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COMPARATIVE ECOPHYSIOLOGY OF NATIVE AND INVASIVE PLANTS ACROSS POPULATIONS, SPECIES, AND CONTINENTS

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ABSTRACT

Invasive species are a persistent threat to biodiversity and agriculture that cost countries millions of dollars each year. Despite the scale and impact of the problem of invasive species, it remains a mystery why some introduced species become invasive and others remain benign. Here, I examined the theory that invasive species possess key traits which make them successful invaders. Specifically, I investigated the hypotheses that 1) plasticity increases invasive species' success compared to native species, 2) invaders evolve rapidly in their new ranges, 3) invasive species avoid tradeoffs apparent in natives related to growth, resource use, or defense. Using 30 species of native and invasive shade tolerant shrub species in a common garden, I tested whether invasive species had leaf traits that were more plastic to seasonal changes in light availability. I found that invasive species were more plastic, but I found no evidence that their plasticity contributed to increased carbon assimilation across the growing season. Next, I compared leaf traits of populations of five invasive species growing in forests from East Asia in their native range (Japan) and their invasive range (Eastern North America). I also compared these five invaders to three native co-occurring North American species. I selected individuals that occurred along a natural light gradient to assess leaf plasticity in response to light availability. I determined that plasticity in invaders partially arose in the native range, and that some plasticity evolved rapidly in the invaded range after introduction. I concluded that many North American species are less adapted for life in high light environments, such as along forest edges, than East Asian invaders; the ability to grow in both low and high-light conditions may give some forest invaders a competitive advantage. Finally, I quantified traits of annual, perennial, coastal salt tolerant perennials, and invasive populations of *Mimulus guttatus* in a greenhouse setting (22 total populations). I decomposed relative growth rates of each group into specific leaf area, photosynthetic rate, and leaf mass fraction to determine which trait contributed most to growth rate within each group of populations. Invasive populations did not have higher relative growth rates, contrary to our predictions, but they did have different ways of achieving high growth rates. I also examined whether tradeoffs associated with global trait patterns such as the leaf economics spectrum apply at the population level. I found that global patterns of plant functional traits may not be applicable when studying traits within species. I conclude that a variety of traits and evolutionary history contribute to the success of invasive species around the globe.

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CHAPTER 1: INTRODUCTION

Invasive species are those species that have been transported outside of their continent of origin by anthropogenic means and have established prolific populations in their new ranges, often to the detriment of native species diversity and productivity of agricultural lands (Elton 1958). Invasive species are opportunities to study foundational concepts in ecology and evolution, such as how organisms come to thrive in novel environments (Sax *et al.* 2007). The economic costs of managing invasive species and mitigating damage they cause, upwards of $336 billion USD in the US, Australia, United Kingdom, South Africa, and India alone (Pimental *et al*. 2001; Moles *et al.* 2012) are increasing each day as the world becomes increasingly interconnected.

Our understanding of which traits or ecological circumstances allow some introduced species to become invasive and preclude establishment of others remains poor. Some have hypothesized that repeated introductions of invasive species, either through contaminated trade vessels or through horticultural means, allows invasive species to establish through repeated introductions to favorable habitats. Others purport that traits of the invasive species themselves, such as high relative growth rate (Baker 1965), high resource use efficiency, or prolific seed production (Von Holle & Simberloff 2005) allow invaders to establish. Still others hypothesize that traits of the invaded communities, like low diversity or high resource availability facilitate invasions (Jeschke *et al.* 2012; Fridley & Sax 2014). Most likely, it is a combination of these three factors that cause some species to become invasive. I focus on testing the general hypothesis that traits of invasive species themselves make them successful in their new, invaded ranges. Specifically, I address two commonly hypothesized traits of invasive species: increased plasticity (Richards *et al.* 2006; Davidson, Jennions & Nicotra 2011) and increased relative growth rate (Baker 1965; Pattison, Goldstein & Ares 1998; Bellingham *et al.* 2004).

High phenotypic plasticity in invasive species is a theory originally proposed by Baker (1965) suggesting invaders possess higher phenotypic plasticity than co-occurring natives, which allows them to colonize more environments and ultimately maintain higher fitness. For example, in the deeply shaded deciduous understory of temperate deciduous forests, phenotypic plasticity in response to light may be advantageous (Delagrange *et al.* 2004; Paquette *et al.* 2012). This is because light levels in the understory are highly seasonally variable (Augspurger, Cheeseman & Salk 2005; Martinez & Fridley 2018) and canopy gaps due to treefall can occur. Plants that can take advantage of these changes in light availability may assimilate more carbon which in turn leads to higher growth rates and seed production. Additionally, the ability to colonize a wide variety of light environments as a result of phenotypic plasticity may allow species to colonize both forest understory interiors and forest edges while native species might frequently be limited to one type of environment (Pattison *et al.* 1998; Leicht & Silander 2006). Invasive species phenotypic plasticity may be the result of millions of years of evolution in the native range (Fridley & Sax 2014), but it may evolve in the new range because of population bottlenecks or differences in selection pressures, such as release from enemies present in the native range but not invaded range (Keane & Crawley 2002). However, where invader phenotypic plasticity arises (native range or invaded range) likely varies by species, so additional studies are needed to understand these mechanisms fully.

One way to test for trait differences between ranges is to perform ‘home’ and ‘away’ studies of populations of invasive species in both their native (home) and invaded (away) ranges. For example, through this type of study, some forest understory invaders have been shown to display increased resource (nitrogen) use efficiency in comparison to co-occurring native species (Heberling & Fridley 2016). Changes in nitrogen use efficiency may come about through changes in nitrogen allocation to various pools within leaf tissue. Allocation to one pool or the other becomes advantageous in differing light environments (Evans 1989), and an optimal allocation scheme exists for each light environment (Hikosaka 2014). For example, N allocation to light cycle reactions may be higher in high light environments relative to shaded environments (Hikosaka 2014).

Phenotypic shifts in the invaded range may also be influential in successful invasions if they impact relative growth rate (RGR), which is often cited as key trait influencing invader success (Baker 1965; Grotkopp, Rejmánek & Rost 2002). However, invasive species cannot also possess high RGR before arriving in their invasive ranges. Three physiological traits underlie RGR: leaf mass fraction (LMF), photosynthetic rate, and specific leaf area (SLA) (Hunt & Cornelissen 1997). Few studies on the influence of each of these traits in invasive plant populations exist. Insights to how invaders maximize RGR, and whether they maximize it in different ways from native populations will allow us to better understand why species invasions exist and what traits facilitate their success in novel environments.

Chapter 2

Woody understory species tend to leaf out earlier than the tree canopy and maintain their leaves after tree canopy senescence in autumn (Fridley 2012). Consequently, they are faced with a physiological challenge: adapt leaf photosynthesis to light environment changes or face decreased carbon returns. I hypothesized that understory invaders would display higher adaptive plasticity in leaf traits in response to seasonal light changes than native species. I monitored leaf production in all individuals to assess the importance of leaf production in trait plasticity within a single plant. In addition, I assessed if plants were plastic via production of new leaves or through variation in leaves produced at the onset of the growing season. Using 17 species of invasive and 13 species of native shrubs in a common garden in Syracuse NY, I monitored seasonal changes in leaf physiology. I found that invaders displayed higher levels of seasonal plasticity with respect to light level changes, but I found no evidence that this plasticity was adaptive in a C gain sense. The only species group for which seasonal plasticity influenced C gain was in native species that continually produced new leaves throughout the growing season.

Chapter 3

When non-native plants first arrive in their new ranges, they may be subject to genetic bottlenecks or artificial selection through horticultural plantings that alter their phenotypes (Dlugosch & Parker 2008). They may also undergo rapid evolutionary change because of exposure to a novel environment or release from natural enemies (Keane & Crawley 2002). Regardless of mechanism, phenotypic changes in the invasive range can facilitate spread and successful invasion. In chapter 3 I examined traits of invasive woody understory species from East Asia in both their native and invasive ranges and compared them to native species in ENA forests. Specifically, I measured ecophysiological traits in four invasive species in their native and invasive ranges occurring along a natural light gradient in forests. I hypothesized that invasive species would have higher leaf trait variability along a light gradient, which would make them more successful in a variety of light environments than native species. I also hypothesized that trait shifts in the invasive range would facilitate carbon (C) gain for invasive species. I found evidence that invaders respond plastically to light availability in their native range, but also that the plasticity may be heightened in their invaded range and may lead to increased C gain. This result is highly trait-dependent – not all traits showed shifts between the native and invasive ranges. Furthermore, I saw trait shifts in leaf traits related to C gain in the invaded range and found evidence that ENA native species are poorly adapted to light conditions outside of deeply shaded, intact understory.

Chapter 4

Global patterns of plant traits predict that long-lived perennial plants should have a different set of traits than short lived annual plants (Wright et al. 2004). For example, according to the leaf economics spectrum (LES) theory, perennial plants should have traits such as low photosynthetic rates and low specific leaf area (SLA), while annual plants should display the opposite traits (Wright et al. 2004). In general, longer lived plants tend to have traits that extend the lifespan of their leaves, while annual plants have leaf traits that instead maximize carbon assimilation during a short growing period.

However, these trait co-occurrences have not frequently been examined within a single species. RGR is thought to be predictive of invasiveness, but the physiological traits that contribute to RGR within a single species containing annual, perennial, and invasive populations has not been studied before (Baker 1965). I sought to decompose RGR within a single species that possesses multiple life history strategies (annual vs. perennial) and that is also invasive. I used approximately 22 populations of *Mimulus guttatus* grown in the greenhouse to assess differences in RGR, leaf photosynthesis, leaf mass fraction ([LMF] allocation to photosynthetic material), SLA, and leaf N content. I hypothesized that annual and perennial populations would divide themselves along the trait axes associated with the LES (Wright *et al.* 2004) and they would have different traits contributing most to their RGR, and that invasive populations would have higher RGR than native populations. I found that traits of annual and native perennial populations were very similar and that the two maximized RGR via similar combinations of traits. RGR of invasive populations was comparable to that of native perennial populations, but they maximized RGR through LMF and SLA, while native populations only showed significant contributions of SLA to RGR. This study suggests that within small scales or within closely related taxa, global patterns such as the LES may not apply. Additionally, RGR does not always increase in the invaded range and may not be the best predictor of invasiveness.

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CHAPTER 2: ACCLIMATION OF LEAF TRAITS IN SEASONAL LIGHT ENVIRONMENTS: ARE NON-NATIVE SPECIES MORE PLASTIC?

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Abstract

1. In temperate deciduous forests, understory light environments vary dramatically throughout spring, summer, and autumn due to tree canopy leaf display. This variability in light level is a physiological challenge for understory species that produce sun-adapted leaves in the spring before being shaded by the tree canopy. Similarly, some understory species display leaves late into autumn after the tree canopy senesces.
2. Many species in North American deciduous forests with extended leaf display are not native to North America. Since many non-native species have been shown to have greater plasticity than natives, we hypothesized that leaves of non-native species may be more plastic with respect to seasonal light changes than natives, and that this plasticity may allow them to assimilate more carbon in the same environment.
3. We measured leaf traits and photosynthetic capacity of 17 native and 13 non-native understory shrub species in a common garden in Syracuse, New York, during spring, summer, and autumn. We tested for the contribution of seasonal mean and variance (plasticity) of leaf traits to a species' average photosynthetic rate and total leaf production. We also analyzed the extent to which leaf adjustments depended on whether plants continued to produce new leaves over the growing season.

4. Leaf traits of both native and non-native species varied seasonally, but plasticity varied in extent and contribution to overall carbon gain. Non-native species had the highest seasonal plasticity, but, contrary to our hypothesis, such plasticity did not contribute to their overall carbon gain. However, leaf trait plasticity was adaptive for native species that continued to produce leaves throughout the year, primarily due to increases in quantum efficiency and electron transport rate in leaves produced mid-year compared to leaves produced early in the year.
5. *Synthesis*. Despite large adjustments in leaf traits across seasonal light environments for both native species and non-native species, we found little evidence that leaf-level plasticity drives non-native invasion or contributes strongly to annual carbon gain or productivity in understory species. Instead, differences in mean leaf traits across seasons are sufficient to explain carbon gain advantages of non-native woody species in deciduous forests.

Keywords

Invasion ecology; leaf traits; plasticity; leaf demography; non-native species; ecophysiology; seasonality; photosynthesis; deciduous understory

Introduction

The light environments of deciduous understories are highly dynamic due to tree canopy leaf display. Light levels peak in late spring, decrease with tree canopy leaf out, and increase again after tree canopy senescence in autumn (Hutchison and Matt, 1977; Fig. 1). This dramatic variation in light availability poses a unique challenge for understory plants: leaves produced in high light levels in the spring, with high photosynthetic rates, may experience negative carbon balances once light levels decrease in the summer (Augspurger, Cheeseman and Salk, 2005). Leaves produced after canopy emergence forego high carbon gain associated with spring light levels. Due to putative tradeoffs in resource allocation between light and dark reactions of photosynthesis, sun and shade leaves often exhibit contrasting metabolic traits (Evans and Poorter, 2001; Hikosaka, 2004; Oguchi et al., 2006). Shade-adapted leaves tend to be thin with high specific leaf area (SLA) and chlorophyll (chl) content, increased levels of light harvesting capacity and efficiency (quantum efficiency [α]), and reduced dark respiration rates (R_d) (Valladares and Pearcy, 1998; Sánchez-Gómez et al., 2006; Valladares and Niinemets, 2008). These traits maximize carbon assimilation rates in low light and reduce tissue maintenance and respiration costs (Lambers et al., 1998; Valladares and Niinemets, 2008). Sun-adapted leaves tend to have the opposite traits: low SLA, high assimilation rates, high respiration rates, high electron transport rates (J_{max}), and high maximum carboxylation capacity of rubisco (V_{cmax}). These traits can be maintained at a positive carbon balance when light is abundant, and together maximize C assimilation in full sun (Abrams and Kubiske, 1990).

Leaf traits often adjust after expansion in response to shifts in irradiance levels, such as the formation of forest gaps (Turnbull et al., 1993; Yamashita et al., 2000; Oguchi et al., 2003; Oguchi et al., 2008) or self-shading due to new growth (Kikuzawa, 1991). This trait adjustment with respect to changes in light availability, herein defined as plasticity within a single leaf or plant, is an important component of understory plant fitness in deciduous forests (Kikuzawa, 1991; Gill et al., 1998; Augspurger et al., 2005).

Within-plant or within-leaf plasticity can become especially important after light level changes due to gap formation or seasonality. Such plasticity may be accomplished by adjusting existing leaves or producing new leaves for plants with indeterminate growth (Augspurger et al., 2005; Kikuzawa and Lechowicz, 2011). Seasonal leaf trait plasticity may allow leaves to maintain positive carbon balance as light levels change after the leaf is produced, and can include both chemical changes (i.e. Rubisco & chl fractions) (Rothstein and Zak, 2001; Niinemets et al., 2003) and physical traits such as SLA or mesophyll cell density (Oguchi, Hikosaka & Hirose, 2003). Leaf chemical changes often involve shifts in nitrogen (N) allocation that reflect different light levels. For example, in the spring more N may be allocated to rubisco, increasing carboxylation (V_{cmax}) levels in high light. In the summer, when light is low, N may be reinvested in chl and light harvesting proteins (Kitaoka & Koike, 2004). Light-saturated electron transport rate (J_{max}) is also maximized in full sunlight, which may determine the light level at which photosynthesis becomes limited by cellular CO_2 concentration instead of light (Farquhar, VonCaemmerer & Berry, 1980; Niinemets and Tenhunen, 1997). Although plasticity with respect to forest gap formation is relatively well understood (Naidu and Lucia, 1997; Kitao et al., 2000; Yamashita et al., 2000; Myers and Anderson, 2003; Oguchi et al., 2006; 2008), studies that have measured leaf trait plasticity of shade-tolerant understory plants with respect to light seasonality are based largely on tree seedlings or herbaceous species in spring and summer (Jurik, 1986; Rothstein and Zak, 2001; Kitaoka and Koike, 2004, 2005; Yasumura, Hikosaka and Hirose, 2006) and have not addressed the significance of autumnal changes, particularly in the context of forest invasions (but see Xu, Griffin and Schuster, 2007). Additionally, the cumulative fitness consequences of leaf trait plasticity over long time scales (single year or multiple years) are not well understood due to limited studies that have linked whole-plant carbon gain to environment-induced plasticity in functional traits.

Another component of seasonal leaf plasticity is leaf demography: the combined patterns of leaf emergence, longevity, leaf cohort production, and leaf fall (Kikuzawa & Lechowicz, 2011). Leaves of understory species produced at different periods in the growing season vary both physiologically and morphologically as a result of the prevailing light environment at leaf birth (Xu et al., 2007; Fridley, 2012). Understory species that produce only one cohort of leaves must either adapt to varying light conditions and sudden bursts of light or restrict leaf activity to certain seasons. One way plants may do this is through delayed spring leaf emergence (Kikuzawa, 1991; Augspurger et al., 2005). In temperate deciduous woody species of Eastern North America (ENA), we have observed three broad leaf demographic strategies: 1) highly shade tolerant, late successional determinant species that produce a single cohort of leaves in conjunction with or after tree canopy leaf out; 2) indeterminant shade-tolerant species that continually produce new leaves until autumn; and 3) species that produce a cohort early in the spring prior to tree canopy leaf out and a second cohort of leaves in early summer or in conjunction with tree canopy leaf out (see also Kikuzawa, 1991; Fridley and Craddock, 2015). Continual leaf production across seasons may be an effective strategy for maximizing photosynthetic capacity across light environments. However, this strategy is likely to come at a significant cost of energy and nutrients used to make new leaves rather than for reproduction or reserve storage (Chapin et al., 1990).

High plasticity in form and function is an oft-cited mechanism of non-native species advantage (Baker, 1965; Sultan, 2001; Callaway et al., 2003; Funk, 2008). Leaf phenology differs strongly between native and non-native ENA forest species, particularly in terms of autumnal senescence (Fridley, 2012). However, it is unclear whether non-native species advantage includes concomitant seasonal plasticity in leaf traits within individual plants. One non-native species in ENA forests, *Berberis thunbergii*, has been shown to shift leaf traits seasonally with respect to changing light availability in the understory more than co-occurring native species. Seasonal plasticity allows this species to take advantage of the high light availability in the spring, while co-occurring native species fail to utilize high spring light availability, and thus assimilate less C overall (Xu et al., 2007). Non-native species may also have different resource-use strategies than natives, such as higher chl:N ratios in shaded conditions, higher photosynthetic rate, and/or higher photosynthetic nitrogen use efficiency (Niinemets et al., 2003; Heberling and Fridley, 2013; 2016). High seasonal plasticity relative to native species, in concert with large seasonal fluctuations in understory light levels, may be one way that non-native species outcompete natives in the understory.

Here, we focus on seasonal plasticity in leaf function as a potential mechanism of non-native species advantage in temperate deciduous forests. This is only one of several potential ways in which plasticity drives forest invasions but likely one of the most significant to the fitness of understory species (Valladares and Niinemets, 2008). Our analysis considers seasonal plasticity at two scales, including variance in a leaf trait within 1) an individual plant (including trait changes associated with the production of new leaves) and 2) an individual leaf, as plant fitness in a heterogeneous light environment may be influenced by both types of adjustments.

We monitored seasonal leaf function in a common garden composed of native and non-native understory shrubs common to ENA. We included measurements taken before (May), during (July), and after (Oct) simulated forest shade. As a proxy of individual fitness, we used two performance parameters related to an individual's energy budget and fitness: 1) mean instantaneous photosynthetic rate (MIPR) across the growing season (related to annual C gain and gross primary productivity, which contributes to fitness in ways beyond leaf growth, such as root growth, survival, and defense, that we did not measure), and 2) aboveground net primary production (NPP), estimated as total annual leaf production as a proxy for annual growth rate. We sought to answer the following questions: 1) Do physiological leaf traits vary seasonally with light environment? 2) Does variation in leaf traits across seasons influence annual C gain or NPP? 3) Do non-native species have more seasonally plastic leaf traits than native species? 4) Do indeterminate plants, with leaves produced in both sun and shade environments due to tree canopy dynamics, exhibit greater plasticity in leaf traits than determinate (single flush) plants? We hypothesized that in the spring and autumn, leaf traits associated with full sun environments (high V_{cmax}, J_{max}, and low SLA) maximize understory shrub C gain, while in the summer, C gain is maximized by efficient light harvesting characteristic of shade-adapted leaves (high α, chl, and SLA). We also hypothesized that non-native species would exhibit higher seasonal leaf plasticity than native species, facilitating their greater overall assimilation and leaf production rates. Lastly, we hypothesized that indeterminate plants exhibit higher seasonal leaf plasticity due to the production of new leaves rather than through adjustments of existing leaves.

Methods and Materials

Study Site and Species

We measured leaf properties across three seasons in a common shade garden in Syracuse, New York, USA (43.0° N, 76.1° W). Our study included 30 species (two to three individuals of each species) of native and non-native deciduous shrubs and lianas of ENA common to forest understories (Table 1). Individuals were planted as saplings in three replicate blocks 7-9 years before this study (Fridley, 2012). Transplants were sourced from local natural areas when possible and otherwise obtained from nursery stock of similar latitude. All selected non-native species are managed as invasive species in ENA (Fridley, 2008) except for naturalized *Viburnum setigerum*. We selected species to include phylogenetically paired native and non-native species to minimize the effect of phylogenetic autocorrelation. Similarly, growth-determinate and indeterminate pairs were selected within genera whenever possible. To simulate a tree canopy a black polypropylene shade cloth transmitting 20% of total radiation (Fig. 1) was placed over the common garden from June 5 to October 1. (Note Fig. 1 shows light levels for a subsequent year of more complete light data, in which plant culture was identical except the shade cloth was removed November 1). Although the light red:far red ratio (R:FR) under black (neutral) shade cloth does not reproduce forest understory R:FR (<1), plants adapted to shaded conditions are generally unresponsive to R:FR (Smith 1982; Schmitt and Wulff, 1993; Morgan and Smith, 2017) and we have not detected phenological differences between shaded and unshaded individuals at the garden (Fridley, 2012). Average midday light conditions in the common garden were 1500-2000 PPFD in the spring, 300-400 PPFD in the summer, and 800-1000 PPFD in autumn (Fig. 1).

