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SURVIVAL IN THE FOREST UNDERSTORY: RESILIENCE AND RESISTANCE TO DAMAGE IN NATIVE AND INVASIVE WOODY PLANTS

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ABSTRACT

Non-native woody plants from Europe and East Asia have invaded intact, low-light forest understories in Eastern North America. Compared to co-occurring native species, invasive species exhibit greater productivity and resource use efficiency, but the extent to which plants are subject to tradeoffs between productivity and survival is unknown. Herbivory and freezing events are common sources of damage in the forest understory, and non-structural carbohydrates (NSCs) and defense compounds support resilience and resistance to damage. In this dissertation, I evaluated the hypothesis that invasive species maintain high productivity at the expense of storage and defense, reducing their survival under severe damage. In the first chapter, I identified differences in seasonal NSC concentrations in 39 species of native and invasive plants and evaluated whether a tradeoff exists between growth and storage. I found no evidence of a growth-storage tradeoff, but native species tended to maintain greater soluble sugar concentrations than invasive species in both stems and roots. However, invasive species had greater root starch during the growing season. In the second chapter, I investigated defense investment in 20 native and invasive species, coupling generalist herbivore feeding trials with leaf defense trait assays. I found that invasive species have higher leaf lignin and nitrogen concentrations than native species, but generalist caterpillars showed no nativity preference, suggesting that invasive species do not benefit from enemy release. In the third chapter, I compared growth and survival responses to varying levels of defoliation in two pairs of native and invasive tree species in the field. Over three growing seasons, I subjected saplings of each species to each of the following treatments: no defoliation control, half defoliation over three years, full defoliation over one year, and full defoliation over two years. Native and invasive species had similar responses to treatments: half defoliation reduced radial growth but did not affect survival, while full defoliation caused greater growth reductions and severe decreases in survival. NSCs in living saplings were not affected by defoliation, suggesting that plants prioritize storage over growth in response to damage. Taken together, these studies show differences in carbon storage and defense in native and invasive woody species, but contrary to my hypothesis, I did not find evidence for direct tradeoffs between growth and survival. Additionally, native and invasive species are equally targeted by generalist herbivores and exhibit similar responses to damage in the forest understory.

SURVIVAL IN THE FOREST UNDERSTORY: RESILIENCE AND RESISTANCE TO DAMAGE IN NATIVE AND INVASIVE WOODY PLANTS

by

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B.S., UC Davis, 2011

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CHAPTER 1: Introduction

The transport of plant species from one place to another is perpetual: wind, water, wildlife, and the movement of continents have provided numerous opportunities for biotic interchange, with each introduction an experiment in colonization, survival, and persistence (Vermeij et al. 1991). Globalization over the past 200 years has increased the frequency of these experiments, giving rise to a high incidence of economically damaging invasive species (Hulme 2009). What are the drivers of non-native plant species spread in novel ecological communities? While disturbance and subsequent resource availability frequently facilitate contemporary invasions (Elton 1958), low resource, intact ecological communities are not resistant to plant invasions (Funk 2013). Despite low light (Finzi and Canham 2000) and historically low nutrient (Vitousek and Howarth 1991) conditions found in temperate deciduous forests, woody plants from Europe and East Asia have successfully colonized forest understories of Eastern North America (ENA) (Martin, Canham, and Marks 2009).

In contrast to accidental introductions associated with agriculture and overseas shipping routes, a majority of ENA invasive woody plants were intentionally brought to North America (Mack 2003, Fridley 2008). European species, including *Acer platanoides*, were popularized in 19th century ENA as attractive, hardy urban trees (Nowak and Rowntree 1990) or hedgerows in the cases of *Rhamnus cathartica* and *Berberis vulgaris* (Kurylo and Endress 2012). East Asian species, on the other hand, like *Lonicera japonica*, were collected and introduced in ENA as exotic additions to ornamental gardens (Mack 2003, Fridley 2008, Rejmanek 2014). Interestingly, most of these species have native ENA congeners, which may support invasion success if phylogenetic constraint conserves traits important for persistence (Cadotte et al. 2018).

How did non-native species transition from manicured garden specimens to invaders in mid- to late-successional hardwood forests?

Invasive woody plants exhibit a suite of traits that likely confer success in the forest understory. Many species display extended leaf phenology, retaining photosynthetically functional leaves for nearly a month later in the fall than native species, affording them greater annual carbon gain (Fridley 2012). Despite increased leaf construction and nitrogen costs, invaders also have greater photosynthetic resource use efficiency, both in common garden and field settings (Heberling and Fridley 2013, Heberling and Fridley 2016). This superiority in resource acquisition increases when integrated over the extended growing season typical of invaders (Heberling and Fridley 2013). Furthermore, ENA invaders tend to have higher leaf and root production than native species and may increase nitrogen cycling in invaded ecosystems (Jo et al. 2015, Jo et al. 2017). Increased productivity supports invasion success in European and East Asian woody species, but it is unclear whether resource acquisition advantages in invaders come at the expense of other important energy pools.

Resource limitation in the forest understory is hypothesized to necessitate tradeoffs between plant growth, energy storage for future use, and defense against herbivores. While growth toward the canopy increases available light for photosynthesis and subsequent production, mortality risk increases if a plant has no means to recover from unpredictable weather events (Zohner et al. 2017), pest and pathogen outbreaks (Weed et al. 2013), and herbivory (Schierenbeck et al. 1994). Non-structural carbohydrates (NSCs), on the other hand, improve survival when carbon demand exceeds supply (Chapin et al. 1990), increase cold tolerance (Poirier et al. 2010), and maintain hydraulic function (Sevanto et al. 2014). However, the sequestration of NSCs likely come at a cost, including the forgone opportunity to grow, acquire more resources, and compete with neighboring species. Similarly, physical and chemical defenses decrease tissue damage by deterring herbivores and reduce plant dependence on NSCs for regrowth, but also incur a resource acquisition cost (Fig. 1). To what extent do these hypothesized tradeoffs apply to native and invasive species in the forest understory? Survival in the forest understory is required for persistence in long-lived woody species that require several years to reach maturity, and observed differences in resource economy between native and invasive species warrant an investigation of resilience and resistance to damage in these two groups.

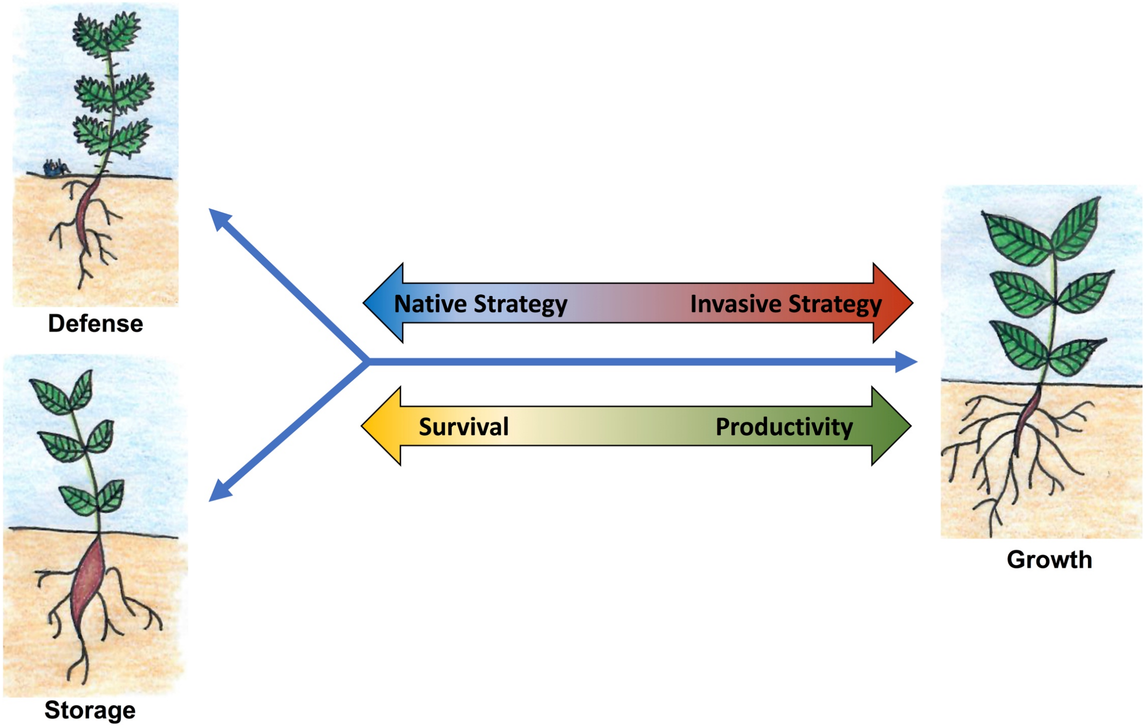

Figure 1. Hypothesized tradeoff between survival and productivity in native and invasive woody species.

In this dissertation, I evaluated whether productivity differences in native and invasive woody plants translate to differences in survival strategies. If native and invasive species are faced with resource constraints, high productivity in invaders could come at the expense of defenses and/or NSCs, while lower productivity in natives may result from increased investment in resource conservation for survival. In this case, invader persistence likely depends upon avoidance of severe damage from herbivory (e.g. enemy release, Keane and Crawley 2002) and/or extreme weather events that necessitate the use of NSCs to regrow. However, greater overall carbon gain in invasive species relative to native species may afford them both higher productivity and equivalent or greater resilience to damage, despite similar relative allocation to growth, defense and storage. Could this mean that invaders are overall both better competitors and survivors in the forest understory? Martin et al. (2010) found evidence for divergence from the growth-survival tradeoff hypothesis in invader *Acer platanoides*. I explored the relationships between production, leaf defenses, and NSCs in the context of survival in native and invasive woody plants.

Chapter 2

In collaboration with Jason Fridley, I assessed growth-storage tradeoffs in 39 species of native and invasive plants co-occurring in a common garden. This study is the first of its kind to compare storage among a large number of phylogenetically paired native and non-native species. I hypothesized that higher productivity in invaders comes at the expense of NSC concentrations. I also hypothesized that leaf phenology affects NSC economy, with determinate ‘single flushing’ species exhibiting a more conservative strategy than indeterminate, continually leafing species. I measured stem and root NSCs (soluble sugars and starch) in spring and fall and determined relationships between storage and leaf production, leaf determinacy, and nativity. I also tested the relative effects of season and tissue type on NSC concentrations. I found no evidence for a tradeoff between leaf production and NSCs in this group of plants. Native species maintained greater soluble sugar concentrations than invaders in stems and roots, but invaders had greater root starch following leaf out. Finally, determinate species possessed greater soluble sugar concentrations than indeterminate species, but indeterminate species maintained higher root starch concentrations. These differences in NSC concentrations are more evidence of divergent resource use strategies in native and invasive species, despite a lack of an apparent tradeoff between growth and storage.

Chapter 3

In collaboration with Jason Fridley and Dylan Parry, I evaluated defense investment in 20 native and invasive species using trait assays and an herbivore feeding trial and assessed whether constraints exist between defense, storage, and growth. I hypothesized that greater productivity in invaders relative to natives comes at the expense of defense investment, perhaps due to enemy release. However, tolerance to herbivory (i.e. refoliation) via stored carbon may mediate the relationship between productivity and defense. I measured leaf lignin, cellulose, nitrogen, total phenolics, and leaf dry matter content in native and invasive species and compared these defense-related leaf traits to the growth rates of two caterpillar species in a no-choice feeding assay. I found that invasive species had greater leaf lignin and nitrogen concentrations than native species, but both groups were equally palatable to insect herbivores. Finally, a constraints analysis that accounted for differences in total carbon gain revealed no clear tradeoffs between growth, storage, and defense in native and invasive species. Results do not support enemy release as an invasion mechanism for European and East Asian species and suggest that predicted increases in pest outbreaks due to climate change will affect native and invasive species equally.

Chapter 4

In collaboration with Jason Fridley, I determined whether native and invasive species exhibit different capacities for survival and growth following varying levels of experimental defoliation in the field. I hypothesized that invasive species would prioritize leaf production over resource conservation to a greater extent than natives. This response, in turn, would make invaders more vulnerable than natives to carbon starvation and mortality following full and repeated defoliation. However, I predicted that both natives and invaders would show similar survival and growth responses to moderate defoliation. The study occurred at two forest sites, each with two co-occurring native and invasive species. Saplings of each species (160 plants in total) were subjected to one of four treatments: no defoliation control, three years of 50% defoliation, one year of 100% defoliation, and two years of 100% defoliation. Leaves were continually removed throughout the growing season as they were produced. I found that native and invasive saplings responded similarly to defoliation treatments. Increasing defoliation intensity reduced radial growth and increased mortality rate across species: few saplings fully defoliated over two years survived. Thus, native and invasive woody plants appear equally vulnerable to varying levels of tissue damage and exhibited similar canopy mortality in response to full defoliation.

In this dissertation I identify key differences in storage and defense traits between native and invasive woody species that could lead to differences in species persistence despite similar palatability to generalist insects and responses to defoliation.

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CHAPTER 2: To spend or to save? Assessing energetic growth-storage tradeoffs in native and invasive woody plants

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Highlighted Student Paper Statement: This comprehensive study characterizes carbohydrate storage allocation across seasons and tissues in 39 species of native and invasive deciduous woody plants and explores a commonly hypothesized tradeoff between storage and growth. We were surprised to find no relationship between leaf production and non-structural carbohydrates, and yet we observed clear differences in storage patterns in closely related natives and invaders, which may stem from differences in historical disturbance regimes between Eastern North America and Europe/East Asia. This research lays the groundwork to evaluate the availability and use of non-structural carbohydrates in natives and invaders in response to disturbance and predict how different species will respond to environmental change.

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[1] Author Contributions: EH and JF worked together to design this research project. EH carried out the data collection and statistical analysis. EH and JF wrote the manuscript.

Abstract

Many non-native woody plants invade low-light forest understories but differ from native species in leaf phenology and seasonality of photosynthesis. It is unknown whether such differences in assimilation patterns are due to contrasting strategies of energy allocation. In a group of native and invasive species in Eastern North America, we hypothesized that invaders employ a grow-first strategy, prioritizing allocation to new structural biomass over carbon storage compared to native congeners. We also hypothesized that species producing a single spring leaf flush exhibit a more conservative carbon storage strategy than species with continuous leaf production. We measured sugar and starch concentrations (non-structural carbohydrates; NSCs) in spring and fall in the stems and roots of 39 species of native and non-native shrubs in a common garden and compared these to patterns of leaf production across species. Native species had higher soluble sugar concentrations than invaders, but invaders tended to store more root starch in spring. We found no difference in leaf production between natives and invaders. Determinate species had more soluble sugars than indeterminate species but had lower root starch. We found no relationship between aboveground productivity and carbon storage. Our results suggest that closely related species with contrasting evolutionary histories have different carbon storage strategies, although not necessarily in relation to their growth potential. The higher soluble sugar concentrations of native species may reflect their evolutionary response to historical disturbances, or different interactions with soil microbes, while increased spring root starch in invaders may support fine root or fruit production.

Introduction

Identifying resource-use strategies of invasive species, and whether they differ predictably from those of co-occurring natives, is a primary objective in invasion biology because it underlies how invasions occur and whether invaders impact ecosystems (Rejmanek and Richardson 1996, Dahler 2003, Funk and Vitousek 2007, Leishman et al. 2010, Funk 2013). Non-native, invasive plants are often more productive than co-occurring native species, even in low resource environments (Funk and Vitousek 2007, Liao et al. 2008, van Kleunen et al. 2010b). For example, in light-limited deciduous forest understories of Eastern North America (ENA), invasive woody species from East Asia (EAS) and Europe (EUR) maintain photosynthetically active leaves for nearly a month longer than native species, which affords them both higher annual carbon gain and higher resource-use efficiencies (Fridley 2012, Heberling and Fridley 2013, Jo et al. 2015, Gallinat et al. 2015, Heberling and Fridley 2016). Why do co-occurring native species not share this high assimilation and growth strategy? One unexplored hypothesis is that native species have evolved a strategy of increased energy storage over growth allocation, which may be advantageous in environments subject to unpredictable disturbances such as extreme weather events (Philippi and Seger 1989, Chapin et al. 1990, Zohner et al. 2017). For example, Zohner et al. (2017) showed that ENA woody deciduous species have delayed spring leaf emergence compared to phylogenetically related species of EAS and EUR, which is associated with higher historical spring temperature variability in ENA. In this context, extreme weather events may have shaped a carbon allocation strategy in ENA lineages that is biased toward storage, constraining their growth phenology to greater extent than those of EAS and EUR (Chapin et al. 1990). Although such carbon stores could confer increased resistance (e.g., freezing protection) and recovery from disturbances (e.g., energy reserves) in native species, they may come at a cost of reduced growth potential if storage and growth are competitive processes (Wiley and Helliker 2012). Other carbon pools, like reproduction, belowground production or defense allocation, could mediate the growth-storage relationship. To our knowledge, the potential for different growth-storage strategies in native and invasive species has not yet been examined.

Soluble sugar and starch (collectively termed non-structural carbohydrates [NSCs]) have myriad roles in plant survival, reproduction, and growth (Dietze et al. 2014). Deciduous species retain large pools of NSCs in winter to support the onset of spring leaf production (Chapin et al. 1990). Following spring leaf production, most species also maintain storage pools to recover from tissue damage due to herbivory, pathogen attack, or extreme weather events (Kozlowski 1992, Kobe 1997). Additionally, NSCs are stored over short periods in roots as a supply to mycorrhizal fungi and/or symbiotic or free-living bacteria in exchange for limiting nutrients (Karst et al. 2016). Carbohydrates travel between plant organs as soluble sugars (Kozlowski 1992), which are also involved in stem freezing protection, both as osmotic solutes and as signaling molecules (Sakai and Yoshida 1968, Ruan 2014). When plant growth is limited, however, newly assimilated carbohydrates may passively accumulate in tissues, and plants may use these carbohydrates for survival when demand exceeds supply (Palacio et al. 2013). We hypothesize that the various roles of NSCs promoting survival suggest that their concentration should be higher in ENA-native lineages shaped by a greater degree of spring weather variability compared to EUR and EAS invaders (Zohner et al. 2017). Alternatively, the greater carbon gain observed in many invaders (Heberling & Fridley 2013) may promote more passive accumulation of NSCs if non-photosynthetic growth limitation (e.g., cold) affects neighboring species equally.

Because carbon in plant tissues accumulates as a function of both supply (photosynthesis) and demand (growth, respiration) processes, there are other woody plant attributes that influence seasonal and tissue-specific NSC concentrations. In particular, structural growth in many species is seasonally restricted: 'determinate' (flush-type) species produce their entire canopy over a brief period at the beginning of the growing season, while 'indeterminate' (continuously leafing) species produce their leaves sequentially or in multiple flushes throughout the growing season (Lechowicz 1984). The lack of midseason allocation to aboveground structural growth in determinate species suggests they should accumulate NSCs to a greater extent than indeterminate species, reaching a maximum in winter before spring leaf emergence. Interestingly, the determinate leafing strategy is relatively rare in invasive species compared to ENA natives (Fridley and Craddock 2015; Martinez and Fridley, unpublished) and is likely associated with an overall carbon allocation strategy biased toward fast growth (see also Iwasa and Cohen 1989). However, there are several widespread invaders in ENA with a determinate leafing habitat (e.g., *Euonymus alatus*), and, given the likely strong role of growth determinacy in NSC levels, it is important to control for such dynamics in native-invader NSC comparisons.

We measured leaf production and seasonal carbon storage in a group of woody species found in temperate deciduous forests of ENA to understand how carbon allocation strategies differ between native and non-native invasive species. We hypothesized that a tradeoff between growth and storage explains potential productivity differences between natives and invaders (Heberling and Fridley 2013). In particular, we hypothesized that ENA native lineages are better adapted to historical disturbance regimes, including extreme events such as late spring frosts, which is most apparent in stem and root soluble sugars following spring leaf production when storage levels in deciduous species are at their annual minimum (Martinez-Villalta et al. 2016).

Remaining stores are available for new leaf production following damage. Alternatively, allocation to reproduction, defenses, or belowground production could mask the relationship between leaf production and carbohydrate storage. Because native species begin leaf senescence on average 28 days earlier than invaders (Fridley 2012), we also hypothesized that they allocate more soluble sugars to stems in autumn for freezing protection. In addition, we hypothesized that, independent of nativity, determinate species store more soluble sugars and starch at the end of the growing season than indeterminate species, in which NSCs fuel new (current season) leaf production. We tested these hypotheses using common garden measurements of leaf production and shoot phenology, along with stem and root soluble sugar and starch samples from phylogenetically paired native and non-native invasive species.

Materials and Methods

We studied 39 species of native and non-native woody shrubs in a common garden at Syracuse University, Syracuse, New York, USA (Table 1). The common garden was established in 2006 and is composed of three replicate blocks per species. To the extent possible, we selected congeneric native/non-native pairs from 13 plant genera. Of the 21 non-native species, 17 are considered invasive in ENA, that is, spreading quickly and having demonstrable impacts on native species or ecosystem properties (Fridley 2008). The remaining four are naturalized; i.e., they survive in natural habitats but have not yet been documented to spread. Because past studies of this group showed that naturalized species tend to exhibit similar traits as invaders (Fridley 2012, Heberling and Fridley 2013, Fridley and Craddock 2015, Yin et al. 2015), for simplicity we treat both naturalized and invasive species as ‘invaders’ throughout the manuscript.

