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Animal aggregations promote emergent aquatic plant production at the aquatic–terrestrial interface

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Abstract. The roles mobile animals and abiotic processes play as vectors for resource transfers between ecosystems ("subsidies") are well studied, but the idea that resources from animals with limited mobility may be transported across boundaries through intermediate taxa remains unexplored. Aquatic plants ("macrophytes") are globally distributed and may mediate transfers of aquatic-derived nutrients from aggregations of aquatic animals to terrestrial ecosystems when consumed by terrestrial herbivores. We used mesocosms (94 × 44 cm) to test whether aquatic animal-generated biogeochemical hotspots increase growth and nutrient content in macrophytes using the macrophyte *Justicia americana* and freshwater mussels. *Justicia americana* biomass production increased and belowground biomass allocation changed with increasing mussel density. At high mussel density, water-column phosphorus increased and carbon:phosphorus ratios in *J. americana* tissues decreased. We deployed motion-sensing cameras to explore herbivory on *J. americana* growing along the margins of the Kiamichi River, Oklahoma, and documented feeding by large mammals (*Odocoileus virginianus*, *Sus scrofa*, and *Bos taurus*). Thus, biogeochemical hotspots generated by aquatic animal aggregations can promote macrophyte production that subsequently is transferred to terrestrial animals. More broadly, this suggests that reductions in aquatic animal biomass may have bottom-up impacts that indirectly affect terrestrial ecosystems via plant–animal interactions bridging ecosystem boundaries.

Key words: aquatic plant; aquatic–terrestrial linkage; biogeochemical hotspots; emergent macrophyte; freshwater mussel; macrophyte herbivory; nutrient subsidy; resource subsidy.

INTRODUCTION

Resource flows between ecosystems ("subsidies") influence ecosystem structure and function reciprocally. Processes that drive variation in terrestrial-to-aquatic resource subsidies are well studied, but recent research highlights the importance of subsidies from aquatic-to-terrestrial ecosystems (Schindler and Smits 2017). Aquatic-to-terrestrial subsidies may be achieved by mobile animals crossing the aquatic–terrestrial interface, such as aquatic insects emerging as flying adults (Baxter et al. 2005), by hydrologic factors like floods (Junk et al.

1989), or by both as in salmon runs (Ben-David et al. 1998). However, the roles that plant–animal interactions play in facilitating cross-ecosystem resource subsidies are not well understood.

The importance of ecological subsidies depends on their quantity, quality, timing, and duration (Subalusky and Post 2019). Aquatic plants (macrophytes) and animals may interact to subsidize terrestrial ecosystems during the growing season. Macrophytes are dominant primary producers in the aquatic habitats embedded within terrestrial ecosystems globally (Chambers et al. 2008) and represent a large and understudied nutrient pool and flux. Nitrogen (N) and phosphorus (P) pools in plant tissues are metrics of food quality, and often correlate with ambient N and P concentrations (Sterner and Elser 2002). Further, macrophytes are thought to be a higher-quality food than terrestrial plants because they have fewer low-quality, carbon-(C-) rich structural compounds (Sterner and Elser 2002).

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In terrestrial, marine, and freshwater ecosystems, nutrient recycling by animal aggregations can create biogeochemical hotspots of N and P (McNaughton et al. 1988, Allgeier et al. 2017, Atkinson et al. 2017). Macrophytes growing near these hotspots should be nutrient rich, which may increase the amount of macrophyte production consumed by herbivores (Cebrian and Lartigue 2004). Shoots of emergent macrophytes extend above the water and are available to terrestrial herbivores, providing an intermediate storage pool between ecosystems for aquatic-derived nutrients. Terrestrial herbivores browsing on macrophytes can translocate nutrients through subsequent egestion, excretion, or mortality on land (Ceacero et al. 2014, Bump 2018), which should have cascading implications for terrestrial ecosystems.

