Nonlinear ecosystem responses to leaf litter subsidies in experimental ponds

Shortened title: Ponds respond nonlinearly to leaf litter

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Abstract. Subsidies of matter between ecosystems can strongly affect trophic interactions in food webs, yet most experiments apply a binary (*i.e.*, subsidy *vs*. no subsidy) approach. This emphasis on extremes implicitly assumes linear relationships and fails to capture potential nonlinear effects in ecosystem responses along gradients of subsidy amount seen in real systems. To examine the effects of such gradients, we manipulated leaf litter biomass across seven subsidy levels in 1000-L replicated experimental ponds. As our subsidy recipient, we used a simple food web that included southern leopard frog tadpoles (*Rana sphenocephala*) and spotted salamander larvae (*Ambystoma maculatum*) as the top vertebrates. We measured temperature, dissolved N and P; dissolved organic carbon (DOC); dissolved oxygen (DO); phytoplankton and periphyton biomass; and amphibian biomass export. Dissolved N, phytoplankton and periphyton biomass were linearly related to subsidy amount, but DOC, periphyton ash-free dry mass, and DO were nonlinearly related to subsidies. Frog biomass export increased as a function of subsidy amount, while salamander biomass export increased up to moderate subsidy levels before decreasing to very low levels at the highest subsidies, suggesting not only a nonlinear response, but a non-monotonic one. Our data suggest that under high subsidies, despite high nutrient levels, the basal resource supply may have switched from an algal to a detrital one. These data show that a dose-dependent approach is fundamental for understanding how subsidy amount affects ecosystems.

Key words: algae; amphibian; canopy cover; dissolved nutrients; salamander; subsidies; tadpole; wetland

Introduction

Ecosystems are often connected by the cross-boundary movement of resources and processes. The movement of nutrients, detritus, prey, and consumers can have important direct and indirect effects on population, community, and food web dynamics (Polis et al. 1997, 2004). Even systems originally thought of as isolated, such as lakes, are highly subsidized by exogenous carbon (Cole et al. 2000; Carpenter et al. 2005). In forested ecosystems, streams and ponds are strongly connected to the terrestrial landscape. Energy and nutrients enter through the soil, falling leaf litter, and deposition of terrestrial arthropods (Wipfli 1997; Wallace et al. 1997; Rubbo et al. 2006).

Temporary ponds can make an excellent system for studying the role of cross-ecosystem subsidies on community processes and ecosystem function because of their small size and high connectivity to the surrounding landscape. In some regions, e.g., the glaciated northern U.S. and Canada, they comprise a major portion of all freshwater ecosystems (Colburn 2004; Calhoun and DeMaynadier 2007). Ponds can function as an abundant source for terrestrial ecosystems (Gibbons et al. 2006) or sink of energy from terrestrial environments (Regester et al. 2006), thus linking aquatic and terrestrial ecosystems. Alterations in forests affect canopy cover around and over ponds and change inputs of light and leaf litter, which form the base energy sources for pond food webs (Colburn et al. 2008). Recent work has suggested that detrital resources can fuel algal production, suggesting brown vs. green energy is false economy (Cottingham and Narayan 2013; Fey et al. 2015; Holgerson et al. 2016), but how leaf litter changes ecosystem dynamics and the balance of green and brown energy needs more exploration. The magnitude of leaf litter effects are particularly significant in ponds (as opposed to lakes) because ponds have a high edge-to-area ratio.

Despite numerous attempts to understand how leaf litter inputs influence ponds (Williams et al. 2008; Stoler and Relyea 2011, 2013; Cohen et al. 2012; Earl and Semlitsch 2013; Schriever and Williams 2013; Stephens et al. 2013; Holgerson et al. 2016), an emphasis on extreme input values (*i.e.*, presence or absence of a subsidy) and a focus on consumer responses have failed to capture potential nonlinear effects of leaf litter subsidy inputs. For example, although many experimental studies have found addition (e.g., Richardson (1991)) or exclusion (e.g., Wallace and others (1999)) of terrestrial leaf litter to streams to be important for consumers and overall productivity, almost all of these manipulations have been presence/absence based (Richardson and Wipfli 2016). This early work has established that leaf litter strongly influences ecosystems, but implicitly assumes a linear relationship between high and low subsidy levels, with intermediate subsidies having an intermediate effect, when in reality a nonlinear effect is just as likely, e.g., Nowlin et al. (2007). A dose response could offer important insights, as ecologists have long hypothesized the existence of a nonlinear "subsidy-stress gradient" and the effects of subsidies may be markedly different at population, community, and ecosystem levels (Odum et al. 1979). High subsidy levels can create an environment that may be toxic to some members of the community (e.g., tannins; Earl and Semlitsch (2015)). Non-linear dose response relationships where non-essential substances are beneficial at low levels and toxic at higher levels are well known in toxicology and human health research, but ecologists typically do not approach questions this way (Costantini et al. 2010).

Testing for a dose response in leaf litter subsidies in an artificial setting where pond characteristics such as size, hydroperiod, community, and nutrients can be controlled allows for better quantification in the absence of background differences among ponds. Here, we used a rigorous replicated design applied to experimental ponds to test for ecosystem responses to a range of leaf litter subsidies, from very low to very high natural levels of leaf subsidies measured in ponds (Earl 2012). If the ecosystem response is linear, increases in subsidy would result in concomitant positive linear responses of the population or ecosystem parameter. This sort of response would be plausible in a system dominated by autotroph energy as the base of the food web. However, in many ecosystems, detritus such as leaf litter supports more complex food webs, a higher diversity of species, and longer food chains than would be supported by autotrophs alone due the added heterotroph energy at the base of the food web (Hairston Jr and Hairston Sr 1993). Moore and Hunt (1988) found that most (72.5%) community food webs they examined (*n* = 40) contained both brown and green energy channels to consumers. In these more complex ecosystems, a nonlinear response to leaf litter subsidies could occur if the high levels of subsidy stress some members of the community (Odum et al. 1979) or because of a switch to a detrital-based food web (Moore et al. 2004).

We use our experimental approach to test the form of the relationships between terrestrial leaf litter subsidies and pond dissolved nutrients, algae, and amphibian larvae. We first tested the bivariate relationships between leaf litter subsidy amount and all response variables using *a priori* hypothesized relationships. Then we built a causal model (Grace 2006) to test how leaf litter subsidies influenced the ecosystem by comparing effects mediated through autotrophy or heterotrophy (**Fig. 1**). This work will help quantify how leaf litter subsidies may impact small aquatic systems and clarify the dose response of various ecosystem (*i.e.*, nutrients, phytoplankton biomass, periphyton biomass, dissolved oxygen, and temperature) and top consumer responses (*i.e.*, tadpoles and salamander larvae).