We examined leaf production and phenology across species in 2008-2010, prior to destructive tissue harvests for NSCs in 2013-2014 on the same individuals. We defined an individual's productivity as the maximum number of leaves produced over the growing season in 5 randomly selected branches per plant across years (2008-2010), multiplied by its mean dry leaf mass. *Cornus racemosa* individuals were growing adjacent to the common garden and their measurements did not include productivity. Patterns of seasonal leaf production and senescence from 2008-2010 in the same individuals (Fridley 2012) were used to categorize species' leafing behaviors as determinate or indeterminate. Species that produced 90% of their leaves prior to June 15 were considered determinate, and indeterminate otherwise.

We collected stem and root samples (total N=639) from three individuals per species following leaf drop in November/December 2013 and following full leaf expansion in May/June 2014. We sampled new (2013) and year-old (2012) stems separately. At each sampling event, we collected three stems of each age from each individual, and two root samples from the root crown. Three native species without invasive congeners (*Cornus florida*, *Cornus racemosa*, and *Hydrangea arborescens*) were only sampled in the fall, and invaders *Elaeagnus angustifolia* and *Frangula alnus* had no spring stems sampled. Samples were placed in a cooler of dry ice and stored at -80 ºC. We freeze-dried tissue samples for 48 hr prior to grinding samples to fit through a 40-mesh screen (Thomas Scientific Mini Wiley Mill, Swedesboro, NJ, USA). Ground samples were stored at 4 ºC until analysis.

Soluble sugar and starch concentrations were determined using a combination of near-infrared spectroscopy and colorimetric methods following Ramirez et al. (2015). All samples were run through a near-infrared spectrometer (Bruker MPA Multi-purpose FT-NIR analyzer, Ettlingen, Germany) and their absorbances measured between 1300 and 2650 nm (Curran 1989).

A subset of 98 of the 639 samples were selected for NSC analysis using the Kennard-Stone algorithm (Kennard and Stone 1969) on NIR spectra. The Kennard-Stone algorithm composes a representative subset by first selecting the two most distant samples from one another in parameter space. It continues selecting additional samples based on their maximum distance from the closest sample already included in the subset. These samples were used to calibrate NSC concentrations determined by wet chemistry to the NIR spectra.

We measured soluble sugar and starch (NSC) concentrations in the calibration sample set using the colorimetric method of Chow and Landhausser (2004). Briefly, soluble sugars were extracted using ethanol and phenol-sulfuric acid and sample absorbance was measured at 490 nm on a microplate reader (BioTek Instruments, Inc, Winooski, VT). Starches were broken down to glucose equivalents using alpha-amylase and amyloglucosidase enzymes, and then treated with PGO color solution + o-dianisidine for spectral analysis at 525 nm. NSC concentrations determined colorimetrically were calibrated with the near-infrared spectra using partial least squares (PLS) regression. The number of components used in the PLS model was determined by the component axis with the minimum coefficient of variation (Ramirez et al. 2015). This calibration model (see Supplementary Materials) was used to estimate the soluble sugar and starch concentrations of the entire sample dataset. We ran 500 iterations of a bootstrapping algorithm that sampled the calibration dataset with replacement and used these values to fit the PLS model to our sample dataset. We then estimated the standard error for each sample and incorporated these values into our statistical models as observation error (see below).

We compared soluble sugar and starch concentrations across species, seasons, and tissues using hierarchical Bayesian regression models (Fridley and Craddock 2015). Because we found no significant differences in NSCs between new and year-old stems (analysis not shown), we took the mean starch and soluble sugar concentrations across stem ages (N=423 samples). Models included a phylogenetic variance-covariance matrix to account for the correlation of observations due to common descent (de Villemereuil et al. 2012), using the approach and phylogeny of Jo et al. (2016). Pagel's λ was used to estimate phylogenetic signal, scaled from 0 (phylogenetic independence) to 1 (Brownian evolution). We first examined the main effect of nativity (0 = invasive, 1 = native) on leaf production, accounting for phylogenetic relatedness. We then used separate models predicting soluble sugars and starches using the same phylogenetic correction (see Supplementary Materials). One set of models compared the overall effect of nativity to the main effects of season (0 = fall, 1 = spring), tissue type (0 = root, 1 = stem), determinacy (0=indeterminate, 1=determinate), and productivity (continuous) on sugar and starch concentrations. We also separated sugar and starch concentrations by season and tissue type to test for nativity and determinacy differences in stem and root storage in specific seasons, resulting in eight additional models. The latter models included tissue diameter to account for differences in NSC concentrations by organ size. We scaled continuous variables to make their effect sizes comparable to categorical variables by subtracting the mean and dividing by two standard deviations (Gelman and Hill 2007). We included observation error as a normal random intercept of mean zero and known standard deviation from the bootstrapped standard error of each NSC observation. All models were run in R (R Core Team 2016) using the R2jags package (Su and Yajima 2015). We ran three Markov chain Monte Carlo (MCMC) chains for 10,000 iterations (after 3,000 burn-in) in the productivity model, 30,000 iterations (after 5,000 burn-in) in the overall models, and 10,000 iterations (after 3,000 burn-in) in tissue and season-specific models (with the exception of the spring stem starch model, which required 50,000 iterations with 10,000 burn-in). Model convergence was determined by the Gelman-Rubin (1992) convergence diagnostic (Rhat<1.1).

We performed a principal components analysis (PCA) to describe relationships between NSC concentrations, productivity, leaf determinacy, and nativity. We used a MANOVA of mean species PC scores from the first and second components to test for multivariate differences between natives and invaders. We used 'prcomp' and 'manova' from the base R package for these analyses (R Core Team 2016).

Results

Natives and invaders did not differ in leaf production (Fig. 1). In an overall model comparing main effects of nativity, season, tissue type, growth determinacy, and productivity, we found strong effects of all factors except productivity on plant soluble sugar and starch concentrations (Fig. 2). Native species overall had significantly higher concentrations of soluble sugars (Fig. 3), as did determinate species of both native and invasive groups (Fig. 4). Starch concentrations showed the opposite pattern with respect to nativity and determinacy (Fig. 2), although their effect sizes were small in relation to root-stem differences (Figs. 3-4). Effect sizes of nativity and determinacy were similar in magnitude to the effects of season and tissue type on soluble sugar concentrations (Fig. 2), and leaf production was neither associated with sugar nor starch concentrations across species. Post-leafout (spring) NSC levels were lower than fall levels for all species (Figs. 3-4).

Nativity and determinacy patterns of NSCs were similar when examined separately by season and tissue type (Figs. 3, 4). The bias of soluble sugars in natives did not depend on season and had similar effect sizes in roots and stems (Fig. 3), nor did the greater sugars in determinate species depend on season or tissue type (Fig. 4). Indeterminate species had substantially larger root starch concentrations in both spring and fall (Fig. 4), but the starch bias in invaders was only significant in spring (Fig. 3). We detected only weak phylogenetic signal in the relationships between seasonal storage, nativity, and determinacy ($\lambda < 0.3$).

Species-level NSC patterns are shown in Supplemental Figure 1. Native species including *Rhamnus alnifolia* and *Euonymus obovatus* had the highest stem NSC concentrations across seasons, while natives *Sambucus racemosa* and *Viburnum lentago,* and invaders *Euonymus phellomanus* and *Lonicera japonica*, consistently had the lowest stem NSCs. *Sambucus racemosa* had the highest root NSCs across seasons. The native *Euonymus atropurpureus* also had very high root NSCs across seasons, but was intermediate for stem NSCs. With the exception of spring stem concentrations, the native N-fixer *Elaeagnus commutata* consistently ranked near the bottom in NSC concentrations. Spring stem rankings reflected leaf determinacy, with indeterminate species occurring primarily at the low end of the spectrum (Figure S1a), while fall stem and root rankings exhibited native dominance at the highest NSC concentrations (Figure S1 b,d).

In multivariate analysis, the first two principal components (PC1 and PC2) accounted for 23% and 17% of the variation in NSC concentrations, respectively, across species, season, and tissue type (Figure 5). Native and invasive species occupied significantly different regions of PC1 and PC2 (MANOVA, Pillai's number = 0.18, F= 8.72, P<0.0005). Root starch and root sugars varied predominantly along PC1, while stem starch and fall stem sugars varied along PC2. Starch and sugar concentrations were negatively associated, particularly in stems.

Discussion

We found significant differences in stored carbon between native and non-native, invasive woody species in ENA, despite high variance in storage within some species (Fig. 1). In contrast to our hypothesis that increased storage in natives is related to their generally more conservative growth phenology, we found no evidence of a negative relationship between carbon invested in annual leaf growth and total NSCs across species. Moreover, our estimates of leaf production rates did not show an invader bias despite large differences in growing season duration (Fridley 2012), assimilation rate (Heberling and Fridley 2013), and estimated annual carbon gain (Fridley 2012). Although we did not measure whole plant carbon budgets in our study, our results point to two non-exclusive, novel contrasts between native and invasive species in ENA forests: 1) the greater carbon gain of invaders may support both growth and storage—that is, greater root starch reserves than natives after spring leaf out may translate to a more rapid response to defoliation (e.g., herbivory), without incurring a growth cost; and 2) natives consistently maintain more soluble sugars in stems and roots, with an effect size that rivals other plant attributes (e.g., determinacy) associated with NSC dynamics (Fig. 2a). We discuss the significance of these differences to forest invasions in turn.

After spring leaf out, invaders contained, on average, about 11% greater root starch concentration than native species, an effect that remained after controlling for both phylogeny and the tendency of invaders to exhibit indeterminate leaf growth (Fig. 3c). Although we did not investigate its potential function in the present study, the role of root starch in woody plants as an energy reserve that promotes resprouting in the event of defoliation is well established (Poorter et al. 2010; Dietze et al. 2014) and suggests that at least part of invaders' enhanced annual carbon gain goes toward survival in addition to growth mechanisms. This is corroborated by studies documenting strong compensatory responses to herbivory in many invaders

(Schierenbeck et al. 1994; Rogers and Siemann 2002; Callaway et al. 2006), although we note that, absent complete defoliation, compensatory responses can also be achieved by higher assimilation rates of surviving leaf tissue (Huang et al. 2010). As our estimates of annual leaf growth do not suggest an overall bias toward invaders, it is unclear why the native-invader root starch difference disappears by fall. One possibility is that energy stored as root starch in spring is allocated to fine roots later in the growing season, which Jo et al. (2015) showed to be greater overall for invaders using individuals from the same common garden. Another possibility is that invaders allocate more energy to fruit production in summer and fall. Although invasive species have generally been shown to produce more offspring than co-occurring native species (Mason et al. 2008; van Kleunen et al. 2010a), we are unaware of studies on reproductive allocation per se in forest invaders.

On the other hand, the higher concentration of soluble sugars in native species was consistent across seasons and tissues (22% greater in roots, and 11% greater in stems; Fig. 3). This difference is in addition to the independent contribution of a determinate leaf habit, which is more common in native ENA species than the overall invader pool (Fridley and Craddock 2015). There are several potential ramifications of this soluble sugar difference to understanding ENA forest invasions. First, these results support our hypothesis that native lineages may be better adapted to historical disturbance regimes than more recently arrived (invasive) lineages. For example, historic early-season freeze events in ENA may require woody species to maintain relatively high stem soluble sugar concentrations to reduce risk of embolism or cell lysis (Sakai and Yoshida 1968, Kozlowski 1992, Ashworth et al. 1993, Travert et al. 1997, Grant and Dami 2014, Zohner et al. 2017). These unpredictable damage events may promote bet-hedging behavior in native species, where plants maintain high soluble sugar in stem and root tissues to minimize variance in performance across years or seasons at the expense of other carbon pools (Philippi and Seger 1989, Philippi 1993, Fridley 2017). This may suggest that increases in unpredictable events that bring high risk of embolism or cell damage may limit the spread of invaders, if survival is promoted by soluble sugar reserves (Morin et al. 2007, Charrier and Ameglio 2011, Trischuk et al. 2013). Second, differences in root sugar concentrations between natives and invaders might also indicate a greater investment in soil microbial interactions in natives or differences in the timing of above/belowground carbon translocation patterns (Kozlowski 1992, Marler et al. 1999, Wolfe et al. 2008, Pringle et al. 2009, Karst et al. 2016). Jo et al. (2015) found large differences in specific root length and fine root growth between this group of native and invasive species that could indicate a tradeoff in nutrient foraging, with nutrient uptake in natives biased toward microbial relationships (requiring carbohydrate transfer) and away from root structural investment. Current studies are underway in this group to document whether such nativity-based systematic differences in soil microbial relationships occur. Third, the greater concentration of soluble sugars in native species may result from passive accumulation due to a shorter period of leaf growth (Fridley 2012), as would be expected in fall NSCs. However, passive sugar accumulation is an unlikely explanation for native-invader sugar differences in spring, shortly after leaf out, particularly given such large differences in both roots and shoots. In this context, functional NSC studies are a clear next step in forest invasion research.

As hypothesized, species with determinate growth had higher soluble sugar concentrations in all tissues, but indeterminate species maintained higher root starch concentrations in spring and fall. Because determinate species do not reinvest photosynthates in additional leaf and stem structural tissue during the growing season, elevated fall sugar concentrations are a straightforward consequence of less overall tissue produced (but see Canham et al. 1999). What is less clear is why determinate species maintain higher soluble sugar concentrations following spring leaf out. One hypothesis is that, like native species overall, leaf determinacy is part of a syndrome of conservative behaviors, including elevated storage allocation. Many determinate species in deciduous forests are shade tolerant, produce long-lived leaves, and do not break bud until later in the spring than indeterminate species (Lechowicz 1984, Navas et al. 2003, Fridley and Craddock 2015). Indeterminate species may ultimately make the same investment in leaves as determinate species, but their relative leaf investment is lower in the first part of the growing season. Thus, we expect determinate species to have a greater incentive to protect their canopy via greater allocation to NSCs and anti-freeze protection (sugars). The large difference in root starch content between indeterminate and determinate species is surprising, and may suggest a direct link between late season carbon gain (through new leaf production in indeterminate species) and root energy storage. In particular, Smith et al. (2014) showed that root production occurred earlier in spring in two indeterminate *Lonicera* species compared to determinate shrubs in the Rhamnaceae. Thus, indeterminate species that allocate carbon to leaf growth in the summer may preferentially reserve root starch in the winter to promote root expansion before spring leaf display. Alternatively, indeterminate species may require greater starch reserves to produce multiple leaf cohorts throughout the growing season.

Despite overall differences in storage patterns between natives and invaders, both groups showed high variation in soluble sugar and starch concentrations (Supplemental Fig. 1). Several factors likely contribute to this variation. First, species in the common garden occur across a large array of latitudes and light environments (e.g., forest edges vs. interiors), which differ in freezing risk and likely have strong influences on phenology, growth patterns, and storage

(Lechowicz 1984, Morin et al. 2009, Panchen et al. 2014, Muffler et al. 2016). Second, because our focal species reach reproductive maturity at different ages and sizes, some individuals in our study showed high reproductive allocation and others none; additional measurements of flower and fruit abundance could shed additional light on growth-storage constraints in these species. Third, given the large taxonomic representation in our study, species likely differ in the chemical form of stored compounds, including lipids and oligosaccharides (Hoch et al. 2003) that we did not measure.

Conclusion

We found differences in carbon allocation to soluble sugars and starches in native and invasive woody plants of Eastern North American forests. Although the function of stored forms of carbon remains unclear, our study provides a foundation for comparative studies of natives and invaders that focuses on survival (e.g., freezing resistance, response to defoliation). In particular, high allocation to soluble sugars in natives may align with a bet-hedging strategy that confers resilience in an unpredictable environment: the benefit of protecting tissues against damage from environmental extremes (like spring freeze events) outweighs the cost of forgoing growth in stable conditions (Zohner et al. 2017). Alternatively, there may be a connection between high soluble sugar concentrations in native species and belowground interactions with microbial mutualists. Measuring carbohydrate mobility and fluxes in native and invasive species, particularly as it relates to frost damage prevention and survival from defoliation, is likely to improve our understanding of invasion mechanisms in a global change context.

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Figure Captions

Figure 1: Mean leaf production and total % NSC (stem + root) in native (white circles) and invasive (black circles) species in (a) spring and (b) fall. Error bars represent +/- one standard error. Refer to Table 1 for species acronyms.

Figure 2: 95% (thin lines) and 80% (thick lines) credible intervals for posterior coefficients of nativity, season, tissue type, leaf determinacy, and productivity in (a) overall soluble sugar and (b) starch models. Season had the strongest effect on soluble sugars—spring concentrations were significantly lower than fall concentrations, while tissue type was the strongest determinant of starch concentration—stems had lower starch concentrations than roots. Native species and determinate species had higher soluble sugar concentrations than invaders and indeterminate species, respectively, but indeterminate species had higher starch concentrations.

Figure 3: Mean soluble sugar and starch concentrations in native and invasive species in (a) spring stems (N=53 invaders, 41 natives), (b) fall stems (69, 49), (c) spring roots (60,44), and (d) fall roots (68,45). Error bars represent +/- SEM. "*" indicates that 95% of the posterior distribution of the native-invader difference did not include zero.

Figure 4: Mean soluble sugar and starch concentrations in determinate ("Det") and indeterminate ("Indet") species in (a) spring stems (N=44 determinate, 50 indeterminate), (b) fall stems (54, 61), (c) spring roots (46,58), and (d) fall roots (53,57). Error bars represent +/- SEM. "*" indicates that 95% of the posterior distribution of the determinate-indeterminate difference did not include zero.

Figure 5: Principal components analysis of soluble sugars and starches, nativity, and productivity values. Points represent species means, but all individual measurements from each species were used in the analysis. Black circles represent invasive species, while white circles represent native species.

Table 1: Focal species along with taxonomic family, nativity status, and leaf production habit.

Family	Species	Invasive Status	Species Acronym	Leaf Determinacy
Adoxaceae	*Sambucus racemosa*	Native	SARA	Indeterminate
	Viburnum dilatatum	Invasive	VIDI	Indeterminate
	Viburnum lantana	Invasive	VILA	Indeterminate
	Viburnum lentago	Native	VILE	Indeterminate
	Viburnum prunifolium	Native	VIPR	Determinate
Berberidaceae	*Berberis thunbergii*	Invasive	BETH	Indeterminate
Caprifoliaceae	*Lonicera canadensis*	Native	LOCA	Determinate
	Lonicera fragrantissima	Invasive	LOFR	Indeterminate
	Lonicera hirsuta	Native	LOHI	Determinate
	Lonicera japonica	Invasive	LOJA	Indeterminate
	Lonicera maackii	Invasive	LOMA	Indeterminate
	Lonicera morrowii	Invasive	LOMO	Determinate
	Lonicera reticulata	Native	LORE	Indeterminate
	Lonicera sempervirens	Native	LOSE	Indeterminate
	Lonicera standishii	Invasive	LOST	Indeterminate
	Lonicera tatarica	Invasive	LOTA	Determinate
Celastraceae	*Celastrus orbiculatus*	Invasive	CEOR	Determinate
	Celastrus scandens	Native	CESC	Indeterminate
	Euonymus alatus	Invasive	EUAL	Determinate
	Euonymus atropurpureus	Native	EUAT	Determinate
	Euonymus bungeanus	Naturalized	EUBU	Indeterminate
	Euonymus europaeus	Invasive	EUEU	Determinate
	Euonymus hamiltonianus	Naturalized	EUHA	Determinate
	Euonymus obovatus	Native	EUOB	Determinate
	Euonymus phellomanus	Naturalized	EUPH	Determinate
Cornaceae	*Cornus florida*	Native	COFL	Determinate
	Cornus racemosa	Native	CORA	Determinate
Elaeagnaceae	*Elaeagnus angustifolia*	Invasive	ELAN	Indeterminate
	Elaeagnus commutata	Native	ELCO	Indeterminate
	Elaeagnus multiflora	Invasive	ELMU	Indeterminate
	Elaeagnus umbellata	Invasive	ELUM	Indeterminate

Hamamelidaceae	*Hamamelis virginiana*	Native	HAVI	Indeterminate
Hydrangeaceae	*Hydrangea arborescens*	Native	HYAR	Indeterminate
Lauraceae	*Lindera benzoin*	Native	LIBE	Determinate
Rhamnaceae	*Frangula alnus*	Invasive	FRAL	Indeterminate
	Frangula caroliniana	Native	FRCA	Indeterminate
	Rhamnus alnifolia	Native	RHAL	Determinate
	Rhamnus cathartica	Invasive	RHCA	Indeterminate
	Rhamnus davurica	Naturalized	RHDA	Determinate