In streams, dense multispecies aggregations of freshwater mussels (Bivalvia: Unionoida) filter suspended material and excrete and biodeposit nutrients near the river bottom, creating biogeochemical hotspots of nutrient cycling (Atkinson and Vaughn 2015). Mussel aggregations are often next to dense stands of the widely distributed macrophyte, American water willow (*Justicia americana*), which has thick root networks that form gravel bars at river margins (Fritz et al. 2004*b*). Terrestrial herbivores forage at gravel bars, and where *J. americana* and terrestrial herbivores co-occur, *J. americana* shoots often show evidence of browsing (e.g., Dick et al. 2004; see Appendix S2), although peer-reviewed evidence is scant. Substrate stability is key to mussel habitat suitability (Allen and Vaughn 2010) and is facilitated by *J. americana* roots (Fritz et al. 2004*b*). In return, *J. americana* derives nutrients from mussel excreta (Atkinson et al. 2014).

It is unknown if mussel-derived nutrients influence *J. americana* productivity, biomass allocation, or nutrient composition. Uptake of mussel-derived N and P may increase nutrient content in plant tissues because autotrophs can store excess nutrients (Sterner and Elser 2002). Although *J. americana* aboveground biomass senesces in the fall, the underground biomass survives in the sediment, providing overwinter storage of resources. Elevated nutrient availability tends to increase total plant biomass and decrease the proportion allocated to belowground structures in herbaceous plants (Müller et al. 2000), and *J. americana* adjusts its biomass allocation based on environmental factors (Fritz et al. 2004*a*). In turn, biomass allocation may influence the availability and palatability of *J. americana* to herbivores.

Here, we asked how mussel-generated biogeochemical hotspots affect *J. americana* biomass production and allocation, nutrient stoichiometry, and consumption by terrestrial herbivores. We simulated *J. americana* gravel bars in a mesocosm experiment with varying mussel densities and tested three hypotheses: (H1) mussels increase ambient availability of N and P by recycling and excreting these nutrients in inorganic forms; (H2a) increased mussel density promotes *J. americana* biomass production and (H2b) shifts biomass allocation away from belowground structures towards aboveground structures because of increased nutrient availability; (H3a) C:P and C:N ratios in *J. americana* decrease as mussel density increases because plant tissues incorporate more P and N associated with mussel nutrient recycling; and (H3b) *J. americana* tissues will exhibit elevated δ^{15}N signatures as mussel density increases because animal-derived nitrogen is enriched in ^{15}N relative to inorganic sources. We documented field herbivory of *J. americana* by terrestrial vertebrates with motion-sensing cameras and used these observations to supplement our experimental findings and make inferences on the potential role of *J. americana* as a mediator of aquatic-to-terrestrial resource subsidies.

METHODS

Experimental design and sampling

Recirculating mesocosms (94 × 44 cm) consisted of molded plastic liners suspended in fiberglass basins (Appendix S1: Fig. S1) that have been used successfully in other experiments examining mussel ecosystem effects (Appendix S1). We sampled water-column nutrients at the beginning (week 1), midpoint (week 5), and end (week 9) of the experiment, prior to water changes. We quantified ammonium (µg NH_4^+ -NL^{-1}) by the phenol-hypochlorite method, and orthophosphate, soluble reactive phosphorus (SRP; µg P L^{-1}) by the molybdate blue method (U.S. Environmental Protection Agency [USEPA] 1983). Mats of filamentous algae formed over the course of the experiment. To account for this, we quantified algal cover at week 9 by photographing each mesocosm with a fixed objective lens placed 1 m above the water surface. Photos were randomized and scored from 0 to 10 based on the surface coverage of algae by five independent observers following Parr et al. (2020).

We quantified total *J. americana* wet biomass in each mesocosm at week 0 and week 9, after manually removing periphyton and dead biomass, and gently spinning plants to remove excess water (Bickel and Perrett 2016). At week 9, we dried plant tissue at 70°C for 72 h and separated aboveground (leaves and stems) and belowground (roots and runners) dry biomass. We were not able to determine initial aboveground versus belowground biomass allocation because sampling was destructive, but we attempted to plant consistent root biomass proportions in establishing the experiment. Flowering was negligible; thus, its contributions were not assessed. Thus, biomass allocation is a static measure at the end of the experiment, and biomass production represents estimated change in biomass over time. We pooled final *J. americana* biomass measurements (n = 38) to create a wet mass–dry mass regression (dry mass = 0.20 × wet mass + 0.14, R^2 = 0.88) to determine initial dry biomass and thus biomass gained during the experiment.

