12-2017

Lethal and Sublethal Effects of Novel Terrestrial Subsidies from an Invasive Shrub (Lonicera maackii) on Stream Macroinvertebrates

Ryan W. McEwan
University of Dayton, rmcewan1@udayton.edu

Kevin W. Custer
University of Dayton

Eric B. Borth
University of Dayton

Sean D. Mahoney
University of Dayton

Lethal and sublethal effects of novel terrestrial subsidies from an invasive shrub (*Lonicera maackii*) on stream macroinvertebrates

Kevin W. Custer[1,2], Eric B. Borth[1,3], Sean D. Mahoney[1,4], and Ryan W. McEwan[1,5]

[1]Department of Biology, University of Dayton, Dayton, Ohio 45469 USA

Abstract: The biology of headwater streams is intimately linked to that of the surrounding terrestrial environment through organic matter subsidies. *Lonicera maackii*, an invasive shrub that is becoming abundant in headwater stream riparian areas, deposits substantial quantities of organic matter into the aquatic system. This organic material has allelopathic effects on terrestrial plants and insects, and a growing body of work suggests strong connections between *L. maackii* invasion and aquatic biota. *Lonicera maackii* deposits fruit and flowers in quantities and timings that are unique, and we tested the hypothesis that these subsidies would negatively affect survival and growth of laboratory-cultured *Hyalella azteca* and field-collected *Anthopotamus verticis* and *Allocapnia* spp. Invertebrates were exposed to a gradient of fruit (reference sediment + 0, 0.31, 0.62, 1.25, or 2.5 g dry mass [DM]) and flower (reference sediment + 0, 0.30, 0.60, 1.2, or 2.4 g DM) biomass in laboratory and field sediment exposure tests. *Hyalella azteca* survival was significantly reduced by exposure to *L. maackii* fruit in the laboratory and in the field exposures, and a negative effect was observed for *A. verticis* ($p < 0.05$). *Lonicera maackii* flower biomass was associated with negative effects on survival of *H. azteca* in the field and laboratory exposures and of *A. verticis* in the laboratory exposure. During the laboratory exposures, dissolved O_2 (DO) and pH were <2 mg/L and 5.5, respectively. In the field exposures, DO and pH were comparable to stream conditions during fruit exposures, declining significantly with increasing flower biomass. Our results suggest that *L. maackii* fruit and flowers, novel subsidies in these systems, can negatively affect benthic organism survival and growth. Research focused on verifying this novel subsidy hypothesis for *L. maackii* and other species could enhance our understanding of invasion biology and terrestrial–aquatic linkages.

Key words: *Lonicera maackii*, Hyalella, sediments, subsidies, headwater streams, invasive species

Organic-matter subsidies from the terrestrial environment are a foundational resource for aquatic food webs in headwater streams (Hawkins and Sedell 2009, Wallace et al. 2015). Aquatic microbial and macroinvertebrate communities use allochthonous inputs as nutrient and energy resources and for habitat (Cummins and Klug 1979, Vannote et al. 1980). Large-scale alterations in riparian plant communities can alter the exchange between aquatic and terrestrial habitats (Naiman and Decamps 1997, Baxter et al. 2005) and influence food webs and nutrient/energy cycling from local-to-watershed scales (Tank et al. 2010). Terrestrial–aquatic interactions are critical to broader biodiversity–ecosystem function relationships (Naiman et al. 1993), and understanding these linkages is important for watershed management (Likens and Bormann 1974, Kominoski et al. 2011).

Headwater streams are a critical component of larger freshwater systems, and a growing body of evidence that suggests the ecological health of freshwater systems is linked to functions provided by headwater streams (Lowe and Likens 2005). Headwater streams are tightly linked to the surrounding landscape through cross-system subsidies and are highly vulnerable to disturbance (Cummins 1974, Vannote et al. 1980, Baxter et al. 2005). For instance, aquatic macroinvertebrate composition is strongly associated with allochthonous alterations of stream chemistry, and significant losses are associated with acidification (Guerold et al. 2000). Aquatic biota are highly responsive to influences associated with urbanization and agricultural development, with diversity positively associated with riparian forests and negatively associated with impervious surfaces (Moore and Palmer 2005). Losses of aquatic biota are a conservation concern and may influence foodweb dynamics (Baxter et al. 2005) and can result in significant indirect effects (Wallace et al. 1989). Negative effects on headwater streams accumulate across larger

E-mail addresses: [2]kevin.custer@wright.edu; [3]mahoneys3@udayton.edu; [4]eborth1@udayton.edu; [5]To whom correspondence should be addressed, ryan.mcewan@udayton.edu

DOI: 10.1086/694895. Received 29 September 2016; Accepted 13 August 2017; Published online 3 October 2017.
Freshwater Science. 2017. 36(4):750–759.

areas and can create watershed-scale effects (Freeman et al. 2007).

Invasions by non-native plant species are an increasingly prevalent feature of riparian areas of headwater streams with potential for significant alterations of stream biota. Invasive species are associated with edge habitats, such as those created by waterways, and fragmented riparian forests near human habitation are particularly vulnerable (Yates et al. 2004, Bartuszevige et al. 2006). Flowing streams may be a dispersal pathway for some invasive species (Planchuelo et al. 2016). Exotic species often possess a suite of unique traits that enable their invasion (Callaway and Ridenour 2004). The creation of dense populations in the riparian areas of streams has substantial potential to influence aquatic biota directly or as plant biomass exhibiting those unique traits is transferred as a subsidy. For example, a significant body of research has explored the potential role of riparian invasion by *Tamarix* in a variety of alterations to aquatic ecosystems (Shafroth et al. 2005, Hultine et al. 2010). Riparian invasion of the exotic shrub *Elaeagnus angustifolia* influences stream nitrogen dynamics (Mineau et al. 2011) and has been associated with a 25× increase in litter that is much more recalcitrant than native species (Mineau et al. 2012). In contrast, *Ailanthus altissima* is an invasive tree that influences stream biology partially via deposition of leaf material that decomposes rapidly (Swan et al. 2008). These studies, among others, provide strong support for the concept that riparian invasion has potential to alter headwater stream biota and suggest the mechanisms are complex and species specific.

The deciduous shrub Amur honeysuckle (*Lonicera maackii* (Rupr.) Herder) is a non-native invasive plant that has proliferated rapidly in eastern North America. Invasion by this species can influence the biology and function of forests (Luken and Thieret 1996, Hutchinson and Vankat 1997, Gould and Gorchov 2000, McNeish and McEwan 2016). An allelopathic relationship between this species and terrestrial insects has been established in field and laboratory experiments (McEwan et al. 2009, Lieurance and Cipollini 2012, 2013a, b), and the leaf chemistry of foliage suggests higher N content and lower lignin than native species (McEwan et al. 2012). *Lonicera maackii* foliage breaks down rapidly in both terrestrial (Arthur et al. 2012, Poulette and Arthur 2012, Trammell et al. 2012) and aquatic environments (McNeish et al. 2012). Colonization of experimental leaf packs strongly indicated that *L. maackii* foliage supports a unique macroinvertebrate community (McNeish et al. 2012), and experimental riparian removal indicated a connection between riparian invasion and aquatic biota. Experimental microcosm assays indicated that *Culex pipiens* larvae survivorship is enhanced by the presence of *L. maackii* foliar leachate (Shewhart et al. 2014). In summary, a variety of data sources suggest a link between *L. maackii* invasion and aquatic macroinvertebrate community composition, but the mechanism(s) of linkage remain unclear. Illuminating this relationship is important for advancing understanding of terrestrial–aquatic linkages and for practical reasons because managing the invasion of *L. maackii* is a primary concern for land managers across vast areas of the American Midwest.