Figure 1

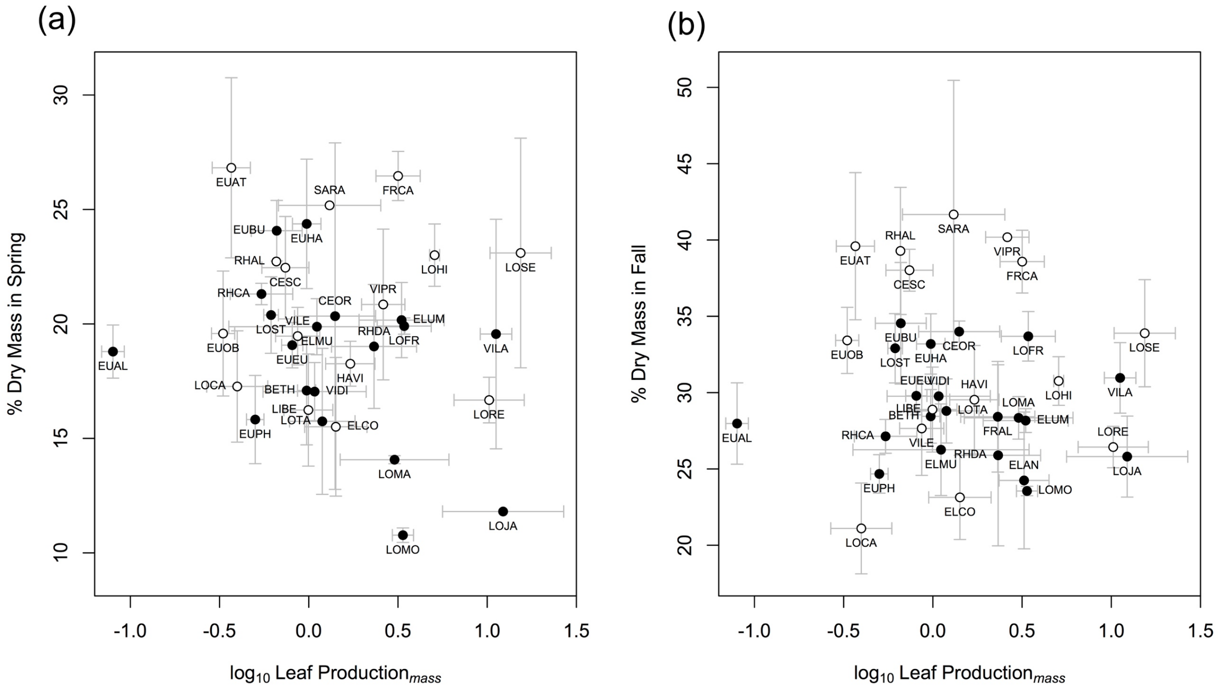

(a)
% Dry Mass in Spring
log$_{10}$ Leaf Production$_{mass}$
10
15
20
25
30
-1.0
-0.5
0.0
0.5
1.0
1.5
EUAT
FRCA
SARA
EUBU
EUHA
LOHI
LOSE
RHAL
CESC
RHCA
VIPR
CEOR
ELUM
VILE
RHDA
LOST
ELMU
LOFR
EUOB
VILA
EUAL
EUEU
HAVI
LOCA
BETH
VIDI
LIBE
LORE
LOTA
ELCO
EUPH
LOMA
LOJA
LOMO
(b)
% Dry Mass in Fall
log$_{10}$ Leaf Production$_{mass}$
20
25
30
35
40
45
50
-1.0
-0.5
0.0
0.5
1.0
1.5
SARA
RHAL
VIPR
EUAT
FRCA
CESC
EUBU
CEOR
LOFR
LOSE
EUOB
EUHA
LOST
LOHI
VILA
EUEU
VIDI
HAVI
LIBE
LOTA
LOMA
BETH
ELUM
EUAL
FRAL
RHCA
VILE
LORE
RHDA
ELMU
ELAN
LOJA
EUPH
LOMO
ELCO
LOCA

Figure 2

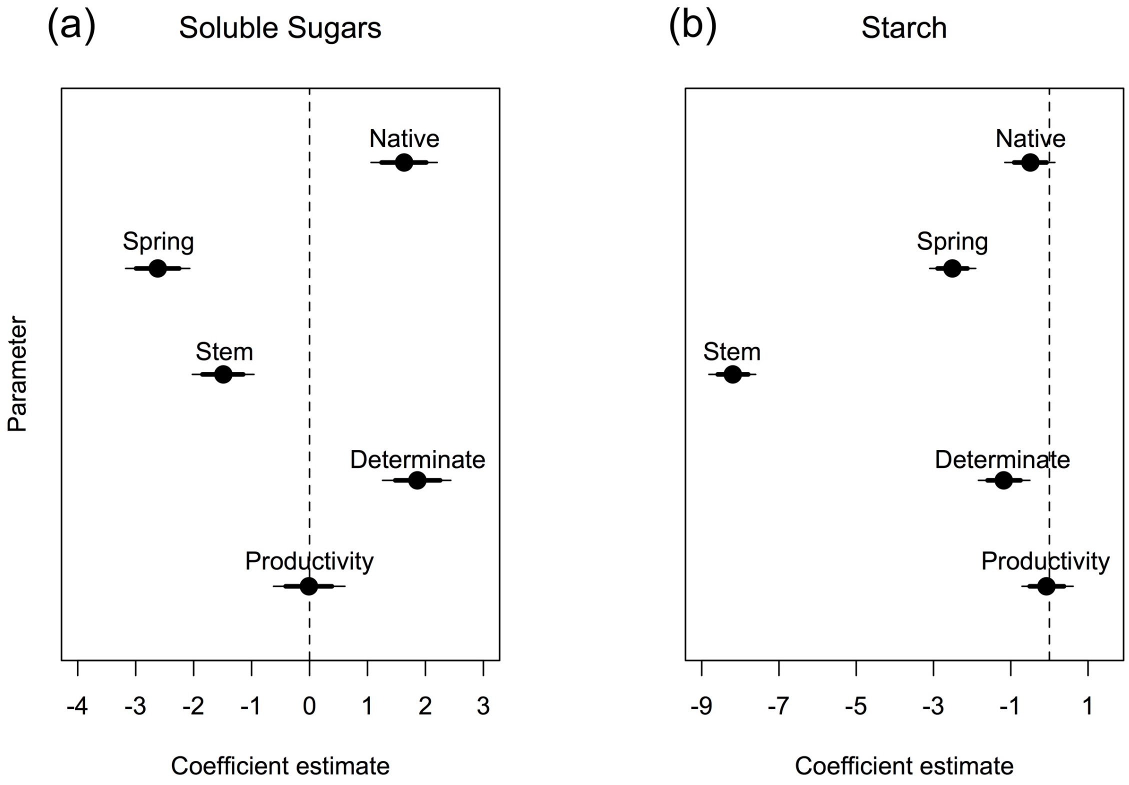
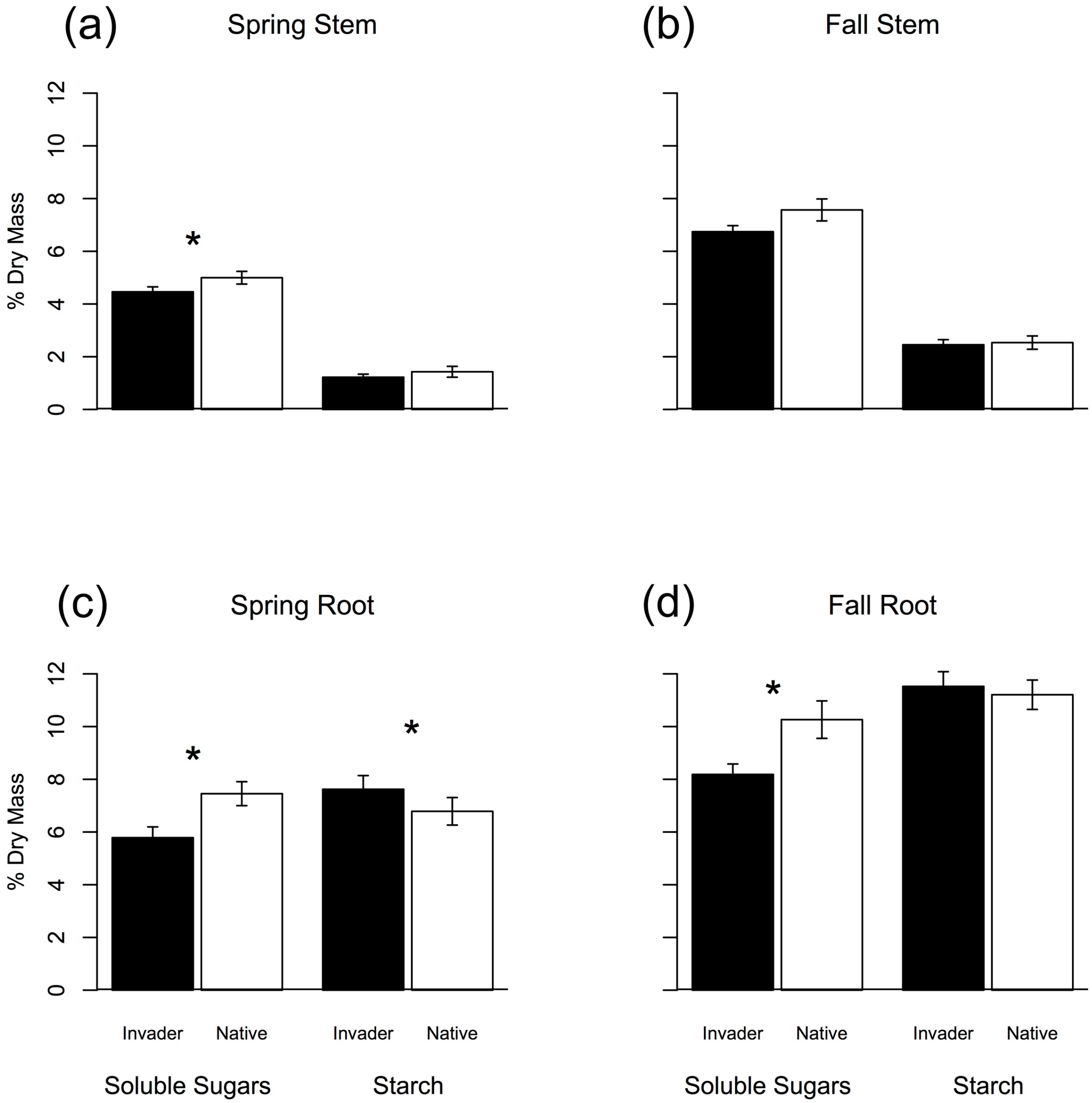

(a)
Soluble Sugars
(b)
Starch
Parameter
Native
Spring
Stem
Determinate
Productivity
Native
Spring
Stem
Determinate
Productivity
-4
-3
-2
-1
0
1
2
3
-9
-7
-5
-3
-1
1
Coefficient estimate
Coefficient estimate

Figure 3



(a)
Spring Stem
(b)
Fall Stem
(c)
Spring Root
(d)
Fall Root
% Dry Mass
% Dry Mass
0
2
4
6
8
10
12
*
Invader
Native
Invader
Native
Soluble Sugars
Starch

Figure 4

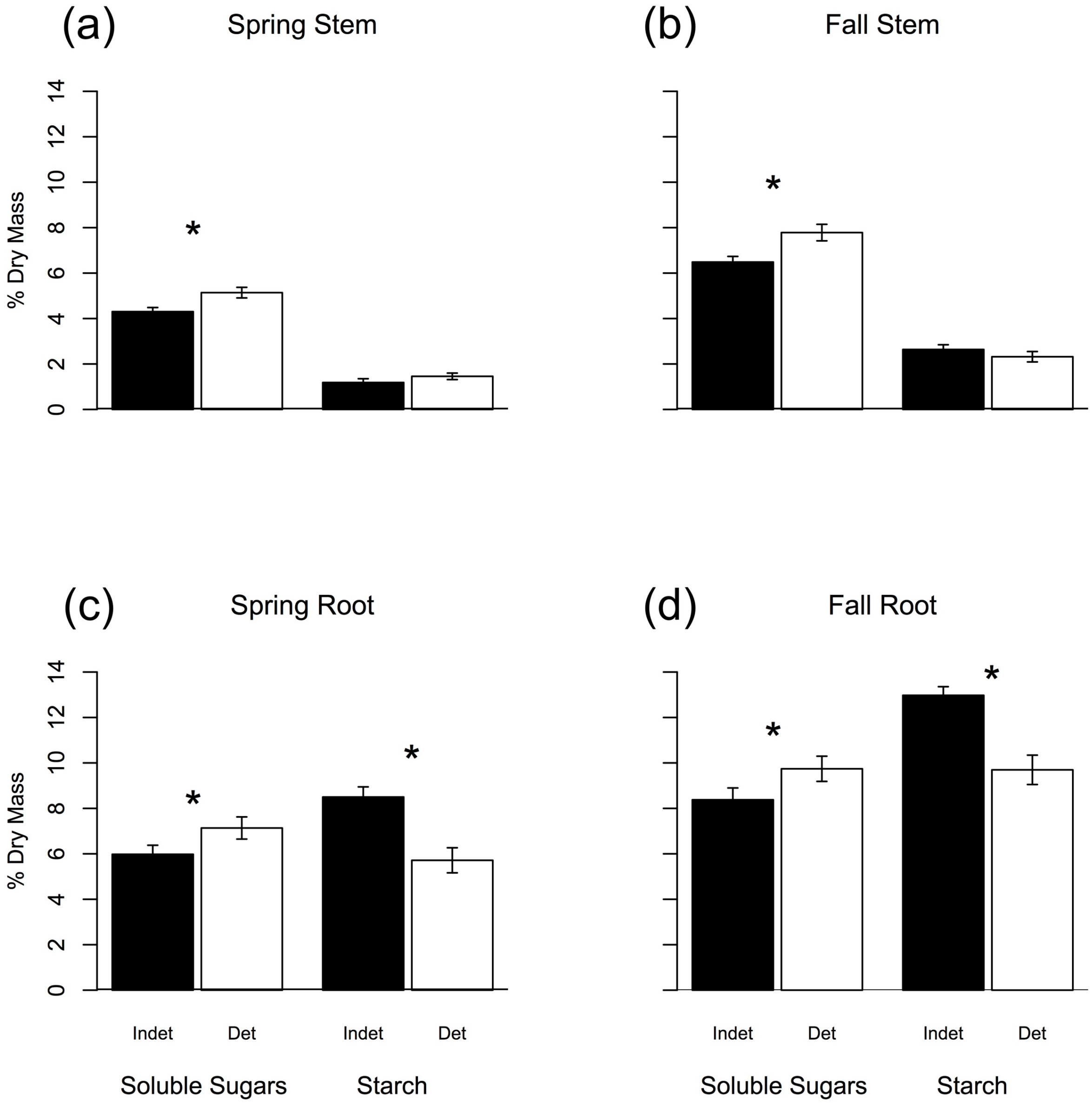
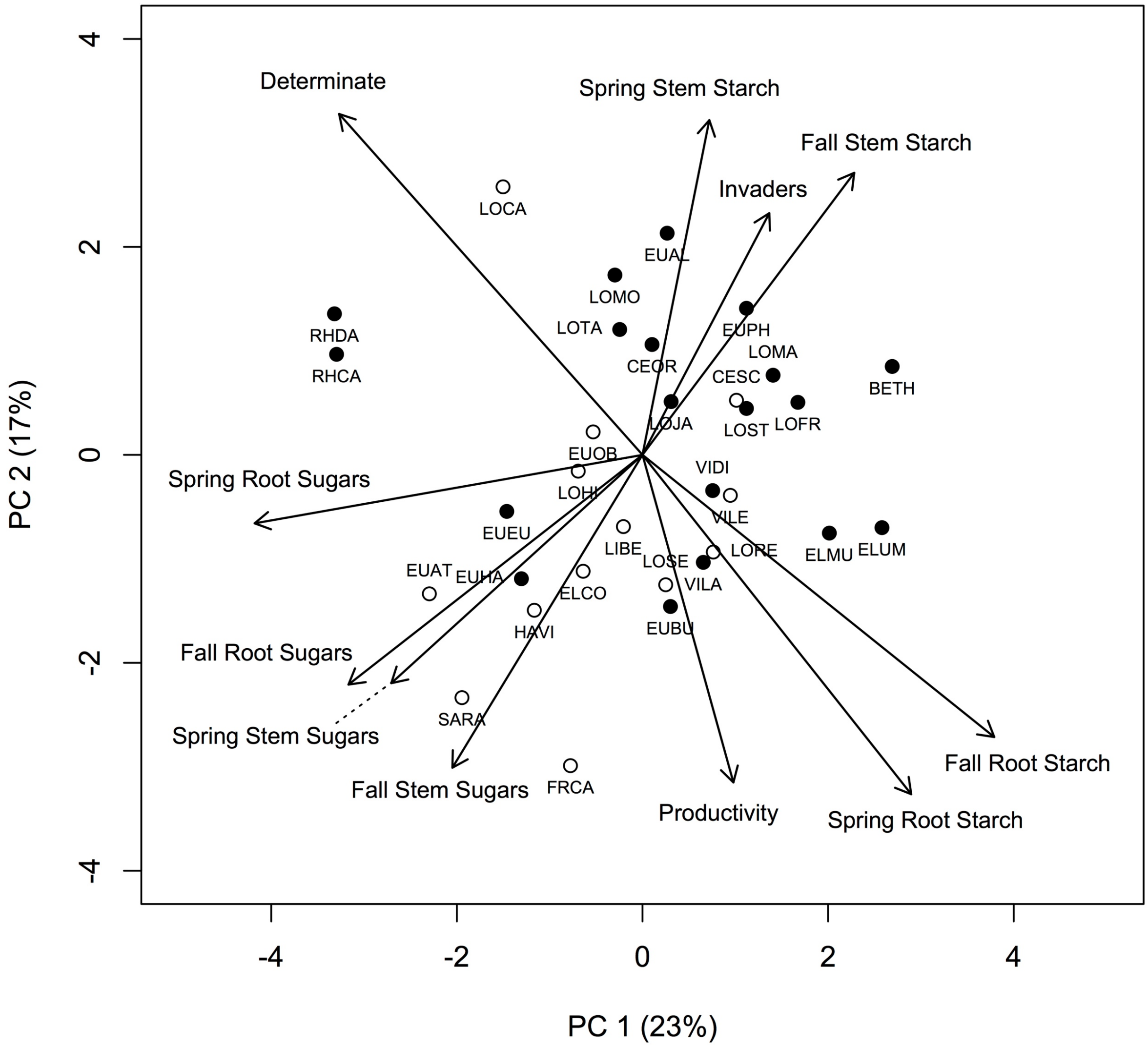

(a)
Spring Stem
(b)
Fall Stem
(c)
Spring Root
(d)
Fall Root
% Dry Mass
0
2
4
6
8
10
12
14
*
Indet
Det
Indet
Det
Soluble Sugars
Starch

Figure 5



PC 1 (23%)
PC 2 (17%)
-4
-2
0
2
4
Determinate
Spring Stem Starch
Fall Stem Starch
Invaders
Spring Root Sugars
Fall Root Sugars
Spring Stem Sugars
Fall Stem Sugars
Productivity
Spring Root Starch
Fall Root Starch
LOCA
EUAL
LOMO
LOTA
RHDA
RHCA
CEOR
EUPH
LOMA
CESC
BETH
LOJA
LOST
LOFR
EUOB
LOHI
VIDI
VILE
EUEU
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ELMU
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EUHA
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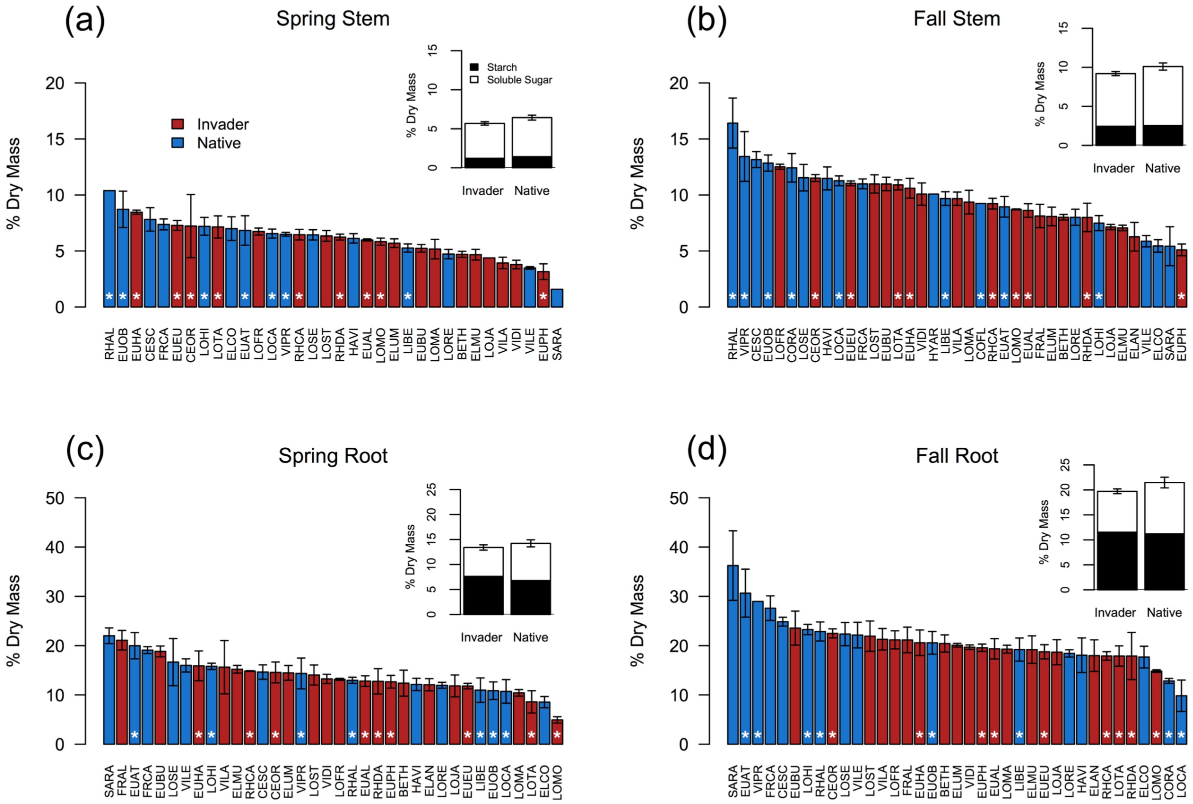
VILA
HAVI
EUBU
SARA
FRCA

Supplemental Information



Supplemental Figure 1: Mean NSC concentrations per species (N=1-3 individuals per species) in (a) spring stems (N=53 invaders, 41 natives), (b) fall stems (N=69 invaders, 49 natives), (c) spring roots (N=60 invaders, 44 natives), (d) fall roots (N=68 invaders, 45 natives). Error bars represent +/- SEM, although some species are only represented by one sample, and thus have no error bars. Red bars correspond to invaders and blue bars correspond to natives. Bars containing an asterisk (*) correspond to determinate species. Inset stacked bar plots show total NSC concentrations in native and invasive groups across season and tissue. Soluble sugar concentrations are represented in black, while starch concentrations are in white. Inset error bars represent +/- SEM.

