



Timing of oviposition influences the effects of a non-native grass on amphibian development

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Abstract

Land-use change can alter the energy dynamics in aquatic systems by changing the subsidies that form the nutrient base within them. However, experimental evaluations of subsidy change often fail to consider how effects, such as differences in individual growth and survival, may differ under varying ecological contexts experienced in the field. We used a mesocosm approach to investigate how litter (Native Prairie or Non-Native Tall-Fescue Grass) surrounding wetlands and timing of oviposition affected larval amphibian development. We found that survival differed between litter types in the Early-Oviposition treatment, with nearly 100% mortality in Fescue treatments. Conversely, survival was similar across litter types in the Late Oviposition treatment (~43%), and larvae in Late-Fescue treatments metamorphosed more quickly and were larger post-metamorphosis than larvae in Prairie treatments. Follow-up experiments confirmed that low dissolved oxygen (DO) was responsible for high mortality in Early-Fescue treatments; high quantities of Fescue resulted in a microbial bloom that reduced DO to <2 mg/L for several days, resulting in low hatching success. This effect was eliminated in treatments with supplemental aeration. Finally, we confirmed that experimentally observed DO patterns also occurred in the field. Context (i.e., timing of inundation relative to amphibian breeding) is critical to understanding the effects of subsidies on amphibian populations; early and explosively breeding species may experience catastrophic mortality due to DO depletion; whereas, species that breed later may experience enhanced fitness of recruits. Considering the effects of non-native species across different ecological contexts is necessary for elucidating the extent of their impacts.

Keywords Agriculture · Dissolved oxygen · Grasslands · Microbial respiration · *Lithobates*

Introduction

Land-use change can have profound effects on ecosystem function, either through direct physical impacts (e.g., habitat loss) or indirect consequences (e.g., climate change; Foster et al. 2003). Land-use can alter spatial subsidies, such as

litter inputs, which often form the base of nutrients available for uptake and assimilation by consumers and detritivores in aquatic ecosystems (Stream Solute Workshop 1990, Polis et al. 1997, Pace et al. 2004, Leroux and Loreau 2008). Specifically, the quality of vegetation entering the recipient ecosystem (Rubbo and Kiesecker 2004, Maerz et al. 2010, Stephens et al. 2013), either through changes in labile properties or nutrient composition, can have strong cascading effects on food webs (Carpenter et al. 1985, 2001).

Vegetation entering the aquatic system from surrounding terrestrial habitat is the dominant type of spatial subsidy in temporary wetlands and can strongly influence microbial growth and algal production (Cohen et al. 2012, Earl et al. 2014). Oak leaves have been shown to support a less dense matrix of microbes compared to maple leaves in tree-hole ecosystems (Fish and Carpenter 1982). Microbial activity is the primary mechanism associated with litter (i.e., vegetation) conditioning, and litter type and quality can affect the rate of conditioning. For example, microbial production

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on oak leaves is low at the beginning of conditioning but increases sixfold over time; in contrast, microbial production on maple leaves is three times higher at the beginning of conditioning, but remains relatively constant over time (Rubbo and Kiesecker 2004). The control litter quality exerts over microbial conditioning has potential ramifications throughout the food web, as microbial carbon is a critical source of nutrition for meiofauna and macroinvertebrates (Meyer 1994), representing a significant contribution toward aquatic secondary production (Hall et al. 2000). Reduced conditioning results in lowered food quality for invertebrates which, in turn, negatively affects detritivore growth, survival, and production (Rubbo et al. 2006, Maerz et al. 2010). Overgrowth of microbial communities can result in hypoxic conditions (Mallin et al. 2006); thus, heterotrophic microbes are key players, albeit indirectly, in the population dynamics of aquatic vertebrate consumers.

Amphibians are often used as model organisms for testing the effects of spatial subsidies on population and community dynamics (Rubbo and Kiesecker 2004, Williams et al. 2011, Cohen et al. 2012) and litter quality has been shown to exert strong bottom-up effects on amphibian production (Maerz et al. 2005, Earl and Semlitsch 2013). Litter can affect amphibians through a variety of mechanisms including algal production, microbial production, tannin production, pH, and dissolved oxygen (DO). Additionally, human-induced changes in litter quality and composition, often resulting from the introduction of invasive plants and loss of native species, have been shown to strongly affect amphibian fitness (Stephens et al. 2013). For example, tannins produced from Eurasian Purple Loosestrife (*Lythrum salicaria*) reduced larval survival by 50% in a bufonid species (Maerz et al. 2005). Extracts from invasive Arum Honeysuckle (*Lonicera maackii*) also reduced larval amphibian survival up to 30% compared to native and control treatments (Watling et al. 2011a). Although several studies have evaluated the effects of non-native subsidies on amphibian larval development, these studies are usually conducted using a limited number of experimental treatments under controlled lab or mesocosm conditions, which avoid much of the variability in environmental conditions present in natural systems. Thus, these studies have limited ability to detect how consistent effects are across a variety of plausible ecological contexts, such as how litter effects might be influenced by the timing of wetland inundation and amphibian breeding. Additionally, most studies on the effects of litter subsidies on amphibian development have focused on vernal pool systems in forests; comparatively little research on the role of subsidies has been conducted in prairie grassland ecosystems, despite their critical conservation status (Samson et al. 2004, Henwood 2010).

