

Effects of plant litter diversity, species, origin and traits on larval toad performance

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Decreased plant diversity is expected to reduce ecosystem function. Although many studies have examined effects of plant species on trophic interactions, information regarding effects of native or non-native plant diversity on performance of individuals of higher trophic levels is limited. We reared larval American toad *Anaxyrus americanus* tadpoles in outdoor mesocosms containing litter of 1, 3, 6 or 12 plant species drawn randomly from a pool of 24 (15 native, 9 nonnative) species. Tadpole performance varied significantly among litter types in single litter treatments and pH and litter C:N were significant predictors of tadpole performance. Metamorphs were larger in mixtures than expected based on performance in single species treatments, suggesting a non-additive effect of diversity. Litter diversity did not affect probability of survival or probability of metamorphosis. Plant origin (native or non-native) had no significant effect on amphibian performance. Our study suggests some benefits to tadpole development at low levels of plant diversity, but questions assumed benefits of increased plant diversity and assumed detrimental effects of nonnative plant species for a common larval amphibian. Presence of specific plant species with strong negative effects on tadpole performance may outweigh diversity benefits in brown food webs.

Extinctions and invasions are reshuffling biotic communities at an unprecedented rate (Barnosky et al. 2011), yet the factors that contribute most significantly to loss of species and ecosystem function remain highly contested, particularly for non-native species (Hof et al. 2011, Davis et al. 2011, Schlaepfer et al. 2011, Vila et al. 2011, Simberloff et al. 2013). This contested terrain is due in part to a lack of scientific investigations into mechanistic explanations (but see DeVore and Maerz 2014), yet the study of effects of species and community changes to ecosystem function is central to ecological theory and to environmental management (Wardle et al. 2011). Current understanding of the relationship between biodiversity and ecosystem function (BEF) is drawn largely from studies of plant communities in temperate grassland ecosystems (Cardinale et al. 2011, Reich et al. 2012). The vast majority of plant material is not consumed green; it enters detrital food webs (Moore et al. 2004), but to date only ~13% of BEF studies have focused on brown food webs (Cardinale et al. 2011).

Ecologists have begun to address this disparity by extending BEF experiments to aquatic ecosystems (Gamfeldt et al. 2008, Kominoski et al. 2009). Much of this work has examined how plant litter diversity affects decomposition processes (Swan and Palmer 2006, Kominoski et al. 2007, Lecerf et al. 2007, Gessner et al. 2010, Lecerf and Kominoski 2010). Meta-analyses comparing top-down and bottom-up effects of diversity report both positive and

negative effects on decomposition rates (Srivastava et al. 2009, Schindler and Gessner 2009). Importantly, changes in plant diversity can have cascading consequences not only for microbial litter processors, but also for higher trophic levels (Thebault and Loreau 2003, Petchey et al. 2004, Duffy et al. 2007, Scherber et al. 2010, Haddad et al. 2011). These consequences include increased invertebrate survival and/or aggregate biomass with increasing litter diversity (Di Giulio and Edwards 2003, Pfisterer et al. 2003, Downing 2005). But studies of the effect of litter diversity on performance (survival, growth, reproduction) of individual consumers are few and studies that exist (Rubbo and Kiesecker 2004, Swan and Palmer 2006, Williams et al. 2008, Reiskind et al. 2009, Stoler and Relyea 2011) either manipulate only a few species or do not vary diversity in ways that would allow to partition diversity effects from compositional effects.

Amphibians are experiencing widespread declines due to interacting effects of disease, climate and land-use change, nonnative species, and habitat exploitation (Hof et al. 2011). Many amphibians depend on freshwater environments for their larval stages where litter and detritus with its associated biofilms and biota are essential components of tadpole diets (Altig et al. 2007, Schiesari et al. 2009). Despite evidence for strong effects of individual litter species on tadpole development in mesocosm experiments (Rubbo and Kiesecker 2004, Maerz et al. 2005, 2010) and in the field (Cohen et al. 2012), only three studies have compared effects of single and

multi-species litter treatments on amphibian larvae. The first two were designed to assess forest succession on aquatic systems. When Rubbo and Kiesecker (2004) assembled single and mixed treatments of red maple *Acer rubrum* and red oak *Quercus rubra*, they found wood frogs *Rana sylvatica* showed decreased larval survival in red maple-only treatments. Williams et al. (2008) report that metamorphs of three anuran species were larger when developing in a mixture of grass species compared to metamorphs developing in a mixture of deciduous litter. The third study was designed to compare effects of individual litter species and mixtures of litter from different forest types (Stoler and Relyea 2011). Grey tree frog *Hyla versicolor* metamorphs developing in 15 litter treatments of either eight broadleaf monocultures, four coniferous monocultures, a mixture of the eight broadleaf species, a mixture of the four coniferous tree species and a mixture of all 12 species, showed lower metamorph mass in the broadleaf–conifer mixture than the mean of the 12 monocultures but no other significant effects of mixtures (Stoler and Relyea 2011). Collectively, these studies demonstrate that litter composition significantly affects amphibian performance. But due to their designs, these studies were unable to distinguish composition effects from diversity effects.