CHAPTER 3: Plant defense against generalist herbivores in the forest understory: a phylogenetic comparison of native and invasive species

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Abstract

Invasive woody plants from Europe and Asia tend to be more productive than co-occurring native species in deciduous forests of Eastern North America (ENA), but community-scale drivers of invasion success remain unknown. If increased productivity in invaders relative to natives comes at the expense of reduced relative allocation to defenses, generalist herbivores may preferentially consume invasive species, potentially reducing the effectiveness of invader growth advantage. We compared leaf traits related to herbivory (nitrogen concentration, total phenolics (%), cellulose and lignin concentrations, and leaf dry matter content) in 20 phylogenetically paired native and non-native, invasive species and evaluated species palatability to two generalist herbivores: one non-native (European gypsy moth, *Lymantria dispar*) and one native (fall webworm, *Hyphantria cunia*). We also evaluated potential physiological constraints between defense-related leaf traits, leaf production, and carbon storage in this group of species. Invasive plants had higher leaf N and lignin concentrations than related native species, but gypsy moth and fall webworm growth were not associated with plant nativity. Plant defense traits did not predict differences in gypsy moth growth, but fall webworm growth was negatively associated with leaf N. We found no evidence of tradeoffs between defense-related leaf traits, leaf production, and carbon storage, indicating a limited role for carbon-based tradeoffs relating to growth and herbivory in these species, although other unmeasured carbon pools including reproduction may impact these relationships. Woody invaders continue to spread in ENA forest understories, but we found no evidence of inhibition or facilitation by generalist insect herbivores, despite differences among species in leaf traits associated with assimilation.

Key Words: generalists, herbivory, defense, traits, palatability, constraints

Introduction

More than a half century ago, Elton (1958) popularized the idea that introduced species gain ecological advantage over co-occurring native species as a result of reduced predation or parasitism; i.e., they experience 'enemy release' (Keane and Crawley 2002). As the science of invasion biology has matured, it has become clear that the Enemy Release Hypothesis (ERH) is at best an oversimplification of native-invader interactions, in part because the significance of top-down control varies along resource gradients (Blumenthal 2006) and interactions themselves are subject to rapid evolution in the invaded range (Blossey and Notzold 1995, Whitney and Gabler 2008, van Kleunen, Bossdorf, and Dawson 2018). Unsurprisingly, support for ERH has varied dramatically by species, region, and trophic level (Davis 2009, Schultheis et al. 2015, Meijer et al. 2016). In particular, the role of generalist herbivores in the spread of invasive plants remains unclear. While some studies show that non-native plants experience lower generalist herbivore attack rates relative to native species (Wolfe 2002, Eschtruth and Battles 2009, Lieurance and Cipollini 2012, 2013), others have argued the opposite, particularly given the relatively high leaf protein content of many invaders (van Kleunen et al. 2010, Heberling and Fridley 2013). Given the importance of generalist herbivores to forest dynamics (Schowalter, Hargrove and Crossley 1986, Haack and Byler 1993, Ayres and Lombardero 2000), improved understanding of the relationship of non-native species and their generalist herbivores is a priority in plant invasion studies.

The ERH predicts that invasive species evade specialist herbivores, but generalist herbivores also influence plant defense allocation in the invaded range. A corollary to ERH that incorporates generalist herbivory is the shifting defense hypothesis (SDH, Müller-Schärer, Shafner, and Steinger 2004, Joshi and Vrieling 2005). SDH states that invasive plant species in their invaded range may shift allocation away from quantitative defenses (i.e. digestion-reducing compounds), which affect both specialists and generalists, in favor of qualitative defenses (i.e. toxins), which are typically less effective against specialists. Qualitative defenses, including alkaloids and phenolic glycosides, typically deter generalists but do not restrict inherent leaf growth rate like lignin (Müller-Schärer, Shafner, and Steinger 2004). The overall reduction in defense investment may give non-native species a competitive edge via increased relative allocation to growth pools (Blossey and Notzold 1995). Indeed, some successful invasive species increase the production of toxins in the invaded range (Joshi and Vrieling 2005, Doorduin and Vrieling 2011), however, Doorduin and Vrieling (2011) found no evidence of reductions in quantitative defenses in the invaded ranges of a variety of invasive forb species. Additionally, few studies evaluate the extent to which SDH influences invader success in other plant growth forms, including long-lived woody species.

Woody invaders in ENA deciduous forests are generally more productive than co-occurring native species, but it is unknown how plant growth traits interact with defense investment. Invaders have greater leaf and fine root production than natives and use leaf resources more efficiently in photosynthesis (Heberling and Fridley 2013, Jo et al. 2015, Heberling and Fridley 2016). Invaders also maintain their leaves for longer duration, affording them higher annual carbon gain (Fridley 2012). High allocation to resource-acquisitive traits in invaders might result from decreased relative investment in physical and chemical defenses (Züst and Agrawal 2017)—a potential outcome of SDH. Slower growing native species, in contrast, may produce more hard-to-digest compounds, including lignin and cellulose, and toxic chemicals, like alkaloids, phenolics, and glycosides to combat herbivory (Bazzaz et al. 1987, Coley 1988, Dudt and Shure 1994, Fraser and Grime 1999, Fine et al. 2006, Endara and Coley

2011, Züst and Agrawal 2017). Indeed, some temperate woody invasive species experience decreased herbivore loads compared to native species (Cappuccino and Carpenter 2005, Cincotta et al. 2009, Lamarque et al. 2011, Lieurance and Cipollini 2012, 2013). However, such studies are limited to prominent ENA invaders and do not associate damage with particular defense traits, making it unclear whether invaders often escape or resist damage. Alternatively, invaders and natives may maintain similar relative investment to productivity and defenses, but the greater annual carbon gain in invaders leads to greater productivity with no decrease in defense against herbivores. These defenses, in turn, may represent novel weapons to which generalist herbivores are not adapted (Callaway and Ridenour 2004). The connection between defense traits and herbivory remains unknown for a majority of invasive shrubs and trees, particularly those of temperate deciduous forests.

Resource limitation in the shaded forest understory is likely to impose tradeoffs in energy investment between defenses and other energy pools important for persistence, such as growth and storage. Whereas growth toward the canopy increases access to light, storage in the form of non-structural carbohydrates (NSCs) enables recovery following damage and protection from freezing (Dietze et al. 2014). Reduced herbivory may prompt invaders to prioritize leaf production over physical or chemical defenses and NSC storage. Native species, on the other hand, may invest more in defense to resist both specialist and generalist herbivores and/or store NSCs to more effectively tolerate damage (Carmona and Fornoni 2013). In the absence of enemy release, however, invader strategies likely mirror natives, with high investment in defenses leading to low attack rates or high investment in storage leading to high regrowth capacity following attack. Studies on native and invasive species responses to herbivory in temperate North American forests report increased compensatory growth (e.g. greater leaf production after defoliation) in invaders (Schierenbeck et al. 1994, Rogers and Siemann 2005, Ashton and Lerdau 2008, Huang et al. 2010). Hinman and Fridley (2018) found that woody invaders in ENA have greater root starch concentrations than natives during the growing season, which might support regrowth following defoliation. However, other resource sinks, including reproduction and microbial mutualisms, may mediate constraints between defense, growth, and storage (Chapin et al. 1990). Identifying allocation constraints in understory woody species will improve our understanding of native and invader responses to disturbance.

We conducted an experimental study of leaf herbivory in phylogenetically paired native and invasive woody plant species of ENA deciduous forests to test whether established differences in productivity and resource economy are associated with defense investment and herbivore performance and the extent to which growth and defense traits reflect energetic tradeoffs at the whole-plant level. Specifically, our objectives were to (1) compare common leaf trait indicators of physical and chemical defenses across 20 species of phylogenetically paired native and invasive shrubs co-occurring in a common garden, (2) determine the association of herbivore growth rates and leaf traits commonly associated with defense in native and invasive woody shrubs using two generalist insect herbivores in feeding trials, and (3) evaluate how putative defensive allocation relates to leaf production and NSC storage allocation in this set of species. Because invaders tend to be more productive than natives, we hypothesized that invaders would invest less in defenses. Reduced defense investment would make invaders more palatable to generalist herbivores if leaves are low in both qualitative and quantitative defenses. We predicted negative associations between herbivore growth rates and defense-related leaf traits. We also hypothesized that resource limitation leads to constraints between productivity, defense, and storage in understory species. Annual carbon gain differs widely between species

(Fridley 2012) and likely affects a plant's absolute capacity for growth, storage, and defense investment. Thus, we expected to find evidence of tradeoffs particularly in species with similar levels of carbon gain.

Methods

Focal species

We selected 20 species of native and non-native woody plants belonging to six families (Table 1). Each plant family was represented by at least one native/non-native pair. All non-native species included in the study are considered 'invasive' in the United States, with the exception of *Euonymus bungeanus*, which is classified as 'naturalized' (Fridley 2008). Mature plant individuals used for trait and palatability assays co-occur in a common garden at Syracuse University (Syracuse, New York, USA; 43° 03' N, 76° 09' W) with the exception of *Acer* species. All plants were of fruiting and flowering age for the duration of data collection. The garden was created in 2006 and consists of three replicate blocks. Blocks were covered with a shade cloth allowing 20% light transmittance June through November to simulate an overstory canopy. Plants within the garden were wild collected from the Syracuse area or obtained from a commercial nursery located at a similar latitude. *Acer* leaf samples were collected from trees in a secondary forest ca. 4 km from the garden (Heath Park, Syracuse, NY; 42° 59' N, 76° 08' W). For more information on planting and garden culture see Fridley (2012), Fridley and Craddock (2015), and Martinez and Fridley (2018). No effort was made to control background herbivory in the garden. In May 2016 a subset of *Euonymus* species in the common garden experienced high levels of insect defoliation, but afflicted species were excluded from the study.

Herbivore feeding trials

We measured leaf palatability of focal species to two generalist caterpillars: the European strain of gypsy moth (*Lymantria dispar L.*), an invasive species resident in ENA for 150 years, and the native fall webworm (*Hyphantria cunea*), a species that has successfully invaded both Europe and Asia. We selected these herbivore species because they are among the most generalist woody plant feeders in the region and are geographically widespread in the northeastern U.S. Larvae emerge at different points in the growing season, with gypsy moth hatching in spring (early May) and fall webworm emerging in early fall (August) at this latitude.

In June 2016, fourth-instar gypsy moth larvae were weighed individually and then placed in 150 mm diameter Petri dishes with ca. 2 g of fresh leaves, including petioles, of each species. Gypsy moth larvae originated from eggs provided by the USDA-APHIS (New Jersey Standard Strain). After hatching, larvae were reared at 23°C (16:8 L:D) in an environmental chamber on standard gypsy moth artificial diet (Frontier Agricultural Services, Newark, DE) in groups of 20 in 118 ml wax paper cups. Newly molted, unfed 4th instars were randomly allocated to treatments. We included 20 gypsy moth replicates per plant species. Branches were collected the previous day from 1-3 plant individuals and cut underwater for overnight storage. Leaf turgor was maintained in Petri dishes by inserting cut petioles or stems in plastic tubes or wrapped in moistened paper towels for species with short petioles. Bundles of petri dishes were wrapped in clear plastic to prevent desiccation and placed in environmental chambers at 23°C and 16:8 (L:D). A pan of water was placed on the bottom shelf of each chamber to maintain humidity. All larvae were allowed to feed for 72 hours. Larvae were then removed, placed individually in microcentrifuge tubes and frozen overnight. Microcentrifuge tubes were uncapped, placed in racks, and held at 60 °C in a drying oven for three days. Larvae were then weighed to the nearest

0.01 mg. Remaining leaf mass in each replicate was dried in a 70°C drying oven for three days and then re-weighed to obtain final leaf dry mass. A subset (n=35) of gypsy moth larva not used in the experiment was used to develop the regression equation $y=0.1077x+1.4462$ ($R^2=0.72$), for estimating the initial dry mass (y) from the initial wet weights (x) of experimental caterpillars. All growth measurements were calculated using dry mass. We calculated relative growth rate (RGR) using the following equation:

$$\mathrm{RGR} = [\ln(M_{\mathrm{final}})-\ln(M_{\mathrm{initial}})]\ \mathrm{time}^{-1}\ (\mathrm{mg\ mg^{-1}\ day^{-1}})$$

We estimated leaf mass consumed per day with regression equations developed using five randomly selected leaves set aside during preparation of the herbivore feeding trial. Leaves were taken from the same branches collected for the feeding trials. Individual leaves were weighed fresh before being placed in a 70°C drying oven for three days. Leaves were re-weighed following drying. We then developed regression equations for each plant species using wet weight and dry weight values. Regression equations were used to calculate the initial dry weight of the leaves used in the feeding trials. We subtracted the final dry weight from the initial dry weight and divided by the length of the experiment in days. Several feeding trial replicates (generally restricted to specific species) appeared to gain mass over the course of the experiment, likely due to herbivore preference for leaf mesophyll and avoidance of vascular tissue. For this reason, leaf mass removed data were only used to verify nativity patterns in insect performance.

The experiment was repeated later in the growing season (August 2016) with fall webworm. In this assay, fourth-instar larvae were collected from wild populations near Oneida Lake, NY. Colonial webs were collected from roadside black cherry (*Prunus serotina*) trees.

Larvae were removed from their webs, combined to ensure genetic homogeneity, and placed in 19 L plastic buckets with black cherry foliage until use in the experiment. We included 10 replicates per plant species with the exception of *Elaeagnus commutata*, which died back during the summer and could not be used in the fall feeding trial. Because fall webworms are colonial, each replicate received four randomly selected larvae and growth rate measurements were conducted on the mean of the group rather than the individual larva. Plants were treated the same as in the gypsy moth assay. A regression equation, y=0.1383x+0.434 (R^2=0.97), where x=initial wet mass and y, the initial dry mass (Post and Parry 2011) was used to estimate the initial dry weight (y) of caterpillars to determine mass change on a dry weight basis over the course of the experiment. We calculated RGR and leaf mass removed in the same fashion as in the gypsy moth assay.

Leaf and whole plant trait measurements

Leaf dry matter content (LDMC) was determined during the herbivore feeding trials using the leaves reserved for wet weight-dry weight regressions. We obtained summer-fall means of leaf nitrogen concentration (hereafter ‘leaf N’) in the same plant individuals (excluding *Acer* species, *Euonymus bungeanus*, and *Lonicera periclymenum*) from Martinez and Fridley (2018), using an elemental CN analyzer (NC 2100, Thermo Quest CE Instruments, Milan, Italy). We determined leaf total phenolic content on a wet weight basis using leaves collected in August 2016. Briefly, we pulverized fresh leaf disks in methanol, and treated the extract with sodium carbonate and Folin-Ciocalteu reagent to break down phenolic compounds into gallic acid equivalents (Ainsworth and Gillespie 2007). Gallic acid equivalents were estimated using a standard curve measured at 765 nm in a microplate reader and then converted to total phenolics (%) by fresh leaf weight.

We measured leaf cellulose and lignin + mineral concentrations using a chemical digestion process on dried leaf material used to determine LDMC. Leaves of each species were ground to a homogenized powder to fit through a 40-mesh screen (Thomas Scientific Mini Wiley Mill, Swedesboro, NJ, USA). We then added 0.5 g of ground leaf material from each species to three replicate ANKOM filter bags per season (ANKOM Technology, Macedon, NY). Bags were soaked in a neutral detergent solution (3% sodium lauryl sulfate, 2% EDTA, 1% triethylene glycol) to remove soluble compounds, followed by an acid detergent solution (3% sulfuric acid) to remove hemicellulose and cell membrane-bound proteins. A 72% sulfuric acid solution was used to release cellulose from the samples, leaving behind lignin and minerals. Following each digestion, the bags were dried and weighed to determine the proportion leaf mass released. Summer and fall cellulose and lignin + mineral concentrations did not significantly differ, so mean seasonal concentrations were used in statistical analyses.

For the constraints analysis, species-level leaf production measurements from 2008-2010 were obtained by multiplying the maximum number of leaves produced during each growing season on 5 random stems multiplied by the average leaf mass for three common garden individuals per species (Fridley 2012). Average annual carbon gain was calculated by Fridley (2012), using photosynthesis and leaf extension data collected monthly from 2008-2010; see therein for modeling methods. Leaf production and carbon gain measurements were averaged across the three years of data. We obtained mean total NSC concentrations (summed soluble sugars and starch) from Hinman and Fridley (2018). Briefly, total NSCs were measured in stems and roots sampled from common garden individuals following leaf out in spring 2014 using a combination of near-infrared spectrometry and chemical extractions. Stem and root NSCs were summed to create one NSC value per individual, and mean NSC concentrations per species were calculated. *Acer* spp. and *L. periclymenum* were missing NSC concentrations and were not included in the constraints analysis.

Statistical analyses

We used the mean values for plant species (n=20) leaf traits, resource allocation traits, and caterpillar RGRs in all analyses. We tested for native-nonnative (hereafter ‘nativity’) differences in average LDMC, leaf N, phenolics, cellulose and lignin + minerals concentrations, gypsy moth and fall webworm relative growth rates (RGRs), and leaf mass loss using linear mixed effect models with a family random effect to account for phylogenetic autocorrelation. Analyses were performed using the “nlme” package (Pinheiro et al. 2018) in R 3.3.2 (R Core Team 2016). We then created hierarchical Bayesian (HB) regression models (Fridley and Craddock 2015) to test the effects of LDMC, leaf N, lignin + minerals, and cellulose concentrations on gypsy moth and fall webworm RGRs using the R2jags package (Su and Yajima 2015). We chose the HB modeling framework because it is capable of approximating missing data in predictor variables by drawing values from a normal distribution composed from existing data whose variance is estimated by the model (Gelman and Hill 2007). We estimated six missing leaf N values using this framework. We found significant correlations between total phenolics and cellulose, and total phenolics and LDMC. As a result, we removed total phenolics from the mixed model to prevent issues related to collinearity among independent variables. The mixed model had the following structure:

$RGR \sim LDMC + \text{leaf N} + \text{lignin and minerals} + \text{cellulose} + (1|\text{family})$

We standardized continuous predictor variables by subtracting the mean and dividing by the standard deviation (Z score) to estimate relative effect sizes. For each larval species model, we ran three Markov chain Monte Carlo (MCMC) chains for 500,000 iterations after 50,000 burn-in. All models converged with the convergence diagnostic Rhat < 1.003 (Gelman and Rubin 1992).

We tested for tradeoffs between defense traits, leaf production, and NSC storage in a constraints analysis by comparing plant relative investment in any combination of two traits (storage/production, defense/storage, or defense/production) in all possible two-species comparisons. Leaf production values had a skewed distribution and were log-transformed. To obtain one overall defense value for each species, defense-related traits (LDMC, leaf N, phenolics, cellulose, lignin + minerals, gypsy moth growth, and fall webworm growth) were ranked 1 (associated with low levels of defense) to 5 (high levels of defense) based on 20% quartiles. Rankings for all traits were summed to determine each species' composite defense score. High concentrations of total phenolics, cellulose, lignin + minerals, and LDMC were classified as high levels of defense, while high leaf N (Mattson 1980), gypsy moth growth, and fall webworm growth were classified as low levels of defense. Three missing values were given a 3, adding no information to the composite metric. To account for differences in the total carbon budget across species, we ran a permutation test that included annual carbon gain as a covariate in the following way. First, we examined the relationship of all pairwise contrasts of two traits (e.g., defense and leaf production) in relation to differences in carbon gain, expecting that greater similarity in carbon gain increases the negative trait association (tradeoff). We then compared the observed pairwise regression to a permutation model in which defense, production, and storage values were randomly permuted (without replacement) 1000 times. Evidence for a non-random tradeoff in species of similar carbon budgets is associated with an observed slope outside the 2.5 and 97.5 quantiles of the permuted slope distribution.

Results

Invaders had marginally greater leaf N (t = -2.15, df = 8, P = 0.06) and more lignin + minerals (t = -3.43, df = 13, P = 0.005) than native species (Fig. 1). However, we found no effect of nativity on cellulose concentrations, total phenolics, or LDMC. Species from the same families tended to show similar defense-related trait values (Fig. 2). Phenolic concentrations were highly variable among species. While *Lonicera japonica* on average had less than 5% total phenolics in fresh leaves, *Acer* species had concentrations of 20-30% of fresh leaf weight and low lignin + mineral content (15% dry leaf mass). We also found a wide range of lignin + mineral concentrations: *Elaeagnus commutata* leaves were less than 10% lignin + minerals by dry mass, while *Euonymus alatus* leaves were nearly 30% lignin + minerals. Both *Frangula* species had high leaf N (over 3% by dry mass).