Leaf Trait Measurements

Leaf traits of each individual were measured in late May, July, and mid-late October of 2015. Gas exchange measurements were performed on cut branches in the laboratory following Niinemets et al., (2005), Heberling and Fridley, (2013), and Heberling et al., (2016). Healthy branches at or near the top of the leaf canopy were chosen for collection. Branches were cut from plants just before dawn on cool mornings to reduce water loss and were then immediately recut under water inside a bucket to restore xylem conductivity. We allowed the cut branches to acclimate in low light to lab conditions for 24-48 hours loosely covered under plastic wrap. Each morning, branches were recut under fresh water. Measured gas exchange parameters after this adjustment period are similar to those measured in the field for these species (Heberling et al. 2016). Leaf gas exchange was measured on one leaf per individual using an LI-6400 portable infrared gas analyzer (Li-Cor, Lincoln, Nebraska, USA) with a set flow rate of 700 µmol s^{-1} at 25 °C. Leaves were allowed to stabilize in the Li-Cor cuvette for at least 15 minutes prior to collecting data. Assimilation rate was measured at CO_2 concentrations in the following order: 400, 300, 200, 100, 50, 400, 400, 600, 800, and 1000 ppm (all at a saturating light level of 1000 PPFD [photosynthetic photon flux density - photons m^{-2} s^{-1}]). Following collection of A/Ci curves, A/q curves were collected using light levels of 200, 150, 100, 50, 20, and 0 PPFD at 400 ppm [CO_2]. Chlorophyll content was measured on two leaves per individual via reflectometry with a portable atLEAF+ meter (GREEN LLC, Wilmington, Delaware, USA). Three chlorophyll readings per leaf were taken, avoiding large veins. Leaves were scanned using a LI-3100 leaf area meter (Li-Cor, Lincoln, Nebraska, USA), dried for 48 hours at 70°C, and weighed to determine specific leaf area (SLA; cm^2 g^{-1}). Following weighing, leaves were ground and analyzed for total C and N content (N$_{mass}$, g m^{-2}; NC 2100, CE Elantech, Lakewood, NJ, USA). We used area-based leaf traits for all analyses. Mass-based leaf traits, such as %N and %C were converted to area-based using SLA.

We marked leaf nodes with colored string to track production of additional leaf cohorts throughout the growing season. In July and October, gas exchange and leaf properties were collected using the same methods as in the spring for each individual. If the individuals produced additional leaves after the placement of the shade cloth, gas exchange data and leaf traits were collected for these new leaves (cohort 2) in addition to the leaves belonging to the cohort produced in full sun (cohort 1). We considered determinate species to be those that did not produce any additional leaves after an initial spring flush and indeterminate species to be those that produced more leaves throughout the growing season. Leafing patterns were determined using both 2015 data and detailed leaf demography surveys from 2008-2010 (Fridley and Craddock, 2015). Leaves of the native species *Lindera benzoin* and *Hydrangea arborescens* were senescent by the October measurements and were given trait values of zero. After removal of the shade cloth in October, no species produced a third cohort of leaves in time for autumn measurements and therefore the maximum number of cohorts in our study is two. Time constraints prevented the monitoring of total leaf production in 2015, so we used estimates derived from a prior (2008-2010) study that tracked the total number of leaves produced per year on five randomly selected branches of each focal plant (Fridley, 2012; Fridley and Craddock, 2015). Annual rates of leaf production (g yr^{-1}) were calculated as the maximum amount of leaves produced per year in 2008-2010 for all five branches, multiplied by a species' average leaf mass.

Data Analysis

Photosynthetic parameters were modeled from gas exchange data by fitting the Farquhar, Von Caemmerer, and Berry (FvCB) model of C_3 photosynthesis in a hierarchical Bayesian framework (Farquhar et al. 1980; Feng and Dietze 2013; Peltier and Ibanez 2015; Heberling and Fridley 2016). We fit a separate hierarchical model for each species-season-leaf cohort combination with a random effect for block. We modeled maximum rate of carboxylation (V_{cmax}), apparent quantum yield (α), maximum rate of electron transport (J_{max}), maximum photosynthetic rate at saturating PPFD (A_{max}), and daytime dark respiration rate (R_d). Values of constants and priors for fixed and random variables are listed in Table 2. R_d was fit using data from both A/Ci and A/q curves, and A_{max} was calculated using the minimum of the light and CO_2-limited photosynthesis equations at 400 ppm [CO_2]. Models were parameterized in JAGS (Plummer 2003) using R2jags (Su and Yajima, 2015) in R (R Core Team, 2016) (JAGS v 3.4.0) (Methods S1&S2). Posterior estimates were derived from model runs with three parallel Markov chain Monte Carlo (MCMC) chains for 80,000 iterations (10,000 burn-in iterations were discarded). Convergence was assessed with the Gelman-Rubin (1992) diagnostic (<1.1). R code is included in Appendix A. Mean values of the posterior distributions of the above parameters were used in linear models to analyze seasonal trait plasticity and in a PCA incorporating lab-measured leaf traits.

We evaluated the contribution of seasonal trait plasticity to a species' overall carbon gain and competitive ability using two performance parameters: a C gain estimate using photosynthetic rates, and leaf production rates. We calculated the first performance parameter, mean instantaneous photosynthetic rate (MIPR), using A_{net} at 1000, 100, and 800 PPFD (at 400 ppm [CO_2]) during spring, summer, and autumn, respectively. Observed A_{net} values for each season were extracted from gas exchange curves, and then an average was calculated for each plant across the three seasons:

$$MIPR = (A_{1000} + A_{100} + A_{800})/3 \tag{1}$$

All other physiological parameters equal, leaf plasticity in response to varying light conditions contributes to fitness if such changes increase a species' overall mean photosynthetic rate across seasons. We thus used a species' MIPR as one measure of the adaptive significance of plasticity. Because annual carbon gain also depends on leaf allocation, we used our estimates of annual leaf production rate as a secondary performance measure related to fitness. These measures can describe plant growth potential in response to leaf plasticity. For both response/performance measures we used a generalized least squares (GLS) model that included, the mean and variance across three seasons of five area-based leaf traits (J_{max}, α, R_d, SLA, and N_{area}) as fixed-effects (eq. 2) using the 'nlme' package in R (Pinheiro et al., 2017). Our model structure took the form:

$$C \sim \mu T_1 + \sigma^2 T_1 + \mu T_2 + \sigma^2 T_2 + \ldots + \mu T_n + \sigma^2 T_n \tag{2}$$

where *C* = MIPR or leaf production rate and *T* = mean leaf trait for a group of native or non-native plants. In this equation plasticity is estimated by the variance of each trait across the entire growing season. The use of trait means and variances in a regression to quantify the adaptive significance of plasticity follows the approach of Lacey et al. (1983). Significant relationships of trait variance to performance parameters related to fitness (here, MIPR and annual leaf production) have been called 'adaptive phenotypic plasticity' (van Kleunen & Fischer 2005), which we estimate here by comparing regression coefficients of standardized mean and variance statistics across seasons for leaf traits in relation to assimilation and growth rates (C) (eq. 2). GLS models included a phylogenetic distance matrix to account for phylogenetic autocorrelation (Fig. S1; phylogeny from Jo et al. 2016). We created separate models for native and non-native species and for each dependent performance variable (MIPR and annual leaf production). For native species, we fit separate models for determinate and indeterminate species. For non-native species there were insufficient determinate species to merit separate models for leafing class. Additional separate models were also created for indeterminate plants including and excluding second cohort leaf traits to examine the adaptive significance of second leaf cohort production in within plant plasticity. V_{cmax} was excluded from GLS models due to high collinearity ($r > 0.7$) with other assimilation parameters.

We compared GLS model fits incorporating phylogenetic autocorrelation with standard linear mixed models (lmer; package 'lme4') that included a random effect for block (Bates et al., 2015). To fit a phylogenetic regression using GLS, we produced correlation matrices using different values of phylogenetic signal (Pagel's λ) and used the correlation matrix producing the lowest regression AIC value ($\lambda = 0.8$). The use of the phylogenetic correlation matrix did not qualitatively alter regression coefficients (Table S5) compared to the random block model, so we report results of the latter model below.

To compare suites of leaf traits between nativity groups and across seasons, we performed a principal component analysis (PCA) on ranked values of all leaf traits and photosynthetic parameters. Multivariate inference was based on Hotelling tests for season, nativity, and their interaction, and post-hoc corrections on individual leaf traits included Hochberg's test (Curran, 2017). Lastly, we performed Wilcoxon rank sum tests between native and non-native and determinate and indeterminate groups to compare differences in MIPR, leaf production rates, and photosynthetic leaf traits. All statistical analyses were performed using R v3.3.1 (R Development Core Team, 2017).

Results

Seasonal variation in leaf traits

All leaf traits varied seasonally (Fig. 2). Overall, non-native species had higher mean trait values than native species. Trait values frequently associated with sun leaves, such as high V_{cmax}, J_{max}, R_d, and A_{max}, were highest in the spring, decreased in summer, and increased or maintained similar values from summer into autumn. Trait values associated with shade-adapted leaves, such as high chl content and α, were highest in the summer (Fig. 2). N_{mass} (not shown) was highest in the spring and decreased gradually until leaf senescence. However, N_{area} stayed relatively constant throughout the year, increasing slightly for non-natives and indeterminate native species consistent with gradual leaf thickening (decreasing SLA; Fig 2). Non-native species had higher trait values associated with high photosynthetic rates (V_{cmax}, J_{max}) in spring and autumn compared to native species than in the summer (Fig. 2). All non-native species maintained higher values of V_{cmax} into autumn, whereas only leaves of the second cohort of native species maintained similar V_{cmax} values into autumn. Similarly, all second-cohort leaves and determinate non-native species maintained higher J_{max} values into autumn than determinate native species (Fig 2). Indeterminate plants had 14% higher MIPR than determinate plants (W=4260, p<0.01; 7.06 vs. 6.21 µmol CO_2 m^{-2} s^{-1}, respectively). Non-native plants had 21% higher MIPR than native plants (W=4987, p<0.001; 7.42 vs. 6.12 µmol CO_2 m-^{2} s^{-1}, respectively). Indeterminate plants had 58% higher leaf production rates than determinate plants (W=4584, p<0.001; 5.66 vs. 3.59 g, respectively). Differences in MIPR between native and non-native species did not translate into differences in leaf production rates (W= 3238, p=0.39).

Influence of trait plasticity on fitness parameters

The contribution of seasonal plasticity to MIPR across species varied by whether a species produced more than one leaf cohort. Overall, determinate (single-flush) species displayed much less seasonal plasticity than indeterminate species (Table 3). For non-native species, no leaf traits were significantly related to MIPR, but effect sizes (standardized coefficients) were largest for variance of α and mean SLA. For native determinate species there were no significant effects of leaf trait means or plasticity on A_{net}, but high SLA and R_d values had relatively strong negative effects on MIPR. For native indeterminate species seasonal plasticity played a more important role in maintaining high MIPR. The analysis including seasonal changes in only the first cohort of leaves suggests seasonal plasticity is not important for indeterminate species as no trait's variance significantly affected MIPR. However, including the second cohort of leaves in the plasticity analysis indicated high individual-level plasticity in α marginally increased MIPR, and high plasticity in R_d decreased MIPR. Additionally, high mean SLA negatively related to MIPR using the second leaf cohort of indeterminate species (Table 3).

The effects of seasonal leaf trait plasticity and leaf trait values were less pronounced on leaf production rates. For non-native species there were no significant effects of mean leaf traits or trait variance on leaf production rate. However, mean N_{area} and variance in N_{area} had the strongest negative effects and mean α had the strongest positive effect. Determinate native species' leaf production was most positively affected by mean α and variance in N_{area}, and most negatively affected by mean N_{area}, though neither were significant predictors. For native indeterminate species variance of SLA of the first cohort of leaves was significantly negatively correlated with leaf production, as was variance in R_d. For the second cohort leaves of indeterminate native species, variance in J_{max} significantly increased leaf production rates, while variance in R_d had a marginally significant negative effect on leaf production rate (Table 3).

Multivariate patterns across seasons

Overall, native and non-native species showed similar directional changes in leaf traits with respect to season and light level (Fig. 3). However, the large spring-to-summer changes in leaf traits present in non-natives were generally absent in natives, and non-native and native species varied significantly both from each other and seasonally in multivariate trait space. From spring to summer, leaves of both non-native and native species showed increased α and decreased A_{max}, J_{max}, and V_{cmax} values. From summer to autumn, non-natives decreased SLA and reduced α and V_{cmax} values. J_{max} was the only trait significant for predicting variation in traits between first and second cohorts of leaves (Fig. 3).

Discussion

Our analysis of leaf traits of ENA deciduous understory species suggests that leaves of non-native species exhibit greater seasonal plasticity than native species. This finding is in agreement with the long-standing hypothesis about 'ideal weeds' originally proposed by Baker that phenotypic plasticity is a proximate driver of species invasions (Baker, 1965; Richards et al., 2006; Funk, 2008; Paquette et al., 2012). Plasticity has been theorized to benefit non-natives by allowing them to maintain 'fitness homeostasis' in a variety of environments (Rejmánek, 2000; Richards et al., 2006). In contrast to this hypothesis, we found no evidence that such leaf plasticity is associated with annual carbon gain in non-native species or their annual rate of leaf production. Non-native species in our study maximized MIPR primarily by maintaining high values of all leaf traits throughout the year, rather than by adjusting leaf traits. While ours is not the first study to conclude that non-native species seasonal plasticity is not always adaptive (Davidson, Jennions & Nicotra, 2011), the relatively large species comparison suggests leaf plasticity per se is not a common mechanism of invasions in temperate forests (but see Xu et al., 2007).

In lieu of leaf adjustments throughout the growing season, our results suggest that the success of non-native forest shrubs is driven by higher values of leaf traits associated with high assimilation rates (Fig 2). Native species' MIPR was more strongly associated with high quantum efficiency (α) in summer, suggesting higher shade tolerance that does not lead to higher overall growth rates. Although high quantum efficiency is not solely associated with shade tolerance, it is commonly found in shade tolerance trait syndromes such as low R_d, high SLA, and low V_{cmax}. The ability of non-native species to maintain higher V_{cmax}, J_{max}, and α values during all seasons may be due to resource allocation tradeoffs associated with defense, leaf toughness, enemy release, leaf N partitioning, and belowground allocation (Jo, Fridley & Frank, 2015) not measured in this study (Feng, Auge & Ebeling, 2007; Feng et al., 2009). Although non-native species in our study system generally have longer leaf lifespan (Heberling and Fridley, 2013), it is yet unclear whether leaf defense- or structure-related chemistry is different across native and non-native groups. Native species in our study may have evolved to have highest leaf function during shaded summer conditions due to historically higher climate variability and extreme events in ENA forests relative to East Asian and European forests (Rahmstorf and Coumou, 2011; Augspurger, 2013; Zohner et al., 2017). This strategy likely allowed natives to avoid risky periods of leaf display in early spring or late autumn when extreme cold events are most likely to occur. Whether climate change will reduce or intensify the occurrence of such extreme events relative to the foliar phenology of native and non-native species is unclear (Rahmstorf and Coumou, 2011; Augspurger, 2013), and likely to influence the relative importance of leaf plasticity versus restrictive phenology to the fitness of deciduous species.

Indeterminate native plants showed a clear photosynthetic advantage over determinate species in our study. The significance of plasticity in indeterminate plants lies in the production of new leaves midseason; no variance terms were predictive for MIPR or leaf production rate when only the first cohort of leaves were analyzed, except for SLA plasticity (Table 3). Plasticity in SLA describes the tendency of leaves of most species to thicken over the growing season. Maximum electron transport rate (J_{max}) and quantum efficiency (α) had the largest effect sizes of the photosynthetic traits for these species most associated with adaptive plasticity, which is reflected in the strong tendency of second cohort leaves to exhibit high J_{max} and low light harvesting in autumn compared to first cohort species (Fig. 2). As high α and J_{max} are often associated with adaptation to low and high light conditions, respectively (Lambers et al., 1998, Valladares & Niinemets, 2008), the production of new leaf cohorts in indeterminate species appears to be an adaptation allowing for renewed high A_{net} late in the season after canopy senescence. However, because a high correlation between J_{max} and maximum carboxylation rate (V_{cmax}) prevented our use of both traits in plasticity analyses, we cannot exclude potential late-season increase in V_{cmax} as an additional feature of adaptive leaf plasticity in indeterminate species. V_{cmax} variation across leaves is driven by investment in rubisco (Niinemets & Tenhunen 1997); isolation of the seasonal concentration of rubisco, chlorophyll, and membrane-associated nitrogen in leaves of understory species (Hikosaka and Terashima, 1996) would therefore help to explain mechanisms of adaptive leaf changes in these species.

One caveat in our interpretation is that the summer light levels present in our common garden (20% of above-canopy radiation) may not be representative of light levels of understories where some determinate species typically grow (<10%, Fig. 1A). Had we subjected plants to lower light levels in the summer, photosynthesis of some determinate natives may have been higher than non-native species that prefer sunnier conditions. Compared to natural forests the shade cloth treatment in our common garden also reduces the occurrence of sunflecks, which drive photosynthesis in some understory species (Way and Pearcy, 2012). Due to pragmatic constrains, we did not measure total or integrated seasonal C gain for our species which may have reduced the power of our adaptive plasticity analyses. Temperature dependency of photosynthesis is also known to vary by species (Berry and Bjorkman, 1980; Battaglia, Beadle & Loughhead, 1996), and we did not account for fluctuating temperatures in the spring and fall that could impact photosynthetic rate (Dougherty et al., 1979). Nonetheless, our results suggest that for a wide variety of deciduous forest species that are not associated with extreme summer shade (Fig. 1), seasonal plasticity in leaf structure or chemistry is not the primary mechanism of understory non-native species' success.

Conclusion

We found that understory shrub leaf traits vary seasonally with light availability in a simulated deciduous forest environment. However, contrary to our expectations, most leaf-level seasonal plasticity did not strongly contribute to photosynthetic rates or leaf growth. We found little evidence that seasonal leaf plasticity is a general explanation for the success of non-natives in deciduous forests. Non-native species did have higher overall photosynthetic traits (V_{cmax}, J_{max}, A_{max}, N_{area}; Fig 2) across seasons than native species. These higher photosynthetic trait values likely contribute significantly to their invasion success in ENA forests. In contrast, we found that seasonal plasticity does contribute to annual leaf production in native indeterminate plant species. Specifically, indeterminate plants adjusted J_{max} and α to their advantage depending on seasonal light availability through the production of mid-season, shade-adapted leaves. Indeterminate species also had high photosynthetic rate overall, indicating an advantage to having an indeterminate growth strategy if resource levels are high enough to support continued leaf production. Seasonal plasticity may drive fitness for some native continuously growing, indeterminate plants, but we found no evidence that seasonal plasticity drives non-native species invasions in ENA forest understories. Instead, fitness for non-native species appears to be driven by maintenance of high photosynthetic trait values throughout the growing season.

Author Contributions

KAM and JDF conceptualized and designed the experiment. KAM collected the data and performed statistical analyses, and led manuscript writing. JDF contributed to statistical analyses and manuscript writing. Both authors approved this final draft for submission.

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Table 1: Study species, *indicates indeterminate plants. NA=North America (USDA, NRCS 2017).

Species	Family	Nativity	Origin
Sambucus racemosa	Adoxaceae	Native*	Circumboreal
Viburnum acerifolium	Adoxaceae	Native	NA
Viburnum dentatum	Adoxaceae	Native*	NA
Viburnum dilatatum	Adoxaceae	Non-native	Asia
Viburnum lantana	Adoxaceae	Non-native	Europe/Asia
Viburnum prunifolium	Adoxaceae	Native*	NA
Viburnum rafinesquianum	Adoxaceae	Native	NA
Viburnum setigerum	Adoxaceae	Non-native*	Asia
Berberis thunbergii	Berberidaceae	Non-native*	Asia
Calycanthus floridus	Calycanthaceae	Native*	NA
Lonicera hirsuta	Caprifoliaceae	Native	NA
Lonicera japonica	Caprifoliaceae	Non-native*	Asia
Lonicera maackii	Caprifoliaceae	Non-native*	Asia
Lonicera reticulata	Caprifoliaceae	Native	NA
Lonicera sempervirens	Caprifoliaceae	Native*	NA
Lonicera tataricum	Caprifoliaceae	Non-native	Asia
Celastrus orbiculatus	Celastraceae	Non-native*	Asia
Celastrus scandens	Celastraceae	Native	NA
Euonymus alatus	Celastraceae	Non-native	Asia
Euonymus atropurpureus	Celastraceae	Native	NA
Euonymus bungeanus	Celastraceae	Non-native*	Asia
Euonymus obovatus	Celastraceae	Native	NA
Cornus amomum	Cornaceae	Native*	NA
Elaeagnus umbellata	Elaeagnaceae	Non-native*	Europe/Asia
Elaeagnus commutata	Elaeagnaceae	Native*	NA
Hamamelis virginiana	Hamamelidaceae	Native*	NA
Hydrangea arborescens	Hydrangeaceae	Native	NA
Lindera benzoin	Lauraceae	Native*	NA
Frangula alnus	Rhamnaceae	Non-native*	Europe
Rhamnus cathartica	Rhamnaceae	Non-native	Europe

Table 2: Bayesian priors and parameter information for the model used to fit photosynthetic parameters.