North America has lost more than 95% of its native grassland habitat (Samson and Knopf 1994, Comer et al. 2018),

due largely to conversion of prairies to agricultural fields. One such crop is Tall-grass Fescue (*Lolium arundinaceum* [Schreb]), with over 86.5 million hectares grown across the United States for feeding livestock and as turf grass (Sleper and West 1996, Cherney 2007). Compared to prairie wetlands, fescue-dominated wetlands might provide a very different habitat for developing larval amphibians. While many studies have shown invasive and non-native vegetation negatively affect tadpole development (Rubbo and Kiesecker 2004, Maerz et al. 2005, Williams et al. 2011), other studies have found that non-native species can have positive effects on larval development (Watling et al. 2011b, Rogalski and Skelly 2012). Additionally, Cohen et al. (2012) suggest that vegetation quality (i.e. nutrient composition) and quantity might play a more important role in amphibian development than the specific vegetation species. Although agricultural ponds have been shown to support amphibian populations (Knutson et al. 2004), as one of the most widely distributed grasses in the world (Miller 2003), the effects of Fescue grass on animal communities have remained understudied. Thus, determining whether Fescue has positive or negative effects on the survival and development of larvae under varying conditions (e.g., quantities and environmental conditions) would provide important information for understanding declines in amphibian species, especially in prairie habitats.

The objective of this study was to evaluate the effects of non-native Fescue grass on the hatching success, larval development, and survival of prairie amphibians. Specifically, we used a series of mesocosm experiments and field studies to address the following questions: (1) What are the long-term effects of litter type on the survival and development of an imperiled frog? (2) How important is litter type and quantity, and its effects on Dissolved Oxygen, in amphibian hatching success? (3) Does litter quality drive observed effects on amphibian development? (4) Do effects observed in mesocosms also occur in the field? We hypothesized that non-native Tall-grass Fescue would increase survival and growth of amphibians due to its expected labile (i.e., more readily broken down) properties.

Methods

Long-term effects of litter type on survival and development of an imperiled frog

We used large mesocosms located at the University of Arkansas Uptown Campus, Fayetteville, AR, to test the effects of two litter treatments (non-native Fescue grass and mixed native Prairie vegetation) on the long-term development and survival of Crawfish Frog (*Lithobates areolatus*) larvae across two years. Native Prairie vegetation contained

a mixture of sedges (*Carex* spp., *Pycnanthemum* spp., and *Eleocharis* spp.) and forbs (*Boltonia asteroides*, *B. diffusa*, and *Periscaria* spp.) collected from seasonally flooded areas within a restored prairie (Woolsey Wet Prairie Preserve [WWPS], Fayetteville, Arkansas; a known Crawfish Frog breeding site), and thus represents typical litter input for ephemeral wetlands in prairies. Fescue litter was collected from seasonally flooded wetlands within a hayfield adjacent to WWPS. We collected both litter types in late February and dried the litter for at least two weeks prior to experiments. The Crawfish Frog was chosen as a study species because it is currently experiencing precipitous declines throughout its range (IUCN 2013). The IUCN (2013) has listed the Crawfish Frog as near threatened; the species is listed as state endangered in Iowa and Illinois, and is a species of greatest conservation need in Arkansas, Oklahoma, Missouri, Kansas, Kentucky, Mississippi, and Louisiana. Additionally, Crawfish Frogs breed in both prairie and agricultural wetlands (Baecher et al. 2018). They are often one of the first Ranid frogs to begin breeding in the spring and will move into a wetland following the first warm rains that inundate depressional wetlands, which in Arkansas usually occur in early March (Trauth et al. 2004).

In 2015, we constructed an array of twelve, 1325-L polyethylene cattle tanks with six replicates of the two litter treatments and added Crawfish Frog larvae 7 days post-litter addition. In 2016, we constructed an array of 24 tanks with 12 replicates of each litter type. Based on the previous year's results (see below), we added Crawfish Frog larvae 22 days post-litter addition in 2016, resulting in two 'timing of larval introduction' treatments that were confounded with year (Early [2015] and Late [2016] Treatments). We filled tanks with 1000 L of tap water and allowed water to stand for 5 days to allow chlorine to dissipate. We added 1 kg (1 g/L) of dried litter to each tank on 25-Mar-2015 and 28-Feb-2016. We selected 1 kg based on previously published experiments (Stoler and Relyea 2011), as well as field measurements of standing litter biomass (Kross et al. unpubl. data) from our litter collection location, which is a known Crawfish Frog breeding site. We inoculated each mesocosm with 0.5 L of concentrated zooplankton collected from Crawfish Frog breeding ponds by straining pond water through 150- μ m mesh to create a concentration of zooplankton. Finally, we covered mesocosms with black 30% shade cloth (PAK unlimited, Inc. Conelia, GA) to prevent colonization by potential competitors (e.g., Grey Treefrog [*Hyla versicolor*]) and predators (e.g., Dragonfly nymphs).

We collected Crawfish Frog eggs at WWPS in March 2015 and 2016. Due to the conservation status of the Crawfish Frog, only two egg masses were collected in 2015 and one egg mass was collected in 2016. We hatched eggs in the lab. After hatching, we separated larvae (Gosner stage [GS] 25–26; Gosner 1960) haphazardly into groups of 40

and added them to each mesocosm on 31-Mar-2015 and 22-Mar-2016.

