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Functional traits and resource-use strategies of native and invasive plants in Eastern North American forests

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ABSTRACT

Despite the presumption that native species are well adapted to their local environment, non-native invaders seem to outperform native plants. Intuitively, it appears paradoxical that non-native species, with no opportunity for local adaptation, can exhibit greater fitness than native plants with this advantage. Here, I compared traits of native and invasive shrub and liana species in Eastern North American (ENA) forests to test the overarching hypothesis that non-native understory species invasive to this region have superior resource-use strategies, or alternatively, they share the same metabolic tradeoffs as the native flora. First, at a global scale, I addressed the largely untested hypothesis that biogeography places significant constraints on trait evolution. Reanalyzing a large functional trait database, along with species' native distribution data, I found that regional floras with different evolutionary histories exhibit different tradeoffs in resource capture strategies. Second, using a common garden to control for environment, I measured leaf physiological traits relating to resource investments, carbon returns, and resource-use efficiencies in 14 native and 18 non-native invasive species of common genera found in ENA understories, where growth is presumably constrained by light and nutrient limitation. I tested whether native and invasive plants have similar metabolic constraints or if these invasive species (predominantly from East Asia) are more productive per unit resource cost. Despite greater resource costs (leaf construction, leaf N), invaders exhibited greater energy- and nitrogen-use efficiencies, particularly when integrated over leaf lifespan. Efficiency differences were primarily driven by greater mean photosynthetic abilities (20% higher daily C gain) and leaf lifespans (24 days longer) in invasive species. Third, motivated by common garden results, I conducted a resource addition experiment in a central NY deciduous forest to investigate the role of resource limitation on invasion success in the field. I manipulated understory light environments (overstory tree removal) and N availabilities (ammonium-nitrate fertilization) to create a resource gradient across plots each containing 3 invasive and 6 native woody species. Invasive species generally exhibited greater aboveground productivity and photosynthetic gains. After two treatment years, invasive species displayed more pronounced trait responses to the resource gradients, primarily light, relative to the weaker responses of native species. Lastly, I asked whether species exhibit similar resource-use strategies in their native and invasive ranges. I measured leaf functional traits of *Rhamnus cathartica* (native to Europe, invasive in ENA) and *Prunus serotina* (native to ENA, invasive in Europe) in populations across central NY and northern France. Notably, I found invasive US populations of *R. cathartica* had markedly greater photosynthetic rates (50% higher) and reduced leaf N resorption rates in autumn (30% lower) than native French populations. Contrastingly, I found minimal leaf trait differences in *P. serotina* between native (US) and invasive (French) populations. Collectively, my results highlight the utility of functional trait perspectives and support a mechanistic explanation for invasion success based on differential abilities of species to convert limiting resources to biomass.

FUNCTIONAL TRAITS AND RESOURCE-USE STRATEGIES OF NATIVE AND INVASIVE PLANTS IN EASTERN NORTH AMERICAN FORESTS

by

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DISSERTATION

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INTRODUCTION.

Understanding modern floristic interchange through functional traits

> *Instead of six continental realms of life [of Wallace (1876)] . . . there will be only one world, with the remaining wild species dispersed up to the limits set by their genetic characteristics, not to the narrower limits set by mechanical barriers.*
> (Elton, 1958)

Historically isolated floras are no longer isolated. Mountain ranges, oceans, and the many other geographic barriers that have restricted plant dispersal in the past are effectively dissolving to create a modern supercontinent akin to Pangea. The globalization of human activities has resulted in the widespread movement of plants around the world that created considerable conservation concern as a major source of global environmental change (Vitousek *et al*., 1997). These ecological consequences have included significant alterations to ecosystem function (Ehrenfeld 2010), changes in local/regional biodiversity (Wilcove 1998; Sax & Gaines, 2003), and the restructuring of regional biota (Lodge & Shrader-Frechette, 2003). Replicated across continents and biomes, plant invasions serve as one of the largest unplanned experiments in ecology and evolution (Sax *et al*., 2007).

The invasion process can be conceptualized along a continuum (Richardson & Pyšek 2000) from 1) introduction to 2) naturalization (*i.e.*, self-sustaining populations, but not spreading) to 3) invasion (actively spreading populations; greatest management concern). Introduced species are released from selection pressures in their home range (*e.g.*, coevolved herbivores) while at the same time confronted with a new set of environmental challenges (*e.g.*, novel climate, competitors). Leading invasion hypotheses invoke escape from natural enemies

(Blossey & Nötzbold, 1995; Keane & Crawley, 2002), weak competition from native residents (Elton, 1958; Levine et al, 2004) and/or novel “weapons” or resource-use strategies (Davis *et al.,* 2000; Callaway & Ridenour, 2004). Mechanistically, successful invasion requires a given species to meet one of three conditions (Hulme & Barrett, 2012): 1) pre-existing traits well suited for the abiotic and biotic conditions of invaded range habitat (pre-adaptation; Mack, 2003; Fridley & Sax, 2014) 2) physiological flexibility to adjust relevant trait values (phenotypic plasticity; Baker, 1965, Davidson *et al*., 2011) and/or 3) ability for rapid trait evolution (local adaptation; Whitney & Gabler, 2008).

In Eastern North America (ENA), the non-native flora comprises around 2,629 taxa and includes representative species from every floristic region except New Zealand. The majority of these non-native plants originated in Europe and commonly inhabit disturbed, open habitats across ENA (Fridley, 2008), which are colloquially considered “weeds.” However, only a restricted subset of non-native taxa (440) is currently recognized as “invasive” (*i.e.*, those with spreading populations). An unexpectedly large fraction of these invasive species consist of East Asian shrubs and lianas that invade ENA forests (Fridley, 2008). Forest invasions provide an important and interesting study system; as growth in temperate forest understories worldwide are limited by light (Pacala *et al*., 1994) and in some cases, soil nitrogen availabilities (Reich *et al*., 1997). Unlike invasions into ecosystems of high resource or anthropogenic disturbance, invasive species in resource-limited systems often exhibit traits associated with resource conservation, such as slow growth, increased tissue investments, and efficient resource use (Funk, 2013). In addition to the novel insights into ecophysiology, ENA forests as a study system provide a broader biogeographic perspective on the invasion process. Plant interchanges between Eurasian and ENA forest ecosystems are highly asymmetric, with East Asia contributing comparatively more invaders to ENA, than ENA species to East Asian forests (Weber *et al.*, 2008; Fridley, 2013).

How do these woody species from Eurasia thrive in ENA forests? More generally, how are non-native species able to thrive in an introduced region and outperform resident species, despite the notion that native species are locally adapted to the environmental conditions? This concept has been deemed the paradox of invasion (*sensu* Sax & Brown, 2000). In essence, testing the "how" and "why" invasion hypotheses require an understanding of trait ecophysiology. Recent developments on the use of plant *functional traits* have advanced community ecology into a more predictive, mechanism-based discipline (Weiher *et al.*, 1999; Díaz *et al.*, 2004; McGill *et al.*, 2006; Westoby & Wright, 2006). In this dissertation, I refer to functional trait as any individual-level morphological, physiological, or phenological feature that indirectly relates to fitness (Violle *et al.*, 2007) and *strategy* as any combination of functional traits that can be linked to physiological performance (Donovan *et al.*, 2011).

Current theories on the evolution of leaf functional traits, developed largely independent from invasion biology, assume strong global convergences in resource-use strategies. This "worldwide leaf economic spectrum" (LES; Wright *et al.*, 2004) runs from fast growing, resource demanding species (e.g., high nutrient concentrations, high photosynthetic capacities, high specific leaf areas, short leaf lifespans) to those species with contrasting traits. This functional convergence has been explained through biophysical limitations and selection (Reich *et al.*, 1999;

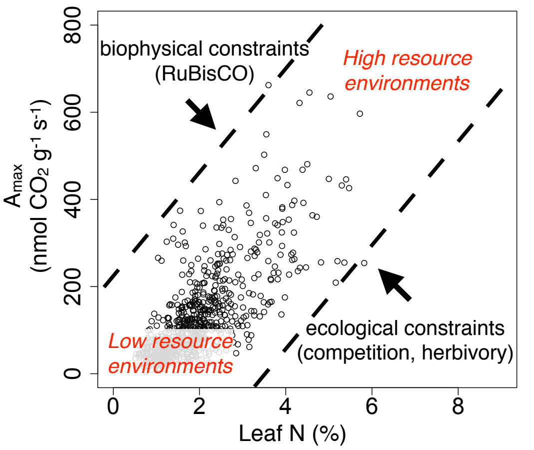

Figure 1. *Relationship between photosynthetic capacity (A_{max}) and leaf nitrogen (N) in a global dataset. R^2 = 0.55, P<0.001. Data redrawn from Wright et al. (2004).*

Donovan *et al*., 2011; Fig. 1). With the recognition of only subtle LES modulation from climate (Wright *et al*., 2005b) and even smaller differences between growth forms (Wright *et al*., 2005a) or biomes (Reich *et al*., 1999), the broad generality of the LES has been presented as a robust framework for plant strategy evolution that is independent of phylogeography. For any given energetic investment in leaf construction, LES theory assumes that species have had sufficient time and genetic variation to evolve an optimum strategy for converting resources into biomass, regardless of evolutionary history (Donovan *et al*., 2011).

What functional traits or strategies are associated with invasiveness? Recent studies have sought to place this reported LES generality within this invasion context. In an effort to identify trait differences that promote successful invasions, the leaf economic strategies between natives and invaders have been compared (*e.g*., Leishman *et al*., 2007, 2010; Peñuelas *et al*., 2009; Ordonez *et al*., 2010). The basis for much of this literature assumes global constraints on LES tradeoffs (Donovan *et al*., 2011). Following LES theory, it is commonly concluded that invasive species do not differ from native species in their fundamental resource capture strategies. Rather, trait differences should be seen along a common resource tradeoff axis (shift along a common slope of LES tradeoffs; Fig. 2a), which largely reflects variation in resource availability among

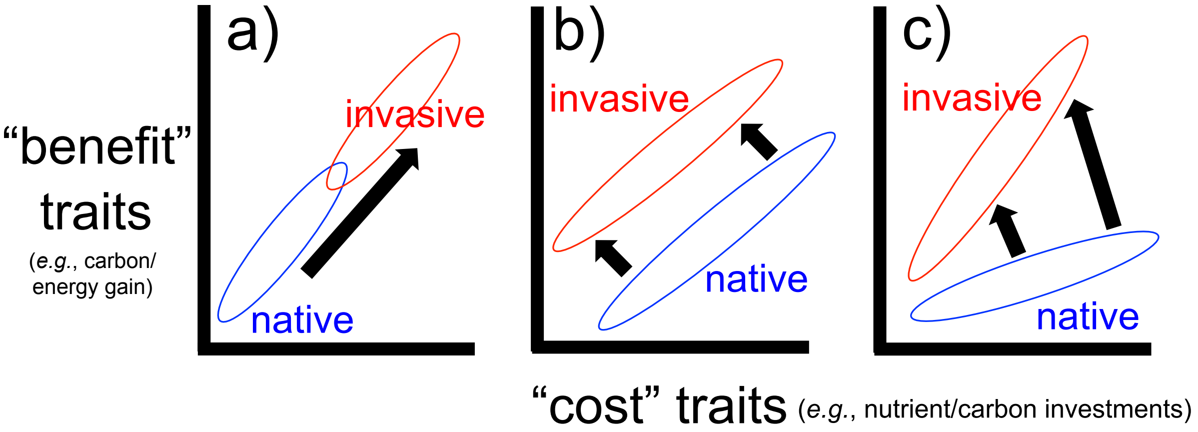

Figure 2. *Hypothesized functional differences between native and invasive species. a) trait shifts along common strategy tradeoff axis (e.g., the LES) b) uniform traits shifts along shared slope (e.g., allocation toward other processes or greater efficiency) c) non-uniform trait shifts indicating fundamentally different functional strategies.*

habitats (Leishman *et al*., 2010). An alternative prediction, particularly in resource-limited systems, is that trait differences between native and invasive species might be intrinsic. In other words, all other factors equal, these invasive species might reap greater carbon benefits at a given resource investment (Fig. 2b,c).

Here, I compared traits of native and invasive shrub and liana species in Eastern North American (ENA) forests to test a mechanistic explanation for successful invasions based on an alternative concept of “metabolic efficiency” – that is, the ability of a plant to acquire and convert limiting resources (e.g., light, nutrients, or water) into biomass (and ultimately, into offspring; “reproductive power” *sensu* Brown *et al*., 1993). While these physiological efficiency differences could be pre-adapted traits from the native range (Mack, 2003), this dissertation first seeks to identify general physiological and efficiency differences between invasive and co-occurring native plants.

In chapter one, at a global scale, I analyzed a large leaf trait database combined with species distribution information to test whether the unique evolutionary histories of regional floras place constraints on global trait evolution. Past analyzes have considered leaf trait relationships according to in biome or climate. This study presents one of the first tests of leaf trait variation as a function of broad scale historical differences. I found substantial variation in leaf trait relationships between the floras of Northern and Southern Hemispheres, East Asia and ENA, and Hawaiian Islands and tropical mainland floras. These differences between floristic regions support an alternative, historical perspective to suggest that species from different evolutionary backgrounds can exhibit different tradeoffs in resource-use traits.

In chapter two, utilizing an established common garden to control for environment, I compared leaf functional traits relating to carbon capture, resource investments, and resource-use efficiencies in 14 native and 18 invasive related species found in ENA forest understories. Despite greater leaf nitrogen and structural investments, invasive species displayed greater resource-use efficiencies (carbon benefits per unit resource cost), particularly when integrated over leaf lifespan. These results suggest these invasive species are not constrained to the same axis of resource-use strategies.

In chapter three, I tested whether the results from the common garden comparison were observed under field conditions to assess the degree to which resource availability affects functional traits and determines invasion success. Unlike in ecosystems with high disturbance, I hypothesized that invasive shrubs in ENA forests would maintain physiological advantages over native species, even under low light and nitrogen conditions. I performed two-year factorial manipulation of light and soil nitrogen in a deciduous forest in central NY to construct and experimental resource availability gradient with three invasive shrubs and six native shrubs/saplings. In agreement with common garden comparisons, I found invasive species to display greater rates of carbon gain and resource investments at all resource levels. However, invasive species tended to be more responsive to light availability in terms of functional trait adjustments and aboveground biomass production.

Lastly, in chapter four, I compared leaf functional traits in both native and invasive ranges to test whether invasive species display traits that are conserved from their native range. I measured traits of *Rhamnus cathartica* (native to Europe, invasive in ENA) and *Prunus serotina* (native to ENA, invasive in Europe) in populations across central NY and northern France. In both species, I found substantial variation in leaf economic traits and trait scaling relationships. *R. cathartica* displayed 50-60% higher photosynthetic rates and 70% higher senesced leaf nitrogen concentrations in its invasive compared to native range, while intercontinental differences in *P. serotina* were less dramatic. These results question the assumption that functional strategies of invasive species are consistently similar in their native range.

Overall, the results and perspectives in this dissertation highlight the utility of using functional traits to ascribe mechanism to plant invasions and emphasize the importance of recognizing fundamental resource-use strategy differences across species.

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CHAPTER 1.

Biogeographic constraints on the worldwide leaf economics spectrum

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ABSTRACT

Aim The worldwide leaf economic spectrum (LES) describes tight coordination of leaf traits across global floras, reported to date as being largely independent of phylogeny and biogeography. Here, we present and test an alternative, historical perspective that predicts that biogeography places significant constraints on global trait evolution. These hypothesized constraints could lead to important deviations in leaf trait relationships between isolated floras that were influenced by different magnitudes of genetic constraint and selection.

Location Global, including floristic regions of the Northern and Southern Hemispheres, Eastern North America (ENA), East Asia (EAS), Hawaiian Islands, and tropical mainland floras.

Methods We use a large leaf trait database (GLOPNET) and species' native distribution data to test for variation in leaf trait relationships modulated by floristic region, controlling for climatic differences. Standardized major axis (SMA) analyses were used to evaluate biogeographic effects on bivariate relationships between LES traits, including relationships of photosynthetic capacity and dark respiration rate (A_{mass}-$R_{d\text{-}mass}$), leaf lifespan and mass per area ratio (LL-LMA), and photosynthetic capacity and nitrogen content (A_{mass}-N_{mass}).

Results Independent of climate or biome, floras of different evolutionary histories exhibited different leaf trait allometries. Floras of the Northern Hemisphere exhibited greater rates of return on resource investment (steeper slopes for the trait relationships analyzed), and the more diverse temperate EAS flora exhibited greater slopes or intercepts in leaf trait relationships, with the exception of the A_{mass}-N_{mass} relationship. In contrast to our hypothesis, plants of the floristically isolated Hawaiian Islands exhibited a similar A_{mass}-N_{mass} relationship to those of mainland tropical regions.

Main conclusions Differences in leaf trait allometries among global floristic regions support a historical perspective in understanding leaf trait relationships and suggest that independent floras can exhibit different tradeoffs in resource capture strategies.

Keywords: functional trait variation, plant strategy theory, biogeography, convergent evolution, plant metabolic efficiency, leaf lifespan, photosynthetic rate, leaf respiration, leaf nitrogen

INTRODUCTION

Wright *et al*. (2004) reported a general tradeoff surface ("leaf economic spectrum"; LES) describing coordinated variation in leaf traits among global floras (see also Reich *et al.*, 1997; Reich *et al.*, 1999; Wright *et al*., 2005a, b). This spectrum runs from species that have a quick return in leaf investment (i.e., low construction cost, low mass per area, short longevity, high photosynthetic capacity, high respiration rate, and high nutrient content) to those with contrasting traits associated with slow return on resource investment. With the recognition of only subtle LES modulation from climate (Wright *et al*., 2005b) and even smaller differences between growth forms (Wright *et al*., 2005a) or biomes (Reich *et al*.,1997; Reich *et al*., 1999), the broad generality of the LES has been presented as a robust framework for plant strategy evolution that is independent of phylogeography. These reportedly widespread general patterns in leaf trait relationships point toward global evolutionary convergence in the fundamental tradeoffs of plant resource economics (Shipley *et al*., 2006; Donovan *et al*., 2011; Tilman, 2011). Several mechanistic explanations of this reported global generality of the LES have been invoked, including the physical constraints of leaf physiology (Shipley *et al.*, 2006; Blonder *et al.*, 2011), genetic constraints, and selection pressures against assumed inefficient trait combinations (Donovan *et al*., 2011).

At its core, the leaf economics spectrum is an argument for the optimization of plant resource foraging across a range of environmental conditions, with different strategies across the gradient driven largely by resource availability (Fig. 1, shift A). Given a position along a resource gradient, LES theory assumes that species have had sufficient time and genetic variation to evolve an optimal strategy for converting resources into biomass through a maximally efficient carbon economy (Reich *et al.*, 1999; Donovan *et al.*, 2011). Although differences in environmental or biotic stresses between regions may require allocation of resources to processes not directly associated with carbon gain (such as defensive chemistry, frost or drought tolerance, etc.; Fig. 1, shift B), it has been argued that an increase in leaf tissue investment should return a similar return in carbon gain, as evinced by a common slope of leaf trait relationships among global floras (Reich *et al*., 1997; Reich *et al.*, 1999; Wright *et al.*, 2004). If LES trait relationships are indeed consistent among floras that have been isolated for long periods of evolutionary time, then either: 1) phylogenetic constraints on leaf-level carbon economy are minor across major plant lineages; 2) optimal carbon economies evolve relatively quickly once plant lineages colonize new areas of novel resource conditions; or 3) the core allometries of LES evolved early in vascular plant evolution and have been preserved as the major lineages colonized the Earth’s land masses (Tilman, 2011).

An alternative hypothesis is that contingencies in the evolutionary development of historically isolated biotas—e.g., the large variation in age, spatial extent, and phylogenetic diversity (number of resident plant lineages) of the world's floras (Takhtajan, 1986)—has precluded the emergence of a canonical, globally consistent set of leaf trait allometries. If an optimized carbon economy is largely the result of selection, with less efficient genotypes being eliminated over the course of plant evolution due to low fitness (Donovan *et al.*, 2011), then the level of efficiency of species' carbon economics should be proportional to the competitive intensity experienced by plants in a particular region, which we reason is in part a function of the size and diversity of the flora (Dobzhansky, 1950). With substantial differences in evolutionary histories between modern global floras (Takhtajan, 1986), we predict that those floras that evolved in isolation should exhibit different sets of strategies in resource economics, beyond ecosystem differences in allocation (Reich *et al.*, 1999; Wright *et al.*, 2001). This difference should be most clearly demonstrated through the existence of different trait allometries (i.e., slopes), where, per unit increase in leaf cost traits, the summed benefits are greater in the flora with greater genetic variation and historical selection pressures for metabolic function (Fig. 1, shift C). A shift in slope for leaf economic traits signifies the regions follow different carbon capture strategies (*sensu* Leishman *et al.*, 2010). LES modulations have been reported in the form of slope or y-intercept differences between sites of different climates (Wright *et al.*, 2005b), but biogeographic differences independent of climate have not been examined.

It is well known that plant and animal lineages released from competition and predation pressures after colonization of oceanic islands evolve along different trait trajectories than mainland lineages (Carlquist, 1974; Brown, 1995; Lomolino *et al.*, 2010), and this ‘naïve’ evolutionary status of island assemblages is a frequent reason given for their high susceptibility to invasion from mainland lineages (Vermeij, 2005). Small or historically isolated floras may also be more susceptible to phylogenetic constraints that limit regional trait evolution, a phenomenon that is well described in the plant invasion literature (Mack, 2003) and could in theory limit the expression of leaf-level trait allometries (Donovan *et al.*, 2011). The degree to which allopatric trait convergence is realized, even within a biome type, can be constrained by these important historical differences between regions (Cody & Mooney, 1978).

We utilized the GLOPNET data set (Wright *et al*., 2004) to analyze plant trait relationships according to their native floristic region. Prior studies have analyzed such relationships in the context of climate (Wright *et al*., 2005b), biome (Reich *et al*., 1997; Reich *et al*., 1999), growth form (Wright *et al*., 2004; Wright *et al.,* 2005a), phylogeny (e.g. Ackerly & Reich,1999; Walls, 2011), or habitat factors (e.g. soil fertility; Ordoñez *et al*., 2009). However, these analyses lack the ability to evaluate differences between global floras as a result of historical influences. Testing for biome-level variation in LES trait relationships (e.g., Reich *et al*., 1999), based solely on ecological similarities that ignore evolutionary differences, obscures the detection of potential differences arising from historical constraints. Potential historical constraints include the presence or absence of given plant clades through evolutionary time (phylogenetic constraints), past physical barriers to dispersal, climatic/geologic events, and other relict biogeographic processes (including past biotic interactions), all of which influence the evolutionary trajectories of distinct floristic regions. Evaluation of the relative influence of biogeography requires comparing climatically similar phytogeographic regions that reflect evolutionarily meaningful units based on shared evolutionary histories (Takhtajan, 1986). Past studies have measured the influence of phylogeny on leaf trait patterns (Ackerly & Reich, 1999; Walls, 2011). However, there have been no studies to date which compare LES relationships at the level of floristic regions, the level that would reflect the larger scale phylogenetic and biogeographic constraints in trait evolution. We expected that isolated floras would exhibit significantly different strategies of leaf carbon economics relative to larger, more connected and diverse floras, which we assume experienced greater competitive intensities for longer periods of evolutionary time (Dobzhansky, 1950; MacArthur, 1972).

We chose three different trait relationships to quantify the potential for carbon gain per resource cost using key leaf-level tradeoffs. These included relationships of leaf photosynthetic capacity and dark respiration (A_{mass}-$R_{d\text{-}mass}$), leaf longevity and leaf mass per area (LL-LMA), and photosynthetic capacity and leaf nitrogen concentration (A_{mass}-N_{mass}). A given flora was said to follow "fundamentally different carbon capture strategies" (*sensu* Leishman *et al.*, 2007; Leishman *et al*. 2010) if resident species displayed a greater increase of a carbon gain trait per unit increase cost trait (i.e., steeper slope; Fig. 1, shift C). Resource allocation differences can also be shown if resident species displayed consistently greater carbon gain trait values at any given value of a cost trait (i.e., greater y-intercept; Fig. 1, shift B). Differential allocation could be due to either abiotic differences between sites or core strategy differences due to historical reasons. Therefore, floras with similar climates could theoretically exhibit slope or y-intercept shifts, if historical differences can explain their leaf physiologies (Fig. 1).

We tested the following hypotheses:

(**H_1**) Northern Hemisphere (NH) floras exhibit a greater slope or intercept in LES trait relationships than those of the Southern Hemisphere (SH), showing significant deviations from the presumed global generality of LES tradeoffs. Controlling for climate differences, this first comparison was motivated by the contrasting evolutionary histories of the predominantly vicariant floras of former Gondwanaland (current SH floras) and the larger, more connected floras of former Laurasia (current NH floras) (Morrone, 2009). With smaller component areas as Gondwana broke apart, lower phylogenetic diversity, and greater isolation relative to the NH, the floras of the SH historically came into contact with fewer plant lineages and may have experienced lower selection pressure for resource-use economy.

(**H_2**) Plants of East Asia (EAS) show greater slopes or intercept in LES trait relationships than plants of Eastern North America (ENA), despite both floristic regions lying largely within the north temperate mesic forest biome. The potential for a more efficient resource economy of EAS plants than those from ENA is suggested by several lines of evidence. A diversity bias exists with disjunct genera between ENA and EAS having twice as many species in EAS than ENA, resulting from large-scale historical differences between the regions rather than habitat differentiation (Qian & Ricklefs, 2000). We reason that the greater diversity of the EAS flora has led to a more intense competitive environment and therefore greater selection pressure for resource-use efficiency in EAS plants. Additionally, there is a clear recorded invader bias with a large proportion of recent invaders to ENA being of EAS origin (Fridley, 2008), but not the converse (Weber *et al.*, 2008). This invader bias may be at least partly attributable to the greater resource economy of EAS species, which provides supplementary evidence to support our prediction of the scalings of LES traits between EAS and ENA.

(**H_3**) The endemic flora of Hawaii exhibits a reduced slope or intercept in LES trait relationships in comparison to tropical mainland floristic regions. This prediction is based on the small area and younger age of the islands, the low phylogenetic diversity of the native flora, and the recognition that Hawaii represents the most isolated floristic region in the world (Takhtajan, 1986). It is also consistent with empirical evidence from controlled studies involving native and alien Hawaiian species (e.g., Pattison *et al.*, 1999; Funk, 2008).

METHODS

Floristic region and leaf trait data

We combined the extensive GLOPNET data set (Wright *et al.*, 2004) with species' native distribution data to test hypothesized differences in the leaf economies among select global floras. The native range of each species was assigned to one or more of the global floristic regions of Takhtajan (1986). This scheme represents the most current understanding of phytogeographic regions based on areas of endemicity and major centers of plant diversification (see Fridley, 2008). Based on core geographic ranges, each species was grouped as a Northern or Southern Hemisphere species. Species with distributions spanning both Northern and Southern Hemispheres were excluded from the hemispheric contrasts due to the inability to assign these pantropical ranges as exclusively within the Northern or Southern Hemispheres. Native species distributions were assigned primarily through floristic information from the USDA Germplasm Resources Information Network (USDA ARS, 2010). Additional source floras were consulted as appropriate for confirmation of the extents of geographic ranges. The original GLOPNET data set (Wright *et al*., 2004) contains 2,548 entries and 2,021 different plant species from 175 sites. Trait data for 346 entries were excluded for the current analysis due to insufficient distribution data, species originating in human cultivation, or an unclear native origin (see Appendix S1 in Supporting Information for floristic region assignments). See Wright *et al.* 2004 for additional details on the specific measurements protocols and compilation of leaf data in the GLOPNET data set.

The trait combinations selected have clear predicted functional meaning without providing redundant interpretations, including the scaling of photosynthetic capacity to dark respiration (A_{mass}-$R_{d\text{-}mass}$; potential carbon gain-carbon maintenance cost), leaf longevity to leaf mass per area (LL-LMA; duration for potential resource gains per proxy measure of leaf cost), and photosynthetic capacity to leaf nitrogen concentration (A_{mass}-N_{mass}; photosynthetic N use efficiency). We interpret leaf-level performance from these traits and can only speculate on whole plant fitness.

Data analysis

Three floristic region contrasts were chosen: 1) the landmasses of the Northern and Southern Hemispheres, 2) the East North America (ENA; Takhtajan region 3, North American Atlantic region) and Eastern Asia (EAS; Takhtajan region 2, Eastern Asiatic region), and 3) the Hawaiian Islands (Takhtajan region 21, Hawaiian region) and mainland tropical regions (aggregated Caribbean, Amazonian, and Malesian regions, Takhtajan regions 18, 23, 25). Because a "mainland" source flora contrast for Hawaii was not intuitively clear, several alternative tropical floras were explored. The flora that colonized the Hawaiian floristic region is thought to have dominantly arisen from the Malesian islands, as well as from tropical mainlands of Central America and Australia (Takhtajan, 1986). Because fitted lines for species of these regions did not significantly differ in slope or elevation (analysis not shown), species from the tropical mainland and island regions of Caribbean, Amazon, and Malesia were aggregated to form the comparison. Some of the species in the dataset had geographic ranges which spanned more than one floristic region. In a few cases, species were excluded from a given floristic analysis if they were represented in both regions of interest (e.g., several circumboreal or cosmopolitan species were excluded from the EAS-ENA comparison).

Controlling for extrinsic factors of climate, biome, and growth form between floras

To ensure LES contrasts between floras were not the result of climatic differences, we used site climate data from Wright *et al.* (2004) to compare climate distributions between regions. If differences were found for a given comparison, we controlled for climate by rerunning the analysis using only data from sites that did not differ significantly in climate. This was only true for the NH-SH comparison (see Results). There was no significant difference in climate variables for sites used in the EAS-ENA analysis (MAT, rainfall, PET; see Table 1 for sample sizes, t-tests, all $P > 0.2$) or the Hawaii-tropical mainland MAT contrast (n_1= 5 sites (HI), n_2= 22 sites (mainland),t = 0.27, df=13, $P = 0.79$). We did not preclude edaphic differences as a driver in potential trait variation for this contrast (see Discussion). In addition, chi-square contingency tests were performed to assess any biases between floristic regions in the composition of woody and non-woody growth forms represented in the dataset. No consistent bias was found (analysis not shown), and growth form is not considered a major driver of these relationships (Wright *et al.*, 2005a).

Testing for differences in resource economics between evolutionarily distinct floras

Leishman *et al.* (2007) advocate the use of scaling relationships to infer metabolic function and "fundamental" resource capture strategies because resource-use efficiency ratios (e.g., photosynthetic nitrogen use efficiency; A_{max}/leaf N) can be affected by differences in slope, intercept, or shifts along a common slope. Allometric relationships for each floristic region contrast were analyzed through standardized major axis (SMA) regression implemented in the smatr package for R (Warton, 2007; R development core team, 2010). SMA regression line fitting minimizes residual variance in both x and y dimensions and is preferred in analyzing bivariate allometric relationships, as opposed to predicting y from x in classical regression (Warton *et al.*, 2003). Testing in the SMA regression routine involves first testing for common slopes between groups. If the slopes do not differ (homogenous), the lines fitted to the groups may represent a shift along their common slope and/or shifts in elevation (y-intercept). The data were $\log_{10}$ transformed to sufficiently meet the assumptions of SMA regression.

Slope homogeneity was tested for each set of floristic contrasts. If the slope differed between floras (heterogeneity), the flora with a greater slope showed a greater increase in trait x per increase trait y. When comparing leaf economic traits, slope differences between two groups has been interpreted as the groups following different strategies of resource capture (Leishman *et al.* 2007; Leishman *et al.* 2010). When the groups shared a common slope, a common slope was fitted. If the common slope relationship differed in elevation (intercept), the flora with the greater y-intercept exhibited greater resource returns at any given value of cost trait x. Likewise, when fitted lines shared a common slope, groups may be shifted along a common axis, where floras follow identical allometry but lie on different points of the same tradeoff surface (Warton *et al.*, 2003). Heterogeneity in slopes or intercepts of lines separately fitted for each region indicated differences among floras in the execution of a common ecological strategy, rather than habitat-driven shifts within floras, for the trait relationships analyzed.

RESULTS

Northern-Southern Hemisphere contrasts

As predicted, slope heterogeneity was detected in all three relationships in the hemispheric contrasts. Comparing potential carbon gain (A_{mass}) to carbon respiratory costs ($R_{d\text{-}mass}$), leaf longevity (LL) to leaf mass (LMA), and potential carbon gain to leaf nitrogen content (N_{mass}), the slopes of the fitted lines for each trait contrasts were significantly different (Table 1, Fig. 2A-C). Species native to the NH exhibited consistently higher slope values in all three trait relationships. This indicates that in the bivariate relationships explored, the scaling of the NH species' traits showed significantly higher returns per increase in resource investment.

Because these slope differences could be function of MAT (Wright *et al.*, 2005b), differences in mean site MAT between regions were tested. Sites in the Southern Hemisphere had higher MAT on average (see Table 1 for sample sizes; 2-sided tests; Fig. 2A: $t = -1.31$, $df = 16.42$, $P = 0.21$; Fig. 2B: $t = -2.99$, $df = 34.15$, $P < 0.01$; Fig. 2C: $t = -3.882$, $df = 34$, $P<0.001$). These mean site differences were caused by several low MAT sites (MAT<0º C, n = 7) in the

Northern Hemisphere and several high MAT sites (MAT>20º C, n = 7) in the Southern Hemisphere. When excluding these sites in the highest and lowest MAT classes (MAT<0º C and MAT>20º C), the resulting sites did not differ in MAT (t-tests; mean n_1= 25.67 (N Hemisphere), mean n_2 = 14.33 (S Hemisphere), all $P>0.1$), and SMA analyses were rerun using this data subset. The conclusions remained the same for all three relationships (tests for SMA heterogeneity, $P<0.05$). Robust to climate, the points in Fig. 2A-C include sites of all climates.

EAS-ENA contrasts

EAS-ENA regional floras exhibited variable results with respect to the direction of the hypotheses (Fig. 2D-F). Notably, despite small EAS sample size (n=12) and sites having similar climates, the A_{mass}-$R_{d\text{-}mass}$ relationship showed a clear shift in elevation. Against our expectation, the slopes of this relationship when fitted separately by region was statistically insignificant, but assuming common slopes, EAS species exhibited consistently higher A_{mass} for a given $R_{d\text{-}mass}$ (Table 1). A significant shift in slope was detected for the LL-LMA relationship in the EAS-ENA contrast (Table 1, Fig. 2E). The SMA line fitted with ENA species had a significantly steeper slope, indicating higher returns in leaf longevity per increase of leaf dry mass production. Common slopes were fit for A_{mass}-N_{mass} fitted lines between EAS-ENA floras. The scaling showed a significant shift in elevation (Fig. 2F). Unexpectedly, plants of EAS had lower average values of A_{mass} at all values of N_{mass} than ENA (Table 1).

