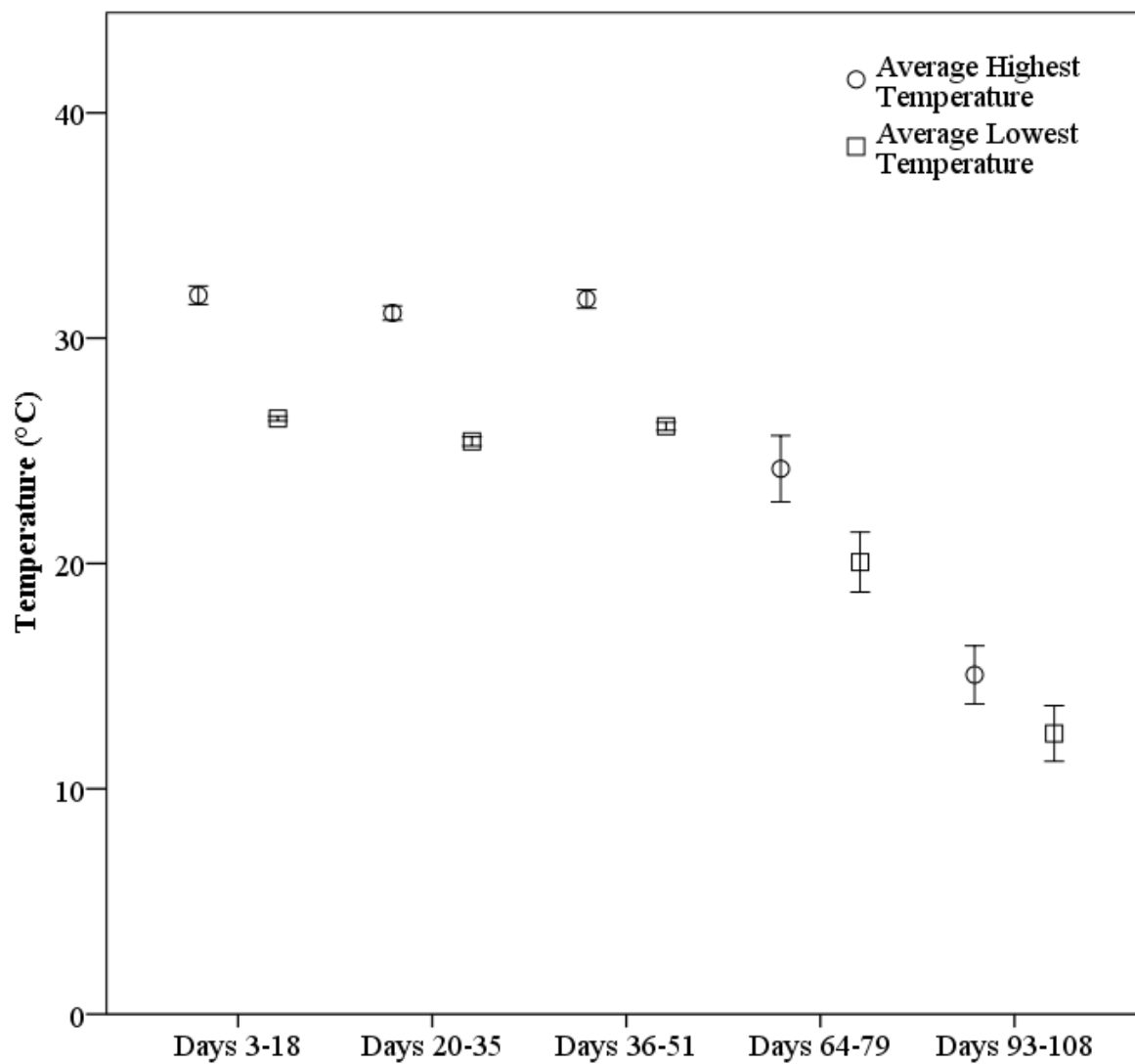
Figure 2: The average highest and lowest temperatures for all treatments on five rotational periods throughout 2018. Each point represents the untransformed treatment mean ± 1 SE.