Symbol	Definition	Term Type	Prior Distribution
R_d	mitochondrial daytime respiration rate	parameter	dnorm(1, 1/variance between replicate individuals)
V_{cmax}	maximum carboxylation rate of rubisco	parameter	dnorm(25, 1/variance between replicate individuals)
α	quantum efficiency of electron transport	parameter	dlnorm(.24, 1/variance between replicate individuals)
A_{max}	Maximum photosynthetic rate	parameter	calculated using modeled photosynthetic data using ambient CO_2 (400 ppm)
J_{max}	Maximum electron transport rate	parameter	dnorm(55, 1/variance between replicate individuals)
Ci	Intercellular partial pressure of CO_2	independent variable	data
Gamma*	CO_2 compensation point	independent variable	data
q	photosynthetic photon flux density (PPFD)	independent variable	data
RE indiv	Random effect for individual plants	parameter	
O	intercellular O_2 partial pressure (kPa)	constant	21
Variance	model variance		
A_{net}	observed net photosynthetic rate	dependent variable	data
α A_{net}	modeled net photosynthetic rate		Either Aj or Av, depending on which was lowest
A_v	carboxylation limited portion of A/Ci curve	parameter	Model fit of V_{cmax}
A_j	electron transport limited portion of A/Ci curve	parameter	Model fit of electron transport rate

Table 3: Results of plasticity regression analysis (gls) on MIPR (μmol CO$_2$ m^2 s^{-1}) and leaf production (g) across three seasons. We used separate models for non-natives, native determinate species and native indeterminate species (first and second leaf cohorts). “ns” = non-significant, “.”= P=0.07, “*” = P<0.05, “**” = P<0.01, “***” = P<0.001. Significant effects are bolded.

	Non-native		*Native Determinate*		*Native Indeterminate – 2nd Cohort*		*Native Indeterminate – 1st Cohort*	
	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P
MIPR								
R_d μ	0.04	ns	-0.73	ns	-0.12	ns	-0.71	ns
R_d σ^2	0.17	ns	0.29	ns	**-0.53**	.	0.08	ns
α μ	0.38	ns	0.37	ns	0.75	ns	0.80	ns
α σ^2	0.28	ns	-0.36	ns	**1.11**	.	0.25	ns
SLA μ	-0.03	ns	-1.15	ns	**-2.44**	*	-0.92	ns
SLA σ^2	0.10	ns	-0.32	ns	0.14	ns	0.93	ns
N g/cm^2 μ	0.27	ns	-0.38	ns	-0.54	ns	0.63	ns
N g/cm^2 σ^2	0.06	ns	0.30	ns	0.51	ns	0.15	ns
J_{max} μ	0.14	ns	0.81	ns	0.40	ns	0.15	ns
J_{max} σ^2	-0.10	ns	-0.55	ns	0.61	ns	0.67	ns
Leaf Production								
R_d μ	-0.52	ns	0.33	ns	-0.24	ns	-1.10	ns
R_d σ^2	0.47	ns	-0.01	ns	**-2.87**	.	1.92	ns
α μ	-0.19	ns	1.69	ns	3.17	ns	0.49	ns
α σ^2	1.90	ns	0.02	ns	2.85	ns	-0.46	ns
SLA μ	1.95	ns	0.17	ns	-0.52	ns	1.19	ns
SLA σ^2	-0.55	ns	-1.10	ns	1.14	ns	**-5.76**	*
N g/cm^2 μ	-1.50	ns	-1.27	ns	1.63	ns	-5.28	ns
N g/cm^2 σ^2	-1.37	ns	1.64	ns	-2.95	ns	2.26	ns
J_{max} μ	0.75	ns	1.33	ns	-1.06	ns	6.79	ns
J_{max} σ^2	0.58	ns	-0.77	ns	**5.03**	*	-1.35	ns

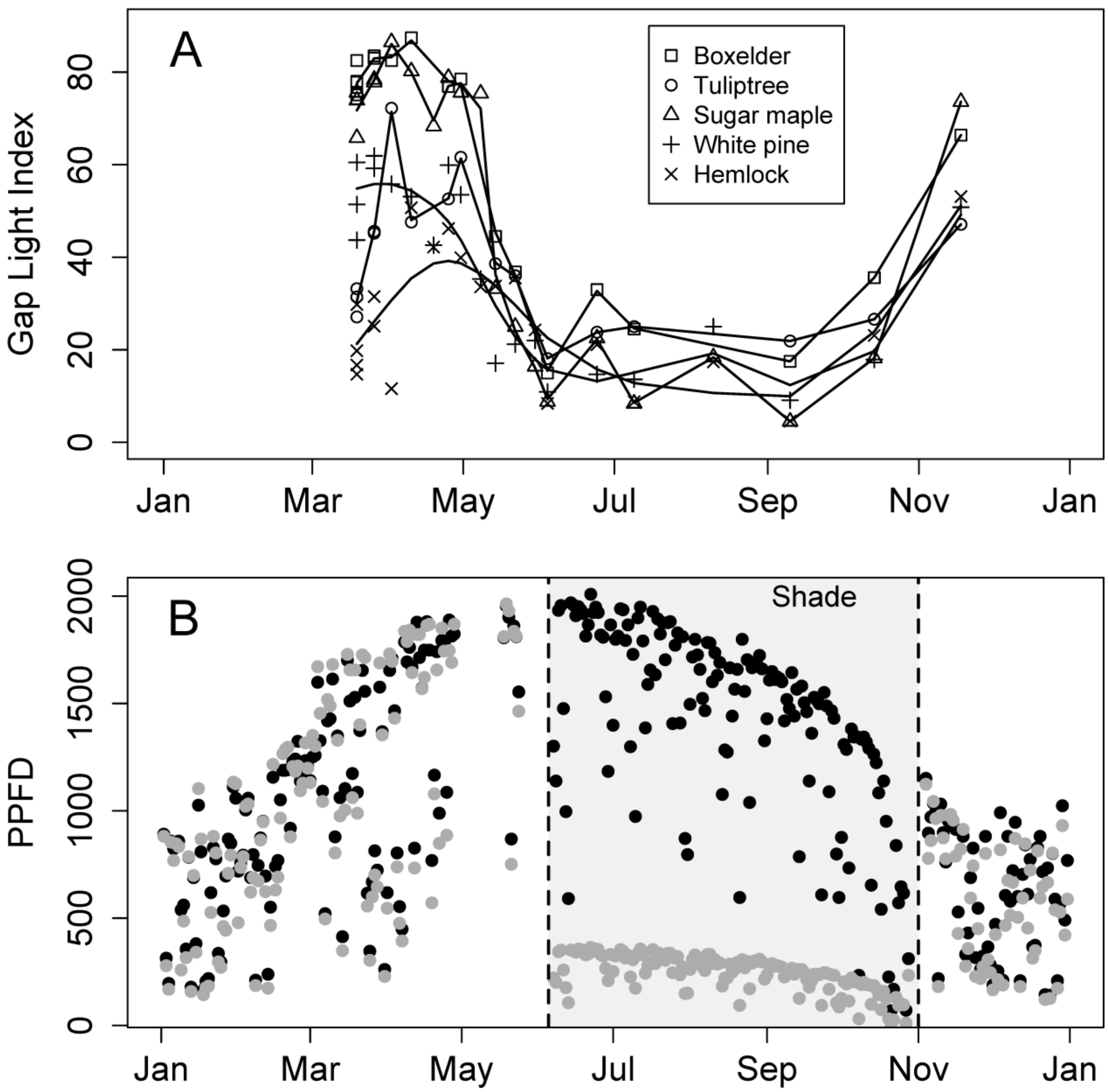
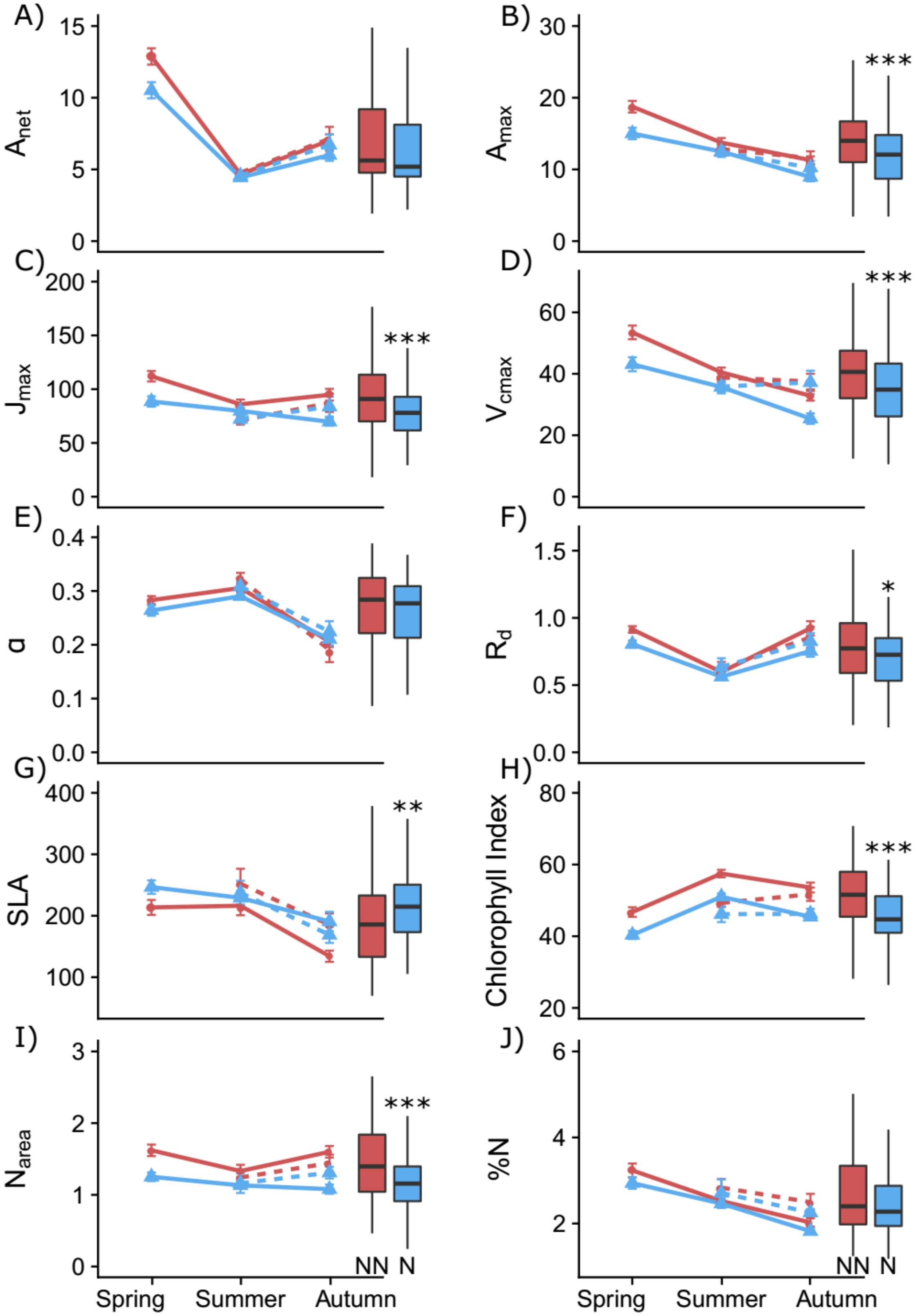


Figure 1: A) Seasonal trajectory of understory light levels in five forest types common to central New York, as measured by the Gap Light Index (Frazer et al. 1999) derived hemispherical photographs taken from Mar to Dec 2016 at a height of 2 m, with loess regression lines. ‘Boxelder’ is a <50 yr stand of deciduous *Acer negundo*; ‘Tuliptree’ is a <80 yr stand of deciduous *Liriodendron tulipifera*; ‘Sugar maple’ is a >200 yr mixed stand dominated by deciduous *Acer saccharum* and evergreen *Tsuga canadensis*; ‘White pine’ is a <100 yr plantation of evergreen *Pinus strobus*; and ‘Hemlock’ is a >200 yr mixed stand dominated by *T.*

canadensis. B) Maximum daily photosynthetic photon flux density (µmol m^{-2} s^{-1}) from quantum sensors located above (black) and below (gray) shade cloth at the common garden study location, in a year subsequent to that of the current study where the shade cloth was deployed from June 5 to Nov 1. In the present study, we removed the shade cloth on Oct 1.



A)
A_{net}
0
5
10
15
B)
A_{max}
0
10
20
30
***
C)
J_{max}
0
50
100
150
200
***
D)
V_{cmax}
0
20
40
60
***
E)
α
0.0
0.1
0.2
0.3
0.4
F)
R_d
0.0
0.5
1.0
1.5
*
G)
SLA
100
200
300
400
**
H)
Chlorophyll Index
20
40
60
80
***
I)
N_{area}
0
1
2
3
***
J)
%N
2
4
6
NN
N
NN
N
Spring
Summer
Autumn
Spring
Summer
Autumn

Figure 2: Mean and standard error of A) observed A_{net} (µmol CO_2 m^2 s^{-1}), B) A_{max} (µmol CO_2 m^2 s^{-1}), C) J_{max} (µmol e^- m^2 s^{-1}), D) V_{cmax} (µmol CO_2 m^2 s^{-1}), E) α (mol e^- mol $quanta^-$), F) R_d (µmol CO_2 m^2 s^{-1}), G) SLA (cm^2/g), H) Chlorophyll index, I) N_{area} (g/m^2) , and J) %N, across 3 seasons and for each leaf cohort. Solid lines indicate values for first cohort leaves, dashed lines indicate means for second cohort leaves. Circles and triangles represent non-native and native species, respectively. Insets report overall means for all leaf cohorts of non-native and native species groups ("NN" and "N", respectively). Asterisks in insets indicate results of Wilcox rank-sum tests; *=p<0.05, **=p<0.0, ***=p<0.001. A_{net}: W=11126; A_{max}: W=12492; J_{max}: W=12553; V_{cmax}: W=12812; α: W=11137; R_d: W=11432; SLA: W=7906; %N: W=10388; N_{area}: W=12284; chlorophyll index: W=14134.

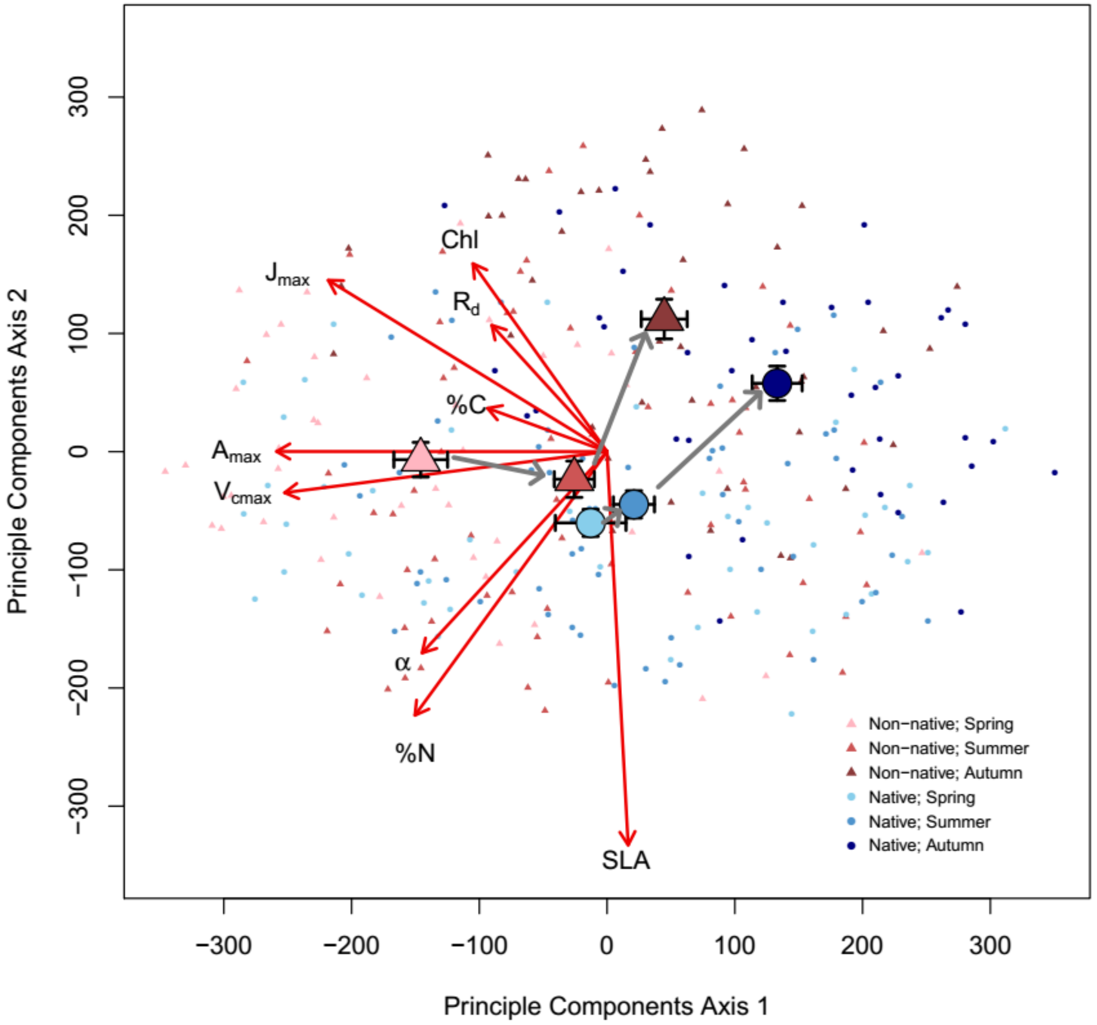


Figure 3: PCA of area-based leaf traits. Large triangles and circles represent nativity-season centroids +/- SE. Small points represent individual leaves. Gray arrows show trajectory of centroids for natives and non-natives. Red arrows represent trait vectors. Axes 1 and 2 explain 38% and 20% of variation, respectively.

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CHAPTER 3: EVIDENCE FOR BOTH PRE-ADAPTATION AND RAPID EVOLUTION IN THE INVADED RANGE OF FOREST INVADERS

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Abstract

Temperate forests are widely invaded by shade-tolerant shrubs and trees, including those of Eastern North America (ENA). However, it remains unknown whether these invaders are 'pre-adapted' for success in their new ranges due to unique aspects of their evolutionary history, or whether selection due to enemy release has driven rapid evolution in the invaded range. We sampled leaf traits of populations of woody understory invaders across light gradients in their native range in Japan and in their invaded ENA range to examine potential phenotypic shifts related to carbon gain between ranges. We also measured leaf traits in three co-occurring ENA native shrub species. In their invaded range, invaders invested significantly less in leaf chlorophyll content (both per unit leaf mass and area) compared to native range populations of the same species yet maintained similar rates of photosynthesis in low light. On the other hand, compared to ENA natives, ENA invaders displayed greater trait variation in response to increasing light availability (forest edges, gaps) in both their native and invasive ranges, giving them a potential advantage over ENA natives in a variety of light conditions. We conclude that both evolutionary history in the native range and new selection pressures in the invaded range underlie the success of many shade tolerant forest invaders.

Keywords

Invasive species, ecophysiology, photosynthesis, chlorophyll content, shade tolerance

Introduction

Temperate deciduous forests are increasingly invaded by introduced woody plants, despite relatively low disturbance rates (Rejmánek 2014). This is particularly true in Eastern North America (ENA), where dozens of species of Old World shrubs and lianas dominate the understory of closed-canopy forests that are otherwise inhabited by mature native trees and shrubs. Many of these species are highly shade tolerant (Fridley 2008; Martin, Canham & Kobe 2010; Heberling & Fridley 2013, 2016; Martinez & Fridley 2018) and some are also highly productive in forest interiors, gaps, and edge habitats compared to native species (Ellsworth, Harrington & James 2004; Leicht & Silander 2006). Mechanisms explaining how these invaders proliferate and outcompete co-occurring native species are not well understood. It is unclear whether non-native forest invaders have evolved competitive phenotypes de novo in their introduced range—for example, due to shifts in resource allocation from defense to growth (Blossey & Notzold 1995)–or whether certain introduced lineages are ‘pre-adapted’ for success as invaders due to unique aspects of their ancestral environments (Fridley & Sax 2014). These two mechanisms of invasion are not mutually exclusive, as both genetic changes in the invaded range and pre-adaptation can occur within multiple traits that affect population growth and potentially fitness. Studies of plant function in native and invaded range populations remain relatively rare but are required to address whether pre-adaptation or evolution in the invaded range contributes to their population growth as invaders (Parker *et al.* 2013). Although traits of invaders have frequently been compared to those of native co-occurring or congeneric species in the invaded range (Niinemets, Valladares & Ceulemans 2003; Hierro, Maron & Callaway 2005; Xu, Griffin & Schuster 2007; Heberling & Fridley 2013, 2016), few studies have compared physiological traits of invaders in their native and invaded ranges, and even fewer studies have involved woody plants (cf. Reinhart & Callaway 2004; Heberling *et al.* 2016).