We found no significant effects of leaf chemical or structural traits on gypsy moth RGR (Fig. 2). However, leaf N had a marginally significant negative effect on fall webworm relative growth rate (posterior mean = -0.040, 95% credible interval = [-0.099, 0.029]). Herbivores showed unique growth patterns across species and families (Fig. 3), but neither gypsy moth nor fall webworm growth rates were related to nativity (Fig. 1). Leaf mass consumed supported these results and was not related to plant nativity in either of the caterpillar feeding assays (results not shown). Both herbivores, on average, had positive growth rates when feeding on the invaders

Acer platanoides and *Viburnum lantana* (Fig. 3), despite high phenolics in *A. platanoides* and high cellulose, lignin, and minerals coupled with low leaf N in *V. lantana.* Invasive gypsy moth and native fall webworm growth was highest on a native shrub and invasive shrub species, respectively: gypsy moth on *Elaeagnus commutata* (0.128 mg mg^{-1} day^{-1}) and fall webworm on *Viburnum lantana* (0.129 mg mg^{-1} day^{-1}). However, there was considerable variation in growth across replicates. *Lonicera* species ranked low in phenolics and consistently were in the mid-range for cellulose, lignin, and LDMC, and yet were unpalatable to gypsy moth but highly palatable to fall webworm. One exception was *L. japonica*, which had the lowest phenolic concentrations (2.06% by fresh mass) and was not eaten by gypsy moth or fall webworm. The invader *Euonymus alatus* was highly palatable only to fall webworm (larvae completely skeletonized leaves), despite having the highest lignin and mineral concentrations in our focal species (28.8% by dry mass). Overall, fall webworm had positive growth rates on a wider range of woody species than gypsy moth, including species in the Sapindaceae, Celastraceae, Caprifolaceae, and Adoxaceae. Neither gypsy moth nor fall webworm grew on species in the Rhamnaceae.

The constraints analysis did not reveal any tradeoffs between leaf production, the defense trait composite variable, and non-structural carbohydrate concentrations after accounting for species differences in carbon gain, and there were no negative relationships between the three variables (Fig. 4). Regression slopes estimated from pairwise contrasts of two traits in relation to carbon gain did not fall outside the 2.5 and 97.5 quantiles of the permuted distribution for any combination of traits (Fig. S1).

Discussion

We examined leaf palatability and defense traits in a set of 20 congeneric native and invasive woody species to determine the extent to which generalist insect herbivory contributes to invader advantage. Although native and invasive plants differed in defense-related leaf traits, the performance of both herbivore species did not vary with nativity. If these patterns are representative of generalist insect folivores in ENA deciduous forests, our findings do not support the hypothesis that non-native shrubs experience different rates of generalist herbivory than related native species. Instead, we found that invaders on average had higher leaf lignin concentrations than natives, a result contrary to the hypothesis that invaders lacking specialist herbivores should invest in qualitative toxins to deter generalists (Müller-Schärer, Shafner, and Steinger 2004). Because most insects cannot digest lignin, it is often thought to deter both specialist and generalist herbivores in a dose-dependent manner (Rhoades 1985, Coley 1988), but we found no such relationship for gypsy moth and fall webworm. Alternatively, invaders may invest in greater leaf structural integrity primarily to prevent pathogen attack, a potential threat to late-season carbon gain observed in many invaders. High lignin content may allow invaders to protect higher leaf N concentrations required for increased productivity. However, the negative association between fall webworm growth and leaf N may indicate the presence of unmeasured N-rich defensive compounds or cell wall proteins that have also been shown to contribute to defense (Feng et al. 2009). Because many invaders assimilate carbon for nearly a month longer than natives (Fridley 2012), greater investment in leaf defenses may be critical to their resource use strategy.

Why did the growth rates of two generalist herbivores respond more to a plant's phylogenetic background than its native/non-native status? One possible explanation is the recent coevolution of insects and non-native plants in the invaded range (Schultheis et al. 2015), as all non-native species in our study were introduced before 1900 (and some were introduced as early as the 17th century; Mack 2003). Further, the European gypsy moth has been in ENA since 1869 (Elkinton and Liebhold 1990) and has presumably coevolved with European invaders (e.g., *Acer platanoides, Viburnum lantana*) in their native range for millennia. Additionally, since native congeners exist in the forest understory, generalists may not perceive differences between closely related native and invasive species if they employ phylogenetically constrained suites of secondary defensive compounds (Dostál et al. 2013, Morrison and Mauck 2007, Wein et al. 2016).

Large discrepancies in gypsy moth and fall webworm growth rates demonstrate that generalists, even within the same feeding guild, are unique in their diet specificity. We did not measure alkaloid or terpene concentrations, which may affect gypsy moth (Barbosa and Krischik 1987) and fall webworm growth rates. However, the chemical basis for gypsy moth (and likely fall webworm) host preference is equivocal (Elkinton and Liebhold 1990) and generalist performance often correlates with other attributes such as leaf morphology, trichome density, and cuticle thickness (Agrawal and Weber 2015). Interestingly, *Viburnum lantana,* a shrub eaten by both caterpillar species, has a high density of leaf trichomes. However, trichomes may primarily deter smaller insect herbivores or earlier larval instars. Additionally, we did not measure inducible defenses, which could have played a role in caterpillar growth differences between plant species (Karban and Myers 1989).

We detected no constraints between leaf production, defense traits, and carbohydrate storage in ENA understory woody plants when controlling for species' differences in annual carbon gain. Despite the expectation that growth-survival tradeoffs are common in resource-limited environments (Huot et al. 2014, Züst and Agrawal 2017), energy allocation patterns are complex in long-lived plants, and our analysis did not include several potentially large pools of carbon. Reproductive allocation, respiration costs, other types of growth (fine roots, cambial expansion, structural growth) and defenses may mediate growth-survival relationships. Furthermore, forest fragmentation and eutrophication, which increase light and nutrient levels, may alleviate constraints between growth, defense, and storage.

Like many controlled palatability studies, our conclusions rely on several assumptions. First, though gypsy moth and fall webworm are common generalist insect folivores in ENA forests, there are a multitude of other generalist herbivores that have significant effects on forest structure. White-tailed deer, in particular, affect tree recruitment (Tilghman 1989, Rossell, Gorsira, and Patch 2005) and are responsible for reducing native species abundance through selective browsing in the understory (Knight et al. 2009). In the insect folivore group alone, leaf miners, gall makers, and leaf chewers have widely different feeding preferences (Agrawal et al. 2005, Kirichenko et al. 2013)—escape from one does not predict escape from other generalist insect herbivores. Although gypsy moth and fall webworm both belong to the leaf chewing guild, they originated on different continents and emerge early and late in the growing season, respectively—differences that likely contribute to divergent performance on this set of woody species. Given seasonal changes in leaf chemistry and structure, from gypsy moth emergence (spring, early summer) to fall webworm emergence (late summer, early fall), associated changes in leaf toughness, water content, leaf N, and secondary chemical composition influence some of the palatability differences between the herbivores. Furthermore, differences in the specific types and concentrations of phenolic and N-containing compounds could have influenced leaf palatability to gypsy moth and fall webworm in this set of species. Nonetheless, our results challenge the hypothesis that commonly measured chemical and physical defense traits reduce the performance of abundant generalist insect herbivores.

Conclusion

We found that native and invasive shrubs in ENA forests have different leaf chemical compositions potentially related to pests and pathogens, but such differences did not translate to the growth rates of two generalist insect herbivores. Although gypsy moth and fall webworm are common defoliators in these forests, they had divergent growth patterns that were not explained by any of the defense traits measured. Our results also suggest that plants produce defenses that may fail to deter some enemies, but we did not find evidence that defensive traits come at the expense of growth or storage. Future investigations of carbon and nutrient allocation in native-invader contrasts that include plant investments in reproduction and belowground function would be a valuable next step in quantifying the impacts of herbivory in the forest understory.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Figure Captions

Fig. 1 Native-invasive comparisons of (a) gypsy moth RGR, (b) fall webworm RGR, (c) leaf N, (d) lignin + minerals, (e) cellulose, (f) LDMC, and (g) phenolics.

Fig. 2 Leaf defense traits in relation to larval relative growth rates (RGR): (a) leaf N, (b) lignin + minerals (c) cellulose, (d) LDMC. Each point represents a species' mean for either gypsy moth (circles) or fall webworm (triangles), with whiskers indicating standard error. White circles represent gypsy moth growth on native species, gray circles represent gypsy moth growth on invaders, white triangles represent fall webworm growth on natives, and gray triangles represent fall webworm growth on invaders. Dotted lines denote zero growth.

Fig. 3 Boxplot comparisons of (a) gypsy moth and (b) fall webworm RGR on native and invasive species.

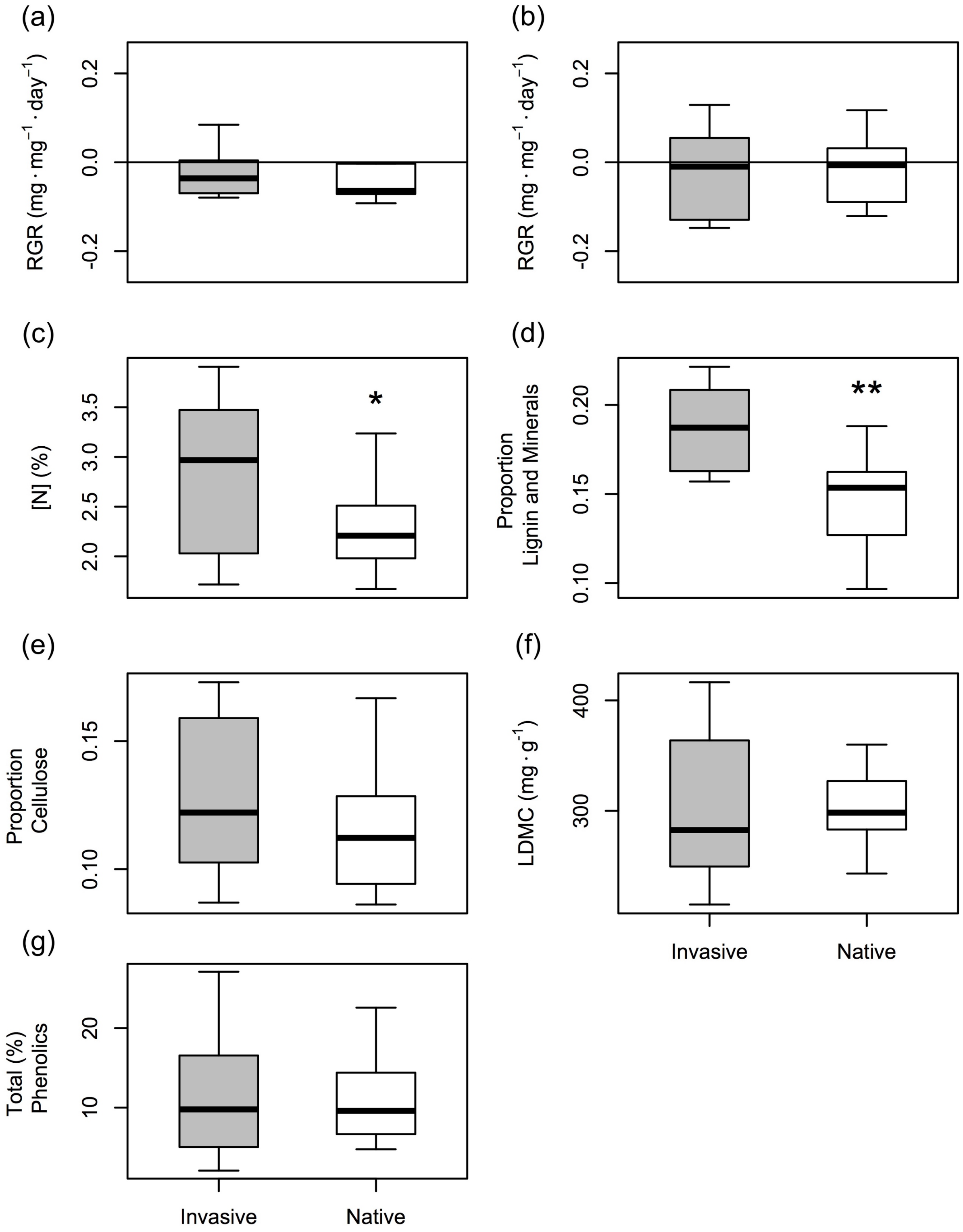
Fig. 4 Relationships of species leaf production, % total nonstructural carbohydrates (spring root + stem concentrations), and composite defense score in natives (white) and invaders (gray). Each point is scaled relative to a species' mean seasonal carbon gain. *Acer spp.* did not have %TNC or leaf production measurements, so they were not included in this analysis.

Table 1 Focal species and their abbreviations, including current status from USDA NRCS (2018).

Family	**Species**	**Species Code**	**Status**
Sapindaceae	*Acer campestre*	ACCA	Invasive
	Acer negundo	ACNE	Native
	Acer platanoides	ACPL	Invasive
	Acer saccharum	ACSA	Native
Elaeagnaceae	*Elaeagnus umbellata*	ELUM	Invasive
	Elaeagnus commutata	ELCO	Native
Celastraceae	*Euonymus alatus*	EUAL	Invasive
	Euonymus atropurpureus	EUAT	Native
	Euonymus bungeanus	EUBU	Naturalized
	Euonymus obovatus	EUOB	Native
Rhamnaceae	*Frangula alnus*	FRAL	Invasive
	Frangula caroliniana	FRCA	Native
	Rhamnus cathartica	RHCA	Invasive
	Rhamnus alnifolia	RHAL	Native
Caprifolaceae	*Lonicera japonica*	LOJA	Invasive
	Lonicera hirsuta	LOHI	Native
	Lonicera periclymenum	LOPE	Invasive
	Lonicera sempervirens	LOSE	Native

Adoxaceae	*Viburnum lantana*	VILA	Invasive
	Viburnum prunifolium	VIPR	Native

Figure 1



(a)
RGR (mg · mg^{-1} · day^{-1})
0.2
0.0
-0.2
(b)
RGR (mg · mg^{-1} · day^{-1})
0.2
0.0
-0.2
(c)
[N] (%)
3.5
3.0
2.5
2.0
*
(d)
Proportion
Lignin and Minerals
0.20
0.15
0.10
**
(e)
Proportion
Cellulose
0.15
0.10
(f)
LDMC (mg · g^{-1})
400
300
Invasive
Native
(g)
Total (%)
Phenolics
20
10
Invasive
Native

Figure 2

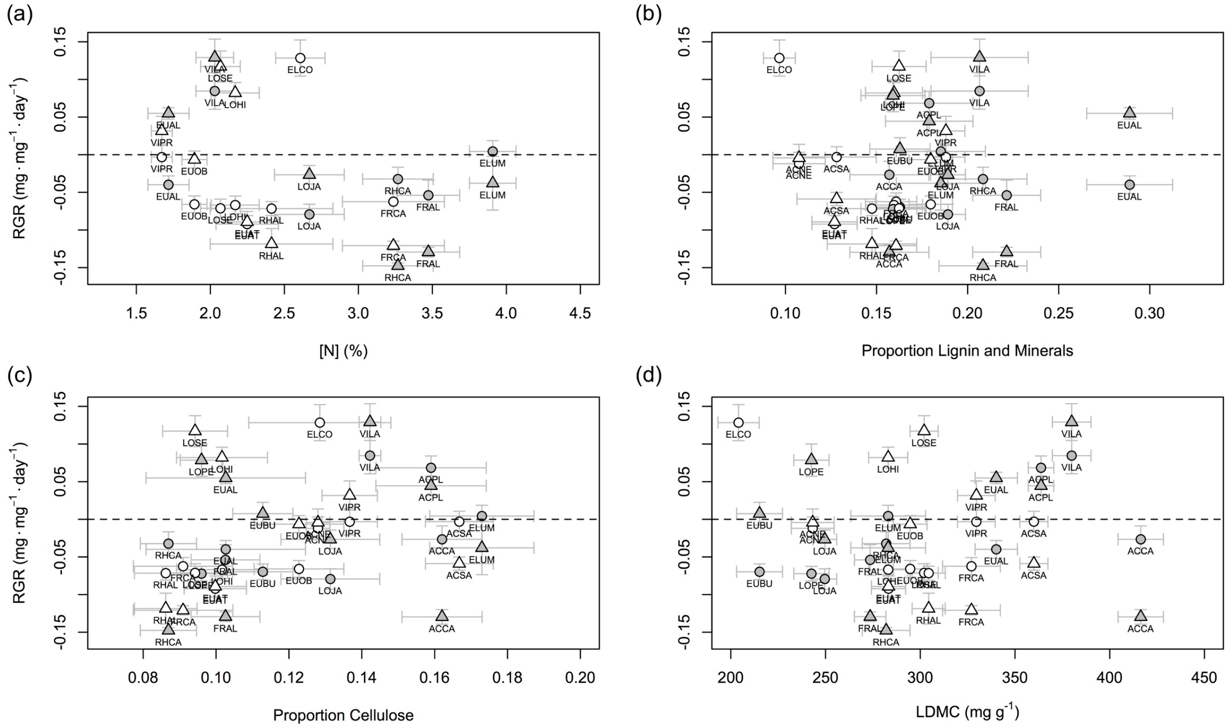

(a)
(b)
(c)
(d)
RGR (mg · mg^{-1} · day^{-1})
0.15
0.05
-0.05
-0.15
[N] (%)
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Proportion Lignin and Minerals
0.10
0.15
0.20
0.25
0.30
Proportion Cellulose
0.08
0.10
0.12
0.14
0.16
0.18
0.20
LDMC (mg g^{-1})
200
250
300
350
400
450
ELCO
VILA
LOSE
LOHI
LOPE
EUAL
VIPR
EUOB
EUBU
ELUM
LOJA
RHCA
FRCA
FRAL
RHAL
EUAT
ACPL
ACNE
ACSA
ACCA

Figure 3

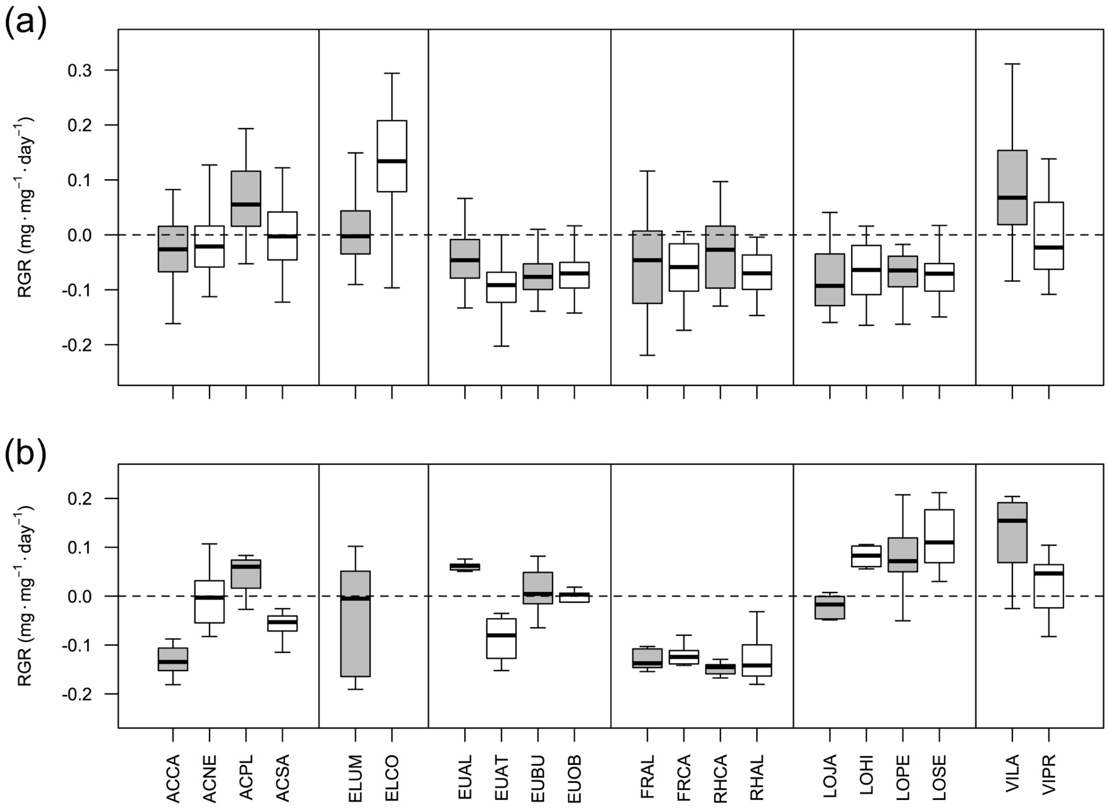
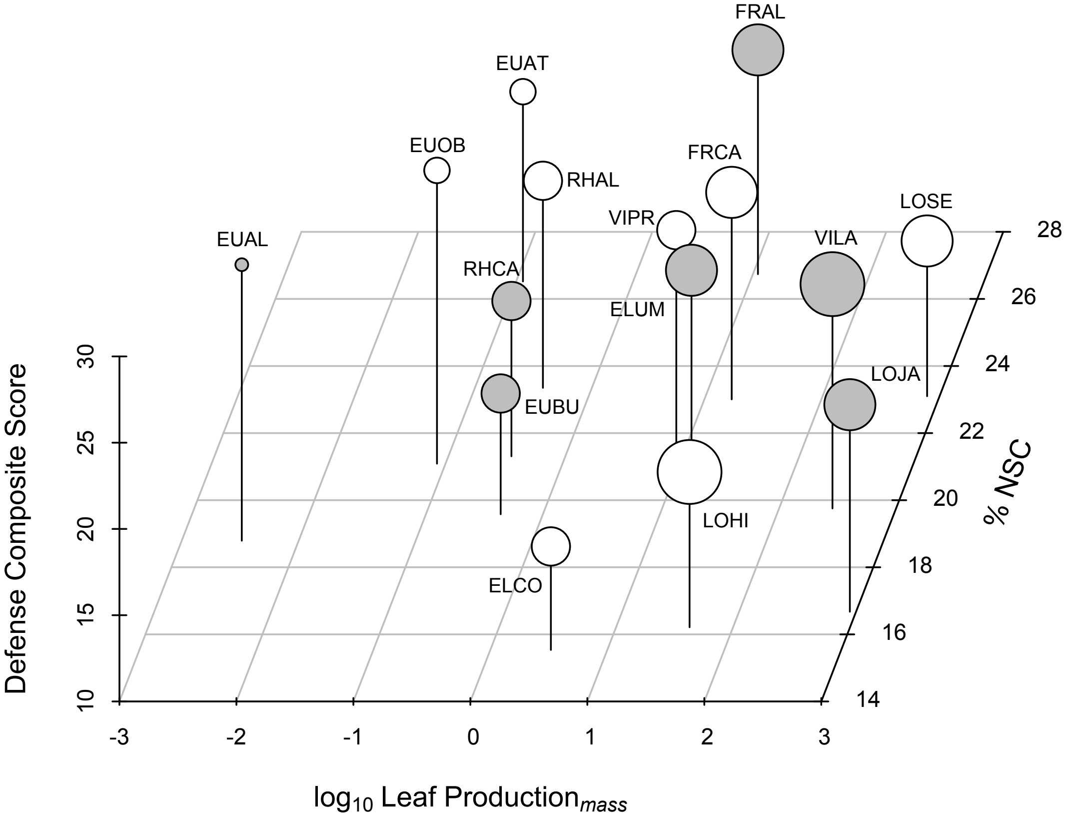