Methods

Experimental design

We created experimental ponds in 1000-L mesocosms (hereafter ponds; 1.8 m^2 surface area) that allowed us to control for features such as age, shape, substrate, photoperiod, temperature, hydroperiod and depth, while retaining microbial, algal, and zooplankton communities similar to natural ponds (Semlitsch and Boone 2009). Previous work has found that processes observed in mesocosms are also important in natural ponds (Resetarits and Fauth 1988; Van Buskirk and McCollum 1999; Van Buskirk 2009). Our approach is not perfect; it is likely that we sacrificed some environmental heterogeneity that may influence the results (Skelly 2002), but our goal was to quantify and compare the effects of leaf litter subsidies on pond ecosystems with the same food web and background conditions. We used a completely randomized design with seven subsidy levels: 0.25, 0.5, 0.75, 1.0, 1.5, 2, and 3 kg of leaf litter per pond. These values were within the range of values seen in an equivalent surface area in natural ponds (Earl 2012), but also represent extremes from very low to very high leaf litter inputs as well. Each treatment was replicated six times for a total of 42 ponds.

We conducted our experiment within a fenced outdoor research facility owned by the University of Missouri, Columbia, MO. Each pond was filled with *c.* 1000 L of tap water on 14 and 15 Mar 2015. After filling, we covered the ponds with high-density polyethylene PAK knit shade cloth (Hummert International, St. Louis, MO, U.S.A.) covers to prevent unwanted colonization of animals and/or escape of experimental animals. This resulted in a 30% reduction in ambient light levels. We allowed the water to dechlorinate for five days before adding leaf litter. We collected leaf litter from a deciduous forest dominated by oak (*Quercus* spp.) and maple (*Acer* spp.) in the Thomas S. Baskett Wildlife Research and Education Area (Ashland, MO, U.S.A.) on 16 March 2015, and added litter to the ponds on 17 March 2015. 200 mL aliquots of bacteria, phytoplankton and zooplankton from local ponds were added on 19 March, 20 March, and 27 March 2015 as inocula to build the base of the pond food web. Pond water was screened through a 1 mm mesh to remove any macroinvertebrate predators and we did not observe any dragonfly nymphs during the experiment. In addition, we added concentrated zooplankton collected with a 60 µm Wisconsin zooplankton net on each of those days. Macroinvertebrates such as chironomids and ostracods either colonized through the lids or with the water, but were present in all ponds during the experiment. On 1 April 2015, we suspended ceramic tiles (22.1 cm^{-2}) midway down the water column along the wall of the northeast side of each pond to monitor periphyton biomass. Pond water depth was maintained at approximately 50 cm through rain water and water amendments during dry periods. To measure temperature, we sunk Thermocron iButtons (model DS1921G, 1.8 cm diameter, precision: 0.5 °C, accuracy: ±1 °C) coated in Plasti Dip (Plasti Dip International, Blaine, MN USA) in the center of every pond on 12 May 2015. We programmed the iButtons to log temperature every four hours until 1 Aug 2016, the day before we ended the experiment.

Study system: top consumers

Southern leopard frogs (*Rana sphenocephala*) and spotted salamanders (*Ambystoma maculatum*) typically co-exist as larvae in ponds in the United States but represent different feeding niches. Southern leopard frogs are mostly primary consumers in ponds — but see Schiesari and others (2009) — whereas spotted salamanders are gape-limited predators of zooplankton, macroinvertebrates, and any other prey small enough for them to consume. We used these two species to build a model food web to examine how leaf litter subsidies would affect some of the most common and largest species occupying ponds in real landscapes.

We collected four *R. spehnocephala* egg masses on 20 March 2015 and *A. maculatum* egg masses on 27 March 2015 from ponds at Fort Leonard Wood, Missouri, USA. We stored the egg masses in a protected area outside and replaced half of the water with fresh pond water every other day until hatching. We allowed tadpoles to develop to the free-swimming stage (Gosner 24–25) (Gosner 1960). We randomly assigned tadpoles to treatments and added eight tadpoles to each pond on 8 April 2015, and an additional 16 on 10 April 2015 for a total density of 24 tadpoles per pond or ~13 tadpoles m^{-2}, well within the natural density for *R. sphenocephala* (F. Rowland, *personal observation*). We allowed salamander hatchlings to develop until they had re-absorbed their egg yolks before adding 12 larval salamanders to each treatment on 20 April 2015. Our salamander densities were within the range of natural densities for spotted salamanders (0.2–80.0 larvae m^{-2} (Figiel Jr and Semlitsch 1990)). We added amphibian larvae at moderate but realistic densities that reflected the population sizes found in ponds near the end of amphibian larval periods.

Sampling and analytical methods

We measured a number of chemical, physical, and biological response variables in the ponds approximately once per month. We sampled all ponds at the beginning (25 May 2015), middle (23 Jun 2015), and near the end of the experiment (20 July 2015) for phytoplankton and periphyton algal biomass (as chlorophyll *a*), periphyton ash-free dry mass (to assess the algal, bacterial, and fungal biomass of periphyton biofilms [AFDM]), water column dissolved organic carbon (DOC), dissolved nitrogen (DN), and dissolved phosphorus (DP).

To determine phytoplankton biomass, we pooled one 200-mL subsample of subsurface water from each cardinal direction of a pond and filtered a known volume through a Pall A/E glass fiber filter (Pall A/E, 1 µm nominal pore size). We stored filters at -20 °C until analyzed for chlorophyll *a* (chl *a*). To determine periphyton biomass, we collected one tile from the mesh and carefully scrubbed a known area onto a Pall A/E glass fiber filter and stored filters at -20 °C until analysis. Chl *a* samples were extracted for 20 minutes in a hot (78° C) 95% ethanol solution, then allowed to cool overnight in the dark before analysis (Sartory and Grobbelaar 1984). We measured pheophytin-corrected chl *a* on a Turner TD-700 fluorometer (Turner Designs, Sunnydale, California USA) calibrated with commercial standards dissolved in ethanol. We used a flow-through method as described in Knowlton (1984).

