Title:

Plant species identity matters when comparing the trophic impacts of native and non-native plants: insights from a community-wide bird-exclusion experiment

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Abstract:

Biological invasions threaten biodiversity by outcompeting native species and disrupting food webs. Invasive species are now ranked as a leading cause for decline of biodiversity and protected species. Non-native woody plants are one group of invasive species that now occupy the majority of terrestrial ecosystems as a result of human activity. In eastern North American temperate forests, understory plant communities are frequently dominated by non-native shrubs. For many species of insectivorous birds and mammals, non-native plants threaten populations by provided less food resources and/or food resources of lower quality. Conservation organizations expend significant effort to remove non-native plants, but evidence that this removal improves food availability to wildlife is lacking. In this project, we examined food webs among four species of non-native shrubs in contrast to six species of native woody plants found in the same habitats. Using a predator-exclusion experiment bird predation effect size, arthropod biomass available to insectivorous birds, abundance of major arthropod taxonomic groups, and the nitrogen content of herbivores and spiders as a proxy for protein availability. All four lines of evidence suggest that non-native plants do not reliably decrease foraging opportunities or quality to songbirds. Compared to nearby native plants, some non-native plant species like Honeysuckles provided higher arthropod biomass with a higher nitrogen content. Conversely, Japanese barberry had fewer arthropods and spider prey had significantly lower nitrogen content. Contrary to predictions, the predation effects of birds were of similar magnitude on both native and non-natives, suggesting insectivorous songbirds actively forage non-native plants for prey. A more nuanced approach to invasive, non-native plant management should be considered that prioritizes species that are low-quality foraging opportunities relative to the local plant community.

Keywords:

Invasive species, invasive plants, non-native plants, insectivores, songbirds, forests, food webs, habitat improvement

Introduction:

Invasive species are a leading cause of biodiversity decline globally (Bellard et al. 2016), with an estimated cost of management totaling $120 billion in the United States (Pimental et al. 2007) as a response to economic impacts averaging $19.9 billion per year (Fantle-Lepczyk et al. 2021). Removal of invasive species is costly, but it can potentially be an effective way to restore ecosystem services. Invasive, non-native plants are particularly challenging to manage in terrestrial ecosystems, with the cost of removal efforts still being difficult to estimate accurately for the U.S. or globally (Rai et al. 2022). Nevertheless, the costs of non-native plant management have not been trivial when quantified, reaching average annual totals of $82 million in California (California Invasive Plant Council, 2022) and $45 million in Florida (Hiatt et al. 2019). In principle, removing non-native, invasive species improves the quality of habitat for native plants (Hartman and McCarthy, 2004) and native wildlife (Schneider and Miller, 2014). Notably, removal of particularly aggressive invasive plant species that form monocultures can drive recovery of arthropod assemblages, which are an important indicator of food availability for wildlife (Gratton and Denno, 2005). However, despite the dramatic efforts to remove established invasive plant populations from managed habitats, there is still not a consensus on whether such practices actually impact the arthropod communities that are prey for wildlife (Robichaud et al. 2021, Traylor et al. 2022). Effective management requires knowledge of when removal of one invasive species may not be justified based on restoration goals (D’Antonio and Meyerson 2002). Decisions about prioritizing some invasive species removal over others is critical since the financial resources for conservation are severely limited relative to the ecological challenge at hand (Arponen 2012, Courtois et al. 2018, Eppinga et al. 2021).