(a)
(b)
RGR (mg · mg^{-1} · day^{-1})
0.3
0.2
0.1
0.0
-0.1
-0.2
ACCA
ACNE
ACPL
ACSA
ELUM
ELCO
EUAL
EUAT
EUBU
EUOB
FRAL
FRCA
RHCA
RHAL
LOJA
LOHI
LOPE
LOSE
VILA
VIPR

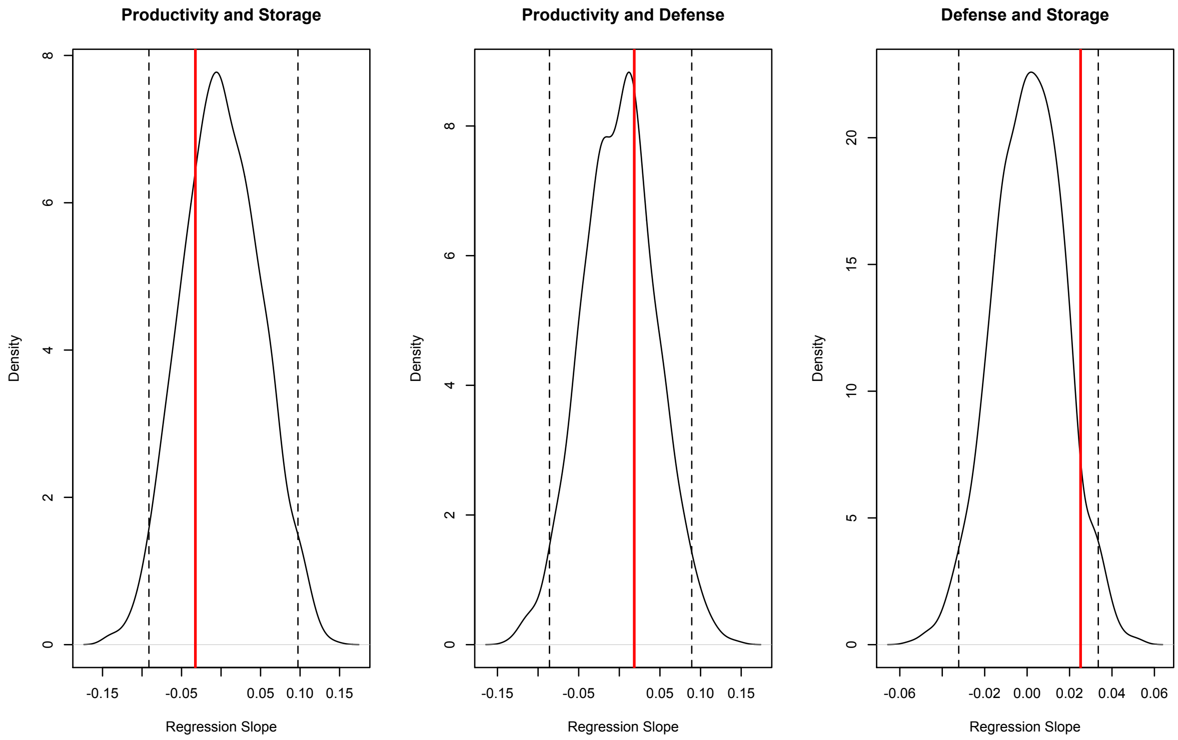
Figure 4



FRAL
EUAT
EUOB
RHAL
FRCA
VIPR
LOSE
EUAL
VILA
RHCA
ELUM
LOJA
EUBU
LOHI
ELCO
Defense Composite Score
10
15
20
25
30
-3
-2
-1
0
1
2
3
log10 Leaf Production_mass
% NSC
14
16
18
20
22
24
26
28

Supplemental Information

Figure S1: Pairwise regressions and distributions formed by permutation models in which defense, production, and storage values were randomly permuted (without replacement) 1000 times. Evidence for a non-random tradeoff in species of similar carbon budgets is associated with an observed slope outside the 2.5 and 97.5 quantiles of the permuted slope distribution.



CHAPTER 4: Impacts of experimental defoliation on native and invasive woody plants in deciduous forests: are native species more resilient to canopy disturbance?

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Abstract

Non-native, invasive woody species in mesic forests of North America are both fast-growing and shade tolerant, but their ability to tolerate disturbances remains unclear. In particular, partial or full defoliation associated with herbivory and extreme weather events may have larger impacts on invasive species if natives maintain greater carbon stores to support rapid regrowth. On the other hand, invasive species may be more resilient to defoliation via upregulation of photosynthesis in existing or regrown leaves. We measured growth, non-structural carbohydrate concentrations (NSCs), survival, and chlorophyll content in response to varying levels of defoliation in saplings of two native and two non-native, invasive species that commonly co-occur in eastern deciduous forests. Individuals were subjected to one of four leaf removal treatments of increasing intensity: no defoliation controls, 50% defoliation over three growing seasons, 100% defoliation over one growing season, and 100% defoliation over two growing seasons. In contrast to our hypothesis, native and invasive species did not differ in defoliation responses, although invasive species experienced more pronounced decreases in leaf chlorophyll following full defoliation. Across species, radial growth progressively decreased with increasing defoliation intensity, and refoliation mass was largely a function of sapling size. Survival rates for half-defoliated saplings did not differ from controls (90% of saplings survived), but survival in fully defoliated individuals over one and two growing seasons was reduced to 45% and 15%, respectively. Surviving defoliated saplings generally maintained control NSC concentrations. Chlorophyll indices in defoliation treatments did not suggest photosynthetic upregulation; instead, fully defoliated saplings showed decreased chlorophyll levels compared to half-defoliated and control individuals. Our results indicate that native and invasive species respond similarly to defoliation, despite the generally faster growth strategy of invaders.

Introduction

Disturbance is a commonly invoked mechanism of plant invasion, freeing up resources that non-native species usurp from native species through rapid growth and reproduction (Davis et al. 2000). However, it is unclear how disturbance affects invasive species following establishment—given their success following disturbance, are invasive species also more resilient to defoliation than co-occurring native species? In Eastern North America (ENA), many shade tolerant, non-native invasive woody plants have spread through forests for over a century (Mack 2003, Fridley 2008). Despite studies commonly highlighting productivity differences between ENA native and invasive woody species (Stewart and Graves 2006, Herron et al. 2007, Lapointe and Brisson 2012, Paquette et al. 2012), few have evaluated differences in survival following defoliation (Gleason and Ares 2004, Vanderklein et al. 2015). Enemy release studies suggest that natives and invaders experience similar attack rates (Morrison and Mauck 2007, Knapp et al. 2008, Hinman, Fridley, and Parry *in prep,* but see Cincotta et al. 2009, Eschtruth and Battles 2009), and extreme events like late- or early-season frosts often cause widespread leaf damage regardless of species' defense investment. Because extreme events, like insect outbreaks, are expected to increase in frequency due to climate change (Dale et al. 2001, Weed et al. 2013), determining a species' capacity for survival and growth following varying levels of defoliation is a critical step toward understanding how intensifying damage events will affect forest composition.

Recovery following total defoliation events requires stored energy, and forest understory plants are often energy (carbon) limited. Carbon limitation, in turn, suggests physiological tradeoffs between growth and energy conservation (Chapin et al. 1990). Many ENA invaders exhibit greater overall carbon gain (Fridley 2012), resource use efficiency (Heberling and Fridley

2013, Heberling and Fridley 2016), and productivity (Jo et al. 2014) than natives. Increased productivity may align with increased tolerance to defoliation: indeed, some understory invaders exhibit high compensatory growth in response to herbivory (Schierenbeck et al. 1994, Rogers and Siemann 2005, Ashton and Lerdau 2007). However, despite increased carbon acquisition in invaders, ENA natives tend to maintain higher carbon storage (non-structural carbohydrates: NSCs) concentrations (Hinman and Fridley 2018), which may confer higher survival by reducing the risk of carbon starvation or hydraulic failure following severe defoliation events (Sala et al. 2012).

The frequency and intensity of defoliation often determines woody plant responses to damage. Partial defoliation rarely causes plant mortality and often does not trigger compensatory leaf production in experimental studies (Hoogsteger and Karlsson 1992, Piper and Fajardo 2014); instead, some plants increase the photosynthetic capacity of remaining leaves to recoup lost carbon (Ovaska et al. 1993, Vanderklein and Reich 1999, Pinkard, Eyles, and O'Grady 2011). Under more intense or complete defoliation, NSCs are required for subsequent leaf production (Dietze et al. 2014), often decreasing immediately following leaf removal (Gregory and Wargo 1985, Vanderklein and Reich 1999, Li, Hoch, and Korner 2002, Jacquet et al. 2014). Beyond tolerance responses to severe defoliation, NSCs also maintain hydraulic function and support cellular respiration under stressful conditions (Hartmann and Trumbore 2016) and could be used to survive periods when refoliation is too costly (e.g. at the end of the growing season). Interestingly, some studies report increases in NSC concentrations following defoliation (Wiley et al. 2013), perhaps due to storage prioritization for survival (Wiley and Helliker 2012). The extent to which native and invasive woody plants use NSCs to tolerate defoliation, or whether damage response strategies differ with defoliation intensity, remains unknown.

In this study, we compared the responses of two species of ENA-native saplings (*Acer saccharum* and *Prunus serotina*) and two species of non-native, invasive saplings (*Acer platanoides* and *Rhamnus cathartica*) to experimental defoliation treatments varying in frequency and intensity over three growing seasons. We hypothesized that invaders compensate for leaf loss to a greater degree than natives while maintaining lower carbon stores (Hinman and Fridley 2018), leading to carbon depletion and increased mortality under repeated defoliation. We also hypothesized that saplings of all species experience lower mortality and growth reductions from frequent but low intensity (50% leaf removal) defoliation than infrequent but high intensity (100% leaf removal) defoliation. We predicted that severe defoliation would lead to reduced growth compared to control trees, but that frequent, moderate defoliation may not stimulate NSC use for refoliation, resulting in similar NSC concentrations in control and 50%-defoliated trees. Both defoliation intensities, however, could lead saplings to increase leaf chlorophyll content (a proxy for photosynthetic activity) in response to reductions in leaf area.

Methods

Our study involved controlled defoliation treatments of four species along light gradients at two field sites in central New York, USA, from 2014 to 2016. At each field site, we selected a native/invasive species pair based on species abundance and co-occurrence in the forest understory. In a mid-successional secondary forest in Pompey, NY (42°55′ N, 76°02′ W), we selected native black cherry (*Prunus serotina*) and invasive European buckthorn (*Rhamnus cathartica*) saplings, while in a second growth maple forest in Syracuse, NY (Heath Park; 42° 59’ N, 76° 08’ W) we selected native sugar maple (*Acer saccharum*) and invasive Norway maple (*Acer platanoides*) saplings. Saplings were generally adjacent or nearby other focal species at each site and were under 4 meters in height.

Within each species, we selected 40 saplings occurring along a natural light gradient. We measured the light environment using canopy photos taken with a hemispherical lens. These photos were analyzed using GLA software to calculate a gap light index (GLI) value (0-100%) (Frazer et al. 1999), which is a measure of the total light transmittance as a percent of the total direct light hitting the canopy over the course of the growing season. Native and non-native species within a site occurred along similar light gradients and did not significantly differ in size (analysis not shown).

Saplings were randomly assigned to one of four defoliation treatments: (1) 50% leaf removal for three years, representing low intensity/high frequency, (2) 100% leaf removal in years one and two followed by no defoliation, representing high intensity/high frequency, (3) 100% leaf removal in year two only, representing high intensity/low frequency, and (4) no-defoliation controls. There were ten replicate individuals of each species occurring across the light gradient within each treatment. Defoliation treatments began in July 2014, continued monthly until fall leaf senescence, and were resumed following leaf expansion in July 2015 and July 2016. Leaves were removed at the petiole, taking care to avoid damaging buds, and defoliation treatments were maintained throughout the growing season (100% defoliated individuals were continually defoliated as new leaves expanded). We achieved 50% defoliation by removing every other leaf on each branch (Piper and Fajardo 2014). Half defoliated saplings did not produce new leaves for the rest of the growing season for any species. We stored leaves removed from each defoliation event in paper bags, dried them at 70 °C for 72 hours, and weighed leaves to determine leaf mass removed and refoliated.

At the beginning and end of each growing season, we measured stem diameter at heights of 10, 100, and 160 cm using digital calipers. We averaged the change in diameter across the three years for statistical analyses. Each month during the growing season, we measured leaf chlorophyll values on four mid-canopy leaves per individual with a handheld meter (atLEAF CHL PLUS, FT Green LLC, Wilmington, DE).

In November 2016, at the end of the manipulative experiment, we randomly selected one branch (connected to the main stem) from each sapling to measure non-structural carbohydrate concentrations. Stems were placed in a cooler on ice and taken back to the lab within 4 hours, microwaved at 600W for 90 seconds to halt enzymatic activity (Popp et al. 1996), dried to a constant weight at 70 °C for 72 hours, and ground to fit through a 40-mesh screen (Thomas Scientific Mini Wiley Mill, Swedesboro, NJ, USA). Bark and interior cork were homogenized in these samples. Ground samples were stored at 4 °C until analysis. We collected stem samples from all individuals at the end of the experiment, but some saplings were dead while others had died back to the root crown, leaving no living stems. Because we could not determine the timing of stem mortality in experimental saplings, NSC concentrations in dead tissues at the end of the experiment likely did not reflect stem carbon balance at time of death. Thus, dead tissues were excluded from NSC analyses.

We determined NSC (soluble sugar plus starch) concentrations in living stem samples using near-infrared spectroscopy coupled with colorimetric methods following the methods of Ramirez et al. (2015). All samples were run through a near-infrared spectrometer (Bruker MPA Multi-purpose FT-NIR analyzer, Ettlingen, Germany) and their absorbances measured between 1300 and 2650 nm (Curran 1989). A subset of 26 of the 68 stem samples were selected for NSC analysis using the Kennard-Stone algorithm (Kennard and Stone 1969) on NIR spectra. The

Kennard-Stone algorithm creates a representative subset by first selecting the two most distant samples from one another in parameter space. It selects additional samples based on their maximum distance from the closest sample already included in the subset. We then measured soluble sugar and starch concentrations in the calibration sample set using the colorimetric method of Chow and Landhausser (2004). Soluble sugars were extracted using ethanol and phenol-sulfuric acid and sample absorbance was measured at 490 nm on a microplate reader (BioTek Instruments, Inc, Winooski, VT). Starches were broken down to glucose equivalents using alpha-amylase and amyloglucosidase enzymes, and then treated with PGO color solution + o-dianisidine for spectral analysis at 525 nm. Because soluble sugars and starches represent potentially available energy for survival, we summed the values to calculate total NSC.

NSC concentrations determined colorimetrically were calibrated with near-infrared spectra using partial least squares (PLS) regression. The number of components used in the PLS model was determined by the component axis with the minimum coefficient of variation (Ramirez et al. 2015). This calibration model was used to estimate the soluble sugar and starch concentrations of all samples composed of living tissue in the dataset. We ran 500 iterations of a bootstrapping algorithm that sampled the calibration dataset with replacement and fit the PLS model to our sample dataset using these values. Bootstrapped NSC values were then used to calculate the standard error of each sample estimate.

We created five mixed effect models to evaluate the interactive effects of defoliation treatment and nativity, and light and nativity on radial growth, stem NSC concentrations, mortality, chlorophyll index, and leaf mass refoliated. In the NSC model, only NSC concentrations in living control and 50% defoliated individuals were included because there were not enough 100% defoliated saplings with living stem tissue at the end of the experiment to include in the analysis. The chlorophyll index model contained a fixed effect for light level to control for variation in chlorophyll due to shading (Logan and Krotkov 1969). In the leaf mass refoliated model, we included mainstem diameter as a fixed effect to account for the effects of sapling size on refoliation and leaf mass was log-transformed to increase response variable normality. Each model had random effects for spatial block nested within species, and the chlorophyll index model included a nested random effect of date to account for repeated measurements across months per individual. We calculated Z scores for continuous parameters light and stem diameter to make their effect sizes comparable to categorical variables. Model structures were as follows:

Radial Growth ~ Treatment x Nativity + Light x Nativity, random=1|Species/Block

NSC ~ Treatment x Nativity + Light x Nativity, random=1|Species/Block

Mortality (0,1) ~ Treatment x Nativity + Light x Nativity, random=1|Species/Block

Chlorophyll Index ~ Treatment x Nativity + Light x Nativity, random = 1|Species/Block/Date

Log_{10}Mass Refoliated ~ Treatment x Nativity + Light x Nativity + Stem Diameter, random = 1|Species/Block

We used the “nlme” package (Pinheiro et al. 2018) in R 3.3.2 (R Core Team 2016) to analyze the linear mixed effect models. We used the “lme4” package (Bates et al. 2018) to construct a generalized linear mixed effect model with a binomial distribution to analyze mortality data (0=dead, 1=alive). In the radial growth model, we removed one outlier representing a large control individual growing in high light to improve model fit. In the NSC model, we removed an outlier with a negative NSC concentration. We ensured normality of the residuals and low correlation among the predictors. We then evaluated pairwise relationships in each of the mixed models using Tukey's Honestly Significant Difference tests in the "multcomp" package (Holthorn et al. 2017).

Results

Increasing defoliation intensity reduced radial growth in all species (Fig 1a). Saplings fully defoliated for two years showed the greatest reduction in growth compared to controls (1.48 mm, $t = -7.49$, $df = 136$, $P < 0.001$), followed by those fully defoliated for one year (0.89 mm, $t = -4.53$, $df = 136$, $P < 0.001$). Saplings half defoliated for three years also experienced reduced growth compared to controls (0.63 mm, $t = -3.22$, $df = 136$, $P < 0.01$) but were not affected as severely as saplings subjected to full defoliation. Nativity and initial sapling size had no effect on radial growth, but native species' growth had a marginally significant positive relationship with increasing light level ($t = 1.75$, $df = 136$, $P = 0.08$). We found no interactions between treatment and nativity on growth. Full defoliation had the strongest effects on radial growth in the year following leaf removal (Fig. S1).

Partial leaf removal had no effect on sapling NSCs at the end of the experiment, and NSC concentrations were not related to nativity (Fig 1b). NSCs showed considerable variation within treatments. However, NSCs and radial growth in living saplings were positively correlated (Fig. 2) ($R^2 = 0.15$, $t = 2.85$, $P < 0.001$).

Sapling mortality was closely related to defoliation intensity (Fig. 1c). Mortality rates in saplings experiencing half defoliation over three years did not significantly differ from background (control) mortality, with roughly 90% of saplings surviving to the end of the study. However, both full defoliation treatments experienced significantly higher mortality rates than controls: 55% of saplings completely defoliated for one growing season died by the end of the experiment, as did 85% of saplings experiencing two years of intense defoliation. Overall, mortality rates were not affected by nativity, light level, or initial sapling size, but light had a marginally significant positive relationship with native sapling survival (z = 1.69, df=149, P = 0.09). *Prunus serotina* had the highest survival rates of the four focal species, and maple species tended to have lower survival rates in older, low-light second growth forests than *P. serotina* and *R. cathartica* growing in younger mid-successional forests.