Hawaiian islands-tropical mainland contrast

Insufficient data for plants endemic to the Hawaiian floristic region precluded analysis of relationships involving LL or R_d. However, SMA regression analysis of A_{mass}-N_{mass} yielded significant results. Against expectation, lines fitted separately for Hawaiian and "mainland" tropical floras exhibited common slopes for A_{mass}-N_{mass} and failed to show a shift in elevation

(Table 1). Rather, a shift along a common slope was detected, with Hawaiian species exhibiting consistently lower values of both A_{mass} and N_{mass} relative to their mainland counterparts (Fig. 2G).

DISCUSSION

The generality of the LES (Wright *et al.*, 2004; Wright *et al.*, 2005a), which predicts commonality of tradeoffs in resource capture between floras, was tested against alternative predictions generated from biogeographic influences on trait evolution. We found evidence against a globally uniform rate of carbon gain per resource invested (a general LES axis), in favor of a historical alternative, which asserts that isolated lineages can follow different metabolic trajectories based on phylogenetic constraints and contrasting selective pressures. In contrast to previous geographic analyses that acknowledge modest LES trait variation of leaf trait as a function of biome (e.g., Reich *et al.*, 1999; Wright *et al.*, 2004) or climate (e.g., Wright *et al.* 2005b), we present a novel biogeographic analysis of global leaf trait data that compares regions with known evolutionary histories. This approach sheds light on biogeographical differences that would otherwise be obscured using approaches that implicitly combine multiple floras that may be similar in terms of broad vegetation types or modern climate regimes but, individually, have distinct evolutionary histories.

Aside from the Hawaiian-mainland comparison, all trait relationships explored supported the broad hypothesis that biogeographic influences can result in different leaf trait relationships (Fig. 1, shifts B and C). This was represented by significant differences in the allometric scaling of slope between floristic regions for key leaf-level resource tradeoffs. The associated biological significance of these statistical differences is in need of further exploration. Significant shifts in slope for NH-SH comparisons and shifts in elevation (intercept) or slope for EAS-ENA comparisons, given no significant differences in climate regimes, indicate the existence of broad-scale differences in plant resource capture strategies (Leishman *et al.*, 2010). These differences were detected despite the inevitable variation in trait measurement procedures and growth conditions between studies represented in the GLOPNET database, and our necessary assumption that a species' current native range accurately reflects evolutionary processes that shaped modern floras as defined by Takhtajan (1986).

What explains deviations from a more general, worldwide LES? We suggest that evolutionary processes operating at the scale of a floristic region—an area of plant diversification that has been isolated from other regions during a large part of its evolutionary history, particularly during the Tertiary (Good, 1974; Takhtajan, 1986)—can either constrain the evolution of metabolic efficiency through a lack of genetic variation, or can slow the adaptive process if competitive intensity is low (Dobzhansky, 1950). For example, the potential for higher carbon assimilation (A_{max}) at a given respiration rate (R_d) is theoretically constrained by the biophysical requirements of photosynthetic machinery (i.e., more carbon must be invested to allow for greater carbon gain; Lambers *et al.*, 2008). It is largely assumed that selective pressures prevent plants with less efficient leaf functioning from persisting (Reich *et al.*, 1999; Donovan *et al.*, 2011), but this perspective assumes past evolutionary forces have acted globally, with sufficient selection leading to the past extinction of all plants in floras with unfit trait combinations. We question this assumption, particularly in light of the evolution of metabolic efficiencies of the flora and fauna of oceanic islands, where lower predation and competition pressures have promoted the evolution of a wide variety of plant and animal physiologies that have never evolved on mainlands (Carlquist, 1975; Brown, 1995). By extension, we argue that continental regions that vary in their historical isolation, area, geologic history, and phylogenetic diversity can exhibit similar variation in floristic trait evolution. Relative to floras of similar environments, plants of a floristic region may theoretically be restricted in their capacity to evolve similarly unconstrained optimal solutions, limiting evolutionary convergence (Cody & Mooney, 1978).

The biogeographic differences in resource capture strategies shown here are in direct contrast to the argument of Tilman (2011), who hypothesized identical tradeoff surfaces for floras worldwide ("universal tradeoff hypothesis") based on the historical lack of extinction after biotic interchange. The patterns we report here argue that either the universal trade-off hypothesis is wrong (in favor of the alternative "biogeographic superiority hypothesis"), or that tradeoffs are sufficiently multivariate so as to preclude testing this hypothesis with bivariate data. Although we cannot exclude this latter possibility with our analysis given the limited trait data currently available, our study is novel in demonstrating incongruent bivariate tradeoff surfaces across different biogeographic regions.

Several case studies provide empirical evidence that highlight regional constraints of phylogeny and floristic-wide evolutionary histories on LES trait relationships. For example, endemic species of the Balearic Islands in the Mediterranean were found to have lower photosynthetic capacity (A_{max}) at a given leaf mass per area (LMA) than non-endemic species, which may reflect their collapsing geographic ranges (Gulías *et al*., 2003). Similarly, in comparison to their native competitors, some plant invasion studies that control for habitat, phylogeny, and ontogeny have found that invasive species have higher resource use efficiencies than natives (Funk & Vitousek, 2007; Funk, 2008), lower construction costs at a given LMA (Osunkoya *et al*., 2010), and higher photosynthetic capacity at a given respiratory cost (Pattison *et al*., 1999). Adding support to these case studies, we have shown differences in the allometric scaling of plant economic traits in large-scale comparisons of entire regional floras of contrasting evolutionary history.

LES differences were particularly evident in the trait scaling between the species of the Northern and Southern Hemispheres, suggesting a less efficient resource economy of Southern Hemisphere floras (Fig. 2A-C). During the break up of the most recent unified landmass of Pangaea roughly 180 million years ago, two large landmasses were formed: Laurasia in the NH and Gondwanaland in the SH (Lomolino *et al*., 2010). Upon further breakup, the landmasses of Laurasia were periodically connected through land bridges, allowing for significant biotic interchange. In contrast, vicariance played a more critical role in the floristic development of the landmasses of Gondwanaland (Sanmartín & Ronquist, 2004; Morrone, 2009). With fewer landmass connections and component landmasses of smaller areas, the current SH floras are a product of reduced phylogenetic diversity compared to the flora of the NH (Takhtajan, 1986). We propose that these Southern landmasses had less competitive biotic environments and therefore experienced weaker selection for efficient resource utilization. Differences in plant physiological functioning between these regions have been shown before with respect to particular stresses; for example, SH treelines generally occur lower in elevation than those in the NH (Körner & Paulsen, 2004) and SH plants exhibit lower frost resistance (Bannister, 2007). We suggest reduced metabolic functioning for SH plants, which continue to evolve largely in isolation from the Northern temperate flora (excepting alien invaders), extends to their overall carbon economy.

Slope differences were clear between Northern and Southern Hemisphere floras, but there appears to be additional differences in the relative locations of the clouds of points illustrating bivariate relationships between groups (Fig. 2A-C). Because slope heterogeneity was detected for all relationships, there was no statistical basis for fitting common slopes to detect a shift along a common axis. However, separate mean values of each variable indicate systematic differences between hemispheres. SH species in the data set had lower mean values of A, R_d, N and higher mean values of LL and LMA. Although we excluded climate biases in the data, the directionality of these differences in mean trait values could be partially explained by the greater preponderance of nutrient poor soils in the SH sites.

Functional differences observed in EAS and ENA can also be explained with respect to their evolutionary histories (Fig. 2D-F). Although natural ecosystems in both regions are dominated by mesophytic forest communities that were colonized by similar lineages throughout much of the Tertiary (Donoghue & Smith, 2004), large changes in lineage representation since at least the Pleistocene in ENA have led to the development of modern-day floras with deep contrasts in species diversity, phylogenetic diversity, and levels of endemism (Qian & Ricklefs, 2000). EAS has roughly twice the number of species as ENA and is represented by 50 more plant families (247 vs. 192; Heywood *et al.*, 2007; including 22 endemic families vs. 1 in ENA; Takhtajan, 1986). Given the historical differences between the two regions, detected differences in leaf physiology (Fig. 2D, 2E) follow the prediction that regions with more competitive evolutionary histories (i.e., EAS) ultimately lead to greater metabolic efficiencies. The slope differences of the analyzed trait relationships in EAS and ENA species are consistent with the recent finding that the most effective natural area invaders in ENA are of EAS origin (Fridley, 2008). The present study suggests that this EAS bias in ENA invasions may be due in part to more effective carbon use strategies by EAS lineages.

In addition to slope differences, elevation (y-intercept) shifts were found for the EAS-ENA comparison (Fig. 2D, 2F). When controlling for ecosystem differences, the ecological meaning of an elevation (y-intercept) difference between floras was not immediately clear. As any allocation differences were presumably not modulated by environmental pressures, this shift between the regions could be due, in part, to the same historical drivers generating slope differences. Consistently greater photosynthetic returns at any given leaf respiration values (Fig. 2D) is consistent with higher metabolic functioning in the EAS flora than that of ENA.

The direction of the shift in elevation for the A_{mass}-N_{mass} relationship for EAS-ENA was contrary to our hypothesis of greater metabolic efficiency of EAS species. This difference was not consistent with the A_{mass}-$R_{d\text{-}mass}$ and LL-LMA patterns for the EAS-ENA contrast (Fig. 2D-E). Whether the discrepancy results from nutrient conditions particular to some of the samples included in the GLOPNET dataset, or from floristic differences in nutrient efficiencies that we have not considered, cannot be determined from the present data. In addition, the Hawaiian-mainland analysis for the A_{mass}-N_{mass} tradeoff suggests a shift along a common slope, rather than the hypothesized differences in slope or elevation, indicating a common resource axis among floras of Hawaiian, Malesian, and tropical mainland regions. This may be indicative of environmental differences between these regions that dominated the expression of trait relationships across floras, as Hawaiian species, having diversified on nutrient poor volcanic soils (Vitousek, 1993), exhibited consistently lower values of both A_{mass} and N_{mass}. Similar findings in shifts along common slopes toward slower returns on leaf economics for key traits were found in a recent study of Hawaiian endemics when contrasted with alien competitors (Peñuelas *et al.*, 2010). However, we note that controlled studies using a common garden approach to trait measurements of Hawaiian native and alien plants reported clear scaling differences in LES trait relationships in the direction of our hypothesis (Baruch & Goldstein, 1999; Pattison *et al.*, 1999; Funk, 2008).

There is broad consensus for a general leaf economics spectrum that places constraints on the evolution of plant form and function and underlies the modern development of plant strategy theory (Grime, 1977; Chapin, 1980; Reich *et al.*, 1999; Reich *et al.*, 2003; Wright *et al.*, 2004; Wright *et al.*, 2005a). Although LES studies have stopped short of defining an absolute set of optimal trait relationships, most studies emphasize the global generality of LES patterns (Wright *et al.* 2005a) and global evolutionary convergence in leaf trait relationships (Reich *et al.*, 1997). In contrast, we assert that deviations from a canonical set of leaf allometric relationships across global floras should be expected given a Darwinian perspective of natural selection operating in isolated regions. Our argument is supported by the present biogeographic analysis of a suite of bivariate trait relationships, which detected significantly different allometries in the scaling of A_{mass}-$R_{d\text{-}mass}$, LL-LMA, and A_{mass}-N_{mass} across floristic regions that share common bio-climatic characteristics. We expect such biogeographic differences to be magnified in trait comparisons that control for other types of site variation (e.g., Pattison *et al.*, 1999; Funk, 2008; Osunkoya *et al.*, 2010). Further mechanistic elucidation of the biogeographic signature of plant resource economics will help to refine our understanding of the historical assembly of regional floras and the continuing re-assembly of global floras through modern invasions.

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SUPPLEMENTARY MATERIAL

Additional Supporting Information may be found in the online version of this article:

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Appendix S1. List of species and floristic region assignments.

Table 1. Standardized major axis (SMA) regression parameters, coefficients of determination, and sample sizes for each floristic contrast and trait relationship. A_{mass} (nmol g^{-1} s^{-1}), leaf photosynthetic rate; $R_{d\text{-}mass}$ (nmol g^{-1} s^{-1}), leaf dark respiration rate; LL (months), leaf longevity; LMA (g m^{-2}), leaf mass per area; N_{mass} (%), leaf nitrogen content.

Floristic region contrast	Photosynthetic capacity-dark respiration ($A_{mass} – R_{d\text{-}mass}$)	Leaf longevity-leaf mass per area (LL – LMA)	Photosynthetic capacity-leaf N ($A_{mass} – N_{mass}$)
Northern Hemisphere (NH)	$\log A_{mass}=1.27\log Rd_{mass}+0.67$; $r^2 = 0.61$; n=112; 11 sites	logLL= 1.85logLMA -2.84; $r^2 = 0.45$; n=394; 34 sites	$\log A_{mass}= 1.84\log N_{mass}+ 1.54$; $r^2 = 0.58$; n=447; 44 sites
Southern Hemisphere (SH)	$\log A_{mass}=0.98\log Rd_{mass}+0.91$; $r^2 = 0.42$; n=134; 11 sites	logLL= 1.42logLMA -1.87; $r^2 = 0.30$; n=157; 15 sites	$\log A_{mass}= 1.39\log N_{mass}+ 1.65$; $r^2 = 0.28$; n=159; 16 sites
East Asia (EAS)	$\log A_{mass} =1.04 \log Rd_{mass} +1.10$; $r^2 = 0.57$; n=12; 6 sites	logLL= 2.25logLMA -3.61; $r^2 = 0.57$; n=59; 14 sites	$\log A_{mass}= 1.54\log N_{mass}+ 1.52$; $r^2 = 0.60$; n=53; 19 sites
Eastern North America (ENA)	$\log A_{mass}=1.31\log Rd_{mass}+0.63$; $r^2 = 0.65$; n=84; 7 sites	logLL= 1.70logLMA -2.53; $r^2 = 0.49$; n=137; 15 sites	$\log A_{mass}= 1.76\log N_{mass}+ 1.63$; $r^2 = 0.48$; n=174; 15 sites
Hawaii	Insufficient data (n=0)	Insufficient data (n=0)	$\log A_{mass}= 1.88\log N_{mass}+ 1.49$; $r^2 = 0.26$; n=22; 5 sites
Mainland tropics	Insufficient data (n=27)	Insufficient data (n=77)	$\log A_{mass}= 1.81\log N_{mass}+ 1.51$; $r^2 = 0.24$; n=67; 22 sites

Figure Legends

Figure 1. Hypothetical differences in leaf economic trait relationships that result from shifts along a common slope (A), a shift in the elevation of a common slope (B), or a shift in slope (C) between plants of different floristic regions. Plants of a given landscape show correlated variation along a common tradeoff axis ("leaf economic spectrum"), with different leaf economic strategies employed in niches along a resource gradient (A). However, when comparing regions with different abiotic conditions, important differences may arise as a function of ecosystem or biome-level adaptations, where at a given leaf cost, species of one environment exhibit consistently greater carbon gain (B). This could be due to adaptive differences in resource allocation (i.e., metabolic versus structural) between environments (e.g., mesic vs. arid conditions, less herbivory vs. more herbivory), while showing the same basic tradeoff (slope). In addition, two isolated floras (solid and dashed lines) may show trait scaling differences, as illustrated through contrasting slopes (C). We hypothesize that after controlling for biome-level variation, regions with contrasting biogeographic histories should show slope differences in particular trait tradeoffs that reflect varying selection intensity for leaf function. In the absence of evidence for allocation differences (e.g., climate adaptations), shift B between regions cannot be ruled out as modulated by historical factors (e.g., herbivore pressure, edaphic differences). However, as interpreted from Leishman *et al.* (2010), the flora with the greater slopes (dashed lines) evolved a fundamentally different resource-use tradeoff, defined by acquiring greater returns per unit resource invested. Therefore, we would expect differences in elevation (shift B) or slope (shift C) when comparing floras in the same biome type but with different historic selection pressures.

Figure 2. LES trait relationships for each floristic contrast and results of tests of differences in SMA slope, elevation, and shift along common SMA slope. For N-S hemisphere floras: A) photosynthetic capacity-dark respiration rate (A_{mass}-R_{d-mass}) B) leaf longevity-leaf mass per area (LL-LMA) C) photosynthetic capacity-leaf nitrogen content (A_{mass}-N_{mass}). For Eastern North America-East Asian (ENA-EAS) floras: D) A_{mass}-R_{d-mass} E) LL-LMA F) A_{mass}-N_{mass} For Hawaiian and aggregated mainland floras: G) A_{mass}-N_{mass}. Only significant ($P<0.05$) test results are shown (* $P<0.01$; ** $P<0.001$).

Figure 1.

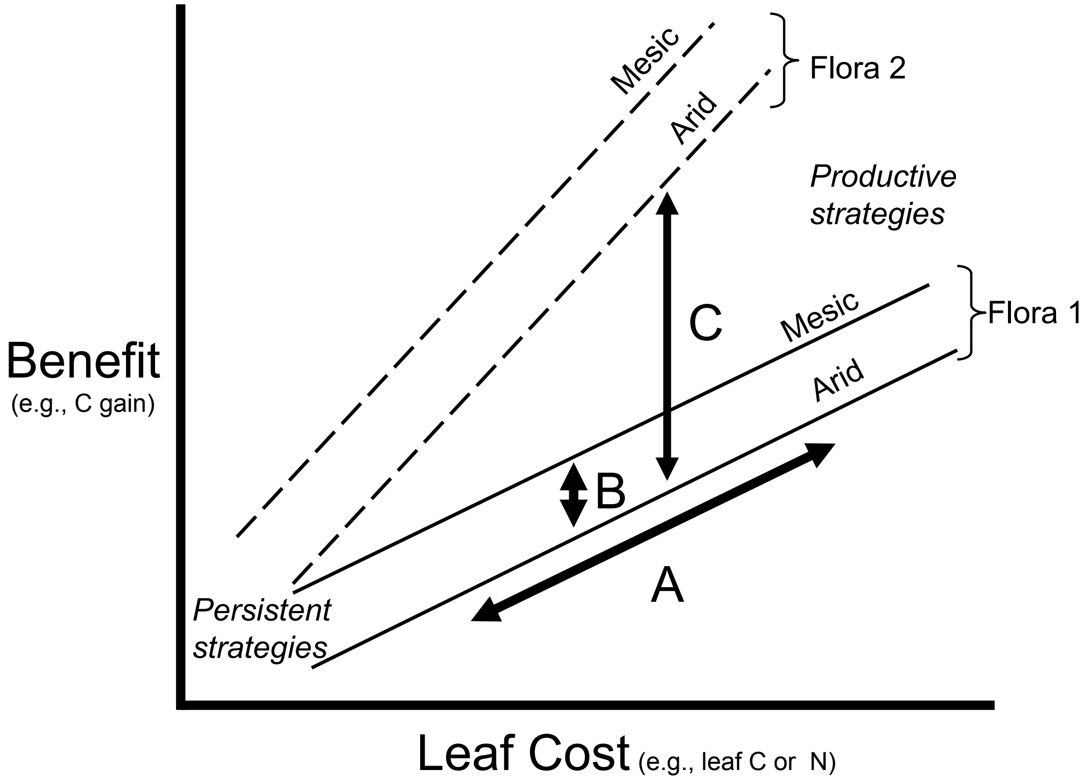
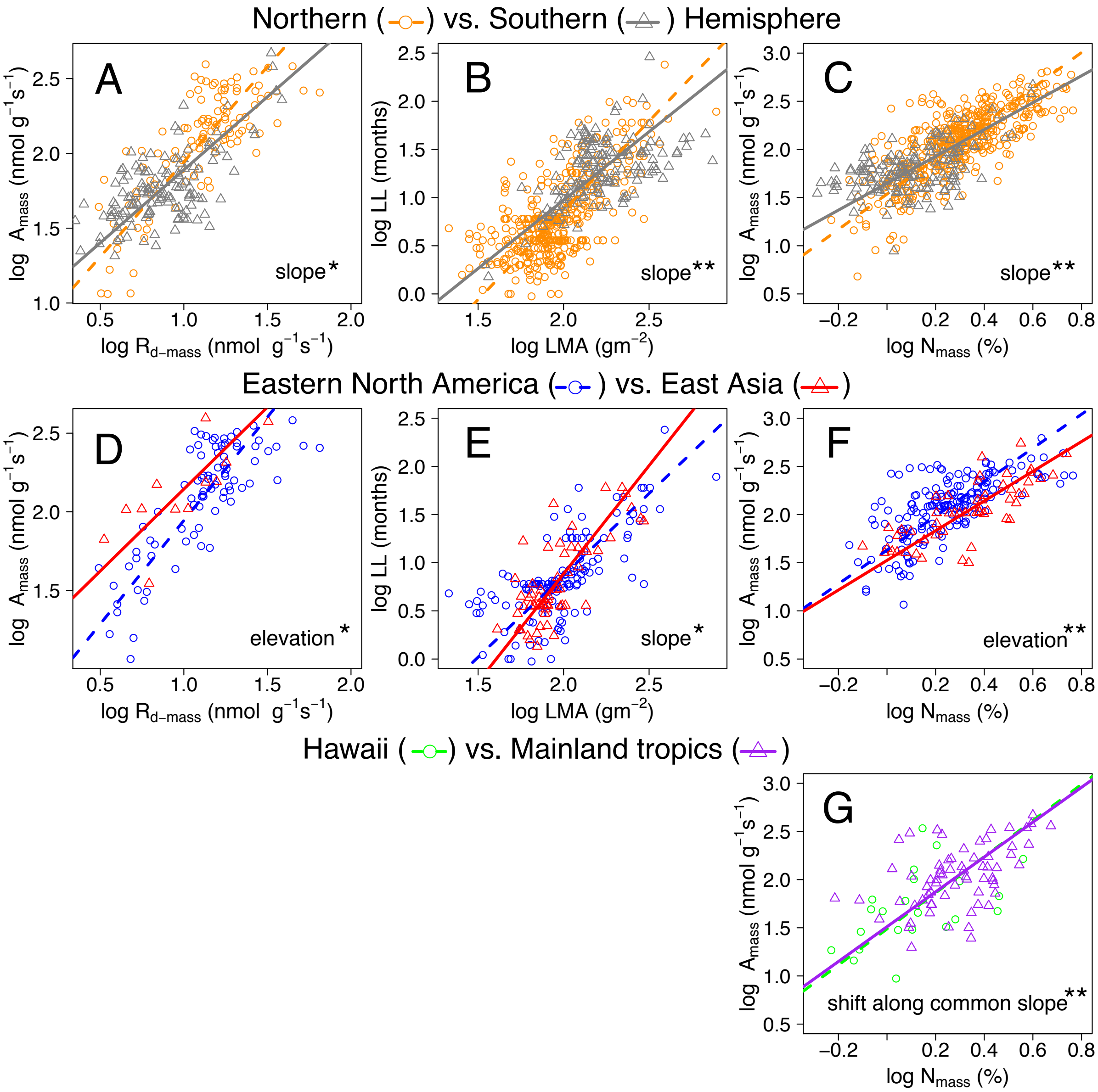

Mesic
Arid
Flora 2
Productive strategies
C
Mesic
Flora 1
Arid
Benefit
(e.g., C gain)
B
A
Persistent strategies
Leaf Cost (e.g., leaf C or N)

Figure 2.


Northern (-○-) vs. Southern (-△-) Hemisphere
A
slope*
log Amass (nmol g-1s-1)
log Rd-mass (nmol g-1s-1)
B
slope**
log LL (months)
log LMA (gm-2)
C
slope**
log Amass (nmol g-1s-1)
log Nmass (%)
Eastern North America (-○-) vs. East Asia (-△-)
D
elevation*
E
slope*
F
elevation**
Hawaii (-○-) vs. Mainland tropics (-△-)
G
shift along common slope**
1.0
1.5
2.0
2.5
3.0
0.0
0.5
-0.2
0.2
0.4
0.6
0.8

CHAPTER 2.

Resource-use strategies of native and invasive plants in Eastern North American forests

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SUMMARY

• Studies in disturbed, resource-rich environments often show that invasive plants are more productive than co-occurring natives, but with similar physiological tradeoffs. However, in resource-limited habitats, it is unclear whether native and invasive plants have similar metabolic constraints or if invasive plants are more productive per unit resource cost—that is, use resources more efficiently.

• Using a common garden to control for environment, we compared leaf physiological traits relating to resource investments, carbon returns, and resource-use efficiencies in 14 native and 18 non-native invasive species of common genera found in Eastern North American (ENA) deciduous forest understories, where growth is constrained by light and nutrient limitation.

• Despite greater leaf construction and nitrogen costs, invaders exhibited greater instantaneous photosynthetic energy-use (PEUE) and marginally greater nitrogen-use efficiencies (PNUE). When integrated over leaf lifespan (LL), these differences were magnified. Differences in efficiency were driven by greater productivity per unit leaf investment, as invaders exhibited both greater photosynthetic abilities and longer LL.

• Our results indicate that woody understory invaders in ENA forests are not constrained to the same degree by leaf-based metabolic tradeoffs as the native understory flora. These strategy differences could be due to pre-adaptation in the native range, although other explanations are possible.

Key words: biological invasions, functional traits, resource-use efficiency, comparative ecophysiology, plant ecological strategies, North American shrubs, leaf economics spectrum

INTRODUCTION

Non-native plant invasions are common in environments of anthropogenic disturbance (Hobbs & Huenneke, 1992), which has led to the generalization that non-native invaders (hereafter "invaders") are most likely to outperform native species in disturbed habitats with high resource availability (e.g., Daehler, 2003). Mechanisms attributed to these disturbance-mediated invasions include broad physiological advantages of invaders over natives following episodic increases in resource availability (Davis *et al.*, 2000). If true, then invaders should exhibit advantages in functional traits that contribute to high productivity given ample resources, such as high specific leaf area, photosynthetic ability, and relative growth rate compared to native competitors (van Kleunen *et al*., 2010; Drenovsky *et al*., 2012). However, it remains unclear if invasion success in resource-limited ecosystems can be explained by mechanisms described for high-resource environments.

Over the past 15 years, there has been substantial development of plant strategy theory and resource use economics (Reich *et al*., 1997; Westoby *et al*., 2002; Wright *et al*., 2004; Wright *et al*., 2005). Wright *et al*. (2004) reported a global pattern of coordinated variation in leaf traits ("worldwide leaf economic spectrum," LES) that invokes general ecophysiological tradeoffs in resource economics as a global axis of variation in plant strategies. This spectrum of strategic variation describes species from slow returns on investments (possessing traits such as low specific leaf area, high construction costs, low photosynthetic rates, and high leaf lifespan) to those at the opposite extreme of quick returns on resource investments. Strategies that lie outside of this general LES are presumed to either be selected against (ecologically constrained by biotic interactions) or biophysically or genetically impossible (Reich *et al*., 1999; Donovan *et al*., 2011).

In an effort to understand invasion processes in light of these developments, studies have explicitly placed invasive plants along a spectrum of leaf trait variation that emphasizes coordinated variation among leaf traits (e.g., Leishman *et al.*, 2007, 2010; Ordonez *et al.*, 2010; Peñuelas *et al.*, 2010; Ordonez & Olff, 2013). In particular, Leishman *et al.* (2010) argued that native and invasive plants share similar carbon capture strategies, with invaders subject to the same tradeoffs between physiological investments and returns (i.e., constrained within the same LES). They concluded that although invasive plants found in disturbed sites had traits that conferred greater productivity, they also experienced higher resource costs relative to natives. Therefore, invasive plants have strategies that correspond to the early successional, fast investment return portion of the LES, a conclusion used to mechanistically explain their dominance in disturbed, high resource ecosystems (Leishman *et al.*, 2007, 2010).

However, ecosystems subject to strong resource limitation are not immune to invasion (Martin *et al.*, 2009), including Eastern North American (ENA) deciduous forests that experience very low light and nutrient levels during the growing season (Fridley, 2008). It is an open question as to whether invasion mechanisms described for high resource environments, such as old fields, anthropogenic sites, and roadsides, are applicable to less disturbed ecosystems of low resource availability (Funk & Vitousek, 2007). It is generally understood that species adapted to resource-poor habitats follow strategies that place a higher premium on efficient use of resources (conservative strategies) at the expense of rapid growth (Aerts & Chapin, 2000).

Demographic studies of temperate forest tree invasions suggest invaders do not necessarily follow demographic or life history tradeoffs evident in the native flora, such as that between low-light survivorship and high-light growth (Martin *et al.*, 2010) and between classic *r*/*K* strategies (Closset-Kopp *et al.*, 2007). Select comparative studies, often in habitats of limited light or nutrients, report invasive plants with seemingly superior performance than natives at a given metabolic or resource cost, including increased growth rates (Osunkoya *et al*., 2010), greater mean performance or trait plasticity (Funk, 2008; Godoy *et al*., 2012; Paquette *et al*., 2012), greater photosynthetic rates at lower respiratory costs (Pattison *et al*., 1998; McDowell, 2002) and greater resource or energy-use efficiencies (Baruch & Goldstein, 1999; Nagel & Griffin, 2004; Funk & Vitousek, 2007; Boyd *et al*., 2009). All else equal, these findings imply that invasive species are not constrained by the same tradeoffs as natives, leading to greater production given similar resource investments. It remains unclear why these seemingly more efficient adaptations are not evident in neighboring native species. Phylogenetic constraints may exist with certain floras never evolving certain trait combinations, which can explain how certain non-native plants with novel resource-use strategies are superior competitors in a new range (Mack, 2003). A recent global analysis of leaf traits supports the possibility that evolutionarily distinct floras within similar biomes may have evolved different tradeoffs in resource capture strategies (Heberling & Fridley, 2012).

In ENA, the naturalized flora includes European forbs that inhabit open, managed, and disturbed sites. In contrast, invasive plants in ENA (i.e., those of highest management concern) are primarily woody species from Central and East Asia that are often invasive in forested habitats (Fridley, 2008). These shade-tolerant plants are particularly troublesome for management because their populations may increase as succession proceeds (Martin et al., 2009). In a recent common garden study of ENA forest species, Fridley (2012) found that invaders exhibit systematic differences in growth phenology, with significantly later leaf senescence for invasive species. It is unclear if any fitness advantage of an extended growing season for invasive species is equalized by tradeoffs at the leaf level such as shorter lifespan (i.e., more rapid leaf turnover) or lower daily productivity.

To test whether invasive plants in ENA forests exhibit different patterns of resource use than natives, we measured leaf-level carbon gains, energy and N investments, and resource-use efficiencies of invasive and native shrubs and lianas found in ENA deciduous forests. All plants were grown in a common garden to concentrate on intrinsic trait differences, rather than those that might arise from environmental differences. We expanded upon other invasion studies (e.g., Leishman *et al*., 2010) to focus on phylogenetically related groups of species found in resource-limited habitats and considered both instantaneous and time-integrated traits (e.g., Funk & Vitousek, 2007). As ENA understory species are constrained by both light and nitrogen (N) availability (Aber *et al*., 1993; Finzi & Canham, 2000), we hypothesized that ENA invaders should have greater carbon gains at lower resource costs. Therefore, we predicted that invasions in ENA forests are not due to greater resource use than natives *per se*, but rather, greater efficiency in the use of those limiting resources (i.e., greater carbon gains per unit resource cost).

MATERIALS AND METHODS

Species selection and sampling protocol

We studied 32 shrub and liana species (14 native, 18 non-native), with 14 non-native species formally recognized as “invasive” in ENA deciduous forests and the remaining four recognized as "naturalized" (Fridley, 2008; Table 1). “Naturalized” refers to non-native species capable of maintaining natural populations without human intervention, whereas “invasive” species refers to a subset of naturalized species with actively spreading populations that have been formally recognized by management agencies. Removing naturalized, but not (yet) invasive, species from the analysis did not affect conclusions (analysis not shown). Therefore, the non-native group is hereafter referred to as "invasive" for simplicity. Because invasive forest species in ENA are biased towards those of East Asian origin (Fridley, 2008), these species were the focus for comparison to native congeners. While the studied species are not equally abundant across ENA, many can co-occur and all species occur in the understory across the Eastern Deciduous Forest biome. Plant material was collected from an experimental garden in Syracuse, New York, USA (43°03′ N, 76°09′ W) established in 2006 in three replicate blocks and a seasonal shade treatment (80%) to simulate forest understory light regimes (see Fridley, 2012). Individuals were originally collected from the wild in the Syracuse region or, if unavailable, acquired commercially from nurseries in North America of similar latitude (Table S1).

From July to mid-August 2011, 2-6 individuals per species were sampled from the common garden for subsequent measurements (mean ± SD: 3.22 ± 1.13 individuals per species). Gas exchange measurements were performed on cut branches, following the protocol of Niinemets *et al*. (2005). We used cut branches for logistical reasons and to ensure measurements were made under consistent environmental conditions. On cool mornings between 06:00-08:00, two upper branches per individual were cut under water. To maintain xylem water potential, the severed ends were retained in water and transported to the laboratory within one hour. The branches were then recut under water and covered in transparent polyethylene plastic to reduce transpiration. Branches were stabilized at room temperature under low light for 1-2 days before recording gas exchange measurements. Each morning, branches were recut under water and the foliage was misted. This pre-conditioning period minimizes temporal and species-level differences in stomatal openness for comparable, consistent photosynthetic measurements (Niinemets *et al*., 2005).

Leaf gas exchange

All gas exchange measurements were made in morning hours on recently expanded, mature leaves using a LI-6400 portable photosynthesis system equipped with CO_2 and temperature control modules (LI-COR, Lincoln, NE, USA). Leaf temperature was maintained at 25º C under ambient humidity throughout measurements with sample chamber flow rate of 700 μmol s^{-1}.

We measured leaf photosynthetic responses to irradiance (PPFD) at 10 steps from 1000 to 0 μmol photons m^{-2} s^{-1} with sample chamber CO_2 concentration at 400 μmol mol^{-1}. All species were light saturated (without photoinhibition) at the highest light levels. Net photosynthetic rate was recorded after equilibrating for at least two minutes at each PPFD and reaching defined stability parameters based on photosynthetic rate and stomatal conductance to water.

Light response curve parameters were estimated through non-linear least squares regression of a non-rectangular hyperbola (Marshall & Biscoe, 1980):

$$A_{net} = \frac{\phi PPFD + \sqrt{(\phi PPFD + A_{max})^2 - 4\theta\phi PPFD + A_{max}}}{2\theta} - R_d \tag{Eqn. 1}$$

where A_{net} and A_{max} are the area-based net and maximum gross photosynthetic rates (μmol CO_2 m^{-2} s^{-1}), respectively, ϕ is the apparent quantum yield (mol CO_2 mol photons^{-1}), R_d is daytime dark respiration rate ($|A_{net}|$ at no light; μmol CO_2 m^{-2} s^{-1}), and θ is curve convexity (dimensionless). Light compensation point (LCP) was estimated from the x-axis intercept, and light saturation point (LSP) was estimated as the PPFD when 75% of A_{max} (model asymptote) was achieved.