In contrast to the aforementioned studies, recent BEF studies allow for partitioning of diversity effects from composition effects (Huston 1997, Loreau and Hector 2001). This is accomplished by constructing multi-species treatments from a large pool of candidate species in a substitutive design. This is the approach we chose to assess effects of plant litter diversity on the performance of American toad *Anaxyrus americanus* larvae using a pool of 15 native and 9 nonnative plant species. We incorporated phylogenetic pairs of native and non-indigenous species into our experiment to assess potential effects based on evolutionary origin (Hof et al. 2011, Simberloff et al. 2013). Plant invasions are altering the composition of wetlands in our study region and across the world (Galatowitsch et al. 1999) and their potential to differ in key leaf traits (Leishman et al. 2007), led some to hypothesize that nonnative species are a lower quality resource for larval amphibians (Maerz et al. 2005, Brown et al. 2006, Watling et al. 2011a). However, plant origin (nativity) does not necessarily predict plant functional traits (Thompson et al. 2006, Meier and Bowman 2008, van Kleunen et al. 2010, Cohen et al. 2012, Violle et al. 2012, Martin and Blossey 2013).

Differences in litter quality (e.g. elemental composition such as C:N, C:P, lignin) can affect quality and quantity of resources available to amphibian larvae and therefore larval performance (Rubbo and Kiesecker 2004, Maerz et al. 2005, 2010, Schiesari 2006, Williams et al. 2008). For example, evidence suggests that certain plant traits predict amphibian larval performance, including various secondary compounds (Martin and Blossey 2013) and litter C:N (Cohen et al. 2012).

We therefore used a traits framework to test for mechanisms explaining tadpole performance in single plant species treatments. We were guided by the following hypotheses: 1) tadpole larval performance will be a function of litter C:N; as C:N increases larval performance will decrease; 2) probability of survival and size at metamorphosis will be a function of diversity, with larval survival and size at metamorphosis

peaking in the most diverse litter treatments; these effects will be non-additive; and 3) in treatments examining phylogenetic pairs, survival and size at metamorphosis will be lower for tadpoles developing in litter of nonnative species compared to treatments with native litter.

Methods

In late October 2009 we collected senesced leaves of 15 native and 9 nonnative plant species common to New York wetlands (Table 1) in the vicinity of Ithaca, NY, USA. For each species we collected from multiple individuals and pooled the litter, storing it in brown paper bags in a greenhouse until spring 2010. We submitted three sub-samples from all plant species for analyses of C and N (Cornell Nutrient Analysis Lab in Ithaca, NY, USA) and lignin and P (Dairy One, Ithaca, NY).

On 28 April 2010 we collected three *Anaxyrus americanus* egg clutches from within the vicinity of the Cornell Resource Ecology and Management Facility ponds (42°44'63.50"N, 76°48'26.89"W). We maintained each clutch individually in 15 l, mesh-covered plastic containers floated in an artificial outdoor pond to buffer containers against rapid temperature fluctuations. We exchanged water in containers every 2–4 days using aged tap water (3–4 days old) and provided hatching tadpoles with dry fish flakes ad libitum until they reached the free-swimming stage Gosner stage 24 (Gosner 1960). Between the free swimming Gosner stage 24 to the end of the experiment, tadpoles consumed algae and biofilms that accumulated in each container. On 5 May 2010 we stocked 1 l plastic containers each with a total of 1 g of dry litter according to treatments and allowed litter to be conditioned for 19 days. The litter amount of 1 g is an approximation of wetland litter inputs in our region (J. Dietrich unpubl.) and similar to amounts used by others (Maerz et al. 2005, Brown et al. 2006). We replicated single species treatments 30 times ($n = 720$ containers; 24 species \times 30 replicates). To construct diversity treatments of 3, 6 or 12 species we used a substitutive design: within each diversity level, we constructed 30 replicates by drawing at random from the pool of 24 litter species ($n = 90$ containers; 3 treatments \times 30 replicates, a substitutive design). Each replicate had a unique species composition with 1 g total dry litter per container (equal amounts for each species in diversity treatments). On 24 May 2010 we transferred a single tadpole (Gosner stage 24) into each container (Gosner 1960). In total we placed 10 individuals from each of the 3 clutches into each treatment.

We arranged all containers in a randomized block design by clutch and treatment in Styrofoam grids of 56 containers that floated in 15 outdoor cattle tanks (1135 liter stock tanks, $1.6 \times 1.8 \times 0.6$ m) to buffer containers against rapid temperature fluctuations. We covered containers with fine mesh and enclosed tanks in walk-in field cages (shade 15%, porosity 1629CFM) roofed with a thin layer of clear plastic to protect larvae from predators and treatments from rain effects.