Leaf mass produced following full defoliation was associated with sapling size (t = 2.63, df = 58, P = 0.0109), and was not related to nativity (Fig. 3) or light level. Furthermore, leaf production did not significantly differ between saplings fully defoliated for one year or two years, as individuals in the latter treatment produced very few leaves the second year of defoliation.

Chlorophyll index over the 3-year experiment varied by treatment and nativity (Fig. 4). Half-defoliated saplings maintained control chlorophyll levels, but fully-defoliated saplings over one or two years had significant reductions in chlorophyll index that were 11-15% below control levels (100% x 1: t = -8.02, df = 1003, P < 0.001; 100% x 2: t = -8.32, df = 1003, P < 0.001). Invasive saplings in the full-defoliation treatments showed greater reductions in chlorophyll index than native saplings (100% x 1: t = 3.64, df = 1003, P < 0.001; 100% x 2: t = 3.75, df = 1003, P < 0.001). Chlorophyll levels were negatively associated with light level (t = - 3.97, df = 1003, P < 0.001), but we found no interactive effects of light and nativity on chlorophyll levels. Species had similar seasonal chlorophyll patterns (Fig. S2), and *Rhamnus cathartica* had the highest chlorophyll levels of the four species.

Discussion

We evaluated the effects of defoliation frequency and intensity on storage, growth, mortality, and photosynthesis in two pairs of native and invasive woody species. Contrary to our hypothesis that invasive species would experience greater mortality than native species due to preferential growth allocation at the expense of NSC stores, we found little effect of nativity on sapling responses. Instead, *A. saccharum, A. platanoides, P. serotina,* and *R. cathartica* appear to respond similarly to varying levels of leaf removal. Carbon limitation in the shaded forest understory coupled with the frequency of tissue damage from extreme weather events or herbivory may similarly constrain tree species responses to defoliation (Mitchell et al. 2006). In line with other studies of woody plant responses to defoliation (Canham et al. 1999), we found that defoliation treatments led to large intraspecific differences in survival, growth, and photosynthesis (but not NSCs) and that responses were similar across all four species.

In the forest understory, resource depletion following intense defoliation is likely a significant source of mortality given our results. Experimental saplings subjected to multiple years of partial (50%) defoliation maintained high survival rates, yet complete defoliation severely reduced survival across all species. Surviving half-defoliated and fully defoliated individuals generally maintained stem NSC concentrations similar to controls, which may indicate that saplings prioritized NSC concentrations over growth in response to stress (Wiley et al. 2012). Indeed, NSCs may prevent freezing damage during the leafless period and are required to maintain cell turgor in the phloem during the growing season. Though we did not monitor NSCs in dead individuals, carbon depletion via refoliation and/or stem respiration in the canopy might explain our observation that many fully defoliated individuals exhibited complete canopy die-off in the seasons following defoliation. Alternatively, reduced NSC concentrations in canopy branches may have increased sapling vulnerability to freezing damage (Klotke et al. 2004) or hydraulic failure (McDowell 2011, Sevanto et al. 2014). Some top-killed saplings survived by sprouting from the mainstem or root crown (Fig. S3; E. Hinman, personal observation), but many did not survive to the end of the experiment. High mortality following full defoliation is surprising given that deciduous species predominantly store C and N in woody tissues (Piper and Fajardo 2014) and must remobilize C and N annually to produce new leaves. Despite documentation that deciduous trees in other temperate environments commonly recover from multi-year growing season defoliation events (Kulman 1971), our defoliation treatments may have been harsh enough to interrupt nutrient mobilization due to cavitation or resource depletion. Saplings limited to sprouts at the root crown were likely unable to restore phloem function to the canopy and died of carbon starvation.

Sapling growth was strongly affected by defoliation treatment. In line with our hypothesis, one and two years of 100% leaf removal reduced radial growth to a greater degree than three years of half defoliation across all species. This is likely a direct consequence of losses to photosynthetic area and indicates that NSCs were either unavailable or not used to support radial growth in the mainstem during the experiment. Additionally, fully defoliated saplings refoliated 5-30% of their initial leaf mass between July and November of the same treatment year, likely reducing carbon pools used to generate next season’s early wood and leading to “missing rings” (Kulman 1971). Interestingly, the mass of leaves refoliated in 100% defoliated individuals was positively correlated with sapling size, indicating that species engage in similar refoliation strategies following complete leaf loss and that the NSC pools used for leaf production scale with sapling size.

Saplings did not appear to upregulate photosynthesis following defoliation by increasing chlorophyll concentrations. Nitrogen (Vitousek and Howarth 1991) or phosphorus limitations could have curtailed photosynthetic upregulation. Additionally, the decrease in chlorophyll index in fully defoliated individuals is likely related to the younger age of the leaves produced following leaf removal. Interestingly, invasive species tended to show a larger decrease in chlorophyll index following full defoliation than native species. This might indicate that native species retain greater N stores to support leaf production in response to defoliation than invasive species. Greater leaf N found in many understory invaders (Heberling and Fridley 2013, Jo et al. 2014, Heberling and Fridley 2016, Hinman, Fridley, and Parry *in prep*) may come at the expense of N stores in woody tissues, and lower leaf N resorption rates in invaders (Heberling and Fridley 2016) could lead to a N deficit following severe defoliation events in which N stores are depleted. Decreased carbon assimilation following defoliation could compromise the exchange of photosynthates and nutrients between plants and soil microbes, resulting in N-deficient leaves.

Despite our observations of clear defoliation effects on growth and survival, our field study has several limitations. First, though native and invasive species differed in chlorophyll levels following severe defoliation, our ability to make generalizations about native-invasive differences is restricted by our use of only four species. Second, species may rely on physical or chemical defenses to avoid intense defoliation events, such that consistent, high levels of leaf loss across species are uncommon in the field (but see Kulman 1971). However, our defoliation treatments were intended to identify thresholds for growth and mortality, independent of current defoliation rates in deciduous forest understories. Finally, stem NSC concentrations are an incomplete sample of the carbon balance of living individuals. It is possible that defoliation led to NSC reductions in other organs, including roots (Landhausser and Lieffers 2011). Depletion of root NSC storage could hinder nutrient foraging due to insufficient fine root production.

Nutrient deficiency, in turn, likely reduces carbon assimilation and sapling survival in the—a potential explanation for decreased chlorophyll content in defoliated invasive species. Greater resolution of NSC and N allocation to leaves, stems, and roots throughout a defoliation event, coupled with C and N tracer methods to uncover the directional movement of nutrients through plant tissues, would identify the resource use strategies behind defoliation responses. Despite these limitations, our data reveal that both half and full defoliation lead to growth reductions in understory species and that full defoliation specifically presents significant survival risks to both native and invasive woody species.

Conclusion

We found that forest understory saplings of native and invasive species tolerate partial defoliation, but full defoliation causes significant decreases in growth, photosynthesis, and survival. Living saplings maintained stem NSCs following defoliation in our experiment, but our understanding of the role NSCs play in survival from damage remains limited. Given the greater productivity typical of forest invaders and similar native and invasive species responses to defoliation, our results suggest that non-native woody species do not face increased survival costs for growth advantages. Predicted increases in climate-related disturbance events, like pest outbreaks or extreme weather, could shift forest composition by reducing abundance of both native and invasive tree species. However, we provide evidence that moderate but frequent defoliation events in the understory do not inhibit the spread of invasive woody species in ENA deciduous forests.

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Figure Captions

Figure 1: Effects of defoliation treatments in native and invasive species on (a) radial growth, (b) NSC concentrations, and (c) mortality. Points indicate species means and lines represent one standard error. Letters refer to the results of Tukey HSD tests, where bars labeled with different numbers are significantly different at $\propto$= 0.05.

Figure 2: Relationship between radial growth and NSC concentrations across treatment and nativity.

Figure 3: Relationship between radial growth and log leaf mass refoliated following 100% defoliation over one (black points) and two (white points) growing seasons. Inset figure shows log leaf mass refoliated in native and invasive groups.

Figure 4: Chlorophyll index across defoliation treatments and (a) nativity, (b) species. Gray boxplots represent invasive species, while white boxplots represent native species

Figure 1

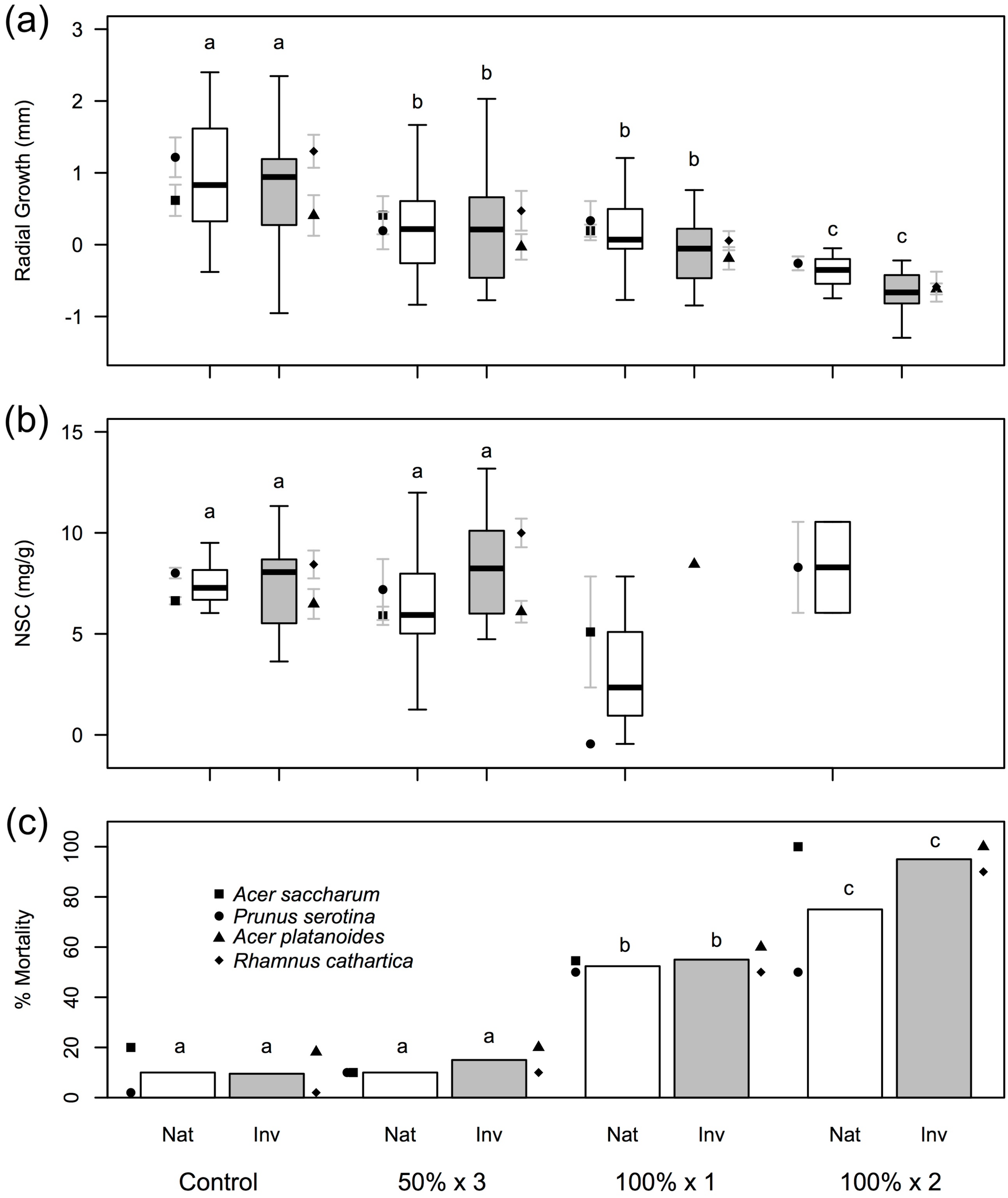
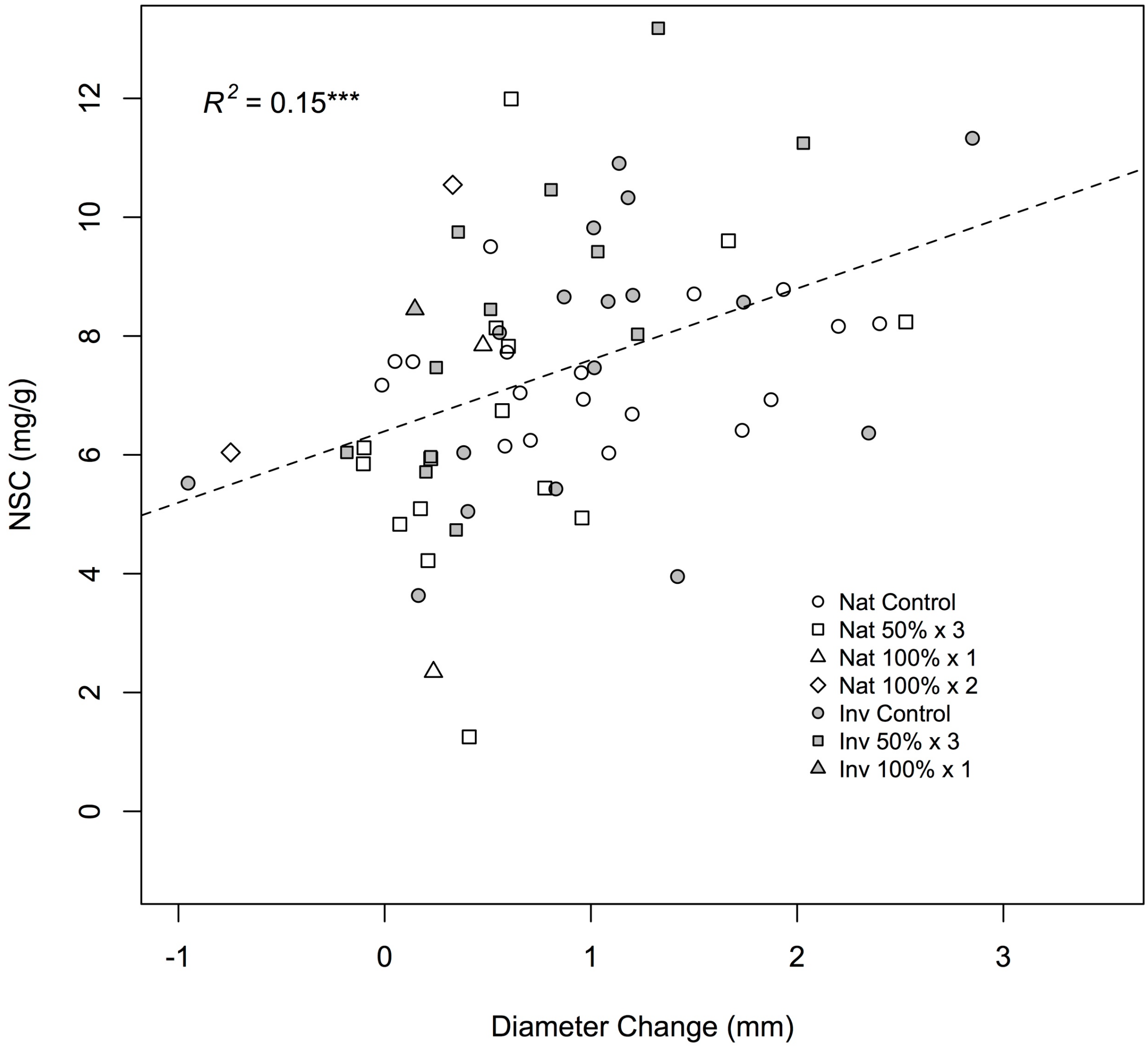
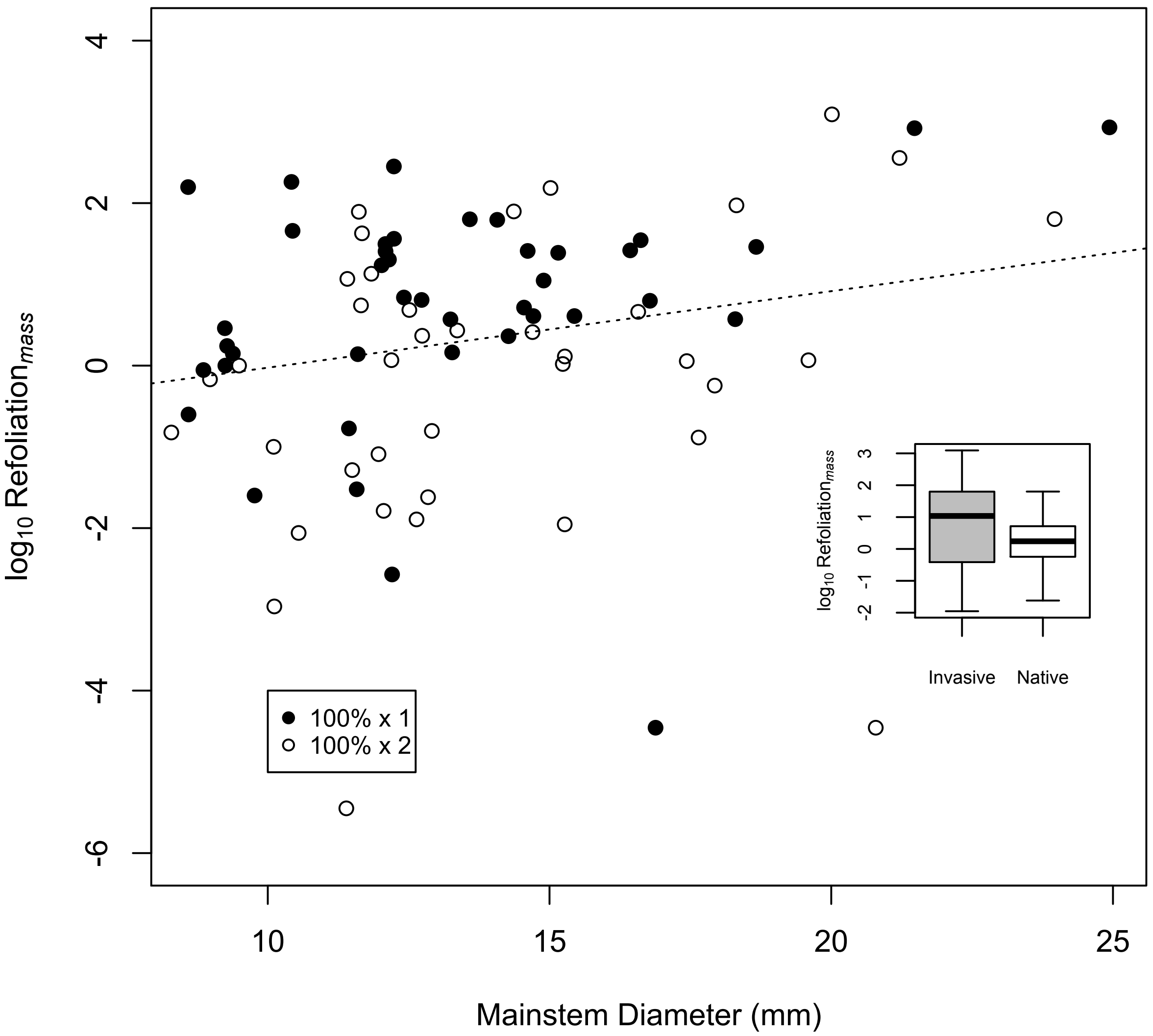

(a)
Radial Growth (mm)
3
2
1
0
-1
a
a
b
b
b
b
c
c
(b)
NSC (mg/g)
15
10
5
0
a
a
a
a
(c)
% Mortality
100
80
60
40
20
0
■ Acer saccharum
● Prunus serotina
▲ Acer platanoides
◆ Rhamnus cathartica
a
a
a
a
b
b
c
c
Nat
Inv
Nat
Inv
Nat
Inv
Nat
Inv
Control
50% x 3
100% x 1
100% x 2

Figure 2



R^2 = 0.15***
NSC (mg/g)
0
2
4
6
8
10
12
Diameter Change (mm)
-1
0
1
2
3
○ Nat Control
□ Nat 50% x 3
△ Nat 100% x 1
◇ Nat 100% x 2
● Inv Control
■ Inv 50% x 3
▲ Inv 100% x 1

Figure 3



4
2
0
-2
-4
-6
log10 Refoliationmass
10
15
20
25
Mainstem Diameter (mm)
● 100% x 1
○ 100% x 2
3
2
1
0
-1
-2
log10 Refoliationmass
Invasive
Native