Relative to co-occurring native shrub species, shade-tolerant invaders often have increased photosynthetic rates (Heberling & Fridley 2013), faster responses to increases in light availability (Yamashita *et al.* 2000), and higher trait variation in response to light (Niinemets, Valladares & Ceulemans 2003b; Funk 2008; Paquette *et al.* 2012). One or a combination of such photosynthetic advantages may lead to increased growth rates and fecundity (Valladares & Niinemets 2008). However, it is unknown whether these traits are also present in the native range of invaders or if selection and admixture (i.e. bottlenecks or genetic drift, repeated introductions) have fostered the evolution of new phenotypes in their invaded ranges (Tsutsui *et al.* 2000; Lee 2002; Maron *et al.* 2004; Puzey & Vallejo-Marin 2014).

Trait variation within and among populations may facilitate species invasions by allowing invaders to colonize a wider variety of environments with greater success than natives (Baker 1965; Richards *et al.* 2006; Funk 2008; Paquette *et al.* 2012; Lamarque *et al.* 2015). Trait variation in response to light availability is often a key contributor to woody forest plant fitness, given the importance of canopy disturbance for long-term survival of most tall-stature plants (Canham 1989). One way that invaders may display higher trait variation than native species is through quickened leaf responses to fluctuating light resources caused by canopy gap formation (Davis, Grime & Thompson 2000). For example, some understory trees are capable of increasing mesophyll surface area after canopy gap formation to increase photosynthetic rate (Oguchi *et al.* 2006). Furthermore, although trait variation can be costly (DeWitt, Sih & Wilson 1998; Relyea 2002; Oguchi, Hiura & Hikosaka 2017), invaders may experience lower energy or nutrient costs in leaf function. These lower costs are a result of reduced need for chemical or structural defenses against specialist herbivores that are lacking in the invaded range. For example, variability of allocation in leaf nitrogen (N) may be beneficial for shade tolerant understory plants if light levels or defense needs are variable, especially since N often limits growth in temperate deciduous forests (Lebauer & Treseder 2016). However, debate remains about the benefits of trait variation to population growth because invader trait variation with respect to some environmental gradients need not be adaptive (Funk 2008, Martinez & Fridley 2018).

Leaf function depends on the allocation of N to three major functions: metabolism (photosynthesis and respiration), defense (N-containing secondary compounds) (Gleadow, Foley & Woodrow 1998), and structure (Evans 1989). In metabolism, N is primarily allocated to either thylakoid membrane proteins and light cycle pigments (i.e. chlorophyll [chl]) or Calvin cycle enzymes, including rubisco. In theory, an optimal partitioning of N exists for each leaf at a given light level that maximizes daily photosynthesis and photosynthetic nitrogen use efficiency (PNUE, carbon assimilated per unit leaf N) (Hikosaka & Terashima 1995). Changes in leaf N allocation and maintenance of high PNUE in response to shifting light availability can enhance carbon gain for invaders relative to co-occurring native species with lower PNUE (Heberling & Fridley 2013). Although some species of woody, shade-tolerant invaders have been found to have higher PNUE in their invasive ranges (Heberling & Fridley 2013; Heberling *et al.* 2016), it remains unclear whether this is an advantage at both high and low light levels. In addition, one study of native and invaded range N allocation in an invasive herbaceous plant, *Ageratina* *adenophora*, showed a decrease in cell wall N and an increase to photosynthetically active compounds in the invasive range (Feng et al. 2009). This change in N allocation may relate to leaf structural defense since cell wall proteins aid in cell wall strength (Feng et al. 2009). If invaders allocate leaf N differently than co-occurring native species, this may explain the photosynthetic advantages found in prior studies of invaders.

The goal of our study was to quantify differences in leaf traits between native and non-native invasive species and estimate their variance across populations of different light levels (hereafter interpopulation trait variability, or ITV). We focused on populations in the native and invaded ranges of four woody, understory invaders native to East Asia (EAS), and compared leaf traits with co-occurring, native Eastern North American (ENA) species (Table 1). Our study is unique in that it includes trait values along an environmental gradient for a suite of invasive species in both their native and invaded ranges. We were specifically interested in understanding how N allocation influences photosynthetic rates in invaders along a light gradient, and the distribution of structural leaf traits associated with herbivore defense in their native and invaded ranges. We hypothesized that invaders 1) invest less in structural defense in the invaded range due to enemy release (low LDMC and high SLA), and as a consequence, 2) have higher PNUE than natives, 3) allocate leaf N differently in their away range (e.g., chl per unit leaf N; Chl_N) that increases PNUE for invaders compared to individuals in the native range (Heberling *et al.* 2016), and 4) display greater ITV than co-occurring natives in N allocation along forest light gradients.

Methods

Study Species

Our focal species included four species native to EAS and invasive in ENA: *Celastrus orbiculatus* (liana in the Celastraceae), *Lonicera japonica* (liana in the Caprifoliaceae), *Viburnum dilatatum* (shrub in the Adoxaceae), and *Euonymus alatus* (shrub in the Celastraceae). Co-occurring ENA natives included *Prunus virginiana* (shrub/tree in the Rosaceae), *Cornus racemosa* (shrub in the Cornaceae), and *Viburnum acerifolium* (shrub in the Adoxaceae). We also included the circumboreally distributed *Sambucus racemosa* (shrub in the Adoxaceae), considered native to both regions. All EAS species are shade tolerant, deciduous plants that occur in forest interiors in both their native and invasive ranges. ENA invaders were selected based on knowledge that they frequently occurred in similar forest types and forest interiors in ENA compared to EAS forests.

Study Sites

We measured leaf traits on ENA invaders in both Japan (native range) and New York (NY)/Pennsylvania (PA) (invasive range) and the same traits of co-occurring ENA native species in NY and PA. All measurements were taken in July of either 2016 or 2017. We selected plants from two to three populations per species in each range (3 sites in Japan, 3 sites in ENA [NY and PA]). Within each location, individuals were selected along a light gradient. Individuals occurring in high light were located near forest edges or large gaps, and individuals in deep shade were in forest interiors. Invaders and *S. racemosa* were measured in forests near Sendai, Japan; in forests in the Hudson Valley, NY (Cary Institute for Ecosystem Studies; Cary IES, 41.79, -73.74; and in State College, PA (Penn State Arboretum, 40.81, -77.87). In Japan, sites included a primary growth forest in the Botanical Gardens of Tohoku University (38.26, 140.85), a secondary forest in the Experimental Station for Medical Plant Studies, Graduate School of Pharmaceutical Sciences, Tohoku University (38.26, 140.84), and a secondary forest in an experimental garden at the Graduate School of Life Sciences, Tohoku University (38.26, 140.83). ENA native species, including *S. racemosa*, were measured at Cary IES, Penn State Arboretum, and in a forest near Pompey, NY (42.92, -76.04). In Japan, six to seven individuals of each invader and *S. racemosa* were sampled across the three field sites and along a light gradient. We attempted to create similar light gradients for each species at each site. For ENA samples, at least six individuals of each species were sampled along a light gradient (Table 1). Hemispherical photographs were taken above each plant using a Canon T2i or Nikon D5300 digital SLR camera and Polaroid .21x super fisheye lens filter attachment.

Leaf Trait Measurements

We measured photosynthetic rates using a portable infrared gas analyzer (LI-6400, Li-Cor, Lincoln, Nebraska, USA). All individuals were at least second-year plants and had woody tissue present. Plant height ranged from 0.3 to 1.5 m. Photosynthetic rates (A_{50} and A_{1000}) were measured at photosynthetic flux densities (PPFD; µmol quanta m^{-2} s^{-1}) of 50 and 1000 because these values bracket typical July light values in deciduous forests (Martinez & Fridley 2018). We selected healthy leaves near the top or outside of the plant canopy for photosynthesis and leaf trait measurements. Concentration of CO_2 was maintained at 400 µmol mol^{-1}, and leaf vapor pressure deficit was kept below 1.5 kPa. In Japan, leaf cuvette temperature was maintained at 30 °C and in ENA, at 25 °C. We chose these temperatures because they were nearest to ambient conditions. All photosynthetic measurements were taken before 1:00 PM.

Eight leaves were collected from each plant for further analysis. We determined specific leaf area (SLA cm2/g), total nitrogen (N) content, and total leaf carbon (C) content using three leaves per individual. Fresh leaves were scanned to determine leaf area, dried for > 48 hours at 65 °C, and weighed. Total leaf N and C content was analyzed using an NC 2100 autoanalyzer for ENA plants (CE Elantech, Lakewood, NJ, USA) and a Vario EL III autoanalyzer for Japan plants (Elementar Analyser Systeme, Hanau, Germany). Leaf N and C content were determined using three leaves per individual. Photosynthetic nitrogen use efficiency (PNUE) was calculated as $A_{1000mass}$ /N_{mass}. Leaf dry mass content (LDMC) was determined using three fully hydrated leaves, with leaves placed in plastic bags with moistened paper towels for 24 hr before weighing fresh mass. Leaf chl content was determined using leaf disk punches with an area of 0.79 or 2.00 cm^2 from one leaf per individual, avoiding large midveins. Leaf disk punches were placed in dimethylformamide for at least 24 hr before chl content was determined spectrophotometrically at wavelengths of 646.8 and 633.8 nm (Porra, Thompson & Kriedemann 1989). We calculated low- and high-light chl use efficiency ($A_{50\ chl}$ and $A_{1000\ chl}$ respectively) by dividing A_{50area} and $A_{1000area}$ by chl_{area} ($\mu g/cm^2$).

Data Analysis

Gap light index (GLI) values (0-1) for each plant were calculated from hemispherical photos using Gap Light Analyzer software v2.0 (Frazer, Canham & Lertzman 1999). To determine differences in photosynthetic trait values for invaders and natives, we grouped plants into three categories: EAS natives (plants native to and growing in East Asia), ENA invaders (East Asian species growing in their invasive ENA range), and ENA natives. *Sambucus* *racemosa* fell in either EAS natives or EAS natives depending on the location of individuals measured.

We used linear mixed models (lme4 in R, Bates *et al.* 2017) to test for differences in leaf traits between each of these three plant groups. Data for LDMC were arcsine transformed. We included log-transformed GLI as a fixed effect, and incorporated species and site nested in continent as random effects. If the interaction terms between GLI and nativity/range were non-significant (*P*>0.05), they were excluded from the final models. Tukey's HSD tests were performed on mixed model results (excluding interaction terms).

ITV with respect to light was evaluated in separate linear mixed models for each plant group (data subsetted by ENA invaders, ENA natives, EAS natives) with a random intercept included for species (leaf trait ~ logGLI + (1|species)). We used a linear mixed model with random effects for species and location to test for differences in GLI between nativity/range groups. Finally, we used a principle components analysis (PCA) to examine multivariate patterns of leaf traits, including Hotelling tests to compare differences between group means (EAS natives, ENA natives, and ENA invaders). All analyses were performed in R v3.3.1 (R Core Team 2017).

Results

Mean trait differences across plant groups

The distribution of light (GLI) values was similar across ENA invader, ENA native, and EAS native populations (*P*>0.05) (Fig. 1). We also did not generally find significant differences between these groups in traits related to structural defense, including both SLA and LMDC, although LDMC was significantly different between ENA invaders and ENA natives (Table 2).

For photosynthetic traits, mean values for PNUE did not vary between EAS native and ENA invaders, but in the full linear mixed models, ENA natives were significantly different – however, this difference was not present in the Tukey's post-hoc tests which excluded GLI interaction (Fig. 1). ENA invaders had significantly higher $A_{50\ area}$ and lower chl_{area} than EAS natives (Table 2).

The results of the Tukey's post-hoc tests on models excluding GLI interactions indicated ENA invaders had higher $A_{50\ chl}$ than both ENA and EAS natives, lower ch_{lN} and chl_{mass} (marginally significant) than EAS natives, and higher $A_{50\ area}$ and $A_{1000\ chl}$ than EAS natives. Both EAS natives and ENA invaders had higher N_{area} than ENA natives. EAS natives had marginally significantly higher chl a:b compared to ENA natives (Fig. 1).

Interpopulation trait variability across light gradients

For all plant groups, light availability (GLI) was positively associated with $A_{1000\ area}$, PNUE, N_{area}, LDMC, $A_{1000\ chl}$, and chl a:b (Table 2, Fig. 1). Conversely, chl_N, chl $_{mass}$, $A_{50\ area}$, SLA, and $A_{50\ mass}$ decreased along the light gradient for all plant groups (Table 2). $A_{1000\ mass}$, chl $_{area}$, N_{mass}, and $A_{50\ chl}$ did not change significantly along the light gradient for any species group (Figure 1, Table 2). To determine whether the response of traits to light (ITV) differed across plant groups, we included GLI and a random species intercept in trait models (separate from the full models that assessed differences in traits between plant groups). The results of these models vary slightly from the full models since the data were subsetted by plant group. These secondary models in addition to the full interaction models indicated that, for $A_{50\ chl}$ and $A_{1000\ chl}$, ENA invaders had increased ITV compared to both EAS natives and ENA natives (Table 2, Fig. 3). ENA invaders also had higher ITV compared to ENA natives in PNUE, chl_N, N_{mass}, LDMC, and chl a:b (Table 2). Counter to our expectation, chl a:b marginally decreased for ENA natives with increasing light (*P*=0.05). ENA natives also had a concomitant decrease in PNUE with increasing GLI, while PNUE of both EAS natives and ENA invaders significantly increased with GLI (Fig. 1 & 3). Interestingly, the slope of N_{mass} across the light gradient for ENA invaders was not different from zero ($P>0.05$), while it was positive for ENA natives ($P<0.001$) (Fig. 3).

Multivariate Differences between Plant groups

When examined across mean values of all traits simultaneously, plant groups occupied significantly different regions of multivariate trait space (Hotelling test; $P<0.001$) (Fig. 2). ENA invaders were associated with higher values of $A_{1000\ area}$, $A_{50\ chl}$, $A_{1000\ chl}$, PNUE, chl a:b, and GLI than either native range group, as well lower N_{mass}. Leaf structural traits, including SLA, LDMC, and C content, separated ENA natives (higher SLA/lower LDMC) from EAS natives (lower SLA/higher LDMC). PCA components 1 and 2 explained 49% of the total variation in traits across populations.

Discussion

In agreement with past studies (Heberling & Fridley 2013, 2016; Martinez & Fridley 2018), we found significant differences in leaf traits between native and invasive species in ENA forests. Unlike other studies, we investigated whether trait values of the invaders expressed in their invaded range were like those in native range populations. We found that leaf trait differences were consistent with both evolutionary change in the invaded range, where invasive range trait values have shifted from home range values, and invader pre-adaptation, where invaders showed advantageous trait values in both the invaded and native range. Among traits showing a shift in the invaded range is chl-use efficiency in high and low light ($A_{1000\ chl}$ and $A_{50\ chl}$), where understory invaders achieved higher rates of assimilation with lower chl $_{area}$ than in their native range. Because quantum efficiency is strongly influenced by chl content (Evans 1989), we expected plants with higher chl content to have higher low-light photosynthetic rates at low light levels. A reduction in chl investment without loss of photosynthetic capacity may explain invaders' greater competitive capacity in a variety of light environments relative to ENA natives (cf. Martinez & Fridley 2018).

We found little support for our second hypothesis that invaders invest more in structural defense traits in their new ranges (e.g. low SLA and high LDMC). LDMC was marginally lower in ENA invaders compared to EAS natives in our full model (Table 2), but the difference is not likely to be biologically significant. It may be that differences in leaf anatomy, such as mesophyll thickness or chloroplast arrangement, or decreased N allocation to chemical defenses may play a role explaining increased allocation to chl but not overall N content between ranges. Also contrary to our hypothesis, we did not find differences in PNUE for invaders across native and invaded range populations, in contrast to a prior study of one invasive ENA species native to Europe (Heberling et al. 2016). However, this could also be explained by differences in N allocation between the two ranges inferred in our $A_{50\ chl}$ and $A_{1000\ chl}$ results—for example, less investment in chl and more investment in dark cycle proteins as light availability increased in the invaded versus home range. Invasive species in both their native and invasive ranges had similar levels of interpopulation trait variability (ITV) across the light gradient for many traits, such as N_{mass}, chl a:b, chl_N, and SLA. However, in agreement with our hypothesis, ITV was greater in the invaded range for some traits relating to N allocation, where invaders captured more light with less chl content in both high and low light ($A_{50\ chl}$ and $A_{1000\ chl}$) than native range populations. This supports the idea that some traits relating to carbon gain have shifted to invaders' advantage in their introduced range, although the reason for the shift remains unclear.

We found partial support for our hypothesis that invaders have higher interpopulation trait variability (ITV) than ENA native species along light gradients. When ENA native species displayed high ITV with respect to light, it was often associated with traits that we would expect to reduce C gain—for example, decreasing PNUE and chl a:b at higher light levels. As an accessory pigment that captures additional wavelengths when light is limiting, chl b expression is generally upregulated in shade, leading to an expected chl a:b decrease with decreasing light availability (Terashima & Hikosaka 1995; Valladares & Niinemets 2008; Hikosaka 2014). Decreasing chl a:b with GLI in ENA native species is therefore surprising and may underlie their reduced photosynthetic rate in high light found in other studies (Heberling & Fridley 2013). Unlike that of ENA invaders, leaf N (N_{mass}) in ENA natives increased in high light, although this increase was not associated with greater photosynthetic capacity. This also suggests the leaf physiology of ENA natives is less adapted to high light than EAS species. The function of this increased leaf N remains unknown, and we hypothesize it has a role in defense or structure (e.g., cell wall N) that was unmeasured in our study.

The greater variability in leaf traits for ENA invader populations across a light gradient suggests that, all else equal, ENA invasive species have a larger niche breadth with respect to light than co-occurring natives, which may support higher population sizes in habitats like secondary forests that contain a variety of light conditions. Studies of invaders in other regions have reached a similar conclusion; for example, five non-native forest species in Hawaii were shown have higher photosynthesis rates in both high and low light than co-occurring native species (Pattison, Goldstein & Ares 1998b). In addition, Hawaiian invaders were found to allocate less leaf N to the cell wall and more N to metabolism than native species (Funk, Glenwinkel & Sack 2013). Similarly, *Celastrus orbiculatus*, a deciduous invasive liana, was found to grow faster in a variety of light levels when compared to *C. scandens*, a native congener, likely due to increased trait variability (Leicht & Silander 2006). In agreement with these studies, our results suggest invaders are more variable in their allocation of leaf N than native species. However, we caution that variable trait responses to light are not necessarily adaptive in woody invaders—in a common garden experiment of 30 native and invasive ENA shrub species, Martinez & Fridley (2018) found that leaf trait plasticity across varying seasonal light environments was not correlated with enhanced annual carbon gain. This underscores the need to link high- and low-light carbon gain to survival and seed production to fully evaluate the role that leaf-level C and N dynamics play in species invasions.

In general, the reduced responses of ENA native leaf traits to light compared to invasive species remained regardless of whether the measurements were taken in the invaded or native range of the invaders. This partially supports the hypothesis that invaders are preadapted for success before introduction, for example because of different selection historical selection pressures in their ancestral environments (Fridley & Sax 2014). Fridley (2013) and Zohner et al. (2017) argued that East Asian climates of the same latitude have been more climatically stable than those of ENA over Pleistocene, facilitating the evolution of less risk-averse phenotypes that have both earlier spring and later fall foliar phenology. One possible ramification of this hypothesis is that EAS deciduous understory species are better adapted to both high light conditions present in early spring / late autumn as well as low light levels midseason, which could explain why we found plants across populations to be more responsive to different light levels. However, this was not true across all traits—for example, leaf N was more variable in ENA natives, although there is little evidence that this played a role in photosynthetic ability.

One caveat to our study is that we did not measure differences in edaphic properties across populations or consider the potential of different soil microbial communities in the invaded range to facilitate more efficient use or uptake of resources for invaders in their new ranges. Evidence of microbial interactions facilitating growth in the invaded range is apparent for some woody species; for example, soil microbial mutualisms have been shown to be of greater benefit to maple (*Acer*) invaders in their non-native ranges due to enemy release (Reinhart & Callaway 2004). Invaders may also be released from soil pathogens in their invaded ranges; with fewer resources allocated to pathogen defense, more resources can be spent on leaf function and metabolism (Dawson & Schrama 2016). In addition, because we sampled only a few populations within the range of each species, our measurements are an underestimate of the total extent of genetic variation in leaf traits within species. However, we believe our inclusion of multiple species helps generalize our results from relatively few populations per species, particularly as observed trait shifts were seen across species. Finally, the differences we describe in low-light photosynthetic performance may not extend to the very low light levels (PPFD<50 μmol photons m^2 s^{-1}) of darker forests and would further be strongly dependent on leaf dark respiration rates that we did not measure. Because invaders have been previously shown to display higher returns on respiratory investment per unit assimilation than native species (Pattison, Goldstein & Ares 1998; Heberling & Fridley 2013), we speculate that carbon gain advantages in very low light by invaders, where present, could be driven more by respiratory processes than the photosynthetic traits we describe here.

Conclusion

We found that photosynthetic traits were more variable along a light gradient for populations of ENA invaders, compared to both co-occurring native species in ENA and conspecific populations in the invaders’ native range in Japan. Higher variability in these traits may be associated with greater overall carbon gain for forest invaders, particularly for populations that inhabit highly heterogeneous light environments, as is common in temperate deciduous forests. Additionally, higher trait variability along a light gradient in their invaded ranges may be allowing invaders to colonize a wider variety of habitats. On the other hand, because higher variation in some leaf traits was seen in both the invaded and native range for invaders, both the evolutionary history of these species and changes in selection pressures in the invaded range appear to facilitate the greater annual carbon gain of invaders. Whether these traits are predictive of invasions requires additional study of the relationship of carbon gain and population growth in understory species.

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Author Contributions

KAM designed the project, collected the data with input from KH and JDF, and analyzed the data. JDF assisted with data analysis. KH, RO, and MA assisted with field and laboratory data collection. KAM wrote the manuscript with input and assistance from JDF, KH, RO, and MA.