Each week we measured temperature, pH, dissolved oxygen (DO), and conductance in five random containers per treatment using a YSI 556 MPS unit. We recorded five

Table 1. Litter species, origin (native or nonnative), dry matter composition (% C, N, P, lignin) and abiotic conditions in mesocosms (conductance, $\mu\text{S l}^{-1}$ pH; DO, mg l^{-1}) for congeneric pairs (listed first), unpaired species, and diversity treatments. Data for elemental composition are means of 3 samples/treatment and data for abiotic conditions in mesocosms are means of all replicates/treatment ($n = 30$).

Code	Litter species	Origin Origin	Elemental composition (%)				Mesocosm conditions		
			C	N	P	Lignin	Cond. ($\mu\text{S l}^{-1}$)	pH	DO (mg l^{-1})
1	<i>Acer platanoides</i>	intro	43.95	1.09	0.14	21.23	0.76	7.65	3.69
2	<i>A. rubrum</i>	native	47.33	0.66	0.57	13.13	0.62	7.50	4.18
3	<i>A. saccharum</i>	native	44.99	0.67	0.17	15.73	0.62	7.59	4.19
4	<i>Alnus glutinosa</i>	intro	46.13	2.54	0.13	15.80	0.69	7.80	4.84
5	<i>A. incana</i>	native	48.56	2.27	0.11	15.33	0.68	7.63	3.66
8	<i>Fallopia cilinode</i>	native	43.30	2.90	0.23	14.90	0.77	7.99	4.90
9	<i>F. x bohemica*</i>	intro	46.76	2.23	–	–	0.65	7.82	5.45
15	<i>Phragmites australis</i>	intro	43.60	1.73	0.10	7.07	0.71	7.78	5.17
16	<i>P. australis americanus</i>	native	42.58	1.45	0.08	5.70	0.70	7.77	5.28
23	<i>Typha angustifolia</i>	intro	48.55	1.24	0.06	17.90	0.63	7.74	6.25
24	<i>T. latifolia</i>	native	47.58	1.61	0.12	13.50	0.65	7.61	4.84
6	<i>Asclepias syriaca</i>	native	47.70	2.82	0.56	11.70	0.75	7.77	5.62
7	<i>Cornus racemosa</i>	native	45.66	0.87	0.19	5.87	0.64	7.52	4.37
10	<i>Impatiens capensis</i>	native	41.82	1.01	0.18	8.50	0.80	7.87	5.33
11	<i>Juncus</i> spp.	native	44.72	0.83	0.13	3.80	0.96	7.85	6.61
12	<i>Liriodendron tulipifera</i>	native	47.77	1.06	0.10	10.50	0.75	7.70	3.59
13	<i>Lonicera</i> spp.	intro	44.44	1.30	0.34	22.27	0.88	7.93	3.99
14	<i>Lythrum salicaria</i>	intro	40.82	1.34	0.21	1.10	0.86	7.45	3.14
17	<i>Pinus strobus</i>	native	50.94	0.73	0.06	21.70	0.60	7.57	6.10
18	<i>Quercus alba</i>	native	47.83	1.02	0.23	23.23	0.57	7.44	4.77
19	<i>Q. rubra</i>	native	48.78	0.95	0.12	21.03	0.66	7.61	4.33
20	<i>Rhamnus cathartica</i>	intro	42.37	1.56	0.14	15.47	0.81	7.84	3.57
21	<i>Rosa multiflora</i>	intro	45.15	2.26	0.19	5.47	0.71	7.62	3.61
22	<i>Tsuga canadensis</i>	native	45.65	0.62	0.10	18.70	0.59	7.85	6.73
25	3 species	–	–	–	–	–	1.24	7.75	4.77
26	6 species	–	–	–	–	–	0.70	7.68	4.07
27	12 species	–	–	–	–	–	0.72	7.73	4.09

*There was insufficient litter collected to test P and lignin.

performance traits: survival and days-to-death for all tadpoles, as well as metamorphosis, days to metamorphosis, and weight at metamorphosis for larvae that metamorphosed. We checked containers for tadpole survival and metamorphs 3–4 times per week. We recorded days-to-death as the time between when the tadpole was placed in the experimental container and the day it was found dead. We measured days-to-metamorphosis as the time between when the tadpole was placed in the experimental container and Gosner stage 44 (emerged and defined forelimbs). We then removed metamorphs from mesocosms and held them individually in containers in the laboratory until their tail had resorbed (1–3 days, Gosner stage 46). We then blotted each metamorph, recorded wet mass and snout-vent length (SVL), and released them back into their natal pond. The experiment was terminated on 12 August 2010.

Analyses

We constructed models of larval performance in JMP 9.0. To test for effects of plant species, clutch, and tank on days-to-death and days-to-metamorphosis, we constructed Cox proportional hazards regression models. This method is semiparametric in that it requires no choice of probability distribution for days-to-death and estimates the effects of covariates by maximum partial likelihood (Cox 1972, Allison 2010). In these analyses a dead or metamorphosing tadpole represented a complete observation, whereas survivors

or larvae that did not metamorphose were right-censored at termination of the experiment. Significant differences between risk ratios were tested with Bonferroni-corrected effect likelihood ratios.