Periphyton AFDM was estimated by Standard Methods 2540 D and E (American Public Health Association et al. 1915). Briefly, we filtered a known area of each periphyton tile onto pre-ashed and weighed Whatman Grade 934-AH filters (1.5 µm nominal particle retention size). We then dried filters at 105° C for an hour and re-weighed them. We ashed the filters (30 minutes at 550° C) to remove organic matter and used the difference between dry and ashed weight to estimate mass of periphyton.

We measured DN, DP, and DOC in filtered pond water (1–1.5 µm retention). We acidified all samples with sulfuric acid to a pH of approximately 2 and stored at 4º C until analysis. We analyzed DN with the second derivative method (Crumpton et al. 1992) after persulfate digestion. For DP, we used the ascorbic acid method following persulfate digestion (Standard Method 4500-P E) (American Public Health Association et al. 1915). We ran both DN and DP on a Spectronic Genesys 2 spectrophotometer (Thermo Fisher Scientific, Madison, WI USA). We filtered water samples for DOC through membrane filters (0.45 µm pore size) and ran samples on a Shimadzu total organic carbon instrument (TOC 5000; Kyoto, Japan). We performed all nutrient analyses in duplicate and averaged the results.

We estimated dissolved oxygen (DO) using a handheld YSI temperature (to nearest 0.1 ºC) and DO (to nearest 0.01 mg L^{-1}) meter during peak sun irradiation (11:00-14:00). We did this on three occasions: 27/28 May, 27/28 June, and 30/31 July 2015. We only collected oxygen measurements when the previous 24 hours and time between peak and pre-dawn measurements had no precipitation, minimal cloud cover, and low wind conditions with no rippling of water in ponds (< 8 mph).

We monitored the ponds daily for metamorphosed frogs (individuals with at least one forelimb) and salamanders (defined as the complete absorption of gills). We recorded date of metamorphosis, wet mass to the nearest 0.01 g, and body length as snout-vent length (SVL) to the nearest mm. We did not weigh and measure frog metamorphs until complete reabsorption of their tails. We ended the experiment on 4 August 2015 when at least three individuals of each species from each pond had metamorphosed. When we drained the ponds, we thoroughly searched the leaf litter for remaining individuals to estimate survival. Non-metamorphosing individuals were not included in any other analyses. These animals represented approximately 2.4% (± 1.0 SE) and 16.6% (± 3.2) of surviving frogs and salamanders, respectively.

Statistical analyses

We performed all statistical analyses in R version 3.6.1 (R Core Team 2019) on mean values of the three sampling periods. We used the *mle2* function within the 'bbmle' package (Bolker and R Development Core Team 2017) to compare the fits of various linear and nonlinear models for algal parameters, nutrients, dissolved oxygen, and amphibian responses using maximum likelihood. For the iButton temperature data, we calculated daily means and the difference between minimum and maximum temperature within each day. Then we fit mixed models with litter treatment, date, and the interaction of litter x date as fixed effects, treating pond as a random effect using the ‘lme4’ package (Bates et al. 2014). We used the *Anova* function in the ‘car’ package (Fox and Weisberg 2018) to calculate χ^2 statistics.

To estimate the effects of leaf litter on pond ecosystem metrics, we used structural equation modeling (SEM) in the ‘lavaan’ package in R (Rosseel 2012). Structural equation modeling is a method for building causal models among intercorrelated variables to explore the relative strength of different relationships. To guide our causal model, we developed a meta-model (**Fig. 1**) representing the constraints of our SEM: (1) Since DOC, DN, and DP were highly correlated ($r > 0.9$), we used principal components analysis (PCA) to collapse them using the *prcomp* function into a common axis (PC1), which explained 97% of the variance in nutrients. (2) Our two metrics of periphyton biomass (AFDM and chl *a*) were similarly collapsed into one axis (which explained 83% of the variance in the original variables) using PCA.; (3) If the ecosystem is primarily driven by algal/green responses, the strongest effects (*i.e.,* highest standardized coefficients) would be mediated through nutrients and algal biomass (**Fig. 1a**). (4) Alternatively, if detrital processes are more important, the strongest links would be mediated through subsidy affecting DO, as an indicator of heterotrophic respiration (**Fig. 1b**). (5) The relationships between leaf litter and nutrients, phytoplankton, periphyton, and DO did not change over the three sampling dates, so we used means of the three sampling dates to estimate a net effect over the entire experiment. (6) We log-transformed frog and salamander biomass, DO, and phytoplankton chl *a* to linearize the relationships for ease of model interpretation.

We used a likelihood-ratio chi-square statistic to test whether the covariance matrix generated by the SEM model differed significantly from the data. A $P > 0.05$ would indicate that the observed and predicted models were not significantly different, and that the fit for our model was adequate (Shipley 2000). We also assessed model fit using the Comparative Fit Index (CFI), which is relatively insensitive to sample sizes (Fan et al. 1999).

Results

Ecosystem responses to leaf litter

Our subsidy gradient resulted in ecosystems varying from oligotrophic to eutrophic in nutrients, spanning a large range of DOC (21–49 mg L^{-1}) and algal biomass responses indicative of oligotrophic to mesotrophic conditions (**Table 1**). Leaf litter subsidy amount had strong effects on the chemical, physical, and biological properties of the pond ecosystems (**Fig. 2**). DN, phytoplankton chl *a*, and periphyton chl *a* all linearly increased with subsidy input (**Fig. 2b, d, and e**; **Table 2**, **Table 3**), but DOC and DP increased nonlinearly (**Fig. 2a, c**; **Table 2, Table 3**). Periphyton AFDM exhibited a nonlinear fit, increasing up until 2 kg of leaf litter, and then decreasing at 3 kg (**Fig. 2f**). The only ecosystem property to show a decline with increased leaf litter subsidy amount was dissolved oxygen, which decreased nonlinearly and precipitously as a function of leaf litter input (**Fig. 2g**, **Table 2**, **Table 3**). The lowest leaf litter subsidy level (0.25 kg) had ~ 5 times more oxygen than the highest (3 kg) subsidy level.

The structural equation model provided a good fit to the data (model $\chi^2 = 6.48$, CFI = 1.00, df = 8, $n = 41$, $P = 0.594$; **Fig. 3**). Leaf litter subsidy amount strongly influenced nutrient concentrations (unstandardized estimate ± SE = 0.858 ± 0.035, $P < 0.001$), and higher nutrient concentrations increased phytoplankton (0.424 ± 0.091, $P < 0.001$) and periphyton biomass (1.195 ± 0.168, $P < 0.001$, **Fig. 3**). Interestingly, phytoplankton biomass as chl *a* (0.053 ± 0.042, $P = 0.207$) and periphyton biomass as PC1 (0.040 ± 0.022, $P = 0.072$) had very little effect on DO, but the indirect effect of leaf litter on DO — a proxy for unmeasured heterotrophic production — showed the strongest effect in the whole model (-0.629 ± 0.034, $P < 0.001$).