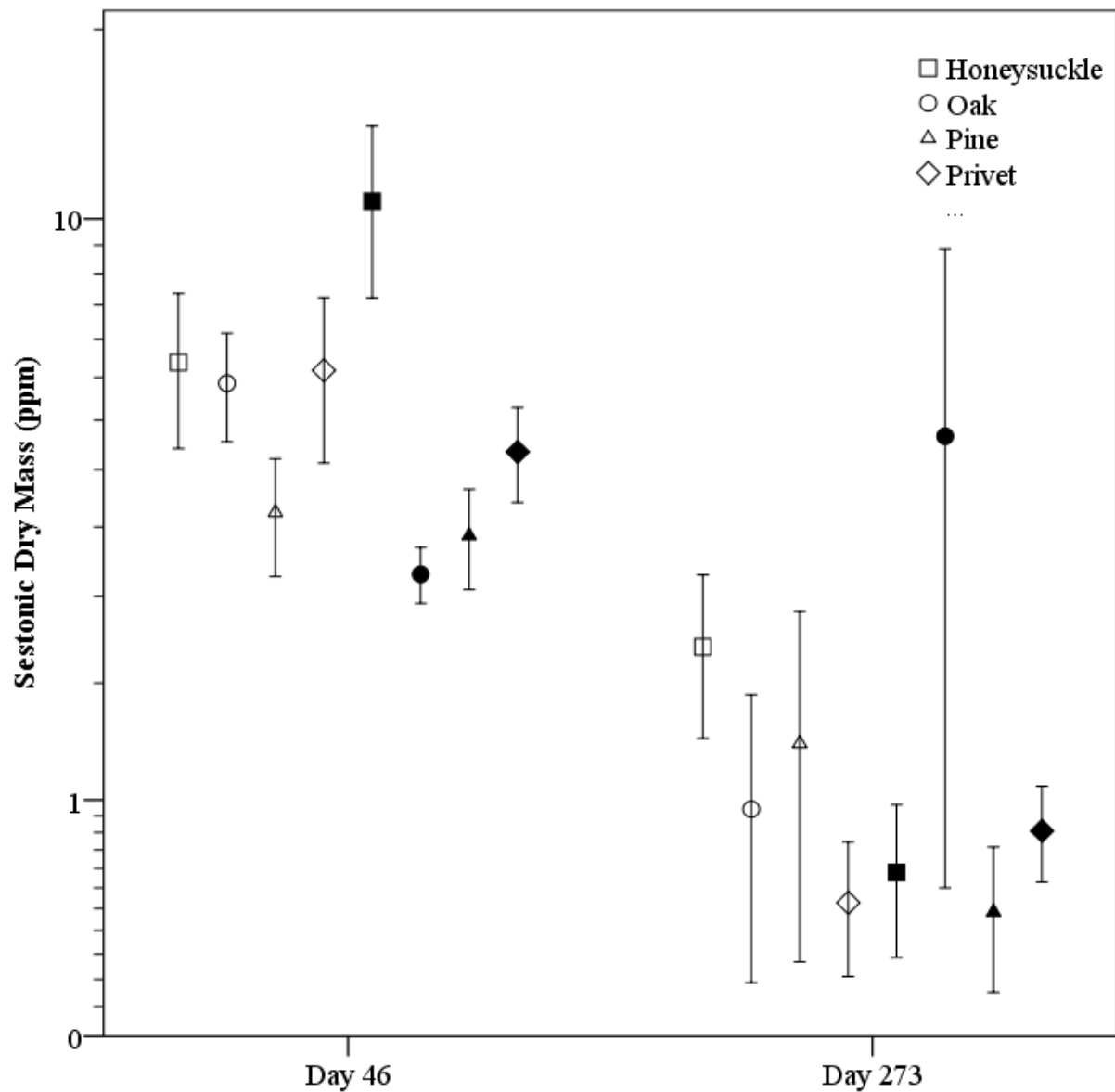
Figure 3: The effects of leaf litter type, time, and tadpole presence on sestonic dry biomass on the two sampling dates (15 September 2018 and 30 April 2019). Filled in shapes