The objective of our study was to characterize selected benthic macroinvertebrate responses to subsidies from *L. maackii*. We assessed the influence of flowers and fruits for several reasons. First, the deposition of these materials is copious and is a unique feature of *L. maackii* invasion because no native riparian species generates a similar subsidy. Second, the timeline of fruit and flower deposition coincides with important periods in macroinvertebrate life cycles. Last, the biology of *L. maackii* fruit is of particular interest because it exhibited strong allelopathic effects on seeds of native plants (McEwan et al. 2010). We used field and laboratory microcosms containing natural sediments to test the hypothesis (H_1) that benthic macroinvertebrates will respond to the presence of *L. maackii* subsidies with increased mortality and decreased growth. We expected the flow-through field microcosms to increase water exchange, thereby affecting the concentration of materials compared to the laboratory system. Therefore, we hypothesized (H_2) that field trials would yield lower negative effects than those conducted in the laboratory. We chose *Hyalella azteca* (Amphipoda:Hyalellidae) because of its status as a model organism for sediment toxicity testing (USEPA 2000, Environment Canada 2013). We added 2 test taxa that are frequent inhabitants of regional streams: *Anthopotamus verticis* (Ephemeroptera:Potamanthidae) and *Allocapnia* spp. (Plecoptera: Capniidae). Given its status as a model organism, we hypothesized (H_3) that *H. azteca* would be the most sensitive species in our trials.

METHODS

Lonicera maackii fruit and flower collection and storage

We collected *L. maackii* fruit and flowers from 1st- or 2nd-order headwater stream riparian areas in southwest Ohio, USA. We collected fruit between 1 October and 9 December 2015 and flowers between 9 May and 6 June 2016. During these periods, we picked fruit and flowers from branches overhanging the stream when their production was high, stored them in plastic bags at 4°C, and used them within 2 d of collection.

Exposures in the laboratory and field

We cultured *H. azteca* under controlled laboratory conditions following recommendations by the USEPA (2000) in dechlorinated City of Dayton tap water. Organisms were between 7 and 14 d old upon exposure initiation. We collected *A. verticis* from the Great Miami River, Ohio, USA (Custer et al. 2016), and *Allocapnia* spp. from a headwater stream in Englewood, Ohio. We transported all field-collected organisms in coolers with site water, and we used native leaves from the stream as substrate during transport. We in-

troduced all organisms to exposure conditions (laboratory or field) <2 h after collection. Laboratory organisms were transported to the field in centrifuge tubes, and organisms were temperature acclimated to ±5°C of stream-water conditions prior to placing them in the chambers.

Sediment-exposure experiments

Experimental design We added the same number of organisms to each replicate in laboratory and field exposures: 10 *H. azteca*, 5 *A. verticis*, and 5 *Allocapnia* spp. Each fruit and flower sediment exposure had 4 treatments plus a reference. Each laboratory and in situ exposure had 4 and 3 replicates/treatment, respectively. From October to December 2015, we ran 6 fruit exposures (*H. azteca* laboratory and field, *A. verticis* laboratory and field, and *Allocapnia* spp. 2 field), and in May and June 2016 we ran 5 flower exposures (*H. azteca* 2 laboratory and 2 field, and *A. verticis* laboratory). For analysis of organism survival and growth, we used only results from exposures with >75% survival in the reference treatment, but we used all exposures to analyze experimental conditions (temperature, dissolved O_2, specific conductivity, and pH), *L. maackii* biomass, and physicochemical relationships.

Exposure sediments We collected sediments from a 2nd-order headwater stream at Englewood Metro Park. We used a hand trowel to collect sediments, transported them to the laboratory on ice, and stored them at 4°C until needed. We measured sediment % solids and total organic C (TOC) following methods by Heiri et al. (2001) and Santisteban et al. (2004). We dried sediments at 105°C for 24 ± 2 h for % solid determination, and then estimated TOC by loss on ignition (LOI) (550°C for 4 ± 0.5 h). We used a correction factor of 0.38 to convert LOI to organic C (Redfield 1934). Sediments were mainly cobble/gravel/sand substrates with 84 ± 1.8% as solids and a total organic C content of 1.3 ± 0.08%. We used them as the reference sediment for all fruit and flower exposures because of high *H. azteca* survival rates (>90%) based on current and previous laboratory sediment toxicity tests (KWC and RWM, unpublished data).

Fruit and flower additions Prior to exposure, we weighed all fruit and flowers to the nearest 0.01 g (wet mass) and added them to beakers or chambers. We made dry mass (DM) corrections by drying replicate samples of fruit and flowers at 105°C for 24 h, and weighing to the nearest 0.01 g. We used this correction factor to present all *L. maackii* fruit and flower biomass. Fruit and flower density estimates were based on DM divided by the surface area (0.003 m^2) of the beaker or in situ chamber.

We loaded *L. maackii* wet fruit biomass into each beaker/chamber at the equivalent of mean DM = 0.31, 0.62, 1.25, or 2.5 g (Table 1). We loaded wet flower biomass into beakers/chambers at the equivalent of mean DM = 0.30, 0.60, 1.2, and 2.4 g DM (Table 2). For exposures carried out later in the fruit and flower seasons, collecting enough biomass that was attached to the shrubs at our established collection sites became difficult. When we were unable to obtain sufficient biomass, we reduced the number of replicates from 6 to 4.

Laboratory exposures We exposed *H. azteca* and *A. verticis* in a standard laboratory sediment toxicity design (USEPA

Table 1. Mean (±SD) values of physicochemical variables in laboratory and field exposures during *Lonicera maackii* fruit sediment exposures. Treatments are fruit dry mass added to microcosms (Reference = no fruit added). DO = dissolved O_2, Cond = specific conductivity, Ortho-P = orthophosphate, Fruit = dry mass of *L. maackii* fruit per area of the bottom of each mesocosm, Wiles = Wiles Creek, nm = not measured.