We assessed C:N:P stoichiometry using molar ratios and the ^{15}N isotopic signature of each biomass

compartment. We quantified C and N content and ^{15}N at the University of Oklahoma Environmental Stable Isotope Lab using a Thermo Isolink CN Elemental Analyzer integrated with a Thermo Delta V Advantage IRMS through a Conflo IV (Thermo Fischer Scientific, West Palm Beach, Florida, USA). δ^{15}N values of samples were calibrated using externally certified standards (U.S. Geological Survey [USGS] 40 and 41a) for δ^{15}N relative to air, and C and N content of samples were calibrated using an algae (*Spirulina*) standard (Elemental Microanalysis Limited, Devon, UK) for C and N content. Total P content was estimated by combustion at 500°C and acid digestion at 105°C followed by SRP analysis as previously described (USEPA 1983).

Camera trap surveys

We placed a motion-sensing game camera (Model TR10i35A-7, Wildgame Innovations, Grand Prairie, Texas, USA) at 10 locations along a 72-km stretch of the Kiamichi River, Oklahoma, USA from 5 August to 10 October 2019 (Appendix S2: Fig. S1). We affixed cameras to tree trunks overlooking *J. americana* stands and set them to record 30s time-stamped videos upon triggering at a range up to ~18 m. To quantify the number of herbivory events caught on camera, we recorded the species-level identity and behavior of terrestrial vertebrate herbivores that visited *J. americana* stands. If herbivores were observed directly consuming *J. americana*, that visit received a binary score of 1, and if not, it received a 0. To prevent double counting, videos showing the same species of the same sex and approximate size within a 1-hr period were assumed to be the same individual and counted as a single herbivory event.

Statistical analyses

We conducted all analyses in R v3.5.1 (R Development Core Team 2018). To evaluate mussel density effects on water-column nutrient concentrations (H1), we evaluated changes in NH_4^+-N by backwards stepwise generalized linear mixed model (GLMM) comparison (package *Rcmdr*) with mussel biomass, *J. americana* biomass production, and final algae cover as fixed effects and with week and water temperature as random effects. SRP showed a nonlinear threshold effect in response to mussel density, so we analyzed this relationship with a piecewise regression (package *segmented*) on $\log_e + 1$ transformed SRP values. We used linear regression to relate mussel biomass to plant biomass production (H2a) and pairwise Spearman correlations to evaluate trade-offs in biomass allocation between tissue compartments, followed by linear regression to determine if significant trade-offs were related to mussel density (H2b). We used linear regression to evaluate relationships between mussel density and *J. americana* C:N, C:P, and N:P ratios (H3a) and δ^{15}N (H3b). We compared δ^{15}N from an initial subsample of *J. americana* from a

reference tank to postexperimental samples and between mussel treatments and controls using Wilcoxon tests. We further analyzed a subset of the data above the threshold breakpoint (*BP*) determined by our piecewise regression (i.e., when mussel density affected SRP). We used median-based robust regression, which limits influence by outliers in small data sets (package *mblm*), to determine if the increase in SRP influenced *J. americana* C:P and N:P (H3a).

Results

Mats of filamentous algae formed in all mesocosms containing mussels, likely seeded from remnant algae on mussel shells despite vigorous cleaning. With one exception, algal mats did not develop in the nonmussel controls. Filamentous algae influence nutrient dynamics in mesocosms (Parr et al. 2020) and thus indirectly compete with *J. americana* for nutrients, so we removed control treatments from our analyses but present their means in Appendix S3: Table S1.

High mussel density increases water-column phosphorus

Ambient NH_4^+-N concentration (±SE) was 82.56 ± 5.41 µg NH_4^+-N/L^{-1}. The best GLMM contained only week as a significant random effect ($P = 0.01$), and thus no response to mussel density (Appendix S3: Fig. S1). Mean SRP (±SE) was 7.55 ± 1.23 µg P L^{-1} and showed a nonlinear threshold effect; there was no relationship between mussel density and SRP at low mussel densities, switching to a log-linear positive relationship at higher mussel densities (Fig. 1a). Piecewise regression analysis estimated the breakpoint (*BP*) and the magnitude of the change in slope ($U = $ slope$_{aboveBP} - $ slope$_{belowBP}$) of the nonlinear SRP response to mussel density (df = 82, *BP* [±SE] = 301.54 ± 114.54 g/m^{-2}, U [±SE] = 0.0033 ± 0.0021, $R^2 = 0.11$; Fig. 1a).

