RESEARCH ARTICLE

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Snail herbivory affects seedling establishment in a temperate forest in the Ozarks

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Abstract

1. Species-specific herbivores are hypothesized to maintain plant diversity by preventing the dominance of any one plant species. However, a large proportion of herbivores have wide host ranges, and these generalists could have similar effects on plant community composition if they exhibit differences in their host preference. Here, we coupled laboratory and field experiments to test whether a common forest-understorey snail (*Neohelix alleni*), a generalist herbivore, has the potential to influence forest composition through differential preference of their plant hosts.

2. We first performed a cafeteria-style experiment to test whether *N. alleni* showed feeding preferences among leaves of five tree species and one shrub common to temperate forests in Missouri, USA. We then conducted a factorial snail and deer exclusion experiment to decouple the effects of snail herbivory from those of white-tailed deer on seedling establishment of 1-month-old newly germinated seedlings of these six woody species in the field. Finally, we examined whether variation in both snail feeding preference and experimentally measured effects of snails on seedling establishment across plant species were related to their relative abundance measured in a 12-ha forest plot.

3. In the laboratory, we found that snails preferred leaves of woody species that were less abundant in the forests relative to those species that were more common. In the forest, we found that experimental exclusion of snails had a stronger positive effect on seedling above-ground biomass and survival over a 1-year period than did exclusion of deer. Plant species found to be more preferred in the laboratory were also those that had lower seedling establishment in the forest due to the negative effects of snails.

4. *Synthesis*. Collectively, our results suggest that greater susceptibility to snail herbivory limits seedling establishment, perhaps contributing to differences in tree species relative abundance. Although less appreciated than their insect and mammal counterparts, herbivory by snails may be significant drivers to the assembly of forest tree communities.

Joint-first authorship between Anna J. Liang and Claudia Stein.

1 | INTRODUCTION

Herbivory is widely known as an important determinant of plant species abundances and species composition (Huntly, 1991; Maron & Crone, 2006; Pacala & Crawley, 1992). Much of the theory on how plant herbivory can serve as a stabilizing mechanism to species diversity assumes that herbivores exhibit strict host specialization (Connell, 1971; Janzen, 1970). In this case, the build-up of herbivores in the vicinity of their preferred host plant species leads to decreased population growth, preventing that plant species from becoming locally dominant. This resulting negative density dependence maintains diversity because non-host plant species are provided a recruitment advantage and are maintained in the system (e.g. Chesson, 2000). However, a large proportion of herbivores do not have strict host specialization, but have wide host ranges (Crawley, 1983; Dearing, Mangione, & Karasov, 2000; Novotny et al., 2002).

The extent to which these generalist herbivores have the potential to regulate plant species abundance and maintain diversity depends on the degree to which they exhibit differing feeding preferences across their palatable host plants (Belovsky & Jordan, 1978; Morrison & Hay, 2011; Sedio & Ostling, 2013). For example, white-tail deer in temperate forest are known to play a keystone role in determining the structure and composition of forest plant communities (Côté, Rooney, Tremblay, Dussault, & Waller, 2004; Horsley, Stout, & DeCalesta, 2003; Rooney & Waller, 2003). By selectively feeding on preferred plant species, deer promote the dominance of unpalatable plant species over more palatable species (Horsley et al., 2003; Rooney & Waller, 2003). Similarly, generalist insects are well known to differ in their effects on hosts, and have been identified as important agents to the diversity of plant communities (Dyer, Letourneau, Chavarria, & Amoretti, 2010; Norghauer & Newbery, 2014; Xiao et al., 2017).

While much attention has been placed on mammal and insect herbivores as potential agents that shape plant diversity, no study has addressed whether herbivory by native gastropods (e.g. snails) has similar impacts on temperate forest ecosystems. We know of only one study assessing the impact of invasive snails and slugs in a Hawaiian forest (Shiels, Ennis, & Shiels, 2014). Even though snails and slugs are known as essential components of forest ecosystems, our current knowledge is largely limited to their importance to forest litter decomposition and nutrient cycling (Mason, 1970; Richter, 1979), or as food sources for many other forest fauna (Digweed, 1993; Graveland, Vanderwal, Vanbalen, & Vannoordwijk, 1994; Nyffeler & Symondson, 2001). This lack of studies is surprising, as many studies performed in grasslands do find strong impacts of gastropod herbivory on plant species composition (Allan

& Crawley, 2011; Buschmann, Keller, Porret, Dietz, & Edwards, 2005; Cleland, Peters, Mooney, & Field, 2006; Hanley, Fenner, & Edwards, 1995) that can even be stronger than the effect of herbivory by vertebrates (Korell et al., 2016). Generally, it is expected that the negative effects of herbivores on seedling establishment lead to greater changes in plant community composition than herbivory on adult plants (Fenner, Hanley, & Lawrence, 1999). Such limitation in seedling establishment can exert dramatic effects on the composition and abundance of plant communities including forest ecosystems (Comita, Muller-Landau, Aguilar, & Hubbell, 2010; Hanley, 1998; Ribbens, Silander, & Pacala, 1994). Whether snails limit the establishment success of seedlings in temperate forests remains untested.