Plant invasions have cascading impacts on ecological communities because they directly modify both above-ground and soil food webs (McCary et al. 2016). Non-native invasive plants (hereafter referred to as ‘non-native’) are particularly prevalent in habitats that have experienced frequent anthropogenic disturbance (Mosher et al. 2009, Wang et al. 2016), meaning their impacts are most pronounced in areas where wildlife may already be distressed. Furthermore, new non-native plant species are expected to continue to accumulate in anthropogenically modified habitats (Seebens et al. 2017). Consequently, understanding the mechanisms by which non-native plants disrupt food webs and wildlife and identifying effective solutions have become priorities for ecologists and land managers. Typically, non-native plants dominate or form monocultures and displace native plant species, negatively impacting native animals indirectly (Fletcher et al. 2019). In habitats dominated by non-native plants, less or lower quality arthropod prey is available to insectivorous birds and mammals (Gerber et al. 2008, Riedl et al. 2018). However, nutritional quality for herbivores is just one of multiple traits of non-native plants that impacts food webs. Compounds released from invasive plants through roots and decaying leaves can impact detritus-based food webs (Robison et al. 2021). Additionally, non-native plants have atypical architecture compared to native plants, leading to different compositions of arthropods independent of the host plant quality (Pearson 2009, Landsman et al. 2020). Each of these trait-based mechanisms provides some insight into the consequences of plant invasion, but cross-species comparisons are needed to help elucidate these pathways. For example, Lind and Parker, 2010 compared a range of plant species testing the hypothesis that non-native plants have significantly different defensive chemistry than natives, but this hypothesis was only supported for a small proportion of the invasive plants examined. For this reason, our study includes a comparison of a community of non-native plants to a community of native plants in a shared environment.

In food web ecology, comparisons are often made between native and non-native species that are in the same genera and are expected to have similar phytochemistry and thus shared defensive traits (Haan et al. 2021, Lampert et al. 2022). Similarly, in comparable habitats, locations dominated by invasive plants often have lower abundances of plant-feeding arthropods, particularly caterpillars (Richard et al. 2019). As a result of lower nutritional quality, invasive plants may have fewer herbivores, shrinking the biomass in the second trophic level (Clark and Seewagen 2019). It is expected that by removing non-native plants, native plants would have an opportunity to recover, thus restoring ecosystem services (Hopfensperger et al. 2017). In landscaping scenarios native plants which provide more insect prey are suggested as replacements for exotic shrubs to help bird populations (Narango et al. 2018, Kramer et al. 2019). By contrast, in managed forests just removal is typically employed without replacement and local native plants recover that were already within that habitat fragment (Flory and Clay 2009, Shields et al. 2015, Farmer et al. 2016, Cutway 2017). Importantly, for wildlife at higher trophic levels the success of this management strategy depends on the presumed superiority of arthropod quantity or quality on native compared to non-native plants. However, this assumption has not been rigorously tested, both in general and particularly in the temperate forests of the northeastern United States.

We tested two hypotheses: (1) a ‘low food availability hypothesis’, and (2) a ‘low food quality hypothesis’. In the low food availability hypothesis, non-native plants are expected to have significantly lower prey available for insectivores compared to native plants in the same environment. Indeed, there is broad, community-level evidence that non-native plants have lower insect abundance and diversity (Tallamy et al. 2020). In the ‘low food availability hypothesis’, taxonomic groups of high value (e.g., caterpillars) are expected to be less available (Narango et al. 2018). Conversely, in the ‘low food quality’ hypothesis, those prey items that are available on non-native plants are expected to have lower in protein content as non-natives are comparably lower quality food sources herbivores themselves (Lieurance and Cipollini, 2013). Since this mechanism has not been tested through nutritional content analysis, we used percent nitrogen content of arthropods as an indicator to protein availability and quality for insectivores (Reeves et al. 2021). Thus, we predicted lower arthropod nitrogen content in non-native plants compared to natives. Finally, in both hypothesis, predators forage on non-native plants less, leading to weaker predator effects on arthropods. Due to lower abundance and quality of prey, insectivorous songbirds will make optimal foraging decisions and invest less effort into finding food on non-native plants (Riedl et al. 2018).

Methods:

*Study System.* We performed a selective predator exclusion treatment on ten woody host plant species at Great Hollow Nature Preserve & Ecological Research center (Fairfield Co., Connecticut, USA; 41.507998 N, -73.530032 W). This 334-hectare forest preserve follows a forest conservation plan managing for wildlife habitat and outdoor education. We intentionally chose closed-canopy plots within this site that would be candidates for non-native shrub removal targeting Japanese barberry (*Berberis thunbergii)*, non-native bush honeysuckles, *Lonicera* spp. (our experiments only sampled *Lonicera morrowii*), burning bush (*Eunonymous alatus*), and autumn olive (*Eleagnus umbellata*). Native understory shrubs and understory trees included striped maple *Acer pennsylvanicum*, shadbush (*Amelanchier canadensis*), musclewood (*Carpinus caroliniana*), and witch-hazel (*Hamamelis virginiana*). Our experiment occurred in areas where the overstory tree composition includes sweet birch (*Betula lenta*) and American beech (*Fagus grandifolia*). As these ten species were the dominant woody plants in the selected habitat, we performed experiments on all to provide a community-wide perspective on the impacts of non-native plants on food webs compared to adjacent native woody plants since this would be the context in which invasive plant management decisions would be made (Westman 1990).