Mussel density increases algal and plant biomass

Algal cover strongly increased with mussel density ($F_{1,30} = 61.15$, $P < 0.001$, $R^2 = 0.66$; Fig. 1b). Mean *J. americana* biomass production was 13.99 ± 0.52 g (4.91 ± 0.27%) and increased linearly with mussel density ($F_{1,30} = 5.18$, $P = 0.03$, $R^2 = 0.12$; Fig. 1c). Pooled aboveground biomass increased marginally ($F_{1,30} = 3.39$, $P = 0.076$, $R^2 = 0.08$; Appendix S3: Fig. S2a) and belowground biomass production increased significantly ($F_{1,30} = 5.28$, $P = 0.029$, $R^2 = 0.12$; Appendix S3: Fig. S2b). Belowground biomass increased at a faster rate, but there was no change in the aboveground:belowground biomass ratio as mussel density increased ($F_{1,30} = 0.30$, $P = 0.59$; Appendix S3: Fig. S2c). Pairwise correlations between the proportional biomass of each tissue compartment (Appendix S3: Fig. S3) revealed no significant trade-offs by *J. americana* in allocation within
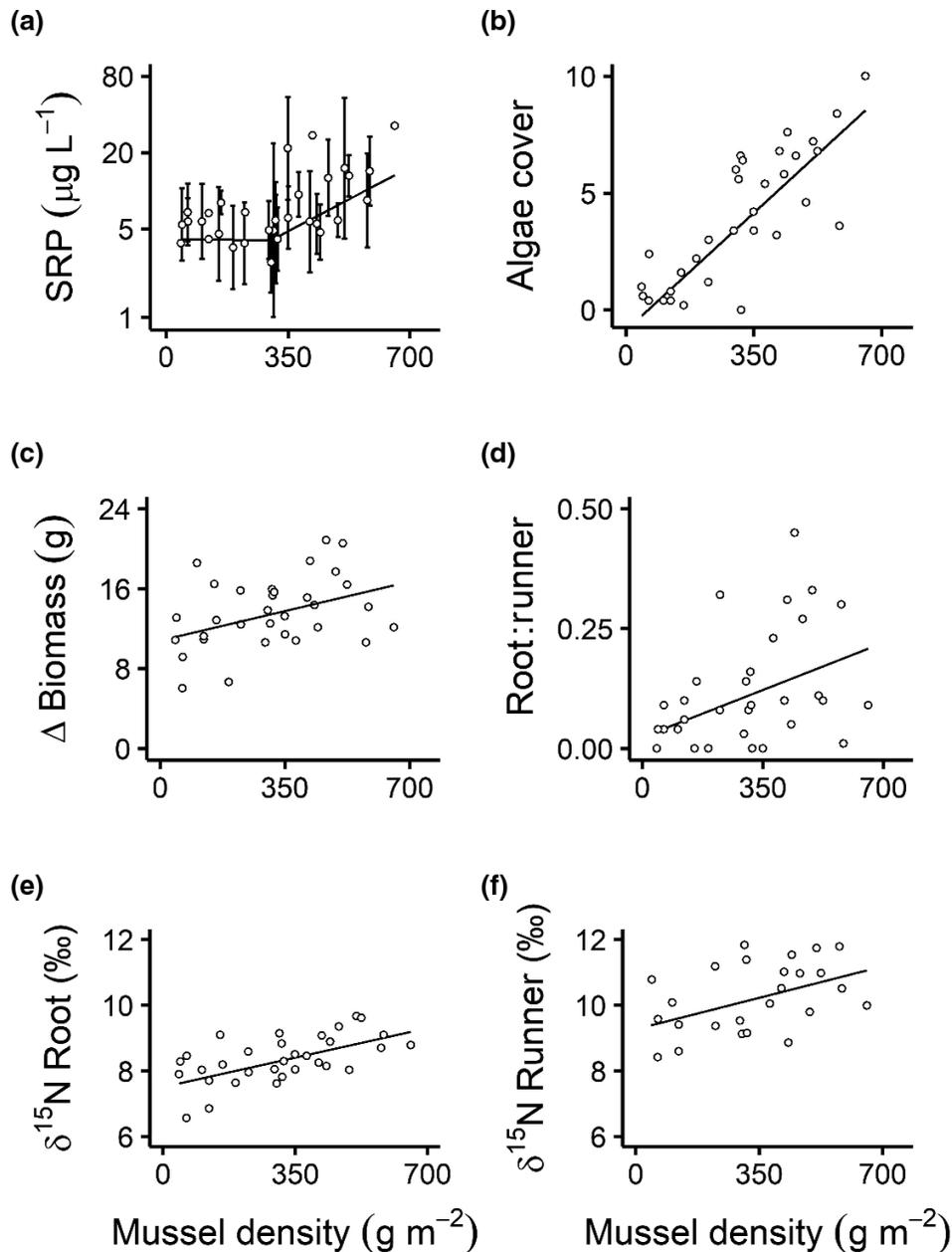
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FIG. 1. The effects of increasing mussel density on ambient nutrient availability and on algal and *Justicia americana* biomass. (a) Soluble reactive phosphorus (SRP) concentration shows no relationship to mussel density at low mussel densities. The relationship becomes positive at approximately 301.54 g/m^2 mussel biomass (Fig. 1a; df = 82, breakpoint [*BP*] [$\pm$SE] = 301.54 $\pm$ 114.54, *U* [$\pm$SE] = 0.0033 $\pm$ 0.0021, R^2 = 0.11). The *y*-axis is on a natural log scale. (b) Algae cover increased linearly as a function of mussel density ($F_{1,30}$ = 61.15, *y* = 0.013*x* − 0.24, *P* < 0.001, R^2 = 0.66). (c) *Justicia americana* biomass production over 9 weeks (Δ biomass) as a function of mussel biomass density ($F_{1,30}$ = 5.18, *P* = 0.03. *y* = 0.0078*x* + 11.17, R^2 = 0.12). (d) Surface runner-to-subsurface root biomass ratio increases with mussel biomass density ($F_{1,30}$ = 5.13, *P* = 0.03, *y* = 0.00026*x* + 0.035, R^2 = 0.12). (e) Roots ($F_{1,30}$ = 15.82, *P* < 0.001, *y* = 0.0024*x* + 7.62, R^2 = 0.32) and (f) runners ($F_{1,23}$ = 5.42, *P* = 0.03, *y* = 0.0025*x* + 9.39, R^2 = 0.16) had significantly enriched δ^{15}N with increasing mussel density. Leaf and stem δ^{15}N showed no relationship to mussel density.