Table and Figure Captions

Table 1: List of species included in the study. Numbers indicate number of individuals studied at each location (109 total).

Table 2: Mixed model coefficients of the effects of light (GLI) and plant group (ENA invaders are reference category) on leaf traits. Interaction between GLI and plant group were removed from models if non-significant, reported as "NA". *** = $P<0.001$; ** = $P<0.01$; * = $P<0.05$; . = $P<0.09$

Table 3: Mean leaf trait values by species. SLA = g/cm^2, LDMC = %, chl$_{area}$=mmol/m^2, chl$_{mass}$=umol/g, A_{50chl} = µmol CO_2 m^{-2} s^{-1}: µmol/m^2; $A_{1000chl}$ = µmol CO_2 m^{-2} s^{-1}: µmol/m^2 ; N_{mass} = % leaf N, N_{area}=g N/cm^2, PNUE= µCO_2 g^{-1} N s^{-1}, chl$_N$ = g chl/g N, GLI = % canopy cover, A_{50}=µmol CO_2 m^{-2} s^{-1}, A_{1000}=µmol CO_2 m^{-2} s^{-1}

Figure 1: a-p) Leaf traits as a function of increasing Gap Light Index (GLI). Points represent individual plants; lines are fitted OLS regression lines within each group. Bars to the right of each scatterplot represent median and upper and lower quantiles for each species grou: p. Letters above bars represent results of Tukey's HSD tests for mixed models without GLI interactions included in the model structure ($P<0.05$, except where noted with * between bars, then $P<0.09$). a) Nmass = % leaf N; b) chl a:b; c) chlN: g chl g^{-1} N; d) SLA: g cm^{-2}; e) PNUE: µCO_2 g^{-1} N s^{-1}; f) LDMC % leaf dry mass content; g) $A_{1000\ area}$: µmol CO_2 m^{-2} s^{-1}; h) $A_{50\ area}$: µmol CO_2 m^{-2} s^{-1}; i) $A_{1000\ mass}$: µmol CO_2 g^{-1}, j) $A_{50\ mass}$: µmol CO_2 g^{-1}, k) chl$_{area}$: mmol m^{-2}, l) chl$_{mass}$: µmol g^{-1}, m)

$A_{50\ chl}$: µmol CO_2 m^{-2} s^{-1}: µmol/m^2, n) $A_{1000\ chl}$: µmol CO_2 m^{-2} s^{-1}: µmol/m^2, o) N_{area}: gN cm^{-2}, p) logGLI: log % canopy cover.

Figure 2: PCA of all measured leaf traits. Bars represent S.E.M. around group centroids. Eigenvalues = 0.31 and 0.20 respectively.

Figure 3: Model coefficients and 95% CIs of traits regressed against (log) Gap Light Index (GLI). Colors denote plant groups as in Fig. 1. Separate models were fit for each species group (ENA invaders, EAS natives, ENA natives), including a random effect for species. Note that these model fits are different from the models used in Table 2. Units and abbreviations same as in Table 3.

Table 1

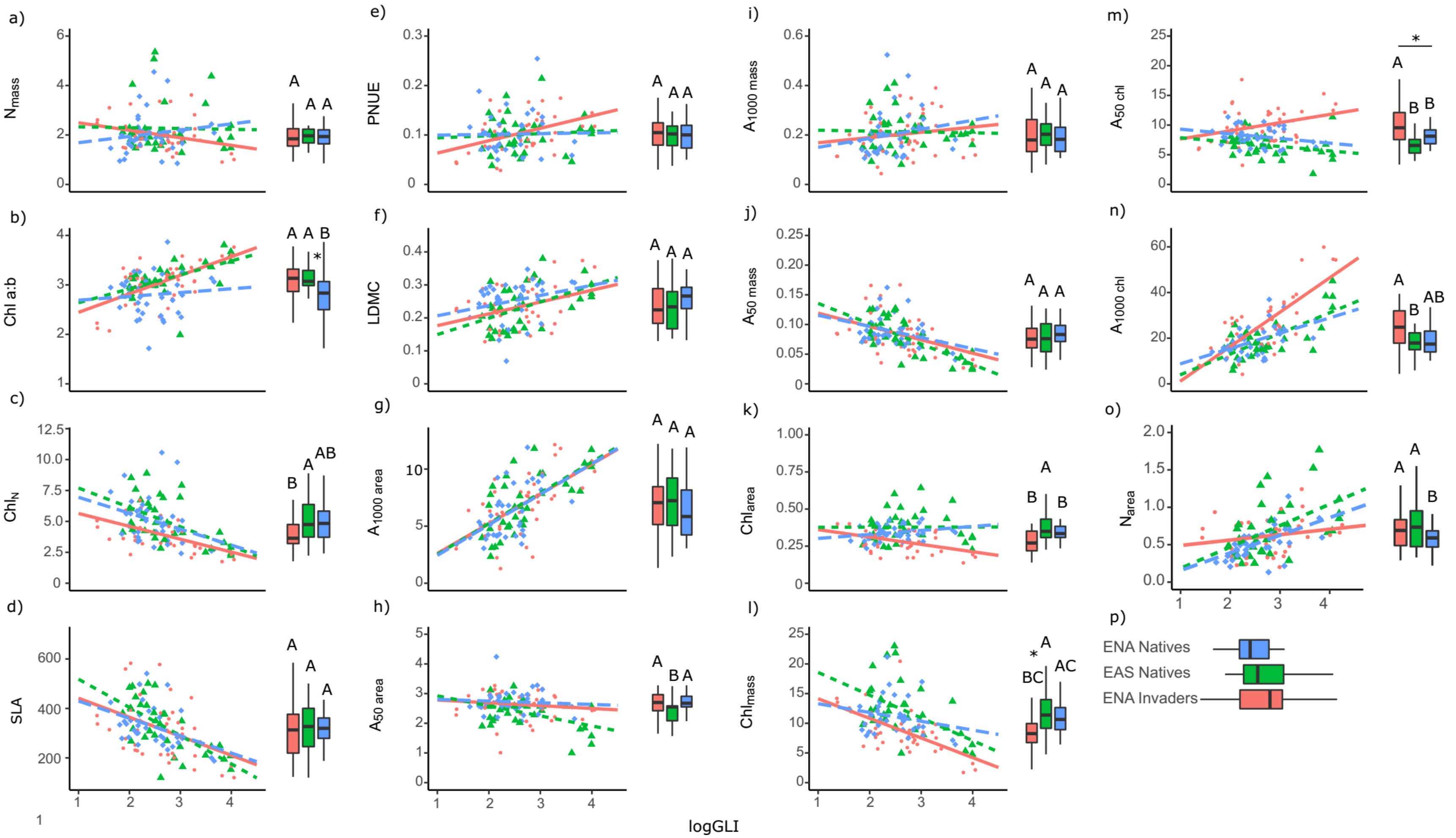
Species	**Family**	**Habit**	**Nativity**	**EAS**	**ENA**	**Total**
Celastrus orbiculatus	Celastraceae	Liana	East Asia	8	12	20
Cornus racemosa	Cornaceae	Shrub	North America	NA	14	14
Euonymus alatus	Celastraceae	Shrub	East Asia	6	12	18
Lonicera japonica	Caprifoliaceae	Liana	East Asia	6	9	15
Prunus virginiana	Rosaceae	Shrub	North America	NA	10	10
Sambucus racemosa	Adoxaceae	Shrub	Circumboreal	7	6	13
Viburnum acerifolium	Adoxaceae	Shrub	North America	NA	6	6
Viburnum dilatatum	Adoxaceae	Shrub	East Asia	7	6	13

Table 2

	Intercept	**EAS natives**	**ENA Natives**	**logGLI**	**EAS native*GLI**	**ENA native*GLI**
N_{mass}	2.95	-0.61	**-2.82*** **	-0.25	0.19	**1.02*** **
Chl a:b	2.08	0.28	0.53	**0.37*** **	-0.09	**-0.29.**
Chl_N	0.7	0.18	**0.47** **	**-0.12** **	-0.02	**-0.14***
SLA	574.1	1.05	40.14	**-106.43*** **	NA	NA
PNUE	3.74	4.23	**12.12** **	**1.92***	-1.37	**-3.93** **
LDMC	0.07	**0.06.**	**0.08***	**0.06*** **	-0.02	**-0.02.**
$A_{1000area}$	-0.91	0.13	0.48	**2.83*** **	NA	NA
A_{50area}	3.05	**-0.27***	0.07	**-0.16***	NA	NA
$A_{1000mass}$	0.14	0.00	**0.06.**	0.01	NA	NA
A_{50mass}	0.16	-0.01	0.01	**-0.03*** **	NA	NA
Chl_{area}	0.31	**0.07***	-0.01	0.01	NA	NA
Chl_{mass}	17.9	2.24	0.6	**-3.08*** **	NA	NA
N_{area}	0.15	0	**-0.24***	**0.24*** **	NA	NA
A_{50chl}	7.12	1.93	3.58	0.86	**-1.82***	**-1.82***
$A_{1000chl}$	-15.76	10.18	**22.22** **	**14.97*** **	**-6.10***	**-9.35** **

Table 3

	N_{mass}	**Chl a:b**	**Chl_N**	**SLA**	**PNUE**	**LDMC**	**$A_{1000\ area}$**	**$A_{50\ area}$**	**Chl_{area}**	**Chl_{mass}**	**N_{area}**	**A_{50chl}**	**$A_{1000chl}$**	**%C**	**GLI**
EAS Natives															
C. orbiculatus	1.76	3.15	0.638	401.62	0.14	0.17	6.59	2.31	0.32	12.66	0.486	7.25	20.69	40.81	16.1
E. alatus	1.92	3.36	0.423	229.53	0.11	0.33	9.12	2.53	0.4	9.2	0.855	6.28	22.65	43.94	19.9
L. japonica	1.98	3.19	0.373	285.87	0.11	0.25	8.03	1.96	0.28	8.11	0.744	6.88	28.19	45.62	29.77
V. dilatatum	1.73	2.85	0.628	307.53	0.08	0.26	5.21	2.55	0.41	12.31	1.156	6.18	12.63	45.08	12.56
S. racemosa	3.94	3.1	0.401	362.44	0.06	0.19	7.21	2.38	0.47	16.81	0.671	5.05	15.31	43.07	18.58
All EAS natives	2.27	3.12	0.529	323.39	0.1	0.23	7.14	2.35	0.38	12.03	0.773	6.21	18.88	43.56	18.97
ENA invaders															
C. orbiculatus	2.51	3.2	0.412	434.28	0.13	0.16	7.6	2.66	0.25	11	0.607	10.5	30.01	44.55	16.37
E. alatus	2.13	2.8	0.356	249.02	0.08	0.28	7.11	2.91	0.34	8.64	0.882	8.52	20.81	45.13	11.77
L. japonica	1.4	3.33	0.386	285.48	0.12	0.25	6.72	2.52	0.21	6.29	0.543	11.91	31.75	44.18	27.82
V. dilatatum	1.68	2.84	0.448	260.29	0.07	0.26	5.27	2.2	0.32	8.33	0.675	6.82	16.34	47.3	12.51
All EAS invaders	2	3.06	0.394	315.4	0.1	0.24	6.88	2.63	0.28	8.72	0.684	9.4	24.59	45.05	17.27
ENA natives															
C. racemosa	1.63	2.86	0.598	289.99	0.13	0.28	7.02	2.7	0.33	9.45	0.551	8.22	21.37	39.26	16.98
P. virginiana	2.09	2.85	0.43	295.39	0.07	0.28	5.17	2.64	0.34	9.9	0.594	7.84	15.35	43.57	9.25
S. racemosa	3.73	2.8	0.443	450.11	0.1	0.15	7.53	2.58	0.36	16.16	0.739	7.12	20.75	43.03	15.56
V. acerifolium	1.79	2.61	0.588	335.01	0.09	0.24	4.77	2.86	0.35	11.59	0.555	8.11	13.52	44.6	10.48
All ENA natives	2.16	2.8	0.524	328.01	0.1	0.25	5.99	2.71	0.34	11.15	0.598	7.95	17.57	41.93	13.23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N_{mass}
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4
6
A
A A
b)
Chl a:b
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2
3
4
A A B
*
c)
Chl_N
0
2.5
5.0
7.5
10.0
12.5
B
A
AB
d)
SLA
200
400
600
A
A
A
1
2
3
4
1
e)
PNUE
0
0.1
0.2
0.3
A
A A
f)
LDMC
0
0.1
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A
A A
g)
$A_{1000\ area}$
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logGLI
i)
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k)
Chl_{area}
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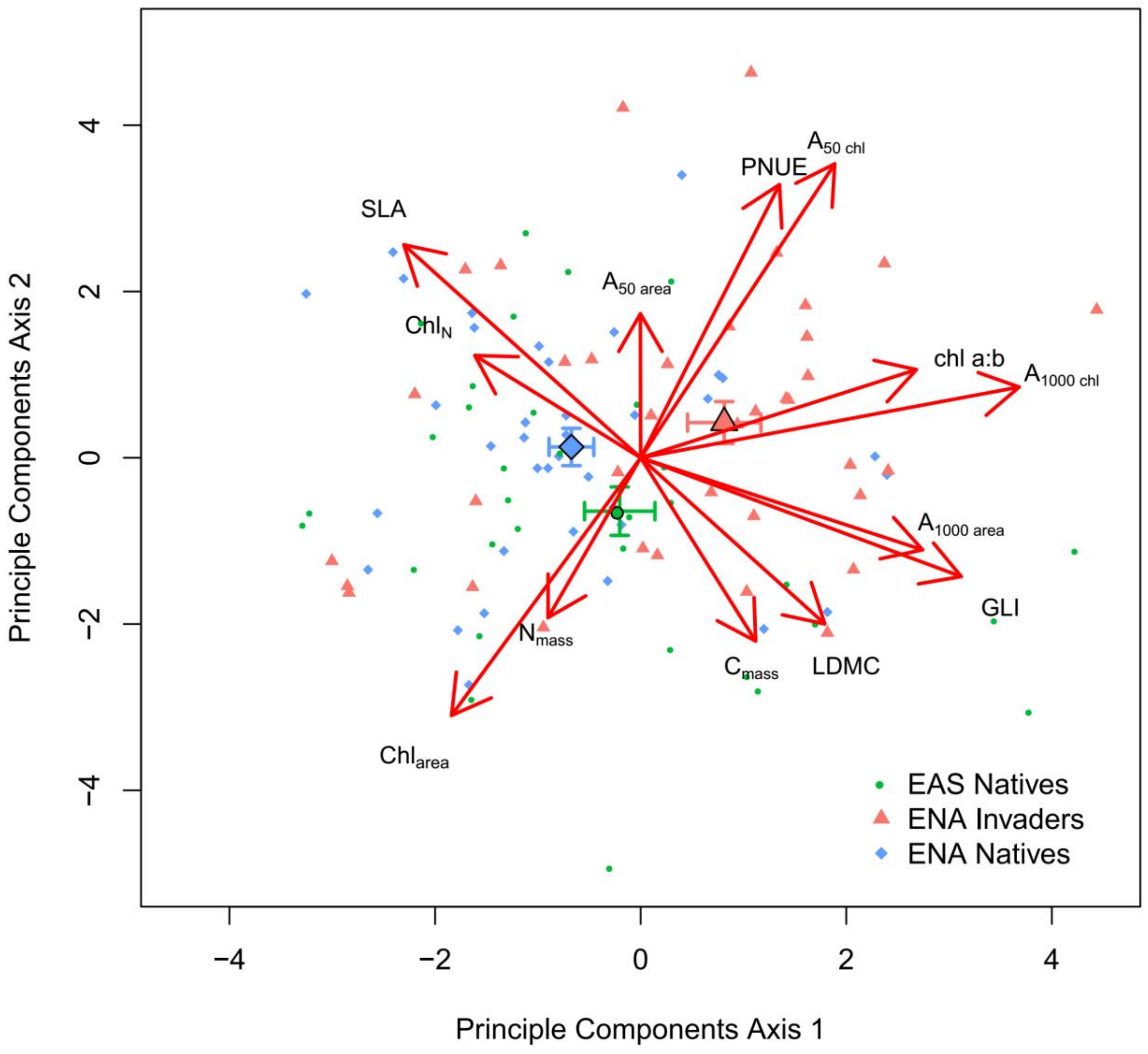
p)
ENA Natives
EAS Natives
ENA Invaders

Figure 1



Principle Components Axis 2
Principle Components Axis 1
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4
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ChlN
A50 area
PNUE
A50 chl
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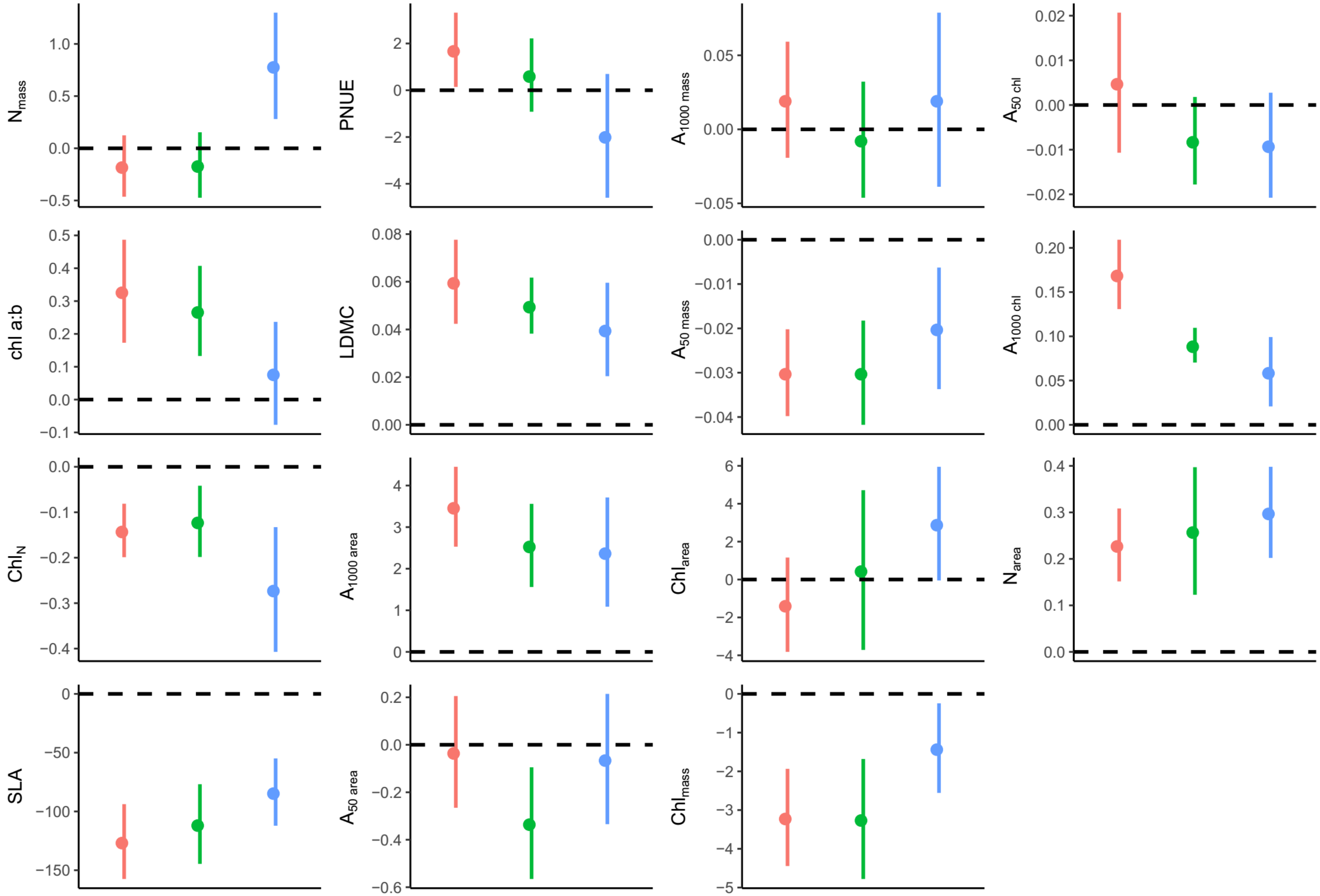
EAS Natives
ENA Invaders
ENA Natives

Figure 2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$A_{50\ chl}$
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0.01
0.00
−0.01
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chl a:b
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LDMC
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$A_{50\ mass}$
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$A_{1000\ chl}$
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0.10
0.05
0.00
Chl_N
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−0.2
−0.3
−0.4
$A_{1000\ area}$
4
3
2
1
0
Chl_{area}
6
4
2
0
−2
−4
N_{area}
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0.3
0.2
0.1
0.0
SLA
0
−50
−100
−150
$A_{50\ area}$
0.2
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−0.4
−0.6
Chl_{mass}
0
−1
−2
−3
−4
−5

Figure 3

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CHAPTER 4: **Divergent growth-related traits characterize native and invasive populations, but not annual and perennial populations of yellow monkeyflower (*Mimulus guttatus)***

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Abstract

Many ecologists have tried to predict suites of plant traits that characterize different life history strategies and distinguish native and invasive plant populations. Growth-related traits may be predictive of invasions at global scales, but within-species studies of these traits may fail to support theories such as the leaf economics spectrum. Furthermore, how growth rate is maximized by invasive species and species possessing multiple life history strategies remains underexplored. Using 22 total populations of an invasive herbaceous species native to the Western United States with annual and perennial phenotypes maintained by a chromosomal inversion (*Mimulus guttatus*), we sought to quantify physiological and leaf traits related to life history strategies and to understand which traits contribute most to relative growth rate (RGR) in native and invasive populations of an herbaceous species. We predicted that perennial populations would display traits related to resource conservation, such as low RGR, low SLA, and low photosynthesis rates, and that annual populations would display the opposite traits. We also predicted that invasive populations would have higher RGR and would maximize RGR through a different mode than native populations. Annual and perennial populations had similar SLA and similar proportional increases in unit RGR per unit SLA. Furthermore, invasive

populations did not have higher RGR than native populations, and RGR in coastal and invasive populations was largely impacted by both SLA and LMF. Our results are evidence that global patterns of leaf traits within a species displaying multiple life history strategies may not be apparent within a single species. We also found evidence that within a single species, RGR may be maximized by different traits, and that RGR may not be a good predictor of invasiveness.