For both the Early and Late Treatment experiments, we measured Dissolved Oxygen (DO) using a YSI 556 MPS handheld meter (YSI Inc. Yellow Springs, OH). In 2015 (Early Treatment), we measured DO haphazardly 5, 8, 56, 71, and 77 days post-litter addition; in 2016 (Late Treatment), we measured DO between 0730 and 0930 h, regularly, but not daily, for 2 weeks, and then measured DO prior to tadpole introduction on day 22.

We checked on larvae every other day, removed larvae from mesocosms at emergence of the first forelimb (GS 42), and held them in the laboratory until full tail resorption occurred (GS 46). At stage 46, we measured snout–vent length (SVL) to the nearest millimeter and mass to the nearest 0.01 g (Mettler ML1502E balance; Mettler-Toledo International, Columbus, OH, USA). We also recorded the date of full tail resorption to determine time to metamorphosis (TTM). For survival, we calculated the proportion of individuals that survived through metamorphosis from each tank, and then calculated an average for each treatment. We used a one-way ANOVA with Timing/Year (i.e., Early vs. Late) as a block to compare their effect on survival. A Tukey's test for non-additivity was used to determine whether there was an interaction between Litter Type and Timing/Year. We analyzed these factors using an ANOVA with a Block due to the confounding of year with the Timing treatment. For body size (SVL and mass) and TTM, we used one-way ANOVAs followed by Tukey's tests for independent contrasts. We performed all data analyses using Program R (3.5.2; R Development Core Team 2018).

Effects of litter type and quantity on amphibian hatching success

Due to the confounding of year with the timing of litter addition, we conducted the following experiment to directly address the relationship between timing of litter addition, DO, and amphibian survival. We conducted a mesocosm experiment, consisting of forty-eight, 18.92-L mesocosms (plastic buckets) with six replicates of eight treatments at the University of Arkansas Uptown Campus in Fayetteville, AR, in spring 2017. Prairies and agricultural fields are managed using a variety of mechanical techniques, such as mowing and fire, which can lower vegetation quantity within the wetland area. Conversely, Fescue can achieve high biomass (500 g–1000 g/m²) in a small area (Kross et al. unpubl. data). Thus, to capture a range of plausible scenarios of wetland provisioning, we used a 2 $\times$ 3 factorial combination of the two vegetation type treatments (Prairie and Fescue) and three litter quantities: Low (0.5 g/l), Moderate (1 g/l), High (2 g/l). Additionally, we included two treatments with High Litter Quantity plus supplemental aeration as a control for

the low DO concentrations expected in High Quantity treatments. We aerated mesocosms using standard aquarium air pumps and air stones. We collected and dried litter as described in the previous experiment.

On 5-Apr-2017, we filled mesocosms with 13.25 L of tap water, allowed water to sit for 48 h for chlorine to evaporate, and then inoculated each with 120 ml of pond water that had been strained through 150 μm mesh to concentrate zooplankton. On 8-Apr-2017, we collected three Leopard Frog (*L. sphenoccephalus*) egg masses from local ponds, and mixed and haphazardly separated them into batches of 20 eggs. We checked all eggs to ensure they were successfully fertilized. Within mesocosms, we placed eggs within a small sub-container (10-cm-diameter PVC tubing with mesh glued to the bottom and polyethylene foam glued to the top as a float) to facilitate observations of hatching success and larval survival. We placed litter directly into the mesocosms simultaneously with egg addition on 8 April to mimic wetland filling co-occurring with breeding following a spring rain event.

We measured DO and water temperature in mesocosms each morning between 0530 h and 0730 h using a YSI 556 MPS handheld meter (YSI Inc. Yellow Springs, OH). We counted the number of larvae that successfully hatched 7 days post addition. By day 7, all unhatched eggs had become cloudy and embryos had become white, indicating mortality. We compared DO concentrations among litter types and quantities 2 days post addition using a two-way ANOVA. To determine if hatching success differed significantly between litter type and mass treatments, we performed a two-way ANOVA. Data did not meet assumptions of normality and homoscedasticity, but attempts to transform data did not alleviate this problem. More conservative non-parametric approaches yielded identical treatment differences, but still suffered from assumption violations. Thus, we interpreted ANOVA results conservatively and focused on differences between treatments that were the strongest.

Litter quality and microbial activity

Litter quality is an important driver of microbial activity and vertebrate production; thus, we determined initial quality for both litter types. We collected six subsamples of litter reserved from litter used in the hatching success experiment. We finely ground each subsample using a Wig-L-Bug ball mill (International Crystal Laboratories, Garfield, NJ) and combusted each at 550 °C for 2 h. We determined phosphorus (P) content by submitting ashed samples to a hydrochloric acid digest at 85 °C before utilizing the ascorbic acid method to determine P (APHA 2005, Rosemond et al. 1993). We determined carbon (C) and nitrogen (N) content in samples with an Isotope Ratio Mass Spectrometer

(Thermo Scientific, Waltham, MA). We compared nutrient data between litter types using *t* tests.