Treatment	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Temperature (°C)	DO (mg/L)	Cond (µS/cm)	pH	Ortho-P (mg/L)	Fruit g/m^2
Laboratory								
Reference	156 ± 16	97 ± 18	23.0 ± 0.4	7.00 ± 0.7	439 ± 22	7.98 ± 0.13	0.02 ± 0.02	0
0.31 g	209 ± 65	161 ± 64	22.9 ± 0.5	4.96 ± 1.8	496 ± 80	7.57 ± 0.32	1.75 ± 1.14	105 ± 0.7
0.62 g	249 ± 93	162 ± 50	22.7 ± 0.4	4.52 ± 2.2	525 ± 116	7.25 ± 0.50	1.80 ± 0.50	209 ± 0.8
1.25 g	322 ± 163	177 ± 76	22.5 ± 0.3	4.11 ± 2.6	589 ± 215	6.94 ± 0.82	1.60 ± 0.36	417 ± 1.1
2.50 g	456 ± 215	242 ± 163	22.5 ± 0.3	3.30 ± 3.1	722 ± 389	6.42 ± 1.06	4.60 ± 3.90	834 ± 2.0
Field								
Reference	347 ± 29	367 ± 25	14.2 ± 1.6	10.17 ± 0.72	1009 ± 23	8.09 ± 0.05	0.23 ± 0.12	0
0.31 g	357 ± 15	398 ± 98	13.9 ± 1.6	9.81 ± 1.15	988 ± 43	8.09 ± 0.05	0.23 ± 0.17	104 ± 0.5
0.62 g	360 ± 24	430 ± 72	14.1 ± 1.6	9.58 ± 0.94	994 ± 46	8.06 ± 0.10	0.23 ± 0.19	208 ± 0.9
1.25 g	348 ± 29	372 ± 62	14.0 ± 1.6	9.03 ± 1.29	1011 ± 40	8.01 ± 0.19	0.20 ± 0.08	417 ± 0.8
2.50 g	359 ± 23	362 ± 27	13.9 ± 1.6	9.32 ± 1.29	1021 ± 26	8.00 ± 0.17	0.17 ± 0.06	834 ± 0.7
Wiles	366 ± 21	373 ± 11	13.8 ± 1.5	10.00 ± 0.87	1040 ± 25	8.08 ± 0.07	0.28 ± 0.08	nm

Table 2. Mean (±SD) values of physicochemical variables in laboratory and field exposures during *Lonicera maackii* flower sediment exposures. Treatments are flower dry mass added to microcosms (Reference = no flowers added). DO = dissolved O_2, Cond = specific conductivity, Ortho-P = orthophosphate, Flowers = dry mass of *L. maackii* flowers per area of the bottom of each mesocosm, Wiles = Wiles Creek, nm = not measured.

Treatment	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Temperature (°C)	DO (mg/L)	Cond (µS/cm)	pH	Ortho-P (mg/L)	Flowers (g/m^2)
Laboratory								
Reference	169 ± 3	97 ± 18	23.1 ± 0.5	6.31 ± 0.7	521 ± 12	7.68 ± 0.24	0.16 ± 0.08	0
0.30 g	176 ± 6	105 ± 15	23.0 ± 0.6	3.70 ± 1.7	535 ± 19	7.54 ± 0.11	0.15 ± 0.08	98 ± 0.1
0.60 g	177 ± 8	116 ± 3	22.9 ± 0.6	2.32 ± 2.0	547 ± 24	7.37 ± 0.14	0.14 ± 0.06	198 ± 0.9
1.20 g	180 ± 12	111 ± 15	22.8 ± 0.5	1.93 ± 1.7	566 ± 35	7.08 ± 0.29	0.16 ± 0.16	394 ± 0.5
2.40 g	233 ± 47	157 ± 56	22.8 ± 0.4	1.74 ± 1.5	692 ± 147	6.41 ± 0.46	0.69 ± 0.78	788 ± 1.0
Field								
Reference	332 ± 8	305 ± 4	15.7 ± 1.7	10.38 ± 0.34	1017 ± 10	8.04 ± 0.06	0.04 ± 0.00	0
0.30 g	333 ± 4	310 ± 0	15.5 ± 1.8	9.75 ± 0.82	1016 ± 8	8.04 ± 0.06	0.14 ± 0.16	99 ± 0.1
0.60 g	353 ±14	326 ± 8	15.4 ± 1.8	10.00 ± 0.40	1017 ± 10	8.05 ± 0.07	0.06 ± 0.05	198 ± 0.2
1.20 g	358 ± 13	332 ± 24	15.4 ± 1.8	9.31 ± 0.63	1021 ± 9	7.99 ± 0.09	0.14 ± 0.01	393 ± 0.9
2.40 g	375 ± 0	351 ± 2	15.4 ± 1.8	8.34 ± 0.87	1022 ± 10	7.87 ± 0.16	0.16 ± 0.13	787 ± 0.8
Wiles	326 ± 23	293 ± 136	16.1 ± 2.1	10.26 ± 0.28	1018 ± 9	8.02 ± 0.04	0.05 ± 0.01	nm

2000). Exposure duration was typically 4 d, with the exception of 1 laboratory exposure that ended after 2 d because of high mortality after 24 h. Each organism was exposed in 300-mL beakers with ~100 mL of reference sediment (Englewood headwater stream; see below) and ~175 mL of overlying water. Each set of beakers received 2-L water changes 2× daily following the methods outlined in USEPA (2000). During the laboratory exposures, we fed *H. azteca* ~0.5 mL $beaker^{-1}$ d^{-1} of a slurry of wheatgrass and fish flake food (TetraMin, Blacksburg, Virginia) and *A. verticis* ~0.5 mL $beaker^{-1}$ d^{-1} of a slurry of stream-conditioned *Platanus occidentalis* (American sycamore) or *Acer saccharum* (sugar maple) leaves. We used dechlorinated City of Dayton tap water for all laboratory exposures (hardness = 144–180 mg/L as $CaCO_3$).

Field exposures We exposed *H. azteca*, *A. verticis*, and *Allocapnia* spp. in the field for 4 to 7 d in chambers (Chappie and Burton 1997, Burton et al. 2005). A full description of the chamber construction was published by Burton et al. (2005). Each chamber had 2 windows covered with nylon mesh (149 µm) to allow water circulation and was capped at both ends to contain the organisms. The chambers were placed vertically to mimic a beaker design, with sediment and subsidies loaded similarly to laboratory beakers. The chambers were deployed at Wiles Creek, Englewood, Ohio, USA (Aullwood Farm), a 2nd-order headwater stream.

Physicochemical monitoring

We monitored physicochemical variables during all laboratory and field exposures. We collected water samples from beakers and in situ chambers with 60-mL syringes before sediment processing for organism survival. In situ chambers were equipped with tubing to enable us to sample water in the chamber while it was deployed in the stream. We measured temperature (°C), dissolved O_2 (DO) (mg/L), specific conductivity (µS/cm), pH, and total dissolved solids (TDS) (mg/L) daily with a YSI Pro Series meter (Yellow Springs Instruments, Yellow Springs, Ohio). We measured total orthophosphate (Ortho-P), hardness, and alkalinity of the beaker or chamber water at the end of each laboratory and field exposure. We analyzed Ortho-P within 48 h with a Hach (Loveland, Colorado) DR 2800. We used titrations to measure hardness and alkalinity, corrected to standards, and presented as mg/L $CaCO_3$ (APHA 1995). We used blanks, standards, and method accuracy checks in Hach nutrient analyses. A blank correction was applied when blank concentrations were higher than the detection limit, and these data are presented as blank-corrected concentrations (mg/L).