represent the presence of tadpoles. Each point represents the untransformed treatment mean ± 1 SE.
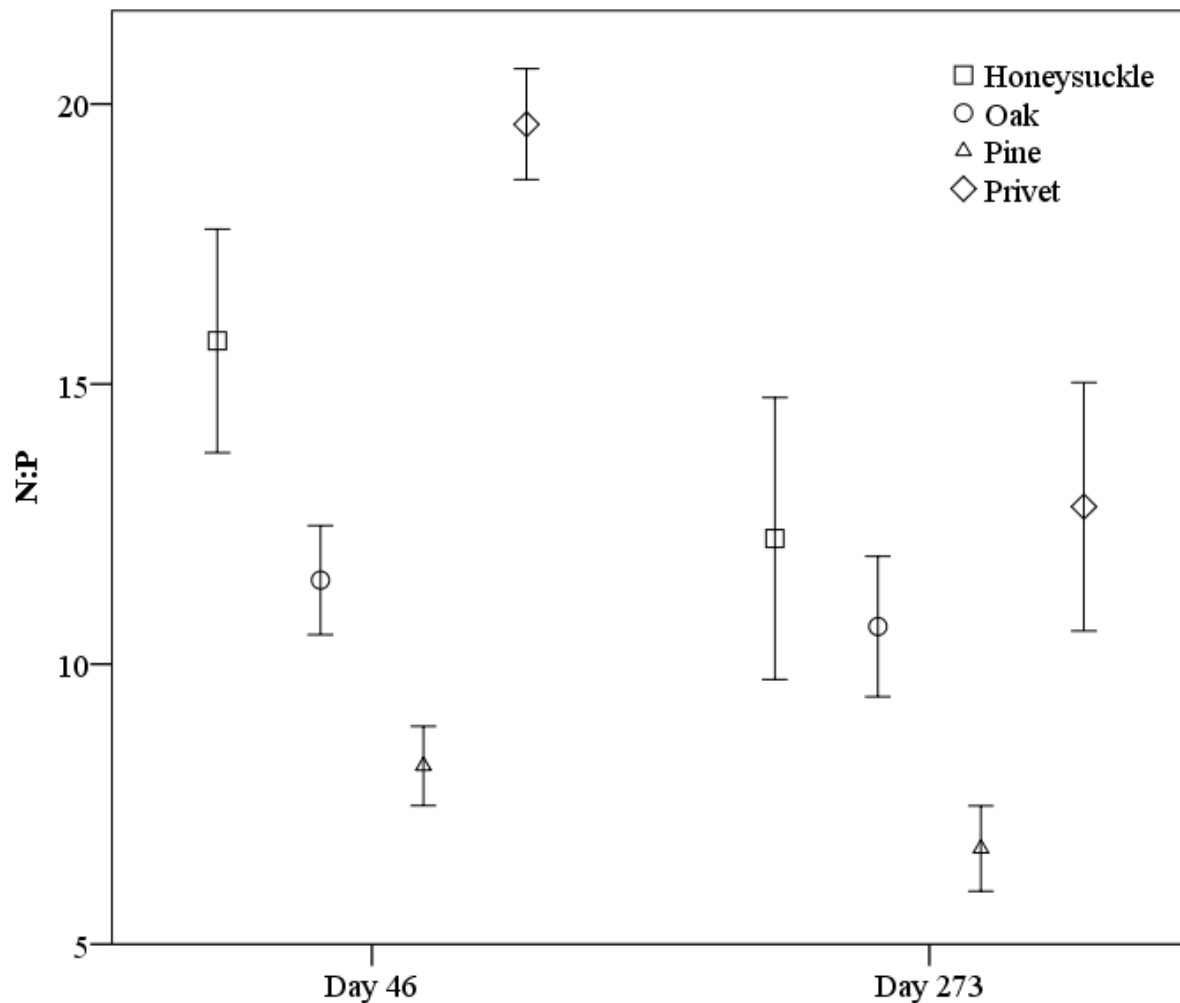
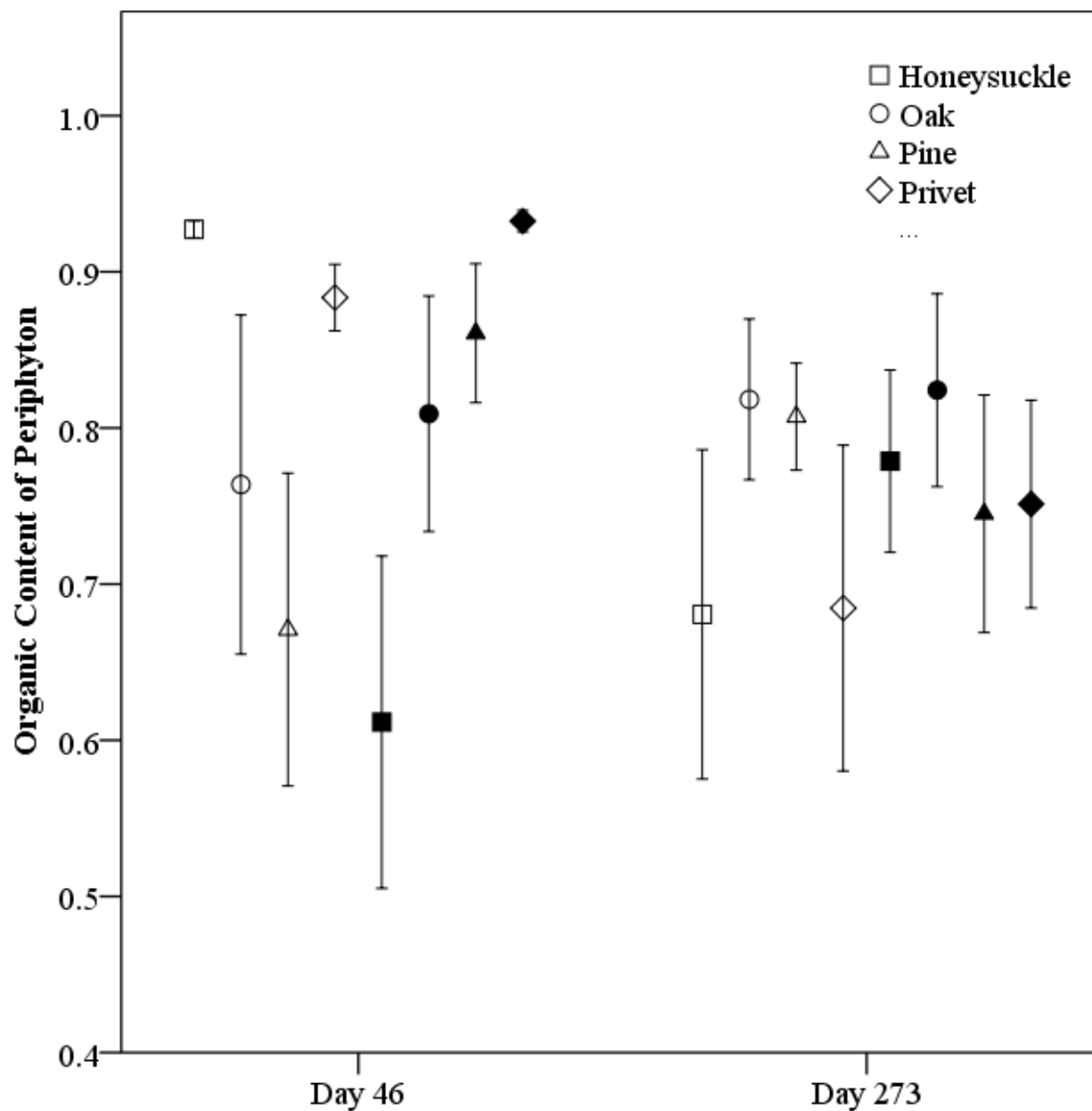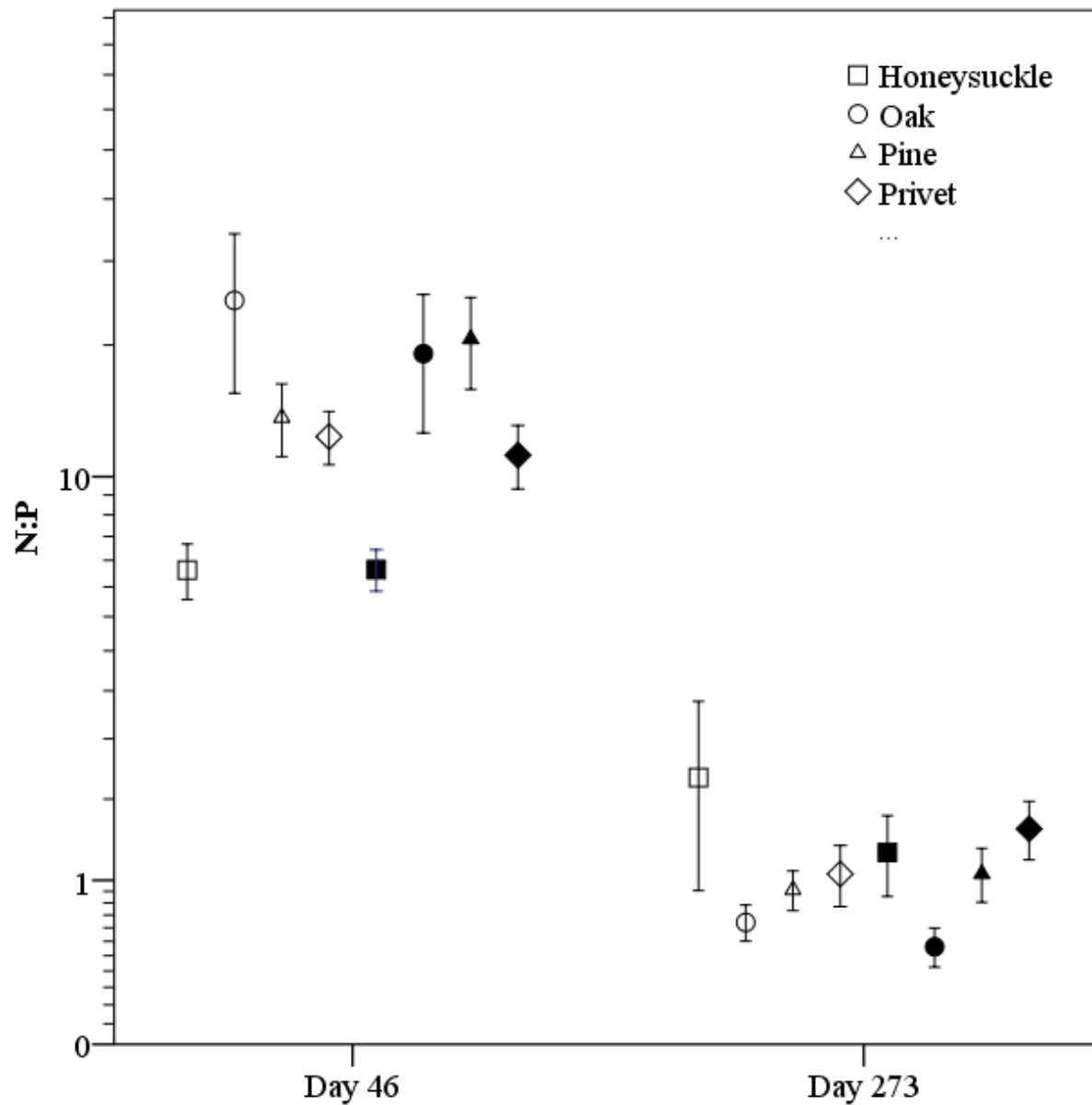**Figure 4:** The effects of leaf litter type and time on seston N:P ratios on the two sampling dates (15 September 2018 and 30 April 2019). Each point represents the untransformed treatment mean ± 1 SE.