In this study, we coupled laboratory feeding preference trials, observational studies and field experiments to test the overarching hypothesis that selective herbivory by a common native forest-understorey snail (*Neohelix alleni* Sampson, Polygyridae) differentially influences the growth and survival of common forest tree and shrub species in a temperate forest in Missouri, USA. We predicted that establishment success of each woody species would be determined by their level of palatability to snails. In a cafeteria-style experiment, we tested the degree to which snail feeding preferences differed among leaves of five tree species and one shrub species. We then determined whether such variation in palatability was linked to seedling establishment in the field, which was determined using a factorial snail and deer exclusion experiment designed to decouple the effects of snail herbivory from those of white-tailed deer (*Odocoileus virginianus* Zimmerman, Cervidae) on seedling growth and survival in the field. Finally, we examined whether variation in feeding preference by snails, and subsequent seedling establishment across species, was correlated with abundance of these six plant species in the forest.

2 | MATERIALS AND METHODS

2.1 | Study system

This study was conducted at the Washington University in St. Louis' Tyson Research Center, located 25 miles (40 km) southwest of Saint Louis, Missouri (38°31′N, 90°33′W; mean annual temperature 13.5°C; mean annual precipitation 957 mm). The research center is located on the northeastern edge of the Ozark ecoregion and is largely dominated by deciduous oak-hickory forest, which is the most common deciduous forest type extending over much of eastern North America. Characteristic plant communities of this region of the Ozarks include dry chert woodlands, dry-mesic limestone/dolomite forests, and open prairies and glades. Soil types

include silty loam and silty clay that develop from shale lime-stone, limestone, and chert formations (Zimmerman & Wagner, 1979). Approximate white-tailed deer densities at Tyson Research Center range from 10–15 deer/km^2 and snail densities range from 0.5–8 snails/5m^2 (C. Stein, unpublished data). Additional information about the study site is available in Spasojevic, Yablon, Oberle, and Myers (2014).

We used six woody species common to Missouri forests for our study (Table 1). Four species are native to the region: *Diospyros virginiana* L. (Ebenaceae), *Frangula caroliniana* (Walter) A. Gray (Rhamnaceae), *Morus rubra* L. (Moraceae) and *Quercus rubra* L. (Fagaceae). The remaining two species, *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) and *Lonicera maackii* (Rupr.) Maxim (Caprifoliaceae), are exotic to the region and established at the study site at least 30–50 years ago (Table 1). *Ailanthus altissima*, known as tree-of-heaven, is native to central China and is considered invasive across most of the United States where it invades a wide range of ecosystems, including forest gaps and forest edges (Knapp & Canham, 2000; Miller, 1990)). *Lonicera maackii*, known as bush honeysuckle, is a shrub native to temperate western Asia that has invaded much of the eastern United States where it occurs primarily in disturbed areas and forest edges (Bartuszevige, Gorchov, & Raab, 2006).

2.2 | Experimental design

2.2.1 | Snail preference trials

We conducted a cafeteria-style preference experiment to test whether the native snail (*Neohelix alleni*) differs in its feeding preferences across our six plant species. Snails were collected from the field in close proximity to the forest plots and fed a controlled diet consisting of lettuce for at least 3 days. Prior to the experiment, snails were starved for 24 hr.

For each plant species, we placed fresh leaf material collected from seedlings growing in forest understorey of our field site (~200 mg each, avoiding the mid-vein) in a 10-cm Petri dish in a circle. A single snail was added to the center of each Petri dish. These arrays were replicated 12 times. To assess the weight loss of the fresh plant material over the duration of the feeding experiment, we set up seven additional Petri dishes filled with the same plant material, but without a snail (controls). Snails were left to feed in dark, moist conditions. After 12 hr, the snails were removed from the Petri dishes and the remaining plant biomass was oven-dried at 60°C for 48 hr and weighed. Relative loss of biomass due to herbivory after 12 hr was used to estimate feeding preference, and was calculated as: Loss of biomass due to feeding (%) = $(DW_{estimated} − DW_{final})/(DW_{estimated})$, where: DW_{final} = dry weight of plant samples after 12 hr of snail feeding. Estimated dry weight ($DW_{estimated}$) was calculated as: $FreshWeight_{initial of each plant in feeding arena} − Loss_{estimated}$, with $Loss_{estimated} = (FreshWeight_{initial of each plant in control} − DW_{final of each plant in control})/FreshWeight_{initial of each plant in control}$.