As microbial activity is an important driver of DO, we measured microbial respiration using twelve, 1 L mesocosms with airtight lids. We filled each mesocosm with 1 L of dechlorinated tap water and recorded DO. We then added 1 g of Fescue litter to each of six mesocosms and 1 g of Prairie litter to the remaining six mesocosms. We inoculated each mesocosm with 1 mL of pond water to introduce microbes. We capped all bottles and placed them in an environmental chamber that cycled from 24 °C for 12 h to 5 °C for 12 h to mimic environmental temperatures in April. We measured DO at 24, 48, and 72 h to quantify net oxygen consumption by microbes. We report respiration values in $\text{mgO}_2 \cdot \text{gAFDM}^{-1} \text{ hr}^{-1}$ (Entekin et al. 2008, Fuell et al. 2013). We compared microbial respiration between litter treatments using a *t* test.

Field observations on the effect of litter type on dissolved oxygen in seasonal wetlands

During the spring of 2017, we collected DO measurements in the field from five Fescue-dominated and five Prairie-dominated seasonally inundated wetlands. All wetlands are part of WWPS, a 48-ha prairie restoration and wetland mitigation site owned by the city of Fayetteville, AR. The Prairie wetlands were located in a restored section of the property, while the Fescue wetlands were located in hay fields adjacent to the restored section (see Baecher et al. [2018] for more site information). Wetlands were dry over the winter and filled on 27-Mar-2017, following the accumulation of 8.48 cm of rain over the previous four days (Drake Field Weather Station, Fayetteville, AR). Wetland areas varied from 0.1 to 0.21 ha and were less than 1 m in depth. We measured DO between 0730 h and 0930 h daily from 30-Mar to 02-Apr-2017 using a YSI 556 MPS handheld meter (YSI Inc. Yellow Springs, OH). During this time, Crawfish Frogs and other early spring breeding amphibians were actively breeding at some of the sampled wetlands and across Northwest Arkansas (Kross and Willson unpubl. data). We measured DO along the shallow edge of each wetland, in water deep enough to completely submerge the probe. We compared the 01 April 2017 DO concentrations between Fescue and Prairie wetlands using a *t* test.

Results

Long-term effects of litter type on survival and development of an imperiled frog

In the Early Treatment (2015) experiment, average DO concentration was less than 1 mg/L in both treatments when

larvae were added to tanks, but lower in the Fescue treatment (Fig. 1a). DO concentration averaged above 3 mg/L towards the third month of the experiment in the Early Treatment and was similar between litter types. In the Late Treatment (2016) experiment, DO concentration decreased sharply after the introduction of Fescue and remained below an average of 3 mg/L for 14 days (Fig. 1b). Dissolved oxygen concentrations in the Prairie treatment decreased gradually and only averaged below 3 mg/L on day 8 post-litter introduction (Fig. 1b). By day 22, DO concentrations had risen, and were similar across both litter types (average Fescue DO = 6.3 mg/L and average Prairie DO = 5.9 mg/L; Fig. 1b).

Litter type and Timing/Year interacted to significantly affect larval survival ($F_{1,33} = 9.36$, $p < 0.01$; Fig. 2a). Only one individual larva completed metamorphosis from the Early Fescue Treatment. Larval survival was similar (~43%) across Early and Late Prairie, and Late Fescue Treatments. Metamorphs from the Late Fescue Treatment averaged 6 mm longer in SVL ($F_{2,26} = 16.74$, $p < 0.001$) and almost twice the mass ($F_{2,26} = 17.26$, $p < 0.001$) of metamorphs from the Early and Late Prairie Treatments (Fig. 2b, c). Additionally, larvae reached metamorphosis 5 days earlier, on average, in the Late Fescue Treatment compared to the Early and Late Prairie treatments ($F_{2,26} = 23.39$, $p < 0.001$; Fig. 2d).

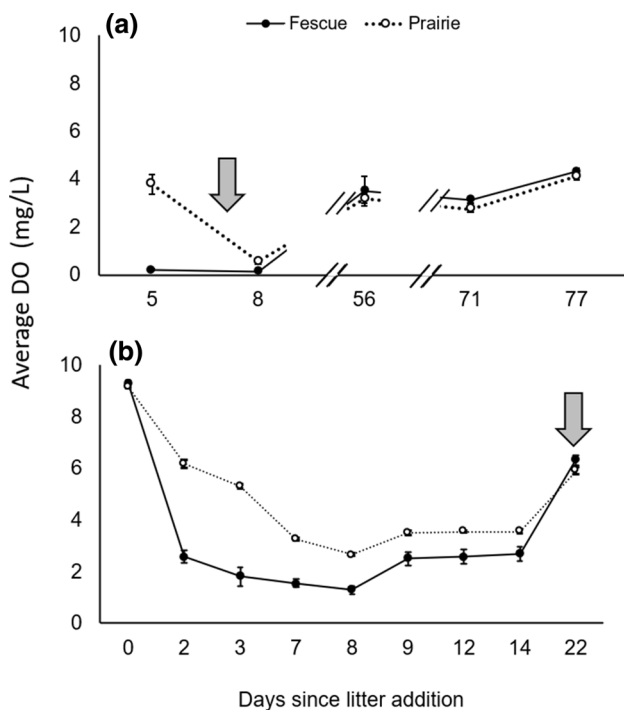


Fig. 1 Average dissolved oxygen (DO) concentration (± 1 SE) in the **a** Early and **b** Late Oviposition Treatments. Arrows indicate when larvae were added to tanks. Solid lines indicate Fescue Treatments (Early $N=6$, Late $N=12$). Dashed lines indicate Prairie Treatments (Early $N=6$, Late $N=12$)