Biomass export was differentially affected by primary production and DO for frogs and salamanders. For salamanders, higher DO had a positive effect on biomass (0.493 ± 0.208, P = 0.018), but both phytoplankton (-0.314 ± 0.162, P = 0.052) and periphyton (-0.160 ± 0.082, P = 0.051) negatively affected biomass export. Frog biomass export was strongly negatively related to DO (-0.611 ± 0.124, $P < 0.001$) and was not predicted by phytoplankton (0.092 ± 0.097, P = 0.340) or periphyton (-0.034 ± 0.049, P = 0.483; **Fig. 3**).

We found that litter subsidy amount altered temperature dynamics (**Fig. 4**). Litter subsidy amount ($\chi^2 = 96.05$, d.f. = 1, $P < 0.001$) and date ($\chi^2 = 4640.33$, d.f. = 1, $P < 0.001$) additively affected mean daily temperature (amount × date interaction: $\chi^2 = 0.13$, d.f. = 1, $P = 0.715$; **Fig. 4a**). The difference between the minimum and maximum daily temperature within each pond exhibited a significant subsidy × date interaction as well ($\chi^2 = 34.81$, d.f. = 1, $P < 0.001$; **Fig. 4b**). For both increased leaf litter subsidy decreased temperature, and the effect was more pronounced as the experiment progressed.

Population-level responses to subsidy amount

Amphibians exhibited mostly nonlinear responses to leaf litter subsidy amount (**Fig. 5**, **Table 2**). All responses were nonlinear, except for frog body condition (**Fig. 5c**), which had the highest support for a linear fit (**Table 2**, **Table 3**). Somewhat surprisingly, frogs and salamanders diverged in their response to subsidy amount; frog biomass export and body condition continued to increase to the highest subsidy level, whereas salamander biomass export and body condition both peaked at a subsidy of 1.5 kg, and decreased at the highest levels (**Fig. 5**). This did not appear to be related to survival, as both species had high average survival rates (frogs 85.8 ± 13% SD; salamanders 83.5 ± 18%) with similar responses to subsidy amount (**Fig. 5e, f**).

Discussion

We observed strong effects of leaf litter subsidy amount on pond ecosystems. Leaf litter subsidies led to decreased temperature, dissolved oxygen (DO), and larval salamander growth and development, but increased nutrient concentrations, algal biomass, and frog body condition and biomass export. We also observed many nonlinear relationships with increased leaf litter. DOC, DP, periphyton AFDM, and most of the amphibian responses were nonlinear, suggesting that high levels of leaf litter subsidy may stress small aquatic ecosystems. Subsidies benefited most aspects of the pond ecosystems up to mid-levels (~ 1.5 kg/1000 L), but became detrimental at the highest levels, perhaps because of greater food web reliance on brown energy sources (Cottingham and Narayan 2013; Fey et al. 2015) or temperature differences. In fact, the strongest standardized path coefficient in the SEM was between subsidy and DO, which is likely an indicator of microbial activity. Our results highlight that presence/absence experiments fail to capture how ecosystem properties and populations respond to subsidy inputs and offer key insights into how aquatic systems might change as subsidies increase or decrease.

Temperature and nutrient dynamics

Leaf litter subsidies induced several physical and chemical changes in our pond ecosystems worth noting. As subsidy amount increased, the mean daily temperature decreased. This was likely due to higher DOC with increased subsidy decreasing light penetration and thereby changing temperature dynamics. Temperature increases can affect ecosystem respiration more quickly than primary production (Yvon-Durocher et al. 2010), and thus the differences observed in our study could have implications for whether small aquatic ecosystems act as a source or a sink of carbon. Furthermore, we observed a temperature buffering capacity of high-subsidy ponds (**Fig. 2b**). Canopy cover over ponds buffers high temperatures during leaf out, but high

DOC released from leaf litter cools the water even further. This could have large implications for ectothermic animals that occupy ponds for one or more of their life stages (which we discuss more below).

Dissolved nutrient concentrations were tightly coupled with leaf litter subsidies in our experiment (**Fig. 4**). Although all dissolved nutrient concentrations increased with subsidy amount (**Fig. 3**), DOC and DP had positive nonlinear relationships with increasing subsidy amount, whereas the response of dissolved N was positive and linear. Nutrient limitation is a fundamental control on primary production, algal biomass, and community composition (Tilman 1976), and we found that nutrients that had leached from the leaf litter subsidy were a strong predictor of algal biomass. Furthermore, the relative balance of N and P could have large implications for the algal ‘food quality’ at the base of the food web and the trophic transfer efficiency of energy (Dickman et al. 2008; Rowland et al. 2015).

Balance of autotrophy vs. heterotrophy

We are just starting to evaluate how detrital processes affect aquatic ecosystem metabolism (Rubbo et al. 2006; Holgerson 2015) and secondary production (Brett et al. 2017). Some components of food webs may have key switching points where reliance on autotrophy *vs*. heterotrophy flips. The nonlinear response of periphyton AFDM in our experiment may underscore this; at low subsidy input algae, fungi, and microbes were all limited by resources, peaked in production at 2 kg, and by 3 kg of subsidy may have switched to a microbial-dominated system. We observed increases in phytoplankton biomass (as chl *a*) with increasing subsidy amount, likely due to increases in dissolved nutrient supplies (**Fig. 3**). However, despite increasing nutrients, we observed larger variation in algal biomass with increasing subsidy, and a highly nonlinear response of periphyton AFDM to subsidies. At low subsidies, primary production appears to be limited by nutrients, but at high subsidies it may be limited by the DOC associated with leaf litter subsidies. Indeed, previous work has indicated that terrestrial DOC can depress primary production by chelating micronutrients (Jackson and Hecky 1980), diminishing enzymatic activity (Hättenschwiler and Vitousek 2000), and attenuating solar radiation (Thrane et al. 2014).

