Interactive effects of deer, earthworms and non-native plants on rare forest plant recruitment

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ABSTRACT

Multiple biotic and abiotic factors, operating at several stages may affect plant demography and recruitment. Across forests in North America increased white-tailed deer (*Odocoileus virginianus*) abundance, non-native earthworms and non-native plant invasions are likely to generate single and interactive effects. We evaluated effects of these stressors on recruitment of three rare plants (*Aristolochia serpentaria*, *Carex retroflexa* and *Trillium erectum*). We conducted a multiple year seed addition experiment using paired open and fenced plots (experimentally excluding deer) at 12 forested sites that differ in earthworm density and non-native plant cover. We found strong microsite limitations for *C. retroflexa*, which completely failed to establish after a 3-year period despite successful germination in greenhouse trials. Addition of *A. serpentaria* and *T. erectum* seed resulted in successful seedling emergence; however, *A. serpentaria* recruitment steadily decreased over the study period. We found no significant effects of non-native *Alliaria petiolata* or *Berberis thunbergii* on seedling recruitment but surprisingly, we found strong positive effects of non-native *Microstegium vimineum* on *A. serpentaria* and *T. erectum*. Deer exclusion resulted in increased *T. erectum* emergence and seedling survival. Earthworm abundance negatively affected survival of *T. erectum* seedlings, and the effect manifested itself through interactive effects with fencing and non-native plants. Comparing recruitment through seeding addition to recruitment through transplants in a parallel study, we identified strong potential for *T. erectum* restoration using seed addition, but this will be dependent upon a significant reduction of deer impacts. Restoration of *A. serpentaria* and *C. retroflexa* will require manipulation of microsite conditions to promote recruitment.

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1. Introduction

Long-term conservation of currently declining or rare, threatened and endangered plant species will depend upon increasing their population growth rates at many locations to ensure local and regional population viability and to prevent demographic bottlenecks (Rodríguez-Pérez and Traveset, 2007; Rother et al., 2013). To achieve this goal, conservation practices should focus on reinstating ecological processes important for plant recruitment. Management may, for example, involve increasing the size of adult populations by managing stressors deemed responsible for poor performance of reproductive individuals. Depending on the species, this may involve management of herbivores, invasive species, anthropogenic disturbances, pollution or harvesting (Mooney and McGraw, 2009; Thomson, 2005; Wall et al., 2012). But increasing the reproductive output of remaining individuals may not always

be sufficient to achieve overall population viability. Furthermore, reestablishing populations through translocation of new individuals or seed addition into apparently suitable habitats is challenging (Holl and Hayes, 2006), often due to a lack of understanding of plant demography (Kwit et al., 2004) and specific habitat requirements.

Successful plant recruitment is determined by the combined effects of multiple biotic and abiotic factors affecting seed production, seed dispersal, seed bank presence, seed germination and seedling establishment (Bricker and Maron, 2012; DiTommaso et al., 2014; Maron et al., 2014; Olsen and Klanderud, 2014). Consequently, recruitment limitations may arise through the interplay of both seed and microsite limitations (Clark et al., 2007; Turnbull et al., 2000). Seed limitation may also occur due to stochastic events, such as dispersal processes, and through deterministic mechanisms that limit seed production, including herbivory and competition (Caughlin et al., 2013; Núñez-Ávila et al., 2013). Microsite limitation refers to biotic and abiotic filters that limit seedling emergence and establishment after seed arrives at

a given location, such as local habitat characteristics (Maron and Simms, 1997). If the net effect of these interactions limits recruitment, plant species persistence is at risk with important consequences for overall community structure and composition (Bricker and Maron, 2012; Bruna, 2003; Rodríguez-Pérez and Traveset, 2007).

Seed addition experiments indicate that most plant populations are both seed and microsite limited (Clark et al., 2007; Turnbull et al., 2000), with microsite limitations of overriding importance in minimally disturbed environments (Clark et al., 2007). Notably, the relative importance of the multiple processes that cause recruitment limitations may vary in space and time (Lortie et al., 2004), are affected by species traits (Moles and Westoby, 2002) and are modulated by interactions of concurrent processes (Maron et al., 2014; Maron and Simms, 1997).

Record high deer densities, non-native earthworm invasions and non-native plants constitute major agents of change in forests in northeastern North America (Côté et al., 2004; Fisichelli et al., 2013; Hale et al., 2006; Mack et al., 2000). The single and combined effects of these stressors may impose local filters that result in recruitment limitations. For example, seed limitation may be the result of chronic deer herbivory reducing flowering probability and seed set of highly palatable species (Knight et al., 2009). Similar effects arise if stressors decrease plant survival or seed production (Clark et al., 2007; Turnbull et al., 2000), likely causing many herbivore-affected populations to be seed limited. Similarly, plant invasions may reduce species richness (Vilá et al., 2011) and reproductive effort of vulnerable species (Levine et al., 2003; Miller and Gorchov, 2004), while negative effects of earthworm abundance on seedling survival (Dobson and Blossey, 2014) may result in fewer flowering individuals and seed set.

These stressors may also increase microsite limitations through habitat alteration. For example, long-lasting changes in forest vegetation composition and structure (Amatangelo et al., 2011; Aukema et al., 2010) due to deer herbivory, earthworm activity or non-native plant invasions (Côté et al., 2004; Hale et al., 2006; Tanentzap et al., 2011; Vilá et al., 2011), may affect abiotic and biotic conditions, including changes in microclimate, light availability, amount and quality of leaf litter, leaf litter invertebrate abundance and composition, as well as soil microbial communities (Eisenhauer et al., 2011; Holdsworth et al., 2012; Suarez et al., 2006). At local scales, deer and earthworms can alter microsite conditions through changes in soil compaction (Kardol et al., 2014; Nuttle et al., 2011), nutrient availability (Bohlen et al., 2004; Ewing et al., 2014; Murray et al., 2013) and mycorrhizal infection rates (Kardol et al., 2014; Lawrence et al., 2003). Similarly, non-native plants may reduce recruitment through allelopathic effects (Cipollini and Flint, 2013; Corbett and Morrison, 2012; McEwan et al., 2010) and reductions in mycorrhizal infection rates (Barto et al., 2011).

The net effect of stressors on demography depends on their effects at each demographic transition. For example, positive earthworm effects on seed germination due to leaf litter depletion and exposure of bare soil (Warren et al., 2012) may be outweighed by negative earthworm effects on seed and seedling survival (Dobson and Blossey, 2014), caused by seed predation (Eisenhauer et al., 2010), seed burial (Lawton, 1994) or fine root consumption (Gilbert et al., 2014). Therefore, to properly assess the role of these factors on recruitment, it is important to conduct long-term seed addition experiments, where the single and combined effect of each stressor can be quantified at different stages of the plant life cycle. However, most seed addition experiments are short-term, following seedlings only during the first year of their emergence (Clark et al., 2007; Turnbull et al., 2000). Moreover, despite a recognized need to quantify interactions among multiple concurrent processes in order to understand the

underlying mechanisms shaping plant populations (Didham et al., 2007), few studies experimentally assess effects of multiple stressors on plant demographic parameters (Crone et al., 2011).

Here we explore the single and interactive effects of deer exclusion, earthworm abundance and non-native plant cover on recruitment of three rare forest understory species: *Aristolochia serpentaria* L., *Carex retroflexa* Muhl. ex Willd and *Trillium erectum* L. (hereafter referred to as *Aristolochia*, *Carex* and *Trillium*). We conducted a five-year seed addition experiment using a network of 12 forested sites that differ in earthworm abundance and cover of three non-native focal plant species [*Berberis thunbergii* DC, *Alliaria petiolata* (M. Bieb.) Cavara & Grande and *Microstegium vimineum (Trin.) A. Camus*]. All native understory target species were present in the region, but absent from our study sites (no extant individuals or presence in the seed bank (Nuzzo et al., 2015)). We addressed the following questions: (1) How is recruitment of *Aristolochia*, *Carex* and *Trillium* affected by the interaction of deer, non-native earthworms and non-native plants? (2) How do these stressors affect the relative importance of microsite limitation on recruitment? (3) Do effects of seedling emergence persist over time resulting in differences in seedling survival and growth? (4) Would restoration be more effective through seeding or seedling transplanting? We addressed the latter question by comparing recruitment success in this experiment to success of transplanted seedlings in a parallel study conducted at the same sites during the same study period (Dávalos et al., 2014).