Organism survival and growth

We recorded survival at the end of each exposure (2, 4, or 7 d) and growth of survivors at the end of 4- and 7-d exposures. We calculated % survival for each replicate by dividing the number of surviving organisms/number of organisms at exposure initiation (n = 5 or 10), then multiplied by 100. We calculated % mortality as the number of dead organisms/number of organisms at exposure initiation, then multiplied by 100. We pooled surviving organisms in each replicate and dried them at 105 ± 2°C for 24 ± 2 h to estimate total DM ± 0.01 mg/replicate and divided by the number of organisms per replicate.

Data analysis

We ran 1-way analyses of variance (ANOVAs) to test survival or growth of each species (*H. azteca*, *A. verticis*, and *Allocapnia* spp.) for each fruit and flower exposure. Species were not combined into beakers or chambers, and fruit and flower exposures were run in different seasons. We used Tukey's multiple comparisons to compare treatment means. We tested ANOVA assumptions with Ryan–Joiner or Kolmogorov–Smirnov tests for normality, and Levene's tests for equal variances. If assumptions were violated, then we used nonparametric Kruskal–Wallis tests. We used linear regression to assess relationships between selected physicochemical parameters and *L. maackii* subsidy biomass added. All statistical analyses were run on Minitab (version 17; Minitab Inc., State College, Pennsylvania).

RESULTS

Aquatic macroinvertebrate responses to fruit

Hyalella azteca responded negatively to increasing fruit biomass in field ($p < 0.001$) and laboratory ($p < 0.001$) exposures (Fig. 1A, B, Table S1). Mortality was greatest (>93%) in the highest fruit biomass treatment in both field and laboratory exposures (Fig. 1A, B). *Anthopotamus verticis* mortality differed between field and laboratory exposures (Fig. 1A, B). During the laboratory exposures, mortality increased to 100% rapidly ($p < 0.001$), and the exposure was terminated after 2 d (Fig. 1B). However, during the field exposure, mortality was much lower (33%) and did not differ among fruit biomass treatments ($p = 0.311$; Fig. 1A). Growth did not differ among fruit biomass treatments (Table S1). *Allocapnia* spp. proved too sensitive for laboratory exposures, and no successful laboratory exposures were achieved. However, *Allocapnia* spp. mortality was low at all fruit biomasses during 4- ($p = 0.838$) and 7-d ($p = 0.903$) field exposures (Fig. 1A), and no growth effects were observed during these exposures (Table S1).

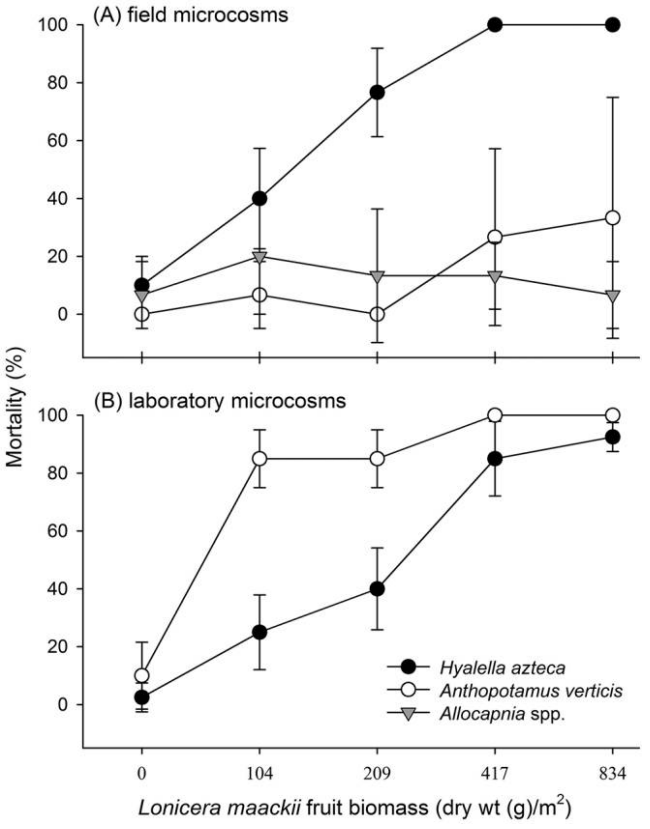

Figure 1. Mean (±SD) % mortality of *Hyalella azteca*, *Anthopotamus verticis*, and *Allocapnia* spp. in response to 4-d field (A) and laboratory (B) sediment exposures to *Lonicera maackii* fruit biomass. *Anthopotamus verticis* exposures were terminated after 2-d because of 100% mortality.

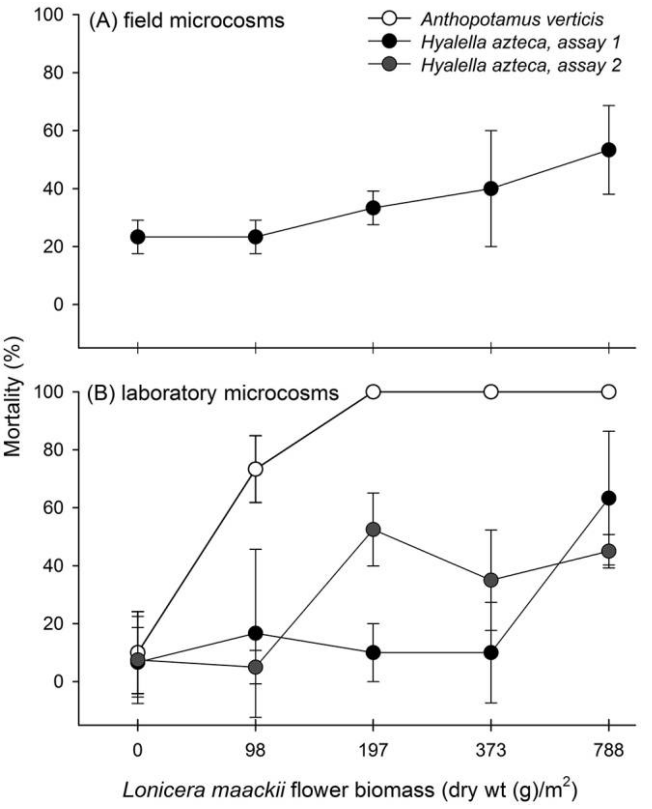

Figure 2. Mean (±SD) % mortality of *Hyalella azteca* and *Anthopotamus verticis* in response to 4-d field (A) and laboratory (B) sediment exposures to *Lonicera maackii* flower biomass.