*Bird exclusion experiment.* From May 4th to May 27th, 2021, we employed a predator exclusion experiment in a paired design (following Singer et al. 2012). Insectivorous birds were prevented from foraging on branches *via* a mesh netting that was draped over branches and affixed to the base of the branch using plastic zip-ties (“- birds” treatment). Each of these branches were paired with a nearby (< 10m) unmanipulated control branch (“+ birds” treatment). We set up treatments on 12 pairs for each of ten focal woody plant species for a total of 240 individual host plants. At the end of the set-up period after May 27th branches were gently tapped to dislodge arthropods from all branches to avoid bias caused by the disturbance of setting up bag treatments. For sampling, arthropod abundance was quantified by collecting all foliage-foraging invertebrates using a branch-beating technique employed for sampling forest caterpillars (Wagner 2005). Each branch was struck with a 0.3m long dowel while hanging over a 1m2 ripstop fabric beat sheet. All invertebrates that landed on the sheet were collected via aspirators or soft-touch aluminum forceps. Each of these 240 plants were sampled in this same manner, every other week for a total of three repeated samples. We combined these repeated samples in all analyses to produce a broader-scale estimate of total arthropod prey availability per plant (Clark et al. 2016).

*Taxonomic identification of arthropods.* All invertebrates collected in the field were transferred immediately to 7 × 3cm plastic vials or 16 × 8cm plastic zip-top bags and preserved in a –80° C lab freezer. Afterwards, specimens collected on entire experimental branches were weighed (wet mass) on a 10-4 g microbalance. All invertebrates were identified to class. Common arthropod species (those observed > 25 times) were then identified to order, and all insects in the orders Lepidoptera, Hemiptera, Hymenoptera were identified to family. True spiders (Araneae) and Opiliones were identified to family as well. Once identifications were complete in August 2021, all taxonomic groups from each individual branch sample and placed into 0.6mL and 2mL Eppendorf tubes kept in a -80C lab freezer. In all, the four numerically dominant taxonomic groupings of arthropods included (1) Lepidoptera (caterpillars), (2) true spiders (Araneae), (3) herbivorous Hemiptera families (Aphidae, Cicadellidae, Membracidae, Miridae, and Pentatomidae), and (4) Orthoptera (families Gryllidae and Tettigoniidae.

*Elemental analysis of arthropods.* Our preliminary analyses suggested that two broad functional groups responded strongly to bird predation effects and varied significantly among native and non-native host plants, each representing a different trophic level above host plants: foliage-feeding herbivores (see Appendix 1a: Selection of herbivores for C:N analysis) and predatory true spiders (Araneae). These two groupings of arthropods are prey for insectivorous birds on trees and their abundances are impacted by experimental manipulation of bird predation (e.g. Gunnarsson et al. 1996). Samples were assayed for C:N ratios as an indirect measure of protein availability (Smets et al. 2021), a nutritional factor which strongly mediates food selection behavior (Robbins et al. 2005). Generally, the insects feeing on individual plants have a similar C:N ratio as their host (Abbas et al. 2014). To assay elemental composition, we first pooled foliage-feeding herbivore taxa and true spiders across sampling periods for each branch in the bird exclusion treatment group. We limited our analyses to branches with birds excluded in order to quantify the nutritional value of the arthropod community as it would be for the first bird foraging on a given branch. We then desiccated arthropod samples for 24 hours in a 60° C drying oven and homogenized samples more than 3 mg in mass with a mortar and pestle. We weighed between 1.5 and 3.5 mg of each sample into tin capsules and evaluated the mass of carbon and nitrogen in samples with a Flash 1112 CHNSO elemental analyzer (CE Elantech inc. Lakewood, NJ, USA) by comparison with an aspartic acid standard curve (Sigma-Aldrich, St. Louis, MO, USA). Percent nitrogen and carbon were calculated by dividing the mass of each element in a sample by total sample mass and we analyzed replicates for a subset of branches, producing mean within-sample coefficients of variation of 4.2% for nitrogen and 2.9% for carbon.