2. Materials and methods

2.1. Study sites

We conducted the study at US Army Garrison West Point (hereafter West Point); a 65 km^2 facility located some 80 km north of New York City within the Hudson Highlands Province of New York State, USA. West Point is covered by upland deciduous forests dominated by oak (*Quercus rubra* L. and *Q. prinus* L.) and/or sugar maple (*Acer saccharum* Marshall). We selected 12 forested sites 1–8 km apart with different percent cover of native and non-native plant species; six sites were located in populations of three focal non-native plant species (*B. thunbergii, A. petiolata* and *M. vimineum*; 2 sites each) and six sites were located in mixed native understory plant communities. All 12 sites vary in invasive earthworm density and biomass (Dávalos et al., 2014). Based on earthworm monitoring results (see Section 2.3 and Dávalos et al., 2015), we classified sites into low and high earthworm abundance categories (measured as function of earthworm density and biomass).

2.2. Study species

We selected three rare herbaceous species based on their occurrence in the study region and conservation status: *Aristolochia serpentaria* (Aristolochiaceae), *Carex retroflexa* (Cyperaceae) and *Trillium erectum* (Liliaceae).

Aristolochia is a perennial herb that grows from a short rhizome with multiple fibrous roots. Stems usually grow singly, reaching 15–60 cm tall, with up to 6–10 alternate leaves per stem. Leaves are hastate shaped, 1–5 cm wide and 1–6 cm long. Maroon s-shaped flowers (1–2 cm long) have a 'trap' design to facilitate insect cross-pollination (Oelschlägel et al., 2009) and are produced singly on short basal stems at or under the leaf litter. Plants rarely have more than two flowers and few plants flower in any given year. The majority of flowers are chasmogamous, but occasionally cleistogamous flowers are produced (Barringer, 1997; Pfeifer, 1966). In our region, flowers are produced June–July and fruits

ripen August–September Capsules are 1–2 cm long with 15–24 seeds. Seeds are gravity-dispersed and most germinate near the mother plant (González and Rudall, 2003). Individual seeds are heart-shaped, 2–3 mm long, lose viability when dried, and typically germinate within the first year (Elliott, 2000). Germinants lack a visible cotyledon (Elliott, 2000). This species appears to have no long-distance dispersal mechanism (Farnsworth and Ogurcak, 2008), is rarely found in disturbed habitats (Farnsworth and Ogurcak, 2008; Landenberger and McGraw, 2004), and has limited ability to recolonize after disturbance (Kirkman et al., 2004). Aristolochia reaches its northern range in NYS where it is listed as Threatened, with six known populations.

Carex is a perennial cespitose sedge with narrow leaves 1.4–3 mm wide. Flowers are produced May–June in several star-like spikes, each with 3–10 perigynea, near the top of a 50–60 cm tall culm. Plants average 12–16 (rarely over 100) flowering culms and culms produce an average of 12 seeds in June–July. Dispersal mechanisms are unknown. Carex prefers early-successional habitat but can persist in late-successional habitat in NYS (New York Natural heritage Program: http://www.acris.nynhp.org/guide.php?id=9511 6 February 2015). The species is listed as Threatened in NYS with 13–35 known populations.

Trillium is a long-lived perennial herb with adult individuals producing three leaves and a single maroon flower at the top of a 20–40 cm tall stem. Plants are usually single-stemmed and grow from a tuber-like rhizome. Plants flower in May, and produce a single maroon fruit per stem in July–August with an average of 14–50 seeds; larger plants produce larger fruits with more seeds than smaller plants (Irwin, 2000). Trillium seeds have an elaiosome and are both ant and gravity-dispersed. Seeds germinate after one or more years of cold stratification; in the first year, a small rhizome is produced and in the second year an above-ground cotyledon emerges that remains present throughout the summer (Case and Case, 2009). The first true leaf is produced the following year, and after one or more years, a single three-leaf stem appears. The first flower appears 5–7+ years after seed germination (Case and Case, 2009). Trillium is considered an indicator of rich mesic forests (Elliott et al., 2014) and is categorized as 'exploitably vulnerable' in NYS.

2.3. Experimental design and data collection

At each site we established paired 30 m × 30 m plots situated 5–50 m apart from each other and randomly assigned one plot to a deer exclusion treatment (open or fenced). We erected deer-proof fences from 7 to 11 July 2008 (Trident extruded deer fence, 2.3 m high, www.deerBusters.com, MD).

We sampled earthworms in mid-July 2008–2011 at 5 random 0.25 m² quadrats per plot (open or fenced) per site/year. We first removed and sifted leaf litter for earthworms, and then extracted earthworms by pouring 3.8 l of mustard solution at 15 g l⁻¹ per quadrat (Frontier Natural Products Co-op, Norway, IA) and captured all earthworms that emerged within a 15 min period (Lawrence and Bowers, 2002). We fixed specimens in 10% formalin, transferred them to 70% ethyl alcohol for storage, weighed each sample and identified each mature individual to species and immature individual to genus. The 12 sites varied in number, biomass and species composition of earthworms and we therefore categorized sites as low abundance (5 sites) or high abundance (7 sites); see (Dávalos et al., 2015) for details.

For each plant species, we established four 20 × 20 cm permanently marked quadrats in each open and fenced plot (20 seeds/quadrat, 4 quadrats/plot, 2 plots/site, 12 sites, total 1920 seeds of each species). At the six sites established in populations of non-native plants, we randomly located the seed quadrats in patches of A. petiolata, M. vimineum and under B. thunbergii

canopy, to test effects of these non-native plants on target plant emergence. In the six sites established in mixed native vegetation we randomly located seed quadrats.

To assess seedling emergence rates under field conditions, we planted fresh seeds of each target species in 2009 or 2010, and monitored seedling emergence above the leaf litter layer from 2010 to 2013. We collected Carex seeds at Wildcat Mountain State Forest and Clearwater Park, New Paltz, NY, on 18 June 2010, and planted seeds 10–11 August 2010. We were unable to collect sufficient numbers of Aristolochia seed locally and therefore purchased Aristolochia seeds from Loess Roots (Stanton, NE). We received seeds 1 October 2009 and planted seeds 4–6 October 2009. We collected Trillium seeds at West Point 6 August 2009 and planted seeds 16–18 August 2009. Trillium has a two year dormancy period (Case and Case, 2009) and we anticipated germination in 2011.

At each planting location, we carefully removed leaf litter before individually planting seeds with tweezers ~2 mm deep (Carex) or 5–8 mm deep (Aristolochia and Trillium) in soil, ~4 cm apart. We then lightly tamped the soil and replaced the leaf litter. We monitored seedling emergence above the leaf litter layer and recorded presence/absence of all species beginning April 2010 through August 2013 at biweekly (2010) or monthly (2011–2013) intervals. We individually marked each seedling emerging from the leaf litter layer (hereafter referred to as emergents) using colored wooden markers. One-year old Aristolochia seedlings could not be distinguished from new emergents; therefore, data after 2010 combine new emergents and surviving seedlings from previous years (hereafter referred to as recruitment). In late August 2011–2013 we also recorded size (height, number of leaves and leaf width) of all surviving Aristolochia. We recorded stage (cotyledon, single- or 3-leaf) of Trillium seedlings in mid-May 2011–2013, and also recorded leaf width in mid-May 2013. We recorded number of culms for each Carex in spring 2011–2013.