Similarly, we measured leaf photosynthetic responses to changing intercellular CO_2 pressure (A/C_i curves) by varying sample chamber CO_2 concentrations between 50 and 500 μmol CO_2 mol^{-1} while maintaining saturating PPFD. A/C_i measurements were taken from the same leaves (or from adjacent nodes) as those for light response curves. Following Wullschleger (1993), maximum carboxylation rates ($V_{c,max}$) were estimated from CO_2 response (A/C_i) curves when intercellular CO_2 partial pressure (C_i) was less than 20 Pa (Rubisco limited). The biochemical photosynthetic model developed by Farquhar *et al.* (1980) was fit using non-linear least squares regression ("segmented regression", see Appendix 1):

$$A_{net} = V_{c,max} \frac{C_i - \Gamma^*}{C_i + K_c(1 + {}^{O}/_{K_O})} - R_{day} \quad \text{(when } C_i \leq 20 \text{ Pa)}$$

(Eqn. 2)

where C_i is intercellular CO_2 partial pressure (Pa), K_c and K_0 are Michaelis-Menten constants for carboxylation and oxygenation (40.4 Pa and 24.8 kPa, respectively), *O* is the O_2 concentration (21 kPa), $V_{c,max}$ is maximum carboxylation rate (μmol CO_2 m^{-2} s^{-1}), R_{day} is the daytime mitochondrial respiration rate (μmol CO_2 m^{-2} s^{-1}), and Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration (3.7 Pa). Rubisco kinetic constants were obtained from von Caemmerer (2000). These constants were assumed to be similar among species (see Sharkey *et al.*, 2007). Calculations using an independent set of literature-derived kinetic constants did not affect the reported conclusions (analysis not shown).

Leaf structural and biochemical characteristics

Following gas exchange measurements, the leaves were harvested, pressed, and oven dried at 60ºC for at least 48 hours. Leaf area was measured using a leaf area meter (LI-3100, LI-

COR, Lincoln, NE, USA) and scanned leaf images. Specific leaf area (cm^2 g^{-1}) was calculated as the leaf surface area per g dry mass. Ground leaf samples were placed in an ashing furnace at 500ºC for 4 hours, and leaf ash concentration was calculated as ash mass divided by sample mass. Duplicate samples were averaged for each individual. Leaf nitrogen (N) and carbon (C) concentrations were determined using an elemental analyzer (CE Elantech, Lakewood, NJ,USA) for leaves collected for photosynthetic measurements and, for a limited species subset, leaf litter samples collected after leaf fall. Leaf lifespan (LL) was determined from 2008-2010 biweekly leaf censuses as described in Kikuzawa (1983) and averaged over years monitored for each species (Fridley 2012).

Leaf construction cost (CC) quantifies the amount of glucose equivalents required to construct a leaf in terms of carbon skeletons, reductant, and ATP, excluding additional costs for maintenance and substrate transport (Williams *et al.*, 1989). Leaf CC_{mass} (g glucose g^{-1}) was estimated using the following equation (Vertregt & Penning de Vries, 1987; Poorter, 1994; Boyd *et al.*, 2009):

$$CC_{mass} = (-1.041 + 5.077C_{mass})(1 - 0.67Ash) + 5.325N_{mass} \tag{Eqn. 3}$$

where C_{mass} is leaf carbon concentration, *Ash* is leaf ash concentration (proxy for mineral concentration; Vertregt & Penning de Vries, 1987), and N_{mass} is leaf nitrogen concentration (all in g g^{-1}). We assumed leaf NO_3^- accumulation is negligible compared to organic N forms, and nitrate is the dominant form of N uptake. The first part of the CC equation above takes into account the carbon costs (empirically determined from the relationship between glucose costs and C content of biochemical compounds; Vertregt & Penning de Vries, 1987). The second part of the first term (including *Ash*) subtracts the mineral component in organic tissue from C cost, as the mineral fraction in organic tissue does not require C skeletons and energy required for their uptake is independent of costs for growth (Poorter, 1994). The last term of the CC equation above accounts for the additional, substantial costs required to reduce nitrate into organic N (proteins).

Metrics of leaf resource-use efficiencies

Resource-use efficiency (RUE) is broadly defined as the amount of carbon assimilated per unit resource (Funk & Vitousek, 2007). Potential photosynthetic energy-use efficiency (PEUE) was calculated as $A_{max,mass}/CC_{mass}$. Time-integrated PEUE was calculated as PEUE x LL, which accounts for the duration of potential returns on initial leaf investment. Potential photosynthetic nitrogen-use efficiency (PNUE) was calculated as $A_{max,mass}$/[leaf N]. For 17 species, time-integrated NUE was calculated as $A_{max,mass}$/[leaf litter N] x LL (Table S1; see Aerts & Chapin, 2000; Hirose, 2012). Since leaf N resorption data were not collected for all species, an additional time-integrated metric of PNUE was calculated as PNUE x LL ("life-span PPNUE" *sensu* Reich *et al.*, 1992). The relationship between PNUE x LL (mol CO_2 g^{-1} leaf N) and NUE (mol CO_2 g^{-1} *unresorbed* N; incorporating nitrogen residence time) scaled in proportion with one another (i.e., isometric; Fig. S1). Therefore, we report our results using PNUE x LL as a robust estimate for time-integrated NUE, since this metric was calculated for the complete dataset. In our dataset, this tight relationship between metrics results from comparable rates of leaf N resorption between our study species ($P>0.1$; Table S2). However, nitrogen resorption can differ between native and invasive species (I. Jo, unpublished data). These potential differences caution that PNUE x LL may not always be a reliable proxy for time-integrated NUE, as N mean residence times can be important in determining overall efficiency (Berendse & Aerts, 1987).

Daily carbon gain was estimated using the light response curves and daily PPFD measured at 30-minute intervals from June to August (see Fridley, 2012), assuming nighttime respiration rate to be 75% of the dark respiration rate measured during the day (Williams *et al.*, 1989). Payback time (days for net carbon gain to equal CC) was estimated as the ratio of CC to average daily carbon gain (Williams *et al.*, 1989) multiplied by $\log_e(2)$ to account for time-discounting effects (Poorter, 1994; Falster, *et al.*, 2012). This estimation of payback time does not account for age-related changes in photosynthesis or maintenance costs but can be considered a relative measure across species.

Data Analysis

Where necessary, measurements were converted between area (i.e., m^{-2} leaf) and mass-based estimates (i.e., g^{-1} leaf) through their corresponding SLA. All statistical analyses were performed in R (R development core team, 2013). We analyzed univariate data with phylogenetic generalized least squares (PGLS) regression models, which accounted for relatedness in the dataset through the phylogenetic distance matrix (see Fig. S2 for tree). We accounted for variation within species by weighting values by their intraspecific precision (standard error^{-1}). For each trait, we compared models with and without a fixed effect of native status (native or non-native) using likelihood ratio tests, following a χ^2 distribution with one degree of freedom. Because the phylogeny was approximate and based on estimated branch lengths, we also performed an analogous analysis using linear mixed-effect models (Bates *et al.*, 2011) that accounted for intraspecific and genus-level variation through random effects. Results of this approach were qualitatively similar to PGLS and are excluded for brevity. When necessary, data were log-transformed to satisfy assumptions of variance homoscedasticity and normality of model residuals.

Bivariate trait relationships were analyzed with standardized major axis (SMA) line fitting implemented with the *smatr* package in R (Warton *et al.*, 2012) using species means (see data Table S1). SMA line fitting minimizes residual variance in both x and y dimensions and is preferred in analyzing bivariate allometric relationships, as opposed to predicting y from x in classical regression (Warton *et al*., 2006). Testing in the SMA routine involves first testing for common slopes between groups. If the slopes do not differ, the lines fitted to the groups may represent a shift along their common slope and/or shifts in elevation (y-intercept).

Lastly, we implemented principal component analysis (PCA) to understand, in a multivariate context, how leaf resource-use efficiency metrics, C assimilation, and leaf longevity were related and if coordinated group variation separated native and invasive plants along axes of physiological functioning. We specifically chose these traits to include in the PCA to summarize the multivariate relationships of our hypothesis that focused on C- and N-use efficiency.

RESULTS

Comparative functioning between native and invasive species

As a group, invaders had greater area-based net photosynthetic rates (A_{net}) than native species, both at saturating and relatively low PPFD (Fig.1). However, area-based A_{net} was comparable between groups at and near 0 μmol photons m^{-2} s^{-1} (Fig. 1; $R_{d,area}$, Table 2). Mass-based dark respiration ($R_{d,mass}$) was moderately greater in invasive species (Table 2). Photosynthetic differences were further reflected through greater maximum photosynthetic rates ($A_{max,area}$, $A_{max,mass}$), maximum carboxylation rates ($V_{c,max}$), and average daily net carbon gains in invasive species (accounting for phylogenetic relatedness; Table 2). Respiration efficiency

($A_{max,area}/R_{d,area}$) was also slightly greater in invasive species, suggesting respiratory costs were lower per unit photosynthetic gain than native species.

Invasive species had significantly greater mean CC_{area}, N_{mass}, N_{area} , similar leaf C and Ash, and subsequently, lower leaf C:N (Table 2). With higher values of both N_{area} and $A_{max,area}$, invasive species were shifted further along a shared tradeoff in the $A_{max,area}$-N_{area} relationship (Fig. 2a). Leaf N was more closely associated with $V_{c,max}$ than $A_{max,area}$, especially within invasive species (Fig. 2b). Among photosynthetic traits, the greatest correlation was between $A_{max,area}$ and $V_{c,max}$ (overall: $r^2=0.49$; $P<0.001$), which describes the recognized relationship between the maximum carboxylase activity of Rubisco and the realized net carbon assimilation rate at ambient CO_2 concentrations. With significantly higher mean values in both traits (Table 2), invasive species were shifted further along a common slope (Fig. 2c).

Considering investments in tissue construction along with the subsequent duration of photosynthetic function, the relationship between daily carbon $gain_{mass}$ and LL was negative, although the correlation was weak (overall: $r^2=0.07$; $P=0.15$). With both shorter LL and lower C assimilation among natives (Table 2), a significant elevation (y-intercept) shift in fitted SMA lines was detected ($P<0.01$; Fig. 3a). Therefore, at a given leaf lifespan, invaders had greater C $gain_{mass}$ (grouped in upper right of Fig. 3a). As expected, the payback time-LL relationship followed the inverse trend of daily C $gain_{mass}$-LL, with a positive, weak association (Fig. 3b). At a given LL, invaders tended to have shorter payback time (SMA elevation test, $P<0.05$), even though invaders also had greater carbon costs in the form of glucose (CC_{area}) to compensate before achieving a net positive leaf carbon balance (Table 2).

Differences in instantaneous and time-integrated resource-use efficiencies

Although invaders exhibited both greater A_{max} and CC_{area} than natives, mean PEUE ($A_{max,area}/CC_{area}$) differed between groups, indicating invaders possessed greater instantaneous carbon returns per unit energy investment. Due to greater LL in invasive species, especially at a given payback time (Fig. 3b), this difference in PEUE was magnified when integrated over time (PEUE x LL; Table 2). In contrast, differences were marginal in PNUE (Table 2, P=0.059), which reflected a proportionately greater mean leaf N and $A_{max,area}$ among invasive species (Fig. 2a). However, due to substantially greater LL in invasive species (Table 2), marginal PNUE differences became significant when integrated over time (PNUE x LL). Conclusions remained the same when considering leaf N resorption rates in a species subset (see Methods; Fig. S1).

Multivariate trends in resource-use efficiencies

The first two axes of a principal components analysis (PCA) using resource-use efficiency traits explained 86% of the variation in the dataset, and separated native and invasive species (Fig. 4a; two-sided t-tests of species' axes scores by native status, Axis 1: t=-2.25, df=28, P=0.03; Axis 2: t=1.89, df=28, P=0.07). Axis 1 (64% variance explained) was positively correlated with daily carbon $gain_{area}$, PEUE, and PNUE, and negatively correlated with payback time, which matches univariate and bivariate testing (Table 2; Fig. 3). Axis 2 (22% variance explained) was most strongly positively correlated with LL (Fig. 4b). LL was orthogonal to daily C $gain_{area}$, which echoes the bivariate tests that indicated commonly held physiological tradeoffs involving LL were less constraining to productivity in invasive species relative to natives (Fig. 2).

DISCUSSION

Do invasive species follow different resource-use strategies?

Much of our current mechanistic understanding of plant invasions stems from studies in anthropogenic, disturbed habitats of high fertility (Martin *et al*., 2009), which show that invaders often exhibit greater resource acquisition rates, not necessarily differences in resource-use efficiency or different constraints in leaf function (e.g., Leishman *et al.*, 2010; Ordonez & Olff, 2013). In other words, native and invasive plants in a given community may follow the same general set of resource capture strategies and trait tradeoffs that have been generalized for species globally (e.g., Reich *et al*., 1997; Wright *et al.*, 2004, Wright *et al.*, 2005). We tested this premise in species common to ENA deciduous forest understories to investigate whether congeneric invasive and native species are similarly constrained to a common set of tradeoffs, or alternatively, whether species invasive in ENA exhibit more efficient resource-use strategies, suggesting greater carbon gain per unit resource invested. Among 32 native and invasive species common to ENA forests, we found evidence that invaders are both more productive and more efficient—they exhibit greater daily C gain and also greater C gain per unit C or N invested in leaf tissue relative to their native competitors (Table 2). Expanding upon past invasion studies (e.g., Baruch & Goldstein, 1999; Funk & Vitousek, 2007; Boyd *et al*., 2009; Leishman *et al*., 2010), these differences were most pronounced when integrated over time.

Invaders as a group exhibited significantly longer LL and greater CC_{area}—traits associated with high resource conservation, low growth rate, and lower competitive ability (Aerts & Chapin, 2000; Westoby, *et al.,* 2002; Wright *et al*., 2004). This finding of longer LL for invasive species builds upon the surprising recent finding that ENA forest invaders have greater relative carbon gains into the autumn, utilizing a temporal niche absent in the native flora

(Fridley, 2012). Plants adapted to low resource environments are expected to exhibit conservative resource-use strategies, but at the expense of fast growth (Aerts & Chapin, 2000). In low resource ecosystems, the success of invasive species may therefore depend on greater resource conservation than native species, but with proportionately slower absolute growth rates. In ENA forests, we found invaders had greater resource-use efficiencies and similar light-use efficiencies compared to native species. Surprisingly, in addition to more conservative resource-use traits, invaders also exhibited traits associated with greater productivity. With both greater photosynthetic gains (Fig. 1) and greater mean LL, invaders as a group exhibited greater energy-use and nitrogen-use efficiencies through time (PEUE x LL; Table 2). LL was weakly related to photosynthetic functioning (Fig. 3) and separated invaders on an axis orthogonal to carbon gains (Fig. 4), which suggests that tradeoffs between resource investments and C returns are distinct between native and invasive species in ENA. Therefore, compared to native species, resource-use strategies of invasive species were not only more efficient but also more productive.

Past studies have found resource-use efficiency (RUE) differences between native and invasive species across a range of habitats (e.g., McDowell, 2002; Nagel & Griffin, 2004; Funk & Vitousek, 2007; Osunkoya *et al*, 2010), including in an ENA forest (Boyd *et al.*, 2009). Funk & Vitousek (2007) found greater instantaneous PEUE and PNUE in invaders in Hawaii across light- and nitrogen-limited habitats. However, they found that natives had comparable resource-use efficiencies when integrated over the lifespan of the leaf. Due to instantaneous differences and time-integrated similarities in RUEs, they reasoned that invasion was driven by dynamics on short (seasonal) timescales. We observed a different pattern in ENA temperate forests, which could be due to the deciduous environment and potential fitness advantages of extended leaf phenology (Fridley, 2012). In ENA, invasion could be explained by later autumn senescence among invasive species (Fridley, 2012) and longer LL that was largely independent of daily C $\text{gain}_{\text{area}}$ (Fig. 3).

Other studies have stressed similarities in leaf resource economics among native and invasive plants (e.g., Leishman *et al*., 2007; Leishman *et al*., 2010; Ordonez *et al*., 2010; Peñuelas *et al*., 2010; Ordonez & Olff, 2013). In a recent study comparing natives and invaders in Australia, Leishman *et al*. (2010) concluded that species from both groups followed metabolic tradeoffs consistent with leaf economics theory. Analyzed as common slopes among leaf traits, they found that across many trait relationships, natives had strategies at the slow returns end of a common, coordinated axis of plant strategies, with lower carbon assimilation rates proportionate with lower resource needs. Similarly, in ENA understory species, we found group shifts along common slopes for instantaneous photosynthetic- and N-related traits (Fig. 2). However, when considering other traits (e.g., CC_{area}, LL), our results suggest that ENA invaders are both more productive and more efficient than natives (Table 2; Figs. 3, 4). Unlike Leishman *et al*. (2007, 2010), we found greater resource-use efficiencies (RUE) among invasive species. Because RUE is a ratio, differences can be found along a common slope in a bivariate relationship, suggesting that an RUE difference alone is not sufficient to conclude a fundamental difference in resource capture strategies (defined as differences in bivariate slope relationships, Leishman *et al*., 2010). Although N-related functional relationships in this study shared slopes between groups (Fig. 2), we found invaders to be more resource-use efficient as a product of greater cumulative C gains per unit invested, which is indicative of different physiological constraints between native and invasive species.

Our conclusions indicating resource-use strategy differences between native and invasive plants contrast with conclusions of these past studies for two possible reasons. First, many studies focused on instantaneous measures and did not incorporate temporal traits. Therefore, we cannot directly compare our results to past studies that do not consider leaf lifespan. Although perhaps less important in disturbed ecosystems, the integration of time into functional comparisons plays a critical role when leaf duration ultimately determines whole plant cumulative carbon gain (Reich, *et al*., 1992; Westoby *et al.*, 2002), especially in light- and nitrogen-limited ENA forests (Aber *et al*., 1993; Finzi & Canham, 2000). Additionally, the conclusions of Leishman *et al*. (2010) may be more representative of invasions in disturbed, fertile habitats, as few invasive species were found in undisturbed habitats in their study. However, Ordonez & Olff (2013) considered trait differences across resource and disturbance gradients and found that, compared to natives, invasive species in high-resource environments had greater mean trait values associated with fast growth, but trait differences between groups were similar across environments. Because individuals in our study were grown in a common environment, we tested inherent physiological differences, rather than *in situ* performance in different habitats.

Unexpectedly, we found ENA invaders had significantly greater CC_{area} and, although not significant, lower mean SLA (Table 2). Low CC has been invoked as a primary measure of invasion potential (Nagel & Griffin, 2001). However, studies have reported mixed results and interpretations, including invasive species with lower CC (Baruch & Goldstein, 1999; Nagel & Griffin, 2001; Boyd *et al*, 2009; Osunkoya *et al*., 2010) and greater CC (McDowell, 2002). Patterns in leaf CC alone may be insufficient to understand invasions across habitats because invested costs are without ecological context unless viewed in light of carbon returns (Griffin, 1994). Williams *et al*. (1989) proposed payback time (PT; days to amortize CC) as a trait to explain ecological variation in LL across habitats. Our measure of PT can be considered a relative estimate across species because we did not directly account for leaf age-related declines in photosynthetic rates. In our data, the PT-LL relationship was weak, although natives and invaders clearly occupied different portions of trait space (Fig. 3b). Further, at a given LL, invasive species tended to have greater carbon returns than natives (elevation shift; Fig. 3b). Because plants should be expected to retain leaves longer than their payback time to achieve a net positive carbon balance (Westoby *et al*., 2002; Falster *et al.*, 2012), it is reasonable to expect the LL-PT relationship to be weak within deciduous species. However, all else equal, the tradeoff between LL and daily C gain among co-occurring plants should be expected to equalize fitness and maintain strategies along that continuum (Falster *et al*., 2012). Tradeoffs with LL were weak in our data, with ENA invaders achieving greater C gains (Figs. 1, 2), greater C assimilation rate at a given LL (Fig. 3a), and greater PEUE through time (Table 2).

Why are East Asian invaders less constrained by metabolic tradeoffs than ENA natives?

ENA invaders appear to be less constrained in their allocation to rapid growth versus long-lived tissues than the native flora, yet it remains unclear why native species would not also exhibit such strategies. One possibility is that we did not measure an important trait that, if measured or integrated over the whole plant, would equalize our reported differences in leaf resource economics. For instance, greater herbivore pressure in natives could lead to lower competitive performance (e.g., Keane & Crawley, 2002), thereby explaining why invasive plants in our study have longer LL without paying the expected metabolic costs. However, given that invasion between the Eurasian and ENA forest ecosystems is asymmetric, with East Asia contributing more invaders to ENA (Fridley, 2008) than vice versa (Weber *et al*., 2008), scenarios that invoke home range herbivory alone are unlikely general for this group of invasive species. Also, East Asian species coevolved with earthworms, which were largely absent from

ENA until recent human introductions (Nuzzo *et al*., 2009). Therefore, the strategies of East Asian species may be better adapted than ENA natives to soil conditions maintained by earthworms, such as increased N cycling. It is uncertain if East Asian species evolved under lower soil nutrient availability to promote increased RUE. However, we found increased C gains for invasive species in addition to greater RUEs, which cannot be explained by historically different soil nutrient conditions alone.

Although tradeoffs in leaf functional traits are broadly consistent worldwide (e.g., Wright *et al*., 2004), there may be important differences in plant functional strategies between regions due to historical constraints (Heberling & Fridley, 2012). Fourteen of the 18 species measured in the current study have native distributions that include East Asia (Table 1). Despite both regions lying primarily in the temperate deciduous forest biome and composed of closely related taxa that diverged in the late Miocene (Donoghue and Smith, 2004), the flora of East Asia has experienced very different environmental conditions over the past several millennia, resulting in higher species diversity, endemism, and phylogenetic diversity (Qian & Ricklefs, 2000). Community-level properties, such as competitive pressure, disturbance, and time for resident species to adapt and fill niches, may make some communities more or less vulnerable to invasion ("community maturity" *sensu* Shea & Chesson, 2002). From this regional perspective, we speculate that invasive plants introduced from East Asia may have "pre-adapted" traits in the native range that confer invasiveness in ENA (Fridley, 2011). However, ecophysiological comparisons in the home range would be needed to confirm this hypothesis.

Conclusions

We found both higher productivity and more efficient resource use in forest understory invaders in Eastern North America compared to their native congeners, and lower metabolic constraints between LL and carbon gain in invaders. From a leaf-level perspective, we found invaders' competitive success to likely be due to comparatively greater carbon gains, and, despite greater leaf N and energy resource investments, a greater duration of returns. This conclusion supports past findings emphasizing the importance of RUE in invasions in resource-limited ecosystems (Funk & Vitousek, 2007) and, in ENA forests, the functional importance for leaf phenology differences in the invasive flora (Fridley, 2012). If these findings are general, then such differences in leaf function of invaders may be expected to drive large shifts in the productive capacities and nutrient budgets of deciduous forest ecosystems.

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Table 1. Woody, deciduous species measured in this study, including current status in Eastern North America (Fridley 2008), general growth form, and biogeographic origin.

Family	**Species**	**Invasive status**	**Growth form**	**Origin**
Berberidaceae	*Berberis canadensis* Mill.[a]	Native	Shrub	
	B. koreana Palib.	Naturalized	Shrub	East Asia
	B. thunbergii DC.	Invasive	Shrub	East Asia
Caprifoliaceae	*Lonicera canadensis* J. Bartram ex Marshall	Native	Shrub	
	L. fragrantissima Lindl. & Paxton	Invasive	Shrub	East Asia
	L. hirsuta Eaton	Native	Liana	
	L. involucrata (Richardson) Banks ex Spreng.	Native	Shrub	
	L. japonica Thunb.	Invasive	Liana	East/Central Asia
	L. maackii (Rupr.) Maxim.	Invasive	Shrub	East/Central Asia
	L. morrowii A. Gray	Invasive	Shrub	East Asia
	L. reticulata Raf.	Native	Liana/Shrub	
	L. sempervirens L.	Native	Liana	
	L. standishii Jacques	Invasive	Shrub	East Asia
Celastraceae	*Celastrus orbiculatus* Thunb.	Invasive	Liana	East/Central Asia
	C. scandens L.	Native	Liana	
	Euonymus alatus (Thunb.) Siebold	Invasive	Shrub	East Asia
	E. americanus L.	Native	Shrub	
	E. atropurpureus Jacq.	Native	Shrub/Tree	
	E. bungeanus Maxim.	Naturalized	Shrub/Tree	East Asia
	E. europaeus L.	Invasive	Shrub/Tree	Eurasia
	E. hamiltonianus Wall. ssp. *sieboldianus* (Blume) H. Hara	Naturalized	Shrub/Tree	East/Central Asia
	E. obovatus Nutt.	Native	Shrub	
	E. phellomanus Loes.	Naturalized	Shrub	East Asia
Elaeagnaceae	*Elaeagnus angustifolia* L.	Invasive	Shrub/Tree	Eurasia
	E. commutata Bernh. ex Rydb.	Native	Shrub	
	E. multiflora Thunb.	Invasive	Shrub	East Asia
	E. umbellata Thunb.	Invasive	Shrub	East Asia
Lauraceae	*Lindera benzoin* (L.) Blume	Native	Shrub/Tree	
Rhamnaceae	*Frangula alnus* Mill.	Invasive	Shrub/Tree	Europe/Central Asia
	F. caroliniana (Walter) A. Gray	Native	Shrub/Tree	
	Rhamnus alnifolia L'Hér.	Native	Shrub	
	R. cathartica L.	Invasive	Shrub/Tree	Europe/Central Asia

[a] Taxonomic classification is unclear. Studied individuals may be hybrids of *Berberis canadensis* and *B. thunbergii*.

Table 2. Mean values (± 1 SE) of photosynthetic, biochemical, structural, and resource-use efficiency leaf traits among native and invasive species. Statistical differences between native and invasive groups were determined using likelihood ratio tests (χ^2 with 1 df) that compared PGLS regression models for each trait with and without a fixed effect of native status. **P*<0.05; ***P*<0.01; ****P*<0.001.

Trait (units) [a]	**Invasive**	**Native**	**Native status (χ^2)**
$A_{max,area}$ (µmol CO_2 m^{-2} s^{-1})	**11.19 ± 0.78**	**8.10 ± 0.71**	**9.53****
$A_{max,mass}$ (µmol CO_2 g^{-1} s^{-1})	**0.2467 ± 0.0272**	**0.1802 ± 0.0181**	**6.84****
$R_{d,area}$ (µmol CO_2 m^{-2} s^{-1})	0.79 ± 0.07	0.73 ± 0.06	2.04
$R_{d,mass}$ (µmol CO_2 g^{-1} s^{-1})	**0.0176 ± 0.0021**	**0.0163 ± 0.0011**	**7.90****
A_{max}/R_d	**17.37 ± 2.06**	**13.52 ± 1.64**	**6.09***
ϕ (µmol CO_2 μmol^{-1} photons)	0.076 ± 0.003	0.074 ± 0.003	2.35
LSP (µmol photons m^{-2} s^{-1})	370.5 ± 92.4	233.4 ± 55.2	<0.01
LCP (µmol photons m^{-2} s^{-1})	10.8 ± 1.0	9.7 ± 0.8	3.53
$V_{c,max}$ (µmol CO_2 m^{-2} s^{-1})	**46.10 ± 2.22**	**32.24 ± 3.00**	**14.71*****
Daily C $Gain_{area}$ (mmol CO_2 m^{-2} d^{-1})	**273.8 ± 9.0**	**223.9 ± 12.1**	**16.94*****
Daily C $Gain_{mass}$ (mmol CO_2 g^{-1} d^{-1})	**6.06 ± 0.53**	**5.00 ± 0.40**	**10.15****
SLA (cm^2 g^{-1})	218.52± 12.75	233.42 ± 8.03	0.25
N_{mass} (%)	**2.97 ± 0.19**	**2.52 ± 0.11**	**14.20*****
N_{area} (g m^{-2})	**1.40 ± 0.08**	**1.11 ± 0.06**	**15.57*****
Ash (mg g^{-1})	91 ± 6	93 ± 8	4.22
C_{mass} (%)	45.00 ± 0.26	44.51 ± 0.40	1.46
C_{area} (g m^{-2})	22.37 ± 1.46	19.80 ± 0.71	3.44
C:N	**16.54 ± 1.05**	**18.60 ± 0.81**	**6.51***
CC_{mass} (eq. g glucose g^{-1})	1.328 ± 0.018	1.384 ± 0.020	1.61
CC_{area} (eq. g glucose m^{-2})	**65.85 ± 4.16**	**56.81 ± 2.28**	**6.13***
LL (days)	**145.1 ± 6.9**	**120.9 ± 6.4**	**7.72****
PT (days)	**6.12 ± 0.54**	**7.25 ± 0.98**	**6.95****
PNUE (µmol CO_2 g^{-1} N s^{-1})	8.28 ± 0.69	7.51 ± 0.67	3.57
PNUE x LL (mol CO2 g^{-1} N)	**101.00 ± 8.61**	**77.73 ± 8.20**	**8.52****
PEUE (µmol CO_2 kg^{-1} glucose s^{-1})	**183.44 ± 19.07**	**137.95 ± 13.03**	**6.38***
PEUE x LL (kmol CO_2 kg^{-1} glucose)	**2.19 ± 0.19**	**1.41 ± 0.13**	**15.29*****

[a] $A_{max,area}$ and $A_{max,mass}$, area- and mass-based light saturated gross photosynthetic rate on an area and mass basis ($A_{max,mass} = A_{max,area}$ x SLA); $R_{d,area}$ and $R_{d,mass}$, area- and mass-based dark respiration rate at ambient [CO_2]; A_{max}/R_d, respiration efficiency; ϕ, apparent quantum yield; LSP, 75% light saturation point; LCP, light compensation point; $V_{c,max}$, maximum carboxylation rate; Daily C $Gain_{area}$ and Daily C $Gain_{mass}$, area- and mass-based average daily carbon assimilation; SLA, specific leaf area; N_{mass} and N_{area}, mass- and area-based leaf nitrogen concentration; Ash, leaf ash concentration; C_{mass} and C_{area}, mass- and area-based leaf carbon concentration; CC_{mass} and CC_{area}, mass- and area-based leaf construction costs; LL, leaf longevity; PT, payback time, days to amortize leaf construction costs (Poorter, 1994); PNUE, potential photosynthetic nitrogen use efficiency; PNUE x LL, "life-span PPNUE" (*sensu* Reich *et al.*, 1992), an index of time-integrated PNUE; PEUE, photosynthetic energy use efficiency; PEUE x LL, time-integrated PEUE.

Figure legends

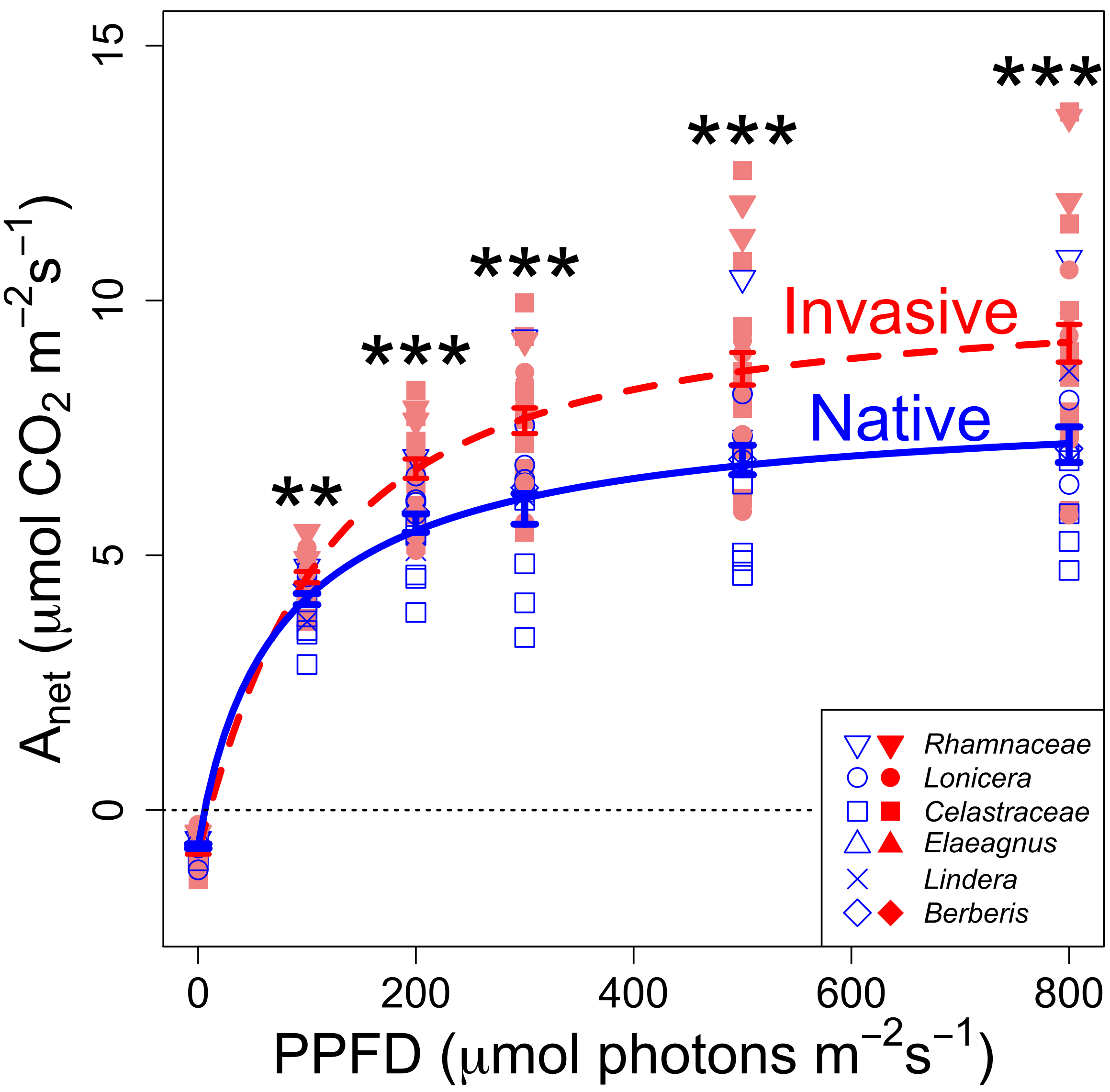
Figure 1. Average modeled light response curves for 12 native (open symbols) and 18 invasive (closed symbols) species. Error bars (group mean ± 1 SE) show empirically measured peak season area-based net photosynthetic rates (A_{net}) at various irradiances (photosynthetic photon flux density; PPFD). Statistical differences are between native and invasive groups evaluated from likelihood ratio tests of PGLS models (**$P<0.01$, ***$P<0.001$). Points indicate species means.

Figure 2. Standardized major axis (SMA) relationships for maximum C assimilation parameters and leaf N. Points indicate species means. a) Light-saturated gross photosynthetic rate (A_{max})-nitrogen concentration (leaf N) all species: $r^2=0.22$, $P<0.01$; native: $r^2=0.01$ $P=0.76$; invasive: $r^2=0.15$, $P=0.12$, b) maximum carboxylation rate ($V_{c,max}$)-leaf N all species: $r^2=0.36$ $P<0.001$; native: $r^2=0.07$, $P=0.42$; invasive: $r^2=0.36$ $P<0.01$, and c) A_{max}- $V_{c,max}$ all species: $r^2=0.49$; $P<0.001$; native: $r^2=0.36$; $P<0.05$, invasive: $r^2=0.38$, $P<0.01$. Significance tests are from SMA for each relationship for differences in slope, elevation (y-intercept), and shift along common fitted slope (**$P<0.01$,***$P<0.001$).