*Statistical analyses.* We employed a series of Generalized Linear Mixed Models (GLMMs) using the *lme4* package (Bates et al. 2015) in R version 4.1.2 (R Development Core Team, 2022). These univariate analyses use the following as response variables for each model: (1) total arthropod biomass sampled per plant in grams, (2) spider abundance (Araneae), (3) caterpillar abundance (Lepidoptera), (4) Hemiptera abundance (5) Orthoptera abundance, (6) N Content of herbivorous insects and (7) N content of spiders. Arthropod biomass (1) was fitted as normally distributed GLMM after a log-transformation and included both host plant species and bird exclusion treatment as fixed effects and branch as random effects. All abundance models were fitted with a negative binomial GLMM. In abundance models, non-native status (yes or no) was a fixed effect along with bird-exclusion treatment. In these models branch and host-plant species were included as random effects. Nitrogen content models were fit as a normal distribution, but since all arthropod samples were pooled across sampling periods and only taken from bagged branches, only host-plant species was used as a main effect (GLM). Posthoc tests comparing changes in biomass, abundance, and nitrogen content were run using the *emmeans* package in R (Lenth 2016). Differences were investigated across all groupings using Scheffe’s method (following Midway et al. 2020) for P-value adjustment in unplanned contrasts. P-values and critical values were determined using the *car* package with analysis of deviance tests and χ2 test statistics (Fox et al 2015).

*Log-response ratios.* A follow-up GLM was employed using the LRR of bag treatments to investigate the interspecific variation in bird predation effects across all host plant species (Singer et al. 2012). Log-response ratios, when used to evaluate the effects of natural enemy exclusion, provide insight into whether the interaction strength of top-down effects vary according to different environmental variables (Chaguaceda et al. 2021, Wooton 1997). In this case, we used a LRR modified from Hedges et al. (1999) as the natural log of the combined arthropod biomass on bagged branches divided by the arthropod biomass on control branches. LLR calculated in this way tests the prediction that bird predation would be weaker on invasive plants, an implicit assumption of both the ‘low food availability hypothesis’ and the ‘low food quality hypothesis’. Birds are expected to forage less on non-native plants compared to natives, thus weakening the impact of the bag-treatment. In addition to comparing predator effects across host plants, this method also facilitates measurement of the relative effect size (e.g. Chaguaceda). LRR values above zero indicate that predator effects are biologically and statistically significant, while those that intersect with zero are not.

Results:

We observed significant variation in the biomass of arthropods among our ten focal host-plant species (Fig. 1, GLMM, *P* = 0.001, χ2 = 26.62, d.f. = 9). Bag treatments significantly increased the biomass of arthropods (GLMM, *P <* 0.001, χ2 = 84.84, d.f. = 1) Native plants did not have significantly higher biomass than non-native plants in a grouped planned contrast (*P* = 0.133, *t* ratio = 1.5, d.f. = 223). Honeysuckle had higher biomass than other non-native plant species, autumn olive, Japanese barberry, and burning bush (Fig 1). Native plants varied in biomass, with musclewood, sweet birch and witch-hazel exhibiting relatively higher biomass then the remaining native plants (Fig 1). Surprisingly, we did not observe statistically significant variation in the effect size of bird predation as measured by LRR (Fig. 2, GLM, *P* = 0.294, χ2 = 10.73, d.f. =9). Furthermore, bird predation LLR was not significantly lower on non-native species compared to native species in a grouped planned contrast (*P* = 0.364, *t* ratio = 0.954, d.f. =106). However, predation effects were above zero for all host-plant species, including native and non-native host plants except for musclewood. In an unexpected result, bagged branches of musclewood were associated with relatively high occupancy of aquatic insects (Fig S1), which may explain the non-significant effect of bird exclusion treatment on arthropod biomass.

