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Living in the litter: the influence of tree leaf litter on wetland communities

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Empirical research in streams has demonstrated that terrestrial subsidies of tree leaf litter influence multiple community factors including composition, diversity and growth of individuals. However, little research has examined the importance of tree litter species on wetlands, which are ubiquitous across the landscape and serve as important habitats for a unique and diverse community of organisms. Using outdoor mesocosms, we assessed the impact of 12 litter monocultures and three litter mixtures (from both broadleaf and conifer trees) on pond communities containing gray tree frog tadpoles *Hyla versicolor*, periphyton, phytoplankton and zooplankton. We found that leaf litter species had substantial and differential impacts on all trophic groups in the community including effects on algal abundance, zooplankton density and amphibian growth. In many instances, patterns of responses were specific to individual litter species yet some responses, including both pH values and periphyton biomass, were generalizable to broad taxonomic groups. In addition, while most responses of litter mixtures were additive, we found evidence for antagonistic effects of litter mixing among responses of periphyton and amphibian body mass. Our results highlight the potential impact of human and naturally driven changes in forest composition on wetland communities through associated changes in leaf litter.

Linkage between aquatic and terrestrial ecosystems allows for frequent exchange of nutrients and organisms which alters local factors such as resource abundance and trophic interactions (Polis et al. 1997). In forested aquatic systems, food webs are generally nutrient-limited (Wilbur 1997), and net ecosystem productivity is driven by external carbon sources (Fisher and Likens 1973, Vannote et al. 1980, Brinson et al. 1981, Polis et al. 1997). For a variety of forested heterotrophic aquatic systems, the bulk of carbon input is derived from plant litter (Nelson and Scott 1962, Minshall 1967, Fisher and Likens 1973, Webster and Benfield 1986) which subsidizes aquatic food web resources (Wallace et al. 1997). In fact, leaf litter originating from trees can account for 99% of total dissolved organic carbon input in stream systems (DOC, Fisher and Likens 1973). Such allochthonous inputs often regulate community productivity by generating more primary and secondary production than available in situ resources can sustain (Polis et al. 1997). Removal of these inputs or shifts in the timing and magnitude of litter decomposition can have strong effects on entire ecosystems.

As a result, the decomposition of plant litter is an essential ecosystem process in forested aquatic systems (Fisher and Likens 1973, Polis et al. 1997, Wallace et al. 1997, Meyer et al. 1998) and the role of leaf litter in stream environments has received increasing attention in the past decade for its strong impacts on ecosystem properties and services (Lecerf and Richardson 2010, Kominoski et al. 2010). While research on the impact of litter is prevalent in lotic stream and riverine systems, studies on lentic ecosystems remain scarce, although leaf litter inputs provide an important carbon source in these systems as well (Bonner et al. 1997, Wetzel 2001, Rubbo et al. 2006, 2008). Such habitats have species uniquely adapted to lentic water, high seasonal productivity, and often stronger trophic interactions than lotic systems (Williams 1996, Wetzel 2001, Shurin et al. 2002). Studies examining litter effects in lentic environments have generally focused on relatively small and ephemeral tree hole ecosystems, where mosquito distribution and performance across three or four litter species is usually dependent on the species of leaf litter present (Fish and Carpenter 1982, Yanoviak 1999, Reiskind et al. 2009). There are a few studies investigating the role of leaf litter in larger, more diverse wetland ecosystems; comparisons of two litter types have found that litter species matters in these systems as well (Rubbo and Kiesecker 2004, Williams et al. 2008).

The quality of litter input for consumers hinges upon the species composition of the litter and the chemistry of constituent species (Brinson et al. 1981, Melillo et al. 1982, Webster and Benfield 1986, Wetzel 2001, Swan and Palmer 2006). Litter chemistry varies widely among tree species (Ostrofsky 1993, 1997) and these chemical differences consequently help determine the food web that can be supported (Webster and Benfield 1986, Facelli and Pickett 1991, Wallace et al. 1997, Werner and Glennemeier 1999). For example, nitrogen and lignin content affect litter quality and palatability (Melillo et al. 1982, Ostrofsky 1997), secondary compounds

such as phenolics can act as anti-microbial agents (Ardón and Pringle 2008), and high litter solubility may darken the water column, consequently imposing limitations on primary production (Karlsson et al. 2009). On a broad scale, single-species inputs of tree litter are generally uncommon in forests; yet understanding the effects of individual litter species provides a basis to understand local ecosystem processes and may offer predictions for consequences of tree species loss or addition.

While understanding the effects single species of litter is an important starting point, the more common scenario in nature involves mixtures of litter species. In stream systems, it is clear that the effects of mixing litter inputs are often different than those expected based on single-species effects (i.e. non-additive). Mixtures of litter may produce synergistic (i.e. greater than additive) or antagonistic effects (i.e. lower than additive) in aquatic ecosystems via interactions at the molecular and microbial level (Kominoski et al. 2007, 2010, Lecerf and Richardson 2010, Swan et al. 2009). A single litter species in a mixture of multiple litter species may facilitate or inhibit the breakdown of other species, thus altering nutrient release and environmental conditions (Swan and Palmer 2006). For example, a recalcitrant litter species (e.g. *Quercus* sp.) may promote growth of consumers within a community by providing a nutrient source that outlasts other litter species (Swan and Palmer 2005), but may also deter growth by lowering the overall nutritional quality of the litter mixture (Rosemond et al. 2010).

The species composition of natural litter mixtures varies widely depending upon climate, geography and succession factors such as disturbance and ecosystem age. However, forests are often identified as coniferous, hardwood, or a mixture of both, and litter chemistry differs strongly between these forest types. For example, coniferous litter is often more refractory than broadleaf litter and is generally lower in nitrogen (Berg and McClaugherty 2008). Such differences between litter in these forest types is likely to have strong impacts on lentic ecosystem processes, yet such effects have not been investigated.

By examining differences between the effects of individual litter species and litter from different forest types, it is possible to understand the effects of litter inputs within and among forests. Achieving this goal is necessary to both enhance our knowledge of ecological processes in forests and to bolster conservation efforts in these systems. Furthermore, such research is becoming more and more critical as natural and anthropogenic disturbance in forests alters tree species composition and associated litter.