Figure 3. Standardized major axis (SMA) relationships between a) average daily C $gain_{mass}$-LL (leaf longevity) (all species: $r^2=0.07$, $P=0.15$, native: $r^2=0.07$, $P=0.41$, invasive: $r^2=0.24$, $P<0.05$) and b) payback time-LL (all species: $r^2=0.02$, $P=0.46$, native: $r^2=0.04$, $P=0.49$, invasive: $r^2=0.09$, $P=0.23$). Points indicate species means. Significance tests are from SMA for each relationship for differences in slope, elevation (y-intercept), and shift along common fitted slope (*$P<0.05$, **$P<0.01$).

Figure 4. Ordination of native (*n*=12; open symbols) and invasive (*n*=18; closed symbols) species using principal components analysis (PCA) based on resource-use efficiency traits: payback time, leaf longevity (LL), indices of time-integrated photosynthetic nitrogen-use efficiency (PNUE x LL) and energy-use efficiency (PEUE x LL), modeled daily C $\text{gain}_{\text{area}}$, PNUE, and PEUE. a) Species scores along axes 1 and 2 and b) trait loadings with vector length and direction corresponding to correlations between the trait and species scores. Axes 1 and 2 cumulatively explained 86% of the variance and separate native and invasive species' resource-use efficiency trait syndromes (two-tailed t-tests: $+P<0.1$, $*P<0.05$). Point symbols follow Fig. 3.

Figure 1.



15
10
5
0
A_{net} (µmol CO_2 $m^{-2}s^{-1}$)
0
200
400
600
800
PPFD (µmol photons $m^{-2}s^{-1}$)
**
***
***
***
***
Invasive
Native
▽ ▼ Rhamnaceae
○ ● Lonicera
□ ■ Celastraceae
△ ▲ Elaeagnus
× Lindera
◇ ◆ Berberis

Figure 2.

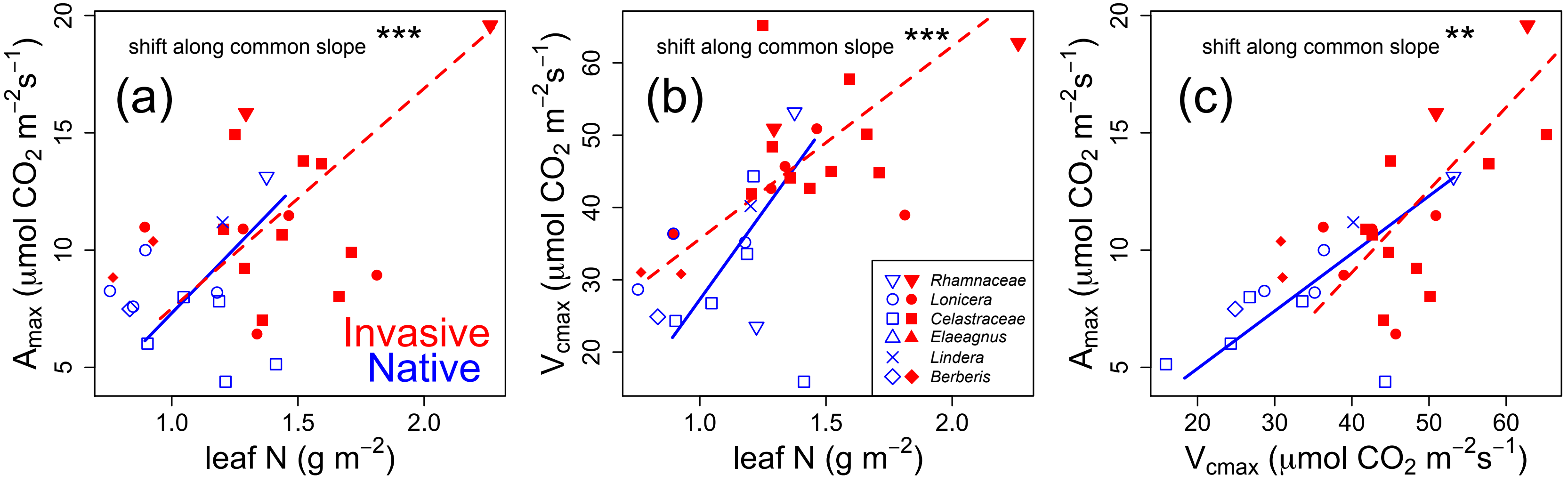

shift along common slope ***
(a)
Invasive
Native
A_{max} (μmol CO_2 $m^{-2}s^{-1}$)
5
10
15
20
1.0
1.5
2.0
leaf N (g m^{-2})
shift along common slope ***
(b)
V_{cmax} (μmol CO_2 $m^{-2}s^{-1}$)
20
30
40
50
60
1.0
1.5
2.0
leaf N (g m^{-2})
Rhamnaceae
Lonicera
Celastraceae
Elaeagnus
Lindera
Berberis
shift along common slope **
(c)
A_{max} (μmol CO_2 $m^{-2}s^{-1}$)
5
10
15
20
20
30
40
50
60
V_{cmax} (μmol CO_2 $m^{-2}s^{-1}$)

Figure 3.

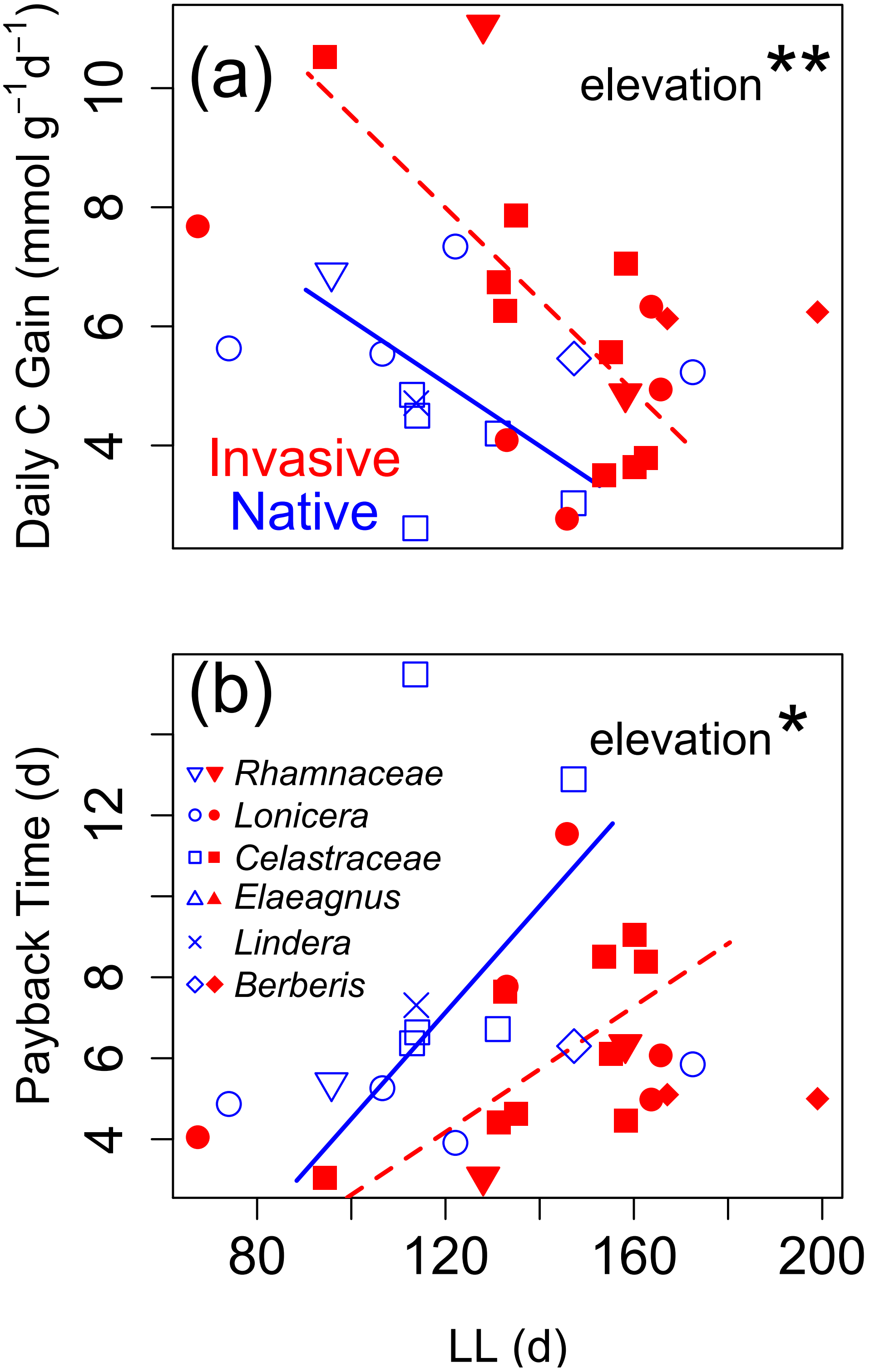
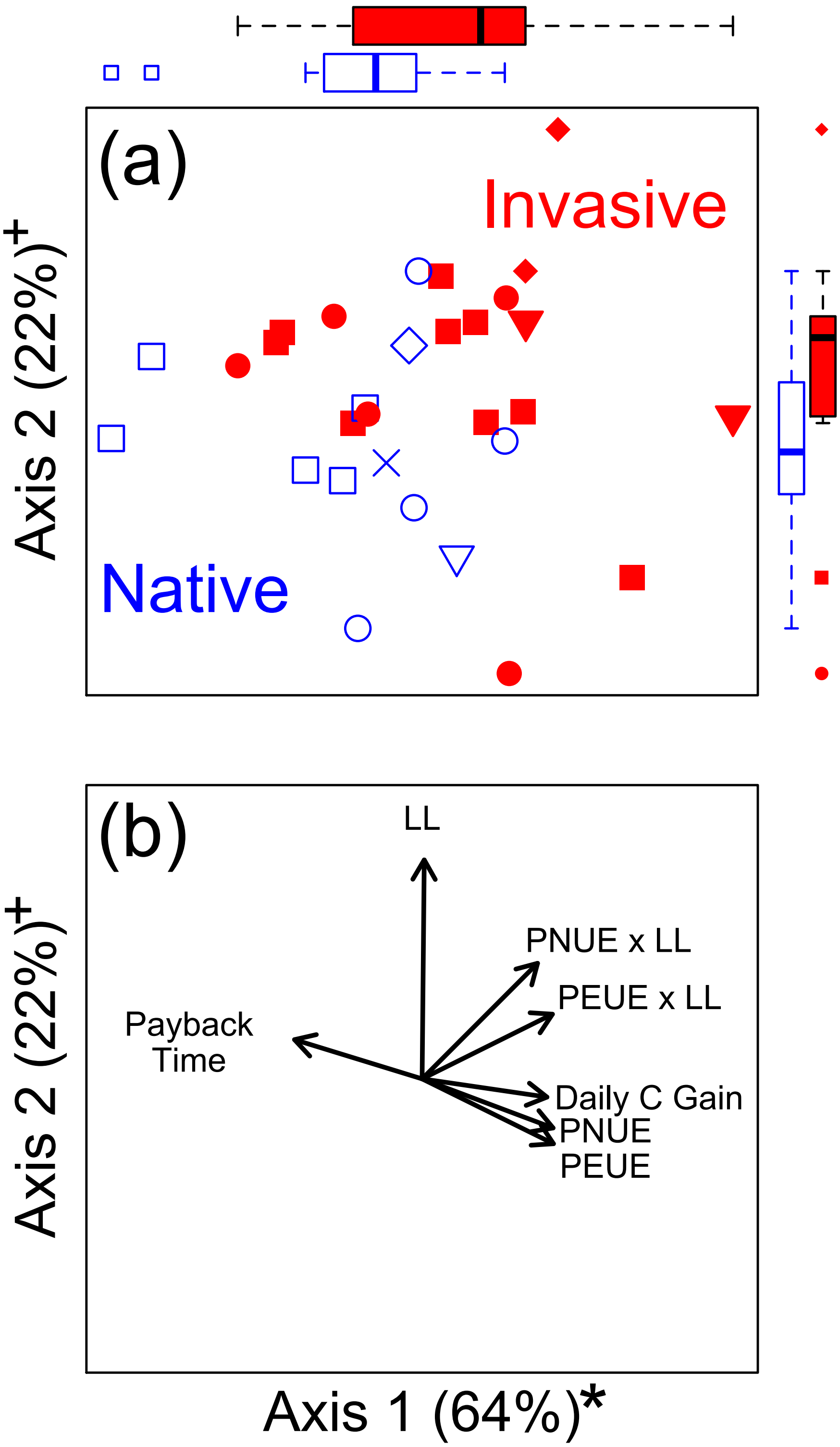

(a)
elevation**
Invasive
Native
Daily C Gain (mmol g^{-1}d^{-1})
4
6
8
10
(b)
elevation*
Rhamnaceae
Lonicera
Celastraceae
Elaeagnus
Lindera
Berberis
Payback Time (d)
4
6
8
12
80
120
160
200
LL (d)

Figure 4.


(a)
Invasive
Native
Axis 2 (22%)+
(b)
LL
PNUE x LL
PEUE x LL
Payback Time
Daily C Gain
PNUE
PEUE
Axis 2 (22%)+
Axis 1 (64%)*

Supporting Information

Table S1. Summary of leaf trait data for each species

Available online: http://onlinelibrary.wiley.com/doi/10.1111/nph.12388/suppinfo

Table S2. Mean values (± 1 SE) of additional nitrogen related variables in a subset of invasive (n=10) and native (n=7) species. Statistical differences were determined using likelihood ratio tests (χ^2 with 1 df) that compared PGLS models for each trait with and without a fixed effect of native status. See Table S1 for data for each species. See also Fig. S1. N resorption data from I Jo (unpublished). +P<0.1; *P<0.05.

Trait (units) [a]	**Invasive (10 spp.)**	**Native (7 spp.)**	χ^2
PNUE (µmol CO_2 g^{-1} N s^{-1})	**8.41 ± 1.02**	6.99 ± 1.04	4.41*
LL (days)	140.4 ± 9.4	128.1 ± 10.5	0.48
PNUE x LL (mol CO_2 g^{-1} N)	**97.69 ± 10.50**	76.27 ± 12.49	6.38*
N resorption (%)	45.00 ± 4.92	54.71 ± 4.18	2.01
MRT (days)	278.59 ± 36.46	298.40 ± 36.50	3.44+
NUE (mol CO_2 g^{-1} *unresorbed* N)	**195.39 ± 30.58**	175.29 ± 32.10	5.25*

[a] PNUE, potential photosynthetic nitrogen use efficiency; PNUE x LL, "life-span PPNUE" (*sensu* Reich *et al.*, 1992), an index of time-integrated PNUE; N resorption, estimate of maximum leaf N resorbed during leaf senescence, calculated as:

$$\frac{[mature\ foliar\ N]-[leaf\ litter\ N]}{[mature\ foliar\ N]}\times 100 \qquad \text{(Eqn. S2.1)}$$

MRT, mean residence time of nitrogen, calculated (see Aerts & Chapin, 2000) as:

$$LL\times\frac{[mature\ foliar\ N]}{[leaf\ litter\ N]} \qquad \text{(Eqn. S2.2)}$$

where LL is leaf longevity;

NUE, nitrogen-use efficiency ("integrated PNUE" *sensu* Funk & Vitousek, 2007) calculated as:

$$MRT\times PNUE \qquad \text{(Eqn. S2.3)}$$

Figure S1. Relationship of time-integrated nitrogen use efficiencies (NUE) metrics among a subset (*n*=17) of the species studied. Maximum NUE (reviewed in Aerts & Chapin, 2000) accounts for nitrogen resorption efficiency and was calculated as mean residence time (MRT) multiplied by PNUE (Table S2). PNUE x LL (A_{max}/leaf [N] x leaf longevity; "life-span PPNUE" *sensu* Reich *et al.*, 1992) serves as a proxy index of NUE. However, there are several potential caveats for its interpretation, including ignoring relocation of nitrogen and effects of leaf aging. However, PNUE x LL is a relatively robust measure of time-integrated nitrogen use efficiency, as leaf economic theory indicates that proportional changes in leaf N resorption do not vary with LL (Table S2) and age related declines in A_{max} are proportional to total LL to negate potential LL driven differences in cumulative lifetime leaf C gain (Reich *et al.*, 1992; Falster *et al.*, 2012). In this data subset with leaf N resorption data, the relationship was isometric, failing to reject $H_0 : \beta = 1$ (test for isometry described in Warton *et al.*, 2006; correlation between residuals and fitted values under H_0: $r = 0.31$, df=15, P=0.22). Therefore, in our dataset, PNUE x LL and NUE scaled in direct proportion to one another ($b = 1$), substantiating our use and interpretation of PNUE x LL.

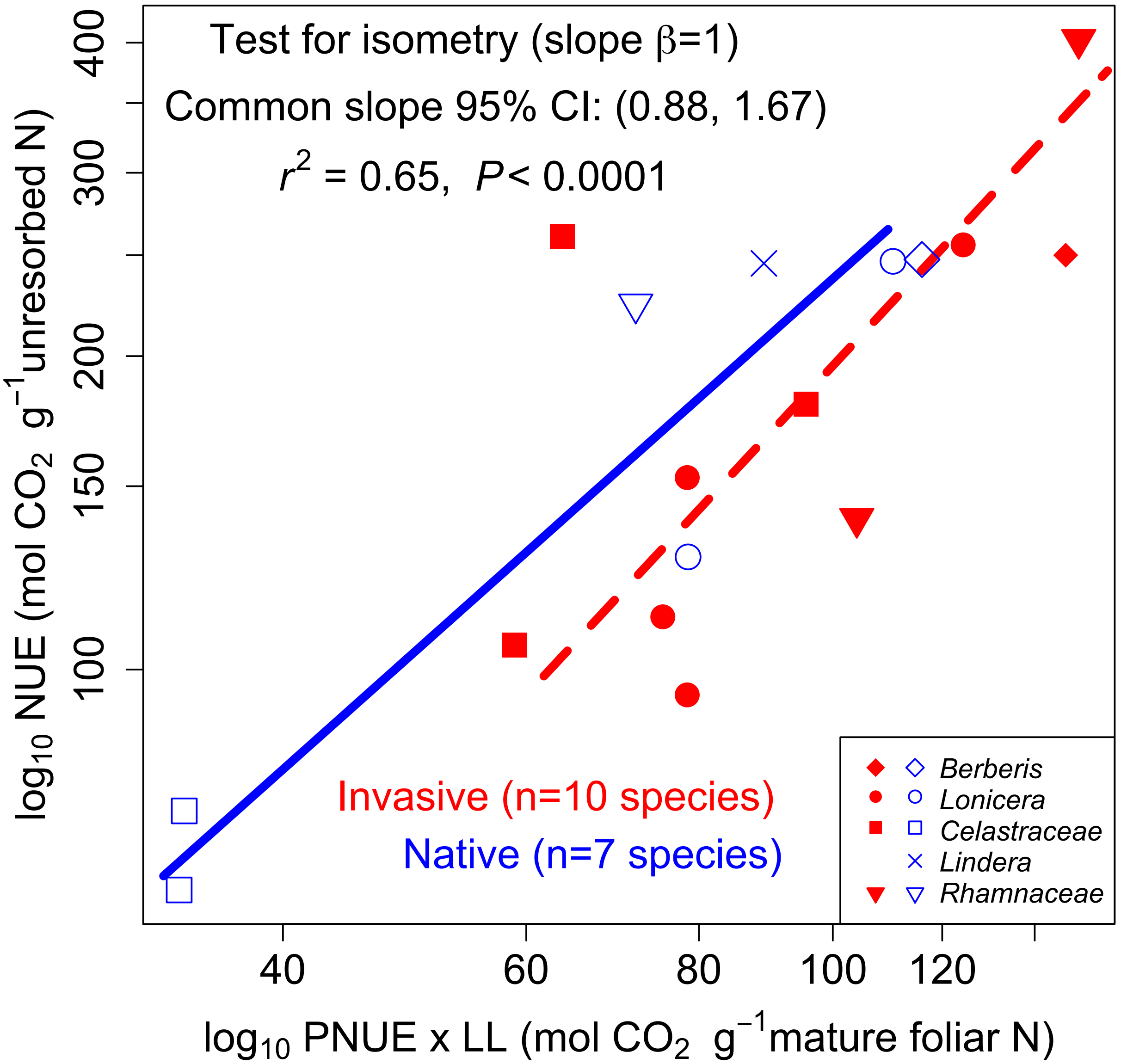
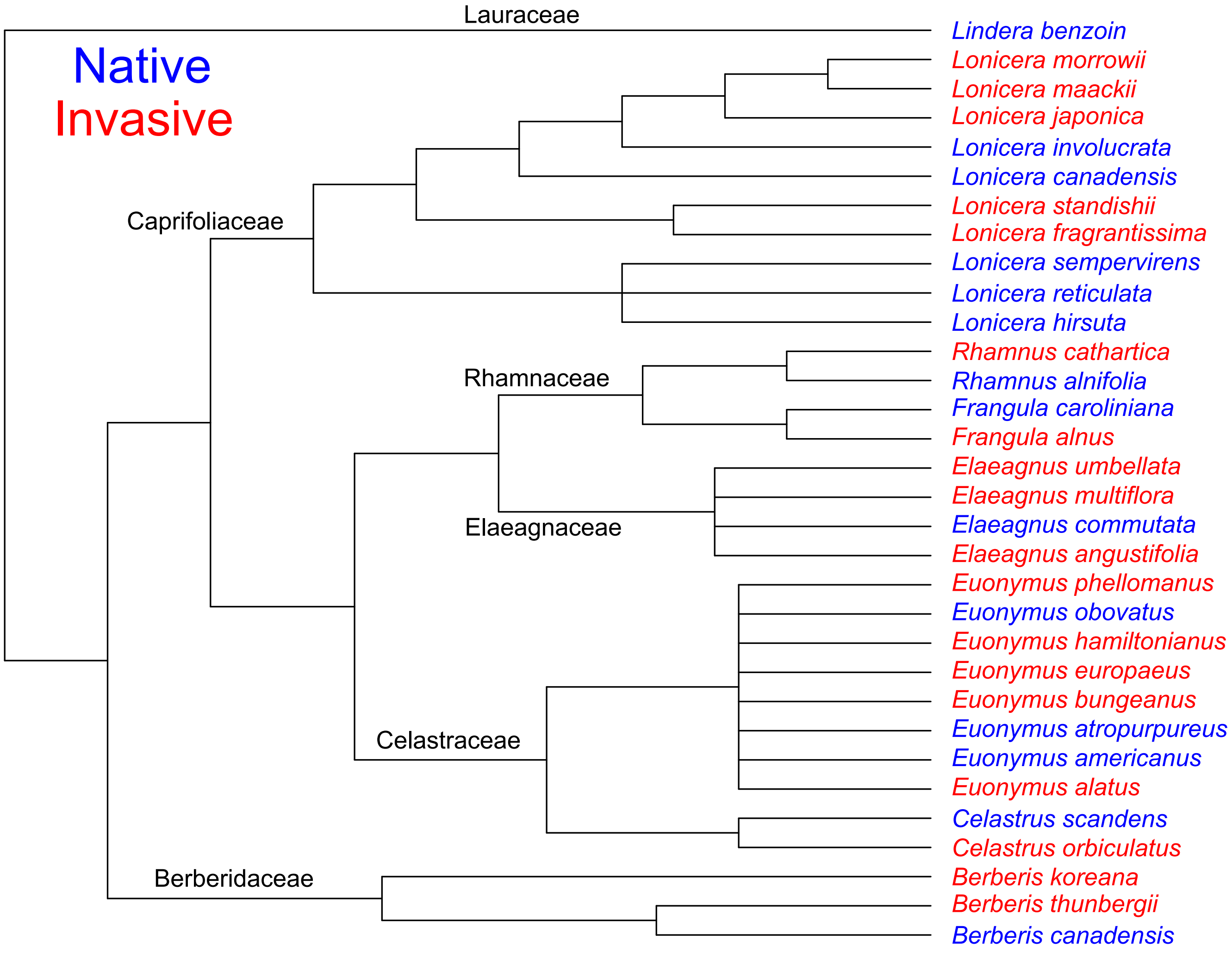

Test for isometry (slope β=1)
Common slope 95% CI: (0.88, 1.67)
r^2 = 0.65, P< 0.0001
Invasive (n=10 species)
Native (n=7 species)
Berberis
Lonicera
Celastraceae
Lindera
Rhamnaceae
400
300
200
150
100
40
60
80
100
120
$\log_{10}$ NUE (mol CO_2 g^{-1}unresorbed N)
$\log_{10}$ PNUE x LL (mol CO_2 g^{-1}mature foliar N)

Figure S2. Phylogenetic tree of the 32 species in this study. Phylomatic was used to build the tree (Webb & Donoghue, 2005). With additional information, polytomies were resolved for *Lonicera* (Rehder, 1903; Theis *et al*., 2005) and *Berberis* (Kim *et al*., 2004). Unknown branch lengths were estimated using Phylocom (BLADJ function; Webb *et al*., 2008) based on the node ages of Wikström *et al*. (2001).



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Rehder A. 1903. Synopsis of the genus *Lonicera*. *Missouri Botanical Garden Annual Report* **1903**:27-232.

Theis N, Donoghue MJ, Li J. 2005. Phylogenetics of Caprifolieae and *Lonicera* (Dipsacales) based on nuclear and chloroplast DNA sequences. *Systematic Botany* **33**:776-783.

Webb CO, Ackerly DD, Kembel SW. 2008. Phylocom: software for the analysis of phylogenetic community structure and character evolution. *Bioinformatics* 24:2098-2100.

Webb CO, Donoghue MJ. 2005. Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes* **5**:181-183.

Wikström N, Savolainen V, Chase MW. 2001. Evolution of angiosperms: calibrating the family tree. *Proceedings of the Royal Society B* **268**:2211-2220.

CHAPTER 3.

Influence of resource availability on understory invasion success: plant ecophysiological responses to an experimental light and nitrogen gradient in an Eastern North American deciduous forest

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ABSTRACT

Non-native, invasive plants are commonly typified by "early successional" trait strategies associated with high resource demands. Subsequently, plant invasions are often thought to be dependent upon site resource availability or disturbance. However, the invasion of shade-tolerant woody species into relatively undisturbed mid- to late-succession forests in Eastern North America (ENA) seem to contradict such generalization, as growth in this ecosystem is presumably constrained by light and nutrient-related limitations. In a factorial manipulation of light and nitrogen availability, we constructed an experimental resource gradient in an ENA forest to test whether these invasive species display increased trait performance and biomass production compared to natives, and whether these predicted differences depend upon resource conditions. As predicted, we found invasive species to exhibit strategies that convey higher rates of carbon gain. Further, we found invasive species to better adjust their traits along the light gradient, while natives were much less responsive. Surprisingly, neither group showed direct trait or growth responses to N additions. However, invasive species increased photosynthetic nitrogen use efficiencies with decreasing N availability, while natives maintained consistent efficiencies. Although natives and invasive species had different responses to resources, our results collectively indicate that these invasive species in ENA forest understories maintain their physiological advantages over co-occurring natives, independent of resource conditions.

INTRODUCTION

Habitats characterized by high resource availabilities, frequent disturbances, or low environmental stresses are generally considered to be more susceptible to invasion by non-native plant species (e.g., Elton, 1958; Burke & Grime, 1996; Hobbs & Hennueke, 2010; Alpert *et al*., 2000; Davis *et al*., 2000). Comparisons with native resident species in these ecosystems indicate invasive species often display “early successional” traits associated with high resource acquisition and increased productivity (Grotkopp *et al.*, 2002; Daehler, 2003; van Kleunen *et al*., 2010; Leishman *et al*., 2010; Ordonez *et al*., 2010). In contrast, environments where light, water, and/or nutrients place significant constraints on plant productivity are often generalized to be more resistant to invasions (e.g., Alpert *et al*., 2000; Daehler, 2003).

In Eastern North America (ENA), many non-native plants inhabit disturbed urban or agricultural habitats (Fridley, 2008). However, a large group of shade-tolerant woody species from East and Central Asia are naturalized and actively spreading (“invasive”) into mid- to late-successional forests in ENA (Fridley, 2008; Martin *et al*., 2009), despite the presumably strong light and nutrient-related constraints on plant growth (Pacala *et al*., 1994; Reich *et al*., 1997; Finzi & Canham, 2000). Recent common garden studies have found these invasive shrub and liana species to senesce their leaves later and assimilate proportionally more carbon into the fall (Fridley, 2012), possess both more productive (photosynthetic C gains) and efficient (C gains per unit resource cost) resource-use strategies (Heberling & Fridley, 2013) and exhibit root traits associated with more efficient soil nutrient foraging (Jo *et al*., 2015) compared to native species. However, it is unclear whether these interspecific strategy differences in a common garden are found in *in situ* resource conditions typical to secondary forests, where resources are heterogeneous and limited. Observational field surveys indicate invasive species abundance is generally increased in forests of high soil nitrate and seasonal light availability (Dreiss & Volin, 2013), but few experimental field manipulations of resources have been performed in the context of native and invasive forest species' ecophysiology and performance (but see Cassidy *et al.*, 2004).

Net primary productivity (NPP) in temperature forest understories is theoretically (co-)limited by biotic and abiotic factors. Current forest succession models highlight strong species-specific responses to light, indicating growth under high light trades off with survival in low light, understory conditions (Pacala *et al.*, 1994; Kobe *et al.*, 1995; Pacala *et al.*, 1996; Walters & Reich, 1996). Shade tolerance is considered a defining feature for strategy variation in forest species worldwide (Valladares & Niinemets, 2008). In addition to light-mediated growth strategies, nitrogen is commonly attributed as a major growth-limiting nutrient in north temperate forests (Reich *et al.*, 1997). Many temperate ecosystems are considered to be particularly nitrogen-limited (Vitousek and Howarth, 1991), with experiments showing increased growth to nitrogen additions duplicated across different ecosystems (LeBauer & Treseder 2008). In contrast to marked light responses, N-related growth limitations in temperate forest are less pronounced and inconsistent across studies and species. Nitrogen addition experiments in temperate forests report complex growth responses to N, with seedling survivorship as a function of both nitrogen and light availabilities (Catovsky & Bazzaz, 2002), no growth or photosynthetic trait differences with increased soil N (Walters & Reich, 1996), or species-dependent contrasting (but subtle) growth responses for early successional species only or dependent on competitive environment (Catovsky *et al.*, 2002; Zaccherio & Finzi, 2007). The majority of these studies used transplanted seedlings. In general, forest growth is more closely correlated to light availability than N availability in ENA woody species (Finzi & Canham 2000).

Given these strong resource limitations in forest ecosystems, it is possible that invasive shrubs are subject to divergent physiological mechanisms than those described for disturbed habitats. Species adapted to resource-poor habitats worldwide tend to consistently follow strategies that convey efficient use of resources at the expense of rapid growth rates (Aerts & Chapin, 1999; Funk 2013). In abiotic contexts where resources are less limiting, efficient use of resources might not convey fitness advantages. Conversely, high growth potential might be irrelevant in light limited understory environments, where maximum photosynthetic and growth rates cannot be achieved. Are invasive species in ENA understories more efficient in their use of limiting resources (*i.e.*, light and nitrogen) as suggested by common garden comparisons; or as suggested by comparisons in disturbed ecosystems, are these species simply shifted along a common plant strategy axis with a more resource demanding physiology that permits proportionally higher rates of productivity?

In a two-year factorial manipulation of light and soil nitrogen in a deciduous forest in central New York, USA, we tested 1) whether invasive species adjust their traits along resource gradients more than co-occurring natives; 2) how potential trait shifts relate to carbon gain and productivity, and 3) the degree to which invasive species maintain physiological advantages over natives under low light and nutrient availabilities. We measured the physiological responses of three invasive and six native woody shrubs or tree saplings common to ENA forest understories along an experimental resource gradient. We hypothesized that invasive species have more physiological flexibility along resource gradients of light and nitrogen availability. Under low resource conditions, we hypothesized that invasive species maintain greater carbon gains than native species and display higher carbon gains per resource cost (resource-use efficiencies). Under high resource conditions, we hypothesized that invasive and native plants may have similar resource-use efficiencies, but invasive species exhibiting trait physiology that confers growth advantages at high resource availability.

MATERIALS AND METHODS

Study site

Plots were selected in a closed canopy secondary forest in central New York (approximately 42°55' N, 76°02' W; town of Pompey, south of Syracuse, NY). The overstory primarily consisted of *Acer saccharum* (sugar maple) and *Fraxinus americana* (white ash), along with other native deciduous tree species, *Prunus serotina* and *Ostrya virginiana*.

Experimental design: light and nitrogen additions

Sixteen 5 m radius plots were selected in summer 2012, following field surveys of species occurrences and light availabilities. Plots were blocked by initial light level as determined through hemispherical photography (described below). Plots were spatially separated but in the same forest. Plots were selected to maximize common species representation and include a natural gradient of light availability. We chose study species based on common occurrence in the forest and region, aiming for maximum representation in plots, with a subset of 3 invasive shrubs and six native woody species in each plot (Table 1). A minimum of two invasive species and three native species were naturally growing in the understory in each plot. At least two plants per species were tagged –one individual for leaf collection and gas exchange measurements on excised branches, and another nearby individual for productivity measurements (described below).

Nitrogen was added in the form of granular ammonium nitrate (NH_4NO_3) in 5 applications from late March to early August for a total of 133 kg ha^{-1} yr^{-1}. This relatively high fertilization rate was chosen to ensure any potential N growth limitations were relieved, but remained consistent with other long and short term understory forest N additions (Aber *et al.*, 1993; Catovsky & Bazzaz, 2002; Cassidy *et al.*, 2004; Fownes & Harrington, 2004; Magill *et al.*, 2004) and roughly double the average N mineralization rates occurring naturally in mesic temperate forests (Reich et al., 1997).

Light availability was increased in half of the plots through overstory canopy thinning or tree felling in November 2012 (post leaf fall). In most cases, experimental gap conditions were achieved after removing one or two trees. Any woody debris was removed from each plot to minimize site disturbances.

Light and soil nutrient availability

Plot- and plant-level light levels were estimated as integrated growing season light availability through canopy photographs taken in the center of each plot in pre-treatment year (2012), treatment year one (2013), and year two (2014), as well as directly above each plant canopy in 2014. Photographs were taken with a camera fit with a fish-eye lens and oriented north. Images were analyzed with Gap Light Analyzer (GLA) software (Frazer et al., 1999) to calculate the “gap light index” (GLI; Canham, 1988), which estimates combined incident diffuse and direct beam radiation over a growing season (% full sun).

Plant available nutrients were quantified using “plant root simulator” (PRS^{TM}) ion exchange probes (Western Ag innovations, Saskatoon, Saskatchewan, Canada) deployed for 4 weeks in May and July 2013. For each deployment, four cation and four anion probes were inserted into the soil of each plot and were pooled for analysis (plot level mean nutrient availability).

Leaf gas exchange

Gas exchange measurements were performed on cut branches, following the protocol of

Niinemets *et al*. (2005), widely used for temperate woody species. At least one individual of each species per plot was tagged for branch and leaf sample collection. Two upper branches per individual were cut in the field between 06:00 and 09:00 h and immediately recut under water. To maintain leaf turgor, the severed ends were wrapped with wet paper towel, lightly misted with water, placed in plastic bags, and stored in a cooler to minimize transpiration until transported to the lab, typically within 1 h. Upon returning to lab, branches were recut and cut stems placed in water, loosely covered in transparent plastic, and stabilized at room temperature under low light for 1-3 d before recording gas exchange measurements. Each morning, branches were recut under fresh water. A subset of measurements was taken *in situ* and these were comparable to those from taken on similar individuals in the lab.