To assess seedling emergence rates under common garden conditions and in the absence of competition or predation we planted additional seeds (40 seeds each for Trillium, 20 seeds each for Aristolochia and Carex) of all three species in five reference germination flats/species, filled with a soil-free potting mix (Pro-Mix® BX Mycorrhizae®, Premier Tech Ltd, Riviére-du-Loup, QC, Canada). We kept flats in shaded cages at an outdoor facility in Ithaca NY and monitored emergence through August 2013. We also assessed Carex emergence under controlled conditions by placing seeds (in gauze bags) under leaf litter on top of soil-free potting mix in two 100 l pots kept in shaded cages outdoors for 30 days of warm moist stratification, followed by 4 months cold moist stratification. On 12–13 January 2011 we placed 25 Carex seeds in petri dishes ($N = 32$) filled with potting mix and held them in temperature (30/10 °C day/night) and light (12 h photoperiod) controlled growth chambers. When visual inspection indicated that the vast majority of seeds had germinated, we recorded emergence 9–10 February 2011.

To evaluate restoration potential through seeding and transplant efforts, we compared survival and growth of emergents to that of transplants used in a parallel study at the same sites. Transplant experiment methods are detailed in (Dávalos et al., 2014) and summarized in A1.

2.4. Statistical analyses

We fitted Generalized Linear Mixed Models (GLMM) with binomial errors to evaluate effects of fencing (fenced or open), vegetation type (native and each focal non-native species), earthworm abundance (low or high) and their interaction on Aristolochia recruitment, Trillium total emergence and survival of Trillium cotyledons. We summarized presence/absence data by quadrat (4

quadrats within each open or fenced plot). Models for multi-year data included site, plot within site, and quadrat within plot and site, as random factors to account for the hierarchical nature of the data. We summarized *Trillium* emergence across years, so in this case models included site and plot within site as random factors. We tested second level interactions only, excluding the plant type x earthworm abundance interaction as we only had one site dominated by non-native vegetation with low earthworm density. Plant invasion and earthworm density effects refer to site level and fencing effects to plot level. Given the low number of *Carex* emergents we did not conduct any formal analysis for this species.

We evaluated the effect of study factors on plant size metrics with Analysis of Variance and a posteriori Tukey tests. Given low *Aristolochia* recruitment after 2010, we evaluated the effect of fencing, vegetation type and earthworm abundance on height, number of leaves and leaf width for 2011 data only. Similarly, by 2013 we recorded sufficient *Trillium* seedlings at only four sites and therefore we evaluated the effect of fencing and life stage (single- or multi-leaf stage) on leaf width at these sites only (including site as a fixed factor).

To compare success rate of seeding vs. transplanting, we compared final emergent vs. transplant survival and size in 2013 with Generalized Linear Models with binomial errors (survival) and linear models (size). We only included 10 study sites in this analysis because we excluded two sites from the transplant experiment in 2013 (Dávalos et al., 2014).

We fitted separate models for each species and measure, and examined and confirmed that model assumptions were met for all cases. We evaluated the explanatory power of competing models with Akaike Information Criterion corrected for small samples sizes (AICc) (Burnham and Anderson, 2002). We ranked candidate models according to ΔAICc (difference between model's AICc and min AICc). We considered all models within 2 AICc to be similar (Burnham and Anderson, 2002). We conducted all tests in R 2.14 (R Core Team, 2014); we fitted mixed models with the lme4 package (Bates et al., 2014).

3. Results

3.1. Seeding experiment results

Carex had very low emergence with just 20 seedlings in 2011 (0.01% emergence rate) and two more in 2012. All emergents produced a single culm (with the exception of one individual with two culms) and all died within 1–2 years. *Carex* emerged at 8 of the 12 study sites and the majority of seedlings were recorded in open plots (15 out of 22, 68%). The very low field emergence contrasts with common garden (43% emergence rate) and incubator trials (78% germination rate).

Aristolochia had low emergence rates in 2010, with 273 seedlings (14% mean emergence across sites). Recruitment (emergence and survival combined) was best explained by one model (wi = 0.73) that included a significant effect of year and vegetation type (Table 1a) and had 6.6 times the explanatory power of the next model (ΔAIC = 3.83, wi = 0.11), which included the interaction between year and vegetation type. Recruitment decreased over time and by 2013 only 19 plants were present. Emergence and recruitment were both significantly higher under non-native *M. vimineum* (30% in 2010) than at sites dominated by native vegetation (11%) or sites dominated by two other non-native species: *A. petiolata* (9%) and *B. thunbergii* (14%; Fig. 1). Low emergence in the field contrasts with good success in a common garden (58% emergence).

Aristolochia height in 2011 (*N* = 91 emergents at 11 of 12 sites) was only affected by vegetation type ($F_{3,322}$ = 6.89, *P* = 0.001):

Table 1

Generalized Linear Mixed Model (binomial) results for the effects of study factors on (a) recruitment of *Aristolochia* (2010–2013), (b) total *Trillium* emergence by 2013 and (c) survival in 2013 of *Trillium* cotyledons emerged in 2011. Seedlings emerged from seeds placed individually into vegetation dominated by either a focal non-native plant (*M. vimineum, A. petiolata, B. thunbergii*, *N* = 2 sites per species) or native species (*N* = 6 sites) at 12 sites at West Point, NY. Sites dominated by *B. thunbergii* were excluded from *Trillium* survival analyses, as only two individuals survived to 2013. Models included site and plot within site and quadrat within plot and site as random factors (the latter for a and c only).

| Factor	Est	SE	Z value	P-value
(a) Aristolochia recruitment				
Intercept	−2.54	0.43	−5.91	<0.001
Year (L)	−1.97	0.15	−13.65	<0.001
Year (Q)	0.37	0.14	2.69	0.01
Year (C)	0.07	0.13	0.56	0.58
Vegetation (not *M. vimineum*)	−1.63	0.47	−3.42	<0.001
(b) Trillium emergence				
Intercept	−1.52	0.25	−6.16	<0.001
Fencing (Open)	−0.40	0.19	−2.06	0.04
Vegetation type (*B. thunbergii*)	−2.36	0.64	−3.67	<0.001
Vegetation type (*M. vimineum*)	1.18	0.50	2.37	0.02
(c) Trillium cotyledon survival				
Intercept	−2.90	0.67	−4.33	<0.001
Fencing (Open)	1.61	0.72	2.24	0.03
Earthworm (Low)	2.44	0.84	2.90	0.004
Vegetation type (*M. vimineum*)	3.63	0.90	4.02	<0.001
Fencing x Earthworms	−2.61	0.94	−2.76	0.006
Fencing x Vegetation	−3.88	0.99	−3.93	<0.001

C cubic, L linear, and Q quadratic.
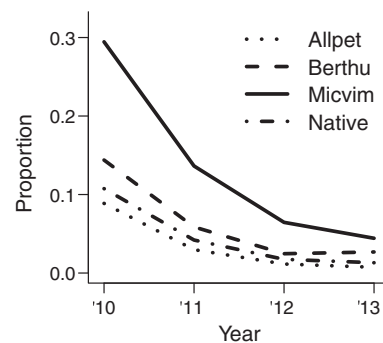
Fig. 1. Recruitment of *Aristolochia* (2010–2013) from seeds placed individually into vegetation dominated by either a focal non-native plant [*A. petiolata* (Allpet), *B. thunbergii* (Berthu), *M. vimineum* (Micvim)] or mixed native species at 12 sites at West Point, NY (*N* = 2 sites per focal species, *N* = 6 for native vegetation). Lines represent Generalized Linear Mixed Model predictions.

seedlings growing under *A. petiolata* (6 ± 0.84 cm) and *M. vimineum* (4.8 ± 0.38 cm) were significantly taller than seedlings under native vegetation (3.4 ± 0.23 cm; mean ± 1SE) but did not differ from seedlings growing under *B. thunbergii* (4.4 ± 0.44 cm; a posteriori Tukey test). Number of leaves and leaf width were not affected by any study variable. *Aristolochia* recruit height (log-likelihood test: X^2 = 15.05, *P* < 0.001), leaf width (X^2 = 5.91, *P* = 0.02) and leaf number (X^2 = 8.20, *P* < 0.004) increased significantly between 2011 and 2013: Surviving *Aristolochia* (*N* = 19 at 4 of 10 sites) were 7.5 ± 0.99 cm tall (mean ± 1SE), with 3.8 ± 0.41 leaves that were 1.7 ± 0.19 cm wide.