2.2.2 | Field experiment

We performed a seedling transplant experiment at eight different sites distributed across the forest of the Tyson Research Center to determine the relative importance of herbivory by snails and by white-tailed deer (*Odocoileus virginianus*) on the growth and survival of the six plant species used in the feeding trials. Each site contained one fenced plot (50 × 50-m wire fence, 2 m in height) designed to exclude deer, and one equally sized adjacent plot that was unfenced and accessible to deer. Fenced and unfenced plots were previously established in 2009, and the distance between each site (fenced and unfenced pair) ranged from 0.4 to 1.1 kilometres. Within each fenced and unfenced plot at each of the eight sites, we established one snail exclosure, one snail enclosure and one unfenced plot (control) naturally accessible to snails. These three snail treatment plots were in close proximity to each other, each separated by one metre. Snail exclosures and enclosures consisted of 2-m × 1-m × 0.2-m solid metal fences (IRKA Schneckenzaun® Typ1, www.der-schnecken-zaun.de) buried 0.1 m below the ground and extending 0.2 m above the ground, with a sharp rim that either curved inwards (enclosure) or curved outwards (exclosure) to prevent snails from climbing out of or into the snail fences. Control plots accessible to snails were of the same size. The inside and outside of both the snail enclosures and exclosures were lined with copper tape (Corry's®) to increase the efficiency of the fences as such physical barriers composed of copper are known to repel terrestrial gastropods (Hata, Hara, & Hu, 1997). To kill snails that accidentally entered snail exclosures, we used slug baits containing the active component, metaldehyde (Ortho 'Bug-Geta'®). To each snail enclosure, we placed two field-collected snails.

In June 2014, locally and regionally collected seeds of our six target plant species (Table 1) that have been collected during the previous fall and winter were first germinated in the greenhouse in a 2:1 field soil and sand mix, and newly germinated seedlings (~1 month old) were removed from the germination containers and transplanted into plots of each snail treatment. Each plot was first cleared from all naturally occurring vegetation by cutting all above-ground vegetation as close to the ground as possible and carefully uprooting the plants to minimize soil disturbances. Each plot was then planted with three seedlings of each of the six species. Seedlings were randomly planted to form a grid, with 30-cm space between each individual. Immediately after planting, we measured initial height of each seedling from the ground to the apical bud. Dead plants were replaced within the first 2 weeks of the experiment. After 1 year in the field, all seedlings were harvested, and above-ground plant biomass was dried for at least 2 days at 60°C and then weighed. We defined a seedling recruit as a germinated seedling that was able to survive without maternal resources for 1 year in the field. In July 2015, this field experiment was then repeated with newly germinated seedlings and was of the exact design as described above.

Relative abundances of the plant species used in the preference trials were obtained from a 12-ha (460 × 260 m) forest plot where all live woody stems with 1-cm diameter or larger at breast height (dbh) were mapped, measured, tagged and identified to species

TABLE 1 Species used in the experiments and their characteristics

	Ailanthus altissima	Diospyros virginiana	Frangula caroliniana	Lonicera maackii	Morus rubra	Quercus rubra
Family	Simaroubaceae	Ebenaceae	Rhamnaceae	Caprifoliaceae	Moraceae	Fagaceae
Origin	Exotic	Native	Native	Exotic	Native	Native
Both exotics are actively spreading at study site	At least present for ≥50 years[d]			1984 first record in Missouri[e]		
Canopy/understorey tree	Canopy	Canopy	Understorey shrub or tree	Understorey shrub	Canopy or understorey	Canopy
Shade tolerance[a]	Intolerant	Tolerant	Tolerant	Intolerant	Tolerant	Intermediate tolerant
Height at maturity [m][a]	16.8	16.8	6.1	4.9	21.3	24.7
Max. dbh at field site [cm][b]	29.1	25.2	12.0	4.7	11.4	90.1
Seed source	Study site	Study site	Study site	Study site	Regional	Regional
Initial height[c] of seedlings 2014 [cm]						
Mean	3.1	11.0	3.8	3.1	35.4[f]	12.9
Median	2.9	11.3	3.7	2.8	41.2	12.4
Min.–max.	1.2–31.7	5–18.5	1–7.7	0.5–6.5	0.4–60.9	5.3–26.7
Initial height[c] of seedlings 2015 [cm]						
Mean	7.6	9.1	5.6	6.2	4.6	10.6
Median	7.1	9.6	5.5	6.6	4.2	10.3
Min.–max.	1.7–17.1	2.2–16.7	0.6–9.8	1.4–10.8	0.8–9.8	6.2–20.0