Aquatic macroinvertebrate responses to flowers

Hyalella azteca and *A. verticis* responded negatively to increasing flower biomass during both laboratory and field exposures (Fig. 2A, B, Table S2). *Hyalella azteca* mortality increased with increasing biomass during both laboratory assays ($p \leq 0.025$); Fig. 2B). *Hyalella azteca* mortality also tended to increase with flower biomass during a field exposure ($p = 0.059$). *Hyalella* mortality was 53% in the highest flower biomass treatment in the field exposure (Fig. 2A)

and 63 and 45% in the 2 laboratory exposures (Fig. 2B). *Anthopotamus verticis* mortality was significantly higher (100%) at the 3 higher flower biomasses than in the reference or the lowest flower biomass treatment in the laboratory exposure ($p < 0.001$; Fig. 2B). During this exposure, one replicate in the reference treatment experienced 100% mortality, unlike the other 2 replicates (0 and 20% mortality). Analyses were run with and without the replicate, and p-values were both significant at $\alpha = 0.05$ ($p = 0.046$ and $p < 0.001$, respectively).

Sediment, water chemistry, and subsidy biomass

Concentrations of DO concentrations fell to <5.00 mg/L during both fruit and flower laboratory exposures and the lowest mean values in our experiments were 1.74 ± 1.5 mg/L in the laboratory flower trials (Tables 1, 2). DO was significantly negatively related to fruit biomass in laboratory exposures ($R^2 = 0.26$ $p = 0.004$), but not in field exposures ($R^2 = 0.07$, $p = 0.153$) (Fig. 3A). Mean DO concentrations were higher (>9.03 mg/L) during fruit field exposures than in the laboratory setting (Table 1). Concentrations of DO

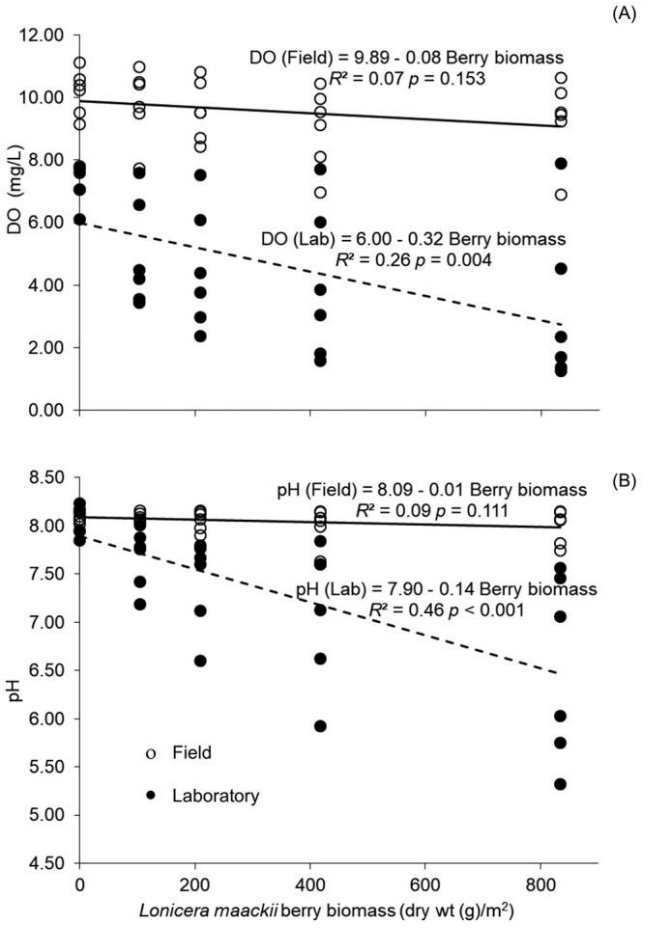

Figure 3. Regressions for dissolved O_2 (DO) (A) and pH (B) across all laboratory (lab) and field sediment exposures as functions of *Lonicera maackii* fruit biomass treatments.

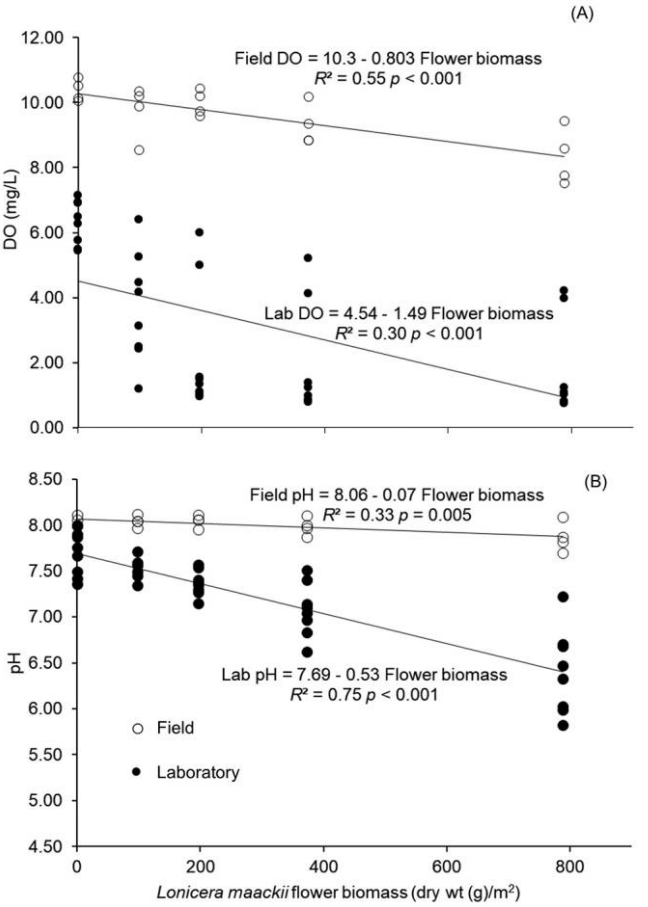

Figure 4. Regressions for dissolved O_2 (DO) (A) and pH (B) across all laboratory (lab) and field sediment exposures as functions of *Lonicera maackii* flower biomass treatments.

were significantly negatively related to flower biomass in both field ($R^2 = 0.55$, $p < 0.001$) and laboratory ($R^2 = 0.30$, $p < 0.001$) exposures (Fig. 4A). In both the field and laboratory flower assays, DO concentration in the highest biomass treatment was lower than the reference; however, this effect was much stronger in the laboratory (Table 2).

Relationships between pH and fruit and flower biomass were similar to those observed with DO. pH declined significantly with increasing fruit biomass during laboratory exposures ($R^2 = 0.46$, $p < 0.001$; Fig. 3B), and mean pH was lowest (6.42 ± 1.06) in the highest fruit biomass treatment (Table 1). pH was not related to fruit biomass in field exposures ($R^2 = 0.09$, $p = 0.111$; Fig. 3B), but was significantly negatively related to flower biomass in both field ($R^2 = 0.33$, $p = 0.005$) and laboratory ($R^2 = 0.75$, $p < 0.001$) exposures (Fig. 4B). The lowest mean pH values (field: 7.87 ± 0.16, laboratory: 6.41 ± 0.46) were observed in the highest flower biomass treatments (Table 2).

In laboratory exposures, conductivity, Ortho-P, hardness, and alkalinity increased as fruit biomass increased (Table 1), whereas conductivity, Ortho-P, hardness, and alkalinity increased as flower biomass increased (Table 2).

However, these water-quality variables were not statistically tested against fruit or flower biomass.