With increased DOC, phytoplankton biomass tends to decrease (Carpenter et al. 1998) and bacterial production to increase (Hessen 1992). Unfortunately, we were unable to quantify heterotrophic production directly, but we observed a large gradient in DOC over the experiment (15.8–64.5 mg L^{-1}), consistent with the range found in wetlands and bogs (Wetzel 2001), and there was a strong indirect effect of leaf litter on DO that was not mediated through nutrients, phytoplankton and periphyton. We suggest that this may be evidence of the importance of the bacterial loop in ponds with high leaf litter subsidies as some studies suggest (Cottingham and Narayan 2013; Fey et al. 2015). The relative contribution of heterotrophic bacteria to overall ecosystem processes increases linearly with DOC (Jones and Lennon 2015). We observed a nonlinear relationship between subsidy amount and DOC, an inverse relationship between DO and DOC, and increase in periphyton AFDM with DOC, suggesting that ponds with higher leaf litter subsidies shift to more heterotrophy. Although we did not alter light directly, increases in DOC caused by leaf litter inputs should strongly alter light availability (Thrane et al. 2014; Jones and Lennon 2015), and ponds surrounded by high canopy cover would have both high leaf litter subsidies and even more pronounced depression of primary production due to light limitation from canopy cover and DOC concentrations. Thus, subsidies of leaf litter can shift relative balance of autotrophy:heterotrophy in ponds, and heterotrophic energy in small aquatic systems may be even more important than in lakes given the high values of DOC measured in small wetlands and marshes. Others have found forested ponds to be highly net heterotrophic (Rubbo et al. 2006; Holgerson 2015).

Consumer responses

Although frog body condition increased linearly with subsidy amount (similar to phytoplankton biomass), all of the other consumer responses were nonlinear. Furthermore, our research suggests that consumer trophic level is an important determinant of the effects of subsidies. For most amphibian species, open canopy conditions consistent with 1 kg or less of leaf litter subsidy generally result in greater species diversity and survival (Skelly et al. 2002; Werner et al. 2007), presumably because higher light promotes higher algal biomass (Skelly et al. 2002). Southern leopard frogs are open-canopy specialists; thus we expected them to do poorly in the high subsidy treatment that is more typical of closed-canopy ponds. However, frog biomass export increased nonlinearly with subsidy, and their body condition was better with higher subsidy as well. We suggest frogs may opportunistically take advantage of whatever resources are available. Some tadpoles can consume detritus (Skelly and Golon 2003; Holgerson et al. 2016), and many species are much more flexible in their diet than previously considered (Schiesari et al. 2009; Whiles et al. 2009).

Despite the fact that previous work on pond-breeding salamanders in the same region has found higher densities in forested ponds (Ousterhout et al. 2015) and better growth in closed-canopy ponds more typical of our high subsidy treatment (Earl et al. 2011), the salamanders in the highest subsidy treatments of the present study grew slowly, had lower survival, and did not metamorphose within our experimental time frame. In theory, predators such as salamanders should be even more constrained by primary producer energy than tadpoles because it has to travel through primary consumers before reaching them (Lindeman 1942). Phosphorus addition, by shifting food webs from detrital to algal energy sources, has experimentally resulted in increased salamander growth (Bumpers et al. 2017), and salamander larvae incorporate less detrital energy than tadpoles (Holgerson et al. 2016). In our experiment the zooplankton community in the highest subsidy switched from *Daphnia*-dominated to copepod-dominated (F. Rowland, *personal observation*) similar to other work (Cottingham and Narayan 2013; Fey et al. 2015). *Daphnia* are richer in N and P than copepods (Andersen and Hessen 1991), so this switch in the prey of salamanders represents a decrease in food quality. Predators may be more constrained under high subsidy conditions, and this deserves further research as to why many salamanders choose to breed in ponds that have lower-quality food supplies for them.

Interestingly, ponds surrounded by forest on a landscape not only receive higher leaf litter subisidies, but will also will dry out faster (Skelly et al. 1999). High leaf litter subsidies reducing growth rates either directly through food limitation or indirectly through decreased temperatures can have strong effects on whether amphibians are successfully able to metamorphose before a pond dries. A species's ability to successfully metamorphose will determine whether a pond functions as a source of energy to terrestrial environments (Gibbons et al. 2006) or as a sink of energy from terrestrial sources (Regester et al. 2006). Understanding the dose-dependent link between leaf litter subsidies and energy flux between aquatic and terrestrial ecosystems is vital to determining this relationship. Our research suggests that high subsidies may benefit omnivorous generalists and increase export, but also inhibit growth of predators. Whether a pond functions as a source or sink of amphibian energy is determined by canopy cover, hydroperiod, and whether the community consists of more anurans or caudates, and this area deserves further attention.

Conclusions

Observational and theoretical modeling approaches need to be linked with experimental approaches to understand how subsidies impact ecosystems (Odum et al. 1979). Our data indicate that small aquatic ecosystems may be switching between autotrophic and detrital/heterotrophic energy sources based on dose of subsidy. The gradient approach we used can provide information to parametrize models and disentangle the complexities of ecosystem response subsidies in ways that would be impossible under the usual presence/absence paradigm. While nutrients increased predictably, we found nonlinear responses of phytoplankton and periphyton, and that generalist consumers are better able to navigate high leaf litter subsidy conditions than predators. We need more research to understand how alterations to landscapes that change the movement of materials affect population, community, and ecosystem processes.

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Table 1. Mean and standard error (SE) values across three sampling points for dissolved nitrogen (DN), dissolved phosphorus (DP), dissolved organic carbon (DOC), phytoplankton biomass (as chl *a*), and periphyton biomass (as chl *a* and ash-free dry mass [AFDM]) as a function of leaf litter subsidy amount.

Litter (kg)	**DN (mg/L)**		**DP (μg/L)**		**DOC (mg/L)**		**Phyto chl *a* (μg/L)**		**Peri chl *a* (μg/cm^2)**		**Peri AFDM (mg/cm^2)**	
0.25	0.38	(0.02)	9.1	(1.08)	21.2	(0.72)	2.6	(0.47)	0.02	(0.002)	0.01	(0.002)
0.50	0.46	(0.01)	15.1	(0.36)	22.3	(0.83)	4.2	(0.59)	0.03	(0.007)	0.01	(0.005)
0.75	0.47	(0.01)	16.9	(0.70)	23.9	(1.13)	3.6	(0.51)	0.03	(0.005)	0.01	(0.001)
1.00	0.51	(0.02)	17.4	(1.14)	24.0	(0.70)	2.6	(0.54)	0.04	(0.007)	0.01	(0.003)
1.50	0.60	(0.02)	23.2	(1.71)	29.3	(1.52)	4.1	(0.70)	0.09	(0.018)	0.02	(0.001)
2.00	0.71	(0.03)	48.9	(4.65)	34.0	(0.61)	8.8	(0.79)	0.22	(0.157)	0.06	(0.009)
3.00	0.88	(0.02)	76.5	(4.15)	48.8	(1.21)	6.3	(1.01)	0.38	(0.139)	0.03	(0.007)

Table 2. Table of candidate models for the effect of leaf litter subsidy amount on pond mesocosms abiotic and biotic variables. Models are ordered based on ΔAIC alongside their respective Akaike weights (w_i). Models with the most support (those < 2 ΔAIC from the model with the lowest AIC) are shown in bold.