[a]Shade tolerance, and height at maturity were obtained from the USDA PLANTS database (https://plants.usda.gov/). Note that height at maturity is an estimate of the median mature height of all plants of a species under ideal conditions defined as soil pH = 5.0–7.8; soil salinity ≤4 mmhos/cm; soil depth ≥40 inches; effective average annual precipitation ≥30 inches; soil texture class = medium; no ponding; rare or no annual flooding; and high water table depth ≥1 foot during plant active growth period. [b]The maximum diameter at breast height (dbh) is the max. dbh in the 2013 census of the ForestGEO plot at the Tyson Research Center. [c]Seedling height was measured in the experiments. [d]Rae Crandall personal observations. [e]Luken and Thieret (1996). [f]In the first field experiment (2014–2015), approximately 5-month-old seedlings of M. rubra were used for the field experiment, as no seeds were available to germinate new seedlings.

following standard protocols (Condit, 1998). Therefore, measured abundances included adults as well as saplings. The census occurred between 2011 and 2012. This plot is part of a global network of forest-ecology plots coordinated through the Smithsonian Institution's Center for Tropical Forest Science (CTFS) and Forest Global Earth Observatories (ForestGEO) (Anderson-Teixeira et al., 2015). Additional information about the census is available in Spasojevic et al. (2014).

2.3 | Statistical analyses

2.3.1 | Snail preference trials

We performed nonparametric Friedman's tests (Roa, 1992) and post hoc multiple comparisons using the Kruskal Dunn test to determine which plant species was preferred over other species. We performed Spearman's rank correlations to determine whether ranked feeding preferences of snails for a given plant species were correlated with that species' relative abundances in the field.

2.3.2 | Field experiment

Our field experiment was analysed as a split-plot design, with deer exclusion as the main-plot level, and snail treatments as the subplot level. Data from the two field transplant experiments were analysed together using sites within year as a random factor. To examine the effects of deer and snail herbivory on seedling establishment of individual tree and shrub species, we used final biomass as the response variable in a linear mixed model (lmer function of the LmerTest package in R). All dead individuals were excluded from the analysis. In the first experiment (2014–2015), only two individuals of *A. altissima* survived, and therefore *A. altissima* was excluded from the analysis. Initial height of seedlings was log transformed and used as a covariate. Deer exclusion, snail treatment, plant species, and their interactions were included into the model as fixed effects. Year, year × site, year × site × deer, year × site × snail × deer and year × site × snail × deer × species were included as random effects. Plant biomass was $\log_{10}$(biomass +0.01) – (–2)-transformed to meet assumptions of normality of the residuals. This transformation is a generalized procedure that preserves the original order of magnitudes in the data and results in values of zero when the initial value was zero (McCune & Grace, 2002). We included two orthogonal contrasts within the species x snail treatment interaction to test the a priori hypothesis that snails negatively influenced growth of each plant species. The first contrast ('added vs. excluded') compared the difference between the effect of the snail addition treatment relative to the snail exclusion treatment. The second contrast ('control vs. excluded') tested the effect of snails in the control treatment relative to the snail exclusion treatment. We then examined log response ratios (Hedges, Gurevitch, & Curtis, 1999) to isolate the effect of snail addition on plant biomass of each species. Log response ratios were calculated as: lnR (i) = ln[$BM_{snail\ addition}$(i)/$BM_{snail\ exclusion}$ (i)], where R (i) is the response ratio of biomass of each species (i),

$BM_{snail\ addition}$(i) is the biomass of plant species i in the snail addition treatment and $BM_{snail\ exclusion}$(i) is its biomass in the snail exclusion treatment, averaged across all study sites and the deer exclusion treatment. Thus, a positive lnR indicates an increase in plant biomass of a species in response to snail addition.

The survival of individual seedlings in response to the different herbivore treatments was analysed using a generalized linear mixed model with binomial error distribution and logit-link function (glmer function of the lme4 package in R). Deer exclusion, snail treatment, and plant species were used as fixed effects. Year, year × site, year × site × deer, year × site × snail × deer and year × site × snail × deer × species were included as random effects. Significance levels were obtained using the Wald chi-squared test (ANOVA function of the car package in R). We used planned orthogonal contrasts within statistically significant snail treatment to test the same a priori hypotheses as described above, that is, (1) '"Added vs. excluded"' and (2) '"Control vs. excluded"'. All analyses were performed using the statistical software R3.3.2 (R Development Core Team; http://www.R-project.org).

3 | RESULTS

3.1 | Snail feeding preferences were correlated with plant species abundances in the field

In the cafeteria-style preference experiment, *N. alleni* exhibited species-specific feeding preferences for seedlings across the six plant species (χ^2 = 24.86, df = 5, p < 0.001, Figure 1a). The most preferred species was the exotic plant species, *A. altissima*, which had an average loss of leaf biomass of 80 ± 0.1% (least square mean ± 1 *SE*) due to snail feeding. The second most preferred species was the native species, *M. rubra*, which had an average loss of 52 ± 0.1%. The least preferred species were *F. caroliniana* and *Q. rubra*, both species experienced no significant reduction in leaf biomass in the cafeteria-style experiment. Ranked feeding preferences were negatively correlated with plant species abundances in a 12-ha tract of temperate forest (Spearman rho = −0.83, p = 0.029). Less preferred plant species were more abundant in the field compared to plant species that were more preferred by snails (Figure 1b).