The majority of *Trillium* seedlings emerged in 2011, two years after planting (359 of 1920 seeds planted in 2009; 18.7%), with only 8 and 2 new seedlings emerging in 2012 and 2013, respectively. Total emergence from 2011 to 2013 was best explained by a model containing fencing and vegetation type effects (Table 1b). Emergence was highest at sites dominated by *M. vimineum*, intermediate at sites dominated by native vegetation or *A. petiolata*
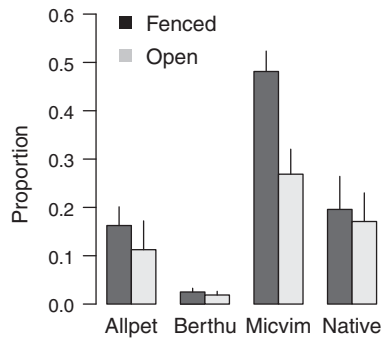
Fig. 2. Emergence (proportion) of *Trillium* cotyledons from seeds placed individually into vegetation dominated by either a focal non-native plant [*A. petiolata* (Allpet), *B. thunbergii* (Berthu), *M. vimineum* (Micvim)] or mixed native species at 12 sites at West Point, NY (*N* = 2 sites per focal species, *N* = 6 for native vegetation). Data are means + 1SE.

and lowest at sites dominated by *B. thunbergii* (Fig. 2). The best model (wi = 0.36) had 1.9 times the explanatory power than the next model, which did not include the effect of fencing (ΔAIC = 1.23, wi = 0.19). Emergence in common garden trials averaged 35%.

Of 359 *Trillium* emergents in 2011 45% survived and transitioned to a one-leaf stage in 2012, with 67% of those (including 8 new emergents) remaining as one-leaf plants and 4% transitioning into a sterile 3-leaf stage in 2013. Survival in 2013 of *Trillium* that emerged in 2011 was best explained by one model (wi = 0.70) that had 3.7 times the explanatory power of the next model (ΔAIC = 2.64, wi = 0.19). The selected model included significant effects of fencing, earthworms and vegetation type, as well as interactions between fencing and earthworms and between fencing and vegetation type (Table 1c). Survival was higher at sites with low compared to high earthworm abundance, and at sites dominated by *M. vimineum* than at sites dominated by native vegetation or *A. petiolata* (Fig. 3). Survival did not differ between sites dominated by native vegetation or *A. petiolata*. Overall fencing had a positive effect on survival, and its effect was more pronounced at low earthworm abundance and at sites dominated by *M. vimineum* (Fig. 3).

Trillium leaf width in 2013 (*N* = 123 at 9 sites, only four sites with >10 individuals included in analyses) averaged 1.7 ± 0.08 cm (mean ± 1SE); leaf width was similar in open and fenced plots (*P* > 0.05) and in both one- and three-leaf seedlings (*P* > 0.05). Leaf width significantly varied across sites ($F_{3,89}$ = 15.43, *P* < 0.001): two sites dominated by native vegetation and low earthworm abundance had narrower leaves (1.2 ± 0.11 cm) than two sites dominated by *M. vimineum* and high earthworm abundance (2.1 ± 0.11 cm; a posteriori Tukey HSD tests).

3.2. Comparison of seed addition vs. seedling transplant for restoration

Carex established successfully only from transplanted seedlings. In 2013, three years after planting, transplants were present at 10 sites averaging 56% survival and were successfully reproducing at 9 sites. In contrast, emergence from seed was only 0.01% and no emergent seedling survived to the end of the study.

Similarly, *Aristolochia* established more successfully from transplants than from seed. Across all sites (excluding 2 sites that were not included in our transplant experiment in 2013), *Aristolochia* emergent survival was significantly lower than transplant survival (*z* = 4.30, *P* < 0.001), but average seedling height did not differ between emergents and transplants ($F_{1,22}$ = 1.79, *P* = 0.20). Emergents were present at 4 of 10 sites included in the analyses (*N* = 20 of 217 emergents), whereas transplants were present at all 10 sites evaluated in 2013 (*N* = 107 of 400 transplants, Table 2).
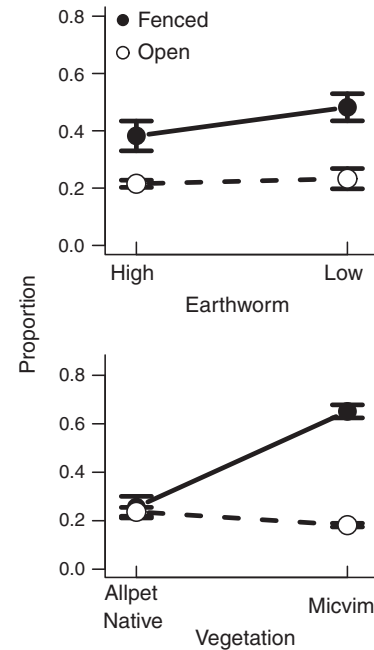

Fig. 3. Proportion of *Trillium* surviving in 2013 as a function of earthworm abundance (top), vegetation type (bottom) and their interaction with fencing. Cotyledons emerged in 2011 from seeds placed individually into vegetation dominated by either a focal non-native plant [*A. petiolata* (Allpet), *B. thunbergii* (Berthu), *M. vimineum* (Micvim)] or mixed native species at 12 sites in 2009 at West Point, NY (*N* = 2 sites per focal species, *N* = 6 for native vegetation). Data are means ± 1SE. Sites dominated by *B. thunbergii* were excluded from analyses because only two individuals survived to 2013.

Trillium established equally well from seed and from transplants. Survival (*z* = 0.72, *P* = 0.47) and leaf width ($F_{1,33}$ = 2.57, *P* = 0.12) of *Trillium* did not differ significantly between emergents (*N* = 90 of 294 emergents) and transplants (*N* = 137 of 400 transplants; Table 2). Emergents and transplants were present at 8 and 10 sites respectively, and while transplant populations were composed of an even proportion of 1-leaf and 3-leaf individuals, emergent populations were mostly composed of 1-leaf individuals (Table 2). Drivers affecting both species survival and recruitment were similar for emergents and transplants (tested in independent models; Table 2).

4. Discussion

Recruitment of two target species (*Aristolochia* and *Trillium*) was seed limited, as seed addition resulted in seedling emergence at our sites. By 2013 *Trillium* establishment averaged 30%, similar to establishment of transplanted seedlings and to seedling emergence in common garden studies, indicating weak microsite limitations for this species. On the other hand, survival of emerging *Aristolochia* seedlings decreased over the course of the study, with only 10% establishing and surviving to 2013. Sustained reductions in survival over time indicate that environmental filters constitute important limiting factors for *Aristolochia* recruitment. In contrast, addition of *Carex* seed did not result in increased seedling emergence and recruitment indicating strong microsite limitations. Our results corroborate findings of other studies showing that the role of microsite limitation increases after initial seedling establishment (Bricker et al., 2010; Olsen and Klanderud, 2014). Our overall results indicate that overcoming seed limitation and dispersal barriers might be sufficient for successful *Trillium* recruitment, but *Aristolochia* and *Carex*, which are strongly limited by suitable site availability, will require additional conservation efforts.

Table 2
Comparison of emergent vs. transplant.

