

# No evidence of co-facilitation between a non-native Asian earthworm (*Amyntas tokioensis*) and invasive common buckthorn (*Rhamnus cathartica*) in experimental mesocosms

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**Abstract** Non-native invasive earthworms are known drivers of forest change in north temperate forests. Much understanding of earthworm invasion is based on species of European origin, but concern about Asian pheretimoid earthworms (e.g. Asian jumping worms, *Amyntas* spp.) is increasing. Some effects of *Amyntas* spp. on soil properties and biota have been studied, but little is known about interaction of *Amyntas* spp. with plants. Potential interaction between *Amyntas* spp. and invasive buckthorn (*Rhamnus cathartica*) is of particular interest given hypothesized co-facilitation between *R. cathartica* and European earthworms—cited by some as components of an “invasional meltdown”. We used reciprocal mesocosm experiments in Wisconsin, USA, to test for co-facilitation between *Amyntas tokioensis* and *R. cathartica*. We asked: (1) Are jumping worms more successful in environments invaded by buckthorn? (2) Does jumping worm activity increase buckthorn germination and establishment? Counter to expectations, co-facilitation was not supported, and we found evidence to the contrary. There was no

increase in litter loss (indicative of consumption by jumping worms) or jumping worm fecundity in buckthorn-invaded environments, and buckthorn germination was unaffected by increased jumping worm densities. Counter to our hypothesis, jumping worm fecundity was greater in buckthorn-free soils than in buckthorn-invaded soils. Our results show no experimental evidence of co-facilitation by either of these invasive species, and highlight potential differences in ecological impact of non-native invasive earthworm taxa that vary in life-history and functional dynamics.

**Keywords** Asian jumping worm · *Amyntas tokioensis* · Earthworms · *Rhamnus cathartica* · Wisconsin

## Introduction

Biotic invasions can considerably alter forest ecosystem structure and function (Vitousek 1990). In north temperate forests, invasive earthworms in particular are gaining attention as a driver of forest change. Prior to European settlement, many north temperate forests of North America lacked earthworm populations, with current earthworm communities dominated by species from Europe and Asia (Hendrix and Bohlen 2002; Bohlen et al. 2004). Invasive earthworms have large ecological impacts on forest ecosystems, including

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mixing organic and mineral soil horizons, decreasing soil carbon storage, and altering nitrogen cycling, soil food webs, and native plant communities (Bohlen et al. 2004; Hale et al. 2006; Blouin et al. 2013).

Much understanding of earthworm invasion is based on studies of European species (particularly family Lumbricidae; Bohlen et al. 2004; Hale et al. 2006), however, managers and ecologists are increasingly concerned about earthworms of Asian origin in temperate deciduous forests (Callaham et al. 2003; Snyder et al. 2011; Greiner et al. 2012; Qiu and Turner 2016). Asian pheretimoid earthworms (e.g. *Amyntas* and *Metaphire* spp.) were first recorded in the United States in 1939 (Gates 1982), and their invasion now spans several northern and southern states (Chang et al. 2016a). While *Amyntas agrestis* is frequently cited as the invader in north temperate forests, recent work shows *A. agrestis* often co-occurs with morphologically similar *Amyntas tokioensis* and *Metaphire hilgendorfi*, with high possibility of misidentification (Schult et al. 2016; Chang et al. 2016a, 2017a). Referred to as “jumping worms” for their characteristic rapid, snake-like movements, these co-invading species are thought to share several traits, and may change forest ecosystems in ways distinct from European species as a result of differences in life-history and functional dynamics (Greiner et al. 2012; Chang et al. 2016b).

*Amyntas agrestis*, *A. tokioensis*, and *M. hilgendorfi* reproduce parthenogenetically, and unlike more commonly studied European species, have an annual lifecycle. They emerge from cocoons in spring and grow rapidly to sexual maturity before reproducing and dying in fall (Burtelow et al. 1998; Greiner et al. 2012). They are epi-endogeic, living in litter and surface soils rather than deep burrows. However, jumping worms are larger than many epigeic species of European origin (Greiner et al. 2010), and live at much higher densities than their European counterparts (Callaham et al. 2003). The combination of greater body size and density and their wide dietary flexibility (Zhang et al. 2010) may increase the effects of jumping worms on ecosystems relative to other earthworm taxa (Chang et al. 2016c). Thus, jumping worms have the potential to markedly change forest ecosystems in ways distinct from European earthworms, yet comparatively less is known about their ecosystem impacts and habitat preferences (Chang et al. 2016a). Initial studies reveal jumping worms can

substantially reduce leaf litter, accelerate decomposition, and alter nutrient pools in forest and prairie soils (Greiner et al. 2012; Qiu and Turner 2016). Invasion of jumping worms also alters organic matter and forest floor structure (Burtelow et al. 1998), and results in a long-lasting granular soil signature (Chang et al. 2016a) that has been hypothesized to negatively impact plant growth.