3.2 | Snails influenced seedling establishment in the field

Snail herbivory significantly influenced seedling establishment, but this effect differed among plant species (Species × snail, $F_{10,\ 318.87}$ = 1.77, p = 0.06, Figure 2). Three of the six species had higher biomass when snails were excluded (Table 2, Figure 2), and log response ratios (lnR) indicated that snail addition in the field negatively affected *L. maackii*, *M. rubra* and *A. altissima* growth (Figure 3). These were the three plant species found to be most preferred in the laboratory (Figure 3). Those species least preferred in the laboratory, *F. caroliniana*, *Q. rubra* and *D. virginiana*, were not differentially influenced by the presence or absence of snails in the field.
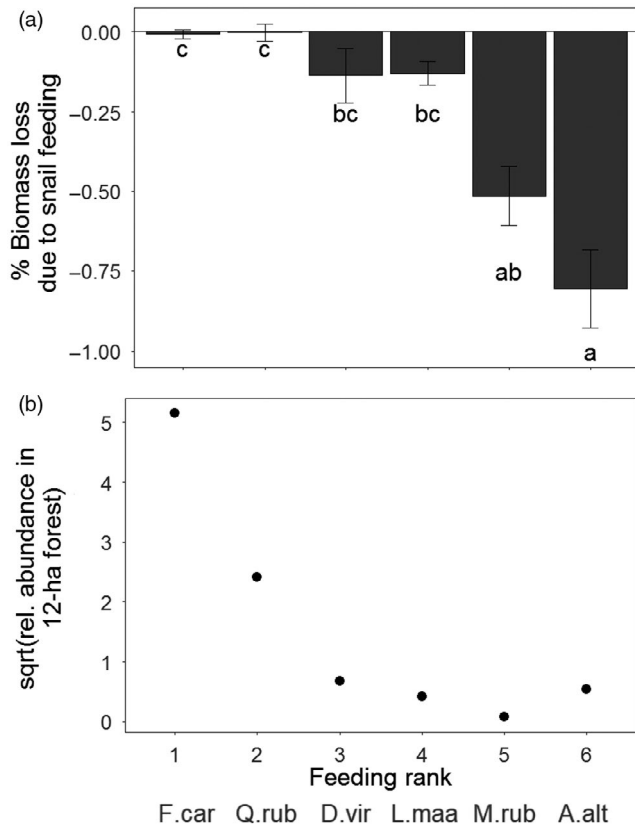
FIGURE 1 (a) Palatability of six different woody species to snails was measured as % loss of biomass in cafeteria-style preference trials using the native snail *Neohelix alleni*. Snail feeding preferences differed significantly across plant species (LSmeans ± *SE*, n = 12). Species (F. car = *Frangula caroliniana*, Q. rub = *Quercus rubra*, D. vir = *Diospyros virginiana*, L. mac = *Lonicera maackii*, M. rub = *Morus rubra*, A. alt = *Ailanthus altissima*) were ranked from left to right by lowest (1) to highest (6) feeding preference. Lower case letters below bars represent means significantly different between species as determined by nonparametric Friedman's tests (χ^2 = 24.86, *df* = 5, *p* < 0.001) and post hoc multiple comparisons using Kruskal Dunn Test. (b) Species palatability was negatively correlated with relative abundances of trees and shrubs, that is, abundances of adults and saplings with a diameter at breast height >1 cm, across a 12-ha forest plot (Spearman rho = −0.83, *p* = 0.029)

Although we did not find that snail addition influenced seedling survival in the field (non-significant 'added vs. excluded' contrast), we did find that survival significantly decreased by about 12% in control plots compared to snail exclusion plots (significant 'control vs. excluded' contrast: *z*-ratio: −2.69, *p* = 0.007), which was consistent across species (lack of a snail × species interaction; Table 3). Survival after 1 year averaged across all plant species was 49.2 ± 4.4% (least square mean ± 1 *SE*) when snails were added, 37.5 ± 4.3% for the control plots and 48.2 ± 4.3% when snails were excluded. Survival did differ significantly among plant species when averaged across all herbivore manipulation treatments (Species χ^2 = 80.18, *df* = 5, *p* < 0.001, Table 3), with *A. altissima* having the lowest survival of 16.0 ± 3.2% (least square mean ± *SE*). Survival for the other plant species was 42.1 ± 5.1% for *L. maackii*, 46.8 ± 5.4% for *M. rubra*,
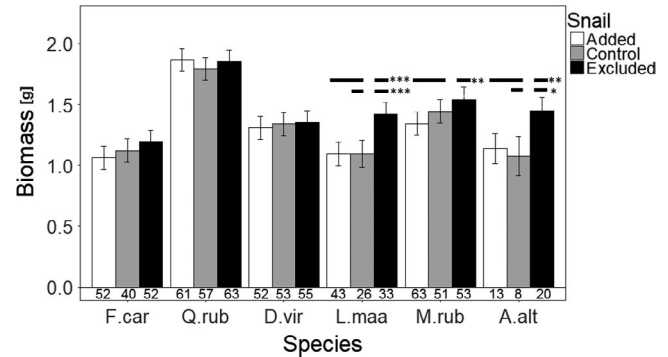