DISCUSSION

Alterations of headwater streams are an increasingly pressing conservation concern especially in relationship to ongoing urbanization and other anthropogenic landscape effects. Invasive species are a particularly complicated kind of anthropogenic effect, wherein native riparian vegetation is replaced by quasi-monocultures of exotic species that often possess a suite of unique traits. Riparian invasion can result in a virtual 'trait monoculture' that shifts the quantity, chemistry, and physical structure of the terrestrial subsidies that influence aquatic communities in headwater streams. Riparian invasion by the exotic shrub *L. maackii* has been linked to changes in the structure and function of aquatic biota (McNeish et al. 2012, 2015). To our knowledge, we are the first to identify novel subsidies from an invasive species (fruits and flowers) as a negative linkage between riparian plant invasion and headwater stream biota.

Lonicera maackii fruit production is copious, and our data suggest that fruit deposition into headwater streams is a negative subsidy for macroinvertebrates. No known native species in the region generates a pulse of fruit biomass as large and as late in the season as that of *L. maackii* (RWM, personal observation and unpublished data). Terrestrial vertebrate species that consume *Lonicera* fruits include rodents (Dutra et al. 2011), birds (Ingold and Craycraft 1983), and deer (Guiden et al. 2015). However, in invaded forests, especially along habitat edges, fruit production is extreme (Lieurance and Landsbergen 2016) and far exceeds the capacity of these species to consume the fruit.

During both field and laboratory exposures, *H. azteca* showed strong negative responses to *L. maackii* fruit. Negative effects of fruit on *H. azteca* survival and growth were stronger in field than in laboratory exposures (refuting H_2), despite the fact that DO concentrations were higher in the field than in the laboratory exposures. This result strongly indicates a direct effect of *L. maackii* fruit on *H. azteca* (discussed below). For reasons we could not ascertain and that were beyond the scope of our study, *Allocapnia* spp. had low reference survival rates during the laboratory exposures. Therefore, we were unable to assess its response to *L. maackii* fruit in a laboratory setting. In the field exposures, *Allocapnia* spp. did not respond negatively to *L. maackii* fruit. Responses by *A. verticis* differed strongly between the field and laboratory exposures. In the laboratory, *A. verticis* did not survive >2 d in the presence of *L. maackii* fruit and demonstrated strong negative effects during those 2 d. However, in the field, *A. verticis* showed no negative effects of exposure to fruit (supporting H_2). In the laboratory, the response of *A. verticis* was greater than that of *H. azteca*, refuting H_3 and suggesting the possibility of an indirect effect of the *L. maackii* fruit extract on both species.

Flowers are a unique subsidy, and we found evidence that *L. maackii* flower deposition into headwater streams negatively affects macroinvertebrates. The timing of flower deposition can differ from that of leaves, and although the biomass may be relatively small in comparison to leaves or other materials, high nutrient content and the timing of this subsidy may make it disproportionately important (Abelho and Graça 1998). In species that flower prolifically, the pulse of rapidly decomposable and high-nutrient litter could create a hot moment in the stream that influences nutrient cycling and aquatic biota (Wantzen et al. 2009).

Our flower assays were limited in scope because the *L. maackii* flowering season is much shorter than its fruiting season. Our flower exposures indicated significant negative effects on *H. azteca* and *A. verticis* (in the laboratory). *Anthopotamus verticis* trials exhibited 100% mortality with increasing flower biomass, but we had difficulty collecting the large number of *A. verticis* required for replicated serial-dilution tests during the period of flower production. Flower production of *L. maackii* is maximized in edge-habitat conditions (Goodell et al. 2010) like those created along headwater streams. The flowers are attractive to pollinators and may positively affect seed set of synchronously flowering nearby native plants (McKinney and Goodell 2011), but our data suggest that spent flowers may be a negative subsidy in headwater streams.

We hypothesize that the source of negative effects of *L. maackii* is associated with 1 of the following 3 processes: 1) inherent chemical toxicity of the flowers and fruits, 2) secondary effects associated with changing water chemistry, or 3) secondary microbial effects. Chemical toxicity of *L. maackii* leaf material to invertebrates has been established in a series of studies. In a laboratory assay, the highly polyphagous caterpillar *Lymantria dispar* exhibited 100% mortality in a no-choice feeding experiment (McEwan et al. 2009). In a series of papers, Lieurance and Cipollini (2012, 2013a, b) identified the possibility of anti-insect-herbivore chemistry in *L. maackii* foliage and identified apigenin and luteolin (flavones) and chlorogenic acid as compounds that might be responsible. More generally, a suite of phenolic compounds, some of which are likely to have anti-insect properties, has been identified in the fruit of the genus *Lonicera* (Jurikova et al. 2012). *Lonicera japonica* flowers have been identified as having significant insecticidal chemistry ostensibly associated with constituent compounds estragole and linalool (Zhou et al. 2012). Zhou et al. (2012) found that essential oil of *L. japonica* had contact and fumigant toxicity against weevils (*Sitophilus zeamais* Motschulsky) and fumigant toxicity against booklouse (*Liposcelis bostrychophila* Badonnel). *Lonicera maackii* is phylogenetically related to *L. japonica*, but we are unaware of any researchers who have identified these compounds in fruit or flowers of *L. maackii*, or what their function might be in an aquatic system. However, the response of *H. azteca* in our trials suggests some mode of direct chemical effect. Future work is

needed to verify this pattern and identify responsible compounds.

A secondary mode of influence was suggested by the response pattern of *A. verticis* to fruit. Mortality was high in the laboratory exposures, but we found no discernable response in the field (supporting H_2). This pattern suggests that changes to water chemistry may be a source of stress in the laboratory assays. Water chemistry responded strongly to increasing subsidy biomass in laboratory microcosms, but was relatively unchanged in the flow-through in situ system used in the field. In particular, we found strongly declining pH and DO in the laboratory fruit exposures and no significant decline in either of these variables in the field. These results suggest that macroinvertebrate responses may be tied to secondary effects associated with changing chemistry in the water system, which in a natural system would cause *L. maackii* subsidy effects to be strongly associated with flow rates in relationship to biomass inputs and most likely to be consequential in pool habitats instead of riffles or runs.

A final potential mechanism for negative effects is microbial activity. *Lonicera maackii* foliage supports a unique microbial community (Arthur et al. 2012), and fruit and flower materials that entered our experimental system could very possibly have been inoculated with this unique microbial flora. During our exposures, we observed that sediments with fruit additions were bubbling, and both fruit and flowers gave off pungent odors as the duration of exposure increased. Fruit exhibited vertical migration both in an upward and downward movement in the beakers, and this movement continued throughout the exposure period. This observation strongly suggests some mode of microbial activity, but ascertaining the biological agent(s) responsible was beyond the scope of our study. Future work focused on the microbial ecology of these subsidies probably would be highly illuminating.