Ecological consequences of Asian earthworm invasions will depend in large part on how they interact with other native and invasive species, as species interactions can amplify or dampen impacts of invasive species on ecosystem function (Simberloff and Von Holle 1999; Richardson et al. 2000; Levine et al. 2003; Brooker et al. 2008). Studies have documented interaction between *A. agrestis* and/or *M. hilgendorfi* and European earthworm species (Zhang et al. 2010; Greiner et al. 2012; Chang et al. 2016b, 2017b; Laushman et al. 2018), as well as a variety of soil and aquatic predators including millipedes, salamanders, and crayfish (Snyder et al. 2011, 2013; Gorsuch and Owen 2014; Ziemba et al. 2015, 2016). However, few studies have investigated potential interaction of Asian earthworms with forest plants (but see Laushman et al. 2018), despite well-documented occurrence of plant-earthworm interactions more generally (Scheu 2003; Bezemer and van Dam 2005; Hale et al. 2006). Of particular interest in Midwestern forests is potential co-facilitation between Asian earthworm species and invasive plants, such as common buckthorn (*Rhamnus cathartica*).

Native to Europe, buckthorn is common in human-dominated landscapes, and is considered among the most abundant and harmful forest invaders in the Midwestern US (Knight et al. 2007; Roth et al. 2015). Its physiology—including rapid growth, shade tolerance, and high photosynthetic rates—and unique phenology allow it to outcompete native plant species (Knight et al. 2007), while modifying soil and leaf litter in ways that encourage further invasion (Heneghan et al. 2006). Buckthorn can fundamentally alter forest structure by forming dense, monospecific thickets (Mascaro and Schnitzer 2007), or by preventing native tree establishment in open sites that would otherwise undergo succession to native-dominated forest. Ultimately, buckthorn dominance can reduce aboveground carbon storage by up to half compared to native communities (Mascaro and Schnitzer 2011).

Additionally, buckthorn is an important overwintering host of the invasive soybean aphid (*Aphis glycines*), an economically important crop pest (Bahlai et al. 2010; Heimpel et al. 2010; Lacasella et al. 2017). Thus, buckthorn impacts several ecosystem services provided by Midwestern landscapes.

European earthworm species (e.g. *Lumbricus terrestris*) are often assumed to interact with buckthorn such that each species facilitates the other (Heneghan 2003; Frelich et al. 2006; Kurylo et al. 2007; Knight et al. 2007, but see Wyckoff et al. 2014; Iannone et al. 2015). This interaction has been proposed as an example of “invasional meltdown”, in which a group of non-native species facilitates each other’s success (Simberloff and Von Holle 1999; Simberloff 2006; Heimpel et al. 2010). European earthworms are hypothesized to aid buckthorn spread via alterations to the forest floor that enhance germination, including thinning of leaf litter, increasing pH, and increasing nutrient cycling and availability (Heimpel et al. 2010; Roth et al. 2015). In turn, buckthorn is hypothesized to facilitate earthworm invasion via nutrient-rich litter, and the creation of a desirable soil environment (Heneghan et al. 2007; Mueller et al. 2018). Supporting this hypothesis, buckthorn and European earthworm populations are positively correlated in woodlands in northeastern Illinois (Heneghan et al. 2007) and Minnesota (Mueller et al. 2018), and removal of buckthorn reduced European earthworm populations by over 60% the following year in Wisconsin forests (Madritch and Lindroth 2009). However, other studies fail to support the putative facilitation. Wyckoff et al. (2014) found greater evidence of negative relationships than positive between buckthorn and earthworms at the prairie-forest border in west central Minnesota. Similarly, with the exception of accelerated litter decomposition, Iannone et al. (2015) found a lack of evidence that buckthorn invasion altered soils—a frequently cited mechanism for earthworm facilitation—in a well-replicated regional study in Illinois. Thus, whether co-facilitation occurs between earthworms and buckthorn remains contested in the literature.

If the hypothesized relationship between European earthworm and buckthorn is correct, co-facilitation might also be expected between jumping worms and buckthorn, with potential consequences for Midwestern landscapes. Because jumping worms occur at higher densities, grow faster, and consume more leaf

litter than European species (Zhang et al. 2010; Greiner et al. 2012; Qiu and Turner 2016), jumping worms could facilitate buckthorn germination and establishment in much the same manner, and perhaps to a greater extent, as European earthworms. Whether the opposite side of this potential relationship—facilitation of earthworms by buckthorn—is likely to hold is less clear, and two alternatives are plausible. Early seasonal decomposition of buckthorn litter (Heneghan et al. 2002) relative to mid-season jumping worm maturity may weaken the effect of buckthorn on jumping worms, as nutrient-rich buckthorn litter is less available as a food source. However, residual pieces of buckthorn litter are still likely to persist in invaded soils, and soil changes including increased nitrogen, calcium, and pH (Mueller et al. 2018) could still influence jumping worm success consistent with expectations based on European earthworms. Contrastingly, faster overall litter decomposition (and thus lower litter quantities) observed in buckthorn-invaded environments could provide a less favorable environment for jumping worms, which consume more litter than their European counterparts.

The presence of both buckthorn and jumping worms at the University of Wisconsin-Madison Arboretum—where *A. tokioensis* and *A. agrestis* were discovered in 2013 (Chang et al. 2016a)—provided an opportunity to test this species interaction. We used mesocosm experiments to ask: Does a positive feedback exist between *A. tokioensis* and *R. cathartica*? Specifically (1) Are jumping worms more successful in environments that have already been invaded by buckthorn? (2) Does jumping worm activity increase buckthorn germination and establishment? We hypothesized increased foliage litter loss in buckthorn-invaded soils due to higher feeding activity of *A. tokioensis* in the presumed more favourable conditions, as well as increased jumping worm fecundity (cocoon production). We further hypothesized increased buckthorn germination and establishment under conditions of higher jumping worm densities, consistent with studies of European earthworms.