FIGURE 2 Biomass of six woody species after one year of growth in experimental field plots with snails either being excluded, added or not manipulated (control). The experiment was replicated in two consecutive years. Bars show LSmeans ± *SE* averaged across deer treatment (excluded/not excluded) and number of replicates is given below each bar. Horizontal lines above bars indicate significant differences among snail treatments (averaged over both deer treatments) for each species separately according to orthogonal contrasts (see Table 2 for details). Significance is indicated by asterisks. *p < 0.05, **p < 0.01, ***p < 0.001

54.4 ± 4.8% for *D. virginiana*, 58.3 ± 4.9% for *F. caroliniana* and up to 60.0 ± 4.8% for *Q. rubra*.

3.3 | Snails had a stronger effect on seedling establishment compared to deer

While snail herbivory did influence biomass of seedlings measured 1 year after transplanting them into the field, deer herbivory did not (Table 2). Biomass averaged across all species was 1.38 ± 0.08 g in deer exclosure plots and 1.34 ± 0.08 g in plots where deer had access. Deer also had no significant effect on survival of seedlings after 1 year in the field (deer exclusion treatment: 49.4 ± 3.4%, deer control: 41.6 ± 3.4%).

4 | DISCUSSION

The seedling stage is one of the most critical phases during a plant's life history, and processes that lead to differences in seedling recruitment across species can determine the composition and species abundance of plant communities (Comita et al., 2010; Hanley, 1998; Ribbens et al., 1994). We show that a species of snail, *Neohelix alleni*, despite being a generalist herbivore, differs in its feeding preferences across host plant species, which translates into consistent differences in seedling establishment in the forest. Seedlings of tree and shrub species found to be less preferred by *N. alleni* in our laboratory experiment were those that were unaffected by our snail manipulations in the field. Those plant species preferred by snails in the laboratory experiment were also those that suffered more from the presence of snails in the field. Although it is well known that snails and slugs have the potential to affect the distribution and abundance patterns of plant species within herbaceous communities (Barlow, Close, & Port, 2013; Hanley et al., 1995; Hanley, Fenner, & Edwards,

Fixed effects	*df* (num, denom)	*F*-value	*t*-ratio[a]	*p*
Initial height	1, 719.17	265.24		**<0.001**
Deer	1, 20.06	1.33		0.262
Snail	2, 366.36	12.99		**<0.001**
Species	5, 357.94	60.77		**<0.001**
Deer × snail	2, 365.01	2.27		0.105
Species × deer	5, 323.75	1.16		0.328
Species × snail	10, 318.87	1.77		**0.064**
Frangula caroliniana				
Added vs. excluded			−1.85	0.065
Control vs. excluded			−1.01	0.316
Quercus rubra				
Added vs. excluded			0.37	0.709
Control vs. excluded			−0.84	0.399
Diospyros virginiana				
Added vs. excluded			−0.62	0.538
Control vs. excluded			−0.42	0.672
Lonicera maackii				
Added vs. excluded			−3.84	**<0.001**
Control vs. excluded			−3.13	**0.002**
Morus rubra				
Added vs. excluded			−3.22	**0.004**
Control vs. excluded			−1.53	0.277
Ailanthus altissima				
Added vs. excluded			−2.13	**0.034**
Control vs. excluded			−2.18	**0.029**
Deer × snail × species	10, 318.64	0.79		0.637

TABLE 2 Results of a linear mixed model testing for the effects of deer and snail herbivory on final biomass of six woody species. Species were transplanted as seedlings into experimental plots in a temperate forest and harvested after one year. The experiment was replicated in two consecutive years. Final biomass was $log_{10}(x + 0.01) + 2$-transformed prior to analyses. Year, deer, snail treatment and species within study sites were used as random effects and initial height of seedlings was used as a covariate

Note. Variance components for random terms are: Year 0.015, year × site 0.001, year × site × deer 0.0002, year × site × deer × snail 0.00, year × site × deer × snail × species 0.042, residual 0.069. Bold values indicate the significance of the *P*-value.

[a]*t*-ratios are given for planned orthogonal contrasts calculated within the species x snail interaction for comparing the snail treatments within each plant species. For details see 'Materials and methods'.