We investigated how multiple coniferous and broadleaf litter species from a diverse, forested landscape influence forested wetland communities. Using outdoor mesocosms, we created replicated wetland communities treated with a variety of common litter species that represented 12 species from six families (alone and in mixtures) and spanned a wide range of leaf structure and chemistry. We investigated two hypotheses: 1) leaf litter species and leaf litter type (broadleaf vs coniferous) will differentially affect the dynamics of producers and consumers in a wetland (i.e. density, body mass and survival); 2) litter mixtures will have non-additive effects on the dynamics of the producers and consumers in a wetland.

Methods

Our experiment was conducted at the Univ. of Pittsburgh's Pymatuning Lab of Ecology. The experiment employed a completely randomized design with 15 treatments that were replicated four times for 60 total experimental units. Each experimental unit was a 100-l polyethylene wading pool, 1 m in diameter and approximately 0.2 m in height, topped with a 60% shade-cloth lid to prevent escape or entry of any organisms and to provide an intermediate level of light penetration relative to the full range of canopy cover under which such ephemeral systems occur (Werner and Glennemeier 1999). We filled each pool with 100 l of well water on 12 June 2006 and added leaf litter on 21 June. The 15 litter treatments included eight broadleaf monocultures, four conifer monocultures, a mixture of the eight broadleaf species, a mixture of the four conifer species, and a mixture of all 12 species (Table 1). All mesocosms contained a total of 100 g of litter, with the mixture treatments using equal portions of all species. This amount is within the range found in nature (Rubbo et al. 2008).

Although litter is typically collected immediately after senescence in the autumn (Rubbo and Kiesecker 2004), we collected fallen leaves and needles in mid-June to simulate a scenario where a dry wetland basin was filled by spring rains. Litter was collected from mostly mesic areas surrounding the field site and from a forested area near

Table 1. Species of leaf litter and amount used in the monoculture and mixture treatments of the mesocosm experiment.

Common name	Family	Latin name	Monoculture (g)	Broadleaf mixture (g)	Conifer mixture (g)	Complete mixture (g)
Black willow	*Salicaceae*	*Salix nigra*	100	12.5		8.3
Big tooth aspen	*Salicaceae*	*Populus grandidentata*	100	12.5		8.3
Northern red oak	*Fagaceae*	*Quercus rubra*	100	12.5		8.3
American beech	*Fagaceae*	*Fagus grandifolia*	100	12.5		8.3
American chestnut (hybrid)	*Fagaceae*	*Castanea dentata* x *mollissima*	100	12.5		8.3
American sycamore	*Platanaceae*	*Platanus occidentalis*	100	12.5		8.3
Red maple	*Aceraceae*	*Acer rubrum*	100	12.5		8.3
Sugar maple	*Aceraceae*	*Acer saccharum*	100	12.5		8.3
White pine	*Pinaceae*	*Pinus strobus*	100		25	8.3
Eastern hemlock	*Pinaceae*	*Tsuga canadensis*	100		25	8.3
Tamarack	*Pinaceae*	*Larix laricina*	100		25	8.3
Norway spruce	*Pinaceae*	*Picea abies*	100		25	8.3

Slippery Rock, PA. We selected species that are common in eastern North American forests and span a range of taxonomic families. We also included several species that were congeners to determine if an effect of one species might be representative of the genus (e.g. red maple and sugar maple). Additionally, several of our selected species are of interest to forest managers including species that have historically declined (e.g. American chestnut), species that are currently declining (e.g. eastern hemlock, red oak), and species that are currently increasing (e.g. red maple) in abundance. Litter was sorted by hand and dried over 24 h by placing it in the sun during the day and in a 30°C room overnight.

On the same day that litter was added (21 June), one unglazed, ceramic tile (divided into two equal sections by drawing a line down the middle) was placed into the middle of each mesocosm and raised off the bottom by approximately 2.5 cm, thereby allowing for the collection of periphyton at two separate times with minimum disturbance to the rest of the mesocosm substrate. On 22 June, we collected, mixed and filtered 60 l water from four nearby wetlands through 21 μm mesh screening, and inoculated each mesocosm with 1 l of water as a source of periphyton, phytoplankton (i.e. producers) and microbial assemblages.

Zooplankton and tadpoles were introduced as primary consumers. On 26 June, we collected zooplankton from natural ponds with a 21 μm mesh zooplankton net and removed large invertebrate predators (primarily *Chaoborus* sp.) to ensure that zooplankton and tadpoles were the top trophic level. An aliquot of zooplankton was distributed to each mesocosm. We added 5 g of rabbit chow (i.e. ground alfalfa) to each mesocosm on 28 June to accelerate algal development. The addition of a nutrient pulse is common practice in mesocosm experiments involving tadpoles (Relyea 2003) and the rabbit chow mass was only 5% of the litter mass.

The grey tree frog tadpoles *Hyla versicolor* were collected as three egg masses in early June. This species oviposits in a wide range of habitats, and its rapid developmental rate allows it to specialize in metamorphosing from highly ephemeral wetlands (Collins and Wilbur 1979). Eggs were hatched and reared in wading pools (containing aged well water, periphyton, and zooplankton), fed rabbit chow ad libitum. After reaching a safe handling mass (Relyea 2003) and allowing plankton and microbial assemblages to develop in the mesocosms for 8 days, 20 tadpoles were haphazardly selected from a mixture of these egg mass (initial mean mass ± 1 SE = 133 ± 15 mg) and added to the mesocosms on 30 June (defined as day 0 of the experiment). This density (25 m^{-2}) is within natural densities (Relyea unpubl.). Twenty tadpoles were set aside to assess mortality due to handling; 24-h survival of this sample was 100%.

Temperature and pH

To assess the abiotic conditions throughout the experiment among treatments, we measured temperature and pH using an electronic water meter. Preliminary analyses indicated no temperature effects among treatments and this factor was removed from the analysis (mid-day measurements among treatments ranged from 28.8 to 29.2°C). Mesocosm pH was recorded on day 7 and day 17 at similar depths and locations across all mesocosms. This factor was retained in the analysis.