Effects of litter type and biomass on amphibian hatching success

Our follow-up experiment on hatching success confirmed that low DO early in development was the driver of low survival observed in the Early Fescue Treatment. Overall, DO concentrations were lower across all Fescue Quantity Treatments and decreased as Fescue Quantity increased (Fig. 3). Litter type and quantity interacted significantly to affect DO 2 days after litter addition ($F_{3,44} = 37.95$, $p < 0.001$). Prior to litter addition, DO concentrations were similar and above 4 mg/L across all treatments. However, within 24 h of litter addition, DO concentration decreased across all non-aerated quantity treatments with steeper declines in higher quantity and Fescue treatments (Fig. 3). Additionally, DO concentrations in non-aerated Prairie treatments were 1.5–5 times higher than DO concentrations in non-aerated Fescue treatments (Fig. 3). For all Low and Moderate treatments, DO concentrations remained relatively stable 24 h post-addition and began to increase 6 days post-addition. For the High (non-aerated) Treatments, DO concentration remained low, but consistent, during the entirety of the experiment. DO concentrations remained high (> 6 mg/L) in the High Aerated Treatments throughout the experiment.

Litter type and quantity interacted to affect Leopard Frog hatching success ($F_{3,40} = 13.882$, $p < 0.001$). Overall hatching success was highest across Prairie Treatments, compared to Fescue Treatments, and decreased as quantity increased (Fig. 4). Zero eggs hatched in the High Fescue Treatment. In the Moderate Fescue Treatment, an average of 35% of eggs hatched. In contrast, the High and Moderate Prairie Treatments had an average hatching success of 53% and 95%, respectively. Hatching success was similar between the Low Prairie (96%) and Fescue (90%) Treatments. High Aerated Fescue and High Aerated Prairie Treatments had an average hatching success of 93%.

Vegetation quality and microbial activity

Both N and P comprised roughly twice as much of initial dry biomass in Fescue than Prairie litter (N: $t_{11} = 25.03$, $p < 0.001$; P: $t_{11} = 3.41$, $p = 0.007$; Table 1). Carbon accounted for significantly greater biomass in Prairie (45%) compared to Fescue (42%) litter ($t_{13} = -35.48$, $p < 0.001$; Table 1). Microbial activity was nearly three times higher in the Fescue Treatment compared to the Prairie treatment after 72 h ($t_{11} = 11.35$, $p < 0.001$; Fig. 5).

Field observations on the effect of litter type on dissolved oxygen

Following a heavy rain event in late March 2017, average DO concentration in Fescue Wetlands was lower than that of

Fig. 2 Average **a** Survivorship to metamorphosis (± 1 SE), **b** Snout–Vent Length at metamorphosis (± 1 SE), **c** Mass at metamorphosis (± 1 SE), and **d** Time to metamorphosis (± 1 SE) of Crawfish Frog (*Lithobates areolatus*) larvae introduced to Fescue and Prairie mesocosms 7 days post-litter addition (Early) and 22 days post-litter addition (Late). Letters indicate significant differences based on an ANOVA and Tukey's HSD test (Fig. 2a: $N=36$) (Figs. 2b–d: $N=30$). Figure 2a does not include letters due a significant interaction effect