the aboveground biomass compartments, or between above- and belowground. However, a strong shift occurred within the belowground biomass. Allocation to sediment surface runners versus subsurface roots (runner:root ratio) increased with mussel density ($F_{1,30}$ = 5.13, *P* = 0.03, R^2 = 0.12; Fig. 1d).

High mussel density alters plant tissue composition

Mean C:N, C:P, and N:P ratios, and percentage of C, N, and P for tissue compartments, are presented in Appendix S3: Table S2. δ^{15}N signatures are detailed in Appendix S3: Table S3. Mean δ^{15}N for leaves, stems,

and roots was significantly higher after exposure to mussels ($P < 0.05$ for each); runners were not compared because they were destroyed during initial field sample collection. Tissue compartments also differed significantly from one another ($P < 0.05$ for each). However, when we compared nonmussel controls to mussel mesocosms, we found that $\delta^{15}N$ of aboveground tissues were not significantly different in the mussel-exposed mesocosms (leaf $P = 0.44$; stem $P = 0.63$). Root $\delta^{15}N$, on the other hand, was significantly higher in mussel mesocosms than controls ($P = 0.014$), and although runners were not significantly higher in ^{15}N than nonmussel runners ($P = 0.14$), the mean was slightly (about 7%) higher (Appendix S3: Tables S1 and S3). Mussel density was positively related to $\delta^{15}N$ enrichment in roots ($F_{1,30} = 15.82$, $P < 0.001$, $R^2 = 0.32$; Fig. 1e) and runners ($F_{1,23} = 5.42$, $P = 0.03$, $R^2 = 0.16$; Fig. 1f), but did not affect $\delta^{15}N$ in stems ($F_{1,30} = 0.10$, $P = 0.75$) or leaves ($F_{1,30} = 1.40$, $P = 0.25$). Variability in *J. americana* C:N:P nutrient content was not well explained by the full mussel density gradient for any of the biomass compartments sampled.