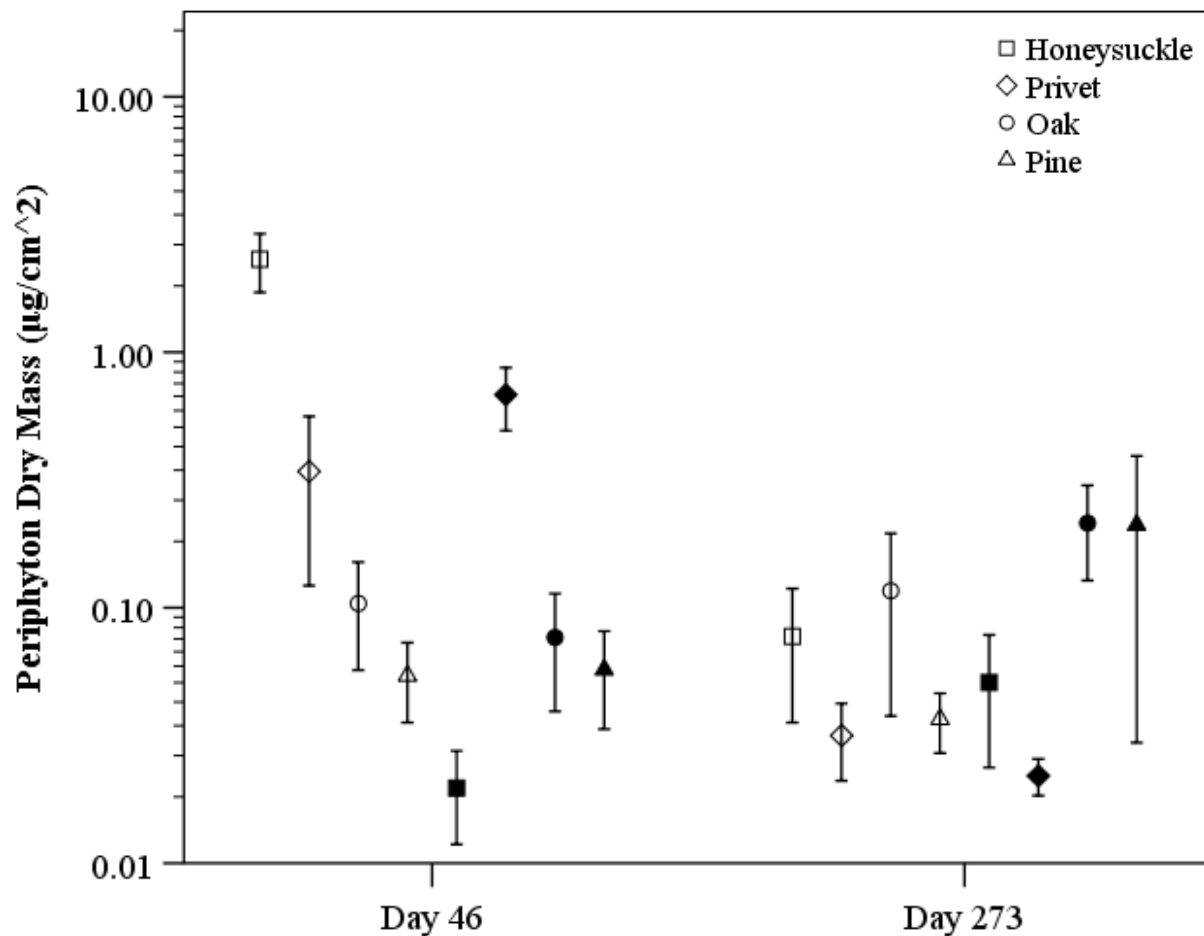
Figure 5: The effects of leaf litter type, time, and tadpole presence on periphyton dry biomass on the two sampling dates (15 September 2018 and 30 April 2019). Filled in shapes represent the presence of tadpoles. Each point represents the log10 transformed treatment mean ± 1 SE.

Figure 6: The effects of leaf litter type, time, and tadpole presence on periphyton percent organic content on the two sampling dates (15 September 2018 and 30 April 2019). Filled in shapes represent the presence of tadpoles. Each point represents the untransformed treatment mean ± 1 SE.