## Methods

### Mesocosm experimental design

We conducted two separate replicated  $3 \times 2$  factorial mesocosm experiments during summer and fall 2016 in the UW-Madison Arboretum's open field facility (adapted from the methods of Qiu and Turner 2016). Mesocosms consisted of intact soil cores (20 cm diameter  $\times$  25 cm depth) collected from forest sites.

### Experiment 1 (Q1)

We tested *A. tokioensis* leaf litter consumption rates and fecundity (cocoon production) in buckthorn invaded versus buckthorn free environments experimentally by manipulating leaf litter mass consistent with variability in buckthorn invaded versus uninvaded sites in the field (online Appendix Table A1), and using soil from paired invaded and buckthorn-free forest sites ( $3 \times 2$  factorial,  $n = 9$  replicates per treatment combination). We aimed to distinguish the hypothesized positive effects of buckthorn soil environments on jumping worm success with the potentially negative effects of reduced litter availability in buckthorn-invaded environments. The mesocosm environment ensured control over worm density. This is important, as variation in worm density, activity and movement could confound results. Additionally, litter remained on top of soil as in natural settings, allowing for continuous assessment.

Intact soil cores ( $n = 54$ ) were collected in late spring from 9 representative forest sites uninvaded by jumping worms in the UW-Madison Arboretum (as per earthworm surveys from the previous summer, Laushman et al. 2018). Each forest site contained a buckthorn invaded and buckthorn free subsite (separated by  $\sim 10$  m), with similar canopy species composition. Within each subsite we recorded diameter at breast height (DBH) and species identity of all saplings and trees ( $> 2.5$  cm DBH) within a  $100 \text{ m}^2$  circular plot. Common overstory species in the study sites included *Acer saccharum*, *Quercus alba*, *Prunus serotina*, *Fagus grandifolia*, and *Carya* spp. We also recorded density of buckthorn seedlings in three  $1 \text{ m}^2$  quadrats—one at the plot center, and two at 1 m from the center in a randomly determined compass direction. In buckthorn-invaded subplots, *R. cathartica* occurred at a mean density of 8.3 stems per  $\text{m}^2$

( $\pm 1.5$  SE). Large buckthorn stems ( $> 2.5$  cm DBH) ranged from 0 (only stems  $< 2.5$  cm present) to 21 per  $100 \text{ m}^2$  plot (mean  $9 \pm 2.7$  SE).

At the center of each circular plot a set of three intact soil cores was collected within a 1 m radius ( $n = 6$  cores per site). All cores were collected at least 1 m from any edge (e.g. walking paths, obvious vegetation transitions). Cores were cleared of leaf litter, woody debris, and undergrowth, and returned to the Arboretum field facility for the remainder of the experiment.

Leaf litter for treatments was collected from non-buckthorn invaded forests surrounding each soil-sampling site (so as not to contaminate non-buckthorn cores with fragments of buckthorn litter and/or soil from heavily invaded areas). Litter from each site was gently mixed to homogenize species composition (litter composition was consistent with common overstory species, above) and sizes of litter pieces, air-dried, and a low (1.5 g), medium (20 g), or high (40 g) litter treatment was randomly allocated to each set of 3 cores from the corresponding collection site. Low, medium, and high litter treatments were representative of field conditions, based on field surveys of litter in buckthorn-invaded and uninvaded environments, whereby leaf litter from 2 randomly located  $30 \text{ cm} \times 30 \text{ cm}$  quadrats within each subsite was collected, dried, and weighed (online Appendix Table A1). Subsamples of litter were oven-dried to establish an oven-dry/air-dry correction factor.

Each PVC core was outfitted with landscaping cloth (bottom), and mesh “worm screens” (top) to prevent worm escape and allow vertical water flow, and the 6 cores for each treatment were placed into completely randomized blocks to account for variation across the field facility. Shade cloth was installed above the facility, and soil piled surrounding each core to mimic forest conditions (online Appendix Fig. A1). Mesocosms were monitored weekly for soil moisture, and deionized water was added as needed to prevent earthworm death by desiccation.

We added five *A. tokioensis* individuals to each core initially, supplemented with 2 additional earthworms 1 month into the experiment to account for possible earthworm death early in the experiment (following Qiu and Turner 2016). All individuals were collected in late June from a known invasion site within the Arboretum using standard mustard extraction methods (Lawrence and Bowers 2002). Densities of up to 7

worms per core, while high, are consistent with natural populations—supported by recorded field densities within the Arboretum (online Appendix Fig. A2, Table A2).

Litter percent cover and depth of surface litter (calculated as an average of 4 random points per mesocosm) were recorded weekly for 18 weeks, ending following the first hard freeze in late October. At 18 weeks, remaining litter was collected from each soil core, oven dried, and weighed to determine total litter mass loss. Percent cover and depth of soil signature—the grainy, visible signature indicative of *Amyntas* activity—was measured within each core following litter removal. We then excavated the top 5 cm of each core in two 2.5 cm increments, and *A. tokioensis* cocoons were isolated and counted by wet sieving through a 1.0 mm standard soil sieve.