Gas exchange measurements were made on recently expanded, mature leaves using an LI-6400 portable photosynthesis system equipped with CO_2 and temperature control modules (Li-Cor, Lincoln, NE, USA). Leaf temperature was maintained at 25º C under ambient humidity throughout measurements with sample chamber flow rate of 500 µmol s^{-1} and reference chamber CO_2 concentration at 400 ppm. Leaves were photoinduced at a moderate irradiance level (300 µmol photons m^{-2} s^{-1}) prior to measuring CO_2 response curves (A/C_i) and light response curves (A/q). Photosynthetically active light levels (q) were then progressively increased until light saturation (800-1,500 µmol photons m^{-2} s^{-1}). All individuals were light saturated at the highest light levels, with no apparent signs of photoinhibition. Light-saturated net photosynthetic rate (A_{sat}) was manually recorded after equilibrating for at least two minutes at high PPFD and reaching defined stability parameters based on photosynthetic rate and stomatal conductance. After achieving light saturation, photosynthetic responses to q (A/q curve) were measured at 5-10 steps (particularly focusing on q <300 µmol photons m^{-2} s^{-1}). Data were logged manually, but ensuring at least 2 min equilibration periods at each light level. Following A/q curves, leaves were stabilized at saturating light level to perform A/C_i curves. In cases where leaves showed decline in photosynthetic rate likely due to A/q measurement stress, we measured an adjacent leaf. We measured photosynthetic responses to CO_2 (A/C_i) at 11 reference chamber CO_2 concentrations (400, 300, 200, 100, 50, 400, 400, 600, 800, 1000, 1500 ppm). Net photosynthetic rates were recorded after equilibrating for 2 min and reaching defined stability parameters based on photosynthetic rate and stomatal conductance.

Leaf structural and biochemical traits

Following gas exchange measurements, leaf thickness was measured using digital micrometer, taking the mean of 15 measurements in recently expanded, mature leaves (avoiding midrib and major leaf veins). Leaves were then scanned for leaf area and oven dried at 60ºC for at least 48 hours. Specific leaf area (cm^2 g^{-1}) was calculated as the leaf surface area per g dry mass. Ground leaf samples were placed in an ashing furnace at 500ºC for 4 hours, and leaf ash concentration was calculated as ash mass divided by sample mass. Duplicate samples were averaged for each individual. Mass-based leaf nitrogen (N_{mass}) and carbon (C_{mass}) concentrations were determined using an elemental analyzer (CE Elantech, Lakewood, NJ, USA) for leaves collected for photosynthetic measurements. Area-based concentrations (N_{area}, C_{area}) were calculated through corresponding SLA. Due to similarity between years and to maximize sample size, we pooled leaf C/N data from two collection years.

To measure leaf N resorption, recently senesced leaves were collected in autumn (October-November 2013) on the same individuals by gently shaking the plant and collecting fallen leaves, aiming to collect leaves from the same cohort as the mature leaf collection. Senesced leaves were oven dried, weighed, and analyzed for C and N. Because leaf mass loss and area shrinkage during senescence can be significant, using uncorrected nutrient concentrations in senesced tissue can lead to an underestimation in calculated nutrient resorption rates (van Heerwaarden *et al*., 2003). Therefore, mass loss was corrected following Vergutz *et al*. (2012). Senesced leaf N concentration (senesced N_{mass}) was calculated as senesced N_{mass} x MLCF, where MLCF is the mass loss correction factor, calculated as the ratio of the dry mass of senesced leaves to the dry mass of mature leaves. Senesced N_{mass} is interpreted as the inverse of leaf N resorption proficiency (Killingbeck, 1996).

Leaf construction cost (CC) quantifies the amount of glucose equivalents required to construct a leaf in terms of carbon skeletons, reductant, and ATP, excluding additional costs for maintenance and substrate transport (Williams *et al*., 1989). Leaf CC_{mass} (g glucose g^{-1} leaf mass) was determined using a biochemical approximation (Vertregt & Penning de Vries, 1987; Poorter 1994; Boyd *et al*., 2009).

Leaf chlorophyll concentration was measured with a chlorophyll meter (atLEAF+, FT GREEN LLC, Wilmington, DE USA), using the mean of 6 readings per leaf (avoiding leaf midrib; measuring 5-10 mature leaves) in from the same leaves with mid-summer photosynthetic measurements. The atLEAF+ measures leaf absorptance difference between 660 nm and 940 nm and has been shown perform similarly to other readers and well correlated to total chlorophyll content (Zhu *et al*., 2012). Species-specific leaf out and senescence dates for each plot were recorded in 2013, as well as biweekly chl measurements from September 2014 through senescence.

Productivity

At least one individual per species per plot was monitored for productivity. Aboveground growth was tracked through tagging growing point terminuses in every branch in early spring

2013 and 2014 (pre-leaf out) using colored tape and re-marked throughout the year. In a few cases where plants were too large to reasonably monitor, a subset of branches were tagged. Leaf counts were done annually to quantify leaf mass production. Aboveground net primary productivity (NPP) was estimated as the dry mass of new woody stems and leaves produced during the study plant from biomass harvests in November 2014. Coarse roots were also harvested to estimate root to shoot ratios. Although likely important on longer time scales and larger plants, we assume radial woody growth was negligible compared to stem elongation and foliage production.

Data analysis

We implemented a version of the classic Farquar, von Caemerrer, Berry (FvCB) photosynthesis model (Farquhar *et al*., 1980) using a hierarchical Bayesian (HB) framework (Patrick *et al*., 2009). We chose the HB framework for several reasons, based on the ability to: 1) efficiently fit a complicated model with multiple parameters relating to biochemical limitations of photosynthesis (Patrick *et al*., 2009) many of which are treated as constants or assigned subjectively in conventional maximum likelihood-based model fitting approaches (Dubois *et al.,* 2007); 2) use data from multiple sources (A/q, A/Ci) simultaneously to inform species and individual-level parameters, rather than fitting data individually curve-by-curve; 3) explicitly incorporate the influence of plant traits on photosynthetic processes to improve model fits and make direct inferences on biologically relevant, mechanistic hypotheses (Feng & Dietze, 2013); 4) estimate posterior probability distributions, as opposed to single point estimates, to directly account for uncertainty in subsequent models; and 5) incorporate prior information to improve model performance for biochemical parameters that are well studied in the plant physiology literature but might be poorly informed by our data alone, while still accounting for this uncertainty based on past interspecific variation for these parameters (Patrick et al., 2009).

We included fixed effects for the influence of functional traits on photosynthetic processes following Feng & Dietze (2013) and included species- and individual-level random effects into the process model. Prior distributions were derived at a broad taxonomic level, centered on literature values (*sensu* Peltier & Ibáñez, 2015) or uninformative. Abbreviations, units, definitions, and distributions are listed in Table 2.

Data model (observation equation)

The likelihood of the photosynthetic data is based on the likelihood of each photosynthetic observation, ($A_{\text{net},i}$), from *i* – N, assumed to follow a normal distribution:

$$A_{net,i} \sim Normal(\mu A_{net,i}, \tau) \qquad \text{Eqn 1}$$

where μA_{net} is the predicted photosynthetic rate and τ is the precision (1/variance) parameter.

Process model (FvCB C_3 photosynthetic model with trait covariates)

$$\mu A_{net} = min\left(A_v, A_j\right) - R_d \qquad \text{Eqn 2}$$

where A_v and A_j are the CO_2 assimilation rate limited by Rubisco (carboxylation) and RuBP (substrate regeneration), respectively; R_d is the daytime mitochondrial respiration rate in the light. Triose phosphate limitation was not included, as the process is considered to not limit photosynthesis in many cases and is commonly not considered in these models (*e.g.*, Dubois *et al.*, 2007; Patrick *et al.*, 2009; Feng & Dietze, 2013; Peltier & Ibáñez, 2015).

Rubisco-limited photosynthesis:

$$A_v = V_{cmax} \frac{C_i - \Gamma^*}{C_i + K_c(1 + {}^{O}/_{K_O})} \qquad \text{Eqn 3}$$

where Vcmax is the carboxylation rate and $\Gamma^*$is CO_2 compensation point in the absence of R_d. $\Gamma^*$is considered to be conserved across species (e.g., Bernacchi *et al.,* 2001) but we chose to model this parameter to improve model estimates (Patrick et al., 2009; Peltier & Ibáñez, 2015) and allowed species-level variation through a random effects term. K_c and K_o are Michaelis-Menten constants for carboxylase and oxygenase activities of Rubisco, presumed to be effectively conserved across species and treated as constants (Table 2).

$$V_{cmax} = V'_{cmax} + \beta_N \left(\frac{N - \bar{N}}{sd_N}\right) + RE_{v,sp} + RE_{v,ind} \qquad \text{Eqn 4}$$

where C_i is the intercellular CO_2 concentration, V'_{cmax} is the overall V'_{cmax} intercept, β_N is slope of fixed effect of leaf N on V_{cmax}, RE is the random effects of species and individuals, respectively. We could have estimated an additional parameter to account for mesophyll resistance (Patrick et al., 2009; Peltier & Ibáñez, 2015). To minimize model parameters, we assume mesophyll conductance is infinite (e.g., Farquhar et al., 1980; Dubois et al., 2007).

RUBP-regeneration-limited (electron transport limited) photosynthesis:

$$A_j = J\frac{C_i - \Gamma^*}{4C_i + 8\Gamma^*} \qquad \text{Eqn 5}$$

where J is the rate of photosynthetic electron transport. J depends on the capacity of electron transport (J_{max}) and photosynthetic photon flux density (q) following Tenhunen *et al.* (1976):

$$J = \frac{\alpha q}{\sqrt{1 + \frac{\alpha^2 q^2}{J_{max}}}} \qquad \text{Eqn 6}$$

where α is the quantum utilization efficiency (initial quantum yield). Similar to Eqn 4, species and individual plant random effects were incorporated into J_{max} (asymptote of J-q curve, varies by individual; e.g., Bernacchi *et al.*, 2001), with specific leaf area (SLA) and leaf chlorophyll (chl) incorporated into α (Niinemets & Tenhunen, 1997; Feng & Dietze, 2013).

$$J_{max} = J'_{max} + RE_{j,sp} + RE_{j,ind} \qquad \text{Eqn 7}$$

$$\alpha = \alpha' + \beta_{SLA}\left(\frac{SLA - \overline{SLA}}{sd_{SLA}}\right) + \beta_{chl}\left(\frac{chl - \overline{chl}}{sd_{chl}}\right) \qquad \text{Eqn 8}$$

Statistical inference

The photosynthetic model described above was implemented for native and invasive species separately and the 95% credible intervals (CI; 2.5% to 97.5% quantiles of posterior distributions) were used to compare parameters by nativity. To test for differences between native and invasive species and the influences of resource availability, the posterior means for each parameter were compared though a HB linear mixed effect models with non-informative priors (with species and plot random effects) and incorporating parameter uncertainty into the data models. GLI and soil N was standardized by corresponding mean and standard error to facilitate comparisons across datasets. Parameter-derived resource-use efficiency traits (Funk & Vitousek, 2007) were modeled as a function of resource availability. Potential photosynthetic energy-use efficiency (PEUE) was calculated as A_{max}/CC. Potential photosynthetic nitrogen-use efficiency (PNUE) was calculated as A_{max}/N_{area}.

Similarly, we tested for differences in NPP as a function of 1) resource availability: light and nitrogen environment (and their interaction) and 2) C gain: photosynthetic capacity (A_{max}; using posterior estimates of model parameters under ambient CO_2 and saturating light) and leaf duration (90% senesced minus leaf expansion dates). Because differences in NPP might be confounded by initial plant biomass, we included starting wood biomass as a covariate in all NPP models.

Analyses were performed in R (R Development Core Team 2015) in JAGS (Plummer, 2014). The final models were run with three parallel Markov chain Monte Carlo (MCMC) chains for 100,000 iterations, discarding the initial 50,000 for burn-in. Trace plots were used to confirm convergence. When the 95% CIs for effects included zero, they were removed from the full model individually, the reduced model was rerun, and the deviance information criterion (DIC) was used to confirm that the model fit was improved (following Feng & Dietze, 2013). Model fits were also evaluated using the R^2 between observed and predicted values.

RESULTS

Experimental resource gradient

Tree removal (light addition treatment) significantly increased plot-level light availability (mean change in GLI ± 1 SE: 14.0 ± 1.5% full sun; Table 3, Fig 1a). As light growth responses are nonlinear, this difference translates to a biologically meaningful shift in light resources (Fig 1a), as forest growth model (Pacala *et al*., 1996; Martin *et al*., 2010) definitions of “deep shade” (GLI = 2%), “moderate shade” (GLI = 5%), “small gap” (GLI = 10%) and “very high light” (GLI = 80%). No plots were in “very high light” but the light gradient spanned moderate shaded conditions to small to medium gap conditions common to deciduous forests (Fig 1a).

Compared to unfertilized plots, fertilized plots increased N availability by roughly five-fold (Fig. 1b). Spring (May) and summer (July) soil nitrogen estimates showed a similar temporal response across all plots (data not shown), so following analyses use May/July mean total soil inorganic N (ammonium plus nitrate). Despite fertilization of equal forms of ammonium nitrate, the N addition treatment had the biggest influence on nitrate availability, which were many orders of magnitude larger than ammonium availability across all treatments (Table 3).

Photosynthetic trait responses

Photosynthetic rates and model parameters showed significant variation across species (Table 4) and resource availabilities (Table 5). Final photosynthesis models for both native and invasive species excluded fixed effect of chlorophyll on α, but included all other fixed and random effects. When included with SLA, β_{chl} 95% CIs included zero and removal of the term substantially improved model fit for both native (DIC=1667 vs. 1609) and invasive (DIC = 2841 vs. 2830) species. Predicted photosynthetic rates (μA_{net}) in final models for both native and invasive species were closely correlated to observed A_{net} ($R^2 > 0.97$).

Leaf nitrogen significantly influenced carboxylation capacity (V_{cmax}) in both native and invasive species, but invasive species were comparatively more responsive (β_N significantly greater; Table 4; Fig. 3a). Invasive species had higher mean V_{cmax} and J_{max} (maximum electron transport rates), but their corresponding 95% CIs overlapped for both parameter intercepts (Table 4). Both native and invasive species had significant but similar SLA influences on α (β_{SLA}).

Native and invasive species displayed important differences across resource availability gradients. In general, invasive species tended to be more responsive to light availability, with greater coordinated variation in photosynthetic parameters (V_{cmax}, J_{max}) and parameter-derived traits (A_{max}, PNUE; Table 5). Invasive species displayed a greater range of A_{max} and significant responses to increases in light availability (β_{GLI} coefficients in Table 4; Fig. 2a). Invasive species also had greater V_{cmax}, J_{max}, and α at a given GLI (Fig 2b,c).

Surprisingly, soil N had no direct influences on any parameter ($\beta_{soil\ N}$ coefficients in Table 5; Fig. 2) and leaf N showed minimal responses to fertilization (Fig 3b). However, the interaction between light and soil $\beta_{soil\ N\ x\ GLI}$ was significant for several parameters (Table 5), with contrasting responses between native and invasive species. Invasive species' responses tended to be negative, whereas native species were positive. As a result, there was a significant negative response of nitrogen availability on PNUE, but no response for natives (Table 4; Fig. 3c; $R^2<0.01$, invasive: $R^2=0.32$). Including only fixed soil N effect ($\beta_{soil\ N}$) confirmed this difference, with 95% CIs for natives essentially centered at zero (-0.003, 0.002) but negative for invasive species (-0.007, -0.001).

Phenological trait responses

As expected, invasive species tended to lose their leaves later into the fall than native species, in terms of 50% and 90% leaf loss (boxplots Fig. 4). Later senescence subsequently permitted invaders to retain significant chlorophyll (chl) later into the fall (Fig. 4). Compared to control plots, light and light+nitrogen addition plots showed slight increases in mid-season chl for invaders, but no response in natives. Leaves in native species showed rapid declines in chl beginning in September. Invasive species maintained mid-season chl levels into mid-October and showed slower declines and higher chl at senescence. In addition to chl, invasive species invested more total nitrogen in their leaves. Similar to chl at senescence, senesced leaf N was greater in invasive species, indicating significantly lower nitrogen resorption proficiencies than natives (Fig. 5).

Structural leaf trait responses

The most consistent response across species was leaf structural adjustments in response to light. SLA declined with increasing GLI (Fig 6a), while LDMC and leaf thickness increased (Fig. 6b,c). Leaf trait responses to the soil N gradient were minimal and inconsistent across species (Fig. 2; Fig. 3b; Fig. 6). At a given light level, invasive species showed greater specific leaf area and lower leaf dry matter content than natives (Fig. 6).

Growth responses

Sapling growth responses in all species were difficult to estimate in the field and were highly variable. Despite this variation, invasive species had the higher maximum rates of net primary productivity (NPP) regardless of treatments, but NPP differences were particularly pronounced at higher light levels (Fig. 7a). Neither invasive nor native species showed NPP responses to increasing soil N (Fig. 7b; Table 6a). As with leaf trait-environment responses, invasive species were more responsive to the light availability gradient, with light-mediated NPP increases in invasive species only (Table 6a). Native and invasive species were influenced by initial plant size in similarly ($\beta_{\text{initial biomass}} \approx 0.2$; Table 6) and native and invasive species did not differ by group in pre-treatment biomass.

Maximum photosynthetic rate (A_{max}) significantly influenced NPP in invasive species but not native species (Table 6b). NPP models that also included potential influences of growing season (leaf duration) showed no response in growing season length in native or invasive species length ($\beta_{\text{leaf duration}}$ was effectively zero in native and invasive species), so the term was removed in the final models.

DISCUSSION

Our current understanding on the processes attributed to plant invasion success is derived to a large degree from foundational perspectives based in resource-rich ecosystems of anthropogenic disturbance (e.g., Elton, 1958). However, habitats that are growth-limited by resources, including water, nutrients, or light, are frequently invaded by species with unique adaptations for these environments (Funk, 2013). In Eastern North America (ENA), many natural area invaders of conservation or management concern are found in forest understories (Fridley, 2008) –an environment where light and/or nitrogen has been epitomized to limit native woody growth and constrain community dynamics (*e.g.*, Bazzaz, 1979; Vitousek and Howarth, 1991; Pacala *et al.*, 1994; Walters & Reich, 1996; Reich *et al.*, 1997; Finzi & Canham *et al.*, 2000; Catovky & Bazzaz, 2002; Zaccherio & Finzi, 2007; etc.). We investigated the effects of resource availability on woody plant ecophysiology and invasion success through an experimental light and nitrogen gradient in an ENA deciduous forest. Supported by common garden interspecific comparisons (Heberling & Fridley, 2013), we predicted that these invasive species should have greater rates of carbon gain and display higher resource-use efficiencies than natives, but these strategy differences might vary along environmental gradients. We found significant differences in the direction and magnitude of physiological responses to resource availability. Most notably, invasive species exhibited more flexible trait responses to increased light. Unlike select previous research that emphasized the importance of disturbance and resource availability in plant invasions (e.g., Burke & Grime, 1996; Davis *et al.*, 2000; Daehler, 2003; Leishman & Thomson, 2005; Leishman *et al.*, 2010), we found invasive species to maintain physiological advantages compared to resident natives, even under low light conditions. Unexpectedly, direct responses to nitrogen availability were negligible in native or invasive species.

The strong influence of site irradiance on plant ecophysiology is reasonably well studied in ENA native tree species (*e.g.*, Niinemets & Tenhunen, 1997), but differences between invasive and native species are less well understood. Compared to common natives, we found invasive species to display highly flexible and more coordinated trait responses to light availability. As predicted by past empirical and theoretical studies (Niinemets & Tenhunen; 1997; Niinemets *et al.*, 1998; Oguchi *et al.*, 2003), leaf anatomical adjustments to irradiance were widespread, consistent across all species (Fig. 6). Across all plots, invasive species tended to have greater photosynthetic rates, but these differences were magnified at increasing understory light levels (Fig. 2). Trait adjustments along the light gradient were frequently in the same direction for both native and invasive species, and these adjustments were stronger for invasive species (Table 5). Invasive species exhibited higher A_{max} at higher light levels (Fig 2, Table 5) and this difference in C gain response led to greater rates of biomass production (Fig. 7;

Table 6). Similar results were found in seedling comparisons of *Acer saccharum* and invasive *A. platanoides* performed in shaded and gap shade-house conditions (Paquette *et al*., 2012).

It was surprising that NPP in native species did not respond to increased light levels in this experiment, given that previous studies on similar species found light responses in radial stem growth (e.g., Pacala *et al*., 1994; Finzi & Canham 2000). Relative to these studies, the highest and lowest light levels were not as dramatic, might not capture variation in native NPP responses. Pacala *et al.* (1994) report growth rate saturation at extremely low light levels for late successional shade tolerant species (e.g., 1% GLI to achieve half its maximum growth rate for *Acer saccharum*). The range of GLI in this study is still quite broad (around 5% to > 30% full sun) and representative of many deciduous forest conditions in the region, supporting the results that invasive species appear more responsive to a wider gradient of *in situ* common understory light levels in these forests.

Unexpectedly, plant responses to soil nitrogen were much less striking, even though N availabilities varied by a factor of five (Fig. 1b). A lack of NPP responses to soil N implies that growth, at least in this forest, was not nitrogen-limited. Responses may become significant if we had reduced N availability to lower than ambient levels, as a previous study found for invasive *Berberis thunbergii* (Cassidy *et al*., 2004). Leaf N significantly influenced carboxylation rates (V_{cmax}) in both groups (Fig. 3a), invasive species displayed comparatively greater photosynthetic adjustments to leaf N, as shown through photosynthesis model fixed effects coefficients (Table 4). While leaf N was significantly higher in invasive species (Fig. 5), neither group showed obvious increases in leaf N with increasing soil N availability (Fig. 3b). This result is in contrast with previous long term N addition studies for the same or similar group of native species that reported increases in leaf N following N fertilization (e.g., Magill *et al*., 2000; Bauer *et al*., 2004). Leaf N differences might become significant over a longer fertilization period. However, even in 14 year N addition experiment in Michigan, Bethers et al. (2009) found no differences in A_{max} for *A. saccharum*. In a related study, this species showed increased in growth to fertilization, but not photosynthetic rates, suggesting soil N might affect C allocation, rather than C assimilation (Talhelm et al., 2011). Additionally, previous seedling field-based (*e.g*., Catovsky & Bazzaz, 2002) or greenhouse-based studies (*e.g*., Fownes & Harrington, 2004) might be less applicable for saplings or mature shrubs than previously thought. Across a natural gradient of N availability, Walters & Gerlach (2013) found minimal within species responses to soil N and unrelated to growth, suggesting intraspecific responses may be less sensitive than those reported in broader scale interspecific comparisons.

Despite no differences in leaf N, there were significant impacts on photosynthetic parameters for light by nitrogen interaction coefficients (Table 5). Surprisingly, these light by nitrogen effects were negative for invasive species and positive for native species. It is unclear why native and invasive species have contrasting responses, and why the response is negative for invasives species. For invasive species measured in the current study, the highest maximum photosynthetic rates are in unfertilized plots (Fig. 2a). A previous study has shown high rates of experimental forest N additions to alter leaf N allocation in native *Pinus resinosa* to result in a 50% decrease in photosynthetic capacity in fertilized trees. It is unclear whether N additions caused similar shifts in allocation in invasive species in the current study.

In addition to differences in biomass and carbon gains, invasive species showed significantly greater photosynthetic nitrogen use efficiencies (PNUE) than native species, but this PNUE difference was only apparent in unfertilized plots (Fig. 3c). There was a positive relationship between PNUE and light availability, but interestingly, the relationship between

PNUE and soil N was negative (Table 5). This PNUE decline was, in part, a product of similar leaf N across treatments combined with lower A_{max} at higher soil N availabilities (Fig. 2a). Decreases in PNUE with increasing soil N might suggest that resource use efficiencies are meaningful only at lower nutrient conditions. Vitousek (1982) argued that nitrogen use efficiency varies as an inverse function of nitrogen availability, reporting that forests of high nitrogen availability have lower nitrogen use efficiencies. This relationship should occur in contexts where nitrogen does not limit biomass production and species have evolved (or can plastically respond) less efficient strategies to increase productivity. An intuitive explanation for our result is that invasive species are able to adjust their strategies along nitrogen gradients, whereas natives' strategies appear to be insensitive. Supporting this notion, common garden species-level comparisons revealed these ENA forest invaders are less conservative with nitrogen (low resorption rates), but have root traits (increased specific root length and fine root production) that enable higher uptake rates and rapid N cycling (Jo *et al.,* 2015).

Does resource availability determine understory invasion success?

Current paradigm in invasion theory places large emphasis on the mediating roles of disturbance and resource conditions (Burke & Grime, 1996; Davis et al., 2000; Leishman & Thompson 2005; but see Ordonez & Olff 2013). Yet, shade tolerant woody species (shrubs, lianas, trees) actively colonize and spread into relatively undisturbed, low light forest understories in ENA. The context of these invasions suggests invaders might substantially differ from other studies that conclude that invasive trait strategies depend upon physiological advantages at high resources (Leishman et al., 2010). To complicate the search for general mechanisms, species-specific case studies suggest that invasion mechanisms for ENA forests invaders may even differ between invasive species and forests. *Ailanthus altissima*, an invasive forest and urban tree in ENA, has been shown to be a “gap-obligate” species that can invade late successional forests, but only in the context of high light availabilities (Knapp & Canham, 2000; Martin *et al*., 2010). It is likely that many forest invaders, particularly those confined to gaps or forest edges follow similar mechanisms reported for invasions into other ecosystems.

Our results are supported by previous studies that implicate fundamental strategy divergence and resource-use efficiency differences between native and invasive species, where invasive species show advantages independent of resource or disturbance conditions. Demographic comparisons with common native ENA tree species indicate that invasive *Acer platanoides* follows a different life history tradeoff between low-light survivorship and growth in high light (Martin et al., 2010). Along with related common garden results (Fridley, 2012; Heberling & Fridley, 2013; Jo *et al.*, 2015), our results build upon this work to experimentally demonstrate in the field that resource availability does not solely determine understory invasion success.

It is intuitively puzzling that native species, which have presumably adapted to local conditions, have not evolved to adapt these “invasive” strategies (Sax & Brown 2000). We argue that these invasive shrubs follow strategies with ecophysiological advantages that are not found in the ENA forest flora. Given that similar forested environments are replicated in isolation across the temperate zone, a logical expectation of natural selection is that certain groups of species might have evolved substantially different physiological solutions to growth under these conditions (Fridley & Sax, 2014). Many of these shrub and liana species that invade ENA forests are native to forests of Central and East Asia (Fridley, 2008). These invasive species may be “pre-adapted” to these environments, with East Asian forests have greater historic selection pressures (higher species and family richness) and more stable climate conditions through evolutionary time for optimizing carbon gain in forest understory niches (Fridley, 2013).

Conclusion

Contrary to the widespread expectation that native species show advantages under certain environmental conditions, we found no evidence that invasive species performed comparatively poorer than natives under the decreased light or nitrogen conditions commonly encountered in ENA forest understories. In fact, our results suggest these invasive species might maintain physiological advantages along the gradient, even under lowest light levels. These results strongly highlight that forest understory invasions do not necessarily depend on resource conditions and may appreciably differ from other invasion mechanisms that invoke fast growth at the expense of proportionally greater resource demands.

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Table 1. Woody, deciduous species measured in this study, including nativity status in Eastern North America and general growth form.

Code	**Species**	**Common name**	**Nativity**	**Growth form**
ACSA	*Acer saccharum* Marsh.	Sugar maple	Native	Shrub, Tree
CORA	*Cornus racemosa* Lam.	Gray dogwood	Native	Shrub
FRAM	*Fraxinus americana* L.	White ash	Native	Tree
LON	*Lonicera* x *bella* Zabel [*L. morrowii* x *tatarica*]	Bell's honeysuckle	Invasive	Shrub
OSVI	*Ostrya virginiana* (Mill.) K. Koch	Hophornbeam	Native	Shrub, Tree
PRSE	*Prunus serotina* Ehrh.	Black cherry	Native	Shrub, Tree
PRVI	*Prunus virginiana* L.	Chokecherry	Native	Shrub, Tree
RHCA	*Rhamnus cathartica* L.	Common buckthorn	Invasive	Shrub, Tree
ROMU	*Rosa multiflora* Thunb.	Multiflora rose	Invasive	Subshrub, vine

Table 2. Model parameters, data, prior distributions and literature sources used in photosynthesis model.

Symbol	**Definition (units)**	**Attribute**	**Distribution (mean μ, sd σ)**	**Literature source**
A_{net}	Net photosynthetic rate (observed) (μmol CO_2 m^{-2} s^{-1})	Dependent variable	Data	-
μA_{net}	Net photosynthetic rate (modeled) (μmol CO_2 m^{-2} s^{-1})	Dependent variable	Predicted value	-
τ	Model precision ($variance^{-1}$)	Parameter	(model $\sigma^2)^{-1}$ $\sigma \sim$ dunif(0,100)	Broad prior
R_d	Mitochondrial daytime respiration rate (μmol CO_2 m^{-2} s^{-1})	Parameter	dnorm(0,10^5)	Broad prior based on Patrick *et al.* (2009)
V_{cmax}	Maximum carboxylation rate of rubisco (μmol CO_2 m^{-2} s^{-1})	Parameter	dnorm(25, 10^5)	Broad prior based on Peltier & Ibáñez (2015)
C_i	Intercellular CO_2 partial pressure (Pa)	Independent variable	Data	-
Γ^*	CO_2 compensation point (Pa)	Parameter	dnorm(4.275,0.01)	Informative prior based on Patrick *et al.* (2009), Bernacchi *et al.* (2001)
O	Intercellular O_2 partial pressure (kPa)	Constant	21	Farquhar et al. (1980)
K_c, K_o	Michaelis-Menten constants for CO_2 (Pa) and O_2 (kPa), respectively	Constant	40.49, 27.84 (adjusted to 25° C)	Bernacchi *et al.* (2001)
β_N	Slope of fixed effect of leaf N on V_{cmax}	Parameter	dnorm(0, 10^7)	Broad prior
N	Leaf nitrogen concentration (g m^{-2})	Covariate	Data	-
Chl	Leaf chlorophyll index (meter	Covariate	Data	-
J_{max}	Maximum electron transport rate (μmol e^- m^{-2} s^{-1})	Parameter	dnorm(55,10^5)	Broad prior based on Peltier & Ibáñez (2015)
$RE_{p,i}$, $RE_{p,i}$	Random individual effects for species, *s*, or individual, *i*, on parameter, *p*	Parameter	(RE $\sigma^2)^{-1}$ $\sigma \sim$ dunif(0,100)	Broad prior
α	Quantum efficiency of electron transport (mol e^- mol^{-1} quanta)	Parameter	dnorm(0.24,0.1)	Informative prior Feng & Dietze (2013)
q	Photosynthetic photon flux density (μmol photons m^{-2} s^{-1})	Independent variable	-	-
SLA	Specific leaf area (m^2 g^{-1})	Covariate	Data	-

Table 3. Summary by treatment for plot-level soil and light conditions.

	Control			**+Light**			**+Nitrogen**			**+Light +Nitrogen**		
Soil	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
NO_3^- (μg 10 cm^{-2} 28 d^{-1})	182	121	240	176	148	216	925	797	1073	713	526	819
NH_4^+ (μg 10 cm^{-2} 28 d^{-1})	3.08	2.40	3.60	2.28	1.35	4.30	3.68	2.40	5.15	2.15	1.70	2.70
P (μg 10 cm^{-2} 28 d^{-1})	1.68	0.35	4.60	0.83	0.35	1.90	0.60	0.40	0.80	0.68	0.50	0.90
Total N (organic/inorganic forms; mg kg^{-1})	0.31	0.28	0.35	0.36	0.33	0.42	0.33	0.27	0.41	0.34	0.32	0.37
Total C (mg kg^{-1})	3.91	3.30	4.66	4.23	3.81	4.94	4.30	3.81	4.79	4.18	3.80	4.56
Organic matter (g kg^{-1}) (loss on ignition)	109	102	120	125	111	146	123	118	130	126	120	134
pH	5.07	4.68	5.41	5.47	4.96	5.99	4.76	4.49	5.17	5.28	4.99	5.64
Light												
GLI (% full sun)	10	7	14	26	24	29	9	5	12	24	17	41

Table 4. Parameter means and credible intervals for photosynthetic models for native and invasive species. Significant differences by nativity (non-overlapping credible intervals) are highlighted in bold. See Table 1 for parameter definitions.

	Native			**Invasive**		
	Mean	**(95% CI)**		**Mean**	**(95% CI)**	
β_N	**5.9**	**2.3**	**9.6**	**16.9**	**12.0**	**21.2**
β_{SLA} x100	1.6	0.2	2.9	1.3	0.1	2.6
V'_{cmax}	30.3	21.2	39.3	49.3	28.9	68.8
J'_{max}	49.2	38.7	58.5	66.5	45.4	82.8
R_d	0.1	0.0	0.2	0.1	-0.1	0.2
α' x10	**2.6**	**2.5**	**2.8**	**3.1**	**2.9**	**3.3**
Γ^*	4.8	4.2	5.3	4.5	3.8	5.1

Table 5. Effects of light (GLI) and nitrogen (N) availability on photosynthetic model parameters (and derived traits) for native and invasive species as assessed through Bayesian linear mixed effects models with species and plot random effects. Significant fixed effects (credible intervals non-overlapping zero) are highlight in bold. Note: slope coefficients are x10.

	Native			**Invasive**		
	Mean	**(95% CI)**		**Mean**	**(95% CI)**	
V_{cmax}:						
β_{GLI}	1.0	-10.0	12.0	**36.9**	**24.0**	**49.3**
$\beta_{soil\ N}$	7.5	-33.4	49.8	-0.8	-67.8	65.9
$\beta_{soil\ N\ x\ GLI}$	1.0	-9.5	11.7	**-27.0**	**-39.0**	**-14.5**
J_{max}:						
β_{GLI}	**-15.4**	**-27.2**	**-3.7**	**107.7**	**89.9**	**125.0**
$\beta_{soil\ N}$	-12.8	-105.3	80.8	-64.9	-147.9	17.7
$\beta_{soil\ N\ x\ GLI}$	**33.7**	**22.2**	**45.8**	**-80.0**	**-97.1**	**-63.2**
α:						
β_{GLI}	**-0.08**	**-0.15**	**-0.01**	-0.07	-0.14	0.00
$\beta_{soil\ N}$	-0.01	-0.09	0.07	0.03	-0.06	0.11
$\beta_{soil\ N\ x\ GLI}$	-0.02	-0.10	0.06	0.02	-0.05	0.09
A_{max}:						
β_{GLI}	-2.8	-5.7	0.2	**18.7**	**14.5**	**22.9**
$\beta_{soil\ N}$	-0.9	-16.0	14.2	-11.2	-26.8	4.1
$\beta_{soil\ N\ x\ GLI}$	**3.7**	**0.9**	**6.6**	**-14.1**	**-18.1**	**-10.1**
PNUE:						
β_{GLI}	3.20	-6.95	13.22	**11.69**	**2.44**	**20.86**
$\beta_{soil\ N}$	-1.42	-11.54	8.92	**-13.00**	**-21.9**	**-4.51**
$\beta_{soil\ N\ x\ GLI}$	1.17	-8.16	10.48	-5.91	-14.52	2.60
PEUE:						
β_{GLI}	-0.15	-1.09	0.80	0.25	-0.29	0.83
$\beta_{soil\ N}$	-0.49	-1.48	0.51	-0.12	-0.90	0.12
$\beta_{soil\ N\ x\ GLI}$	-0.40	-1.37	0.58	-0.38	-0.90	0.12

Table 6. Effects of a) light (GLI) and nitrogen (N) availability and b) photosynthesis on net primary productivity (NPP; g new biomass over 2 year treatment period) as assessed through Bayesian linear mixed effects models with random effects for species. Pre-treatment biomass was included as a covariate ($\beta_{\text{initial biomass}}$). Significant fixed effects (credible intervals non-overlapping zero) are highlight in bold. Note: slope coefficients are x10 and NPP was log transformed.