Primary production

Phytoplankton was sampled on two occasions (day 12 and day 21). Using a tube sampler, we removed four 200-ml samples of water from different locations within each mesocosm and combined them into a single sample. We filtered this water through GF/C glass filters, wrapped the filters in aluminum foil and stored the samples at –20°C. For each sample, chlorophyll a concentration was measured as a surrogate for total phytoplankton biomass. Flourometric analysis was performed on each sample using the calculations and a modified version of the EPA method 445.0 (Arar and Collins 1997). To extract chlorophyll a, we incubated the filters in 90% ethanol in the dark at –20° C for 48 h (stirred vigorously on two occasions) and performed the recommended correction by acidification. The concentration of chlorophyll a was then determined for each sample using a fluorometer. Three samples had to be discarded due to handling accidents, but this did not remove more than one replicate of any treatment.

We assessed periphyton biomass twice during the experiment (on day 11 and day 18) using the clay tile that was placed into each mesocosm. On each sample date, periphyton was scrubbed from half of the ceramic tile into a tub of clean water and this mixture was filtered through preweighed, oven-dried GF/C glass filters. These filters were oven dried for 24 h at 80°C and reweighed to determine the dry biomass of the periphyton.

Primary consumers

Near the end of the experiment (day 20), we assessed zooplankton density. We collected a total of 800 ml of water (200 ml of water from four locations) from each mesocosm using a tube sampler. This sample was then filtered through a 62 μm Nitex screen and the zooplankton collected were preserved in 70% ethanol. Total zooplankton density was determined for each mesocosm.

We also assessed tadpole body mass at two times during the experiment (day 7 and day 17) by haphazardly sampling five tadpoles from each mesocosm, gently patting them dry, and recording their cumulative biomass. After weighing, these animals were returned to the mesocosms. Since tadpoles stop foraging once metamorphosis begins, we chose not to record tadpole body mass after the first metamorph appeared on day 17. Tadpoles with fully formed forelimbs and less than 1 cm of tail length were collected and stored in 1-l containers containing wet sphagnum moss until complete tail resorption (Gosner stage 46; Gosner 1960). The duration of time from the start of the experiment to the day that a frog achieved stage 46 was defined as the time to metamorphosis. Metamorphs were euthanized in a 2% MS-222 solution and preserved in 10% formalin for subsequent weighing to determine the mass at metamorphosis. Survival, mean time to metamorphosis, and mean mass at metamorphosis

for all frogs in a given mesocosm served as our amphibian response variables. The experiment lasted 52 d, at which point all tadpoles had metamorphosed.

Statistical analyses

We employed general linear models (GLMs) to assess community responses to leaf litter composition. Although the assumption of homogeneous variances was not met for all response variables (including zooplankton, mass at metamorphosis, both periphyton samples, and both pH samples), GLMs are generally robust to violations of this assumption (Sokal and Rohlf 1995). When necessary, data were transformed to meet the assumption of normality. Four response variables (tadpole mass, periphyton biomass, chlorophyll a concentration and pH) were measured at multiple times during the experiment and on different dates, allowing us to assess whether responses to litter composition effects changed over time. For these responses, we conducted repeated-measures analyses of variance (rm-ANOVA) for each variable. After finding significant univariate treatment effects, we then conducted mean comparisons using Tukey's HSD to test for differences among treatments. Three responses were measured only once (amphibian survival, time to metamorphosis, and zooplankton population size). For these responses, we performed a MANOVA followed by univariate analyses. Again, we conducted mean comparisons using Tukey's HSD for all significant univariate effects.

Because our approach of running multiple rm-ANOVAs as well as a MANOVA risks conducting a type I error, we performed an additional analysis of variance that analyzed all variables measured late in the experiment (i.e. mass at metamorphosis, time to metamorphosis, survival to metamorphosis, phytoplankton, periphyton, zooplankton and pH). After finding a significant multivariate effect, we then analyzed all significant univariate responses by univariate ANOVA's for single-measured responses and rm-ANOVA's for repeated-measure responses. This analysis confirmed all of the conclusions of the previous analysis (MANOVA not shown).

For each significant univariate effect, we also conducted planned comparisons. The first three comparisons compared the observed and expected additive response of a given mixture treatment to determine if responses were additive, synergistic, or antagonistic. Expected, additive responses were calculated as the mean treatment response of all monoculture species found in each mixture. The first planned comparison contrasted the observed and expected conifer mixture responses. The second planned comparison contrasted the observed and expected broadleaf mixture responses. The third planned comparison contrasted the observed and expected broadleaf–conifer mixture responses. A fourth planned comparison, contrasting the average response of all broadleaf monoculture treatments with the average response of all conifer treatments, tested if responses differed between our two broad taxonomic groups of litter. This comparison allowed for evaluation of average species effects without the influence of potential non-additivity inherent in our previous comparison of the broadleaf and conifer mixture treatments.

Results

Primary producers

The rm-ANOVA on phytoplankton density detected no treatment effect, but there was a time effect and a treatment-by-time interaction (Table 2). Separate ANOVAs showed no treatment effect on day 12 ($F_{14,43} = 1.455$, $p = 0.170$), but a significant treatment effect on day 21 ($F_{14,44} = 2.602$, $p = 0.008$; Fig. 1a). Norway spruce litter supported significantly lower amounts of phytoplankton compared to red oak litter ($p = 0.049$) and marginally lower amounts compared to bigtooth aspen ($p = 0.060$), but there were no other significant differences among treatments within the sample date ($p \geq 0.138$). Except for Norway spruce, phytoplankton density generally increased over time, ranging from a 1.6-fold increase in black willow mesocosms to nearly a 17-fold increase in bigtooth aspen mesocosms. Responses to mixtures did not differ from any respective monoculture responses ($p \geq 0.539$). Planned comparisons did not reveal any differences between the expected and observed mixture responses ($p \geq 0.301$), suggesting an additive effect of all leaf mixtures. There was also no difference between the average of broadleaf and conifer monoculture treatment responses, suggesting no effect of taxonomic group ($p = 0.135$).