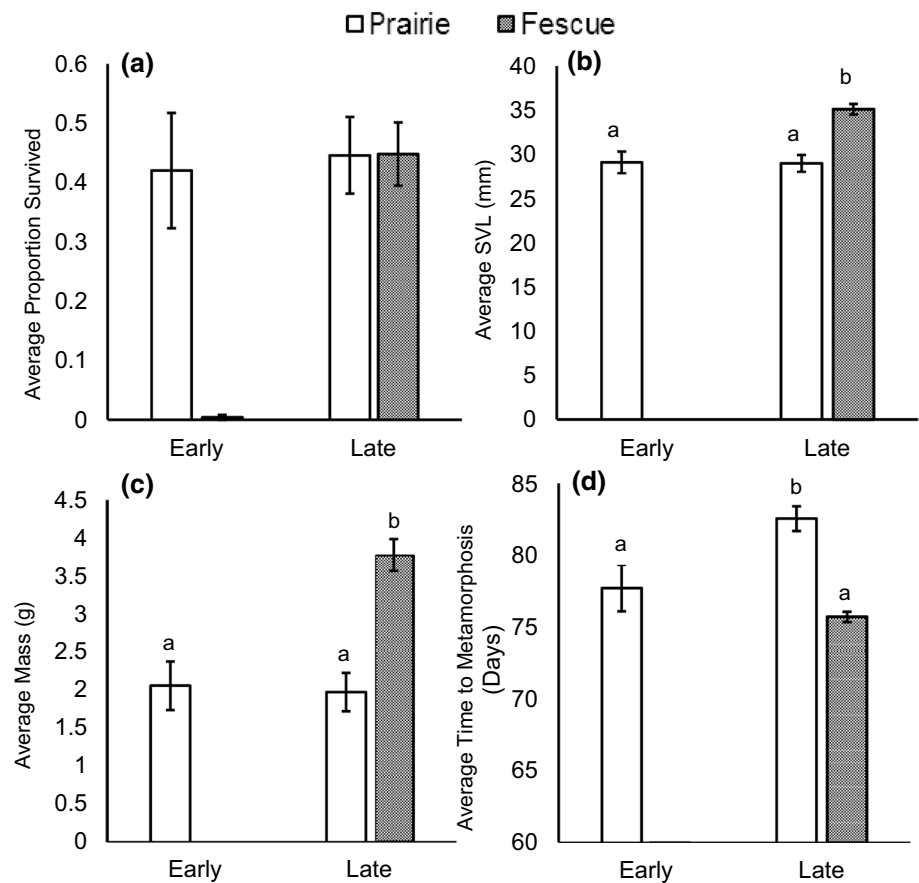
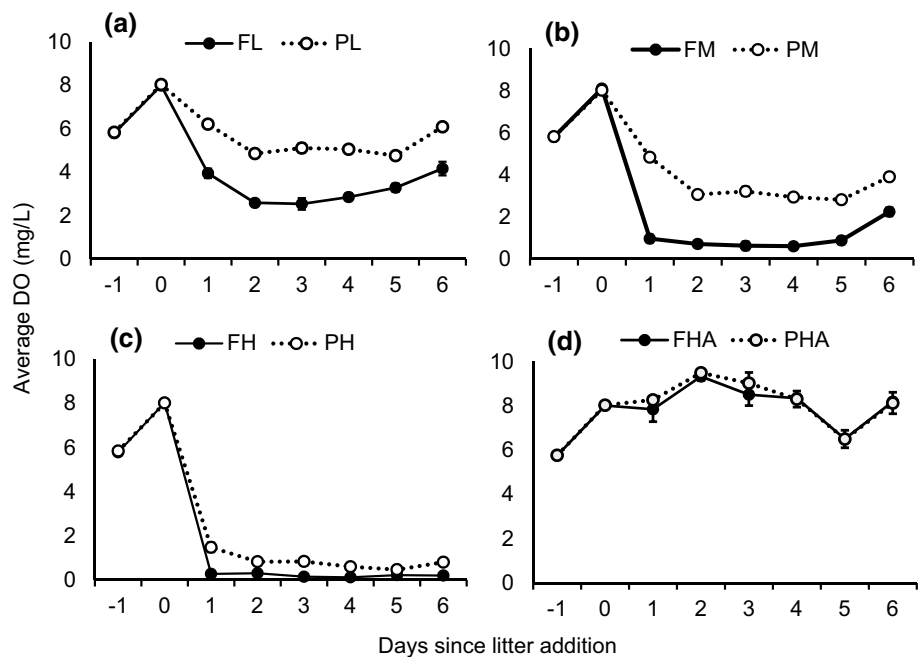


Fig. 3 The effect of litter type and quantity (H=High; M=Moderate; L=Low) on average dissolved oxygen (DO) concentration (± 1 SE) over six days in 18.92 L mesocosms. **a** Low Quantity (0.5 g/L), **b** Moderate Quantity (1.0 g/L), **c** High Quantity (2.0 g/L), **d** High Quantity with Aeration. Solid lines indicate Fescue (F) Treatments. Dashed lines indicate Prairie (P) Treatments ($N=8$ per treatment)



Prairie Wetlands ($t_9 = 10.38$, $p = 0.01$) and followed a similar pattern of decrease observed in mesocosms (Fig. 6). In Fescue Wetlands, DO decreased by 2 mg/L over the sampling

period and averaged 2.05 mg/L 72-hour post-wetland filling. In Prairie Wetlands, DO decreased by 3 mg/L and averaged 4.5 mg/L 72-hour post-wetland filling.

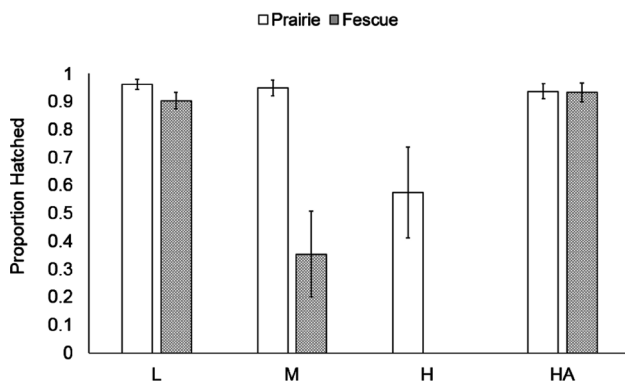


Fig. 4 Average hatching success (± 1 SE) of Leopard Frog (*Lithobates sphenoccephalus*) eggs among eight litter type and quantity treatments ($N=8$ per treatment). Vegetation quantities are indicated by an L (low), M (moderate), H (high), HA (high with aeration)

Table 1 Nutrient content of Prairie and Fescue litter collected in Arkansas, USA ($N=6$ per treatment)

Plant species	Mean ± 1 SE		
	%C	%N	%P
Native prairie grass mix	45.8 $\pm$ 0.06	0.62 $\pm$ 0.02	0.07 $\pm$ 0.03
Non-native fescue	42.5 $\pm$ 0.07	1.42 $\pm$ 0.04	0.18 $\pm$ 0.02