Factor	Aristolochia		Trillium	
	Emergents	Transplants	Emergents	Transplants
Survival[a]	10%	27%	30%	34%
No. sites present[b]	4	10	8	10
Mean no. per site[b]	5 ± 1.47	10.7 ± 3.08	13.2 ± 3.91	13.3 ± 1.29
Mean size (cm)[c]	6.6 ± 1.36	8.2 ± 0.83	1.7 ± 0.08	1.6 ± 0.05
Age distribution[d]			1.6%, 92.7%, 5.7%	0%, 43%, 57%
Drivers[e]				
Vegetation type (V)	+M. vimineum	+Non-native[f]	+M. vimineum	NS
Earthworms (E)	NS	NS	−	−
Fencing (F)	NS	NS	+	+
Interactions	NS	V ∗ Fertilization[g]	F ∗ V	V ∗ Slug[g]
			F ∗ E	E ∗ Slug[g]

NS not significant.
  [a] Averaged and tested across 10 study sites.
  [b] At end of study period in 2013; N = 10 sites.
  [c] Height (cm) for Aristolochia and leaf width (cm) for Trillium; N = 10 sites.
  [d] Percent of Trillium cotyledon, one-leaf and three-leaf individuals in 2013, respectively.
  [e] For recruitment and survival only.
  [f] Non-native category included sites dominated by non-native species A. petiolata, B. thunbergii and M. vimineum (N = 6, 2 sites per species).
  [g] Fertilization and slug exclusion effects were only evaluated for transplants.

We found strong microsite limitations for Carex and Aristolochia that cannot be solely explained by our selected study factors (deer, earthworms and non-native plants). Small-seeded species, such as Carex, often perform better on bare and/or disturbed soil (Willis et al., 2015), with higher germination and survival on sites lacking leaf litter (Vellend et al., 2000). We replaced the original leaf litter after planting Carex and this likely limited emergence. Aristolochia, on the other hand, has a large seed and seeds likely are unaffected by leaf litter (particularly as the fruit ripens on or under leaf litter).

Our selected stressors exerted important single and interactive effects on recruitment of our target plant species. The three focal non-native plant species exerted different effects: M. vimineum had a strong and unexpected positive effect on seedling emergence, A. petiolata had a neutral effect (not differing from native vegetation effects) and B. thunbergii exerted a strong negative effect, but only on Trillium. Neutral A. petiolata effects, at least in part, might be explained by a marked reduction in A. petiolata density throughout the study period at our sites (Nuzzo et al., unpublished data). The different effects of non-native plant species on native species of conservation concern emphasize the need to evaluate introduced species based on their impacts and not their origin (Cohen et al., 2011; Davis et al., 2011; Martin and Blossey, 2013).

Non-native M. vimineum had a positive effect on emergence of two of three species, Aristolochia and Trillium. A similar positive effect of M. vimineum was recorded for A. petiolata growth and reproduction, probably through reduction of common competitor abundance (Flory and Bauer, 2014). At our sites that were dominated by M. vimineum, the annual M. vimineum only reached peak abundance in late summer, not in early spring and early summer when most Trillium and Aristolochia growth occurs. Therefore, M. vimineum might provide a temporal refuge by reducing abundance of competitive species, but exerting reduced competitive effects during peak productivity of our target species. In addition, leaf litter accumulation at M. vimineum sites was lower (Nuzzo et al., unpublished data), likely providing ideal germination conditions and facilitating seedling emergence. Lastly, M. vimineum, which has lower allelopathic potential than the native species Ageratina altissima (Corbett and Morrison, 2012), may exert weaker allelopathic effects than resident vegetation, therefore eliminating an important filter for germination and seedling establishment. However, other studies comparing allelopathic potential of non-native species indicate that M. vimineum has a high inhibitory potential similar to that of A. petiolata (Pisula and Meiners, 2010).

Alliaria petiolata effects are dependent on tissue, target species (Cipollini and Flint, 2013) and invasion history (Lankau, 2012) and while these factors have not been studied for M. vimineum, they are likely to affect germination of resident species.

Deer exclusion, through fencing, resulted in higher emergence, survival and growth of Trillium, but did not affect Aristolochia. These results highlight the importance of non-consumptive deer effects on native vegetation and add to the growing evidence documenting positive and negative effects of deer on non-palatable plant species or on plant stages that are not susceptible to deer consumption (Dávalos et al., 2014; Heckel et al., 2010; Kalisz et al., 2014; Lucas et al., 2013). Adults of this species and of the related species Trillium grandiflorum are highly palatable to deer resulting in reduced individual height and seed set (unpublished data, Knight et al., 2009). Trillium seedlings, on the other hand, are shorter than deer minimum browse height and therefore presumably escape deer herbivory. As such, it has previously been assumed that deer effects on Trillium seedlings are negligible and have a small effect on population persistence overall (Knight, 2004). However, our results clearly show that deer may limit recruitment not only through reduction of flowering probability and seed set, but also through negative effects on seedling establishment. These results suggest that deer impacts on this genus are even stronger than previously estimated. Importantly, our results indicate that in order to capture non-consumptive deer effects it is not sufficient to quantify deer impacts solely through browse indexes or assessment of unbrowsed specimens (Morellet et al., 2007).

Earthworms consume and relocate seed (Eisenhauer et al., 2010; Forey et al., 2011), suggesting that they may negatively affect seed survival and germination. On the other hand, earthworms reduce leaf litter volume (Suarez et al., 2006), which may facilitate seedling establishment (Bartuszevige et al., 2007; Warren et al., 2012). In addition, both beneficial seed dispersers and seed consumers reside in the leaf litter layer, which is greatly reduced or nearly eliminated by earthworm invasions. These opposing processes of earthworms on various abiotic and biotic conditions on the forest floor may cancel out, resulting in the net neutral effect of earthworms on seedling emergence we observed in our field experiment. In this study, we recorded negative earthworm effects on seedling survival and growth, results that are confirmed in a larger study (Dobson and Blossey, 2014). Earthworm impacts are likely occurring at later growth stages through

consumption of fine roots (Gilbert et al., 2014) and leaves (Eisenhauer et al., 2010; Griffith et al., 2013), alteration of root growth (Cameron et al., 2014), decrease of mycorrhizal infection rates (Lawrence et al., 2003) or changes in soil chemical and physical properties that lead to root desiccation or seedling uprooting (Hale et al., 2006).

Negative earthworm impacts on *Trillium* manifested through an interaction with fencing, such that earthworms exerted stronger effects in open than in fenced plots. At our sites, and in a large regional survey, earthworms were more abundant in open than fenced plots (Dávalos et al., 2015), documenting facilitative effects of deer on earthworm populations. Higher earthworm abundance in the presence of deer, which is associated with lower seedling survival (Dobson and Blossey, 2014), is likely the cause of interactive effects between deer and earthworms observed at our sites.

The importance of postdispersal seed predators, such as rodents, birds, ants, ground-dwelling beetles and slugs, has long been recognized (Crawley, 2014), but their effect is highly dependent on seed traits, such as size, as well as on vegetation structure, which may facilitate predator populations (Bricker et al., 2010). For example, wild turkeys consume *Aristolochia* spp. seeds (Mosby and Handley, 1943) and rodent seed predation of *Trillium ovatum* increased in fragmented forests where rodents occurred at significantly higher densities (Tallmon et al., 2003). However, in that study *T. ovatum* seeds were placed above the ground, whereas we placed seeds directly into the upper soil layer and covered them with existing leaf litter, likely concealing seeds from rodents and birds thus reducing potential predator effects. Similarly, our methodology probably reduced seed relocation by ants; *Trillium* seeds are commonly dispersed by ants, which are attracted to seed elaiosomes, a lipid-rich seed attachment (Turner and Frederickson, 2013). Ants may carry seeds to more favorable germination sites and consume elaiosomes, thereby prompting germination (Gunther and Lanza, 1989; Kwit et al., 2012). However, we did not record *Trillium* or *Aristolochia* recruits within our study plots (30 × 30 m), suggesting limited short-range seed relocation.