Analysis of periphyton biomass revealed a significant treatment effect as well as a time effect and a treatment-by-time interaction (Table 2). Separate ANOVAs revealed significant treatment effects on both sampling dates (day 11, $F_{14,44} = 5.885$, $p < 0.001$; day 18, $F_{14,45} = 2.377$, $p = 0.014$; Fig. 1b). On day 11, the black willow mesocosms contained more periphyton than all other

Table 2. Results of ANOVAs and repeated-measure ANOVAs that examined how abiotic and biotic factors changed in response to leaf litter treatment.

	Treatment			Time			Treatment × time		
Response variable	F	p	DF	F	p	DF	F	p	DF
pH	2.389	<0.001	14,45	49.378	<0.001	1,45	1.234	0.286	14,45
Phytoplankton	1.466	0.167	14,42	148.7	<0.001	1,42	2.962	0.003	14,42
Periphyton	5.236	<0.001	14,45	8.085	0.007	1,45	4.104	<0.001	14,45
Amphibian body mass	3.541	0.001	14,45	84.309	<0.001	1,45	4.821	<0.001	14,45
Amphibian survival	0.937	0.529	14,44						
Time to metamorphosis	0.855	0.609	14,44						
Zooplankton	3.883	<0.001	14,44						

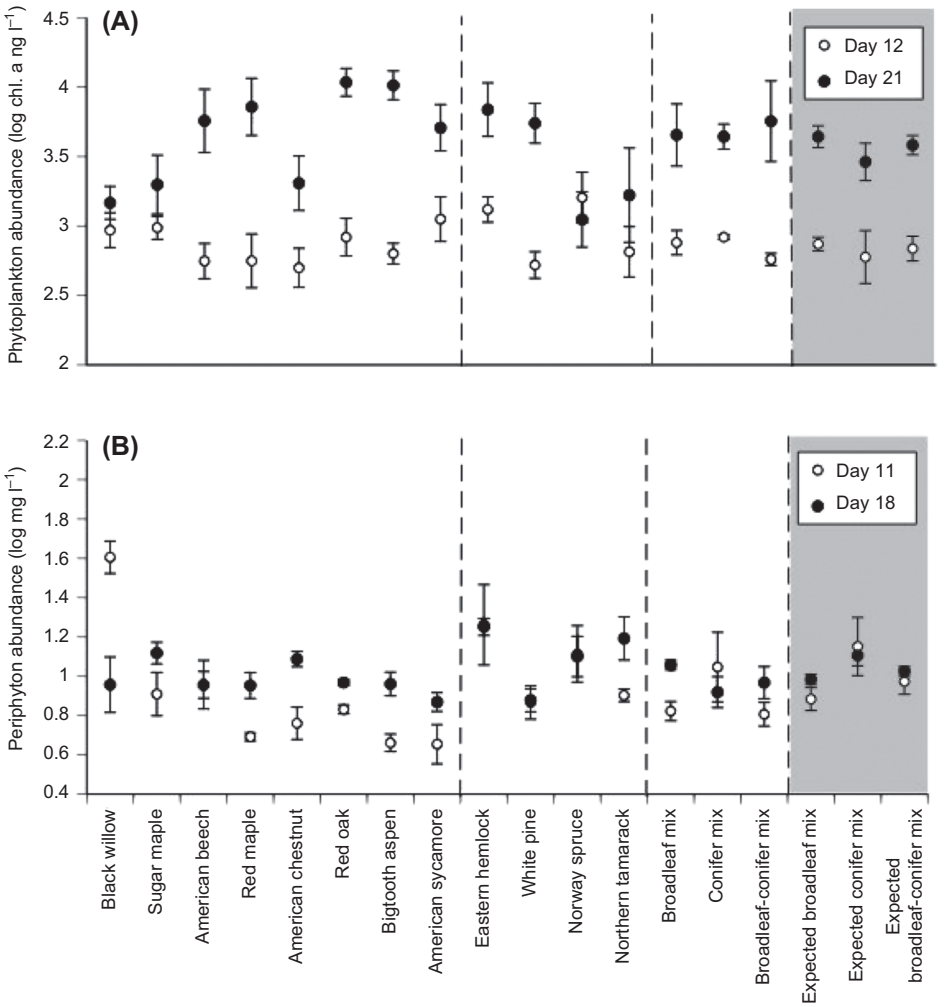

Figure 1. Phytoplankton concentration (measured as chlorophyll a; panel A) and periphyton biomass (panel B) in wetland mesocosms containing 12 leaf litter monocultures and three leaf litter mixtures. In both graphs, the gray area contains expected means of the mixture treatments, based on additive values derived from respective monoculture responses. Data were log transformed and means ± 1 SE are presented.

treatments ($p \leq 0.029$) except eastern hemlock ($p = 0.570$) and Norway spruce ($p = 0.091$). Black willow mesocosms also contained more periphyton than both mixture treatments containing black willow litter (i.e. broadleaf and broadleaf–conifer mixtures; $p < 0.001$). Periphyton in Norway spruce mesocosms was similar to all treatments ($p \geq 0.091$) whereas hemlock periphyton was higher than that of aspen, red maple and sycamore treatments ($p \leq 0.023$) and marginally higher than chestnut mesocosms ($p = 0.077$), but similar to all other treatments including both mixture treatments containing hemlock (i.e. conifer and broadleaf–conifer mixtures; $p \geq 0.157$). By day 18, periphyton in black willow monocultures had decreased from the first sample value ($p = 0.049$) and was similar to all other monocultures and mixture treatments ($p \geq 0.126$). Eastern hemlock continued to support relatively high periphyton levels relative to white pine ($p = 0.049$) and sycamore ($p = 0.039$), but was similar to all other treatments including both mixture treatments containing hemlock ($p \geq 0.126$). Neither white pine nor sycamore supported periphyton growth which differed from other treatments ($p \geq 0.153$). Except for black willow, periphyton biomass either increased from the first to second sample date (aspen, chestnut, red maple, sugar maple, oak and tamarack) or remained the same through time (beech, hemlock, white pine and Norway spruce). Periphyton in the broadleaf and broadleaf–conifer mixture also increased over time while it remained the same in the conifer mixture. Planned comparisons of litter mixtures indicated no differences on either sample date between observed and expected responses ($p \geq 0.243$) except on day 18 when the conifer mixture produced less periphyton than the expected response ($p = 0.028$). In addition, the average periphyton production among conifer monocultures was higher than the average of the broadleaf monocultures (day 11, $p = 0.021$; day 18, $p = 0.010$), suggesting a significant difference between our two taxonomic groups of litter.