Because metamorph mass and SVL were strongly correlated ($\text{weight} = 0.157 \times \text{SVL} - 0.074$, $R^2_{\text{adj}} = 0.747$, $F_{1,211} = 627.88$, $p < 0.0001$), we represent tadpole size as a body condition index (BCI), where $\text{BCI} = \text{mass} / \text{SVL}$ (Karraker and Welsh 2006). To test whether metamorph BCI differed among treatments, we constructed a mixed model ANOVA with treatment and clutch as fixed effects and tank as a random effect, then evaluated significant differences between treatments with Tukey's HSD.

For the single species treatments we used an information theoretic approach to determine 1) which Cox proportional hazards models best explained days-to-death and days-to-metamorphosis; 2) which logistic regressions best explained probability of survival and metamorphosis; and 3) which linear regression models best explained metamorph BCI. We included clutch, tank, mean temperature, conductance, dissolved oxygen, pH, litter C:N ratio, C:P ratio, lignin concentration, and plant origin (native or nonnative) as independent variables in all models. We tested for collinearity with a Pearson test and found no correlation, consequently we retained all variables (Supplementary material Appendix 1 Table A1, Fig. A1). To avoid conflating effects of plant origin with variation due to phylogenetic distance (Agrawal and Kotanen 2003) we used phylogenetic controls and tested for

effects of genus and origin nested within genus in a subset of five congeneric pairs. We used Akaike's information criterion (AIC) to compare potential models and considered all models within 2 AIC of the top model (Burnham and Anderson 2002). We assessed the relative importance of each variable by subsequently evaluating the change in AIC value between the top model and the model minus that variable (Burnham and Anderson 2002).

In order to test for a diversity effect, we constructed Cox proportional hazards models, logistic models, and linear models as described above, but included diversity level instead of plant species as the treatment. We also used tadpole performance in single species treatments to predict tadpole performance in mixtures to determine whether plant litter diversity additively or non-additively impacted tadpole performance. We calculated diversity effect as deviation from the tadpole BCI expected given the average of tadpole BCIs in the constituent single species treatments. A positive value indicates that tadpole BCI was greater in mixtures than expected based on performance in single species treatments. We then compared diversity treatments with one-way ANOVA and independent contrasts.

Results

Single species treatments

Our experimental design supported high rates of larval survival, at least when the litter species used as basal energy

resource was beneficial. Larval survival in single species treatments ranged from 0% (*Rhamnus cathartica*) to 96% (*Pinus strobus*). Tadpoles also failed to metamorphose in *Acer rubrum*, *Cornus racemosa*, *Rosa multiflora* and *Tsuga canadensis* (Fig. 1). Percent metamorphosis was highest in nonnative *Lonicera* spp. (76.7%), native *Phragmites australis americanus* (73.3%), nonnative *P. australis* (60.0%), and nonnative *Alnus glutinosa* (60.0%) (Fig. 1). Days to metamorphosis differed by plant species ($F_{18,363} = 10.38$, $p < 0.0001$) and clutch ($F_{2,363} = 5.82$, $p = 0.0033$). Mean metamorph BCI ranged from 0.038 (*Lythrum salicaria*) to 0.084 (*Fallopia cilinode*), and differed by plant species ($F_{18,194} = 5.47$, $p < 0.0001$) but not clutch ($F_{2,194} = 1.98$, $p = 0.141$) (Fig. 1). Days-to-death significantly differed by plant species ($F_{23,680} = 10.81$, $p < 0.0001$) and clutch ($F_{2,680} = 4.62$, $p = 0.011$; Supplementary material Appendix 1 Fig. A2). In most treatments, mortality occurred gradually; however, in all *R. cathartica* treatments, mortality occurred within the first two weeks (Supplementary material Appendix 1 Fig. A2).

Survival did not differ between species within congeneric pairs (Fig. 1). Probability of metamorphosis was reduced in native *Acer saccharum* (and no tadpoles survived in native *A. rubrum*) compared to nonnative *A. platanooides*, and in native *Alnus incana* compared to nonnative *A. glutinosa* (Fig. 1). Mean BCI was greater in native *F. cilinode* than in nonnative *F. bohemica* (Fig. 1).