In summary, our data suggest that flower and fruit subsidies to headwater streams present a negative, and potentially, toxic subsidy for selected macroinvertebrates. The precise mechanism for this effect was not identified, and more testing is needed across a broader suite of aquatic species, but our data provide a basis for a new hypothesis linking *L. maackii* and headwater streams. Even though the temperate deciduous forests invaded by this species are species rich, the flower and fruit inputs from *L. maackii* are unique in terms of timing, quantity, and chemistry, and our data suggest that these novel subsidies are a particularly important connection between the terrestrial and aquatic habitats. Tests of this novel subsidy hypothesis for other species may provide useful insight for categorizing and managing invasive organisms and for broader understanding of terrestrial–aquatic linkages.

ACKNOWLEDGEMENTS

Author contributions: KWC and RWM designed the research. KWC, SDM and EBB executed the experiments and collected data. KWC and RWM analyzed the data. KWC and RWM wrote the paper with contributions from EBB.

We gratefully acknowledge the contributions made to this project by Sarah Alverson, Albert Burky, Taylor Buskey, Julia Chapman, Shante Eisele, Sarah Frankenberg, Lucas Gaynor, Mitchell Kukla, Corey Kuminecz, Nick Kunce, Meg Maloney, Joseph Murphy, Erin Rowekamp, Charlotte Shade, and Valerie Vlk. We thank the Five Rivers Metroparks and Aullwood Audubon Center for allowing us access to their properties for biomass collections and in situ experiments. Jim Lazorchak and Kimberly Wyatt provided *Hyalella* starter cultures. Allen Burton, David Dominic, and Shelly Hudson graciously supplied the in situ chambers and sediment testing equipment. This research was funded by the National Science Foundation (DEB 1352995).

LITERATURE CITED

Abelho, M., and M. A. S. Graça. 1998. Litter in a first-order stream of a temperate deciduous forest (Margaraça Forest, central Portugal). Hydrobiologia 386:147–152.

APHA (American Public Health Association. 1995. Standard methods for the examination of water and wastewater. 19 edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

Arthur, M. A., S. R. Bray, C. R. Kuchle, and R. W. McEwan. 2012. The influence of the invasive shrub, *Lonicera maackii*, on leaf decomposition and microbial community dynamics. Plant Ecology 213:1571–1582.

Bartuszevige, A. M., D. L. Gorchov, and L. Raab. 2006. The relative importance of landscape and community features in the invasion of an exotic shrub in a fragmented landscape. Ecography 29:213–222.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology 50:201–220.

Burton, G. A., M. S. Greenberg, C. D. Rowland, C. A. Irvine, D. R. Lavoie, J. A. Brooker, L. Moore, D. F. N. Raymer, and R. A. McWilliam. 2005. In situ exposures using caged organisms: a multi-compartment approach to detect aquatic toxicity and bioaccumulation. Environmental Pollution 134:133–144.

Callaway, R. M., and W. M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2:436–443.

Chappie, D. J., and G. A. Burton. 1997. Optimization of in situ bioassays with *Hyalella azteca* and *Chironomus tentans*. Environmental Toxicology and Chemistry 16:559–564.

Cummins, K. W. 1974. Structure and function of stream ecosystems. BioScience 24:631–641.

Cummins, K. W., and M. J. Klug. 1979. Feeding ecology of stream invertebrates. Annual Review of Ecology and Systematics 10: 147–172.

Custer, K. W., G. A. Burton, J. P. Kochersberger, P. D. Anderson, K. J. Fetters, and S. Hummel. 2016. Macroinvertebrate responses to nickel in multisystem exposures. Environmental Toxicology and Chemistry 35:101–114.

Dutra, H. P., K. Barnett, J. R. Reinhardt, R. J. Marquis, and J. L. Orrock. 2011. Invasive plant species alters consumer behavior by providing refuge from predation. Oecologia 166: 649–657.

Environment Canada. 2013. Biological test method: test for survival and growth in sediment and water using the freshwater amphipod *Hyalella azteca*. EPS 1/RM/33. 2nd edition. Method Development and Application Unit, Science and Technology Branch. Ottawa, Ontario.

Freeman, M. C., C. M. Pringle, and C. R. Jackson. 2007. Hydrologic connectivity and the contribution of stream headwaters to ecological integrity at regional scales. Journal of the American Water Resources Association 43:5–14.

Goodell, K., A. M. McKinney, and C. Lin. 2010. Pollen limitation and local habitat-dependent pollinator interactions in the invasive shrub *Lonicera maackii*. International Journal of Plant Sciences 171:63–72.

Gould, A. M. A., and D. L. Gorchov. 2000. Effects of the exotic invasive shrub *Lonicera maackii* on the survival and fecundity of three species of native annuals. American Midland Naturalist 144:36–50.

Guerold, F., J. P. Boudot, G. Jacquemin, D. Vein, D. Merlet, and J. Rouiller. 2000. Macroinvertebrate community loss as a result of headwater stream acidification in the Vosges Mountains (N-E France). Biodiversity and Conservation 9:767–783.

Guiden, P., D. L. Gorchov, C. Nielsen, and E. Schauber. 2015. Seed dispersal of an invasive shrub, Amur honeysuckle (*Lonicera maackii*), by white-tailed deer in a fragmented agricultural-forest matrix. Plant Ecology 216:939–950.

Hawkins, C. P., and J. R. Sedell. 2009. Longitudinal and seasonal changes in functional organization of macroinvertebrate communities in four Oregon streams. Ecology 62:387–397.

Heiri, O., A. F. Lotter, and G. Lemcke. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of Paleolimnology 25:101–110.

Hultine, K. R., J. Belnap, C. Van Riper, J. R. Ehleringer, P. E. Dennison, M. E. Lee, P. L. Nagler, K. A. Snyder, S. M. Uselman, and J. B. West. 2010. Tamarisk biocontrol in the western United States: ecological and societal implications. Frontiers in Ecology and the Environment 8:467–474.

Hutchinson, T. F., and J. L. Vankat. 1997. Invasibility and effects of Amur honeysuckle in southwestern Ohio forests. Conservation Biology 11:1117–1124.

Ingold, J. L., and M. J. Craycraft. 1983. Avian frugivory on honeysuckle (*Lonicera*) in southwestern Ohio in fall. Ohio Journal of Science 83:256–258.

Jurikova, T., O. Rop, J. Mlcek, J. Sochor, S. Balla, L. Szekeres, A. Hegedusova, J. Hubalek, V. Adam, and R. Kizek. 2012. Phenolic profile of edible honeysuckle berries (genus *Lonicera*) and their biological effects. Molecules 17:61–79.

Kominoski, J. S., L. B. Marczak, and J. S. Richardson. 2011. Riparian forest composition affects stream litter decomposition despite similar microbial and invertebrate communities. Ecology 92:151–159.

Lieurance, D., and D. Cipollini. 2012. Damage levels from arthropod herbivores on *Lonicera maackii* suggest enemy release in its introduced range. Biological Invasions 14:863–873.

Lieurance, D., and D. Cipollini. 2013a. Environmental influences on growth and defence responses of the invasive shrub, *Lonicera maackii*, to simulated and real herbivory in the juvenile stage. Annals of Botany 112:741–749.

Lieurance, D., and D. Cipollini. 2013b. Exotic *Lonicera* species both escape and resist specialist and generalist herbivores in the introduced range in North America. Biological Invasions 15:1713–1724.