Primary consumers

Individual amphibian body mass was affected by treatment, time, and a treatment-by-time interaction (Table 2). There was no treatment effect when tadpoles were weighed on day 7 (ANOVA, $F_{14,45} = 1.576$, $p = 0.124$) but there was an effect on day 17 ($F_{14,45} = 4.131$, $p < 0.001$;

Fig. 2). On this date, tadpoles in sugar maple or black willow litter were significantly larger than tadpoles in tamarack, white pine, aspen, chestnut and sycamore treatments ($p \leq 0.043$), marginally larger than tadpoles in Norway spruce, conifer mixture, broadleaf–conifer mixture treatments ($p \leq 0.082$), but were similar in size to all other treatments ($p \geq 0.157$). Planned comparisons revealed no difference between observed and expected responses of mixture treatments ($p \geq 0.306$). There was also no difference between the average of the broadleaf and conifer monoculture treatments for either sample date ($p \geq 0.089$).

Litter also had an effect on amphibian mass at metamorphosis (ANOVA, $F_{14,45} = 6.538$, $p < 0.001$; Fig. 2). Metamorphs in black willow monocultures were larger than individuals from all monocultures ($p \leq 0.022$) except for sugar maple ($p = 0.471$) and eastern hemlock treatments ($p = 0.547$). Individuals from black willow mesocosms were also larger than metamorphs emerging from both broadleaf and broadleaf–conifer mixtures; $p < 0.001$). Individuals from sugar maple monocultures were also larger than those from other monocultures ($p \leq 0.031$) as well as broadleaf mixtures and broadleaf–conifer mixtures ($p \leq 0.013$), but were similar in size to individuals in beech monocultures ($p = 0.168$). Individuals from conifer mixtures did not differ in body mass from individuals in any conifer monoculture ($p \geq 0.531$). Planned comparisons revealed that metamorphs in the broadleaf–conifer mixture were smaller than expected ($p = 0.042$), indicating an antagonistic effect of litter mixing. There were no differences between expected and observed conifer or broadleaf mixtures (conifer comparison, $p = 0.722$; broadleaf comparison, $p = 0.193$), suggesting additive effects in these two mixtures. There was also no difference between the average of all conifer and broadleaf monoculture treatments ($p = 0.127$).

The MANOVA examined those response variables that were only measured once including final zooplankton density, amphibian survival, and time to metamorphosis. There was a significant, multivariate effect of litter treatments (Wilks' $F_{14,125} = 1.577$; $p = 0.028$). At the univariate level, there was no effect of the treatments on amphibian survival or time to metamorphosis (Table 2). In contrast, zooplankton density strongly responded to litter treatments (Table 2, Fig. 3), primarily due to the effect of black willow monocultures, which produced larger zooplankton populations than all other treatments ($p \leq 0.016$) except hemlock and sugar maple ($p \geq 0.093$). Zooplankton populations in black willow litter were also larger than populations from all three mixture treatments ($p \leq 0.009$). Planned comparisons indicated no differences between observed and expected mixture treatments ($p \geq 0.208$), suggesting additive effects. Additionally, there was no difference between the average of broadleaf and conifer monocultures ($p = 0.593$).

pH

Analysis of pH indicated that there was a treatment effect and a time effect, but no treatment-by-time interaction (Table 2). Post-hoc Tukey's comparisons on average pH responses across both sample dates revealed that Norway spruce litter was associated with a significantly higher pH than black willow, beech, and red oak litter ($p \leq 0.023$), and a marginally significant higher pH than conifer and broadleaf–conifer mixtures treatments ($p \leq 0.077$, Fig. 4). All treatments were associated with a slightly higher pH from the first to second sample, except for red maple, tamarack,

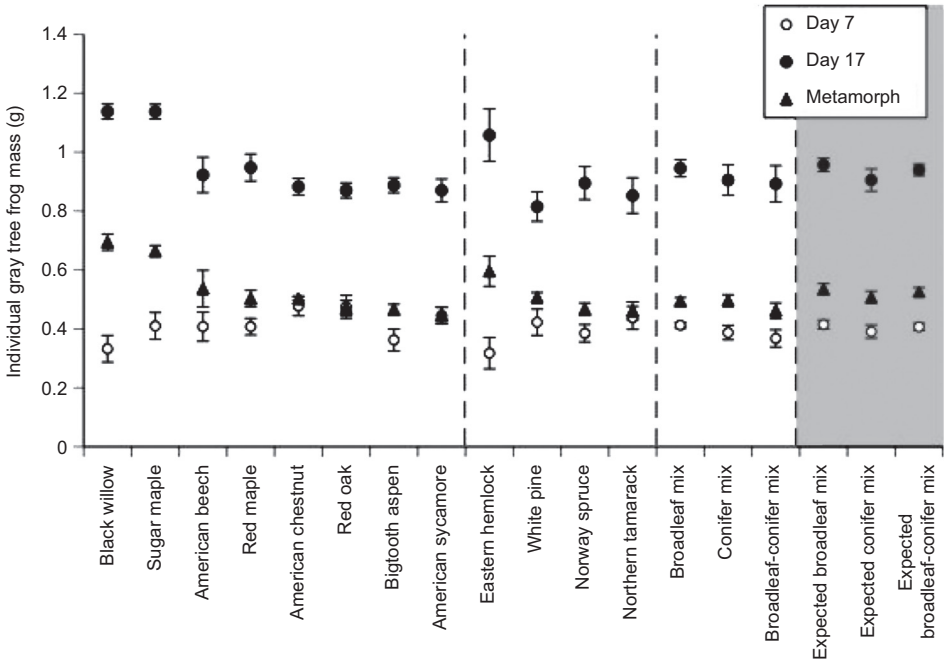

Figure 2. The average body mass of 5 haphazardly selected gray tree frog tadpoles on day 7 and day 17 and the average body mass of all surviving metamorphs when reared in mesocosms containing 12 leaf litter monocultures and three leaf litter mixtures. In both graphs, the gray area contains expected means of the mixture treatments, based on additive values derived from respective monoculture responses. Data are means ± 1 SE.