### Experiment 2 (Q2)

Buckthorn germination trials were also conducted in mesocosms ( $n = 30$ ) as described in experiment 1. Here we again varied litter mass (consistent with experiment 1) and also varied worm density ( $3 \times 2$  factorial experiment, 5 replicates per treatment combination). Reduced litter is the primary mechanism by which earthworms are hypothesized to increase buckthorn germination and establishment. Intact cores (same size as above) were collected from 5 representative buckthorn-free forest sites in the Arboretum (buckthorn completely absent from a 10 m radius surrounding site, to ensure buckthorn seeds were not present in the seed bank, dominant canopy species consistent with above). Within each site, 6 intact soil cores were collected. Cores were collected in 3 pairs of two within a 1 m radius, such that each pair could be randomly allocated to one of three litter treatments, and *A. tokioensis* allocated to one core of each pair while the other served as a control. Paired cores were collected from no more than 15 cm apart. With the exception of earthworm allocation to only one of each pair, cores and experimental setup were treated as described above. Unfortunately, we became aware throughout the course of the experiment that the sampling region assumed uninvaded by *A. tokioensis* based on the most recent survey of earthworm distribution (Laushman et al. 2018) had become invaded prior to soil collection, such that a low number of jumping worms were present in the

“control” cores. Given the life history of the organism, this incipient invasion was undetectable until after the experiment had already started. Thus, we refer to earthworm treatments as “low density” and “high density” throughout the remainder of the work. While precise measurement of jumping worm densities was not possible without destructive sampling, no more than one jumping worm per core was noted visually in low-density treatments throughout the course of the summer.

Representing natural seed rain, we sowed 40 buckthorn seeds [cold stratified for 42 days, (Stewart and Graves 2005)] on top of the litter or soil in each mesocosm, and recorded germination and survival weekly for 18 weeks. Cumulative buckthorn germination was calculated as an indicator of germination success, and final number of surviving seedlings as an indicator of establishment success (Roth et al. 2015). As with Experiment 1, litter percent cover and depth were measured weekly, and remaining litter was collected from each soil core, oven dried, and weighed at the end of the experiment to determine total litter mass loss. We measured percent cover and depth of soil signature following litter removal as an indicator of earthworm activity, confirming the modified low- vs. high-density earthworm treatments.

### Data analysis

To evaluate effects of buckthorn invasion on jumping worm success (Q1), we used generalized linear mixed effects models (GLMMs; using the lme4 package in R, Bates et al. 2015) to test for significant differences in litter mass loss (total and percent loss between end and initial measurements), depth and percent cover of soil signature (log and logit transformed, respectively), and fecundity (number of cocoons produced and cocoon presence/absence) (Table B1). For all models, soil origin and litter mass were included as fixed effects, and site was included as a random effect to account for treatments nested within each sampling site.

To evaluate effects of jumping worm activity on buckthorn germination and establishment (Q2), we used GLMMs to test for significant differences in litter mass loss (total and percent loss between end and initial measurements), depth and percent cover of soil signature (log + 1 and logit transformed, respectively), and buckthorn germination (number of

buckthorn seeds germinated throughout the experiment, log + 1 transformed) and establishment (buckthorn seedlings surviving at the end of the experiment) (Table B2). Here, earthworm density and litter mass were included as fixed effects, and site was again included as a random effect. To account for challenges in this data (including many zeros in buckthorn counts) we also tried multiple alternative model types, including zero-inflated models using the R package `glmmTMB`. All models resulted in similar findings, thus we present results of the more easily interpretable GLMMs here.

All analyses were performed using R statistical software (R Development Core Team 2009). Models were fit using maximum likelihood and the significance of fixed effects was evaluated using type II Anova (using the `car` package in R, Fox and Weisberg 2011), which generates *P* values using Type II Wald chi-square tests. We use a statistical significance level of  $\alpha = 0.05$  to indicate strong support, and  $\alpha = 0.1$  to indicate marginal support. Model residuals were visually inspected for normality and homogeneity of variance. Count data were analyzed using a Poisson distribution where it improved model fit (cocoon number and buckthorn establishment), and cocoon presence was analyzed using a binomial distribution. All materials potentially contaminated with jumping worm cocoons were appropriately disposed of following the experiment.

## Results

### Effects of buckthorn invasion on jumping worm success (Q1)

There were no significant effects of initial litter mass ( $\chi^2(\text{df} = 2) = 0.259$ ,  $P = 0.88$ ) or soil origin ( $\chi^2(\text{df} = 1) = 1.25$ ,  $P = 0.26$ ) on percent litter mass loss over the season (Fig. 1a). Total litter mass loss was positively correlated with initial litter mass, with greater litter loss from mesocosms with higher initial litter treatments ( $\chi^2(\text{df} = 2) = 512$ ,  $P < 0.001$ ; Fig. 1b). There was also a marginal positive effect of soil origin on total litter mass loss, with higher litter loss in mesocosms containing soil from buckthorn-free areas, particularly in medium and high litter treatments ( $\chi^2(\text{df} = 1) = 3.19$ ,  $P = 0.07$ ; Fig. 1b).

Soil signature depth (indicative of earthworm activity) was greater in mesocosms containing higher initial litter mass ( $\chi^2(\text{df} = 2) = 10.6$ ,  $P = 0.005$ ), as well as in mesocosms containing soils from buckthorn-invaded areas ( $\chi^2(\text{df} = 1) = 4.81$ ,  $P = 0.03$ )—with differences driven largely by the lowest litter treatment (Fig. 1c). Percent cover of soil signature showed similar patterns, with greater soil signature in higher initial litter conditions ( $\chi^2(\text{df} = 2) = 27.2$ ,  $P < 0.001$ ) and marginally greater soil signature in buckthorn-invaded soils ( $\chi^2(\text{df} = 1) = 3.34$ ,  $P = 0.07$ ) (Fig. 1d).