Water pH and litter C:N were included in the greatest number of larval performance models, though other factors were also influential (Table 2, Fig. 2; see Table 1 for ranges). In the top model increased DO levels best explained

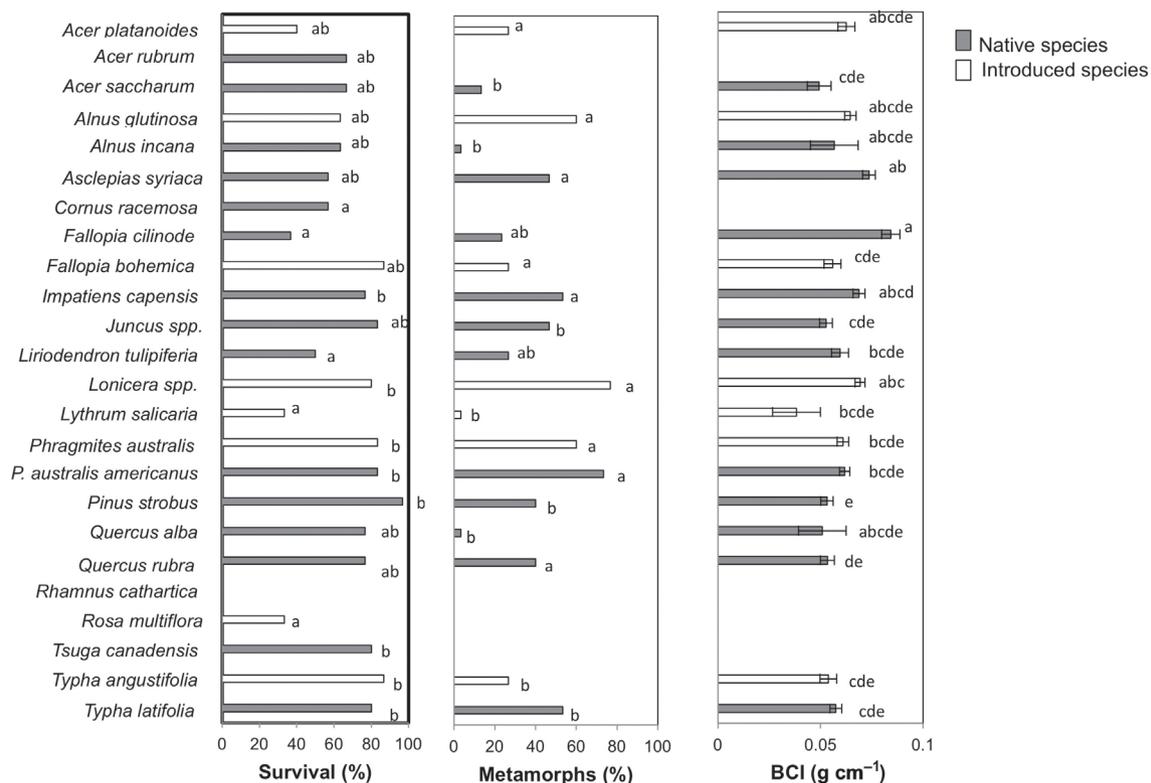


Figure 1. Tadpole survival (%), tadpoles developing into metamorphs (%), and metamorph body condition index (BCI; g cm⁻¹) of *A. americanus* reared in litter of native (gray bars) and introduced (white bars) plant species. Tadpole survival and percent metamorphosing data are based on n = 30 individually reared tadpoles/treatment. Data for mass are means ± 1SE of 1–23 metamorphs/treatment. Treatments identified by the same letter are not significantly different from each other ($p > 0.05$).

Table 2. Constituents of top logistic and linear models for predicting larval toad performance.

Response variable	Model parameters	χ^2 or t	p (> z or t)	% AIC
Probability of survival	DO	67.40	<0.0001	89.6
	lignin	6.25	0.0124	5.8
	pH	3.82	0.0506	2.4
	clutch	5.66	0.059	2.2
Days-to-death	C:N	-1.98	0.048	63.3
	C:P	1.77	0.078	36.7
Probability of metamorphosis	DO	18.58	<0.0001	29.3
	C:N	12.24	0.0005	18.1
	conductivity	11.64	0.0006	17.0
	clutch	11.41	0.0033	12.9
	C:P	9.29	0.0023	12.8
	lignin	5.85	0.0155	6.7
	pH	0.25	0.6143	3.2
Days-to-metamorphosis	C:N	4.45	<0.0001	48.5
	DO	3.33	0.001	30.1
	pH	-2.96	0.0034	21.3
Metamorph BCI	pH	5.44	<0.0001	37.8
	C:N	-5.02	<0.0001	32.2
	lignin	3.73	0.0003	16.8
	origin	-3.35	0.001	13.2

increased survival (Table 2, Fig. 2). Days-to-death increased with increased C:N (Table 2, Fig. 2). As with survival, models of probability of metamorphosis were most influenced by DO levels, with higher probability of metamorphosis at higher DO (Table 2). Litter C:N had the largest effect on the top model for days to metamorphosis (Table 2, Fig. 3). Finally, water pH had the largest effect on the top model for metamorph BCI (Fig. 3), and this was the only performance metric for which plant origin had a significant effect on the top model (Table 2, Fig. 2).