By following emerging seedlings over multiple years, we show that stressors exert different effects on each life stage transition. For example, the initial positive effect of *M. vimineum* on *Aristolochia* decreased throughout the study, such that, at the end of the experiment, recruitment under *M. vimineum* was only slightly higher than under native vegetation or under *A. petiolata* (Fig. 1). Similarly, earthworm abundance did not affect *Trillium* emergence but negatively affected seedling survival. Furthermore, we found interactive effects on *Trillium* seedling survival that did not affect *Trillium* emergence. Overall, our results corroborate other findings that indicate that direction and magnitude of single and interactive effects change with plant ontogeny and that opposite-sign effects may cancel out when integrated across the whole plant life cycle (Bricker et al., 2010; Martorell and Freckleton, 2014; Rodríguez-Pérez and Traveset, 2007). These results highlight the need to conduct long-term seed addition experiments and the need to study and incorporate all demographic transitions (Kwit et al., 2004) in order to truly understand factors limiting plant recruitment and develop effective conservation practices. Long-term studies are particularly important when establishing new populations of rare plants (Drayton and Primack, 2012) as initial survival is often low and successful establishment rates tend to decline over time for the majority of experimental populations (Drayton and Primack, 2012; Godefroid et al., 2011).

4.1. Restoration approach

It is increasingly recognized that in order to preserve and maintain biodiversity, restoration practices cannot focus only on restoring rare or endangered species populations, but should also strive to restore ecological interactions and mechanisms (Tylianakis et al., 2010). Consequently, understanding limitations to plant recruitment is critical to develop successful restoration strategies. For example, if populations are seed-limited then successful restoration requires seed addition and simultaneous manipulation of factors that led to seed limitation. On the other hand, if populations are microsite limited then restoration requires microsite manipulation in order to release recruitment filters.

For our species, restoration success through seed planting or seedling transplanting depended on whether species were seed or microsite limited. Establishment of *Carex* through seeding was completely unsuccessful but transplants successfully established and reproduced at 10 of 12 sites, achieving the highest survival among four transplanted plant species (Dávalos et al., 2014). If other woodland *Carex* species respond in a similar fashion, using seedlings rather than seeds is likely to result in higher establishment rates, an approach recommended by Godefroid et al. (2011). Similarly, seeding of *Aristolochia* was less successful than transplanting but neither approach resulted in viable established populations, indicating limited opportunities for successful restoration at West Point. Even though we found strong microsite limitations for *Carex* and *Aristolochia* at our sites, we consider that there is potential for restoration in the region, as established and viable populations exist in the area, sometimes in close proximity (50 m). Nevertheless, success is dependent on identifying, and successfully manipulating, the underlying processes affecting recruitment limitations.

We found strong restoration potential for *Trillium* through both seed addition and seedling transplant efforts. While both methods yielded similar recruitment rates, *Trillium* restoration through seeding requires significantly less financial and human resources, and less time investment. Given the current financial and time constraints on conservation efforts, restoration through seeding is the suggested approach for this species. It is likely that other woodland *Trillium* species, such as *T. grandiflorum*, may have similar success using seed rather than seedlings to establish new populations.

Our finding that *Trillium* is seed limited indicates that successful restoration of this species must go beyond seeding new populations, and that it is essential to implement measures to promote increased seed production in extant populations; that is, to restore the mechanisms necessary to guarantee population persistence. Detailed demographic studies of *T. erectum* and *T. grandiflorum* show that deer herbivory significantly reduces reproductive effort, resulting in negative population growth rates (unpublished data, Knight, 2004; Knight et al., 2009). Our results demonstrate that non-consumptive effects of deer can further limit recruitment by negatively affecting seedling establishment, and indicate that *Trillium* restoration will require reduction of deer impacts at the landscape level. Taken together, our results show various paths to maintain and restore populations of currently rare or declining plant species through active intervention. Management efforts ideally should target species-specific recruitment limitations and integrate the abiotic and biotic complexities affecting current population growth rates. Successful reestablishment of rare plant populations requires awareness of the effects of concurrent stressors, as well as multiple introductions at larger spatial and temporal scales and knowledge of the habitat requirements of each target species (Godefroid et al., 2011).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2015.04.025.

References

Amatangelo, K.L., Fulton, M.R., Rogers, D.A., Waller, D.M., 2011. Converging forest community composition along an edaphic gradient threatens landscape-level diversity. Divers. Distrib. 17, 201–213.

Aukema, J.E., McCullough, D.G., Von Holle, B., Liebhold, A.M., Britton, K., Frankel, S.J., 2010. Historical accumulation of nonindigenous forest pests in the continental United States. Bioscience 60, 886–897.

Barringer, K., Whitteremore, A.T., 1997. Aristolochiaceae. In: Flora of North America Editorial Committee (Ed.), Flora of North America North of Mexico (1993+, 18+ vols.), vol. 3. Oxford, New York, pp. 44–58.

Barto, E.K., Antunes, P.M., Stinson, K., Koch, A.M., Klironomos, J.N., Cipollini, D., 2011. Differences in arbuscular mycorrhizal fungal communities associated with sugar maple seedlings in and outside of invaded garlic mustard forest patches. Biol. Invasions 13, 2755–2762.

Bartuszevige, A.M., Hrenko, R.L., Gorchov, D.L., 2007. Effects of leaf litter on establishment, growth and survival of invasive plant seedlings in a deciduous forest. Am. Midl. Nat. 158, 472–477.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2014. lme4: Linear Mixed-Effects Models Using Eigen and S4. R Package Version 1.1-7. <http://CRAN.R-project.org/package=lme4>.

Bohlen, P.J., Scheu, S., Hale, C.M., McLean, M.A., Migge, S., Groffman, P.M., Parkinson, D., 2004. Non-native invasive earthworms as agents of change in northern temperate forests. Front. Ecol. Environ. 2, 427–435.

Bricker, M., Maron, J., 2012. Postdispersal seed predation limits the abundance of a long-lived perennial forb (Lithospermum ruderale). Ecology 93, 532–543.

Bricker, M., Pearson, D., Maron, J., 2010. Small-mammal seed predation limits the recruitment and abundance of two perennial grassland forbs. Ecology 91, 85–92.

Bruna, E.M., 2003. Are plant populations in fragmented habitats recruitment limited? Tests with an Amazonian herb. Ecology 84, 932–947.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer-Verlag, New York, USA.

Cameron, E.K., Cahill J., J.F., Bayne, E.M., 2014. Root foraging influences plant growth responses to earthworm foraging. PLoS One 9, e108873.

Case, F.W., Case, R.B., 2009. Trilliums. Timber Press, Portland, Oregon.

Caughlin, T.T., Ferguson, J.M., Lichstein, J.W., Bunyavejchewin, S., Levey, D.J., 2013. The importance of long-distance seed dispersal for the demography and distribution of a canopy tree species. Ecology 95, 952–962.

Cipollini, K.A., Flint, W.N., 2013. Comparing allelopathic effects of root and leaf extracts of invasive Alliaria petiolata, Lonicera maackii and Ranunculus ficaria on germination of three native woodland plants. Ohio J. Sci. 112, 37–43.

Clark, C.J., Poulsen, J.R., Levey, D.J., Osenberg, C.W., 2007. Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. Am. Nat. 170, 128–142.

Cohen, J.S., Maerz, J.C., Blossey, B., 2011. Traits, not origin, explain impacts of plants on larval amphibians. Ecol. Appl. 22, 218–228.

Corbett, B.F., Morrison, J.A., 2012. The allelopathic potentials of the non-native invasive plant Microstegium vimineum and the native Ageratina altissima: two dominant species of the eastern forest herb layer. Northeastern Nat. 19, 297–312.

Côté, S.D., Rooney, T.P., Tremblay, J.-P., Dussault, C., Waller, D.M., 2004. Ecological impacts of deer overabundance. Annu. Rev. Ecol. Syst. 35, 113–147.

Crawley, M.J., 2014. Seed predators and plant population dynamics. In: Gallagher, R. (Ed.), Seeds: The Ecology of Regeneration in Plant Communities, third ed. CABI, Wallingford, UK, pp. 94–110.