Parameter	**Model**	**ΔAIC_i**	**w_i**
DOC	**quadratic**	**0.0**	**1**
	linear	20.2	<0.01
	Michaelis-Menton	75.4	<0.01
DN	**linear**	**0.0**	**0.75**
	quadratic	2.1	0.25
	Michaelis-Menton	54.4	<0.01
DP	**quadratic**	**0.0**	**0.91**
	Michaelis-Menton	4.5	0.09
	linear	13.0	<0.01
Phytoplankton	**linear**	**0.0**	**0.46**
	quadratic	**1.0**	**0.28**
	Michaelis-Menton	**1.2**	**0.26**
Periphyton	**linear**	**0.0**	**0.75**
	quadratic	2.2	0.25
Periphyton AFDM	**third-order polynomial**	**0.0**	**0.99**
	Michaelis-Menton	13.4	<0.01
	quadratic	14.1	<0.01
	linear	14.8	<0.01
Dissolved oxygen	**quadratic**	**0.0**	**0.69**
	linear	**1.6**	**0.31**
Frog export	**quadratic**	**0.0**	**0.94**
	linear	5.5	0.06
Frog body condition	**linear**	**0.0**	**0.77**
	quadratic	2.4	0.23
Frog survival	**quadratic**	**0.0**	**0.98**
	linear	8.5	0.02
Salamander export	**quadratic**	**0.0**	**0.94**
	linear	5.7	0.06
Salamander body condition	**quadratic**	**0.0**	**0.84**
	linear	3.3	0.16
Salamander survival	**quadratic**	**0.0**	**0.80**
	linear	2.8	0.20

Table 3. Top models describing leaf litter subsidy amount (kg) effects on ecosystem properties. Each equation was fit to seven levels of leaf litter times six replicates across 42 mesocosms. Each data point used represented a mean value across three sampling periods. Key: phyto = phytoplankton, peri = periphyton, sal = salamander.

			Fit	
Variable	**Equation**	**n**	**r^2**	***P* value**
DOC (mg L^{-1})	$20.84 + 1.35 \text{ x litter} + 2.66 \text{ x litter}^2$	42	0.94	<0.0001
DN (mg L^{-1})	$0.35 + 0.18 \text{ x litter}$	42	0.93	<0.0001
DP (μg L^{-1})	$8.69 + 4.49 \text{ x litter} + 6.176 \text{ x litter}^2$	42	0.92	<0.0001
Phyto chl *a* (μg L^{-1})	$2.44 + 1.68 \text{ x litter}$	42	0.34	<0.0001
Peri chl *a* (μg cm^{-2})	$-0.06 + 0.15 \text{ x litter}$	42	0.43	<0.0001
Peri AFDM (mg cm^{-2})	$0.03 – 0.09 \text{ x litter} + 0.09 \text{ x litter}^2 – 0.02 \text{ x litter}^3$	42	0.57	<0.0001
Dissolved oxygen	$8.09 - 3.15 \text{ x litter} + 0.45 \text{ x litter}^2$	42	0.94	<0.0001
Frog export (g $pond^{-1}$)	$12.21 + 22.59 \text{ x litter} – 3.85 \text{ x litter}$	42	0.70	<0.0001
Frog condition (g cm^{-1})	$0.38 + 0.18 \text{ x litter}$	42	0.85	<0.0001
Frog prop. survival	$19.57 + 4.21 \text{ x litter} – 1.80 \text{ x litter}^2$	42	0.39	<0.0001
Sal export (g $pond^{-1}$)	$6.90 + 4.17 \text{ x litter} – 1.98 \text{ x litter}^2$	42	0.38	<0.0001
Sal condition (g cm^{-1})	$0.28 + 0.08 \text{ x litter} – 0.03 \text{ x litter}^2$	42	0.16	0.0307
Sal prop. survival	$10.73 + 0.89 \text{ x litter} – 0.76 \text{ x litter}^2$	42	0.49	<0.0001

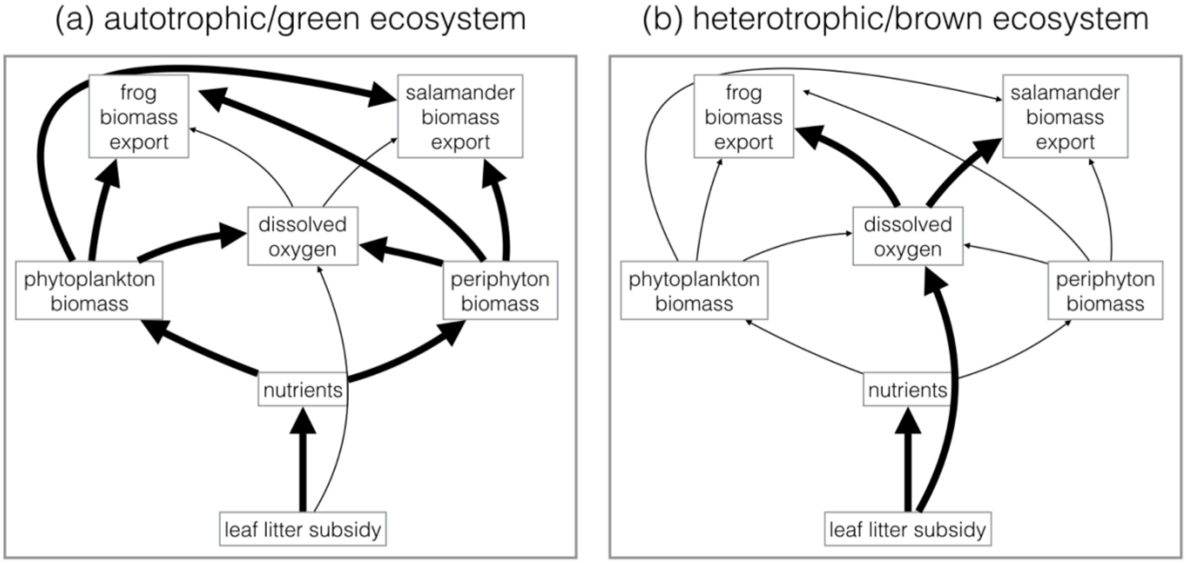

Fig. 1 The meta-model guiding the structural equation modeling. The boxes represent conceptual variables and the arrows indicate the relationships and direction of the relationships. An (a) algal-based ecosystem would have stronger relationships mediated through phytoplankton and periphyton and (b) a detrital-based ecosystem would have responses more strongly mediated through dissolved oxygen.