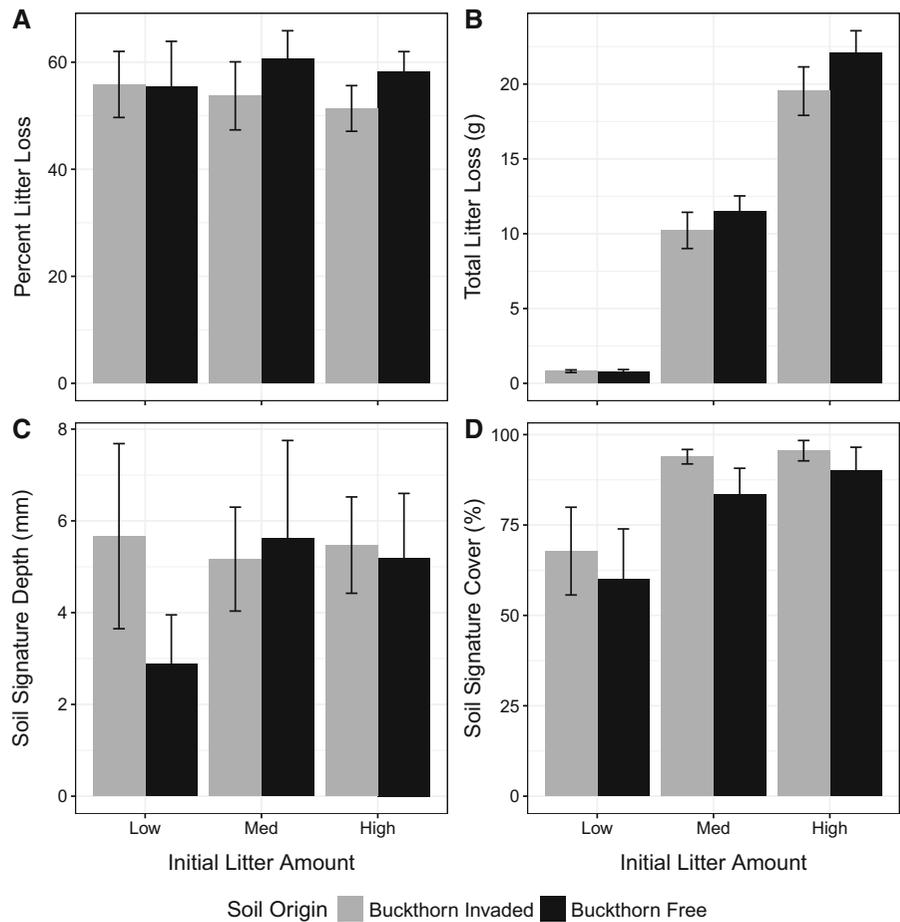
Cocoons were more likely to be present in cores with higher initial litter mass ( $\chi^2(\text{df} = 2) = 7.11$ ,  $P = 0.03$ ), although there was no difference in cocoon presence in buckthorn-invaded vs. buckthorn free soils ( $\chi^2(\text{df} = 1) = 0.866$ ,  $P = 0.40$ ) (Fig. 2b). The number of cocoons produced varied among cores (range 0–51, mean  $5 \pm 1.2$  SE). Cores with higher initial litter mass contained a greater number of cocoons on average ( $\chi^2(\text{df} = 2) = 117$ ,  $P < 0.001$ ), as did cores containing soil from buckthorn-free areas ( $\chi^2(\text{df} = 1) = 15.6$ ,  $P < 0.001$ ) (Fig. 2a). No more than 4 cocoons were found in any mesocosm under a low litter treatment. There was no difference in cocoon presence or number in the top 2.5 cm layer of soil compared to the next deepest 2.5 cm.

There was no significant interaction between litter and soil treatments for any of the variables measured.

### Effects of jumping worm activity on buckthorn germination and establishment (Q2)

Both percent and total litter loss were greater in mesocosms containing a higher initial litter mass (percent  $\chi^2(\text{df} = 2) = 15.2$ ,  $P < 0.001$ ; total  $\chi^2(\text{df} = 2) = 782$ ,  $P < 0.001$ ), and greater in the high-density earthworm treatment compared to low-density (percent  $\chi^2(\text{df} = 1) = 5.52$ ,  $P = 0.02$ ; total  $\chi^2(\text{df} = 1) = 6.99$ ,  $P = 0.008$ ). Litter loss ranged from 17 to 63% in low-density earthworm treatments, and 32–72% in high-density earthworm treatments. On average, litter mass loss was 14% greater in the high-density earthworm treatment across all initial litter mass treatments. Soil signature depth (Fig. 3a) and percent cover (Fig. 3b) were both greater in high-density earthworm conditions (depth  $\chi^2(\text{df} = 1) = 80.7$ ,  $P < 0.001$ ; cover  $\chi^2(1) = 42.2$ ,  $P < 0.001$ ) and in mesocosms with higher initial