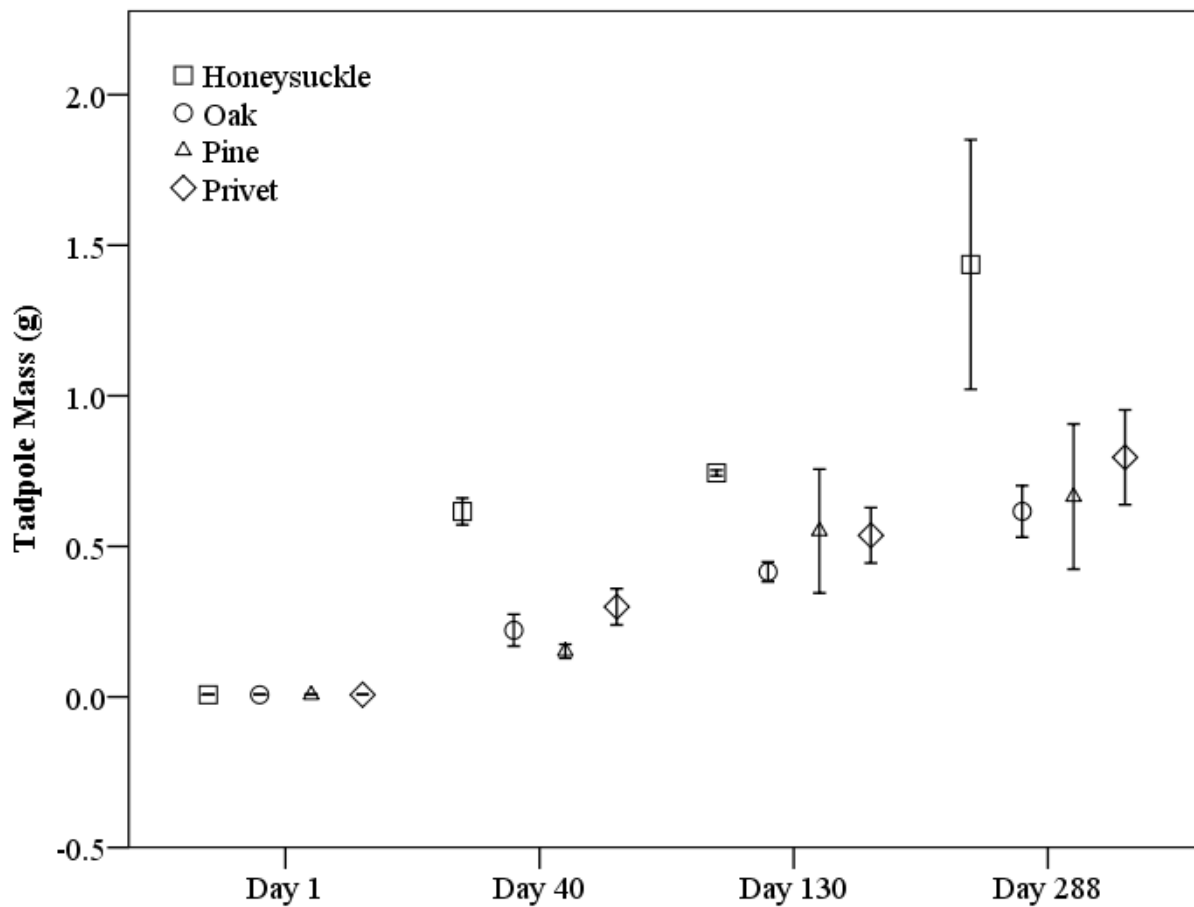
Figure 7: The effects of leaf litter type, time, and tadpole presence on periphyton N:P ratios on the two sampling dates (15 September 2018 and 30 April 2019). Filled in shapes represent the presence of tadpoles. Each point represents the untransformed treatment mean ± 1 SE.

Figure 8: The effects of leaf litter type and time on individual tadpole mass on the four sampling dates (July 31, 2018, September 9, 2018, December 8, 2018, and May 15, 2019). Each point represents untransformed treatment mean ± 1 SE.
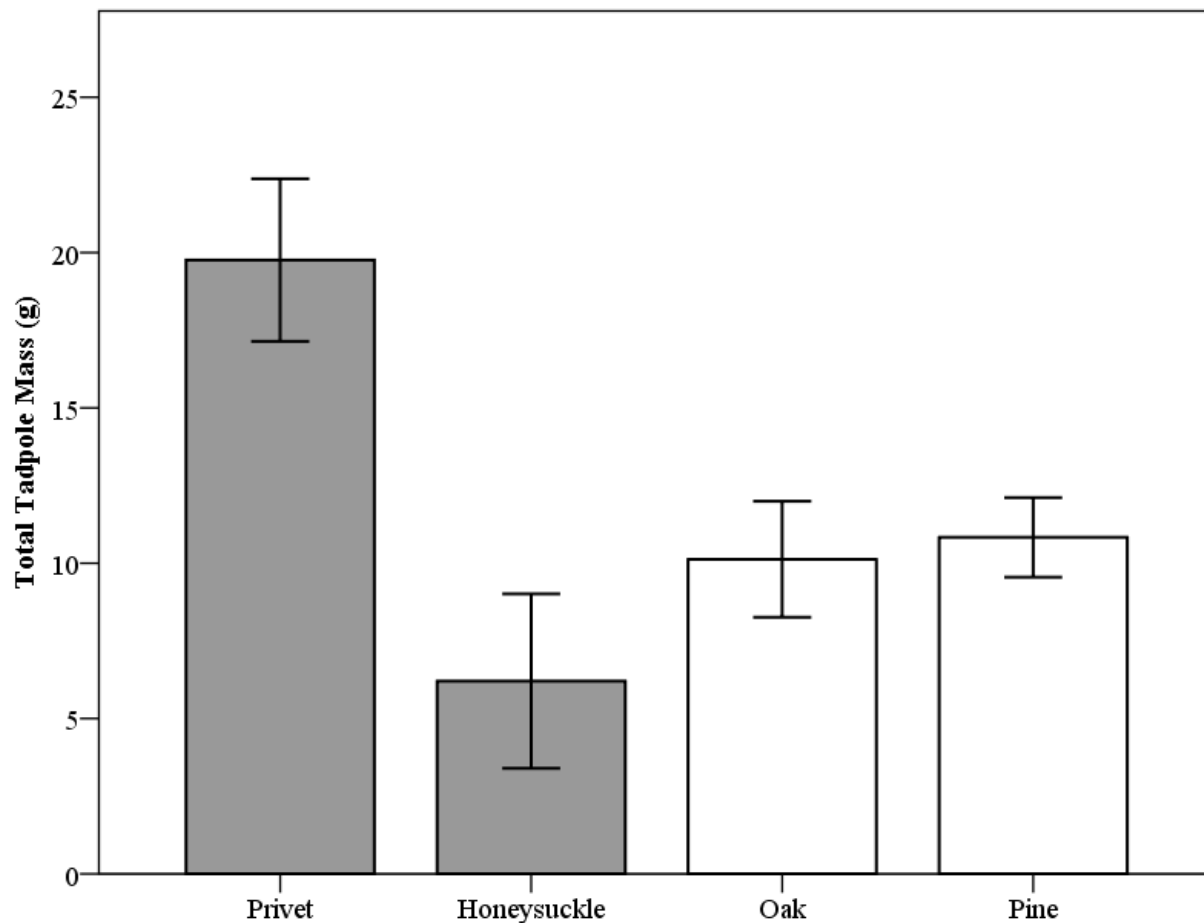