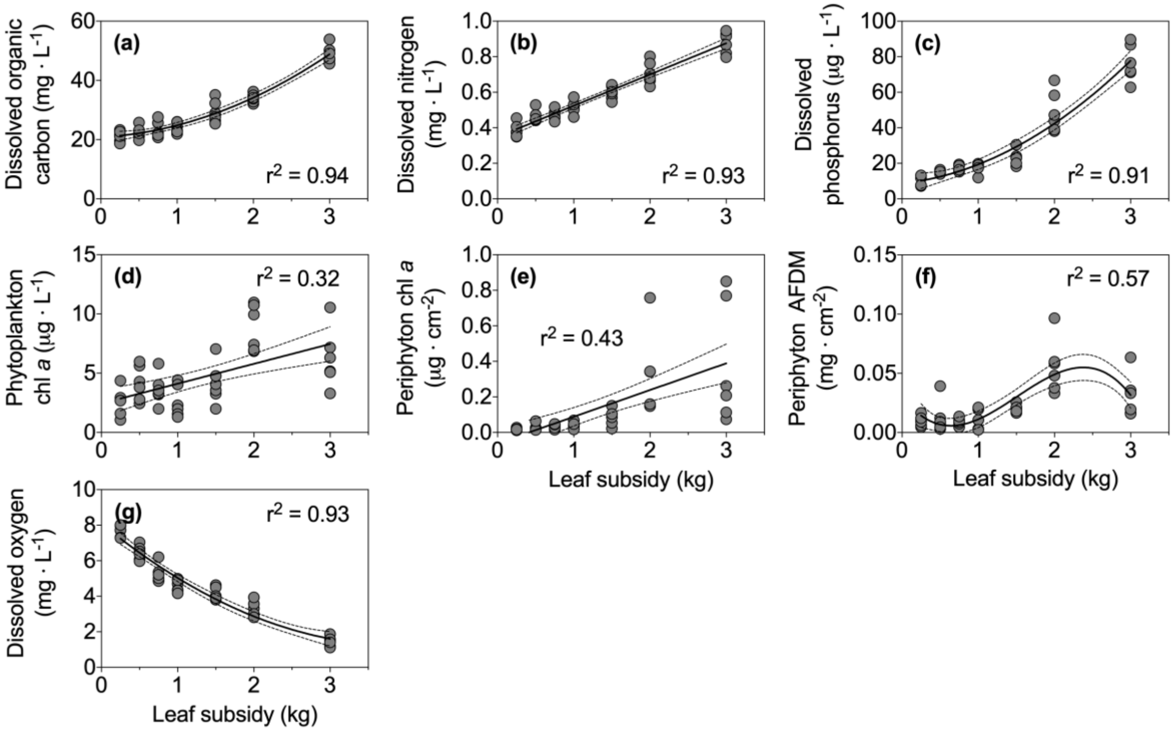

Fig. 2 Ecosystem responses to leaf litter subsidy amount: dissolved organic carbon (a), dissolved nitrogen (b), dissolved phosphorus (c), phytoplankton chl *a* (d), periphyton chl *a* (e), periphyton AFDM (f), and daytime dissolved oxygen (g). Lines represent best fit ± 95% confidence intervals. n = 6 replicate ponds per treatment.

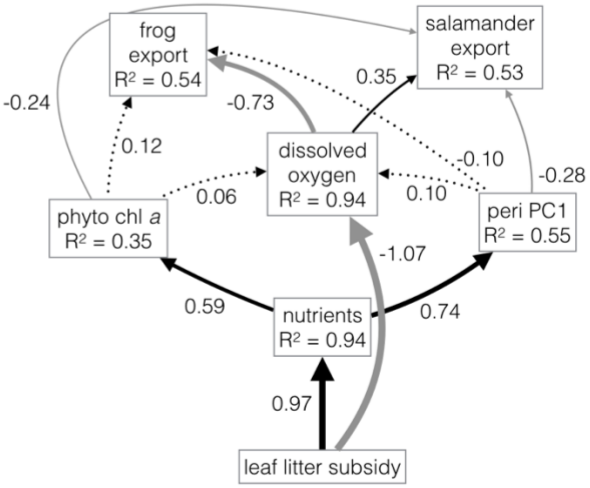

Fig. 3 Ecosystem responses to leaf litter subsidy as assessed by a structural equation model of experiment means. Arrows thickness is proportional to the standardized path coefficient. Black arrows indicate a positive coefficient, gray arrows a negative coefficient, and dotted arrows are weak pathways (*P* > 0.05). Nutrient measurements (DOC, DN, and DP) were modeled using a PCA loading of ‘nutrients’ and periphyton AFDM and chl *a* were similarly condensed into PC1. Frog and salamander export represent the wet sum of all metamorphs from a pond.