Diversity treatments

Litter diversity had no effect on probability of survival (DF = 3, $\chi^2 = 3.63$, $p = 0.304$), days-to-death (DF = 3,

$\chi^2 = 3.01$, $p = 0.390$), probability of metamorphosis (DF = 3, $\chi^2 = 1.21$, $p = 0.751$), or days to metamorphosis (DF = 3, $\chi^2 = 2.09$, $p = 0.553$). Metamorphs reared in mixtures had a higher BCI than predicted by their performance in single species treatments (Fig. 4; three species: $F_{1,17} = 4.78$, $p = 0.0430$; six species: $F_{1,25} = 4.70$, $p = 0.0401$; 12 species: $F_{1,17} = 10.81$, $p = 0.0046$). However, differences in BCI among the 3, 6 and 12 species treatments were not significant ($F_{2,28} = 0.259$, $p = 0.7739$) and slopes of different diversity treatments were not significantly different (Fig. 4b; interaction term: $t = -0.48$, $p = 0.6805$).

Discussion

Tadpole survival, ability to metamorphose, and BCI varied greatly among the 24 single species treatments. When conditions were favorable, 60% or more of tadpoles metamorphosed (Fig. 1). Mesocosm conditions were important predictors, with DO level – a function of plant material, colonizing biofilms, and larval respiration – positively correlated with tadpole survival. Other studies indicate that DO levels below 2.5–4 ppm can negatively affect tadpole behavior or performance (Wassersug and Seibert 1975, Ultsch et al. 1999). Low DO may have been a cause of mortality in some of our treatments, although survival was relatively high in some mesocosms with low DO. We also found significant negative effects of pH in a range in which other studies suggest no negative effects on producers (Havas and Rosseland 1995, Stoler and Relyea 2011). Our experiment used a large number of plant species, establishing a large range in litter traits with potential interactions that previously had not been explored. Our results highlight the potential importance of interactions among plant litter traits to explain mesocosm quality and amphibian performance.

Variation in *Anaxyrus americanus* larval performance was partially explained by variation in litter traits (particularly C:N and pH), confirming our first hypothesis. Out of 24 species, tadpoles failed to metamorphose in five (native *A. rubrum*, *C. racemosa* and *T. canadensis*; and nonnative *R. multiflora* and *R. cathartica*) but only in *R. cathartica* did all tadpoles die (Fig. 1). Secondary compounds in leaf tissue of *R. cathartica*, particularly emodin, deter herbivores

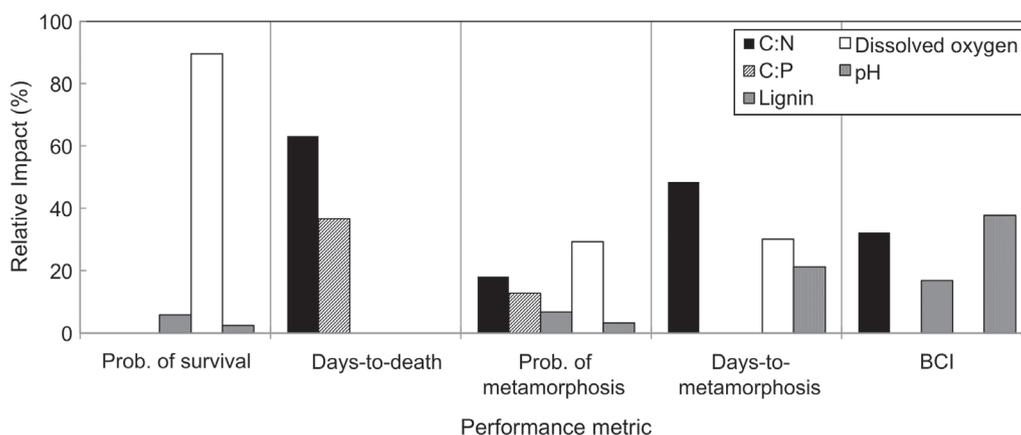


Figure 2. Relative impact (% Δ AIC) of constituent plant litter and mesocosm parameters of top regression models predicting five measures of *A. americanus* larval performance.

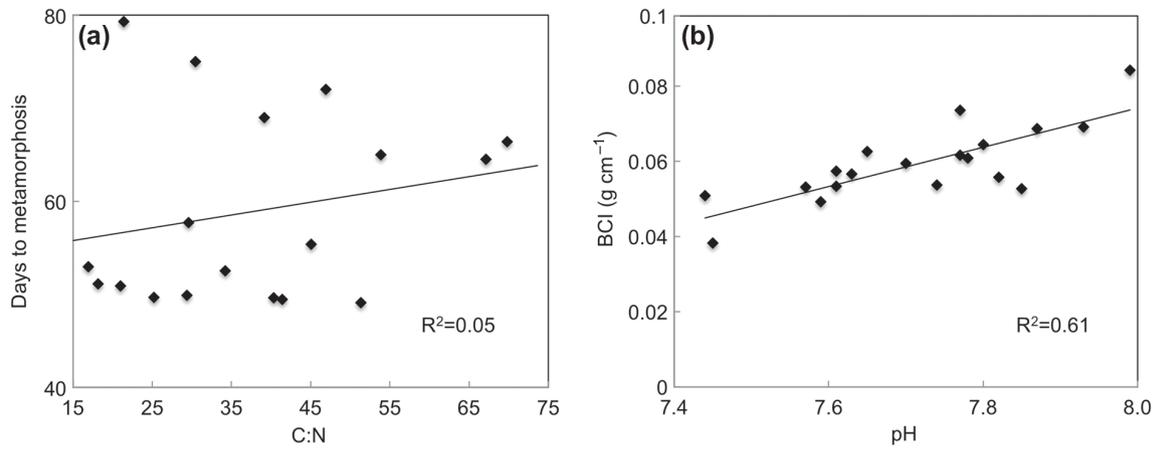


Figure 3. Days to metamorphosis as a function of litter C:N (a), and metamorph BCI as a function water pH (b) in different litter treatments for *A. americanus*.