At high mussel densities (those greater than piecewise regression *BP*) SRP concentration significantly decreased *J. americana* C:P ratios in leaves ($V_{17} = 35$, $P = 0.01$; Fig. 2a), stems ($V_{17} = 30$, $P = 0.007$; Fig. 2b), and roots ($V_{17} = 37$, $P = 0.02$, Fig. 2c). Runner C:P showed no relationship with SRP ($V_{13} = 85$, $P = 0.17$; Fig. 2d). N:P ratios also decreased significantly in leaves ($V_{17} = 20$, $P = 0.001$; Fig. 2e), stems N:P ($V_{17} = 32$, $P = 0.009$; Fig. 2f), and roots ($V_{17} = 44$, $P = 0.04$; Fig. 2g). Runner N:P did not respond to increasing SRP ($V_{13} = 59$, $P = 0.98$; Fig. 2h).

Terrestrial vertebrate herbivores regularly consume J. americana

Of 10 motion-sensing camera traps, 5 were destroyed by flooding in August 2019. From the five recovered cameras, terrestrial vertebrate herbivores visited *J. americana* stands on 190 separate instances (Appendix S2: Table S1). We observed 85 herbivory events by wild vertebrates and 25 herbivory events by domestic cattle (*Bos taurus*) for a total of 110 herbivory observations. 72% of herbivory events occurred in August, 28% in September, and 0% in October (Appendix S2: Table S2). Of the 85 wild vertebrate herbivory events, 45% were by white-tailed deer (*Odocoileus virginianus*) and 55% were by wild boar (*Sus scrofa*). Examples for each species can be found in Videos S1–S4.

DISCUSSION

Our results support the hypothesis that dense aggregations of aquatic animals increase emergent macrophyte production, as increasing mussel density promoted *J. americana* biomass production. We did not find support for an increase in NH_4^+-N concentrations as mussel
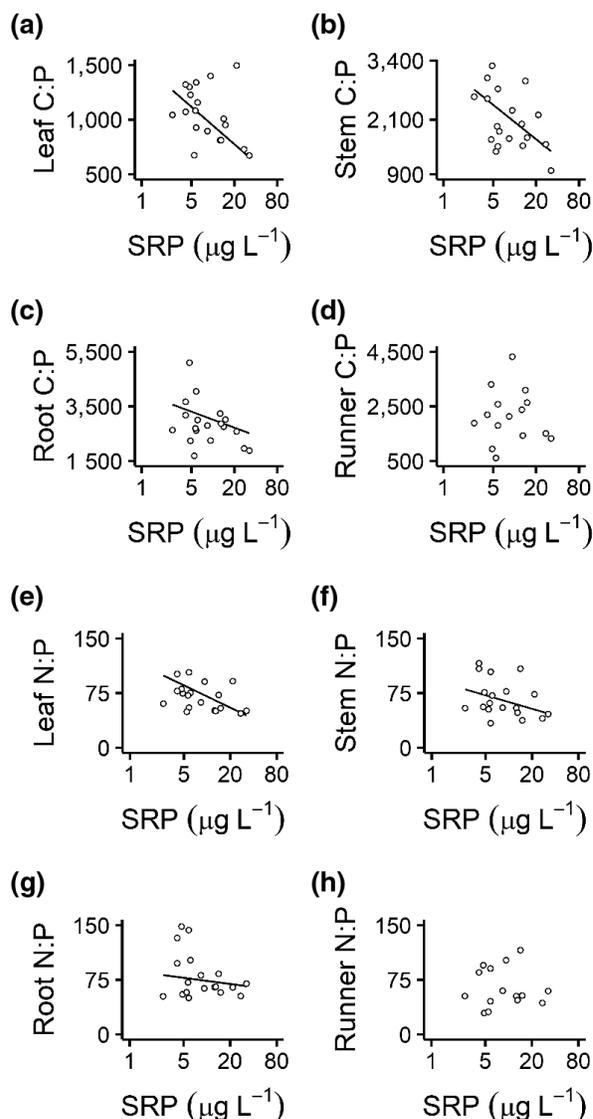