Crone, E.E., Menges, E.S., Ellis, M.M., Bell, T., Bierzychudek, P., Ehrlén, J., Kaye, T.N., Knight, T.M., Lesica, P., Morris, W.F., Oostermeijer, G., Quintana-Ascencio, P.F., Stanley, A., Ticktin, T., Valverde, T., Williams, J.L., 2011. How do plant ecologists use matrix population models? Ecol. Lett. 14, 1–8.

Dávalos, A., Nuzzo, V., Blossey, B., 2014. Demographic responses of rare forest plants to multiple stressors: the role of deer, invasive species and nutrients. J. Ecol. 102, 1222–1233.

Dávalos, A., Simpson, E., Nuzzo, V., Blossey, B., 2015. Non-consumptive effects of native deer on introduced earthworm abundance. Ecosystems (in press).

Davis, M.A., Chew, M.K., Hobbs, R.J., Lugo, A.E., Ewel, J.J., Vermeij, G.J., Brown, J.H., Rosenzweig, M.L., Gardener, M.R., Carroll, S.P., Thompson, K., Pickett, S.T.A., Stromberg, J.C., Tredici, P.D., Suding, K.N., Ehrenfeld, J.G., Philip Grime, J., Mascaro, J., Briggs, J.C., 2011. Don't judge species on their origins. Nature 474, 153–154.

Didham, R.K., Tylianakis, J.M., Gemmell, N.J., Rand, T.A., Ewers, R.M., 2007. Interactive effects of habitat modification and species invasion on native species decline. Trends Ecol. Evol. 22, 489–496.

DiTommaso, A., Morris, S.H., Parker, J.D., Cone, C.L., Agrawal, A.A., 2014. Deer browsing delays succession by altering aboveground vegetation and belowground seed banks. PLoS One 9, e91155.

Dobson, A., Blossey, B., 2014. Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of northeastern North America. J. Ecol. 103, 153–164.

Drayton, B., Primack, R.B., 2012. Success rates for reintroductions of eight perennial plant species after fifteen years. Restor. Ecol. 20, 299–303.

Eisenhauer, N., Butenschoen, O., Radsick, S., Scheu, S., 2010. Earthworms as seedling predators: importance of seeds and seedlings for earthworm nutrition. Soil Biol. Biochem. 42, 1245–1253.

Eisenhauer, N., Schlaghamersky, J., Reich, P.B., Frelich, L.E., 2011. The wave towards a new steady state: effects of earthworm invasion on soil microbial functions. Biol. Invasions 13, 2191–2196.

Elliott, D., 2000. Virginia Snakeroot Aristolochia serpentaria. Planting the Future: Saving our Medicinal Herbs. Healing Arts Press, Rochester, VT.

Elliott, K.J., Vose, J.M., Rankin, D., 2014. Herbaceous species composition and richness of mesophytic cove forests in the southern Appalachians: synthesis and knowledge gaps. J. Torrey Botanical Soc. 141, 39–71.

Ewing, H., Tuininga, A., Groffman, P., Weathers, K., Fahey, T., Fisk, M., Bohlen, P., Suarez, E., 2014. Earthworms reduce biotic 15-Nitrogen retention in northern hardwood forests. Ecosystems, 1–15.

Farnsworth, E.J., Ogurcak, D.E., 2008. Functional groups of rare plants differ in levels of imperilment. Am. J. Bot. 95, 943–953.

Fisichelli, N.A., Frelich, L.E., Reich, P.B., Eisenhauer, N., 2013. Linking direct and indirect pathways mediating earthworms, deer, and understory composition in Great Lakes forests. Biol. Invasions 15, 1057–1066.

Flory, S.L., Bauer, J.T., 2014. Experimental evidence for indirect facilitation among invasive plants. J. Ecol. 102, 12–18.

Forey, E., Barot, S., Decaens, T., Langlois, E., Laossi, K.R., Margerie, P., Scheu, S., Eisenhauer, N., 2011. Importance of earthworm-seed interactions for the composition and structure of plant communities: a review. Acta Oecol.-Int. J. Ecol. 37, 594–603.

Gilbert, K.J., Fahey, T.J., Maerz, J.C., Sherman, R.E., Bohlen, P., Dombroskie, J.J., Groffman, P.M., Yavitt, J.B., 2014. Exploring carbon flow through the root channel in a temperate forest soil food web. Soil Biol. Biochem. 76, 45–52.

Godefroid, S., Piazza, C., Rossi, G., Buord, S., Stevens, A.-D., Aguraiuja, R., Cowell, C., Weekley, C.W., Vogg, G., Iriondo, J.M., Johnson, I., Dixon, B., Gordon, D., Magnanon, S., Valentin, B., Bjureke, K., Koopman, R., Vicens, M., Virevaire, M., Vanderborght, T., 2011. How successful are plant species reintroductions? Biol. Conserv. 144, 672–682.

González, F., Rudall, P.J., 2003. Structure and development of the ovule and seed in Aristolochiaceae, with particular reference to Saruma. Plant Syst. Evol. 241, 223–244.

Griffith, B., Turke, M., Weisser, W.W., Eisenhauer, N., 2013. Herbivore behavior in the anecic earthworm species Lumbricus terrestris L.? Eur. J. Soil Biol. 55, 62–65.

Gunther, R.W., Lanza, J., 1989. Variation in attractiveness of Trillium diaspores to a seed-dispersing ant. Am. Midl. Nat. 122, 321–328.

Hale, C.M., Frelich, L.E., Reich, P.B., 2006. Changes in hardwood forest understory plant communities in response to European earthworm invasions. Ecology 87, 1637–1649.

Heckel, C.D., Bourg, N.A., McShea, W.J., Kalisz, S., 2010. Nonconsumptive effects of a generalist ungulate herbivore drive decline of unpalatable forest herbs. Ecology 91, 319–326.

Holdsworth, A., Frelich, L., Reich, P., 2012. Leaf litter disappearance in earthworm-invaded northern hardwood forests: role of tree species and the chemistry and diversity of litter. Ecosystems 15, 913–926.

Holl, K.D., Hayes, G.F., 2006. Challenges to introducing and managing disturbance regimes for Holocarpha macradenia, an endangered annual grassland forb. Conserv. Biol. 20, 1121–1131.

Irwin, R.E., 2000. Morphological variation and female reproductive success in two sympatric Trillium species: evidence for phenotypic selection in Trillium erectum and Trillium grandiflorum (Liliaceae). Am. J. Bot. 87, 205–214.

Kalisz, S., Spigler, R.B., Horvitz, C.C., 2014. In a long-term experimental demography study, excluding ungulates reversed invader's explosive population growth rate and restored natives. Proc. Natl. Acad. Sci. 111, 4501–4506.

Kardol, P., Dickie, I.A., St John, M.G., Husheer, S.W., Bonner, K.I., Bellingham, P.J., Wardle, D.A., 2014. Soil-mediated effects of invasive ungulates on native tree seedlings. J. Ecol. 102, 622–631.

Kirkman, L.K., Coffey, K.L., Mitchell, R.J., Moser, E.B., 2004. Ground cover recovery patterns and life-history traits: implications for restoration obstacles and opportunities in a species-rich savanna. J. Ecol. 92, 409–421.

Knight, T.M., 2004. The effect of herbivory and pollen limitation on a declining population of Trillium grandiflorum. Ecol. Appl. 14, 915–928.

Knight, T.M., Caswell, H., Kalisz, S., 2009. Population growth rate of a common understory herb decreases non-linearly across a gradient of deer herbivory. For. Ecol. Manage. 257, 1095–1103.

Kwit, C., Horvitz, C.C., Platt, W.J., 2004. Conserving slow-growing, long-lived tree species: input from the demography of a rare understory conifer, Taxus floridana. Conserv. Biol. 18, 432–443.

Kwit, C., Marcello, G.J., Gonzalez, J.L., Shapiro, A.C., Bracken, R.D., 2012. Advantages of seed dispersal for a myrmecochorous temperate forest herb. Am. Midl. Nat. 168, 9–17.