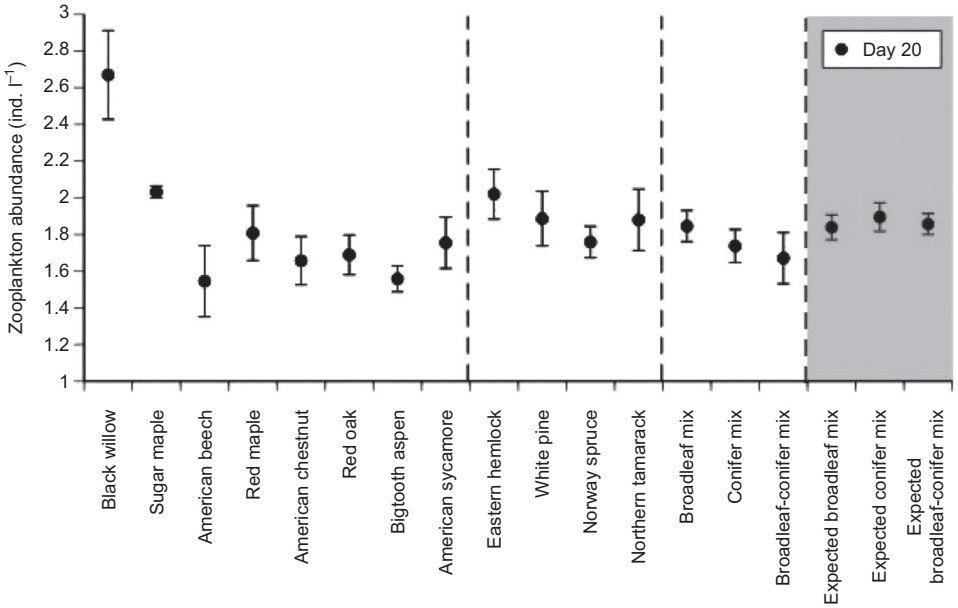

Figure 3. Density of zooplankton (cladocerans and copepods) in mesocosms containing 12 leaf litter monocultures and three leaf litter mixtures (measured on day 20). In both graphs, the gray area contains expected means of the mixture treatments, based on additive values derived from respective monoculture responses. The y-axis is on a log scale and the data are means ± 1 SE.

oak and aspen treatments which appeared to retain the same pH across sample dates. Mixture treatments also supported a higher pH from the first to second sample. Planned comparisons for both sampling days revealed no differences between expected and observed mixture responses ($p \geq 0.110$), suggesting additive mixture effects. Comparisons also revealed that conifer monoculture treatments were associated with a greater average pH than broadleaf treatments during the first sample date ($p = 0.039$), but not on the second date ($p = 0.096$).

Discussion

The results of this study indicate that different species of tree litter can have important impacts on wetland communities. Leaf litter species affected the density of phytoplankton, periphyton and zooplankton as well as the growth of amphibians through their larval stage until metamorphosis. Moreover, mixtures of litter species caused both additive and non-additive changes in the community, demonstrating that effects may be specific to both monocultures of

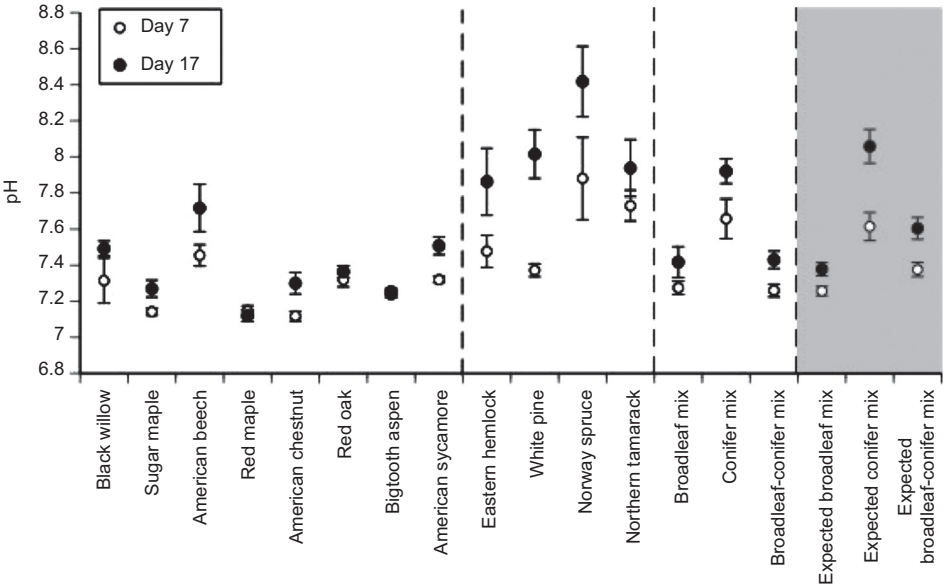

Figure 4. The pH of water in mesocosms containing 12 leaf litter monocultures and three leaf litter mixtures. Measurements were taken on two dates (day 11 and day 17). In both graphs, the gray area contains expected means of the mixture treatments, based on additive values derived from respective monoculture responses. Data are untransformed means ± 1 SE.

litter as well as particular compositions of litter. Finally, we also observed subtle, yet significant differences between broadleaf and coniferous litter types, suggesting that the effect of leaf litter on wetland communities may not only be species-specific.