FIG. 2. *Justicia americana* C:P and N:P ratios decreased in three of the four biomass compartments sampled above the threshold in mussel density where soluble reactive phosphorus (SRP) increases. As ambient SRP concentration increased, *J. americana* C:P significantly decreased in (a) leaves ($V_{17} = 35$, $P = 0.01$, $y = -176x + 1{,}266.5$), (b) stems ($V_{17} = 30$, $P < 0.01$, $y = -389.7x + 2{,}758.5$), and (c) roots ($V_{17} = 37$, $P = 0.02$, $y = -301.9x + 3{,}560.3$). C:P of runners (d) was not significantly related to SRP ($V_{13} = 85$, $P = 0.17$). As ambient SRP concentration increased, N:P of (e) leaves ($V_{17} = 20$, $P = 0.001$, $y = -15.78x + 99.1$), (f) stems = ($V_{17} = 32$, $P = 0.009$, $y = -9.48x + 79.83$), and (g) roots ($V_{17} = 44$, $P = 0.04$, $y = -4.36x + 81.271$) also significantly decreased. (h) N:P of runners was not significantly related to SRP ($V_{13} = 59$, $P = 0.98$). Note the different scales on some *y*-axes. The *x*-axes are on a natural log scale.

density increased, but SRP concentrations increased at high mussel densities, partially supporting the hypothesis that mussels increase ambient nutrient availability. At elevated SRP concentrations, the C:P ratio of aboveground tissues decreased, indicating that animal

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aggregations may increase *J. americana* tissue nutrient content. In addition, increased *J. americana* biomass in response to increasing mussel biomass will result in overall higher N and P storage in *J. americana*, even where tissue nutrient content varied little. This suggests that macrophyte stands growing near animal-generated biogeochemical hotspots may store greater quantities of aquatic-derived nutrients than stands in other areas. Finally, our field observations revealed frequent herbivory by terrestrial vertebrates on *J. americana*, indicating that these animals play a significant role in transferring mussel- and macrophyte-derived resources to adjacent terrestrial ecosystems.

Although relative allocation to aboveground *J. americana* biomass did not increase as we hypothesized, *J. americana* did alter allocation within its belowground biomass in response to mussel density—relatively more biomass to runners at the sediment–water interface than subsurface roots as mussel density increased. Terrestrial plants can grow larger runners in response to nutrient additions, enhancing acquisition of light and nutrients (Dong and de Kroon 1994, Nicholls 2011), so we suspect mussel-derived nutrients drove increased runner production. Runners help in vegetative propagation and help form the biomass network that stabilizes river sediments in *J. americana* stands. Increased runner production may enhance substrate stability in stream reaches with mussel beds, a positive feedback that would promote mussel abundance. Although all biomass compartments were enriched in ^{15}N at the end of the experiment, likely because of evaporation and fractionation in our water source, roots and runners were further enriched in high-density mussel treatments. Either the initial transplant into the mesocosm or mussel burrowing may have destroyed some belowground biomass, and *J. americana* may have increased N uptake to regenerate these lost tissues. Furthermore, *J. americana* does not have the ability to fix N, relying totally upon bioavailable N from the water. Root and runner regeneration would thus increase mussel-derived N in belowground tissues, contributing to a lack of response in ambient NH_4^+-N and a positive response in δ^{15}N, as animal-derived N tends to be enriched in ^{15}N (Post 2002). The structural demands of regeneration may thus have prevented *J. americana* from storing excess N, which would have been necessary for tissue C:N ratios to decrease.