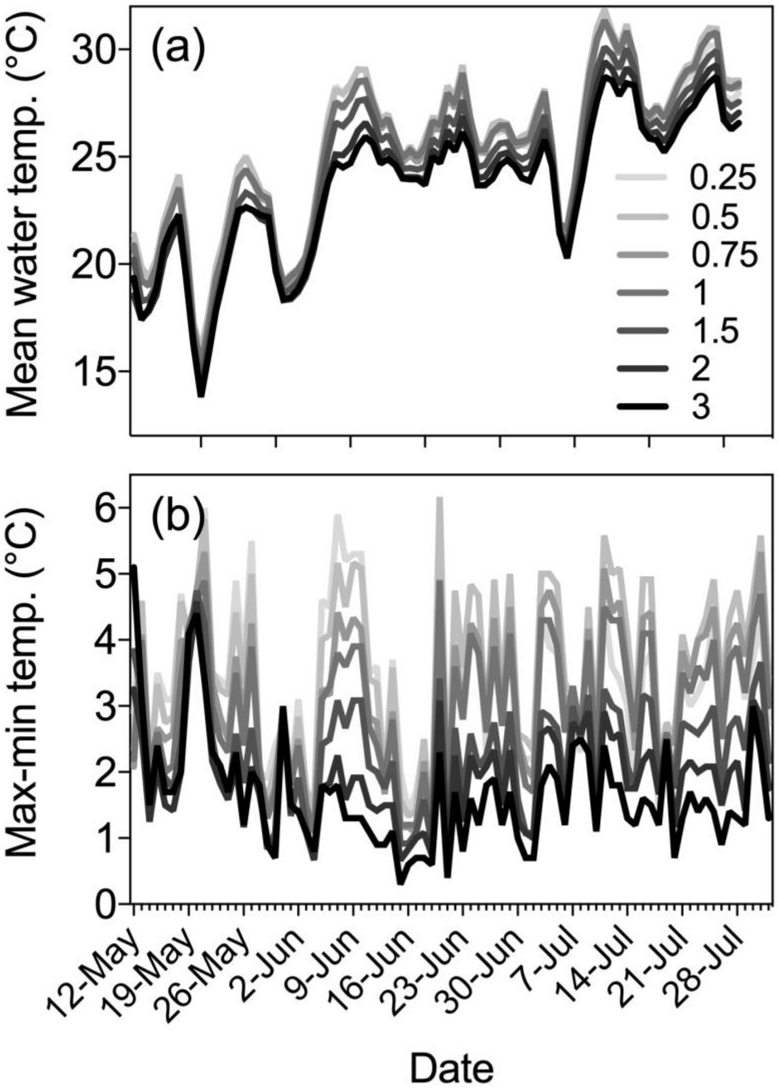

Fig. 4 Temperature responses of experimental ponds to leaf litter subsidy. (a) Daily mean temperature and (b) daily temperature range (º C). Each line represents the mean value for a leaf litter subsidy treatment. n = 5 replicate ponds for 0.25, 1, and 3 kg subsidies (due to iButton failure or inability to find at end of experiment). n = 6 replicate ponds for 0.5, 0.75, 1.5, and 2 kg subsidies. Subsidy amount (in kg pond^{-1}) is represented by gray shading.

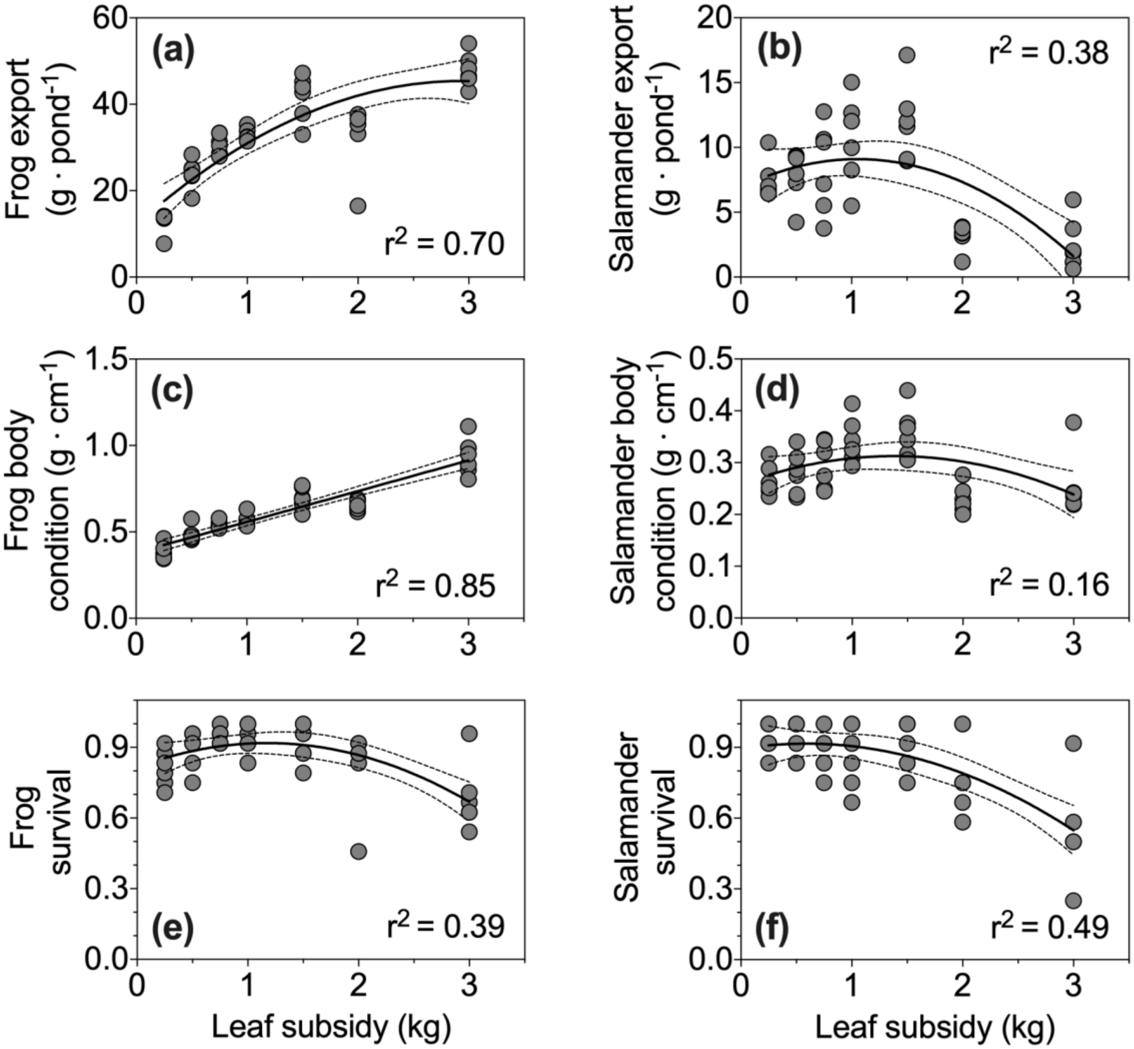

Fig. 5 Responses of frogs (left side) and salamanders (right side) to leaf litter subsidy amount. Biomass export (a, b) is the sum of all individuals reaching metamorphosis. Body condition is mass divided by snout-vent length at metamorphosis (c, d). Proportional survival represents the fraction of original population at the end of the experiment (e, f). The solid line represents the top model and 95% confidence intervals are denoted with dashed lines. n = 6 replicate ponds per treatment.