Effects of different litter monocultures

We observed significant differences in pH among litter treatments, although the range of values (7.04–8.49) is not likely to have strong impacts on producers and consumers in our communities (Havas and Rosseland 1995). Natural ecosystems with years of litter accumulation and decay and different environmental conditions relative to our artificial mesocosms (e.g. light intensity) may experience a larger range of pH values. Furthermore, work on temporary ponds and tree hole communities suggests that litter-driven changes in pH may physiologically constrain the performance of inhabitants (Fish and Carpenter 1982, Bonner et al. 1997). In our experiment, we observed elevated pH in beech, hemlock, pine, spruce, and tamarack treatments. While chemical and physical variation among litter types are the likely mechanism for this result, we did not measure litter chemistry and mechanisms must be inferred from past research. Previous work details the various differences in chemistry for most of the species used in our study (Ostrofsky 1993, 1997, Berg and McClaugherty 2008) and describes potential ways which leaf chemistry may alter the environment (Webster and Benfield 1986). Organic acid leachate from freshly senesced litter such as sugar maple, red maple, chestnut can significantly lower pH (Webster and Benfield 1986, Facelli and Pickett 1991, Ostrofsky 1993, C. Rosenberg pers. comm.). However, acid content of litter was likely reduced after undergoing approximately eight months of decomposition between initial senescence and our collection period, and may not have played a significant role in pH determination. Alternatively, stimulation of algal periphyton growth can significantly increase pH of wetlands during summer months by photosynthetic removal of carbonic acid (Wetzel 2001). This latter mechanism likely generated the subtle shifts in pH which we observed, as pH tended to be higher in treatments where periphyton growth was higher.

Among the 12 monocultures, periphyton biomass increased in both black willow and eastern hemlock treatments while all other treatments supported similar and relatively low periphyton biomass. Among the broadleaf litter found in eastern North American forests, black willow has a particularly high nitrogen content (Ostrofsky 1997) which may fertilize nitrogen-limited algae, such as *Cladophora* (Kupferberg 1997). Likewise, sugar maple has a higher phosphorus content than any other species used in this experiment. While our measurements did not indicate elevated periphyton biomass in this treatment, the increase in consumer mass (i.e. amphibians) suggests that sugar maple actually supported a large quantity of highly palatable periphyton resources. Surprisingly, eastern hemlock treatments induced high periphyton biomass, although hemlock is not known as a particularly nutrient-rich litter species. Furthermore, while hemlock forests are known to promote increased macroinvertebrate diversity in streams, they also tend to have lower total biomass than mixed broadleaf forests although is only known for stream systems and may be different for lentic habitats (Snyder et al. 2002).

Effects of litter species on periphyton biomass were also dynamic, either increasing or decreasing over time depending on the treatment. There are multiple explanations for temporal shifts in algal abundance. First, amphibian consumers grew substantially between the first and second sample date, thereby increasing foraging pressure. Treatments that support the growth of larger tadpoles are likely to see greater reductions in periphyton over time, as was the case with black willow in our experiment. Additionally, differences in consumer survival or time to metamorphosis may have significant, top–down consequences for periphyton growth, yet neither of these responses differed significant among our treatments and likely had little effect in our experiment. Alternatively, increasing algal growth over time may result from increasing nutrient availability as structural compounds (e.g. lignin) binding to nutrients in the litter degrade. Litter with elevated lignin content slows nutrient release and delays growth of primary and secondary production (Webster and Benfield 1986), which agrees with our observations among treatments with high-lignin including chestnut, oak, aspen, sycamore and tamarack treatments. Litter with higher levels of lignin, such as beech, hemlock, pine and spruce litter, may have suppressed periphyton growth for longer than the duration of our study, potentially explaining why periphyton biomass in these treatments was constant and low for both sample dates. Such slow release of nutrients can reduce performance of mosquitoes in tree hole systems (Yanoviak 1999) and may have strong impacts on zooplankton and amphibians in aquatic habitats.

Despite significant shifts in periphyton biomass, we saw almost no difference in phytoplankton among treatments on either sample date, nor was there any clear evidence of association between phytoplankton and periphyton responses. This may result from differential nutrient limitation between periphyton and phytoplankton. Under low nutrient loads, periphyton may dominate (Sand-Jensen and Borum 1991, Carpenter et al. 1996), while phytoplankton may outcompete periphyton at high nutrient loads by shading the benthos and preventing periphyton growth (Gliwicz 1990, Sand-Jensen and Borum 1991). The shallow depth of our mesocosms (~15 cm) may have prevented such interactions from having an effect, and may have given a constant competitive advantage to the periphyton community. In natural systems, deeper water and greater humic accumulation may provide for increased nutrients and greater surface area for periphyton growth, which may alter this outcome.

The lack of treatment differences in phytoplankton abundance could also have been caused by the differences in densities of the zooplankton. Zooplankton can exert strong pressure on phytoplankton blooms, and large zooplankton populations can indirectly indicate high phytoplankton productivity without changes in standing crops (Gliwicz 1990). In our study, zooplankton populations were significantly larger in both black willow and sugar maple treatments, suggesting the presence of high phytoplankton productivity in these treatments. Higher phytoplankton productivity may also have been facilitated by tadpoles grazing periphyton. Such grazing would alleviate competitive pressure from periphyton on the phytoplankton (Sand-Jensen and Borum

1991) and translocate nutrients to the pelagic zone (Iwai et al. 2009). Such trophic interactions may partially explain fluctuations in zooplankton growth, despite a lack of phytoplankton response.

Likewise, we observed increased tadpole growth with and without noticeable changes in periphyton. Black willow and eastern hemlock litter supported large tadpoles and metamorphs, and was associated with increased periphyton biomass early in the experiment, although this latter effect diminished by the second sample date, likely due to increased consumer grazing pressure. This further suggests that both litter species may be associated with high periphyton productivity and high algal nutritional quality for consumers. Larger amphibians were also supported by sugar maple litter, although we observed no similar increase in periphyton standing crop. Nevertheless, recent work demonstrates that tadpoles can graze litter fragments as well as periphyton on the litter surface (Iwai et al. 2009), suggesting that the high nutrient content in sugar maple and black willow (Ostrofsky 1997) may directly support tadpole growth

This should not suggest that litter nutrient content is always immediately associated with consumer growth. In fact, Rubbo and Kiesecker (2004) found the opposite effect, demonstrating that survival and mass at metamorphosis was higher for amphibians in mesocosms with red oak relative to mesocosms with maple litter, which has substantially higher nitrogen and phosphorus content. Their results are particularly intriguing, as increased amphibian survival generates increased competition, suggesting that oak litter was associated with extremely abundant or very high quality periphyton resources. Since Rubbo and Kiesecker (2004) used different anuran species, and larger and more complex communities, it is not surprising that our results differed from those of Rubbo and Kiesecker (2004). Furthermore, they used freshly senescent leaf litter instead of decayed litter collected during the spring, which likely introduced the effects of increased acids in their systems. However, both studies demonstrate that predictions from stream literature may not apply to lentic ecosystems. Differences between the two experiments also demonstrate that responses to litter inputs may depend on a variety of factors including physical wetland characteristics and biological species identity. In fact, Schiesari et al. (2009) has recently demonstrated that some anuran species may be particularly well-adapted to the relatively low food quality of litter-supported environments.