Our analysis of bird predation effects among native-and non-native plants differed for each of four separate taxonomic groups. Araneae (true spiders) were more common on non-native plants overall (Fig. 3A, GLMM, *P* < 0.001, χ2 = 19.19, d.f. = 1), while bird effects were significant on both native and non-native plants (Fig. 3A, GLMM, *P* < 0.001, χ2 = 57.18, d.f. = 1). Hemiptera were not significantly higher or lower non-native plants (Fig 3B, GLMM, *P* = 0.488, χ2 = 0.479, d.f. = 1), and bird predation effects were not significant for this group either (Fig. 3B, GLMM, *P* = 0.141, χ2 = 2.15, d.f. = 1). Fitting with predictions from other studies on caterpillar abundance, we observed fewer Lepidoptera on non-native plants (Fig. 3C, GLMM, *P* = 0.022, χ2 = 5.19, d.f. =1). Despite the lower abundance, bird predation effects were significant for lepidoptera (Fig. 3C, GLMM, *P* < 0.001, χ2 = 25.7, d.f. = 1), and bird predation effects did not significantly differ among natives or non-natives (GLMM interaction term for native vs. non-native plants and bird predation effect, *P* = 0.614, χ2 = 0.25, d.f. =1). Finally, we observed similar abundances of Orthoptera on both native and non-native plants (Fig. 3D, GLMM, *P* = 0.941, χ2 = 0.005, d.f. = 1). Bird significantly reduced the abundance of orthoptera on both plant groups (Fig. 3D, GLMM, *P* < 0.001, χ2 = 15.6, d.f. =1).

We observed significant variation in the %N content by mass for herbivores among the ten sampled host-plant species (Fig. 4A, GLM, *P* < 0.001, χ2 = 38.4, d.f. = 9). For non-native plants, our grouped planned contrast suggested significantly higher %N content by mass compared to natives (Fig 4A, planned contrast, *P* = 0.001, *t* ratio = -3.33, d.f. = 341). Mirroring our observed higher arthropod biomass result, we saw the highest N content on honeysuckle (Fig 4A). For spider %N content by mass, we also saw significant variation among the ten sampled host-plant species (Fig. 4B, GLM, *P* < 0.001, χ2 = 59.61, d.f. =9). Conversely, spider %N content was significantly lower on non-native plants compared to native plants (Fig 4B, planned contrast, *P =* 0.002, *t* ratio = 3.19, d.f. =341), and %N content appeared to be dramatically lower on Japanese barberry compared to all other plant species (Fig 4B).

Discussion:

Introduced organisms have had a dramatic impact on ecosystems and economies, with some conservative estimates for monetary impacts of $1.26 trillion US dollars in North America since 1960 (Crystal-Ornelas et al. 2021). A problem of this scope requires careful strategic planning in which the most damaging non-native species are prioritized. Our results suggest important contexts in which non-native plants are poorer food opportunities for wildlife. First, while three of our focal non-native plants had relatively low arthropod biomass, our study revealed surprisingly high food availability on honeysuckle (Lonicera) compared to natives, contradicting the ‘low food availability hypothesis’. Second, we saw important differences in the composition of prey on non-native plants, with caterpillars being rarer and less available to birds, directly supporting the ‘low food availability hypothesis’ for this taxonomic group, but a reverse pattern was observed for spiders. Support for ‘low food quality hypothesis’ was mixed, with extremely variable nitrogen content of the arthropod community. For herbivores nitrogen content as significantly higher on invasive plants, but for spiders it was significantly lower. Investigation of host-plants specific patterns suggest that the variance in food quality on non-native plants encompasses the range of quality of food found on native plants in the same habitat.