Consumer-driven nutrient recycling in mussel beds has well documented bottom-up effects on aquatic and riparian food webs (Allen et al. 2012, Atkinson et al. 2014). Here, the development of filamentous algae mats across treatments likely limited our ability to detect nutrient responses in both the water and *J. americana* tissues at low mussel densities. Competition between the two autotrophs (algae and *J. americana*) likely suppressed ambient concentrations of both SRP and NH_4^+-N. However, at higher mussel densities SRP availability probably exceeded the combined demand of the competing autotrophs—mussel excretion N:P ratios tend to be

far lower than algal N:P ratios and the *J. americana* N:P ratios we observed here (Sterner and Elser 2002, Atkinson et al. 2013). *Justicia americana* would thus have relatively greater demand for N, further contributing to the absence of patterns in NH_4^+-N despite the observed increase in SRP and the corresponding decrease in C:P and N:P of *J. americana* tissues.

Our results suggest that macrophytes may form a globally distributed conduit for nutrients concentrated by animals at aquatic biogeochemical hotspots to subsidize nearby terrestrial ecosystems. We observed numerous instances of herbivory on *J. americana* by terrestrial herbivores. Nearly 45% of the wild herbivores we observed feeding on *J. americana* via game camera belonged to Family: Cervidae (*O. virginianus*). These mammals are abundant and widely distributed. *Odocoileus virginianus* populations in Oklahoma alone are estimated at 500,000–750,000 individuals (D. Barber, ODWC, *personal communication*), and the herbivore responsible for most other herbivory events, *S. scrofa*, is a pervasive invader across the globe (Barrios-Garcia and Ballari 2012). If browsing on emergent macrophytes is as common and widespread in other systems as it is on the Kiamichi River, there is great potential for terrestrial mammalian herbivores to transfer aquatic-derived resources from *J. americana* to the surrounding landscape.

In light of the results of this study, we revisit the potential importance of macrophytes as an intermediary for aquatic-to-terrestrial resource subsidies using the quantity, quality, timing, and duration framework (Subalusky and Post 2019). Because macrophytes occur at the aquatic–terrestrial interface at a global scale (Chambers et al. 2008), the quantity of aquatic-to-terrestrial resource transfer via macrophytes is likely high. Consumer-driven nutrient recycling may add to the quantity of macrophyte biomass at animal-generated hotspots. The quality of macrophytes as a food resource is likely to be high as well, due to the high nutrient content of aquatic primary producers relative to terrestrial plants (Sterner and Elser 2002). This quality may be further increased by aquatic animals that recycle nutrients into plant-available forms. Our experiment showed an increase in plant P at high mussel densities, a key nutrient for bone and antler formation in vertebrates such as cervids and for fast-growing organisms like insects (Sterner and Elser 2002). As discussed above, cervids are widely distributed, important terrestrial game species and create a conduit for aquatic-to-terrestrial nutrient subsidies by foraging on macrophytes (Ceacero et al. 2014, Bump 2018). Although the duration of most macrophyte production in temperate zones is limited to the summer, this is the time of greatest effect for stream biogeochemical hotspots and a period of great metabolic demand for many animals. Nutrient effects generated by hotspots may subsidize this summertime demand via macrophytes.

Thus, ongoing declines in aquatic animal biomass may have bottom-up impacts that reverberate into terrestrial

ecosystems (Knight et al. 2005, Dirzo et al. 2014, Schindler and Smits 2017). The multistep linkage between biogeochemical hotspots generated by aquatic animals, macrophytes, and terrestrial herbivores needs further study, especially in the field, to draw more robust conclusions about the generality and magnitude of such resource subsidies. We must also expand our knowledge of the flexibility in elemental composition exhibited by macrophytes; for example, if *J. americana* responds to altered mineral concentrations caused by the buildup of mussel shells in gravel bar sediments. Beyond N and P, mineral nutrients like sodium and calcium can influence the consumption of macrophytes (Ceacero et al. 2014). In places where macrophytes provide key nutrients and minerals in the diets of game species or livestock (e.g., Bump 2018), this may create new conservation imperatives for aquatic species. Quantitative field studies on the spatial extent and the nutrient and mineral composition of aquatic biogeochemical hotspots and macrophytes, and on the intensity and frequency of herbivory by terrestrial herbivores on aquatic plants are needed. The effects of biogeochemical hotspots on flowering and pollination in *J. americana* and other macrophytes also deserve attention as a vector for aquatic-to-terrestrial subsidies. Our results suggest that the importance of plant–animal associations that bridge ecosystem boundaries may be underappreciated, and that multistep linkages such as the one we explored here are fundamentally important in conducting resource flows between ecosystems.

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