Landenberger, R.E., McGraw, J.B., 2004. Seed-bank characteristics in mixed-mesophytic forest clearcuts and edges: does "edge effect" extend to the seed bank? Can. J. Bot.-Revue Can. Bot. 82, 992–1000.

Lankau, R.A., 2012. Interpopulation variation in allelopathic traits informs restoration of invaded landscapes. Evol. Appl. 5, 270–282.

Lawrence, A.P., Bowers, M.A., 2002. A test of the 'hot' mustard extraction method of sampling earthworms. Soil Biol. Biochem. 34, 549–552.

Lawrence, B., Fisk, M.C., Fahey, T.J., Suarez, E.R., 2003. Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). New Phytol. 157, 145–153.

Lawton, J.H., 1994. What do species do in ecosystems? Oikos 71, 367–374.

Levine, J.M., Vila, M., D'Antonio, C.M., Dukes, J.S., Grigulis, K., Lavorel, S., 2003. Mechanisms underlying the impacts of exotic plant invasions. Proc. Roy. Soc. London B 270, 775–781.

Lortie, C.J., Brooker, R.W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F.I., Callaway, R.M., 2004. Rethinking plant community theory. Oikos 107, 433–438.

Lucas, R.W., Salguero-Gomez, R., Cobb, D.B., Waring, B.G., Anderson, F., McShea, W.J., Casper, B.B., 2013. White-tailed deer (*Odocoileus virginianus*) positively affect the growth of mature northern red oak (*Quercus rubra*) trees. Ecosphere 4, art84.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., Bazzaz, F.A., 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecol. Appl. 10, 689–710.

Maron, J.L., Simms, E.L., 1997. Effect of seed predation on seed bank size and seedling recruitment of bush lupine (*Lupinus arboreus*). Oecologia 111, 76–83.

Maron, J.L., Auge, H., Pearson, D.E., Korell, L., Hensen, I., Suding, K.N., Stein, C., 2014. Staged invasions across disparate grasslands: effects of seed provenance, consumers and disturbance on productivity and species richness. Ecol. Lett. 17, 499–507.

Martin, L., Blossey, B., 2013. Intraspecific variation overrides origin effects in impacts of litter-derived secondary compounds on larval amphibians. Oecologia 173, 449–459.

Martorell, C., Freckleton, R.P., 2014. Testing the roles of competition, facilitation and stochasticity on community structure in a species-rich assemblage. J. Ecol. 102, 74–85.

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

Miller, K.E., Gorchov, D.L., 2004. The invasive shrub, *Lonicera maackii*, reduces growth and fecundity of perennial forest herbs. Oecologia 139, 359–375.

Moles, A.T., Westoby, M., 2002. Seed addition experiments are more likely to increase recruitment in larger-seeded species. Oikos 99, 241–248.

Mooney, E.H., McGraw, J.B., 2009. Relationship between age, size, and reproduction in populations of American ginseng, *Panax quinquefolius* (Araliaceae), across a range of harvest pressures. Ecoscience 16, 84–94.

Morellet, N., Gaillard, J.-M., Hewison, A.J.M., Ballon, P., Boscardin, Y., Duncan, P., Klein, F., Maillard, D., 2007. Indicators of ecological change: new tools for managing populations of large herbivores. J. Appl. Ecol. 44, 634–643.

Mosby, H.S., Handley, C.O., 1943. The wild turkey in Virginia; its status, life history and management. Pittman-Robertson Projects, Division of Game, Commission of Game and Inland Fisheries, Richmond, Va.

Murray, B., Webster, C., Bump, J.K., 2013. Broadening the ecological context of ungulate-ecosystem interactions: the importance of space, seasonality, and nitrogen. Ecology 94, 1317–1326.

Núñez-Ávila, M.C., Uriarte, M., Marquet, P.A., Armesto, J.J., 2013. Decomposing recruitment limitation for an avian-dispersed rain forest tree in an anciently fragmented landscape. J. Ecol. 101, 1439–1448.

Nuttle, T., Yerger, E.H., Stoleson, S.H., Ristau, T.E., 2011. Legacy of Top-Down Herbivore Pressure Ricochets Back Up Multiple Trophic Levels in Forest Canopies Over 30 Years. Ecosphere 2, art 4.

Nuzzo, V., Dávalos, A., Blossey, B., 2015. Invasive earthworms shape forest seed bank composition. Divers. Distrib. 21, 560–570.

Oelschlägel, B., Gorb, S., Wanke, S., Neinhuis, C., 2009. Structure and biomechanics of trapping flower trichomes and their role in the pollination biology of *Aristolochia* plants (Aristolochiaceae). New Phytol., 184.

Olsen, S.L., Klanderud, K., 2014. Biotic interactions limit species richness in an alpine plant community, especially under experimental warming. Oikos 123, 71–78.

Pfeifer, H.W., 1966. Revision of the North and Central American hexandrous species of *Aristolochia* (Aristolochiaceae). Ann. Mo. Bot. Gard. 53.

Pisula, N.L., Meiners, S.J., 2010. Relative allelopathic potential of invasive plant species in a young disturbed woodland. J. Torrey Bot. Soc. 137, 81–87.

R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.

Rodríguez-Pérez, J., Traveset, A., 2007. A multi-scale approach in the study of plant regeneration: Finding bottlenecks is not enough. Perspect. Plant Ecol., Evol. Syst. 9, 1–13.

Rother, D.C., Jordano, P., Rodrigues, R.R., Pizo, M.A., 2013. Demographic bottlenecks in tropical plant regeneration: a comparative analysis of causal influences. Perspect. Plant Ecol., Evol. Syst. 15, 86–96.

Suarez, E.R., Fahey, T.J., Yavitt, J.B., Groffman, P.M., Bohlen, P.J., 2006. Patterns of litter disappearance in a northern hardwood forest invaded by exotic earthworms. Ecol. Appl. 16, 154–165.

Tallmon, D.A., Jules, E.S., Radke, N.J., Mills, L.S., 2003. Of mice and men and *Trillium*: cascading effects of forest fragmentation. Ecol. Appl. 13, 1193–1203.

Tanentzap, A.J., Bazely, D.R., Koh, S., Timciska, M., Haggith, E.G., Carleton, T.J., Coomes, D.A., 2011. Seeing the forest for the deer: do reductions in deer-disturbance lead to forest recovery? Biol. Conserv. 144, 376–382.

Thomson, D.M., 2005. Matrix models as a tool for understanding invasive plant and native plant interactions. Conserv. Biol. 19, 917–928.

Turnbull, L.A., Crawley, M.J., Rees, M., 2000. Are plant populations seed-limited? A review of plant sowing experiments. Oikos 88, 225–238.

Turner, K.M., Frederickson, M.E., 2013. Signals can trump rewards in attracting seed-dispersing ants. PLoS One 8, e71871.

Tylianakis, J.M., Laliberte, E., Nielsen, A., Bascompte, J., 2010. Conservation of species interaction networks. Biol. Conserv. 143, 2270–2279.

Vellend, M., Lechowicz, M.J., Waterway, M.J., 2000. Germination and establishment of forest sedges (Carex, Cyperaceae): tests for home-site advantage and effects of leaf litter. Am. J. Bot. 87, 1517–1525.

Vilá, M., Espinar, J.L., Hedjda, M., Hulme, P.E., Jarošik, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y., Pyšek, P., 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol. Lett. 14, 702–708.

Wall, W.A., Hoffmann, W.A., Wentworth, T.R., Gray, J.B., Hohmann, M.G., 2012. Demographic effects of fire on two endemic plant species in the longleaf pine-wiregrass ecosystem. Plant Ecol. 213, 1093–1104.

Warren II, R.J., Bahn, V., Bradford, M.A., 2012. The interaction between propagule pressure, habitat suitability and density-dependent reproduction in species invasion. Oikos 121, 874–881.

Willis, J.L., Walters, M.B., Gottschalk, K.W., 2015. Scarification and gap size have interacting effects on northern temperate seedling establishment. For. Ecol. Manage. http://dx.doi.org/10.1016/j.foreco.2015.1002.1026.