Figure 9: Total tadpole mass for each leaf litter type on the final sampling date (May 15, 2019). Filled in bars represent non-native leaf litter species. Each point represents the untransformed treatment mean ± 1 SE.
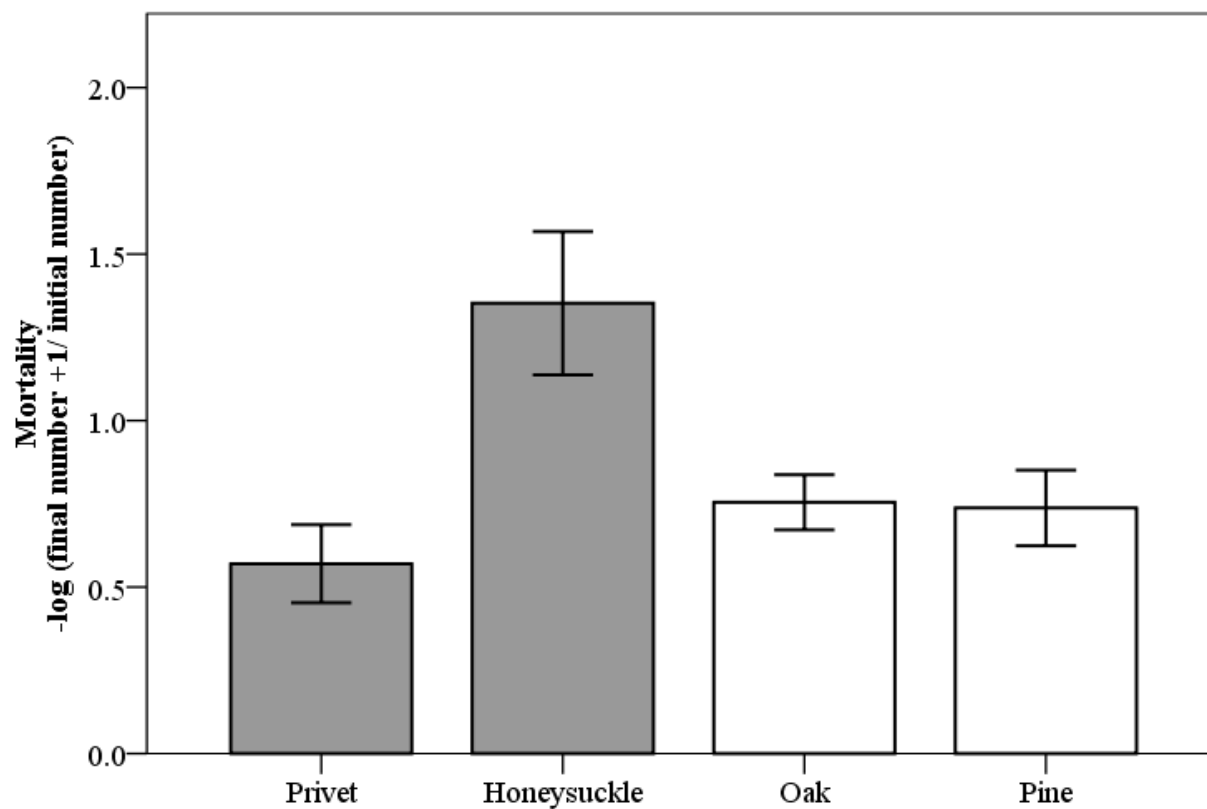
Figure 10: Tadpole mortality for each leaf litter type on the final sampling date (May 15, 2019). Filled in bars represent non-native leaf litter species. To account for tanks with no survivors, one was added to all the survivor proportions then the -log was taken to estimate the instantaneous mortality rate. Each point represents the -log transformed treatment mean $\pm$ 1 SE.