Figure 4

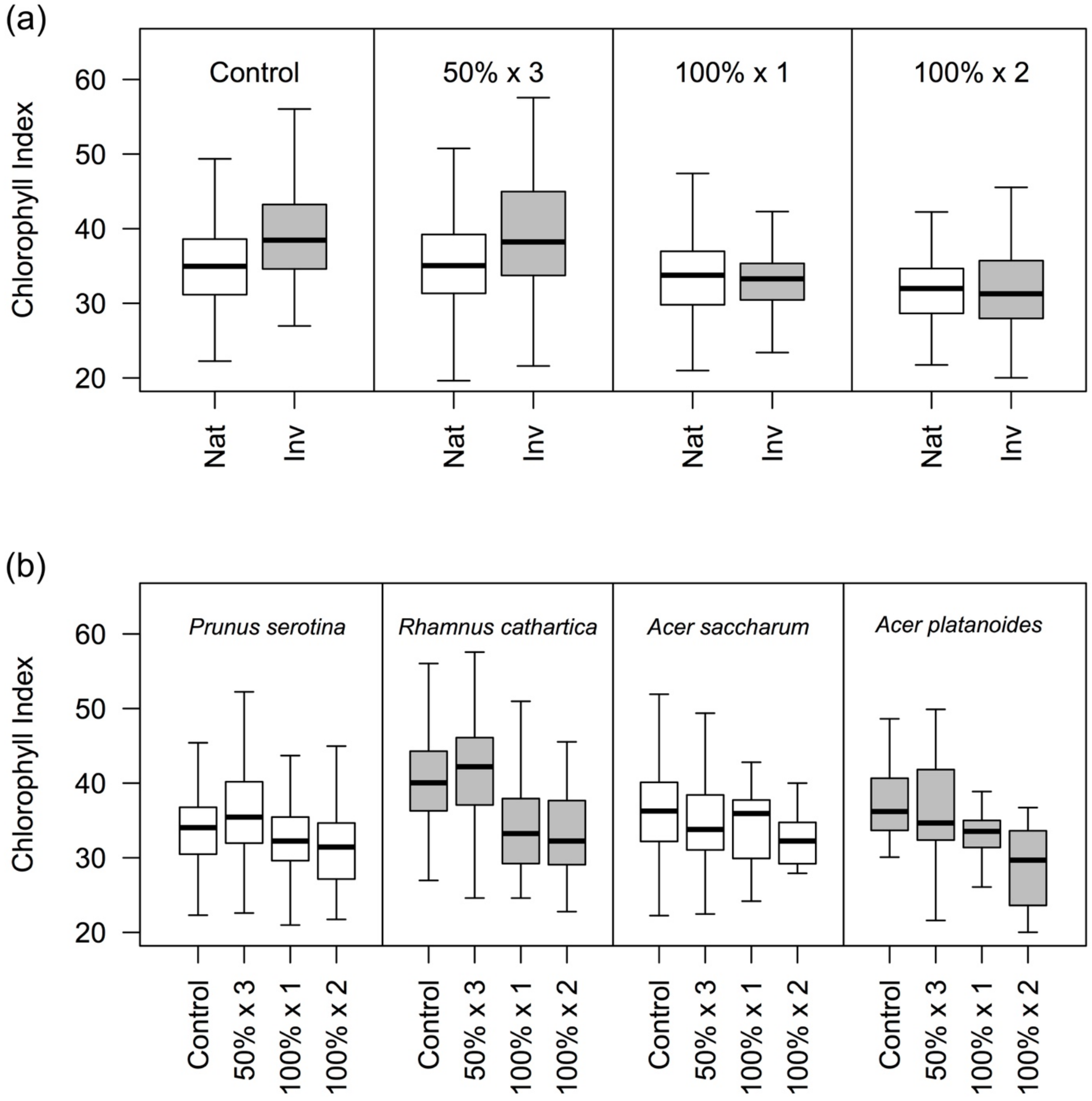

(a)
Chlorophyll Index
20
30
40
50
60
Control
50% x 3
100% x 1
100% x 2
Nat
Inv
Nat
Inv
Nat
Inv
Nat
Inv
(b)
Chlorophyll Index
20
30
40
50
60
Prunus serotina
Rhamnus cathartica
Acer saccharum
Acer platanoides
Control
50% x 3
100% x 1
100% x 2
Control
50% x 3
100% x 1
100% x 2
Control
50% x 3
100% x 1
100% x 2
Control
50% x 3
100% x 1
100% x 2

Figure S1: Radial growth in native and invasive species across defoliation treatment in (a) 2015 and (b) 2016.

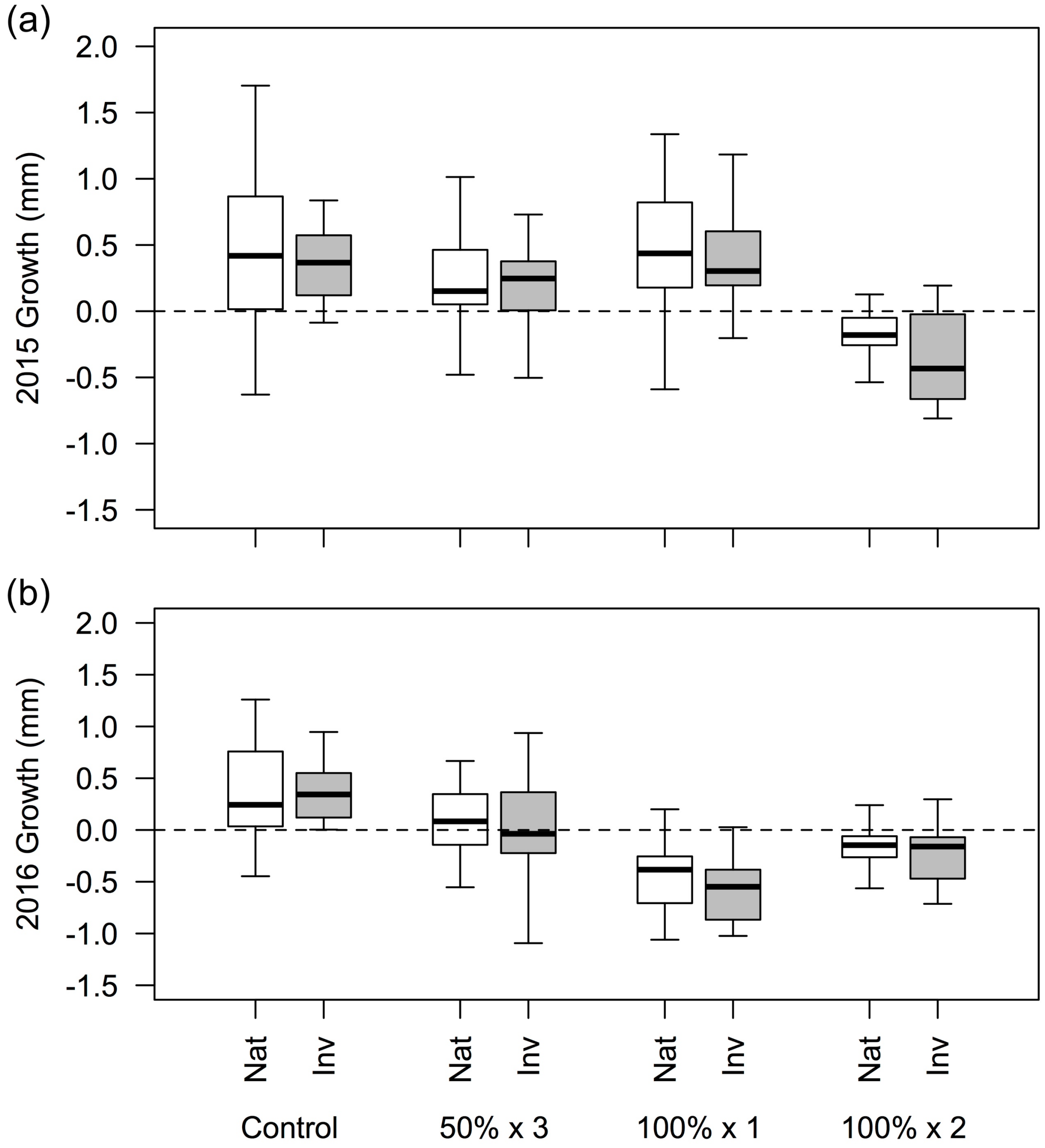


Figure S2: Chlorophyll index for each defoliation treatment over three growing seasons in (a) *Acer platanoides*, (b) *Acer saccharum*, (c) *Rhamnus cathartica*, and (d) *Prunus serotina.*

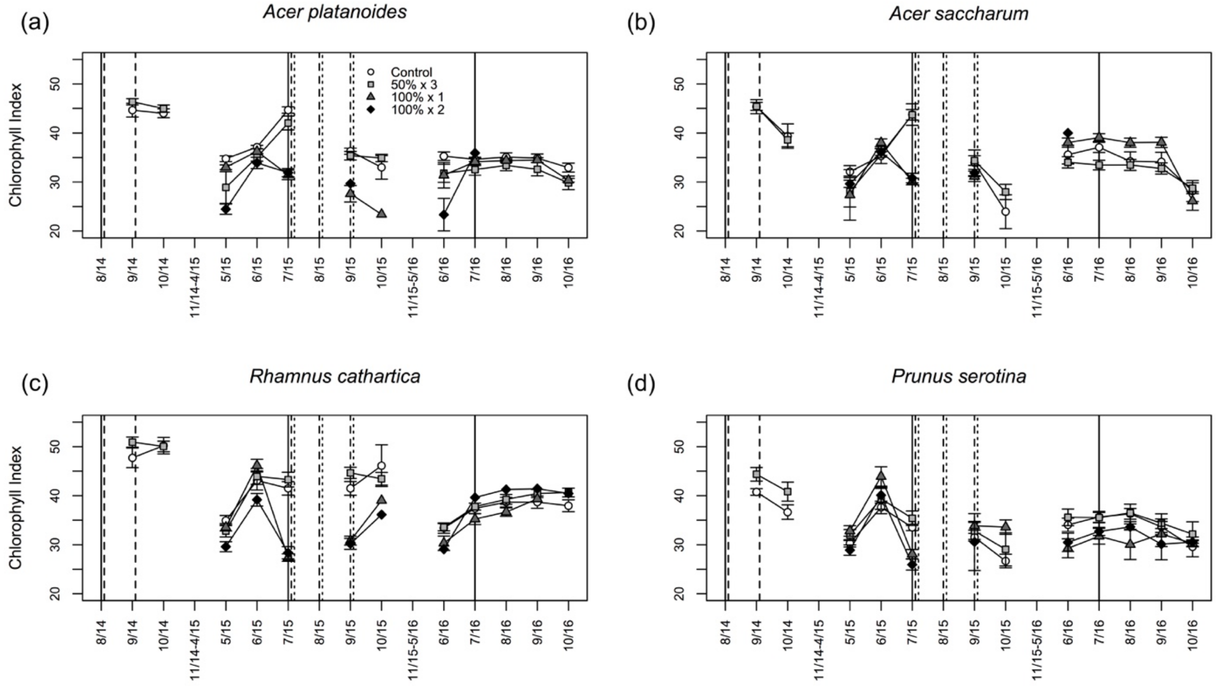


Figure S3: *Prunus serotina* sapling sprouting from main stem and root crown in response to full defoliation.

CHAPTER 5: Synthesis

European and East Asian woody plant species have successfully invaded intact, low light deciduous forests of Eastern North America (ENA) (Mack 2003, Fridley 2008, Martin et al. 2009) despite the historical perspective that invasions thrive under high resources and disturbance (Davis et al. 2000). Why are invaders successful in low resource understory environments? Trees and shrubs invasive in ENA forests often exhibit higher resource use efficiencies than native species (Fridley 2012, Heberling et al. 2013, Heberling et al. 2016) leading to greater overall carbon gain and productivity (Jo et al. 2014). However, competition for resources is but one facet of plant persistence. Survival is a critical factor for long-lived species in the understory (Canham et al. 1999), and we lack a clear understanding of native and invasive species' capacities to avoid, resist, and tolerate damage. Must invaders sacrifice some aspect of their physiology to achieve greater productivity (and thus a competitive edge) over native species? A fundamental element of this dissertation is the hypothesis that plant species are subject to tradeoffs between growth, storage and defense (Chapin et al. 1990, Zust and Agrawal 2017), such that all three pools compete for the same resources. Under resource constraints, high allocation to growth and resource acquisition, as observed in invaders, presumably occurs at the expense of the other pools. In forest understory environments where damage by herbivores and late spring freezing events are not uncommon (Ayres and Lombardero 2000, Augspurger 2013, Zohner et al. 2017), low allocation to defense and/or storage to resist or tolerate damage could result in plant mortality, especially when damage is severe (e.g. entire canopy defoliation). Following low to moderate tissue damage, however, high resource use efficiency in invaders could support a tolerance strategy despite low allocation to defense and storage. In this dissertation, I evaluated the extent to which physiological tradeoffs mediate survival strategies in native and invasive woody species.

In chapters two and three, I assessed allocation to storage (i.e. non-structural carbohydrates; NSCs) and leaf defenses in a large group of phylogenetically paired native and invasive woody species, and determined whether tradeoffs exist between leaf production, NSCs, and commonly measured leaf defenses. I found clear differences in seasonal NSC concentrations between the two groups (Hinman and Fridley 2018). Native species maintained greater soluble sugar concentrations, leading to greater overall NSCs in both spring and fall, which may indicate that natives have a greater capacity for recovery than invaders. However, invaders had higher root starch concentrations following spring leaf out, which could represent a long-term, well-protected storage pool for recovery from tissue damage during the growing season. Regarding leaf defenses, invaders had higher lignin and nitrogen concentrations than native species, despite similar leaf dry matter content, cellulose, and phenolics concentrations (Hinman, Fridley, and Parry *in review*). Storage and defense differences in natives and invaders might indicate that natives have a greater capacity to tolerate damage with overall higher NSC concentrations, while invaders have a greater capacity to resist it with higher leaf lignin. Combining these data with previously collected leaf production rates (Fridley 2012), I tested for constraints between the three allocation pools, accounting for differences in overall carbon gain between species. Contrary to my hypothesis, I found no evidence for tradeoffs between growth, storage, and defense across all species. Instead, other resource pools, including flower and fruit production (Obeso 2002), fungal mutualisms (Kozlowski 1992), and maintenance functions (Fridley 2017) likely mediate growth-storage-defense relationships and deserve further inquiry in this group of species. Furthermore, in this dissertation, we could not determine intended uses of NSCs because the residence time, transport, and functions of NSCs in native and invasive species remains unknown. Carbon tracer experiments would reveal whether non-structural carbohydrate pools in invaders turnover quickly, mirroring their competitive resource acquisition strategies, or compose a stable, long-term pool of energy for when plant demand outstrips supply. Additionally, home and away studies of NSCs in invaders that incorporate carbon tracers could explore whether allocation strategies change from the native to the invaded range. Finally, evidence suggests that NSCs are critical to maintaining hydraulic function (Sevanto et al. 2014), and their function as osmoticants during hardening/dehardening and freezing events in ENA woody species deserves further study.

In chapters three and four, I supplemented the trait-based approach with experiments that assessed native and invader palatability to generalist insect herbivores and plant responses to varying levels of experimental defoliation. I expected that differences in storage and defense allocation observed in trait assays would inform susceptibility to and responses following defoliation, but surprisingly, native and invasive woody plants were, on average, equally poor generalist insect hosts and had similar responses to moderate (50% leaf removal) and severe (100% leaf removal) damage. Despite the small number of species represented in the experimental defoliation study (two native, two invasive), it highlights that dominant understory species are well-equipped to tolerate multi-year, moderate damage at the expense of radial growth, but are generally unable to survive complete defoliation. These results, coupled with the palatability study, suggest that understory species do not frequently experience the large-scale insect outbreaks common in the forest canopy (Logan et al. 2003). However, deer and other herbivores exert differential and often severe pressure on understory plants (Eschtruth and Battles 2008), the repercussions of which are unknown for most understory native and invasive woody plants. Few have explored the effects of herbivory on survival, growth (but see Knapp et al. 2008), and particularly reproduction in our focal species.

Why are woody invaders successful in understory environments? With greater resource use efficiency and overall carbon gain than natives (Fridley 2012, Heberling et al. 2013, Heberling et al. 2016), lower seasonal NSC concentrations (Hinman and Fridley 2018), and more physically defended, nutritious leaves (Hinman, Fridley, and Parry *in review*), ENA invaders experience similar risk of generalist insect herbivory and exhibit similar responses to both moderate and severe defoliation. Results suggest that invaders may subscribe to a fundamentally different set of constraints than natives (*sensu* Martin et al. 2010), potentially due to divergent evolutionary histories (Fridley and Sax 2014). Future work could place defense, storage, and growth in the context of other pools likely imposing energy constraints, including microbial interactions and, critically, reproductive allocation and output (Fig. 1). Fundamental questions, including plant age or size at reproduction, frequency of flowering/fruiting, and proportion of

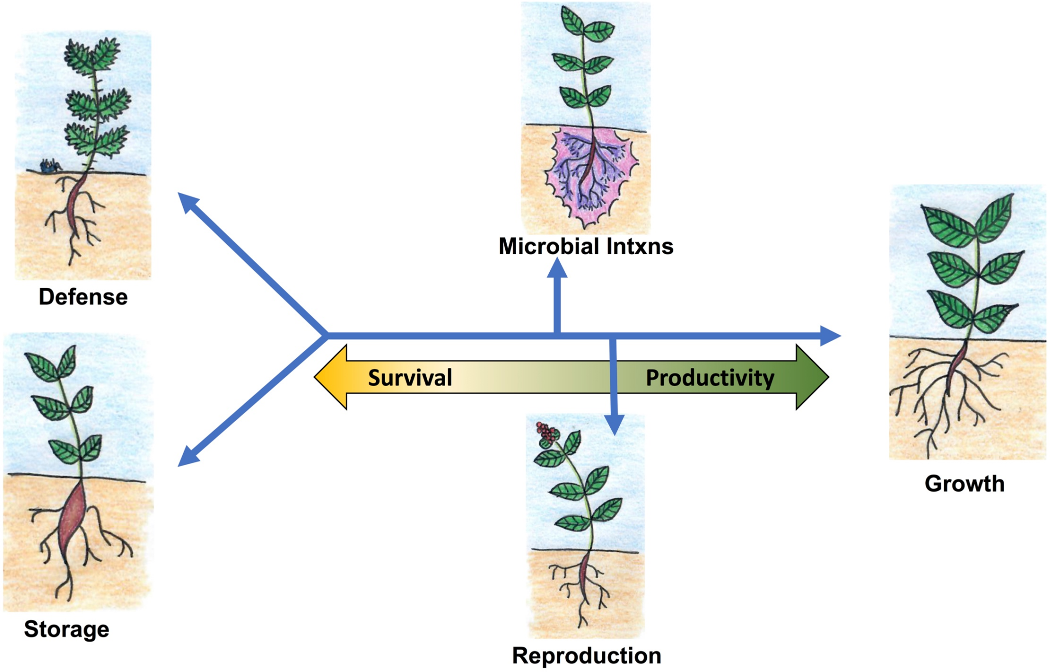


Figure 2: Updated hypothesized tradeoffs between growth, survival, and persistence.

biomass allocated to fruit remain unanswered for a majority of understory woody species (but see Mason et al. 2008). These data, coupled with field observations of the causes of native and invader mortality in the understory, will reveal the extent to which species must defend, store, and grow to successfully reproduce. It is possible that more efficient invaders reach reproductive maturity earlier than native species, which decreases their risk of experiencing a severe damage event prior to flowering and thus reduces invader investment in storage. Studies using both home and away populations would illuminate whether native/invader differences were acquired in the native or invaded ranges. A more complete picture of energy allocation and plant responses to damage is needed to deduce the source of native-invader differences in resource allocation and how these differences will affect species persistence as forest conditions change.

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2007-2011	B.S. in Environmental Science and Management, UC Davis

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Publications

Hinman, E. D., & Fridley, J. D. (2018). To spend or to save? Assessing energetic growth-storage tradeoffs in native and invasive woody plants. *Oecologia*, 1-11.

Hinman, E.D., Fridley, J.D., Parry, D. Defense investment and herbivore preference in the forest understory: a phylogenetic comparison of native and invasive woody plants. *In review for Biological Invasions.*

Hinman, E.D., Fridley, J.D. Impacts of experimental defoliation on native and invasive woody plants in deciduous forests: are natives more resilient to canopy disturbance? *In prep for Tree Physiology.*

Presentations

Hinman, E. D., Fridley, J.D. August 2018. Connecting defense-related leaf traits in 20 species of native and invasive woody plants to the growth of two generalist herbivores in a controlled feeding assay. Oral presentation at the Ecological Society of America Conference in New Orleans, LA.

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Hinman, E., Fridley, J. D. August 2015. Carbon allocation strategies in native and invasive woody plants. Poster presented at the Ecological Society of America Conference in Baltimore, MD.

Hinman, E. September 2012. Project: Mission Blue, An update on the status of the Lupine Diversification Project. Presented at the California Garden Club Conference in San Mateo, CA.

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2017-2018	Community Engagement Coordinator, Cottonwood Canyons Foundation, Holladay, UT
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2013	Ecological Restoration Technician, Marin Municipal Water District
2011	*Mimulus* Demography Field Technician, Angert Lab, Colorado State University
2010-2011	Undergraduate Researcher, Collaborative Learning at the Interface of Mathematics and Biology (CLIMB), UC Davis
2010	Intertidal Diversity Field Technician, Stachowicz Lab, UC Bodega Marine Laboratory
2010	Environmental Compliance Intern, California Army National Guard

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2011 Recipient, Highest Honors (*summa cum laude*), UC Davis

2011 Recipient, Departmental Citation, Department of Environmental Science and Policy, UC Davis

Teaching

2017 & 2015 Anatomy and Physiology Teaching Assistant, Biology Department, Syracuse University

Co-taught a weekly 2-hour laboratory and led a weekly 80 minute recitation section. Graded quizzes, exams, and laboratory assignments.

2016 Global Change Ecology Laboratory Teaching Assistant, Biology Department, Syracuse University

Co-taught a weekly 4-hour field lab focused on data collection and analysis. Assisted in the design of assignments and a final project.

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