**Fig. 1** Effects of *A. tokioensis* on litter loss and soil signature following an 18 week mesocosm experiment. Bars represent mean changes compared to initial conditions for mesocosms containing soils from buckthorn invaded areas (grey) and adjacent buckthorn free areas (black). Error bars are standard errors. **a** Percent litter loss does not differ significantly with initial litter mass or soil origin; **b** total litter loss increases significantly ( $P < 0.001$ ) as initial litter mass increases, and is marginally higher ( $P = 0.07$ ) in buckthorn-free soils; **c** soil signature depth is significantly higher with higher initial litter mass ( $P = 0.005$ ) and in buckthorn-invaded soils ( $P < 0.03$ ); **d** soil signature percent cover is significantly higher with higher initial litter mass ( $P < 0.001$ ) and marginally higher in buckthorn-invaded soils ( $P = 0.07$ )

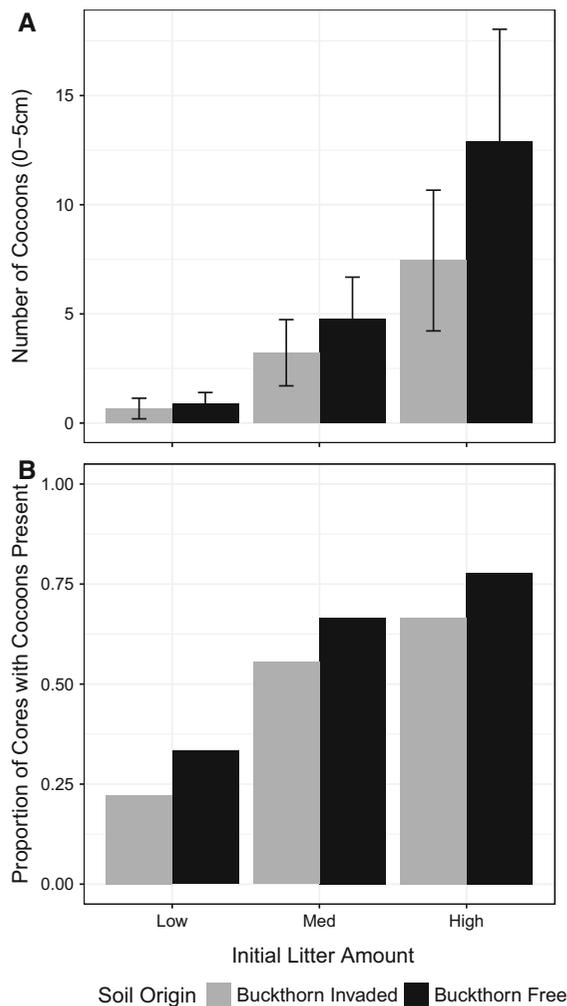


litter mass (depth  $\chi^2(df = 2) = 17.3$ ,  $P = 0.001$ ; cover  $\chi^2(df = 2) = 18.6$ ,  $P < 0.001$ ).

Buckthorn germination was generally low, with a maximum of 5 seeds germinating in any individual mesocosm over the course of the experiment (12.5% germination success). Germination occurred in 15 out of 30 mesocosms. Fewer seeds germinated in mesocosms with a higher initial litter mass ( $\chi^2(df = 2) = 10.7$ ,  $P = 0.005$ ), but earthworm density did not affect buckthorn germination ( $\chi^2(df = 1) = 2.04$ ,  $P = 0.15$ ; Fig. 3c). Buckthorn establishment followed similar patterns to germination. Establishment ranged from 0 to 3 seedlings. Again, fewer seedlings established in mesocosms with a higher initial litter depth ( $\chi^2(df = 2) = 7.01$ ,  $P = 0.03$ ), but earthworm density did not affect buckthorn establishment ( $\chi^2(df = 1) = 1.17$ ,  $P = 0.28$ ; Fig. 3d). There was no significant interaction between litter and earthworm treatments for any of the variables measured.

## Discussion

We found no evidence of positive feedbacks between common buckthorn and jumping worms in our mesocosm experiments, contrary to expectations based on well-studied European earthworm species (Heneghan 2003; Heneghan et al. 2007; Madritch and Lindroth 2009; Heimpel et al. 2010; Roth et al. 2015; Mueller et al. 2018) but consistent with Wyckoff et al. (2014) and Iannone et al. (2015). Soil and litter conditions representative of buckthorn-invaded areas did not facilitate *A. tokioensis* litter consumption or reproduction, and increased *A. tokioensis* density did not affect buckthorn germination or establishment. Our work suggests that although jumping worms and buckthorn each present conservation challenges, feedbacks (e.g. the foundation of an “invasional melt-down”, (Simberloff and Von Holle 1999; Heimpel et al. 2010)) are unlikely in our system. Additionally,