and have allelopathic effects on nearby plants (Knight et al. 2007). Similarly, toxic secondary compounds could explain lack of metamorphosis in the other four plants; for example

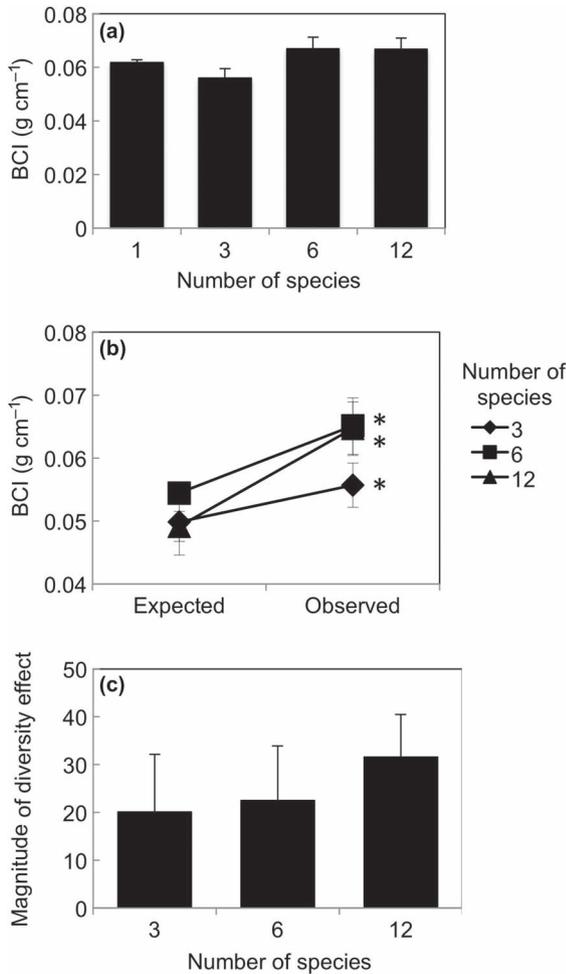


Figure 4. Effects of plant litter diversity on mean *A. americanus* metamorph BCI (a); expected versus observed BCI (b); and overall diversity effects on BCI (c). Data shown are means \pm 1SE. Asterisks identify significant differences from expected values ($p \leq 0.05$) in panel (b). There were no significant differences among treatments in panel (c).

A. americanus did not perform well in tannin-rich *L. salicaria* and *A. rubrum* (0–6% metamorphosed) confirming previous reports of similar effects (Maerz et al. 2005, Brown et al. 2006, Watling et al. 2011b, Martin and Blossey 2013). The importance of secondary litter chemistry in explaining the afterlife effects of litter in aquatic food webs calls for additional mechanistic experiments (Martin and Blossey 2013).

As predicted, metamorphs were larger in diverse treatments than predicted by their performance in single species treatments, and this effect was non-additive, suggesting a true diversity effect. However, litter diversity did not affect tadpole survival or the probability of achieving metamorphosis, thus the overall benefit of increased diversity on *A. americanus* tadpoles is relatively small. This result is consistent with analyses that reveal that most ecological processes saturate at relatively low levels of diversity; the majority of function is achieved by adding 2–4 species to monospecific treatments (Cardinale et al. 2006, 2011, Schmid et al. 2009). The phenomenon of potential saturation is still an open research area. It has been suggested that presence of at least one of each different type of species or functional type, is sufficient to saturate ecosystem properties (Hooper et al. 2005). Where relationships saturate depends on the degree of niche overlap among species, but this would eliminate further beneficial effects of increases in species richness (Hooper et al. 2005). It is possible that in our experiment, on average, three species capture the necessary diversity to promote a nutrient rich biofilm.

Our finding of a positive, non-additive effect of litter diversity differs from results of Stoler and Relyea (2011) and Rubbo and Kiesecker (2004), who report largely additive effects of species mixtures on *H. versicolor* tadpoles and metamorph mass. However, Stoler and Relyea (2011) also report antagonistic effects with metamorph mass lower in a mixture of litter from 12 broadleaf and conifer species than the expected mean of single species treatments. Similarly, Rubbo and Kiesecker (2004) found that combining oak with maple litter in pond systems decreased primary production, zooplankton density, and larval amphibian performance relative to oak litter alone. An examination of their results suggest that certain species, such as black willow *Salix nigra* and sugar maple *Acer saccharum*, may have driven responses

by *H. versicolor* to species mixtures. Unlike our experiment, theirs was not designed to separate diversity effects from species composition effects.