1996; Motheral & Orrock, 2010), our study provides one of the first experimental demonstrations of the importance of native snail herbivory to seedling growth and survivorship in a temperate forest.

We found that feeding preference of a given plant species was correlated with the abundance of that species in the field; however, the causal direction of this relationship is unknown. Plant species that were less common in the forest were those that were found to be more palatable in the laboratory experiment, and had lower seedling establishment in the presence of snails in our field experiment. The consistent link between feeding preferences in laboratory and experimentally determined seedling establishment in field suggests that snail herbivory could directly determine the abundance of tree and shrub species in the forest. Alternatively, other mechanisms could determine species abundance and snails may feed preferentially on locally rare species as a strategy to avoid secondary defence chemistry of abundant species. Locally rare plant species would have

novel secondary metabolites, and preferential consumption of these rare species may serve to spread the toxin load over different detoxification metabolic pathways (Marsh, Wallis, Andrew, & Foley, 2006; Verhoeven, Biere, Harvey, & Putten, 2009). For example, generalist mammal herbivores have been found to forage across a broad range of plant species as to minimize the consumption of any single toxin (Foley & Moore, 2005). Future studies that examine how snails respond to defence chemistry are required to determine the importance of snails in regulating tree species abundance.

There are many plant characteristics that contribute to the palatability of a plant species. In general, functional plant traits related to faster growth rates, enhanced resource allocation patterns and nutrient uptake are known to characterize high palatable plant species. For five of our six plant species, Spasojevic et al. (2014) measured leaf size and specific leaf area on seedlings at our study site. The plant species most preferred by snails, *A. altissima*, is the species
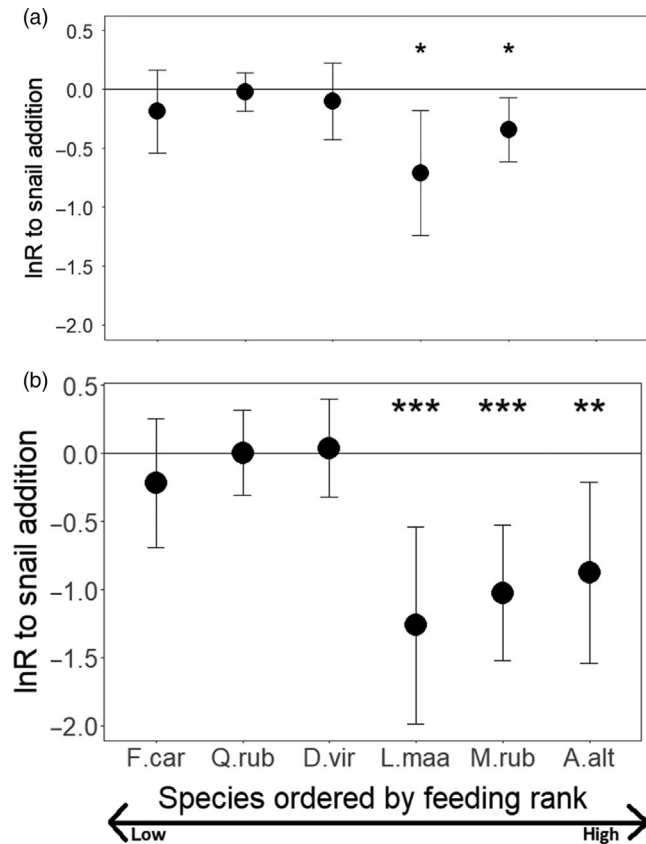
FIGURE 3 The influence of snail herbivory on the biomass of plant species for the species ranked by their feeding preferences as determined by our cafeteria feeding trial (from low to high feeding preference (F. car = *Frangula caroliniana*, Q. rub = *Quercus rubra*, D. vir = *Diospyros virginiana*, L. mac = *Lonicera maackii*, M. rub = *Morus rubra*, A. alt = *Ailanthus altissima*). The experiment was repeated in two consecutive years (a) 2014–2015 and (b) 2015–2016. Survival of *A. altissima* was too low in the first round (2014–2015) to be included in the analysis. Log response ratio (lnR) represents the relative response of species biomass (with 95% confidence interval) to the "snails added treatment relative to the snails exclusion" treatment (averaged over eight study sites). A negative lnR indicates that the biomass of a plant species decreases due to snail herbivory. Significance is indicated by asterisks. *p < 0.05, **p < 0.01, ***p < 0.001

TABLE 3 Analysis of deviance table of a generalized linear mixed effects model (type II Wald chi-square test) testing for the effects of deer and snail herbivory on the survival of individual seedlings from six woody species. Species were transplanted as seedlings into experimental plots in a temperate forest and harvested after 1 year. The experiment was replicated in two consecutive years