	Native			**Invasive**		
NPP as function of:	**Mean**	**(95% CI)**		**Mean**	**(95% CI)**	
a) Resource availability						
β_{GLI}	0.48	-0.40	1.38	**1.61**	**0.43**	**2.76**
$\beta_{\text{soil N}}$	-0.83	-1.74	0.08	0.21	-0.93	1.25
$\beta_{\text{soil N x GLI}}$	-0.3	-1.27	0.67	-0.41	-1.47	0.62
$\beta_{\text{initial biomass}}$	**2.73**	**1.87**	**3.62**	**1.65**	**0.42**	**2.92**
b) Photosynthesis						
β_{Amax}	0.22	-0.62	1.07	**1.19**	**0.24**	**2.15**
$\beta_{\text{initial biomass}}$	**2.03**	**1.22**	**2.83**	**2.30**	**1.39**	**3.20**

Figure 1. Plot-level resource availabilities by experimental treatment. a) Light levels pre- and post-treatment (year 1) and b) plant available nitrogen (nitrate + ammonium) quantified using soil nutrient probes deployed for 4 weeks. Points denote plot level means.

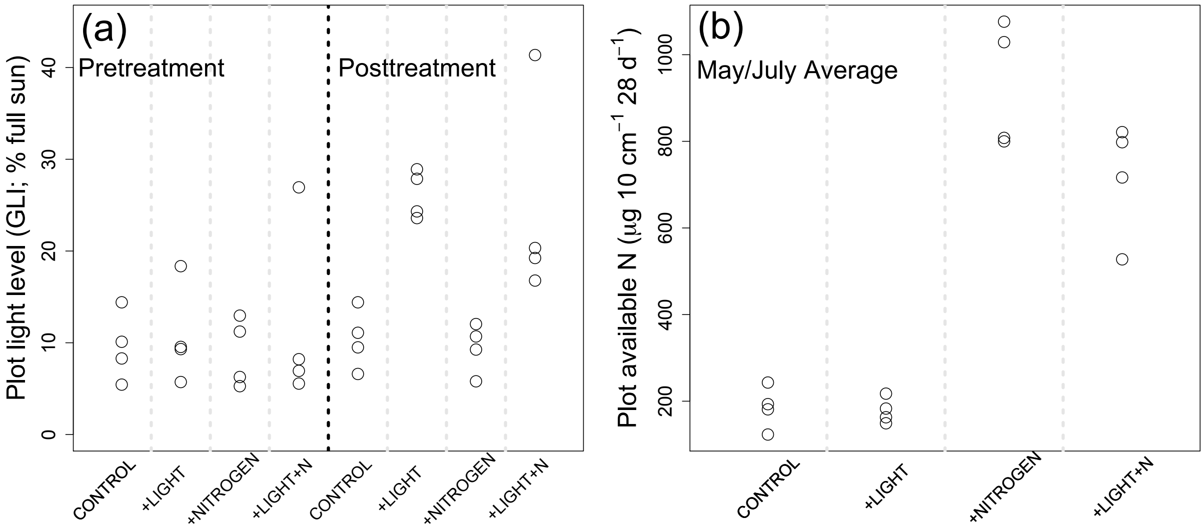


Figure 2. Photosynthetic model parameters as a function of light availability ("gap light index;" GLI) for a) maximum photosynthetic rate (A_{max}) b) maximum carboxylation rate (V_{cmax}) c) maximum electron transport rate (J_{max}) and d) quantum utilization efficiency (α). Light grey error bars denote 95% credible intervals on posterior means. Closed points show individuals in N addition plots for invasive (red, circles) and native (blue, triangles) species.

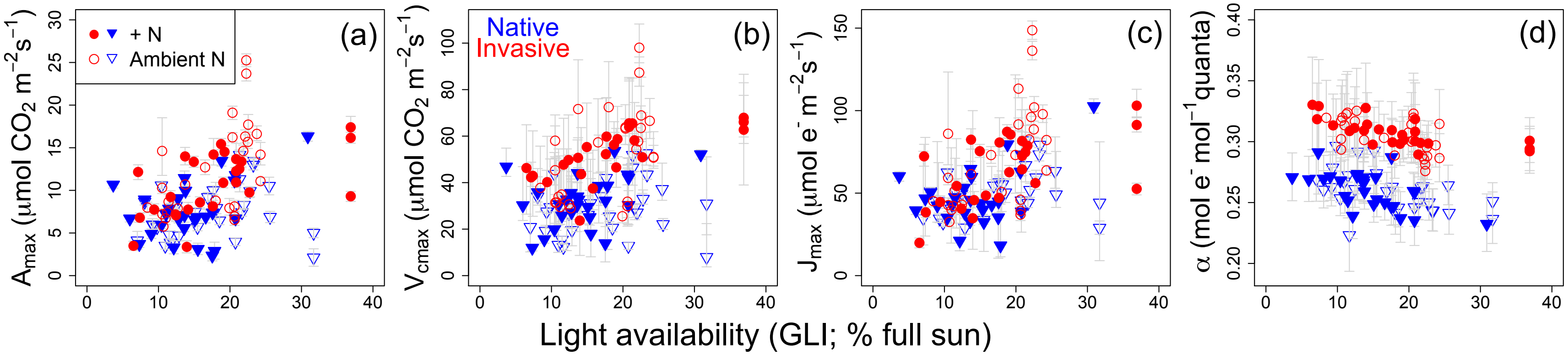


Figure 3. Functional relationships between leaf nitrogen, photosynthesis, and available soil N. a) Carboxylation capacity-leaf N (native: r=0.54, *P*<0.001; invasive: r=0.88, *P*<0.001) b) Leaf nitrogen-available soil N (native: r=0.12,*P*=0.36; invasive: r=0.10, *P*=0.50) and c) PNUE (photosynthetic nitrogen use efficiency)-available soil N (native: r=-0.03, *P*=0.85; invasive: r=-0.42, *P*=0.002). Fitted lines are from ordinary least square regressions (native: dashed, blue; invasive: solid, red). Light grey error bars denote 95% credible intervals on posterior means.

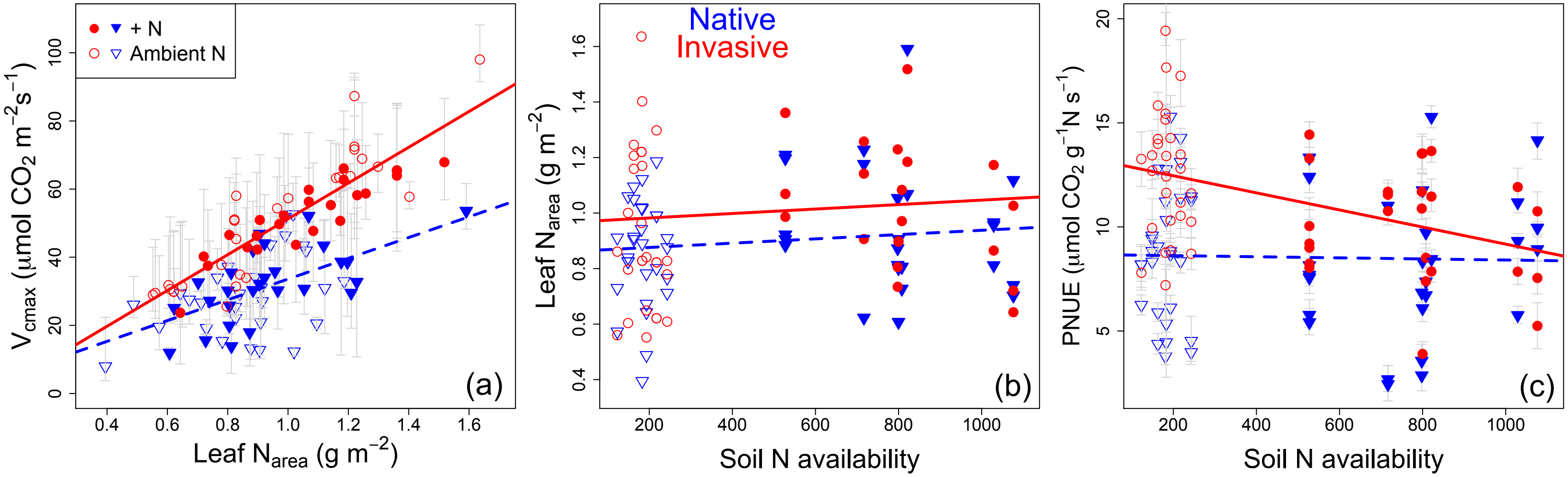


Figure 4. Seasonal trajectory of relative leaf chlorophyll concentration (handheld meter readings) across a) control b) light c) nitrogen and d) light + nitrogen treatments for native (blue, dashed line) and invasive (red, solid line) species. Points represent mean (± SE) by group. Boxplots denote dates of approximate 90% leaf loss as determined through weekly censuses.

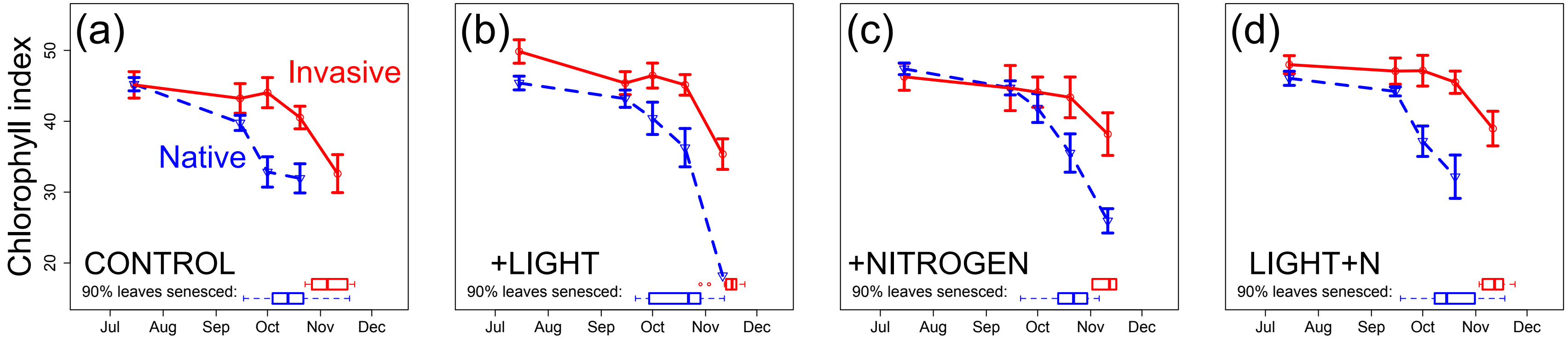


Figure 5. Leaf nitrogen concentrations for mature (filled boxplots) and senesced (open boxplots) for native (left, blue) and invasive (right, red) species. Senesced leaf N was corrected to account for leaf mass loss at senescence. See Table 1 for species codes.

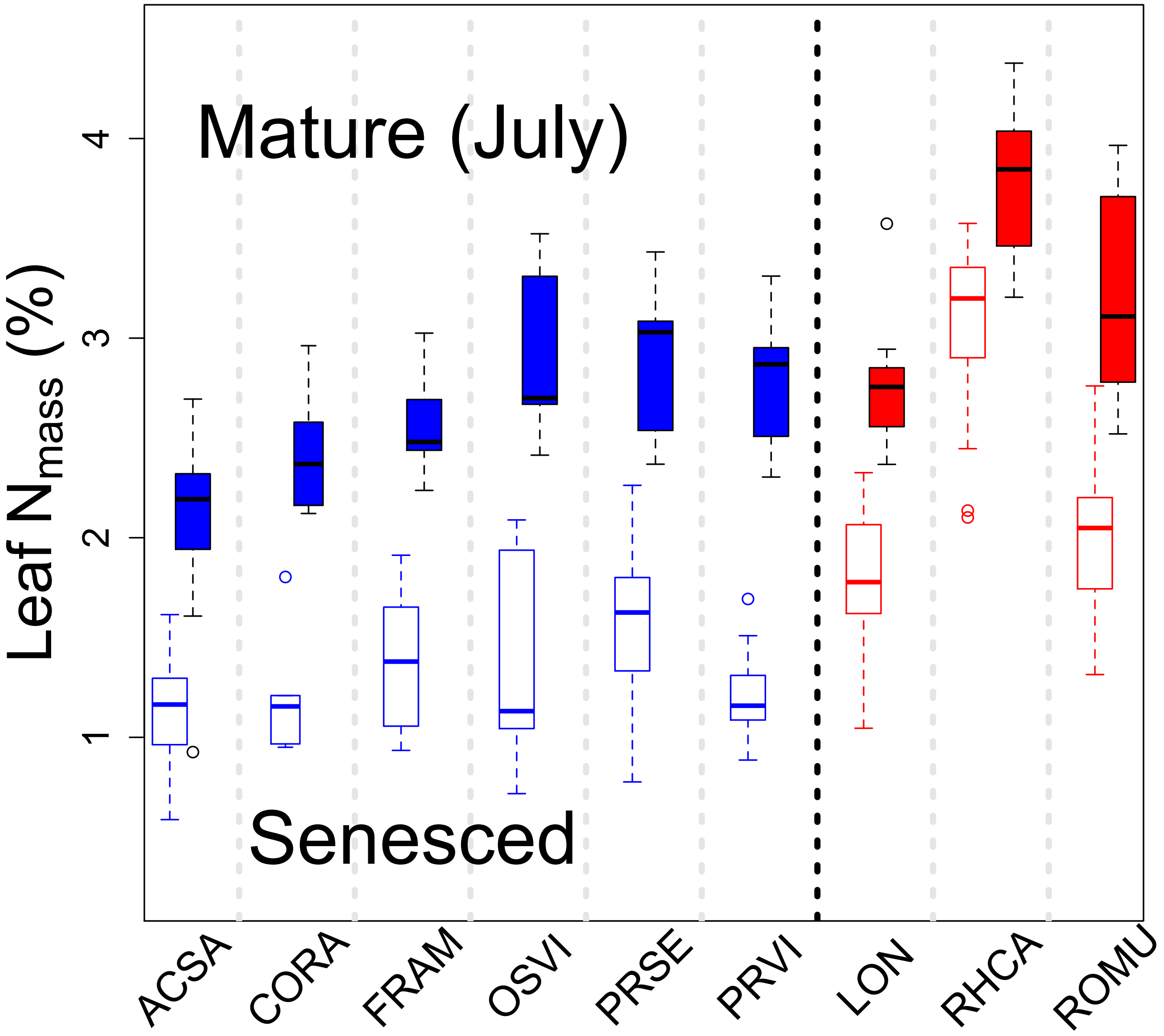


Figure 6. Leaf structural traits as a function of light availability. Fitted lines are ordinary least squares regressions for native (blue, triangle, dashed) and invasive (red, circle, solid lines) for GLI and a) specific leaf area (SLA) (invasive: R^2=0.50, *P*<0.001; native: R^2=0.27, *P*<0.001), b) leaf dry matter content (LDMC; fresh mass/dry mass) (invasive: R^2=0.23, *P*<0.001; invasive: R^2=0.18, *P*<0.001), and c) leaf thickness (invasive: R^2=0.12, *P*<0.05; invasive: R^2=0.06, *P*=0.08). Solid points denote individuals in N addition plots.

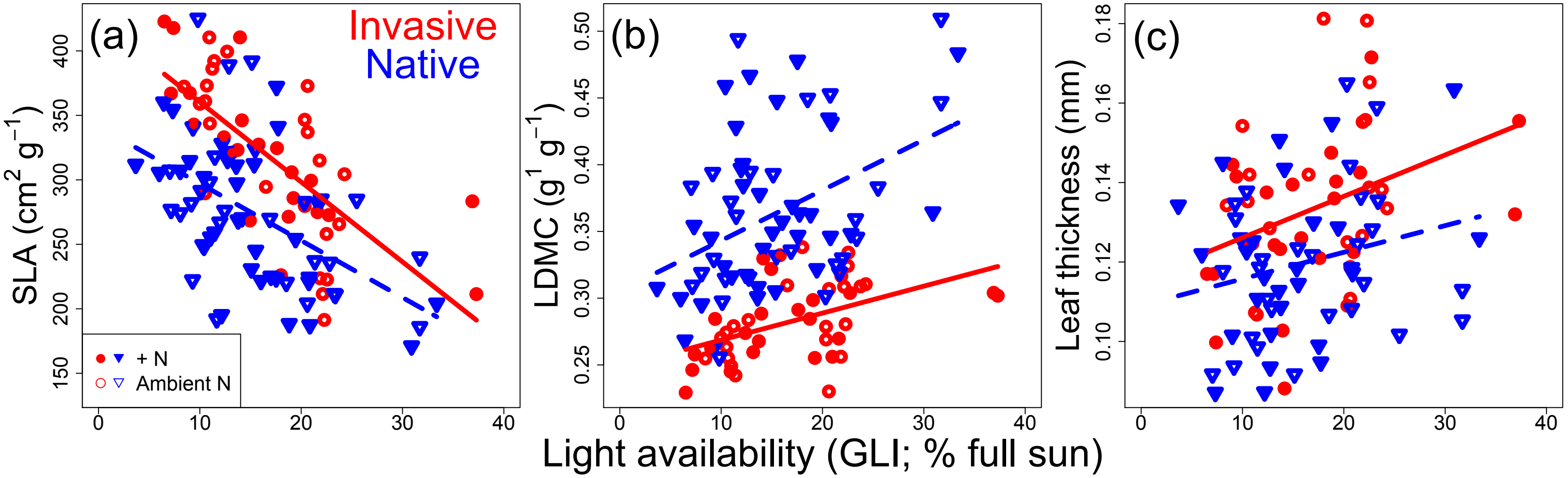
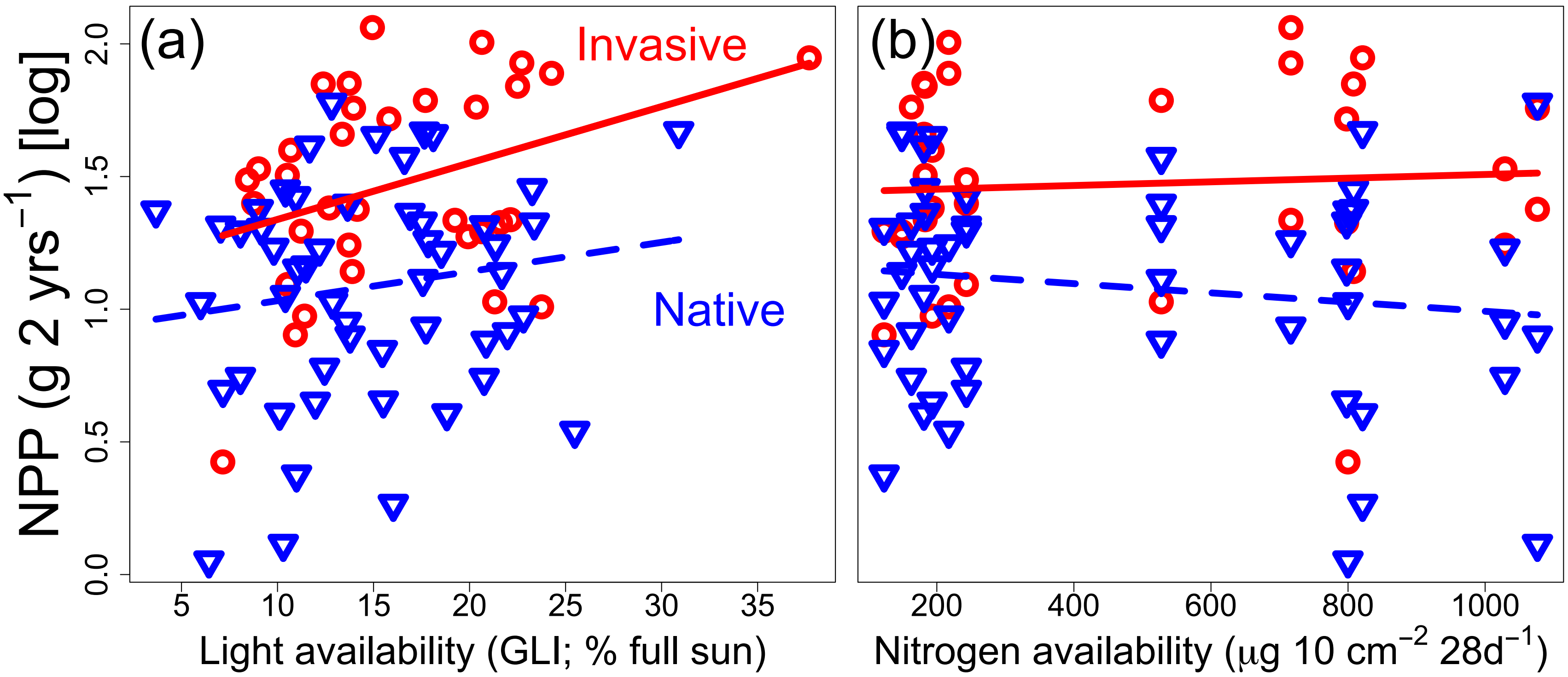


Figure 7. Net primary productivity (NPP) responses measured after 2 years of treatment across a) light levels (invasive: R^2=0.13, $P < 0.05$; invasive: $R^2 = 0.02$, $P > 0.1$) and b) soil nitrogen (invasive: R^2<0.01, P>0.7; invasive: $R^2 = 0.02$, $P > 0.3$).



CHAPTER 4.

Plant functional shifts in the invaded range: a test with reciprocal forest invaders of Europe and North America

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SUMMARY

1. Comparative trait analyses of native and invasive plant species have enriched our understanding on the strategies that make plants successful in particular contexts. However, it is generally unknown whether traits associated with invasiveness arise *de novo* in the introduced range or represent a case of 'pre-adaptation' of some species to become invasive.

2. We compared the functional strategies of two invasive species, *Prunus serotina*, a tree native to Eastern North America (ENA) and invasive in European forests, and *Rhamnus cathartica*, a shrub native to Europe and invasive in ENA, in both their native and invasive ranges. We measured leaf functional traits related to plant carbon and nitrogen economics in populations across northeastern ENA and northern France. This reciprocal field approach is unique, comparing *in situ* physiology within and between each species' shared ranges.

3. Across both species, we found striking differences in leaf economic traits and intraspecific trait scaling relationships. *P. serotina* exhibited similar photosynthetic rates in ENA (native) and France (invasive), but French populations had significantly greater leaf carbon investments in the form of increased leaf respiration, construction costs, and carbon concentrations. *R. cathartica* exhibited 50-60% higher photosynthetic rates in ENA (invasive) than France (native), along with increased leaf nitrogen costs. ENA populations also had substantially lower nitrogen resorption efficiency prior to fall senescence.

4. Intraspecific trait differences between native and invasive ranges indicate shifts in resource-use strategies might be common in invasive species. While further investigations would be needed to determine if our reported strategy differences result from pre-adaptation, post-introduction evolution, and/or phenotypic plasticity, our results question the assumption that functional strategies of invasive species are conserved from the native range and highlight the

utility of measuring *in situ* functional strategies to provide mechanistic evidence for invasion hypotheses.

Key-words: biological invasions, introduced range, leaf economics spectrum, leaf nitrogen, native range, nitrogen resorption efficiency, nitrogen resorption proficiency, photosynthetic rate

INTRODUCTION

Research on plant invasions has increasingly focused on functional traits of non-native plant species in efforts to understand the physiological underpinnings behind invasion success (Daehler 2003; van Kleunen, Weber & Fischer 2010). Many studies have compared particular traits or coordinated trait syndromes (strategies) of invasive species in their introduced range with those common in the native resident flora, with invaders biased toward more productive traits, such as high photosynthetic capacities (A_{max}), specific leaf area (SLA), and leaf nutrient investments (*e.g.*, Leishman et al. 2007; Tecco et al. 2010; Penuelas 2010; Ordonez & Olff 2013). Despite these advancements, relatively few studies have addressed whether the functional strategies of invasive species changes in their invaded range compared to those of their native range (Hierro, Maron & Callaway 2005).

Intraspecific home-and-away trait comparisons have several potential outcomes. First, a species might exhibit significantly different trait values or resource-use strategies in their invasive range compared to their native range, due to enemy release (*e.g.*, reduced herbivory and/or resource re-allocation towards growth) or a novel competitive environment (weak biotic resistance). This shift could be due to evolution in the new range (*e.g.*, local adaptation, genetic drift, admixture, founder effects) and/or phenotypic plasticity. Alternatively, a species might possess similar ecophysiologies in both native and invasive ranges, indicating pre-adaptation and niche similarities between ranges (Fridley & Sax 2014). Lastly, both post-introduction evolution and pre-adaptation can occur jointly, where species or populations with particular pre-adapted traits successfully naturalize, with post-introduction evolutionary fine-tuning to local environments in the new range (*e.g.*, Henery et al. 2010). A growing number of common garden seedling studies of native and invasive populations, primarily comparing morphological traits of herbaceous species, have demonstrated important shifts of invaders toward competitive phenotypes (*e.g.*, Blossey & Nötzold 1995; Zou et al. 2007; García et al. 2013). Fewer studies compare physiological strategy differences measured in the native and invasive range habitats.

Here, we investigate range-level trait differences in north temperate deciduous forests in Europe and Eastern North America (ENA). Recent research on shrubs and lianas in ENA forests has demonstrated that woody invaders from a wide variety of taxonomic groups exhibit extended foliar phenology and contrasting resource-use strategies compared to woody native species. Compared to ENA native congeners, invasive species, most of which originate from East Asia or Europe, tend to senesce leaves later into the fall (Fridley 2012), possess higher photosynthetic abilities and resource-use efficiencies (Heberling & Fridley 2013) and exhibit lower leaf N resorption and root traits associated with more effective nutrient foraging (Jo et al. 2015). In an analysis of invasion patterns across the Northern Hemisphere, Fridley (2013) suggested that these forest invaders may have ‘pre-adapted’ traits in their native range that confer invasiveness in ENA. Invasion patterns indicate directionality toward ENA forest invasions by shade tolerant East Asian species. European species tend to be more invasive in disturbed/anthropogenic habitats, whereas ENA species tend to invade European and East Asian meadows. However, forest understories in Europe are not immune to invasion (*e.g.*, Closset-Kopp et al. 2007). As European forests have a long anthropogenic history, ENA forests have also been increasingly modified through anthropogenic disturbances, including deer overpopulation (Côté et al. 2004), eutrophication (Aber et al 1989), and non-native earthworm invasions (Bohlen et al 2004), suggesting fitness advantages for species functional shifts toward productive phenotypes in this environment. However, it is unclear if the functional strategies shown in introduced populations are consistent with those expressed in their native ranges.

To determine whether carbon (C) capture and resource-use strategies differ between native and invasive ranges, we measured *in situ* leaf-level C gains, energy and N investments, resource-use efficiencies and leaf N resorption strategies in two woody species found in north temperate deciduous forests, *Prunus serotina* (Rosaceae; black cherry) and *Rhamnus cathartica* (Rhamnaceae; common buckthorn). We sampled individuals from multiple populations of each species in Europe (northern France) and ENA (central New York, USA) across forest edge and understory environments. This transatlantic comparison is unique in that the two species are reciprocally invasive (*i.e.*, one species is native where the other is invasive, and vice-versa). Because the species occur in the same stands or within the same region in both areas, this approach helps disentangle trait responses due to particular regional conditions (e.g., climate) versus whether the species is invasive in the region.

Two explanations have been proposed to explain the general success of *P. serotina* in European forests. In its native range (ENA), high seedling mortality as a result of soil pathogens near mature trees limits population densities (Packer & Clay 2000). However, in Europe, this pathogen-mediated negative soil feedback is absent (Reinhart et al 2003), which supports the enemy release hypothesis. Second, it has been suggested that *P. serotina* exhibits a unique mid-successional strategy with a relatively shade-tolerant, long-lived sapling bank but fast growth upon gap formation. This particular strategy is largely absent in the European flora but common in native range competitors, thereby conferring a demographic advantage in French forests (Closset-Kopp et al. 2007). As a result from release from soil pathogens and pre-adaptation, we hypothesized that invasive (French) populations should exhibit more productive traits (e.g., photosynthetic capacity) compared to those measured in the native range (ENA).

Similarly, we hypothesized that *R. cathartica* in the invaded range (ENA) should exhibit traits related to greater resource gain (*e.g.*, leaf N, photosynthetic capacity) compared to populations sampled in similar habitats in the native range (France). In its invasive range, *R. cathartica* displays increased leaf N, greater photosynthetic capacity, extended leaf longevity and reduced leaf N resorption prior to fall leaf senescence compared to resident natives (e.g., Harrington, Brown & Reich 1989; Fridley 2012; Heberling & Fridley 2013; Jo et al. 2015). Knight (2006) hypothesized that the relatively high N in leaf litter (*i.e.*, low N resorption proficiency) in ENA is related to its extended leaf lifespan into fall, as the timing of leaf senescence places physiological constraints on the efficiency of N resorption prior to abscission (Niinemets & Tamm 2005). This hypothesis would be supported if leaf N resorption proficiency were greater in native European populations, where extended phenology has not been reported (Knight et al. 2007). Alternatively, as symbiotic N-fixing association with *Frankia* is an ancestral character in Rhamnaceae (but not exhibited by *R. cathartica*; Soltis et al. 1995), a relatively wasteful plant N economy often associated with N-fixers may be intrinsic to the clade. Indeed, non-native *R. cathartica* individuals showed similarly low N resorption proficiency to N-fixers compared with other non N-fixing taxa (Stewart et al. 2008). This phylogenetic constraint hypothesis argues similar N conservation strategies in both native and invasive populations.

MATERIALS AND METHODS

Study species

Prunus serotina Ehrh. (Rosaceae; black cherry) is a mid-successional tree native to ENA and invasive throughout European forests. This gap-dependent species follows a "sit-and-wait" strategy, where it can form a relatively slow-growing, long-living, shade-tolerant sapling bank under closed canopy conditions. Upon gap formation, the species exhibits rapid growth and reproduction, and can resprout from stumps and roots to revert back to shade tolerant stage (Closset-Kopp et al. 2007). It was introduced to Europe for ornamental purposes in the 17th century, became naturalized in the 19th century, and has been recognized as invasive since the mid 20th century (Starfinger 1997).

Rhamnus cathartica L. (Rhamnaceae; common buckthorn) is a large shrub or small tree native to Europe and western Asia and invasive across ENA (Knight et al. 2007). It is regarded as shade tolerant in both its native (Grubb, Kollmann & Wilson 1996) and invasive (Knight 2006) ranges. Introduced as early as the late 1700s (Kurylo & Endress 2012), buckthorn invades a diversity of habitats in ENA, including open fields, forest edges, and shaded understories. In contrast, despite its relative shade tolerance, it is primarily limited to open areas or forest edges in its native range (Kurylo et al. 2007).

Sampling protocol

Eight spatially separated populations of each species were sampled in ENA and Europe in June and July 2013 (Table S1). In ENA, we chose four locations in central New York, USA where both species co-occur. Since the two species rarely co-occur in Europe, we chose four locations per species in France. We sampled saplings of 1-2 m height for access to crown foliage.

Ten individuals of each species were sampled per site. To the extent possible, individuals of each population were sampled equally across forest edge, gap and closed canopy understory conditions. To account for light-mediated trait variation, "Gap Light Index" (GLI; Canham 1988) was calculated using hemispherical photographs taken directly above the canopy of each individual (Gap Light Analyzer; Frazer, Canham & Lertzman 1999).

Gas exchange measurements were performed on cut branches, following the protocol of Niinemets et al. (2005), widely used for temperate woody species. Two upper branches per individual were cut in the field and immediately recut under water. To maintain xylem water potential, the severed ends were wrapped with wet paper towel, placed in plastic bags, and stored in a cooler to minimize transpiration until transported to the lab, typically within 2 h (for distant populations, within 4-6 h). Upon returning to lab, branches were recut and cut stems placed in water, loosely covered in transparent plastic, and stabilized at room temperature under low light for 1-3 d before recording gas exchange measurements. Each morning, branches were recut under fresh water.

Leaf gas exchange

Gas exchange measurements were made on recently expanded, mature leaves using an LI-6400 portable photosynthesis system equipped with CO_2 and temperature control modules (Li-Cor, Lincoln, NE, USA). Leaf temperature was maintained at 25ºC under ambient humidity throughout measurements with sample chamber flow rate of 500 μmol s^{-1} and sample chamber CO_2 concentration at 380 μmol mol^{-1}. Leaves were photoinduced at a moderate irradiance level (300 μmol photons m^{-2} s^{-1}) until equilibration. Light levels were then progressively increased until light saturation (800-1,500 μmol photons m^{-2} s^{-1}). All individuals were light saturated at the highest light levels, with no apparent signs of photoinhibition. Light-saturated net photosynthetic rate (A_{sat}) was recorded after equilibrating for at least two minutes at each PPFD and reaching defined stability parameters based on photosynthetic rate and stomatal conductance to water. After achieving light saturation, light levels were decreased incrementally to 200 μmol photons m^{-2} s^{-1} and assimilation rate (A_{200}) was again recorded following equilibration. Lastly, the light source was shut off to measure daytime dark respiration (R_d). Area-based maximum gross photosynthetic rate (A_{max}) was calculated as the sum of A_{sat} and R_d.

Leaf structural and biochemical characteristics

Following gas exchange measurements, at least five leaves per branch were scanned for leaf area and oven dried at 60ºC for at least 48 hours. Specific leaf area (cm^2 g^{-1}) was calculated as the leaf surface area per g dry mass. Ground leaf samples were placed in an ashing furnace at 500ºC for 4 hours, and leaf ash concentration was calculated as ash mass divided by sample mass. Duplicate samples were averaged for each individual. Mass-based leaf nitrogen (N_{mass}) and carbon (C_{mass}) concentrations were determined using an elemental analyzer (CE Elantech, Lakewood, NJ, USA) for leaves collected for photosynthetic measurements.