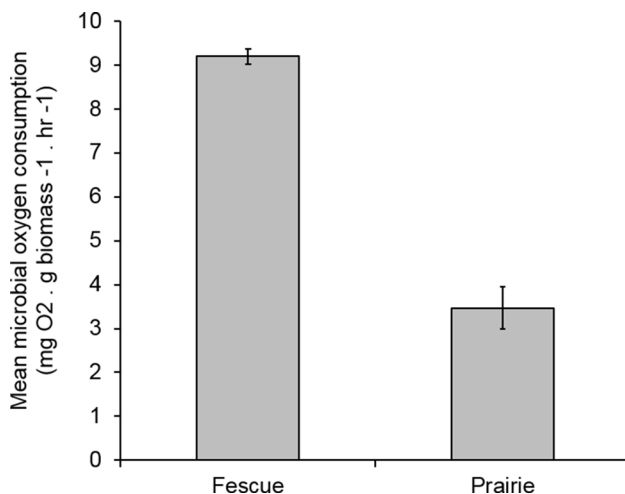


Fig. 5 Average microbial respiration (± 1 SE) in mesocosms containing Fescue or Prairie litter ($N=6$ per treatment)

Discussion

Changes in vegetation community composition and the alteration of subsidies forming the nutrient base of ephemeral aquatic systems can have mixed effects on vertebrates, making it difficult to determine how land-use change might

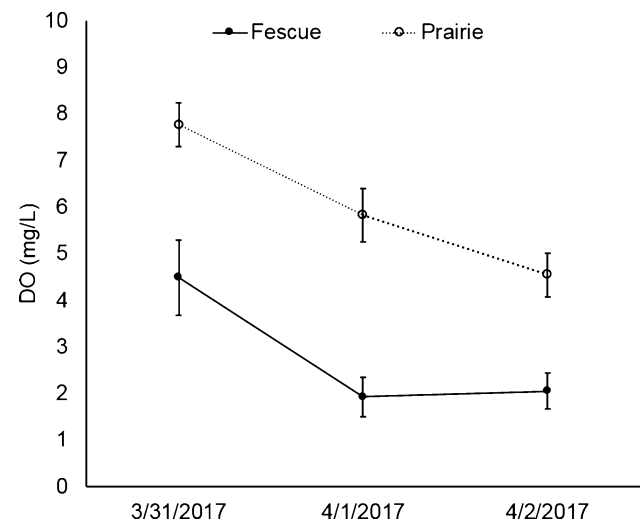


Fig. 6 Average Dissolved Oxygen (DO) concentration (± 1 SE) in Fescue- (solid line) and Prairie-dominated (dashed line) wetlands ($n=5$ per treatment) in Northwest Arkansas following a heavy (8.48 cm) rain event in April 2017

affect wildlife populations. Our experiments demonstrate that non-native Fescue vegetation can have either positive or negative effects on anuran hatching success and larval development, depending on when wetlands are filled and breeding occurs. When eggs and larvae were reared in adequately oxygenated Fescue Treatments, eggs hatched successfully (Low Quantity and Aeration Treatments) and larvae metamorphosed 7 days earlier and had twice the mass of larvae reared in Prairie Treatments (Late Oviposition Treatment). In contrast when eggs and larvae were introduced to mesocosms at the same time as litter, DO concentrations crashed, resulting in fewer or no eggs hatched (Moderate and High Quantity treatments), and lower survival to metamorphosis (Early Fescue Treatment). Low DO conditions were driven by the higher nutrient concentrations found in Fescue, which increased microbial activity. The pattern of the short-term DO depletion in Fescue Treatments was observed in wetlands in the field. Collectively, our data suggest that Fescue could have positive effects on the development of some amphibians, but could cause mass mortality for species that exhibit explosive breeding or early-spring breeding coincident with wetland inundation.

The results of the Late litter type and timing experiment and litter quality comparison generally supported our hypothesis that Fescue would result in increased growth of developing larvae. Larvae in the Late Fescue Treatment reached metamorphosis 7 days faster than individuals from the Late Prairie Treatments, but had similar survival. Metamorphs in the Late Fescue Treatment were nearly twice as large as metamorphs from the Early and Late

Prairie Treatments. In the microbial activity experiment, we observed higher microbial respiration rates in the Fescue Treatment, which suggests that Fescue is more labile than Prairie grass (Wilcox et al. 2005). Metamorph size differences were likely due to the higher N and P content found in Fescue litter, which makes it more labile for conditioning. Labile litter is more readily broken down and used by microbes and algae, which can then transfer those nutrients to higher order consumers (Webster and Benfield 1995, Hall and Meyer 1998). However, these same attributes that benefit development might also reduce survival under certain conditions.

In the Early litter type and timing experiment, we observed near complete mortality of Crawfish Frog larvae reared in Fescue, which was unexpected. We also observed that DO was below 1 mg/L after larvae were added, which likely caused the mortality. In the hatching success experiment, we found that Fescue in Moderate and High Quantities caused a DO crash that lasted for multiple days, resulting in reduced hatching success or total mortality in those treatments. However, mortality was completely ameliorated by adding supplemental aeration. These results are similar to a study that found high quantities (2 and 4 g/L) of Chinese Tallow (*Triadica sebifera*), an invasive species, reduced DO to below 2 mg/L and resulted in complete mortality of Leopard Frog eggs (Adams and Saenz 2012).