Introduction

Suites of traits related to plant growth strategies, including relative growth rate, have been of interest to ecologists since the birth of the field. The three main traits underlying RGR are specific leaf area (SLA), leaf mass fraction (LMF), and net assimilation rate (NAR, A_{net}, or unit leaf rate) (Briggs, Kidd & West 1920; Hunt & Cornelissen 1997; Poorter & van der Werf 1998). All four traits (SLA, LMF, photosynthesis, and RGR) are predicted to vary along life history axes. For example, RGR is a fundamental trait in Grime's characterization of plant strategies along two axes relating to environmental stress and disturbance (competitor, ruderal, stress tolerator) (Grime 1977). Plants capable of tolerating extreme stress tend to grow slowly, while ruderal and competitive species tend to grow quickly (low vs high RGR, respectively) (Grime 1977). Similarly, on a global scale, leaf traits fall out along the Leaf Economics Spectrum (LES) (Wright et al. 2004) – as leaf lifespan and need to tolerate stress increases, leaf nutrient content and photosynthetic rates decrease and leaf thickness increases. However, some have argued that the patterns seen in schemes like the global LES and Grime's triangle are less applicable on small scales or within herbaceous species or genera (Mason & Donovan 2015; Anderegg *et al.* 2018). For example, within the genus of sunflower, *Helianthus*, SLA was uncorrelated with leaf lifespan (Mason & Donovan 2015), opposite of the prediction of the LES that SLA decreases with leaf lifespan (Wright *et al.* 2004).

Within herbaceous plant taxa, both annual and perennial growth forms exist. According to theories of life history strategies, we expect annual plant species to adapt a 'live fast, die young' strategy and occur in areas where resources are abundant but perhaps occur in seasonal pulses, while we expect perennials to exhibit slow-growth, resource conservative strategies because they more frequently occur in lower resource areas. Groups of traits associated with annual and perennial growth strategies have been examined across taxa (Grime 1977; Wright *et al.* 2004), and within a single genus (Garnier 1992; Mason & Donovan 2015; Pilote & Donovan 2016). Within congeneric grass species pairs, annual species were found to have higher RGR than perennial species, mostly explained by differences in SLA (Garnier 1992). Garnier (1992) also reported that annual species had higher SLA than perennial species, which affords them high RGR. In contrast, another study assessing RGR components in different light levels in grass species pairs found that photosynthetic rate and proportion of leaf mass influenced RGR the most depending on light availability – for shade-tolerant species, photosynthesis (NAR) was the most important, and for species of high light environments, LMA was most influential (Ryser & Wahl 2001). Interestingly, multiple life history strategies (e.g., annual vs. perennial) do sometimes occur within a single species and can remain fixed within populations (Vickery 1952; Hitchcock & Cronquist 1973). Further study of co-occurring traits within species can offer insights in to evolutionary patterns in co-occurring traits that are not visible when looking across large scales (Ackerly & Donoghue 1998; Muir *et al.* 2017). For example, direct selection may be acting on one trait strongly correlated with another trait (e.g., SLA and RGR), causing indirect selection and change on the correlated trait, even though there are no obvious selection pressures on the correlated trait (Conner 1988).

Relative growth rate and its underlying physiological drivers are also of interest in invasion ecology (Baker 1965). This is because invasive species often display higher RGR than both co-occurring and native species from the same genus (Pattison, Goldstein & Ares 1998; James & Drenovsky 2007, cf Bellingham *et al.* 2004). Populations of invasive species have also been shown to display higher RGR in the invasive range when compared to populations from the native range (Graebner, Calloway & Montesinos 2012). RGR is thought to contribute to invader success, especially after disturbance (Baker 1965), since high RGR increases resource uptake (Grime & Hunt 1975). However, the physiological mechanisms responsible for how invaders maximize RGR are not well understood. More specifically, we do not know if populations of invasive species maximize RGR through variation in SLA, photosynthesis, and LMF in different combinations than native populations. Previously, invasive populations of woody species have been shown to have higher photosynthetic resource use efficiency and high photosynthetic rates overall compared to native populations of the same species (Heberling *et al.* 2016), but it remains unknown how these traits influence RGR. By studying native and invasive populations of plants within the same species, we can specifically test for which traits, if any, have changed to maximize RGR in invasive populations.

We aimed to answer the following questions in this study: 1) Are there groups of traits associated with different life history strategies in a single species? 2) Which traits maximize RGR and is RGR maximized via different traits among populations of a single species? 3) Do invasive populations have higher RGR than native populations with different underlying traits accounting for increases in RGR? We hypothesized that 1a) annuals would possess leaf traits associated with a resource acquisitive strategy: high SLA, high leaf N content, high instantaneous photosynthetic rates, low allocation to roots which drive high RGR; 1b) perennials would possess leaf traits associated with long leaf life span and a slower resource acquisition strategy: low SLA, lower instantaneous photosynthetic rates, and high allocation to roots, which culminate to lower RGR than annual populations; 1c) populations with physiological tolerances to stressors (e.g. coastal salt spray) would show similar allocation patterns as perennials, but have decreased instantaneous photosynthetic rates, low SLA, and lowest RGR due to physiological costs of stress tolerance; 2) native populations would maximize RGR differently than invasive populations, but that SLA would be an important driver of RGR in all life history groups; and 3) invasive perennial populations would have the highest RGR and maximize RGR using LMF and photosynthetic rate, while native populations would use SLA.

Methods

Study System

Mimulus guttatus (aka *Erythranthe guttata*; yellow monkeyflower) is an herbaceous plant in Phyrmaceae native to Western North America. It is highly phenotypically variable and primarily lives in seeps and seasonally wet habitats (Hitchcock & Cronquist 1973). Two life history strategies (annual and perennial) in the species are maintained via a chromosomal inversion (Twyford & Friedman 2015). The annual form lives in habitats that are wet in spring but dry out over the summer, while the perennial form lives in areas that are wet year-round (Hitchcock & Cronquist 1973). The annual form flowers before its habitat dries out during the summer, while the perennial form tends to produce both flowers and multiple stolons that may persist multiple years. A third group of native *M. gutattus* populations exists in coastal cliff areas on the coast of California that displays high salt tolerance (Lowry, Rockwood & Willis 2008). The populations are fully compatible and gene flow is high between populations of all life history strategies (Twyford & Friedman 2015).

The perennial form of *M. guttatus* is invasive in the United Kingdom (UK) and New Zealand (NZ) (Vallejo-Marin & Lye 2013). In its invasive range, *M. guttatus* occurs in mesic to wet habitats, such as roadside ditches, streams, and seeps. *M. guttatus* is thought to have been introduced multiple times to these continents through horticultural plantings (Puzey & Vallejo-Marin 2014). In its invasive range, *M. guttatus* spreads both through sexual and vegetative reproduction. Previous studies have found invasive populations of *M. guttatus* to be genetically distinct from native populations (Puzey & Vallejo-Marin 2014).

Population Sampling and Data Collection

We selected maternal families of field-collected seed from 22 populations of *M. guttatus* across its native and invasive ranges in Western North America and the Aleutian Islands (native), and NZ and the UK (invasive). Specifically, we grew seeds from 4 annual populations across the Western US, 9 perennial populations, 4 populations in the coastal perennial phylogenetic group (Twyford & Friedman 2015), 3 invasive populations from NZ, and 3 invasive populations from the UK (Fig. 1). A total of 450 plants were grown in a greenhouse during January-May 2018. The plants were grown in two time 'blocks' consisting of 250 and 200 plants. We grew 3 to 5 maternal families per population (most populations had 5 families, but one population did not germinate well so it only had 3 families). Each family had two or four replicates (depending on germination success) and a total of 10 plants per population. Seeds were cold stratified for 5-7 days before being moved to a greenhouse with a 14-hour daylength. Plants were watered daily using a flood bench.

On the first set of ~250 plants, total leaf area, leaf mass, total plant biomass, photosynthetic rate (A_{net}), root biomass, total leaf nitrogen (N), photosynthetic nitrogen use efficiency (PNUE), and days to flowering were collected on half of the plants (one per each family). The remaining plants continued to grow until flowering, and then the same measurements were repeated on these post-flowering plants to calculate relative growth rate and average pre- and post-flowering trait values for each family. To calculate total leaf area we scanned fresh leaves and used the R package 'LeafArea' to calculate total leaf area per plant (Katabuchi 2017). After scanning, leaves were dried at 65ºC for at least 48 hours before weighing to determine leaf mass and specific leaf area (SLA; cm^2/g). Roots were cleaned using tap water and dried and kept separate from leaf, stem, and reproductive material to determine total biomass, root mass fraction (RMF) and leaf mass fraction (total aboveground green biomass (LMF)). Photosynthetic rates (A_{net}) were measured using a portable infrared gas analyzer (LI-6400, Li-Cor, Lincoln, Nebraska, USA). Leaves acclimated in the cuvette for 5 minutes before photosynthetic rate was recorded at 300 PPFD (photosynthetic photon flux density - µmol quanta $m^{-2} s^{-1}$), which was the approximate light level in the greenhouse. We maintained leaf temperature at 25 ºC for all measurements, and flow rate was adjusted to maintain leaf vapor pressure deficit (VPD) below 2.0. Total leaf N and C content was determined from a homogenized sample of at least three leaves per plant using an NC autoanalyzer (NC 2100; CE Elantech, Lakewood, NJ, USA). PNUE was calculated by dividing area-based photosynthetic rates by N content per unit leaf area.

A second set of ~200 plants from the same families (excluding invasive populations) were grown from March-May to determine stem area and its contribution to carbon gain for each family. Stem area data was collected on pre- and post-flowering plants using the length/height of each stolon and stem and its associated diameter. We used the formula for the surface area of a cone minus basal area to determine the area of each stem or stolon. Stem area was added to total leaf area for each plant to determine a 'whole-plant' photosynthetic rate per area. We assumed stem tissue photosynthesized at the same rate as leaf tissue. Relative growth rate (RGR) was calculated for each family using the total biomass of two family replicates harvested pre- and post-flowering ($[\log(mass_{postflower})-\log(mass_{preflower})]$/days between harvest).

Data Analysis

We tested for differences between RGR, A_{net}, whole-plant photosynthetic rate, LMF, RMF, leaf N, and SLA between life history groups, using linear mixed models with latitude as a fixed effect, and population as a random effect nested in life history group. The dependent variable was an average of the first and second harvested plant for each family. We used the lme4 package in R to create all mixed models (Bates *et al.* 2017). Model structure was as follows:

$$Trait \sim Life\ History + Latitude + (1|Life\ History\ Group/Population)$$

Using Tukey's post hoc pairwise tests, we tested for differences between life history groups. Similarly, we also ran mixed models for each life history group with RGR as the dependent variable and SLA, RMF, LMF, and A_{net} as independent variables with a random population effect to determine the contribution of each trait to RGR (R Core Development).

Using a PCA of each family-averaged leaf trait, we assessed trait variation of life history groups in multivariate space. We performed Hotelling tests to test for differences between life history means in multivariate space (Curran 2017). Finally, to compare variance in traits at multiple scales, we performed linear mixed model analyses with an intercept and random effects of life history and population nested within life history. Proportion of variance for each level was determined by dividing each levels' variance by the total dependent variable variance. Residual variance quantifies within-population variance. All analyses were performed in R v. 3.4.0.

Results

Traits varied by life history group, and the different groups had different combinations of traits that maximized RGR. Invasive populations did not have significantly different RGR from any other life history groups aside from annual populations (Fig. 2). Annual populations had significantly higher A_{net} and RGR than all other life history groups (Table 1, Fig. 2), but the lowest whole plant photosynthesis due to lower stem surface area and leaf area. Annual populations had the highest leaf N content. SLA did not vary significantly among the life history groups, except for coastal perennial groups, which had lower SLA than the other three groups. We found that coastal perennial populations had the lowest RGR, but not significantly lower than invasive populations. PNUE was highest in annual and perennial plants, although perennial populations did not have significantly higher PNUE than invasive populations. Coastal and invasive populations had similar values of PNUE (Fig. 2). We did not find large differences in allocation to roots across life history groups, except for coastal perennials having marginally significantly higher RMF than annual populations.

Annuals and perennials primarily maximized RGR through the same trait: increased SLA (Figs. 3, 4). However, coastal perennial populations and invasive populations increased RGR by increasing LMF and SLA (RGR slope Figs. 3, 4). Although invasive populations did not have significantly different RGR from perennial populations, the mechanism by which they achieved equal RGR differed – both LMF and SLA contributed to RGR in invasive populations, while only SLA contributed significantly to RGR in perennial populations.

The results of the linear mixed models for each life history group with RGR as the response variable returned significant relationships between SLA and RGR for both perennial and invasive populations. For invasive populations, RMF was also significantly related to RGR. No other traits were related to RGR for any of the other life history groups (Table 3).

Both SLA and PNUE displayed negative relationships with latitude across life history groups (Table 2). No other traits showed a relationship with latitude. Proportion of trait variance encompassed by each grouping level (life history, population, and within population) varied by trait, but SLA (both leaf level and on a whole-plant basis) had the highest proportion of variance explained by population and life history, A_{net} had the highest proportion of variance explained by population, while LMF and RMF had the highest proportion of variance within population or due to residual model variance (Fig. 5).

Multivariate analysis results indicated that RGR, SLA, leaf photosynthesis (A_{net}), and leaf N were all highly correlated. Annual populations primarily differentiated themselves from the other three life history groups along the axis associated with increases in these traits. The remaining life history groups varied along the axis associated with biomass allocation (LMF vs. RMF). Coastal perennials allocated more biomass belowground than both perennial and invasive populations. Perennial populations were on the other side of this continuum, with higher allocation aboveground than both coastal and invasive populations. All pairwise Hotelling tests of multivariate means of life history groups were significant ($P<0.05$) (Fig. 6).

Discussion

Traits of life history strategies

Contrary to our hypothesis that perennial populations have traits associated with the opposite side of the LES and Grime's classification of plant traits, we found that perennial plants had similar SLA values to annual plants and only marginally lower RGR (Fig. 2). In perennial populations, the need for low SLA leaves to extend leaf longevity may be avoided by production of trichomes or anti-herbivory secondary compounds (Holeski 2007a). Interpopulation variation in trichome production is high and thought to be both genetically determined and inducible, and populations that produce the most trichomes are also thought to experience the most herbivory (Holeski 2007b). The need for leaves that are well defended with low SLA may also be negated by shortening the lifespan of any given leaf in trade for production of new leaves and senescence of older leaves, but to our knowledge, no studies have recorded leaf demography in *M. guttatus*. When corrected for leaf and stem area, perennial populations also had higher total plant photosynthetic rates than annual populations (Fig. 2). However, as predicted by life history theories, leaf-level photosynthetic rate and leaf N content was higher for annual populations (Fig. 2; Table 2). We also expected that annual populations would have higher resource use efficiency (PNUE) than annual populations because this trait is associated with fast growth and short-lived plants in both Grime's classification of life history and the LES. Surprisingly, annual and perennial populations showed no differences in resource use efficiency (PNUE). Thus, at small taxonomic scales, trait differences associated with life history strategies on a global scale are not necessarily always present. A similar result was found by Anderegg et al. (2018) in a metanalysis of approximately 2000 species, where trait patterns did not sort out along global patterns predicted by the LES. For example, the global relationship between decreasing SLA and increasing leaf lifespan was not supported within species; rather, the opposite relationship between SLA and leaf lifespan was found (Anderegg et al. 2018).

As predicted, coastal perennial populations displayed low RGR, SLA, and PNUE, which may reflect the potential costs of physiological tolerance to salt spray (Fig. 2) (Kozlowski 1997; Munns 2002). Compared to populations of non-halophytic *M. guttatus*, these traits fit onto the axes of traits predicted by life history strategy, such as the one outlined by Grime (1974). Plants with high physiological stress tolerance tend to grow slower than those with lower stress because they must invest resources in mitigating damage from stressors. To tolerate salt, plants must reduce absorption of salt into their tissues and/or find a way to contain and remove it from their tissues after uptake (Munns 2002). Since we did not expose our plants to salt, we assume reduced

RGR in coastal populations was due to costs of plant mechanisms that attempt to reduce salt entry into plant tissue. For example, thick, low SLA leaves with waxy cuticles may reduce the amount of salt exposure (Ahmad & Wainwright 1976; Kozlowski 1997). Halophytes also have reduced photosynthetic rates even when not exposed to salt, which perhaps partially explains the reduction in PNUE in coastal populations (Kozlowski 1997). We expect that reduced PNUE is also a factor of reduced SLA, since thick, dense leaves are not conducive to maximum gas diffusion rates through leaf tissue.

Decomposition of RGR by life history

We found that annual and perennial populations maximized RGR through similar values of the same trait, SLA (Fig. 5). This is contrary to other studies of congeneric pairs of annual and perennial plant species, where SLA explained differences in RGR between pairs of annual and perennial species from the same genus. Whereas annual plants have higher SLA than perennials, which contributes greatly to increased RGR in annuals (Garnier 1992). The amount of gene flow amongst populations of *M. guttatus* may be prohibiting annual and perennial plants from diverging in traits that strongly impact RGR (Twyford & Friedman 2015). Furthermore, the previously mentioned traits that perennial *M. guttatus* populations use to defend their leaves against herbivory might negate the need for thicker SLA leaves, thus making the relationship between SLA, LMF, and photosynthesis similar for both annual and perennial populations.

In coastal perennial populations, proportional increases in LMF had the highest increases in RGR. The relationship between SLA and RGR for coastal perennial populations was like that of both annual and perennial populations (Fig. 5). We suspect that large proportional increases in RGR with LMF in coastal populations are explained by the cost of a trait associated with salt tolerance. Coastal populations of *M. guttatus* are also highly morphologically divergent and genetically differentiated from inland populations (Lowry *et al.* 2008), so it is not surprising that LMF contributes differently to RGR for this group of populations than inland perennials and annuals. However, LMF in coastal populations was only marginally different from LMF in annual populations, and not different from either perennial populations or invasive populations (Fig. 2). Variance in LMF was also high within populations (Fig. 5), which also reduces the relationship between it and RGR. From this, we conclude that reduced SLA most strongly contributes to reduced RGR in coastal populations.

Traits of Invasive populations

Contrary to our fourth hypothesis, invasive populations did not have higher RGR than native populations. The lack of difference in RGR between native and invasive populations is contrary to many studies of invasive species that have shown invaders to possess higher RGR than native species (Pattison *et al.* 1998; Grotkopp *et al.* 2002; van Kleunen & Fischer 2008). Because invaders do not display higher RGR than native populations, we speculate that other circumstances facilitated successful invasion of *M. guttatus* in the UK and NZ. For example, enough repeated introductions of a species will likely allow it to establish and become invasive if the environment is similar enough to its home range even if it does not have a novel combination of traits. It also may be that the high level of leaf production and clonal nature of invasive populations gives them a competitive advantage for sunlight over co-occurring native plants. Similarly, increased clonal reproduction in the invasive range is predicted to enhance invasion success in an invasive grass, *Phalaris arundincea* (Lavergne & Molofsky 2007), and an invasive aquatic plant, *Butomus umbellatus* (Brown & Eckert 2005). However, invasive populations appear to be constrained by low leaf photosynthetic rates (Fig. 2) which may reduce RGR. Reduced photosynthetic rate may be a tradeoff with increased LMF when resources are limiting (e.g. N). It may also be that in specific environments in the invaded ranges or in competition with other plants (Graebner, Callaway & Montesinos 2012), invasive populations of *M. guttatus* have higher RGR than surrounding native species. Whole plant photosynthetic rates were unmeasured on invasive populations, but given their high number of stolons and leaves, we predict that they would be like rates seen in native perennial populations.

Also contrary to our prediction that invasive populations would have higher PNUE than native populations, invasive populations had lower PNUE than perennial native populations (Fig. 2). We based this prediction on previous studies of populations of invasive species in both ranges, where invasive populations had higher PNUE than native populations (Heberling et al. 2016). The broader implications of our result remain unclear, since albeit non-significant, invasive populations in our study had slightly lower SLA than native populations, which could explain reduced PNUE in that leaf thickness reduces gas diffusion through leaf epidermis.

No matter the ecological and anthropogenic circumstances of successful *M. guttatus* invasion in the UK and NZ, our results are evidence RGR alone may not be an adequate predictor of invasiveness of species when comparing between native and invasive populations of the same species or closely related taxa. Although this result is not the norm (Pattison *et al.* 1998b; Grotkopp *et al.* 2002; Graebner, Callaway & Montesinos 2012), other studies have made similar conclusions that RGR does not accurately predict invasiveness (Bellingham et al. 2004). Among 33 species of native and invasive woody species in New Zealand, seedlings of invasive species did not display higher RGR than native species (Bellingham et al. 2004). Furthermore, Bellingham et al. (2004) concluded that invasive status of woody species in New Zealand was likely highly related to human introductions, which we predict is also at play in our study of *M. guttatus*.