To measure leaf N resorption, recently senesced leaves were collected in autumn (October-November) on the same individuals by gently shaking the plant and collecting fallen leaves, aiming to collect leaves from the same cohort as the mature leaf collection. Senesced leaves were oven dried, weighed, and analyzed for C and N. Because leaf mass loss and area shrinkage during senescence can be significant, using uncorrected nutrient concentrations in senesced tissue can lead to an underestimation in calculated nutrient resorption rates (van Heerwaarden, Toet & Aerts 2003). Therefore, mass loss was corrected following Vergutz et al. (2012). Senesced leaf N concentration (senesced N_{mass}) was calculated as senesced N_{mass} x MLCF, where MLCF is the mass loss correction factor, calculated as the ratio of the dry mass of senesced leaves to the dry mass of mature leaves. Senesced N_{mass} is interpreted as the inverse of leaf N resorption proficiency (Killingbeck 1996). Similarly, the percent reduction of leaf N between mature and senesced leaves, leaf N resorption efficiency (NRE), was calculated as [1- (senesced

N_{mass}/mature N_{mass})MLCF] x 100.

Leaf construction cost (CC) quantifies the amount of glucose equivalents required to construct a leaf in terms of carbon skeletons, reductant, and ATP, excluding additional costs for maintenance and substrate transport (Williams, Field & Mooney 1989). Leaf CC_{mass} (g glucose g^{-1} leaf mass) was determined using a biochemical approximation (Vertregt & Penning de Vries 1987; Poorter 1994; Boyd; Xu & Griffin 2009):

$$CC_{mass} = (-1.041 + 5.077C_{mass})(1 - 0.67Ash) + 5.325N_{mass}$$

where C_{mass} is leaf carbon concentration, *Ash* is leaf ash concentration (proxy for mineral concentration; Vertregt & Penning de Vries 1987), and N_{mass} is leaf nitrogen concentration (all in g g^{-1}). We assumed leaf NO_3^- accumulation is negligible compared to organic N forms, and nitrate is the dominant form of N uptake. The first part of the CC equation above takes into account the carbon costs (empirically determined from the relationship between glucose costs and C content of biochemical compounds; Vertregt & Penning de Vries, 1987). The second part of the first term (including ash) subtracts the mineral component in organic tissue from C cost, as the mineral fraction in organic tissue does not require C skeletons and energy required for their uptake is independent of costs for growth (Poorter 1994). The last term of the CC equation above accounts for the additional, substantial costs required to reduce nitrate into organic N (proteins).

Resource-use efficiency (RUE) is broadly defined as the amount of carbon assimilated per unit resource (Funk & Vitousek 2007). Potential photosynthetic energy-use efficiency (PEUE) was calculated as $A_{max,mass}/CC_{mass}$. Potential photosynthetic nitrogen-use efficiency (PNUE) was calculated as $A_{max,mass}/N_{mass}$.

Data analysis

Where necessary, measurements were converted between area (*i.e.*, m^{-2} leaf) and mass-based estimates (*i.e.*, g^{-1} leaf) through their corresponding SLA. All statistical analyses were performed in R (R Development Core Team 2014). Trait differences between regions were assessed with linear mixed-effect models (Bates et al. 2014) that accounted for population-level variation through random effects. To control for possible effects of light environment, GLI was included in all models as a fixed effect covariate. For each trait, we compared models with and without a fixed effect of region (native or non-native) using likelihood ratio tests, following a χ^2 distribution with one degree of freedom. When necessary, data were log-transformed to satisfy model assumptions. Bivariate relationships were analyzed with standardized major axis (SMA; Warton et al. 2006) line fitting implemented with the *smatr* package in R (Warton *et al*. 2012).

RESULTS

Comparative functioning between native and invasive ranges

Both species showed significant functional trait differences between their native and invasive ranges (Table 1). However, the magnitude and direction of trait shifts were not consistent across ranges of each species. In general, *R. cathartica* exhibited greater inter-population trait separation than *P. serotina.*

R. cathartica in ENA (invasive) had significantly greater potential photosynthetic rates than French (native) populations, at both low (A_{200}) and saturating ($A_{max,mass;}$ $A_{max,area}$) light levels (Table 1). Leaf respiration rates ($R_{d,area}$, $R_{d,mass}$) were variable among individuals (Fig 1a), but the region-level means were similar across ranges (Table 1). Therefore, with higher $A_{max,area}$ in ENA but similar $R_{d,area}$ in both regions, respiratory costs were lower per unit photosynthetic gain in invasive populations. As expected with increased photosynthetic capacity, invasive ENA populations had greater leaf N investments (N_{mass}, C:N; Table 1). Further, ENA populations displayed much lower leaf N resorption proficiencies (i.e., higher senesced N_{mass}) and efficiencies (percent N_{mass} resorbed prior to leaf senescence; Table 1). Each population included a relatively wide range of leaf N resorption efficiencies, but ENA populations were consistently lower (Fig. 2a). After including the effects of light environment on SLA ($r=-0.60$, $P<0.001$), there was no indication of differences in C investment traits by region, although French populations invested more in leaf carbon relative to nitrogen than invasive ENA populations (C:N, Table 1). There were no consistent inter-population differences in leaf construction costs (CC_{area}, Fig. 1c) or SLA (Fig. 1d).

In contrast, *P. serotina* showed nearly identical photosynthetic rates across invasive French and native ENA populations (Table 1, Fig. 1e-h). Mean C gain rates ($A_{max,mass;}$ $A_{max,area;}$ A_{200}) for *P. serotina* were slightly lower in France (invasive) than ENA (native), but these differences were on the order of 3-12% and statistically insignificant ($P>0.1$, Table 1). Likewise, *P. serotina* exhibited similar N investments (N_{mass}, N_{area}, C:N) and resorption rates (Senesced N_{mass}, N resorption efficiency, Fig. 2b). However, unlike *R. cathartica*, *P. serotina* had significantly greater C investments in the sampled invasive range populations (France), including greater CC_{mass} and mass-based leaf C concentration (C_{mass}, Table 1). Respiratory costs ($R_{d,mass}$, $R_{d,area}$) were also greater in France compared to sampled individuals in native ENA. Consequently, respiration efficiencies (A_{max}/R_d) were lower in the invaded range (Table 1).

Carbon gain tradeoffs with resource investments

Considering bivariate cost-benefit trait tradeoffs in $A_{max,area}$ (C gain potential) with associated resource investments, *R. cathartica* exhibited strong intraspecific correlations (Fig. 1a-d), both within ranges and overall. In contrast, trait relationships were weak for *P. serotina* (Fig. 1e-h), with insignificant bivariate trends ($R^2<0.10$, $P>0.1$). Results were broadly similar when traits were expressed on a mass-basis (Fig. S1).

Invasive *R. cathartica* exhibited greater C returns per increase in respiratory costs than native populations (slope shift in A_{max}-R_d, Fig. 1a, Fig. S1). Similarly, invasive *R. cathartica* populations were shifted further along a shared tradeoff (slope) in the $A_{max,area}$-N_{area} relationship (Fig. 1b) and $A_{max,area}$-CC_{area} (Fig. 1c). Although there were no range-level mean differences in SLA (Table 1), at a given SLA, individuals measured in ENA had consistently greater $A_{max,area}$ than those in native France.

In addition to mid-season mature leaf N (N_{area}, N_{mass}), maximum photosynthetic rate ($A_{max,mass}$) was closely correlated to N concentration in senesced leaves (senesced leaf N_{mass}) in both species (Fig. 3). As expected from N resorption efficiency differences (Fig. 2), invasive *R. cathartica* was shifted along a common slope, with both greater $A_{max,mass}$ and greater senesced N_{mass} (Fig. 3a). In contrast, there were no scaling differences in this relationship for *P. serotina*, despite significant trait correlation (Fig. 3b).

Range-level differences in instantaneous resource-use efficiencies

Although invasive populations exhibited consistently greater A_{max} (*R. cathartica*) and leaf CC (*P. serotina*), only *R. cathartica* displayed differences in nitrogen and energy-use efficiencies. Mean photosynthetic nitrogen-use efficiency (PNUE) was greater in ENA for invasive *R. cathartica*, despite lower N investments in French populations (Table 1, Fig. 3a). Also, as a result of greater A_{max} in ENA than France, but with similar CC, photosynthetic energy-use efficiency (PEUE) was greater in ENA (Table 1).

DISCUSSION

Do species follow different resource-use strategies in their invasive ranges?

Our current understanding on the functional ecology of invasive plants has largely been informed from studies that compare non-native species with resident native species (Van

Kleunen et al. 2010). These interspecific contrasts often highlight trait-based strategy differences, including those associated with increased carbon capture abilities (*e.g.*, Leishman, Thomson & Cooke 2010), faster growth rates (*e.g.*, Grotkopp, Rejmanek & Rost 2002), greater nutrient demands (*e.g.*, Penuelas et al. 2010), or higher resource-use efficiencies (*e.g.*, Funk & Vitousek 2007). However, it remains unknown whether the traits that confer greater relative plant success in their invasive range are similarly observed in their native range.

We measured *in situ* leaf traits of *Prunus serotina* (black cherry; invasive in France, native in ENA) and *Rhamnus cathartica* (common buckthorn; invasive in ENA, native in France) to test whether these species manifest similar traits in their native ranges, or alternatively, if resource-use strategies differ in the invaded range. We hypothesized that both species would display traits that confer a more productive strategy in their invasive range. Our results strongly support the notion that invasive species follow different resource-use strategies in their native and invasive ranges. Interestingly, the nature of these trait shifts was not consistent between the two species studied, with invasive *P. serotina* populations in France shifted towards increased carbon investments and invasive *R. cathartica* in ENA shifted towards increased carbon gains and nitrogen demands.

We hypothesized that *P. serotina* would have greater metabolic rates in its invasive range (France), due to escape from native soil pathogens absent in Europe (Reinhart et al. 2003). Contrary to this hypothesis, *P. serotina* showed very similar maximum photosynthetic rates (A_{max}) across regions (Table 1, Fig.1e-h). Unexpectedly, relative to ENA, invasive populations (France) exhibited greater leaf CC_{mass} and leaf C_{mass}, indicating increased leaf energy investments. Additionally, we found average increases of 44% and 63% in area- and mass-based leaf respiration rates (R_d), respectively. These differences indicate an increase in leaf carbon investments in the invaded rage.

Further, we hypothesized that *R. cathartica* in its invasive range (ENA) should display increased photosynthetic functioning, at the expense of high leaf nitrogen costs, which have been reported in ENA relative to co-occurring natives (Knight et al. 2007, Heberling & Fridley 2013). Our results support this prediction. Range-level differences were striking, with nearly 50% and 57% higher maximum photosynthetic rates in ENA relative to France for $A_{max,mass}$ and $A_{max,area}$, respectively (Table 1, Fig.1a-e). Further, ENA plants exhibited increased gains per unit respiratory costs compared to French plants (A_{max}-R_d slope shift, Fig. 1a), which has been previously reported for invasive species compared to co-occurring natives (Pattison, Golstein & Ares 1998, McDowell 2002). In other words, per unit respiratory cost, ENA plants exhibited increased photosynthetic benefits. Additionally, ENA invaders invested more in leaf N, with appreciably lower leaf N resorption efficiency and proficiency (Fig. 2). Despite this, PNUE was greater in ENA due to larger proportional increases in A_{max}. A fundamental tradeoff between high leaf nutrient acquisition and low internal conservation is predicted by plant resource-use strategy theory (Aerts & Chapin 1999) and has been supported in global analyses (Kobe, Lepczyk & Iyer 2005, Vergutz et al. 2012). Further, mid-season A_{max} and fall senesced N_{mass} (inverse of nutrient proficiency) was closely correlated in both species (Fig 3.). No differences were detected for *P. serotina* in this tradeoff, while *R. cathartica* in ENA was shifted further along a common tradeoff, with greater A_{max} and N in senesced leaves (Fig. 3a).

Several studies have compared functional traits in native and invasive ranges. Leishman, Cooke & Richardson (2014) measured leaf traits in populations across multiple ranges of 13 invasive species in the Southern Hemisphere and found consistent trait shifts in the invasive populations toward faster growth strategies associated with reduced leaf herbivory. In contrast, Ordonez (2014) analyzed literature-derived species trait values for species measured in native and introduced ranges and concluded that traits were consistent. However, this meta-analysis found large variation in the direction and magnitude of differences, with both increased and decreased trait values in their invasive ranges. Similarly, another meta-analysis, including both plants and animals, reported that individuals had higher mean performance traits (*e.g.*, biomass) than in the invaded range, but noted that nearly the same number of species exhibited no change (Parker et al. 2013). Our study highlights specific differences in resource-use strategies in home and away ranges through a unique comparison of a reciprocal invasion in a temperate forest ecosystem, where growth is strongly limited by light availability and, in some cases, soil nitrogen (Reich et al. 1997; Finzi & Canham 2000).

Why are in situ resource-use strategies different in the introduced range?

Despite marked functional differences we found in the invaded range, it is unclear whether these trait shifts primarily result from pre-adaptation, post-introduction adaptation, and/or environmental variation. Since the direction and magnitude of trait changes were not consistent in two species that were reciprocally invasive (i.e., one species invades where the other is native), we argue that environmental differences between France and ENA alone cannot explain the strategy shifts. Environmental differences might be important, as there are undoubtedly differences in climate (oceanic France vs. continental ENA), site edaphic characteristics, and photoperiod. Although we carefully accounted for obvious abiotic differences and population-level variation, it is possible we have not captured all of the range-level variation, especially considering large geographic native ranges of both *R. cathartica* (Kurylo et al. 2007) and *P. serotina* (Pairon et al. 2010). Reciprocal common garden experiments are needed to disentangle genetic differences from phenotypic plasticity. However, an advantage of our *in situ* approach is empirical insight into the proposed explanations for increased success in the introduced range.

In the native range, *P. serotina* densities are suppressed through high soil pathogen-mediated mortality in seedlings near mature trees (Reinhart et al. 2003). Although soilborne pathogens have been suggested to suppress juvenile growth (Packer & Clay 2003), our lack of decreased photosynthetic function in ENA compared to France indicate limited effects of pathogen attack on the physiology of the saplings we measured. Likewise, the high population densities observed in France likely do not translate to individual-level growth. Further, *P. serotina* occupies a particular mid-successional niche that may be absent in the European tree flora (Closset-Kopp et al. 2007), which suggests pre-adaptation. Our combined results support these proposed explanations.

In ENA, *R. cathartica* invades open sites, forest edges, and shaded forest understories. Interestingly, the species is primarily considered a forest edge species in Europe (Kurylo et al. 2007), despite its moderate shade tolerance (Grubb et al. 1996). Compared to native ENA species, *R. cathartica* exhibits extended leaf phenology (Harrington et al. 1989; Fridley 2012), high photosynthetic rates, high leaf N (Heberling & Fridley 2013), and low leaf N resorption (Jo et al. 2015). However, in Europe, the phenology of *R. cathartica* does not differ appreciably from co-occurring natives (Knight et al. 2007) or considered to have long lasting leaves compared to other European shrubs (Kollman & Grubb 1999). Lower nitrogen resorption efficiency (Fig. 2a) and proficiency (Fig. 3a) in ENA compared to France supports the prediction of Knight (2006), who hypothesized that the extended phenology strategy in ENA inhibits nutrient resorption prior to fall senescence. Lower N resorption in ENA may be a plastic response or selected trait for increasing leaf longevity and C gain.

Why would a low N conservation strategy for *R. cathartica* be advantageous in ENA, but not Europe? Several studies have associated *R. cathartica* invasions with a local abundance of non-native earthworms, which may promote a more wasteful plant N economy through altering N mineralization rates (e.g., Heneghan, Steffen & Fagen 2007, Madritch & Lindroth 2008, Roth et al. 2015). However, since earthworms are native in Europe, their presence in ENA alone cannot explain range-level trait differences. Similarly, temperate forests across both regions have been subjected to increased rates of anthropogenic N deposition (Galloway et al. 2004; Holland et al. 2005). Compared to native shrubs and lianas, other ENA invasive species with extended leaf phenology also showed higher rates of N uptake and lower resorption (Jo et al. 2015). Our results indicate this strategy of low N conservation is not present in the native range, or at least less pronounced. Given the prevalence of this strategy in ENA forest invaders (Jo et al. 2015), N-based shifts in plant resource economy between ranges might be a more general, but unexplored, phenomenon.

Conclusions

We found that invasive species can follow different resource-use strategies in their invaded and native ranges. Such large divergences highlight the importance of considering intraspecific variation in functional trait analyses (Donovan et al. 2014, Niinemets 2015) and question the assumption that species mean trait values are conserved across native and invasive ranges (Ordonez 2014). The degree to which these range-level differences are genetic versus plastic remains unknown. Rapid trait evolution in plant invasions is more common than previously thought (Buswell, Moles & Hartley 2011), but given the geographic ranges of many invasive species span large environmental gradients and biotic contexts, phenotypic plasticity likely plays a role in range-level differences. Common garden studies are needed to assess the roles of pre-adaptation, evolution, and plasticity in generating range-level differences. Nonetheless, our results provide an important step toward establishing and understanding how resource-use strategies found in the invaded range compare to those in the native range.

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DATA ACCESSIBILITY

The complete trait dataset is available in Table S1.

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FIGURES

Figure 1. Standardized major axis (SMA) relationships for area-based maximum photosynthetic rate (A_{max}) and leaf cost traits (a,e) dark respiration ($R_{d,area}$), (b,f) nitrogen concentration (N_{area}), (c,g) construction cost (CC_{area}) and (d,h) specific leaf area (SLA). *Rhamnus cathartica* (RHCA, a-d) and *Prunus serotina* (PRSE, e-h) individuals are plotted by region: USA (red circles, solid line); France (blue triangles, dashed line). SMA analyses performed only for relationships showing at least moderate correlation ($R^2>0.10$, $P<0.1$). Significance tests indicate differences in slope, elevation (y-intercept), or shift along common slope (*$P<0.05$, ***$P<0.001$). (a) overall: $R^2=0.14$ $P<0.01$; invasive: $R^2=0.36$, $P<0.001$; native: $R^2=0.48$, $P<0.001$ (b) overall: $R^2=0.56$, $P<0.001$; invasive: $R^2=0.59$, $P<0.001$; native: $R^2=0.19$, $P<0.05$ (c) overall: $R^2=0.41$, $P<0.001$; invasive: $R^2= 0.49$, $P<0.001$; native: $R^2= 0.11$, $P=0.11$ (d) overall: $R^2=0.35$, $P<0.001$; invasive: $R^2=0.57$, $P<0.001$; native: $R^2=0.12$, $P<0.10$ (e-h) all $R^2<0.10$, $P>0.1$.

Figure 2. Leaf N resorption efficiencies (NRE) by native- and invasive-range populations for a) *R. cathartica* (RHCA) and b) *P. serotina* (PRSE). Acronyms denote each population. Points indicate individual measurements. Error bars represent 95% confidence intervals for each range-level mean. Statistical differences between native and invasive ranges for each species were evaluated from likelihood ratio tests of linear mixed models that include population random effects (**, $P<0.01$)

Figure 3. Standardized major axis (SMA) relationships for mass-based maximum photosynthetic rate (A_{mass}) and leaf N in senesced leaves (senesced N_{mass}) for ENA (red circles, solid line) and French (blue triangles, dashed line) individuals. a) *Rhamnus cathartica* (RHCA): overall $R^2=0.39$, $P<0.001$; France $R^2=0.04$, $P=0.29$; ENA $R^2=0.22$, $P<0.01$ b) *Prunus serotina* (PRSE):

overall $R^2 = 0.28$, $P<0.001$; France $R^2 = 0.23$, $P<0.01$; ENA $R^2 = 0.36$ $P<0.001$. Significance tests indicate differences in slope, elevation (y-intercept), and shift along common slope (***$P<0.001$)

TABLES

Table 1. Mean values (± 1 SE) by species for photosynthetic, biochemical, structural, and resource-use efficiency leaf traits among native and invasive ranges. Statistical differences between native and invasive populations were determined using likelihood ratio tests (χ^2 with 1 df) that compared models for each trait with and without a fixed effect of range (invasive or native) with light environment as a fixed effect and population as a random factor. Significantly greater mean values ($P<0.05$) are indicated in bold. $^+P<0.1$; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

SUPPORTING INFORMATION

Fig S1. Bivariate relationships by range for mass-based leaf traits.

Table S1. Leaf trait dataset and associated metadata.

Table 1. Mean values (± 1 SE) by species for photosynthetic, biochemical, structural, and resource-use efficiency leaf traits among native and invasive ranges. Statistical differences between native and invasive populations were determined using likelihood ratio tests (χ^2 with 1 df) that compared models for each trait with and without a fixed effect of range (invasive or native) with light environment as a fixed effect and population as a random factor. Significantly different values ($P<0.05$) by range are indicated in bold. $^+P<0.1$; $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

	Prunus serotina (PRSE)			*Rhamnus cathartica* (RHCA)		
Trait (units) [a]	Invasive (France)	Native (USA)	Native status (χ^2)	Invasive (USA)	Native (France)	Native status (χ^2)
$A_{max,area}$ (μmol CO_2 m^{-2} s^{-1})	6.61 ± 0.32	6.75 ± 0.35	0.15	11.12 ± 0.88	7.09 ± 0.47	3.53+
$A_{max,mass}$ (nmol CO_2 g^{-1} s^{-1})	178.2 ± 10.8	200.1 ± 11.3	0.13	**306.4 ± 12.8**	**205.5 ± 12.2**	**6.78****
$A_{200,area}$ (μmol CO_2 m^{-2} s^{-1})	4.45 ± 0.20	5.03 ± 0.21	1.72	**6.57 ± 0.27**	**4.98 ± 0.31**	**4.74***
$R_{d,area}$ (μmol CO_2 m^{-2} s^{-1})	**0.65 ± 0.05**	**0.40 ± 0.04**	**7.44****	0.37 ± 0.05	0.51 ± 0.05	0.24
$R_{d,mass}$ (nmol CO_2 g^{-1} s^{-1})	**16.3 ± 1.0**	**11.3 ± 0.8**	**8.99****	10.7 ± 0.7	14.4 ± 1.3	0.66
A_{max}/R_d	**11.93 ± 0.92**	**22.77 ± 2.42**	**4.62***	**34.43 ± 3.19**	**14.82 ± 1.90**	**8.00****
SLA (cm^2 g^{-1})	263.16 ± 13.68	301.15 ± 10.54	0.10	323.59 ± 20.39	287.57 ± 7.42	0.62
Mature N_{mass} (%)	2.79 ± 0.10	2.91 ± 0.07	0.17	**3.92 ± 0.10**	**3.11 ± 0.11**	**7.69****
Mature N_{area} (g m^{-2})	1.21 ± 0.10	1.02 ± 0.04	<0.01	1.41 ± 0.09	1.10 ± 0.04	2.87+
Mature C:N	18.60 ± 0.73	16.18 ± 0.43	1.19	**11.71 ± 0.32**	**15.31 ± 0.63**	**7.40****
Senesced N_{mass} (%)	1.32 ± 0.11	1.36 ± 0.07	<0.01	**2.86 ± 0.08**	**1.69 ± 0.04**	**22.38*****
N resorption efficiency (%)	58.43 ± 4.18	61.83 ± 2.47	0.09	**34.06 ± 2.03**	**48.96 ± 2.75**	**9.50****
Mature C_{mass} (%)	**49.44 ± 0.16**	**46.17 ± 0.48**	**6.65****	44.74 ± 0.26	45.29 ± 0.23	1.42
Mature C_{area} (g m^{-2})	21.15 ± 1.25	16.46 ± 0.78	0.53	16.92 ± 1.26	16.15 ± 0.40	<0.01
Ash (mg g^{-1})	**50 ± 1**	**66 ± 2**	**5.46***	106 ± 3	108 ± 3	<0.01
CC_{mass} (eq. g glucose g^{-1})	**1.567 ± 0.009**	**1.400 ± 0.025**	**8.93****	1.349 ± 0.016	1.334 ± 0.015	0.25
CC_{area} (eq. g glucose m^{-2})	65.92 ± 4.00	50.00 ± 2.49	3.16+	51.03 ± 3.93	47.52 ± 1.22	0.02
PNUE (μmol CO_2 g^{-1} N s^{-1})	6.88 ± 0.54	6.88 ± 0.36	0.88	**7.89 ± 0.35**	**5.63 ± 0.43**	**4.84***
PEUE (μmol CO_2 kg^{-1} glucose s^{-1})	114.07 ± 7.24	144.19 ± 8.56	1.74	**227.78 ± 11.03**	**134.29 ± 10.44**	**7.47****

[a] $A_{max,area}$ and $A_{max,mass}$, area- and mass-based light saturated gross photosynthetic rate on an area and mass basis ($A_{max,mass} = A_{max,area}$ x SLA); $A_{200,area}$, area-based net photosynthetic rate at lower photosynthetic photon flux density (200 μmol photons m^{-2} s^{-1}); $R_{d,area}$ and $R_{d,mass}$, area- and mass-based dark respiration rate; A_{max}/R_d, respiration efficiency; SLA, specific leaf area; Mature N_{mass} and N_{area}, mass- and area-based leaf nitrogen concentration of mature foliage; Senesced N_{mass}, mass-based terminal leaf nitrogen concentration of freshly abscised leaves in autumn; C_{mass} and C_{area}, mass- and area-based leaf carbon concentration; Ash, leaf ash concentration;

CC_{mass} and CC_{area}, mass- and area-based leaf construction costs; PNUE, potential photosynthetic nitrogen use efficiency; PEUE, photosynthetic energy use efficiency

Figure 1.

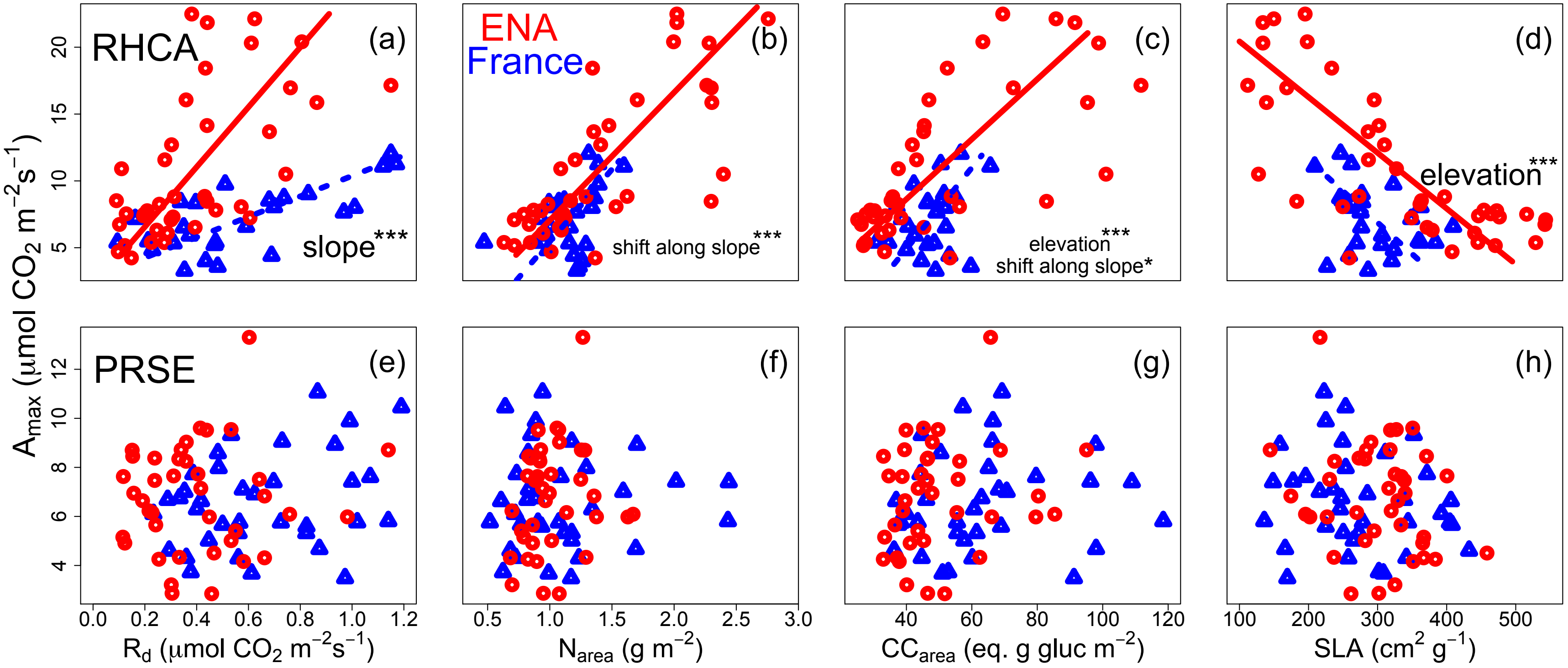

RHCA
(a)
slope***
ENA
France
(b)
shift along slope***
(c)
elevation***
shift along slope*
(d)
elevation***
PRSE
(e)
(f)
(g)
(h)
A_{max} (μmol CO$_2$ m^{-2}s^{-1})
5
10
15
20
4
6
8
10
12
0.0
0.2
0.4
0.6
0.8
1.0
1.2
R_d (μmol CO$_2$ m^{-2}s^{-1})
0.5
1.0
1.5
2.0
2.5
3.0
N_{area} (g m^{-2})
40
60
80
100
120
CC_{area} (eq. g gluc m^{-2})
100
200
300
400
500
SLA (cm^2 g^{-1})

Figure 2.

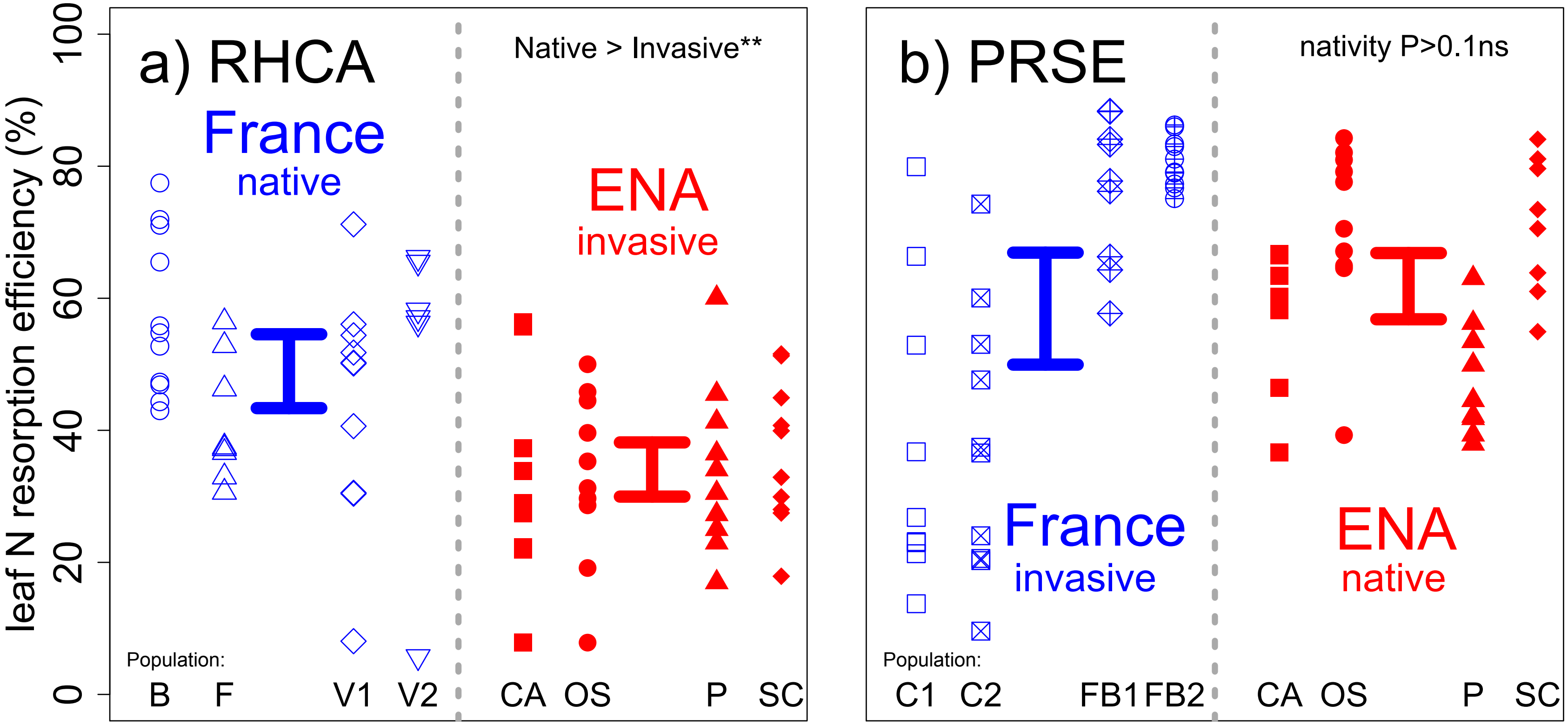

leaf N resorption efficiency (%)
0
20
40
60
80
100
a) RHCA
France
native
ENA
invasive
Native > Invasive**
Population:
B
F
V1
V2
CA
OS
P
SC
b) PRSE
nativity P>0.1ns
France
invasive
ENA
native
Population:
C1
C2
FB1
FB2
CA
OS
P
SC

Figure 3.