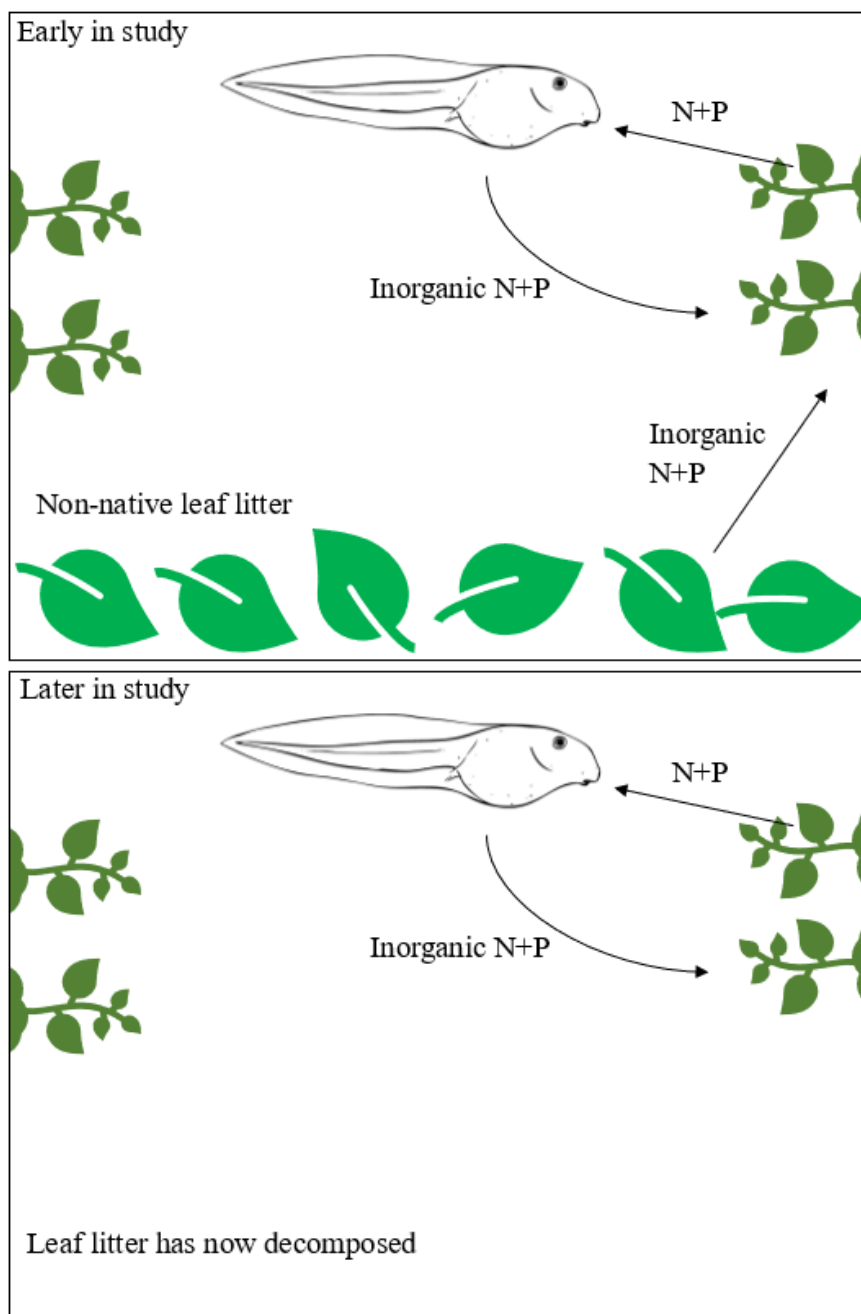
Figure 11: A feedback loop between tadpole grazing and excretion of inorganic nutrients. The top figure represents the non-native treatments in the beginning of the study. The non-native leaf litter was decomposing rapidly and providing a large amount of nutrients to fertilize periphyton. The bottom figure represents the Japanese honeysuckle treatment towards the end of the study. The leaves have fully decomposed leaving the periphyton to fully rely on the excretion of nutrients from tadpoles. As the tadpoles grow and form tissues for growth, less nutrients are excreted back into the system, limiting the periphyton growth over time. The figures can represent the trend seen in the natives if looked at in the opposite order.