Higher-level taxonomic differences

While our study demonstrates that many community factors are controlled by species-specific litter inputs, some factors may also be a result of higher-level taxonomic litter classification, representative of dominant forest types. On average, conifer treatments supported higher periphyton biomass and were associated with higher pH than broadleaf treatments. These effects likely arise from the physical differences between conifer and broadleaf litter. Coniferous litter has relatively impermeable, cutaneous layers that slow leaching rates relative to most broadleaf litter species (Webster and Benfield 1986, Berg and McClaugherty 2008), thereby providing fewer nutrients to the environment. Under such conditions, algae may dominate the periphyton community (Rier and Stevenson 2002) and consequently raise pH levels (Wetzel 2001). Indeed, periphyton appeared much greener in coniferous treatments relative to broadleaf treatments, indicating increased periphyton algal content. Levels of pH were also higher in coniferous treatments. Interestingly, these differences in algal periphyton and pH between broadleaf and coniferous treatments did not induce parallel effects among primary consumers at this level, although effects may exist in more natural systems.

Despite significant differences of pH and periphyton between classes of tree litter in our experiment, responses among litter species within both family and class strongly differed, suggesting that litter has highly species-specific effects. For example, among members of the Salicacaeae family, willow litter supported greater periphyton biomass than aspen litter early in the experiment, consequently producing larger tadpoles, metamorphs and zooplankton populations. Among members of the Aceraceae family, sugar maple produced larger metamorphs than red maple litter. Among the members of the Pinaceae family, white pine litter resulted in lower pH relative to Norway spruce early in the experiment, and lower periphyton growth later in the experiment relative to hemlock. These differences are not entirely surprising, as members within families often have widely differing chemistry (Ostrofsky 1993, 1997). For example, black willow has roughly 250% greater nitrogen than bigtooth aspen, yet both come from the same family (Ostrofsky 1997). Similarly, sugar maple has about 270% greater phosphorus than red maple (Ostrofsky 1997). These comparisons strongly suggest that the effect of litter on aquatic communities is often species-specific.

Effects of litter mixtures

We found few differences between observed and expected responses to mixtures, suggesting that mixtures had mostly additive effects on the community. However, we did observe two antagonistic effects of mixing litter, demonstrating that altering litter species richness may have some ecologically important, non-additive impacts on aquatic ecosystem functions. Specifically, periphyton biomass was reduced in the conifer mixture treatment compared to the expected mean of the four conifer monocultures. Similarly, metamorph mass in the broadleaf–conifer mixture was lower than the expected mean of the 12 monocultures. Antagonistic effects may be observed on periphyton productivity when palatable species are mixed with less palatable species due to preferential periphyton growth on more labile species (Swan and Palmer 2005). Periphyton biomass was also reduced when all coniferous litter species were mixed, suggesting that combining relatively recalcitrant litter species decreases the overall productivity resulting from the mixture. A reduction in periphyton may further explain why metamorph mass was lower than expected in the broadleaf–conifer mixture. Given that size at metamorphosis is a critical determinant of adult fitness (Semlitsch et al. 1998), this effect has substantial, negative implications for amphibian populations colonizing mixed-litter habitats.

Rubbo and Kiesecker (2004) found similar effects in ephemeral pond systems, demonstrating that combining oak with maple litter decreased primary production, zooplankton density and larval amphibian performance relative to

oak litter alone. Both our study and that of Rubbo and Kiesecker (2004) suggest that non-additive effects of mixing litter are antagonistic with regard to primary production and consumer development. Stream studies suggest this might result from preferential microbial colonization of more labile litter species or negative effects on microbial production resulting from the relatively high phenolic content of maple litter (Ostrofsky 1993, Ardón and Pringle 2008). Further work should be performed in this area to elucidate exact mechanisms of non-additive effects of litter mixing, particularly since species mixtures are generally the rule across forested landscapes. Furthermore, non-additive mechanisms are particularly worth investigating, considering the recent attention given to the role of leaf litter and leaf litter mixtures in forested stream ecosystems and their relevance to ecosystem function (Kominoski et al. 2010, Lecerf and Richardson 2010).

The existence of such mixture effects as well as single species effects on ecosystem dynamics is of interest to both theoretical and applied ecology. Loss of biodiversity is presumed to have negative consequences for ecosystem functions, such as nutrient mineralization and primary production. Our results, while certainly not a formal test of the relationship between biodiversity and ecosystem function, demonstrate only antagonistic effects of litter mixing relative to single-species effects and consequently suggest that aquatic habitats with one species may occasionally exhibit increased function than diverse species-mixtures. As this effect was only observed in a small number of comparisons, it strongly suggests the importance of individual species on ecosystem function rather than total biodiversity. Hence, while our study says little about the effects of increased biodiversity, it does indicate potentially dramatic shifts in ecosystem function which may be concurrent with rapid shifts in forest composition caused by anthropogenic involvement. For example, in eastern United States, oak species are intensely browsed by over-populated white-tailed deer, while the invasive wooly adelgid *Aldeges tsugae* is advancing upon and decimating dense stands of eastern hemlock. Maples are also displacing oak species, resulting in a dramatic shift in the chemical and physical structure of litter. As demonstrated by this study, such changes in forest composition may alter the functioning of pond and wetland systems. Further experiments designed to specifically to address biodiversity effects on ecosystem function are needed to understand the full role of tree biodiversity on forest communities and processes.

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