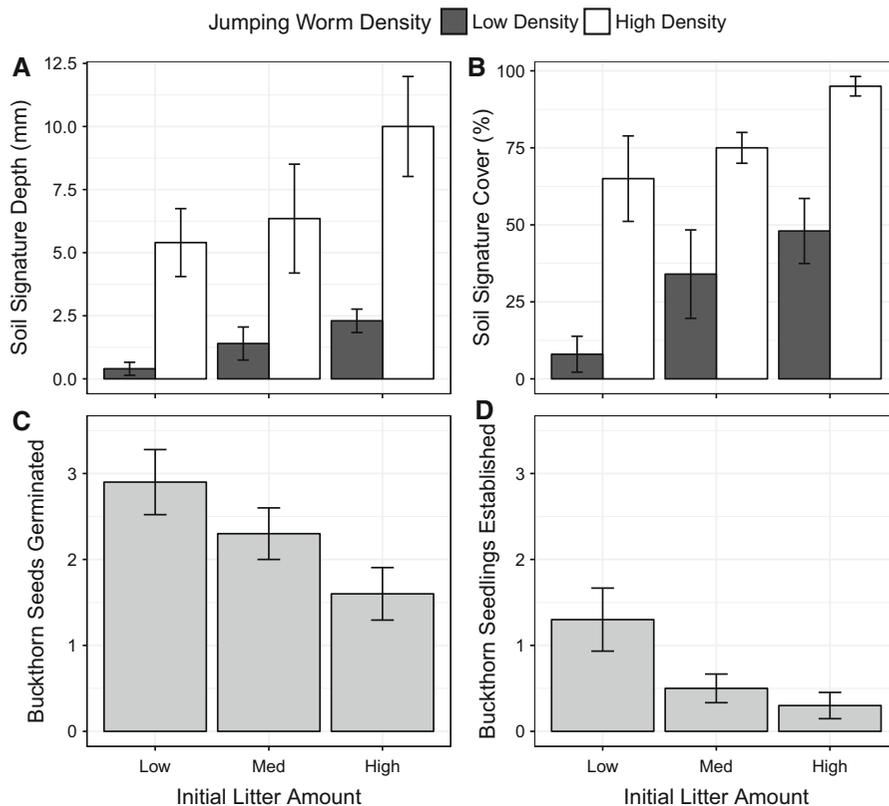
**Fig. 2** Number and presence of *A. tokioensis* cocoons following an 18 week mesocosm experiment. Bars represent mean number of cocoons (a) or proportion of cores containing cocoons (b) in mesocosms containing soils from buckthorn invaded areas (grey) and adjacent buckthorn free areas (black). Error bars are standard errors. a Cocoons are present in significantly higher numbers with higher initial litter mass ( $P < 0.001$ ), as well as in soils from buckthorn-free areas ( $P < 0.001$ ); b cocoon presence is significantly more likely with higher initial litter mass ( $P = 0.03$ ), but unaffected by soil origin

results support recent findings that Asian earthworms may have distinct ecological consequences from those of European origin (Laushman et al. 2018; Qiu and Turner 2016)—cautioning against basing understanding of earthworm invasions on European species alone.

Litter loss—indicative of litter consumption by earthworms—was not increased in areas previously

invaded by buckthorn as hypothesized, and was actually slightly lower than in soils from buckthorn-free areas (Fig. 1b). Interestingly, soil signature—a more direct indicator of earthworm activity—was often higher in buckthorn-invaded soils despite the lower litter loss (Fig. 1c, d), perhaps indicating that jumping worms were feeding on other sources of organic matter in addition to litter. Zhang et al. (2010) highlight the dietary flexibility of *A. agrestis*, including soil organic matter and soil biota, and Snyder et al. (2013) note *A. agrestis*' direct consumption of the FH (i.e. surface organic) soil horizon. Thus, it may be that soil under buckthorn stands supports broader dietary options for *Amyntas* due to differences in soil properties (Heneghan et al. 2002; Knight et al. 2007). However, differences in both litter consumption and earthworm activity were slight in our experiment, and a more direct study of dietary habits would be needed to confirm this hypothesis. Additionally, recent studies have questioned the extent to which soil properties differ systematically under buckthorn invaded and buckthorn-free stands (Iannone et al. 2015), and a lack of strong differences may have contributed to our results.

Our measurements of jumping worm fecundity provide additional evidence refuting the hypothesis of increased earthworm success in buckthorn-invaded areas. Although highly variable, mean cocoon density was highest in soils from buckthorn-free areas (Fig. 2a). Possible mechanisms for this difference are unclear, although previous work shows that differences in faunal or microbial communities may influence jumping worm fecundity (Snyder et al. 2013). In addition to effects of soil origin, cocoons were more than twice as likely to be present in higher litter areas, and occurred in higher numbers when present (Fig. 2). These results are intuitive—higher resource availability leads to higher fecundity—and are consistent with previous findings of minimal *A. agrestis* cocoon production in soil alone compared to treatments containing both litter and soil (Ikeda et al. 2015). However, these results contrast those of Snyder et al. (2013) who found no significant difference in *A. agrestis* cocoon production in mesocosms with litter and the soil FH horizon compared to only the FH horizon. In our experiment, lower fecundity in low-litter environments is further evidence against earthworm facilitation by buckthorn, given that low litter conditions characterize many buckthorn-invaded



**Fig. 3** Effects of *A. tokioensis* on soil signature and *R. cathartica* germination and establishment following an 18 week mesocosm experiment. For **a**, **b**, Bars represent mean changes compared to initial conditions for mesocosms containing low density (dark grey) and high density (white) earthworm treatments. For **c**, **d**, bars represent mean changes compared to initial conditions averaged across earthworm treatments. Error bars are standard errors. **a** Soil signature depth is significantly greater under high jumping worm density ( $P < 0.001$ ) as well as

higher initial litter mass ( $P = 0.001$ ); **b** soil signature percent cover is significantly greater under high jumping worm density ( $P < 0.001$ ) as well as under higher initial litter mass ( $P < 0.001$ ); **c** *R. cathartica* germination was lower with higher initial litter mass ( $P = 0.005$ ), but did not significantly differ with jumping worm density; **d** *R. cathartica* establishment was lower with higher initial litter mass ( $P = 0.03$ ), but did not significantly differ with jumping worm density

areas due to the replacement of native understory species with rapidly-decomposing buckthorn (Henehan et al. 2002; Table A1).