Two broad mechanisms cause non-native plants to have different above-ground arthropod communities compared to natives, and these mechanisms may explain the patterns we saw with bird predation and arthropod communities. First, the nutritional quality of these plants is considered lower compared to native plants. By virtue of having less nitrogen or other nutrients, or increased defensive chemistry, herbivores on non-native plants are less abundant and less diverse (van Hengstum et al. 2013). Second, the branch architecture or leaf shape provide novel microhabitat for arthropods and thus creating a distinct community from those found on native plants (Bultman and DeWitt 2008, Landsman et al. 2021). Fitting with observations on other woody non-native plants, we observed higher spider abundance on our non-native plants. Likely differences in twig and leaf architecture provide superior scaffolding and hunting territory (Bultman and DeWitt 2007). Conversely, herbivores like caterpillars (Lepidoptera) were less abundant on non-native plants, likely owing to their inferior nutritional quality, but other mechanisms, such as increased threat from spider predation may also be important. Equivalent abundances of another major herbivore functional group, the Orthoptera, suggest that mobile grazing generalists may not be as constrained by the lower nutritional quality of non-native plants as they can forage on both types of hosts.

One of the gaps in past research on non-native plant invasions is the limited ability of previous studies to assess how much invader-driven changes in arthropod communities translate into altered interactions between arthropods and their predators. Our study allows us to investigate this question by combining quantification of the arthropod community on a range of host plants with a predator exclusion experiment to quantify top-down effects. Moreover, we facilitated this investigation by considering trends in broad taxonomic groups, which can be informative for aggregating effects over complex systems (*sensu* Wagner et al. 2021). Accordingly, we did in fact find differences between native and non-native plants in the abundances of caterpillars and spiders as well as differences in nitrogen content of those groups ranging from around 0.5% in aggregate to 1% in specific contrasts. These differences in nitrogen content translate to differences in protein content of approximately 3 – 6% (McDonald et al. 2011, Smets et al. 2021), which, while not extreme, are detectable by songbirds and can result in substantial differences in body condition (Bairlein 1998, Klasing 1998, Razeng and Watson 2015). Nevertheless, despite these differences we were surprised to see comparably strong predation effects on native and non-native plants, indicating that birds actively forage on and take prey from all available host species. Thus, it appears that the introduction of non-native plants has not greatly impacted the interactions among higher trophic levels at our study site. It is important to note, however, that in our specific case study, some high-quality native plants are rare or absent from the understory in which non-native plants were dominant (Tarr 2022), potentially increasing the overlap in quality between the native and non-native shrub communities. Similarly, at present it remains unclear whether there are any notable downstream nutritional consequences of shifts in arthropod abundance and nitrogen content for songbirds, even in the absence of changes in predatory behavior.

One of the key priorities for invasive species research includes understanding the context of the environments that are being invaded or have been invaded (Ricciardi et al. 2021). Non-native plants are prevalent in these environments, in part, due to ecological disturbances caused by human activity (Homes et al. 2021). Current management practices attempt to ameliorate the impacts of non-native plants on wildlife through physical or chemical removal (Weidlich et al. 2020). However, our results suggest that native plant community is a critical comparison point. For example, forest composition and structure may be more important for some arthropod groups for determining density and abundance even on non-native plants (Traylor et al. 2022). It should be established in a given region whether native woody plants are superior foraging opportunities for songbirds, especially since invasive plant removal tactics themselves can have unintended, negative impacts (Kettenring and Adams 2001). One particularly surprising observation in this study was the range in quality of the arthropod community supported by our focal native plants. While, for example, witch hazel supported a greater total biomass, shadbush was substantially lower and largely indistinguishable from non-natives in both of those measures. Consequently, the relative value of removing a non-native shrub will depend on the particular pairwise comparisons being made at a given site. Overall, our results suggest that a more nuanced management strategy for habitat improvement goals in northeastern USA forests in which the native plant community is considered as the reference point in invaded habitats rather than assume that all non-natives are poorer foraging opportunities for wildlife.

Acknowledgements: The bird exclusion experiment and taxonomic identification of insects were completed by three dedicated and talented summer technicians at Great Hollow: Max Kirsch, Chris Tait, and Joan Tremblay. Volunteers Joe McLaughlin, Ethan Mackenzi, and Alexa Dattner provided valued support for fieldwork in 2021. Helen Poulos and Paul Kraut provided help on elemental analysis in 2022. We thank Tim Farkas and Ian Cranston for advice on statistical analyses and data manipulation over the course of this project.