Predictor variables	df	χ^2	z-ratio[a]	p
Initial height	1	51.24		**<0.001**
Deer	1	2.58		0.108
Snail	2	8.12		**0.017**
Added vs. excluded			0.27	0.787
Control vs. excluded			−2.69	**0.007**
Species	5	80.18		**<0.001**
Deer × snail	2	0.46		0.795
Species × deer	5	1.27		0.938
Species × snail	10	8.29		0.600
Deer × snail × species	10	9.19		0.514

Note. Variance components for random terms are: Year 0.029, year × site 0.00, year × site × deer 0.117, year × site × deer × snail 0.00, year × site × deer × snail × species 0.497.
[a]z-ratios are given for planned orthogonal contrasts.

with the highest specific leaf area (362.76 cm²/g) and the smallest leaf size (32.58 cm²). In comparison, *Q. rubra*, a species that was non-palatable to snails in our experiment, had the lowest specific leaf area (159.61 cm²/g) and the largest leaf size (137.72 cm²). These results indicate that palatability of a plant species to snails increases with higher specific leaf area and decreases with leaf size. A finding that is in line with results from a previous study in which leaf thickness has been shown to influence palatability in the way that plants with the thinnest leaves were most preferred by gastropods (Dirzo, 1980). Future studies are needed to assess which traits determine gastropod feeding preferences for seedlings of woody species.

One of the leading hypotheses proposed to explain the success of invasive plant species is that these species have an advantage because they can escape their natural enemies of their native ranges

(Keane & Crawley, 2002). However, two of the three plant species that showed the strongest responses to the presence of snails in the field were invasive species (*A. altissima* and *L. maackii*). Instead, this finding is in line with the biotic resistance hypothesis which proposes that resident herbivores (and other antagonists such as competitors) can decrease invader success (Levine, Adler, & Yelenik, 2004; Maron & Vilá, 2001). Our finding that native snails reduce establishment of these invasive plant species is in contrast with previous studies that suggest that snails do not differentially prefer native or exotic grassland and woody plant species (Buschmann, Edwards, & Dietz, 2006; Korell et al., 2016; Motheral & Orrock, 2010; Shiels et al., 2014). In addition to snail herbivory, the low abundance of both invasive species might also be explained by the lack of major disturbances within the 12-ha forest plot. Both species are early successional and are known to establish only in forest edges and disturbed areas (Bartuszevige et al., 2006; Knapp & Canham, 2000).

We also found that the effects of deer on young seedling establishment were negligible relative to that of snails. Deer are well known to have both negative and positive effects on forest communities as seed and sapling predators (Côté et al., 2004; Rooney & Waller, 2003) and as seed dispersers (Myers, Vellend, Gardescu, & Marks, 2004). However, the exclusion of white-tailed deer did not affect biomass or survival of young seedlings as did the exclusion of snails in our experiment. This is consistent with previous studies in grasslands that showed invertebrate herbivory to be more influential to seedling establishment than vertebrate herbivory (Allan & Crawley, 2011, Korell et al., 2016, but see Hulme, 1994).

We suggest that the relative importance of snails and deer to establishment will likely change as tree seedlings mature to saplings. Our experiment only tested for the effects of snails and deer on seedling

establishment after 1 year. During this time, seedlings of our experiment were likely too short to be consumed by white-tailed deer. Previous studies have shown that deer select taller plants over shorter plants (Anderson, 1994). For example, a study on *Quercus buckleyi* found a negative effect of white-tailed deer only on saplings taller than 20 cm (Russell & Fowler, 2004). Because snails prefer seedlings and young plant material to mature plants (Fenner et al., 1999), the negative effects of deer relative to snails are expected to increase at the sapling stage.

Future studies are needed to assess if the negative correlation between snail feeding preferences and woody species abundances in the field is observed for other species and in other regions. In our study, we only examined six woody species, which limits the generalization of our results. Further, quantifying the degree of snail herbivory in comparison to deer herbivory on seedlings in the field would allow a more rigorous test of the importance of snail herbivory for the establishment of forest species. For our study, we cannot completely exclude the possibility that the snail fences and copper barriers might have repelled other seedling enemies such as non-volant insects. However, the significant reduction in seedling growth in plots where we experimentally added snails compared to snail exclosures suggests that the majority of effects were due to snails.

In summary, our findings highlight the importance of gastropods in temperate forest systems. We present strong experimental evidence that snails have the potential to shape woody species community composition in a temperate forest through their differential feeding preferences at the seedling stage. Our study suggests that snails may be important drivers to tree species abundance, but follow-up experiments are required to confirm this hypothesis.

AUTHORS' CONTRIBUTIONS

C.S. conceived the idea. C.S., A.J.L., S.A.M. designed the study. A.J.L., E.P. and C.S. conducted the experiments. J.A.M. and R.M.C. contributed essential species abundance data. C.S. analysed the data with assistance from A.J.L. A.J.L., C.S. and S.A.M. wrote the manuscript with input from all authors.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.v0t4qm3 (Liang et al., 2019).

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