Decomposition of RGR in Invasive Populations

Interestingly, there were large proportional increases in RGR per unit increase in LMF for invasive populations (Fig. 4). A cline in vegetative growth and reproduction – a large component of LMF - with increasing latitude has been documented in *M. guttatus*, such that plants in higher latitudes tend to produce vegetatively instead of sexually (van Kluenen & Fischer 2008). We hypothesize that the large effect of increases in LMF in invaders may be related to increased vegetative reproduction because of the high latitude of origin of our invasive populations, rather than their invasive status. Decreased allocation to sexual reproduction and floral structures may increase resources available for vegetative growth and increase returns on leaf production. Invasive populations were unique in this aspect since no other life history groups showed any significant relationship of RGR with LMF. This suggests that invasive populations may be avoiding a tradeoff between SLA (leaf thickness) and LMF to which other populations may be restricted. Further evidence of this tradeoff avoidance is that invasive populations also displayed a large proportional increase in RGR with increasing SLA compared to native perennial populations (Fig. 4). However, it should be noted that the relationship between RGR and LMF was not significant in our mixed model analysis (Table 3).

Local and Latitudinal Patterns

We found evidence of the potential for local selection on SLA, RGR, and photosynthetic rates, since these traits had the highest amount of variance explained by life history strategy and population. However, LMF, RMF, and leaf N displayed the highest variance within populations, indicating there is either substantial genetic variance for these traits within populations, or they are plastic and determined by a microenvironmental variable that we did not measure in this study (Kawecki & Ebert 2004). We caution that high within-population variance in LMF and RMF may be the result of the difficulty of washing peat-based potting mix from plant roots. Had we used a different growing medium, we expect that population level variance in these traits might be reduced. However, RMF has been shown to vary at least two-fold in some *M. guttatus* populations (Harper, Smith & Macnair 1997), so our results may have been similar had we used a different potting medium. This result of high within population variance for some traits, such as vegetative traits, and minimal variance within population for other traits, specifically flowering time (Hall & Willis 2006), has been found before in *M. guttatus*.

Further evidence of local selection is shown by an effect of population latitude in some traits. Specifically, SLA and PNUE were negatively associated with increasing latitude across all populations (Table 2). We speculate that this SLA pattern may be driven by increased precipitation, longer growing seasons, and thus need for leaves with a longer lifespan in higher latitudes than those growing in lower latitudes. Because precipitation increases along our latitude gradient, so does growing season length and herbivore pressure, indicating that leaf defense may also be driving the latitudinal decrease in SLA (Holeski 2007a, Kooyers, Blackman & Holeski 2017). Combined, increased herbivore pressure and leaf longevity necessitate thicker more well defended leaves, which may explain the decrease in SLA with increasing latitude. Interestingly, more northern populations, such as Northern California (not those as far north as Alaska), also exhibit higher trichome density, which acts as additional herbivore defense (Holeski 2007b). Decreases in PNUE with increasing latitude may also be explained by the same drivers, such that more N needs to be allocated to production of trichomes and other defensive compounds instead of photosynthesis.

Our study and interpretation of results are not without minor limitations and caveats. We caution that all our populations were grown under the same constant photoperiod (14 hr). In a natural setting, some of our more northern populations (especially those from the Aleutian Islands) may be exposed to much longer and rapidly changing photoperiods, which has the potential to induce physiological changes unobserved in this study. Environmental conditions such as light availability or soil nutrient content may play a large role in the proportional contributions of plant traits to RGR as well (Poorter & VanderWerf 1998; Ryser & Wahl 2001; Shipley 2002).

Conclusion

Plant traits contributing to whole-plant growth rate, including photosynthetic rate, leaf dry matter partitioning, specific leaf area, and leaf N content, varied across native and invasive populations of a single species, *Mimulus guttatus,* in ways that were not consistent with global plant trait relationships. Furthermore, while relative growth rate is typically predictive of invasiveness, we did not find any differences in growth rate between native and invasive populations. Thus, we also conclude that relative growth rate is not an adequate predictor of invasiveness in herbaceous taxa and that special care should be taken when generalizing about traits that correlate with invasion success or when comparing traits among populations of the same species.

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Table and Figure Captions

Table 1: Populations used in study.

Table 2. F-statistics from trait mixed models, includes population as random effect nested in life history group. *** = $P<0.001$; ** = $P<0.01$; * = $P<0.05$; . = $P<0.09$.

Table 3. F-statistics from RGR mixed models (RGR dependent variable) for each life history type. *** = $P<0.001$; ** = $P<0.01$; * = $P<0.05$; . = $P<0.09$. (Total photo is whole-plant photosynthesis, except for invasive populations that we did not measure stem surface area on, so A_{net} is substituted).

Figure 1. Map of populations included in study in degrees, minutes, seconds. (Exact coordinates of New Zealand populations are unknown, but all are sourced from Otago).

Figure 2. Boxplots of traits by nativity/life history group. Black lines indicate median values. Letters above graphs display results of Tukey's posthoc comparisons of mixed models (no interaction terms included; * indicates Tukey's pairwise comparison is marginally significant, $P<0.1$). RGR: relative growth rate (log(g) d-1); SLA: specific leaf area (cm2 g-1); LMF: leaf mass fraction (proportion of biomass allocated to leaves); Photo/Anet: leaf-based photosynthetic rate (µmol CO2 m-2 s-1); Whole plant photosynthesis (µmol CO2 s-1); RMF: root mass fraction (proportion of biomass allocated to root mass); %N: % leaf N content; PNUE: Photosynthetic nitrogen use efficiency (µmolCO2 g N-1 s-1).

Figure 3. Relative growth rate (RGR) vs. measured leaf traits. Colors and units are same as Fig. 2. Lines are fit using OLS, no random effects included.

Figure 4. Decomposition of relative growth rate (RGR) into its components: LMF, SLA, and photosynthesis. Model coefficients extracted from logRGR ~ log(LMF) + log(Photosynthesis) + log(SLA). These coefficients explain relative contribution of each plant trait to RGR. Trait units are the same as in Fig. 2.

Figure 5. Proportion of variance explained by each grouping/level determined by model: Trait ~ 1 +(1|Life hist. group/population). Abbreviations are same as Figs. 2 and 3.

Figure 6. PCA of observed leaf traits. Eigenvalues for PCA 1 and PCA 2 were 0.42, and 0.20 respectively. Circles represent mean and SE of life history groups.

Figure 6. PCA of observed leaf traits. Eigenvalues for PCA 1 and PCA 2 were 0.42, and 0.20 respectively. Circles represent mean and SE of life history groups.

Figure 7. Days to flowering and leaf photosynthesis (A_{net}). $P<0.05$ for overall slope of all points.

Tables and Figures

Table 1

Population	Lat	Lon	Life History	Nativity	Location
BCB	36.03.771	121.35.532	Coastal Perennial	Native	Big Creek Reserve, CA
BHI	41.09.295	123.53.378	Annual	Native	Redwood NP, CA
CER	53.0	3.32.564	Perennial	Invasive	Cerrigydrudion, Denbigshire, Wales
DBL	56.11.492	3.57.54	Perennial	Invasive	Dunblane, Perthshire, Scotland
FEV	44.24	122.18	Annual	Native	Linn County, OR
G27	35 58.290	118 28.359	Perennial	Native	Sequoia National Forest, CA
GBM	41.22.718	124.04.175	Coastal Perennial	Native	Gold Bluffs Marsh, Redwood NP, CA
HEC	44.08	124.07	Coastal Perennial	Native	Heceta Beach, OR
HOC	47.23.124	123.08.8837	Perennial	Native	Hood Canal, WA
KCG			Perennial	Native	Aleutian Islands, AK
LCA	40.2	121.12	Perennial	Native	CA ??
LMC	38.51.839	123.05.035	Annual	Native	Lower Mendocino County, CA
MUB	45.47	113.36	Perennial	Native	Mussigbrod Creek, MT
NKL	50.21.503	126.55.839	Annual	Native	Nimpkish Lake, Vancouver Island, BC
NZ1			Perennial	Invasive	Otago, New Zealand
NZ3			Perennial	Invasive	Otago, New Zealand
NZ4			Perennial	Invasive	Otago, New Zealand
SLS			Perennial	Native	Aleutian Islands, AK
STB	37.53.479	122.38.085	Coastal Perennial	Native	Stinson Beach, CA
SWB	39.02.159	123.41.428	Coastal Perennial	Native	Sperm Whale Beach, Irish Beach, CA
TOM	57.15.18	3.22.48	Perennial	Invasive	Tomintoul, Moray, Scotland
TRR	39.18	120.12	Perennial	Native	Truckee River, CA
TSG	53.25.130	131.54.944	Perennial	Native	Ted's Spring, Queen Charlotte Islands, BC

Table 2

	Life History	**Latitude**
SLA	**15.43*** **	**6.86***
Photo	**6.51****	2.69
LMF	**2.77.**	0.25
RMF	**2.84.**	0.01
RGR	**8.10****	2.42
%N	**4.31***	0.24
PNUE	**7.10****	**6.02***
Total Photo	**3.98***	0.44

Table 3

	SLA	**LMF**	**RMF**	**Total photo**
Annual	2.22	0.47	2.69	0.05
Coastal	1.53	0.34	0.29	0.47
Perennial	**6.56***	0.35	**4.83***	0.60
Invasive	**4.81***	0.11	0.23	0.24

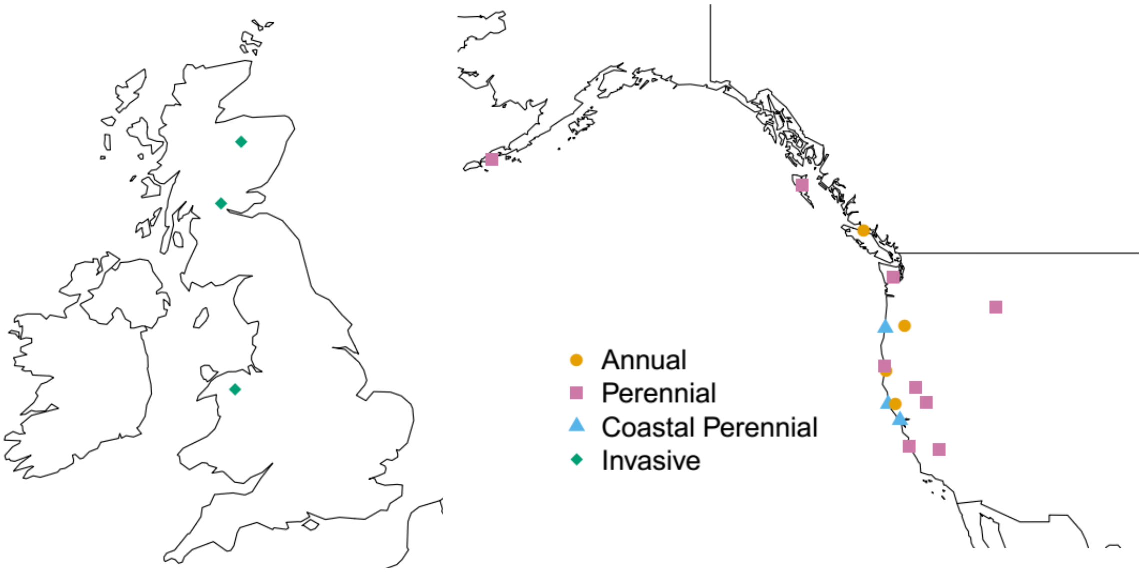


Figure 1

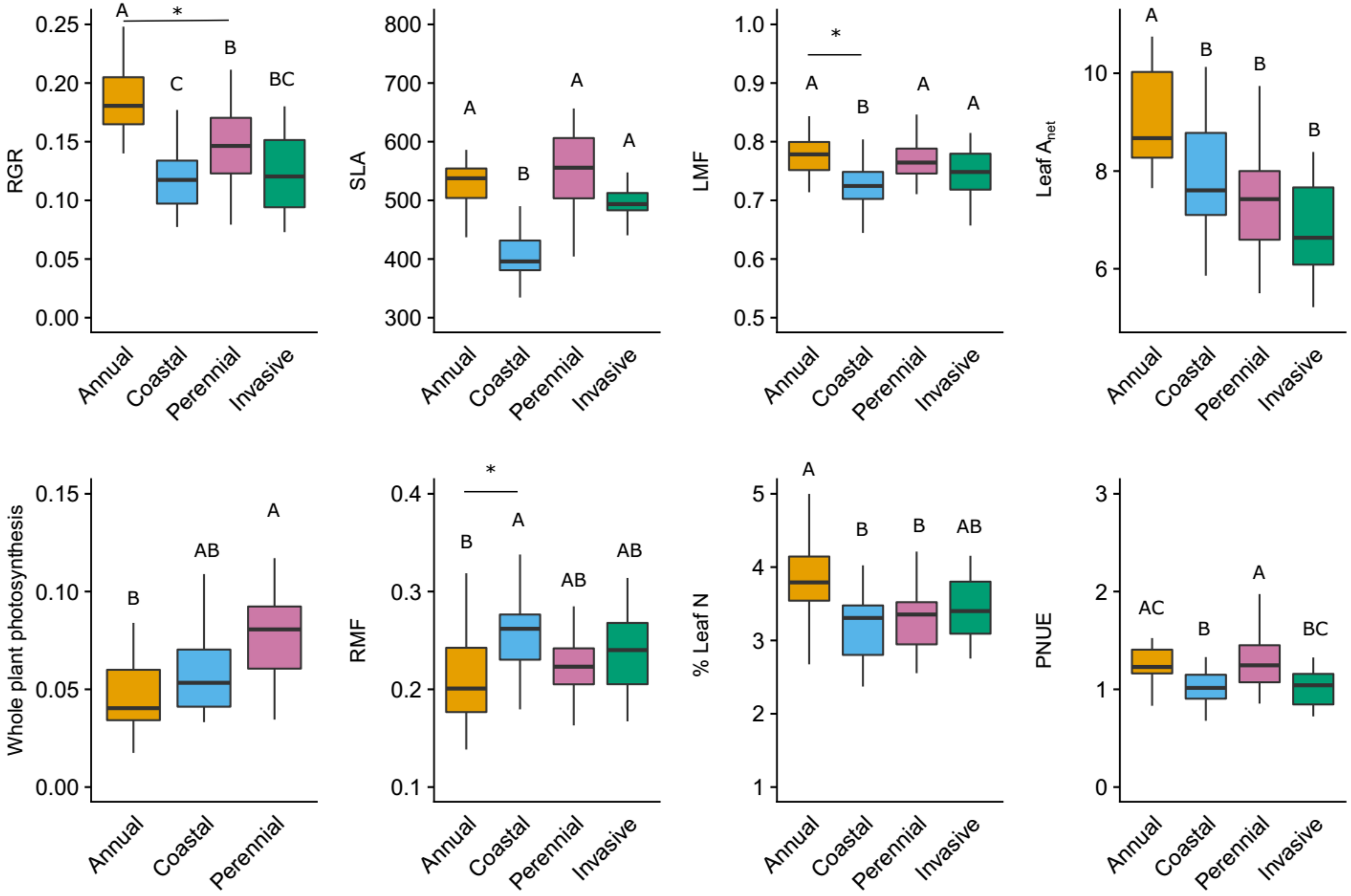

RGR
0.25
0.20
0.15
0.10
0.05
0.00
A
*
C
B
BC
Annual
Coastal
Perennial
Invasive
SLA
800
700
600
500
400
300
A
B
A
A
Annual
Coastal
Perennial
Invasive
LMF
1.0
0.9
0.8
0.7
0.6
0.5
*
A
B
A
A
Annual
Coastal
Perennial
Invasive
Leaf A_{net}
10
8
6
A
B
B
B
Annual
Coastal
Perennial
Invasive
Whole plant photosynthesis
0.15
0.10
0.05
0.00
B
AB
A
Annual
Coastal
Perennial
RMF
0.4
0.3
0.2
0.1
*
B
A
AB
AB
Annual
Coastal
Perennial
Invasive
% Leaf N
5
4
3
2
1
A
B
B
AB
Annual
Coastal
Perennial
Invasive
PNUE
3
2
1
0
AC
B
A
BC
Annual
Coastal
Perennial
Invasive

Figure 2

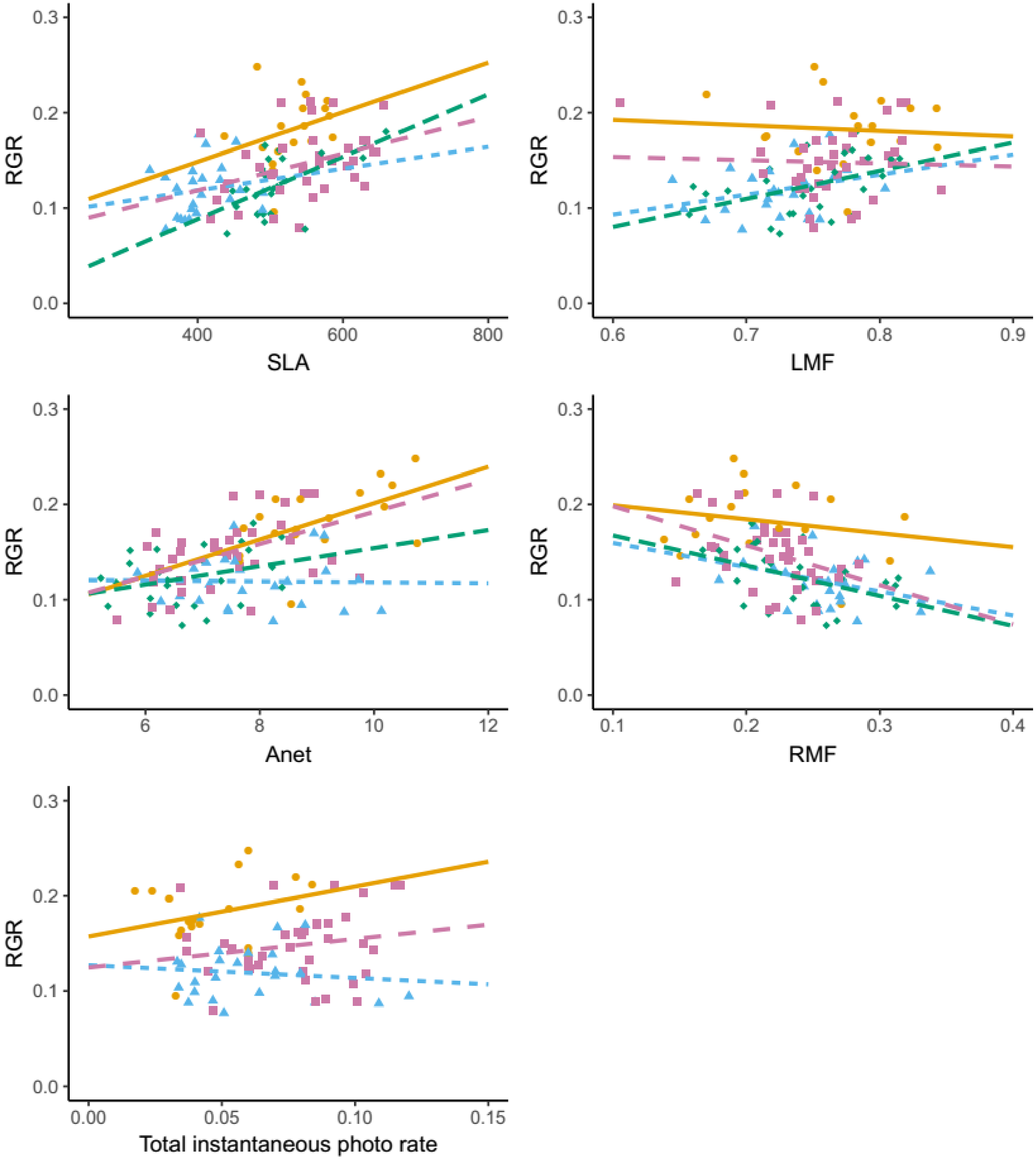

RGR
0.3
0.2
0.1
0.0
400
600
800
SLA
RGR
0.3
0.2
0.1
0.0
0.6
0.7
0.8
0.9
LMF
RGR
0.3
0.2
0.1
0.0
6
8
10
12
Anet
RGR
0.3
0.2
0.1
0.0
0.1
0.2
0.3
0.4
RMF
RGR
0.3
0.2
0.1
0.0
0.00
0.05
0.10
0.15
Total instantaneous photo rate