Overall, results from our first experiment imply a lower likelihood of *A. tokioensis* population growth and survival in buckthorn-invaded areas than those free of buckthorn. The relative lack of cocoons in low litter conditions also implies a possible dependence of *A. tokioensis* on leaf litter. While some research implies that *A. agrestis* are not obligate litter feeders (Zhang et al. 2010; Snyder et al. 2013), other studies find litter an important food source (Ikeda et al. 2015). In our system, litter seems to play an important role in the lifecycle of *A. tokioensis*. Reproduction was below replacement in all low-litter mesocosms (only 4 of 18

low-litter mesocosms contained cocoons at all, and no more than 4 cocoons per mesocosm were recovered for 7 initial earthworms), either due to lack of resources to allocate to reproduction in these conditions, or failure of *Amyntas* individuals to survive to reproduction. This may be a result of an important microhabitat or refuge provided by leaf litter as well as a food source for this largely surface-dwelling earthworm. Further research on the habitat preferences of jumping worms is needed, particularly as they continue to expand their range in the US. Additionally, future research should investigate the potential for cocoons deeper in the soil profile—which was logistically infeasible in the present study.

Our hypothesis of increased buckthorn germination and establishment under higher jumping worm activity was also unsupported. The limited germination and establishment under medium and high litter treatments confirmed existing knowledge regarding the negative effect of leaf litter depth on buckthorn germination (Knight et al. 2007; Roth et al. 2015). However, increased earthworm activity did not moderate this effect as in other studies (e.g. Roth et al. 2015). Epigeic earthworms are hypothesized to increase germination through changes to soil environment and nutrient availability (e.g. patches of nutrient rich castings) (Asshoff et al. 2010), but in the case of buckthorn primarily through the reduction of leaf litter (Roth et al. 2015). Even under high-density earthworm treatments we saw less litter loss than anticipated based on field observations and previous studies (e.g. 32–72% loss vs. 84–95% loss in Qiu and Turner 2016). It may be that the jumping worms are eating more than just litter (Zhang et al. 2010; Snyder et al. 2013), or that the field conditions were unsuitable. The 2016 summer and fall were uncharacteristically cool and rainy, which may have influenced *A. tokioensis* feeding behaviour—particularly in mesocosms where options to seek more desirable conditions are limited. Alternatively, it may be that *A. tokioensis*, which is smaller than the more commonly studied *A. agrestis*, has less impact on litter than previously assumed. Future studies should investigate potential differences in the ecological impacts of different Asian jumping worm species (Chang et al. 2016b).

The absence of facilitation may also be a consequence not of total litter loss but of timing. Given the annual lifecycle of *Amyntas* spp., most litter loss occurs in the late summer/early fall (Qiu and Turner 2016). This contrasts with the more consistent feeding of European earthworm species throughout the growing season. While buckthorn does germinate throughout the summer and even into the fall in our study region, peak buckthorn germination in this region is mid-summer (Susan Carpenter, University of Wisconsin Arboretum, *personal communication*). Thus, even in cases where jumping worms ultimately consume enough litter to alter germination conditions, there is likely to be a mismatch in timing whereby the peak germination period has already passed by the time of peak litter consumption. However, yearly temperature variability may alter this relationship, with *Amyntas* maturation highly variable among

years (e.g. mature worms present May 2017 versus July 2016 in the UW-Madison Arboretum, *personal observation*).

Even in the lowest litter conditions, where neither amount nor timing of litter loss presents an obstacle, buckthorn germination and establishment were not significantly increased under high earthworm densities (Fig. 3). However, germination was much lower than anticipated in our experiment overall (Stewart and Graves 2005). Low germination can be partially attributed to ongoing issues with moulding seeds in very wet, rainy conditions during our experiment. There is also potential that earthworm predation of seeds and seedlings may have reduced germination and establishment rates (Eisenhauer et al. 2010; Cassin and Kotanen 2016), although we are unaware of any studies that look at seed or seedling predation by Asian earthworms specifically. Our findings should be confirmed in studies with higher germination success, as well as under more natural conditions. We also encourage future studies to consider a gradient of earthworm densities, including a true control treatment, and studies of movement patterns and rates of spread. Given the absence of a “no jumping worm” treatment in our study, we cannot rule out potential issues of non-linear or threshold effects. It may be that low-density and high-density jumping worm treatments tested here have similar ecological effects.

From a conservation and management perspective, these results are encouraging—although longer term monitoring is warranted to confirm our experimental findings. While buckthorn and jumping worms are both found in abundance in southern Wisconsin, and each provides challenges, there does not appear to be risk of co-facilitation. The presence of buckthorn throughout southern Wisconsin is unlikely to facilitate jumping worm spread, particularly given the unfavorable low-leaf litter conditions characteristic of buckthorn stands. Similarly, the incipient jumping worm invasion is unlikely to worsen the challenges already posed by buckthorn, as jumping worms do not seem to be decreasing leaf litter enough, or at the right time, to facilitate buckthorn invasion. Future research should focus on determining the habitat preferences of *Amyntas* to better understand factors driving the spread and success of this rapidly expanding species.

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