Lieurance, D., and K. Landsbergen. 2016. The influence of light habitat on the physiology, biomass allocation, and fecundity of the invasive shrub Amur honeysuckle (*Lonicera maackii*, Caprifoliaceae). Journal of the Torrey Botanical Society 143: 415–426.

Likens, G. E., and F. H. Bormann. 1974. Linkages between terrestrial and aquatic ecosystems. BioScience 24:447–456.

Lowe, W. H., and G. E. Likens. 2005. Moving headwater streams to the head of the class. BioScience 55:196–197.

Luken, J. O., and J. W. Thieret. 1996. Amur honeysuckle, its fall from grace. BioScience 46:18–24.

McEwan, R. W., M. A. Arthur, and S. S. Alverson. 2012. Throughfall chemistry and soil nutrient effects of the invasive shrub *Lonicera maackii* in deciduous forests. American Midland Naturalist 168:43–55.

McEwan, R. W., L. G. Arthur-Paratley, L. K. Rieske, and M. A. Arthur. 2010. A multi-assay comparison of seed germination inhibition by *Lonicera maackii* and co-occurring native shrubs. Flora—Morphology, Distribution, Functional Ecology of Plants 205:475–483.

McEwan, R. W., L. K. Rieske, and M. A. Arthur. 2009. Potential interactions between invasive woody shrubs and the gypsy moth (*Lymantria dispar*), an invasive insect herbivore. Biological Invasions 11:1053–1058.

McKinney, A. M., and K. Goodell. 2011. Plant–pollinator interactions between an invasive and native plant vary between sites with different flowering phenology. Plant Ecology 212:1025–1035.

McNeish, R. E., M. E. Benbow, and R. W. McEwan. 2012. Riparian forest invasion by a terrestrial shrub (*Lonicera maackii*) impacts aquatic biota and organic matter processing in headwater streams. Biological Invasions 14:1881–1893.

McNeish, R. E., and R. W. McEwan. 2016. A review on the invasion ecology of Amur honeysuckle (*Lonicera maackii*, Caprifoliaceae) a case study of ecological impacts at multiple scales. Journal of the Torrey Botanical Society 143:367–385.

McNeish, R. E., E. M. Moore, M. E. Benbow, and R. W. McEwan. 2015. Removal of the invasive shrub, *Lonicera maackii*, from riparian forests influences headwater stream biota and ecosystem function. River Research and Applications 31:1131–1139.

Mineau, M. M., C. V. Baxter, and A. M. Marcarelli. 2011. A non-native riparian tree (*Elaeagnus angustifolia*) changes nutrient dynamics in streams. Ecosystems 14:353–365.

Mineau, M. M., C. V. Baxter, A. M. Marcarelli, and G. W. Minshall. 2012. An invasive riparian tree reduces stream ecosystem efficiency via a recalcitrant organic matter subsidy. Ecology 93:1501–1508.

Moore, A. A., and M. A. Palmer. 2005. Invertebrate biodiversity in agricultural and urban headwater streams: implications for conservation and management. Ecological Applications 15: 1169–1177.

Naiman, R. J., and H. Decamps. 1997. The ecology of interfaces: riparian zones. Annual Review of Ecology and Systematics 28: 621–658.

Naiman, R. J., H. Decamps, and M. Pollock. 1993. The role of riparian corridors in maintaining regional biodiversity. Ecological Applications 3:209–212.

Planchuelo, G., P. Catalán, and J. A. Delgado. 2016. Gone with the wind and the stream: dispersal in the invasive species *Ailanthus altissima*. Acta Oecologica 73:31–37.

Poulette, M. M., and M. A. Arthur. 2012. The impact of the invasive shrub *Lonicera maackii* on the decomposition dynamics of a native plant community. Ecological Applications 22:412–424.

Redfield, A. C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. Pages 177–192 *in* R. J. Daniel (editor). James Johnstone Memorial Volume. Liverpool University Press, Liverpool, UK.

Santisteban, J. I., R. Mediavilla, E. López-Pamo, C. J. Dabrio, M. B. R. Zapata, M. J. G. Garcia, S. Castaño, and P. E. Martínez-Alfaro. 2004. Loss on ignition: a qualitative or quantitative method for organic matter and carbonate mineral content in sediments? Journal of Paleolimnology 32:287–299.

Shafroth, P. B., J. R. Cleverly, T. L. Dudley, J. P. Taylor, C. Van Riper, E. P. Weeks, and J. N. Stuart. 2005. Control of *Tamarix* in the western United States: implications for water salvage, wildlife use, and riparian restoration. Environmental Management 35:231–246.

Shewhart, L., R. W. McEwan, and M. E. Benbow. 2014. Evidence for facilitation of *Culex pipiens* (Diptera : Culicidae) life history traits by the nonnative invasive shrub Amur honeysuckle (*Lonicera maackii*). Environmental Entomology 43:1–10.

Swan, C. M., B. Healey, and D. C. Richardson. 2008. The role of native riparian tree species in decomposition of invasive tree of heaven (*Ailanthus altissima*) leaf litter in an urban stream. Ecoscience 15:27–35.

Tank, J., E. Rosi-Marshall, N. A. Griffiths, S. A. Entrekin, and M. L. Stephen. 2010. A review of allochthonous organic matter dynamics and metabolism in streams. Journal of the North American Benthological Society 29:118–146.

Trammell, T. L. E., H. A. Ralston, S. A. Scroggins, and M. M. Carreiro. 2012. Foliar production and decomposition rates in urban forests invaded by the exotic invasive shrub, *Lonicera maackii*. Biological Invasions 14:529–545.

USEPA (US Environmental Protection Agency). 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. 2 edition. EPA/600/R-99/064. Office of Research and Development and Office of Water, US Environmental Protection Agency, Washington, DC.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130–137.

Wallace, J. B., S. L. Eggert, J. L. Meyer, J. R. Webster, and W. V. Sobczak. 2015. Stream invertebrate productivity linked to forest subsidies: 37 stream-years of reference and experimental data. Ecology 96:1213–1228.

Wallace, J. B., G. J. Lugthart, T. F. Cuffney, and G. A. Schurr. 1989. The impact of repeated insecticidal treatments on drift and benthos of a headwater stream. Hydrobiologia 179:135–147.

Wantzen, K. M., C. M. Yule, J. M. Mathooko, and C. M. Pringle. 2009. Organic matter processing in tropical streams. Pages 43–64 *in* D. Dudgeon (editor). Tropical stream ecology. Elsevier, London, UK.

Yates, E. D., D. F. Levia, and C. L. Williams. 2004. Recruitment of three non-native invasive plants into a fragmented forest in southern Illinois. Forest Ecology and Management 190:119–130.

Zhou, H. Y., N. N. Zhao, S. S. Du, K. Yang, C. F. Wang, and Z. L. Liu. 2012. Insecticidal activity of the essential oil of *Lonicera japonica* flower buds and its main constituent compounds against two grain storage insects. Journal of Medicinal Plants Research 6:912–917.