Figure 3

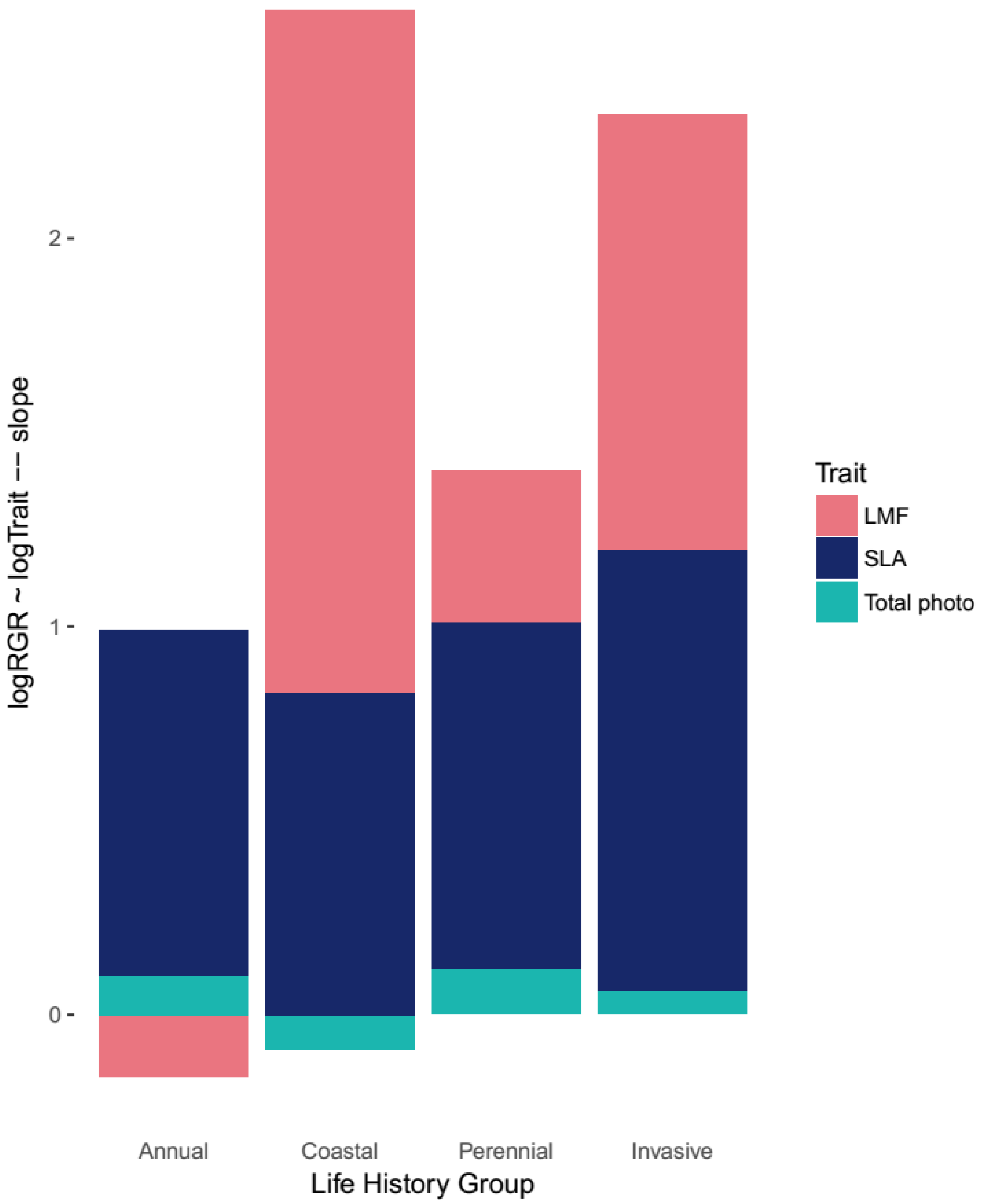

logRGR ~ logTrait -- slope
2
1
0
Annual
Coastal
Perennial
Invasive
Life History Group
Trait
LMF
SLA
Total photo

Figure 4

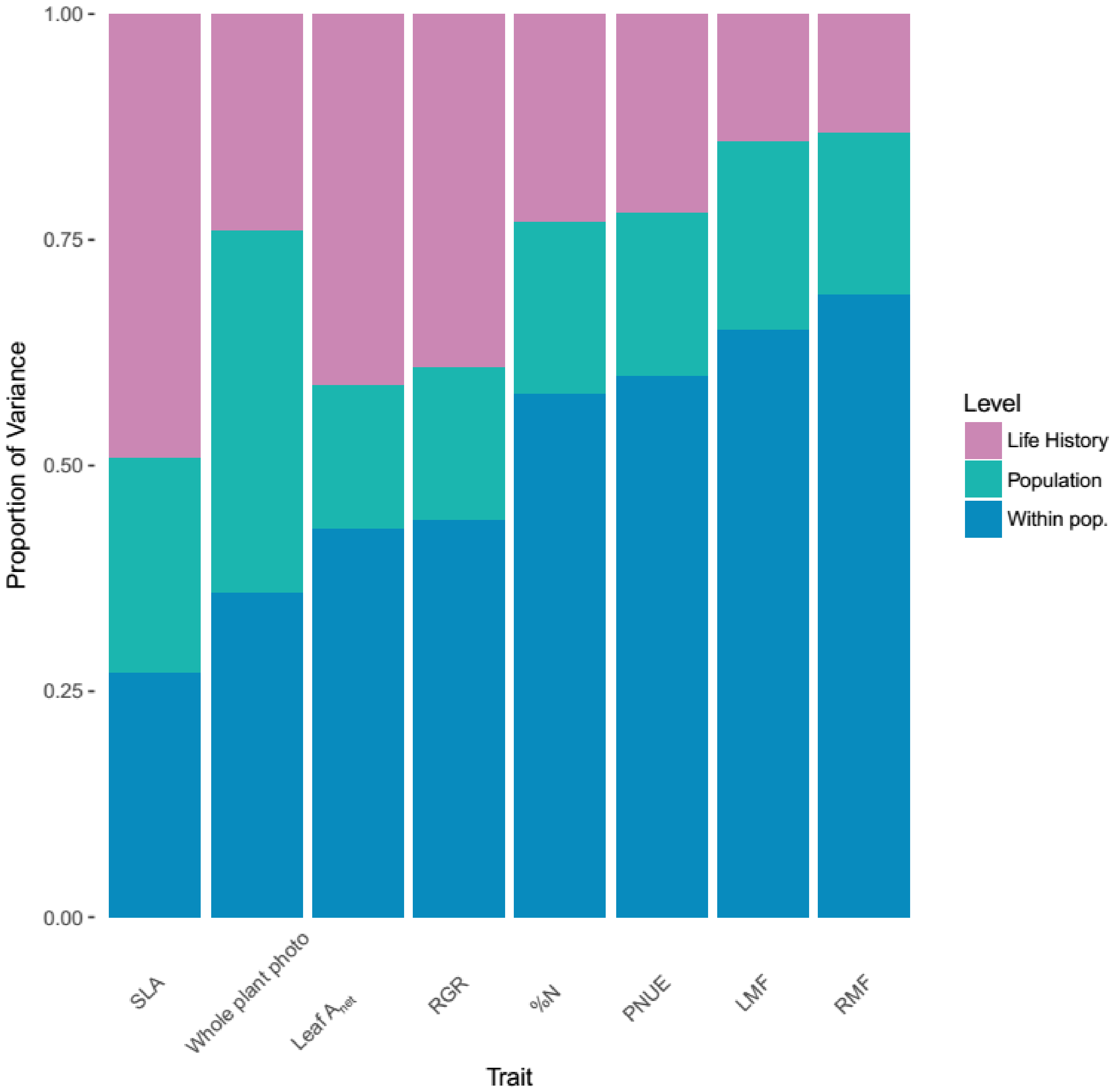
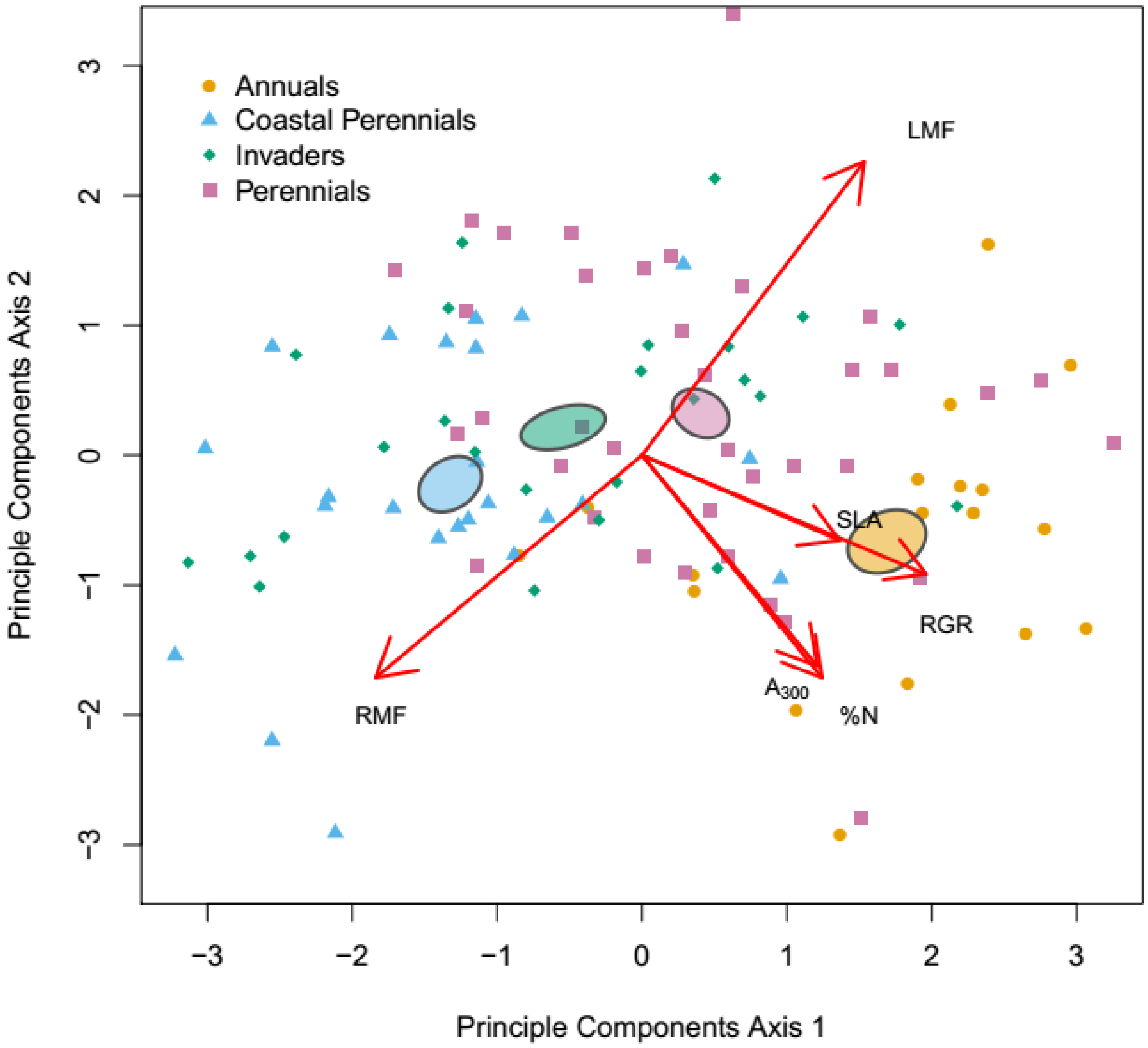

Proportion of Variance
1.00
0.75
0.50
0.25
0.00
SLA
Whole plant photo
Leaf A_{net}
RGR
%N
PNUE
LMF
RMF
Trait
Level
Life History
Population
Within pop.

Figure 5



Annuals
Coastal Perennials
Invaders
Perennials
LMF
SLA
RGR
A_{300}
%N
RMF
Principle Components Axis 2
Principle Components Axis 1
3
2
1
0
−1
−2
−3
−3
−2
−1
0
1
2
3

Figure 6

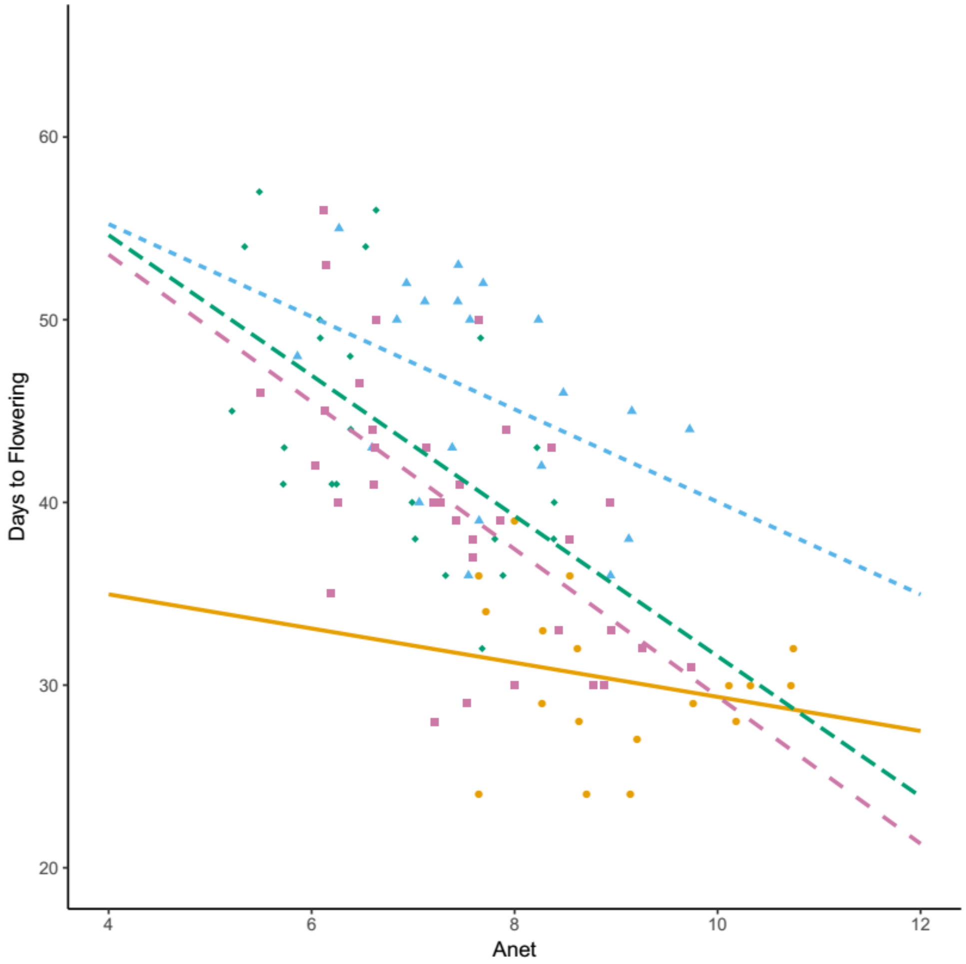


Figure 7

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CHAPTER 5: SYNTHESIS

Invasive species are a major threat to biodiversity, and their threat will continue increasing as a result of anthropogenic activity and global change (Moles *et al.* 2012). Therefore, it is imperative that we understand how they become invasive and how they operate in their new ranges if we are to mitigate their damaging effects. There are both demonstrated and hypothesized plant traits that contribute to invader success. For example, invasive species may be more plastic in response to fluctuating resources (Baker 1965; Funk 2008), they may be more efficient with limiting resources (Heberling & Fridley 2016), or they may have developed traits that allow them to avoid tradeoffs exhibited by native plants (Martin *et al.* 2010). Here, I focused on illustrating trait-based mechanisms that may facilitate invasions and whether those traits originated in the species' historic range or the new, invaded range. I also looked for ways that invasive species may avoid tradeoffs related to key traits, such as relative growth rate, to which native species are subject.

First, I tested whether invasive species have higher plasticity than native species. I studied how 30 native and invasive understory shrub species showed plastic responses to seasonal fluctuations in light availability (Ch 2). I found that invasive species were more plastic than native species, but that plasticity in photosynthetic traits was not adaptive in a carbon gain sense. This result was surprising and questions the importance of the broader significance and adaptiveness of plasticity: just because plasticity exists doesn't necessarily mean it is an important aspect of fitness in the invasive species' new range. Examining the adaptiveness of plasticity should be a goal of studies on invaders in the future.

Second, I found evidence that plasticity in invasive species may arise from processes in both the species' native range and its invasive ranges. This result was significant because the field of invasion ecology still lacks information about how traits of invasive species arise. In my study of five invasive shrub species native to East Asia (Ch. 3), I found that some traits were plastic in response to light availability in the understory in the native range, but new, additional traits were plastic in response to light when looking in the species' invasive range. I conclude that a mix of pre-adaptation from evolutionary history and life history tradeoff avoidance in the invasive range make an invasive species a successful invader.

Lastly, I examined patterns of leaf traits in different life history strategies of a single invasive species (*Mimulus guttatus*) to test the applicability of theories such as the Leaf Economics Spectrum at the species level (Wright et al. 2004). I also decomposed relative growth rate (RGR) of populations of *M. guttatus* with different life history strategies and invasive status to determine if invasive populations maximize RGR differently. By measuring RGR, I was also able to test the theory that RGR is a good predictor of invasiveness within a single species. To do this, I performed a greenhouse study of 22 populations of *M. guttatus*. The 22 populations included perennial, annual, coastal perennial, and invasive populations. I decomposed RGR into its component traits: specific leaf area, photosynthetic rate, and leaf mass fraction (Poorter & VanderWerf 1998). Using this decomposition, I determined that proportional increases in some traits led to much larger increases in relative growth rate for invasive populations than they did for native populations of *M. guttatus*. I also found evidence that, on small taxonomic scales, co-occurring traits predicted by life history theories like the Leaf Economics Spectrum (Wright *et al.* 2004) do not hold true. For example, short-lived annual plants are predicted to invest less in leaf construction and have higher SLA than long-lived perennials. However, in my study, annual and perennial populations had similar values of specific leaf area, and additionally had similar decompositions of relative growth rate. However, I did not find that invasive populations had higher RGR than native populations. Within a single species, RGR may not be a good predictor of invasiveness.

In sum, I found that a variety of approaches are necessary to understand how and why invasive species become successful in their new ranges. Continued study of invasive species in their native habitats will allow us to determine if invaders truly are pre-adapted for success or evolve rapidly in their invaded ranges. On the other hand, controlled experiments in common gardens or greenhouses allow for environmental noise reduction, which allows us to see true inter-species or inter-population variation in traits. I theorize that invasion success is determined on a species-by-species basis, and that while larger patterns across taxa may improve our general understanding of how invasive species come to be, to gain a true mechanistic understanding of invader success, we must study them on small taxonomic levels. For example, my dissertation results indicate the importance of growth in invasiveness varies taxonomically. While growth rate appears important for predicting invasiveness in shade tolerant shrubs, the importance of growth rate was much less important for populations of an invasive herbaceous plant like *M. guttatus*. These discrepancies in my results may stem from the differences in habitat and presence of competitors for these species. Shade tolerant shrubs in Eastern North America start life in a shaded environment and must constantly compete for light, while fast-growing herbaceous annuals grow up in full sunlight. Furthermore, large, slow-growing woody plants and small herbaceous plants vary greatly in the time that they take to reach maturity and reproduce: *M. guttatus* reaches maturity in 2-3 months, while shade tolerant woody shrubs take years to reach reproductive age.

Differences in traits relating to invasiveness in my target woody and herbaceous species may also stem from the use of field versus controlled (greenhouse) environments. Had I studied

M. guttatus plants in their natural habitats, I predict I would have seen a greater signal of growth in invasive populations, since many populations grow in harsh low resource conditions in the desert of Western North America. Observing invaders in their natural environments would also take into account processes like enemy release or extended phenology (Fridley 2012) that might be increasing the growth invasive species. To obtain a fuller understanding of the importance of growth, leaf traits, and pre-adaption of invasive species, more reciprocal transplant studies of invasive species in the habitats in which they occur and along resource or competition axes are needed.

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Education

Syracuse University, Syracuse, NY: Biology PhD candidate, advised by Dr. Jason Fridley; anticipated defense: Oct. 2018

Southern Illinois University, Carbondale, IL: MS, Plant Biology 2014, advised by Dr. David Gibson

Knox College, Galesburg, IL: BA, major: Environmental Studies; minor: Anthropology & Sociology; Honors: Magna cum Laude, Phi Beta Kappa

Publications

Martinez KA, Fridley JD (2018) Acclimation of leaf traits in seasonal light environments: are non-native species more plastic? Journal of Ecology, in press.

Martinez KA, Gibson DJ, Middleton BA (2015) Core-satellite species hypothesis and native versus exotic species in secondary succession. Plant Ecology, 216: 419-427.

Gibson DJ, **Martinez KA** (2014) Shawnee National Forest Vegetation Plot Analysis.

Manuscripts in Prep

Martinez KA, Fridley JD, Oguchi, R, Aiba, M, and Hikosaka K. Evidence for both pre-adaptation and rapid evolution in the invaded range of forest invaders

Fellowships & Awards

2018	Student Travel Award – Ecophysiology Section of ESA ($500)
2018	Travel Grant – Graduate Student Organization, Syracuse University ($450)
2016-2018	STEM Fellow, Syracuse University ($58,000)
2016	NSF EAPSI Japan Fellow ($12,000)
2015-2016	Dept. of Biology TA, Syracuse University ($58,000)
2014-2015	McNair Fellow, Syracuse University 2014-2015 ($65,000)
2013-2014	Dept. of Plant Biology TA, Southern Illinois University ($20,000)
2012-2013	Southern Illinois University Masters Fellow ($20,000)
2011	Undergraduate thesis research grant, Knox College ($1000)

Presentations

2018	**Martinez KA**, Fridley JD. Seasonal plasticity of shade tolerant shrubs. Ecological Society of America, New Orleans, LA
2016	**Martinez KA**, Hikosaka K, Fridley JD. Ecophysiology of deciduous forest invasions: variation in leaf traits home and away. NSF/JSPS Research Symposium. Tokyo, Japan.

Teaching Experience

2017-2018	Undergraduate Honors Research Advising; Lauren Woolhiser, B.S. Syracuse University
2016	General Biology Teaching Assistant, Syracuse University
2015	Global Change Biology Teaching Assistant, Syracuse University
2013-2014	General Biology Teaching Assistant, Southern Illinois University

Other Research and Field Experience

2011	McHenry County Conservation District: Natural Resource Management Intern
2011	School for Field Studies – Yungaburra, Queensland; semester abroad
2010	Green Oaks Term, Knox College;

Service Positions and Affiliations

2017-2018	Syracuse University Biology Graduate Student Organization, Vice President
2017-2018	Syracuse University Graduate Student Senate, Senator
2015-2018	Sigma Xi, member
2013-2014	Southern Illinois University Plant Biology Graduate Student Organization, Vice President
2013-2014	Southern Illinois University Restoration Club, President
2012-2018	Phi Beta Kappa, member

References

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