Multiple mechanisms could explain the non-additive effect of plant diversity in our experiment. Litter diversity could allow for colonization of a wider variety of algae, bacteria, and fungi, thereby serving as a diversified diet base that could result in higher quality food, but this appears to be realized at a rather low diversity level and does not show improvements as diversity increases beyond three litter species. Alternatively, litter diversity could dilute effects of “toxic” species (Pennings et al. 1993, Speiser and Rowell-Rahier 1993). Taken together, our results suggest that diversity effects of litter on consumers in brown food webs may be significant but less than reported effects of plant diversity on plant community standing biomass in which the most diverse polycultures produce 1.43 times more biomass than average monocultures (Cardinale et al. 2011). Tadpoles in our highest diversity treatments were 0.42 times larger than predicted by single species treatments.

We tested for effects of origin to address concerns over effects of plant invasions on amphibian performance (Brown et al. 2006, Maerz et al. 2006, 2010, Hof et al. 2011, Watling 2011a, b, DeVore and Maerz 2014). We reject the hypothesis that tadpole performance would be reduced in litter of nonnative species; for closely related species effects of nonnative and native species were indistinguishable. These results highlight the importance of considering multiple native and nonnative species when designing studies regarding potential impacts of nonnative species. Due to wide variation in treatment effects, simply picking a native and nonnative species can introduce selection bias and may result in potentially widely misleading data.

Our results point instead to the importance of litter traits (particularly C:N) in determining tadpole performance. While we did not directly measure biofilm productivity in our treatments, previous studies have demonstrated that low C:N litter can decompose faster and support biofilms of higher nutritional quality than higher C:N litter (Wetzel 1995, Cebrian and Lartigue 2004, Schiesari 2006). Our results are consistent with other laboratory and field studies that have shown that aquatic heterotrophs perform better in low C:N litter (Skelly et al. 2002, Tuchman et al. 2002, 2003, Rubbo and Kiesecker 2004, Schiesari 2006, Williams et al. 2008, Cohen et al. 2012). Such litter traits can vary as much among populations of a single species as they can among species – intraspecific variation accounts for as much as 40% of overall trait variation (Kattge et al. 2011). For example, intraspecific variation in litter traits of native and nonnative *Phragmites australis*, not litter origin, determined larval spotted salamander *Ambystoma maculatum* performance (Martin and Blossey 2013). We recognize that mesocosms can magnify the strength of ecological relationships (Skelly 2002), but results of similar experiments conducted in our group were confirmed in the field (Cohen et al. 2012). A recent study of algal communities in US lakes indicated diversity–biomass relationships within the range that has been characterized by more than 600 BEF mesocosm experiments (Zimmerman and Cardinale 2013) lending support to the notion that mesocosm results can predict field performance.

Further studies of the effects of plant community composition on amphibian performance should incorporate a trait framework to help advance both BEF theory and wetland conservation. Amphibians are sensitive to changes in species composition, habitat, temperature and chemical stressors (Hof et al. 2011). Consequently, changes to wetland plant communities that result in litter inputs with changed elemental composition, increased C:N ratio, or a decrease in plant diversity, whether due to plant invasions or shifts among native plant species, have the potential to reduce amphibian production and performance (DeVore and Maerz 2014). But larval amphibians also vary greatly in larval physiology and feeding behaviors (Altig et al. 2007). It is therefore likely that amphibians will respond in species-specific ways to changes in litter input. This experiment utilized *A. americanus*, a generalist found in high abundance across central New York State (Gibbs et al. 2005) that may be less sensitive to litter quality than other amphibian species. Results using other amphibian species may produce different results, as species-specific interactions are common (Cohen et al. 2012). However, it is also clear that origin (native or not) is not a useful predictor of amphibian performance. The generally assumed overwhelming negative effects of non-native species on individual native species (Hof et al. 2011, Vila et al. 2011, Simberloff et al. 2013) may not hold up to close scrutiny, especially for non-native plants (Davis et al. 2011, Schlaepfer et al. 2011).

Our results suggest plant–amphibian interactions are important when considering amphibian conservation. While introduced diseases, predation, climate change, habitat loss and alteration are primary stressors contributing to amphibian declines (Collins and Storfer 2003, Hof et al. 2011), habitat restoration attempts that overlook plant traits may create impediments for amphibian recovery. For example, in this experiment origin (native or nonnative) was not a useful ‘trait’ predicting larval amphibian habitat quality. But plant species identity and even differences among plant populations (Martin and Blossey 2013) clearly matter. We are only at the beginning stages of a full understanding of mechanisms that underlie plant–amphibian interactions and we consider this as a fertile field for future inquiry.

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Supplementary material (available online as Appendix oik.01745 at <www.oikosjournal.org/readers/appendix>). Appendix 1.