Litter quality (i.e. Nitrogen [N], Phosphorus [P], Carbon [C] content) is an important factor associated with amphibian production (Cohen et al. 2012). Stephens et al. (2013) observed that amphibians reared in mesocosms with litter that had higher percent N were larger (i.e., increased SVL and mass) than those reared in litter with low percent N. Others have found that litter quality influences microbial activity, and phosphorus content can exert strong controls on the early stages of decomposition (Aerts and de Caluwe 1997). While we did not manipulate nutrient levels to determine the specific nutrient controlling microbial activity, our results do indicate that higher litter quality may influence microbial oxygen consumption. Heterotrophic microbes are key players in the breakdown of decomposing organic matter such as leaves and grasses (Battle and Golladay 2001). Their primary role is in mineralization and conditioning, which aids in the breakdown of recalcitrant compounds and creates a more suitable, nutritious substrate for higher trophic levels. Given the higher nutrient content of Fescue, areas dominated by Fescue may be more susceptible to DO crashes, as high-quality food supports greater microbial production and, thus, respiration. We conclude that high N content and the labile properties of Fescue results in a microbial bloom that depletes DO and can cause mass mortality of eggs and early-stage amphibian larvae. The particular attributes of Fescue (i.e., labile, high nutrient composition) that are beneficial for

larval development can also drastically reduce egg and larval survival by inducing a DO crash.

While many amphibian studies record DO, most only report that DO varied across experimental treatments (e.g., Maerz et al. 2010; Earl and Semlitsch 2013; Stephens et al. 2013). As a result, the effects of litter inputs on DO and larval anuran survival have not received much attention (but see Adams and Saenz 2012). This is largely due to the fact that most larvae of most anuran species have lungs. However, lungs are not fully functional immediately after hatching or during early larval stages for many species (Burggren and West 1982). Although the effect of decreased access to oxygen on larval amphibian respiration has been examined (Feder 1983a, b), few studies have reviewed the effect of low DO on anuran hatchling and early larval (GS 26-27) survival. Additionally, the duration of low DO conditions may influence hatching success and larval survival. In our experiment, low DO (<2.0 mg/L) persisted for up to 7 days and at levels below 4.0 mg/L for up to 14 days. Additionally, we observed low DO in recently inundated Fescue-dominated wetlands in the field. A prolonged effect of low DO immediately following inundation might disproportionately affect amphibians that breed explosively or early in the spring.

Amphibian populations are declining due to anthropogenic habitat changes; our field and experimental results highlight yet another pathway that could contribute to population declines. Low DO concentrations in wetlands have been shown to have negative effects on amphibian egg and larval survival (Bradford 1983, Sacerdote and King 2009). We were able to confirm that the DO reductions we observed in our experiments also occur in nature. Thus, there is an increased risk of reproductive failure for the many amphibian species that breed concurrently with wetland filling in grassland habitats, such as the Crawfish Frog, Gopher Frog (*L. capito*), Spadefoot Toads (*Spea spp.* and *Scaphiopus spp.*), and the Small-mouthed Salamander (*Ambystoma texanum*). Salamander species may be particularly sensitive to the low DO conditions we observed in Fescue wetlands. Sacerdote and King (2009) found that Spotted Salamander (*A. maculatum*) eggs did not hatch if DO was below 4.0 mg/L. Many salamanders, including all pond-breeding species, have gills throughout their larval period; thus, they require high concentrations of DO to meet their metabolic needs. In contrast, species with prolonged breeding activity or those that breed after DO has returned to levels above 4.0 mg/L might benefit from larger body sizes and shorter times to metamorphosis observed in Late Fescue treatments. Larger body sizes at metamorphosis have been linked to increased survival to reproduction (Smith 1987), reduced time to first reproduction (Semlitsch et al. 1988), and improved juvenile foraging ability (Cabrera-Guzmán et al. 2013). A reduction in time to metamorphosis can also be important for species

that breed in temporary wetlands, where hydroperiod can be an important factor associated with survival. If a pond dries prior to metamorphosis, larvae will desiccate, sometimes resulting in complete reproductive failure (Semlitsch 1983, 1987). Our results suggest that interpreting the effects of non-native vegetation on amphibian survival and development is complex and dependent upon timing of wetland inundation, oviposition, and vegetation quality and quantity.

Ecosystems have been substantially modified by anthropogenic activities and understanding the implications of such changes is one of the greatest challenges facing ecologists. Non-native species introductions and propagation can have unforeseen effects on native species, such as positive and negative effects of *Fescue* on tadpole development, which serve to further complicate our understanding of ecosystem function and conservation. Investigating the effects of non-native species introductions and abiotic factors under different ecologically relevant contexts (e.g., breeding, migration, growth) and the mechanisms that drive those effects is critical for understanding population and community ecology in changing landscapes (Agrawal et al. 2007). Here, we described a potential mechanism that may exacerbate current amphibian population declines by reducing reproductive success through increased variation in breeding wetland suitability. Further work modeling the population-level consequences of these effects are needed, especially for species that are explosive or early spring breeders. More broadly, our results suggest that realistic assessment of the effects of non-native species should incorporate treatments that consider a variety of plausible ecological interactions, as well as comparison with field data to assess consistency of effects observed in experimental mesocosms.

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Author contribution statement CSK and JDW conceived the ideas and designed methodology; CSK, AKD, and PLM collected the data; CSK and AKD analyzed the data, CSK led the writing of the manuscript, comments from JDW improved the manuscript. All authors gave final approval for publication.

Data availability The datasets generated during the current study are available as an electronic supplement with this manuscript.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Animal rights All applicable institutional and national guidelines for the care and use of animals and were approved and followed (IACUC protocol #'s 15033 and 17079).

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