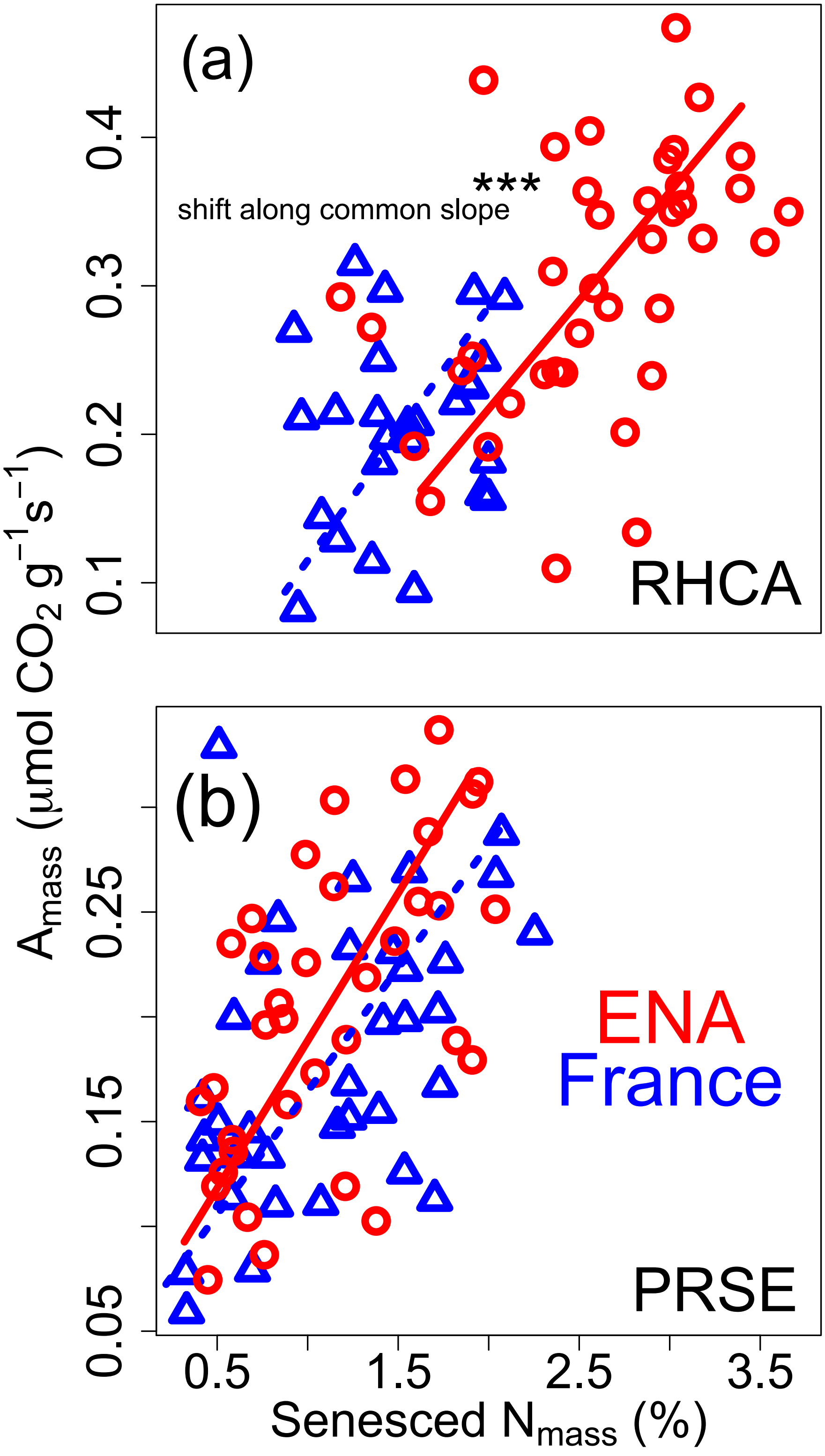
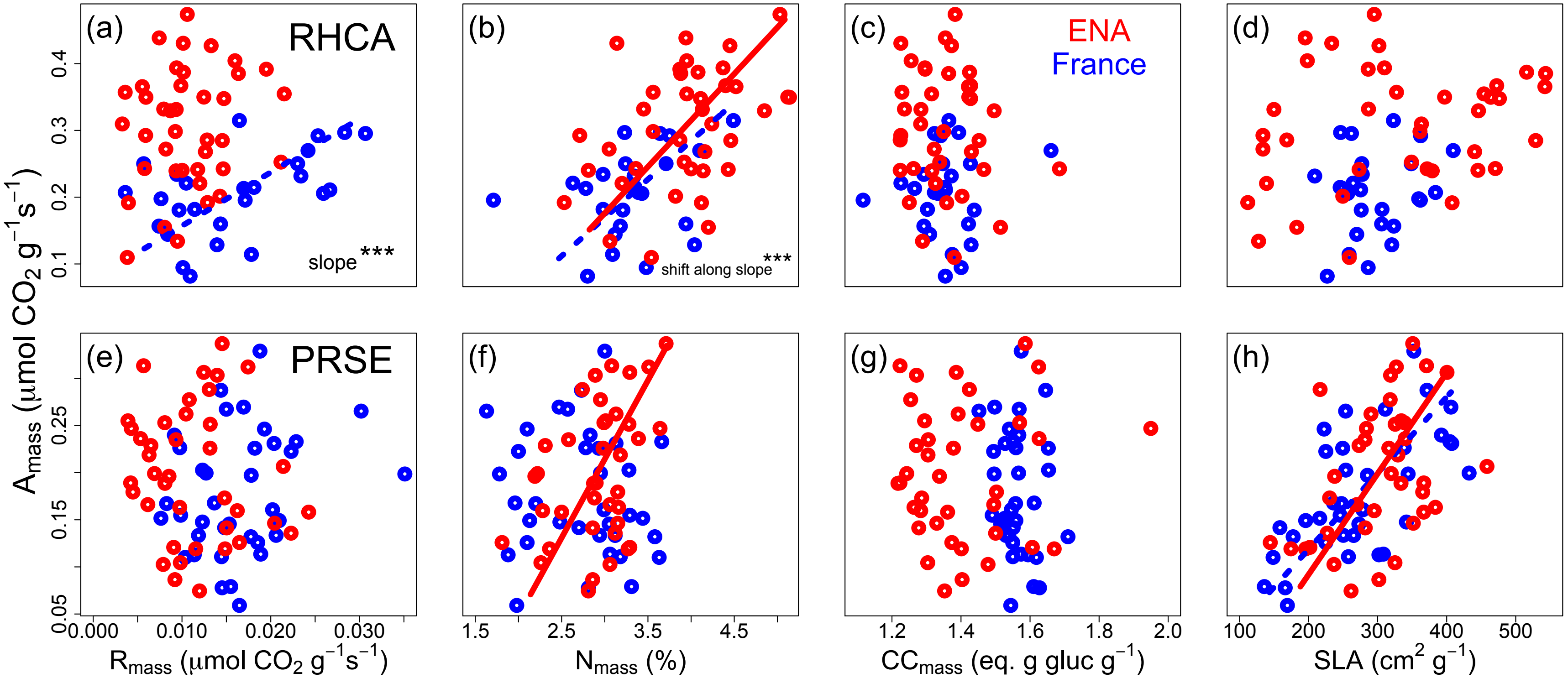

(a)
shift along common slope
***
RHCA
(b)
PRSE
ENA
France
A_{mass} (µmol CO_2 $g^{-1}s^{-1}$)
0.1
0.2
0.3
0.4
0.05
0.15
0.25
0.5
1.5
2.5
3.5
Senesced N_{mass} (%)

Figure S1. Standardized major axis (SMA) relationships for mass-based maximum photosynthetic rate (A_{mass}) and leaf cost traits (a,e) dark respiration (R_{mass}), (b,f) nitrogen concentration (N_{mass}), (c,g) construction cost (CC_{mass}) and (d,h) specific leaf area (SLA). *Rhamnus cathartica* (RHCA, a-d) and *Prunus serotina* (PRSE, e-h) individuals are plotted by region: USA (red circles, solid line); France (blue triangles, dashed line). SMA analyses performed only for relationships showing at least moderate correlation (R^2>0.10, *P*<0.1). SMA tests indicate differences in slope, elevation (y-intercept), or shift along common slope (****P*<0.001). (a) overall: R^2<0.01 *P*=0.93; invasive: R^2=0.03, *P*=0.31; native: R^2=0.26, *P*<0.01 (b) overall: R^2=0.26, *P*<0.001; invasive: R^2=0.16, *P*=0.01; native: R^2=0.09, *P*=0.14 (c) all R^2<0.05, *P*>0.05 (d) overall: R^2=0.08, *P*=0.02; invasive: R^2=0.06, *P*=0.13; native: R^2=0.04 *P*=0.33 (e) all R^2<0.10, *P*>0.1 (f) overall: R^2=0.02, *P*=0.24; invasive: R^2=0.01, *P*=0.50; native: R^2=0.15 *P*=0.02 (g) all R^2<0.10, *P*>0.1 (h) overall: R^2=0.29, *P*<0.001; invasive: R^2=0.42, *P*<0.001; native: R^2=0.18 *P*<0.01 .



SYNTHESIS.

Proximate and ultimate mechanisms of plant invasions

> *I suspect that this preponderant migration [of plants from Northern to Southern Hemispheres] . . . is due to the greater extent of land, and to the northern forms having existed in their own homes in greater numbers, and having consequently been advanced through natural selection and competition to a* ***higher stage of perfection or dominating power****, than the southern forms . . .* [emphasis added] (Darwin, 1859)

Natural selection can only act on local standing variation. Evolutionary processes do not occur in a global context. Well before Darwin, natural historians have long recognized that distant regions often consist of very different species, despite similar environmental conditions (Buffon, 1761). By extension, it is logical to expect that these isolated species are the product of the evolution of different strategies towards optimizing fitness, despite similar current environmental conditions. Fossil evidence of biotic interchanges over deep time document large-scale extinctions, with organisms from one region largely displacing those from another (Vermeij, 1991). Although happening at substantially different rates and in novel anthropogenic contexts, the core ecological processes that dictate modern invasions should be no different than historical interchanges (Vermeij, 2005). Many hypotheses have been posited to explain the success of invasive plants, with emphasis on the roles of escaping enemies (*e.g.*, Keane & Crawley, 2002), rapid evolution (Blossey & Nötzbold, 1995), disturbance regimes (*e.g.*, Hobbs & Huenneke, 1992), and resource/niche opportunities (*e.g.*, Davis *et al*., 2000; Shea & Chesson, 2002). However, few modern invasion perspectives explicitly invoke historical differences between regions (but see Fridley, 2011, 2013; Fridley & Sax, 2014).

Ascribing the ultimate (evolutionary) basis for particular plant invasions first requires an understanding of potential strategy differences between native and invasive species (proximate causes). Predicting the general traits that confer invasiveness have long been of interest, both for applied conservation and basic ecology theory (*e.g.*, Baker 1965, Rejmanek & Richardson 1996, van Kleunen *et al*., 2015). Studies often report invasive plants to exhibit traits that are associated with fast growth, including high carbon assimilation rates, specific leaf areas, and leaf nitrogen (*e.g.,* van Kleunen *et al.*, 2010). A common expectation in many studies is that native species should have a competitive advantage over invasive species in some environmental contexts (Daehler, 2003). Otherwise, why would native species not also have these "invasive" traits? It has been argued (and demonstrated empirically in some habitats) that invasive species are shifted towards strategies that confer faster growth than natives, but this comes at the expense of high resource investment (Leishman *et al*., 2010; Ordonez *et al*., 2010). In other words, native and invasive species follow the same fundamental physiological tradeoff surface.

A "perfect" organism would be one that has maximized fitness through having low resource requirements, high growth rate, early reproduction, infinite fecundity, and unbounded lifespan (*i.e.*, "Darwinian demon" *sensu* Law 1979). Yet no organism is perfect. Species are constrained by ecophysiological tradeoffs. Therefore, natural selection is about strategy optimization, not maximization. But, are some species closer to this hypothetical "Darwinian demon" than currently appreciated?

In this dissertation, I compared plant functional traits and resource-use strategies at several taxonomic and geographic scales. First, in a global dataset, I found evidence to support that species from regions of different evolutionary histories follow different resource-use strategies. Motivated by global differences, I investigated whether invasive plants follow strategy differences from natives to test the hypothesis that some invasive species might exhibit more efficient resource-use strategies. Alternatively, invasive species might be constrained to the same tradeoffs as native species, but merely shifted towards faster growth that trades off with proportionately greater resource demands. To test these ideas, I focused on native and invasive woody species of Eastern North American (ENA) forests. In my second study, I performed an interspecific trait assay in a common garden to compare ENA native and invasive shrubs/lianas. Despite greater leaf nutrient and energy costs, I found that invasive species were more resource-use efficient. Third, I extended this comparison to performance in the field to show that, within and between species, invasive plants maintain physiological advantages across light and nitrogen gradients. Last, I compared the functional strategies of two species from the previous study, in both native and invasive range populations.

Overall, the research in this thesis supports the use of functional traits to understand strategy differences between species. Through trait comparisons in different contexts, I found support that a group of ENA forest invaders show unique advantages over the native species. Further, these invasive species' strategies are not constrained by shared trait tradeoffs with in the native flora. A major finding in this research is that woody invasions into shaded ENA forest understories do not follow the same mechanisms described for ecosystems of high disturbance or resource availability.

The approach of this thesis contributes to the recognition of regional constraints on global plant trait evolution, provides a mechanistic framework for why certain invaders frequently out-compete native floras into particular habitat and resource conditions, and could have broader applications for global assessments of invasion risk. It remains unclear whether the strategies differences reported here are common for other temperate forest invasions worldwide. The potential role of home range pre-adaptation versus properties and processes that occur post-introduction is also understudied. Future work is needed on the biogeography of plant function to address the relative importance of universal tradeoffs versus fundamental differences in resource-use strategies.

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APPENDIX 1. A practical guide to measuring leaf-level photosynthesis

With the development of portable systems for infrared gas analysis over the past decades, the measurement of photosynthetic traits has grown in ecological studies. The conceptual and technical considerations to measure leaf gas exchange can be initially daunting. Here, I provide a practical level of detail that is necessary for meaningful measurement without overwhelming a new user. It can be adapted for the objectives of a given study. Annotated R code for fitting photosynthetic response curves with sample data are also provided. Comments and suggestions are welcome.

General information on gas exchange measurement methods

- Protocol by Evans & Santiago (2010) on Prometheus Wiki found here
- Also see other protocols related to gas exchange on Prometheus Wiki (here), including chlorophyll fluorescence, leaf respiration, temperature response, humidity response, and other lab/field protocols
- Pérez-Harguindeguy *et al*. (2013) handbook of functional plant traits (available here)
- Review of gas exchange measurements by Long & Bernacchi (2003)
- Review of chlorophyll fluorescence by Murchie & Lawson (2013)
- Li-cor 6400 reference manual (available here) with protocol suggestions, general troubleshooting, programs, and maintenance. *Definitely read the first chapter on the equations used and the basics how the system works.* The technical support team is incredibly helpful, too.

Sampling considerations

1. *In situ measurement vs. detached foliage measurement*: If possible, measuring leaf gas exchange on intact plants is best, but given logistical constraints (e.g., field accessibility and power source), it is often necessary to sample twigs (in the lab or field). The good news is that for most woody plants, detached foliage measurements are similar to *in situ*, or in some cases, may be preferred to ensure maximal stomata conductance, which might vary day-to-day under field conditions. This protocol is well described and validated by several studies (e.g., Niinemets *et al*., 2005, Peñuelas *et al*., 2010, Heberling & Fridley, 2013). Twigs are cut from upper branches (early in the morning or when water stress or VPD is low), immediately recut under water (to maintain xylem tension), and brought to lab (preferably within an hour or two). Photosynthetic rates/stomata openness remains high upon initial cutting but then functioning decreases until stabilized in lab. The twigs are recut under water in the lab, stored at low-moderate light conditions, and stored in vases in DI water (or tap). Leaves should be covered with clear plastic to maintain humidity and stomata openness. Water should be changed daily and stems recut under water. After 1-2 days (stabilization period), measurements can be taken.
2. *Leaf cohort/season:* Photosynthesis often changes seasonally (especially in temperate environments) and by plant/leaf age. Standard protocols for most traits are on "recently expanded, mature leaves" that are exposed to the sun (or for understory plants, those leaves most exposed to light). It is also standard to measure at "peak" growing season

(somewhat subjective). If you are limited to measuring at one time point, changes in leaf/plant age and season should be recognized.

3. *Time of day/plant status*: A common concern is mid-day suppression of photosynthesis (or early morning/late day, for that matter). However, some studies randomize their replicate measurements throughout the day, to account for possible error. Also, plant status also affects instantaneous plant metabolic performance. It is not uncommon for a plant or leaf to give questionable data that might be unrepresentative, in which case move on to the next plant and revisit at another time. It happens.

General operating instructions for Li-Cor 6400

The instructions below were developed using Li-Cor 6400 portable photosynthesis system (software OPEN 6.2.3) with the standard chamber (3x2 cm), temperature control module, LED light source and CO_2 mixer to measure broadleaf deciduous shrubs, but the guidelines are similar for other systems and can be configured for needle-leaved taxa.

Machine set-up

- Plug in AC power and 1 battery or if in the field, two batteries
- Flip on power switch
- Highlight “LED lightsource.xml” (*or other configuration*) and press enter
- “Is chamber/IRGA connected” – press “Y” (check everything is connected)
- Press f4 “New Measurements” – system will begin warming up

*Daily preparation checklist (*see Li-Cor manual 4-2 for further explanations)*

During warm up:

1. Install CO_2 cartridge. Use a new o-ring each time to prevent leaks. Cartridge lasts around 8 hours after cartridge is pierced.
2. Check temperatures (display h): block, air, and leaf should be similar and reasonable (within a few °C of each other).
3. Check light source/sensors (display g): external PAR (remove protective cap), ParIn, ParOut
4. Check pressure sensor: should be near 100 kPa or reasonable (display g)
5. Check leaf fan (2 > f1): press letter “O”; listen for fan to stop (confirms that fan was indeed functioning); press 5 to turn back to fast
6. Check flow control: change flow to 1000 μmol s^{-1} (press 2 > f2>T> 1000). Make sure flow is able reach at least 750 μmol s^{-1} (display b). Turn soda lime to full scrub. Flow should only drop 15 μmol s^{-1}. Turn dessicant to full scrub. Flow should only drop another 15 μmol s^{-1}. Turn flow back to 500 (press 2 >f2>T> 500).

After warm up and IRGAs are ready (about 10 min after powering on):

7. Check flow zero: turn off fan (2>f1>O) and pump (2 > f2>O). The flow (display b) should drop to within 1 – 2 μmol s^{-1}.
8. Check CO_2 IRGA zero: Close chamber (adjust screw for seal around gaskets, but not too tight). With mixer off (2 > f3 > N), fan on (2>1>5), and flow at 500 (2 >f2>T> 500), watch CO_2R and CO_2S (display a). Turn soda lime to full scrub and desiccant to full bypass. CO_2R should quickly fall to within 5 ppm of 0 (CO_2S will follow). If not, soda lime may need to be replaced or CO_2 IRGA calibrated.

9. Check H_2O IRGA zero: With turn CO_2R near 0, turn desiccant to full scrub. H_2OR (display a) should fall within 0.3 of mmol mol^{-1} of zero. If not, desiccant may need to be replaced or IRGA calibrated. The desiccant does not seem to last long, but replacement depends on how much humidity control your protocol requires.
10. Check T_{leaf} zero: unplug leaf temperature thermocouple connector (purple). Make sure leaf and block temperatures (display h) are within 0.1 degrees of each other. Replug thermocouple connector. Gently touch thermocouple – T_{leaf} should quickly increase.
11. Turn on mixer (2 >f3>) and set to desired CO_2S or CO_2R (e.g., 350 - 400 ppm). Controlling CO_2R is advantageous (esp. for A/Ci curves) because system can reach set point much quicker, but controlling CO_2S may be preferred in order to keep leaf environment constant.
12. Turn on light source (2>f5>PAR>800) and set to desired PAR (e.g., 800)
13. Check stomata ratio (press 3>f2): 0 for stomata only on one side of leaf, 1 for equal stomata density on each side. (affects stomata conductance parameters)
14. Clamp first leaf of day. Adjust screw so gaskets are sealed snugly around leaf, but not overly tight to affect the leaf. Wait for system to equilibrate (a "*" indicates the system has not yet reached set point for that parameter).
15. Input leaf size (3>f1). Does the leaf fill the chamber? (Area=$6cm^2$) or enter estimate. This user-defined constant can be changed after download, and values recomputed. An initial estimate now is helpful for quality assurance during the measurement. Filling the chamber is desirable for improved accuracy. For small leaves, consider filling the chamber with more than one leaf (but avoiding overlap can be difficult).
16. Match IRGAs (1>f5): should be done throughout day; autoprograms can be set to do before each reading. Definitely match after clamping first leaf of the day and wait to match when system is in equilibrium (i.e., CO_2S and CO_2R reasonably stable).
17. Verify no air leaks: lightly exhale near chamber (don't blow). Watch CO_2S – it should remain fairly stable.
18. Begin measurement protocol.

Power off and storage

- Flip switch to off (no special way to shut down system)
- Unplug battery/power source. Replace protective cap on external par sensor.
- Store with both chemical tube knobs to midway (loose). Make sure foam chamber gaskets are not stored compressed (i.e., chamber open or loosen screw for storage)

Maintenance notes

- Be sure to replace CO_2 mixer filter every 25 cartridges (about every box). Licor cartridges are great, but I also use other brands (e.g., "sport-grade" brands such as Daisy, Gamo) bought at any sporting goods store (*much* cheaper at ~$20 for 25). However, check the mixer filter more often, as they may have more oil/grease than Licor brand cartridges. Licor has cautioned me against using Crosman brand, as they may be oily and clog mixer.
- Keep a log of routine maintenance and other issues.
- Check manual for annual maintenance (air filter replacement, gaskets replaced, etc.). This can quickly get out of hand and can cause bigger issues.
- It is recommended to calibrate at factory every few years (expensive at ~$1000 plus shipping), but this depends on your use and required precision.

Quick approach to measuring carbon assimilation rates

- *Measure saturated photosynthetic rate (A_{sat}) and leaf dark respiration (R_d)*
- *Maximum gross photosynthetic rate (A_{max}) estimated as $A_{sat} + R_d$*
- *Saturating light levels differ by species/individuals (but quick to determine)*
- *Measuring A_{net} at ambient or low light might be more ecologically relevant than A_{max}*

1. Open log file (1>f1). Enter file name (e.g., "date_plantID") and remarks to refer to later if desired.

2. Set starting parameters:

- Turn dessicant knob to midway (*depends on humidity*) and soda lime to full scrub
- PAR = 1000 µmol m^{-2} s^{-1} (2>f5>PAR>T>1000) (*this might cause photoinhibition for shade plants with low photosynthetic rates; if so, acclimate more slowly*)
- CO2S = 380 µmol mol^{-1} (wait for machine to reach before clamping first leaf) (2>f3>Sample CO2>Target>380) (*assuming 380 ppm is ambient*)
- Flow = 700 µmol s^{-1} (press 2 >f2>T>700) (*or 500 or lower in dry environments*)
- Temp (leaf) = 25º C (press 2 > f4>>Leaf Temperature> T>25) (*or ambient*)
- Stomata ratio: generally 0 (stomata on one side only; 1 for equal stomata density on each side) (press 3>f2>0)

3. Clamp leaf

- choose young BUT fully expanded, mature leaf
- ensure a good seal around leaf (need not be too tight; tighten/loosen screw as needed)
- leaf chamber is filled = 6 cm^2 (or for smaller leaves, change area: 3>f1)
- leaf not wrinkled and clean of debris

4. Define stability (5>f4>labels>f4 >Typical (*or your definition*) >labels>f5)

- Stability parameters can help you systematically decide when to log data (or when autoprogram logs).
- *Define stability that works for your application. Examples of common stability definitions: Photo and Conductance (mean or coefficient of variation less than set threshold), DCO2 (difference between sample and reference chamber CO2 coefficient of variation <0.3% or <1%).*

5. Adjust PAR if needed (2>f5>PAR>T>enterNewValue) to ensure photosynthesis is saturated (without photoinhibition, where photo declines at high light).

6. Log point when reasonably stable and leaf fully induced/acclimated to light (wait *at least* 5 minutes).

- Track stability by pressing "[" and for other graphs "]"
- Manually log data point (1>f1 hear beep)

7. To measure R_d, repeat steps 2-6 above but with light source shut off (2>f5>O). Stabilization might take a while, if leaf was not adapted to dark conditions. Measuring dark respiration with confidence can be a challenge. Given the low rates (and potentially high relative error), matching is essential before logging.

Light response curves (A/*q*): measurement

CO_2 concentration is kept constant and irradiance (PAR) is varied to measure the response of photosynthesis (carbon assimilation; Photo; A). There are a few approaches and schools of thought. You can either start at high PAR and decrease PAR (generally quicker response times for leaf to be induced to light) OR start at low PAR and slowly increase PAR (slower stabilization times but has advantages). Acclimation of the leaf to high light (photoinduction) is critical before logging. On the other hand, it is also important to avoid high light stress (photoinhibition). Data are logged when photosynthetic stability is reached after a waiting period at a given PAR. Licor manual nicely outlines different approaches and additional considerations.

1. Set starting parameters:
- Turn dessicant knob to midway (*depends on humidity*) and soda lime to full scrub
- PAR = 1000 µmol m^{-2} s^{-1} (2>f5>PAR>T>1000) (*this might cause photoinhibition for shade plants with low photosynthetic rates; if so, acclimate more slowly*)
- CO2S = 380 µmol mol^{-1} (wait for machine to reach before clamping first leaf) (2>f3>Sample CO2>Target>380) (*assuming 380 ppm is ambient*)
- Flow = 700 µmol s^{-1} (press 2 >f2>T>700) (*or 500 or lower in dry environments*)
- Temp (leaf) = 25º C (press 2 > f4>Leaf Temperature> T>25) (*or ambient*)
- Stomata ratio: generally 0 (abaxial stomata only; 1 for equal stomata density on each side) (press 3>f2>0)

2. Clamp leaf
- choose young BUT fully expanded, mature leaf
- ensure a good seal around leaf
- leaf should not be clamped too tightly (tighten/loosen screw as needed)
- leaf chamber is filled = 6 cm^2 (or for smaller leaves, change area: 3>f1)
- leaf not wrinkled and clean of debris

3. Define stability (5>f4>labels>f4 >Typical (*or other definition*) >labels>f5)
- Stability parameters can help you systematically decide when to log data (or when autoprogram logs). *Define stability that works for your application.*
- *Examples of common stability definitions: Photo and Conductance (mean or coefficient of variation less than set threshold), DCO2 (difference between sample and reference chamber CO2 coefficient of variation <0.3% or <1%).*

4. Wait to allow leaf to begin to equilibrate (few min)
- press "[" to monitor stability and "]" for real time graphs
- Make sure in line j, machine components are "OK" and fan reads "Fast"

5. Open program
a. Press 5>f1>LightCurve2
b. Name file (date_plantID)
c. Enter remarks or if none, just hit enter

d. Set PAR lamp settings (for a “good” curve, 10 steps is reasonable; for a “great” curve, consider more)
   example: “800 1000 1300 1000 800 500 300 200 100 50 20 0”
e. Min wait time = 120s and press enter
f. Max wait time = 5000 s and press enter (will not log values until stability is reached)
g. Set when to match: e.g., “If one of…” elapsed time >30min,CO2 change >100, ΔCO2<10
h. Is stability definition ok? Press enter (*or change stability definition*)

6. Run: program will run automatically. There is no need to constantly watch, but checking for reasonable values throughout the program would be a good idea. If curve is irregular for some reason, abort program (press escape > A), and start again on new leaf or switch plants. Press k to monitor how much time is left. Press 1>f2>importGraph>lightcurve to view logged points.

Light response curves (A/*q*): parameter estimation

See annotated R code (page 14) with sample data (“sample_lrc.txt”).

Light response curve parameters can be estimated through non-linear least squares regression of a non-rectangular hyperbola (Marshall & Biscoe, 1980):

$$A_{net} = \frac{\phi PPFD + \sqrt{(\phi PPFD + A_{\max})^2 - 4\theta\phi PPFD + A_{\max}}}{2\theta} - R_d$$

where A_{net} and A_{max} are the area-based net and maximum gross photosynthetic rates (µmol CO_2 m^{-2} s^{-1}), respectively, ϕ is the apparent quantum yield (mol CO_2 mol photons^{-1}), R_d is daytime dark respiration rate ($|A_{net}|$ at no light; µmol CO_2 m^{-2} s^{-1}), and θ is curve convexity (dimensionless).

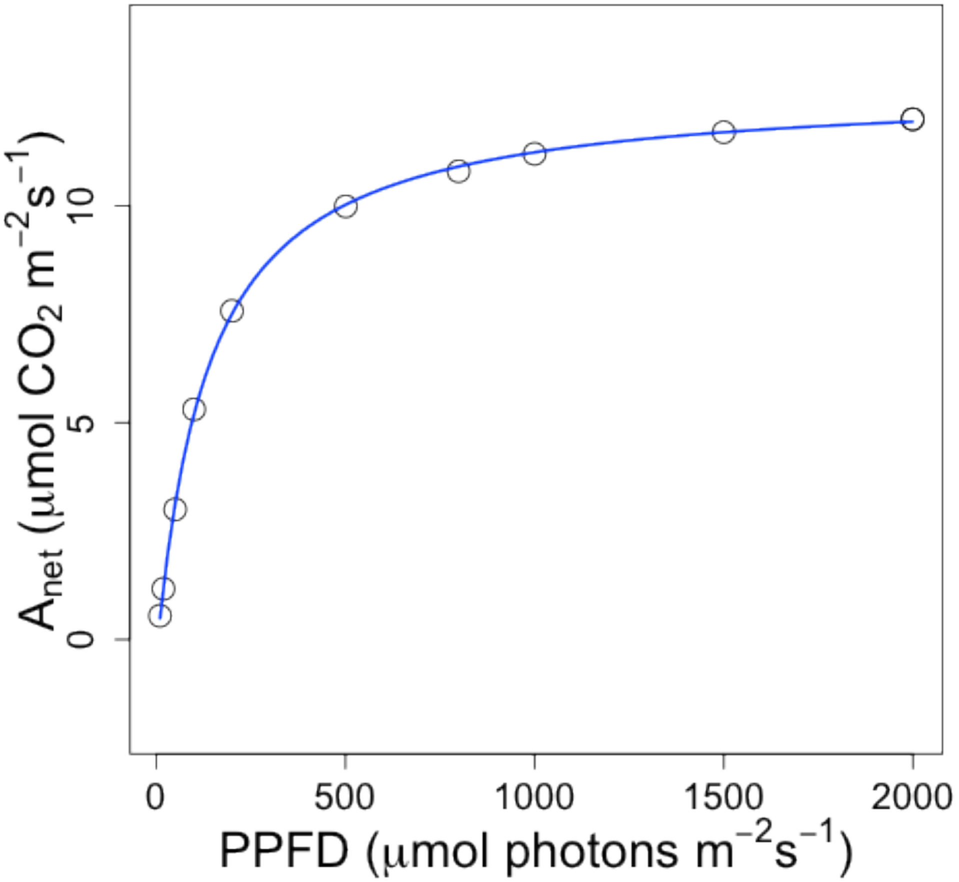

Figure A1.1. *Sample A/Q data with fitted curve. Fitted parameter values (± 1 se):*
A_{max} = 13.13 ± 0.20 µmol CO_2 m^{-2} s^{-1})
AQY = 0.0943 ± 0.0087 mol CO_2 $photons^{-1}$
R_d = 0.39 ± 0.13 µmol CO_2 m^{-2} s^{-1}
θ = 0.084 ± 0.132

Leaf respiration: some considerations

Measuring leaf respiration (R_d) can be conceptually and technically troubling. Respiration can be suppressed in the light (Kok effect; Kok 1948). R_d can also be affected by photosynthate supply, with some models assuming night respiration to be 75% of respiration during the day (e.g., Williams *et al*., 1989). "Day" respiration usually refers to mitochondrial respiration in the light (i.e., excluding photorespiration). Like $A_{max,}$ which erroneously is often used synonymously with A_{sat} or A_{net}, R_d in the literature may not always refer to the same measure. Leaves can be measured as described above using dark-adapted leaves. Leaves can be detached and placed in the darkness in a bag and measured in 1 hour (e.g., Reich *et al*., 1998). Respiration in the light versus dark can also be estimated through curve fitting ("Kok Method," e.g., Nagel & Griffin, 2004; Atkin *et al*., 2013) or other methods (see Heskel *et al*., 2013), which might differ from empirical measurements. I have also measured leaf respiration at 2 am using a black light so I can see (but mostly outside the range of PAR). The methods will depend on what aspect of leaf respiration is being measured. Methods of R_d estimation (curve fitting, empirical measurement during day/night) can be different enough to warrant caution if R_d is a trait of focused interest.

Leaf acclimation, frequent matching and IRGA calibration is a must, given accuracy issues related to such low R_d for many plants (e.g., shade tolerant shrubs often < 1 µmol CO_2 m^{-2} s^{-1}). See Atkin *et al.* (2015) for a comprehensive summary of R_d across taxa and environments.

Carbon dioxide response curves: measurement

Light level is kept constant and CO_2 concentrations are varied to measure the response of photosynthesis (carbon assimilation; A) to changing intercellular CO_2 concentrations (C_i; µmol *CO_2* mol air^{-1}*). If you want C_i measured as a partial pressure (Pa), you will need to program for "Ci_Pa" to log.*

1. Set starting parameters:

- Turn dessicant knob to midway (*depends on humidity*) and soda lime to full scrub
- PAR = 1000 µmol m^{-2} s^{-1} (2>f5>PAR>T>1000) (*acclimate to whatever is saturating)*
- CO2R = 400 µmol mol^{-1} (wait for machine to reach before clamping first leaf) (2>f3>Reference CO2>Target>400)

NOTE: Targeting reference chamber CO2 rather than sample CO2 allows machine to reach targeted CO2 concentrations more quickly (reference chamber is smaller than sample chamber). However, CO2S can instead be targeted, but equilibration takes longer.

- Flow = 500 µmol s^{-1} (press 2 >f2>T>500) (*or different*) (faster flow rates = faster equilibration; however, high flow rates may decrease humidity)
- Temp (leaf) = 25° C (press 2 > f4>Leaf Temperature> T>25) (*or ambient*)
- Stomata ratio: generally 0 (abaxial stomata only; 1 for equal stomata density on each side) (press 3>f2>0)

2. Clamp leaf

- choose young BUT fully expanded, mature leaf
- ensure a good seal around leaf
- leaf should not be clamped too tightly (tighten/loosen screw as needed)
- leaf chamber is filled = 6 cm^2 (or for smaller leaves, change area: 3>f1)
- leaf not wrinkled and clean of debris

3. Define stability (5>f4>labels>f4 >Typical (*or your definition*) >labels>f5)

- Stability parameters can help you systematically decide when to log data (or when autoprogram logs). *Define stability that works for your application.*
- *Examples of common stability definitions: Photo and Conductance (mean or coefficient of variation less than set threshold), DCO2 (difference between sample and reference chamber CO2 coefficient of variation <0.3% or <1%).*

4. Wait to allow leaf to equilibrate to starting conditions (few min)

- press "[" to monitor stability).
- Make sure in line j, machine components are "OK" and fan reads "Fast"

5. Open program

a. Press 5>f1>A-CiCurve2
   Name file (date_plantID)

b. Enter remarks or if none, just hit enter
c. Set CO2R settings (for a "good" curve, 8-10 steps is reasonable; for a "great" curve, consider more, especially at lower CO2) (I prefer to start at ambient, decrease, allow leaf to equilibrate at ambient, and then increase – there is a nice description of this in the Licor manual)
   example: "400 300 200 100 50 400 400 600 800 1000"
d. Min wait time = 60s and press enter
e. Max wait time = 120 s and press enter (log even if stability is not reached)
f. Set when to match: Match before= "always"
g. Is stability definition ok? Press enter (*or change stability definition)*

6. Run: program will run automatically. There is no need to constantly watch, but checking for reasonable values throughout the program would be a good idea. If curve is irregular for some reason, abort program (press escape > A), and start again on new leaf or switch plants. Press k to monitor how much time is left. Press 1>f2>importGraph>C-Ci to view logged points.

Carbon dioxide response curves: parameter estimation

See annotated R code (below) with sample data ("sample_aci.txt").
Classic model developed by Farquhar et al. (1980).

There are several approaches to fitting carbon dioxide response curves. A brief outline of the model and fitting techniques are included in the annotated R code at the end of this protocol. Additional perspective can be found in Dubois *et al.* (2007), Sharkey *et al.* (2007), Miao *et al.* (2009), and Niinemets *et al*. (2009). A succinct overview of the model and fitting techniques can be found in Diaz-Espejo *et al.* (2012).

The original Farquhar-von Caemmerer-Berry (1980) model was developed for C_c (chloroplastic CO_2 concentration) not C_i (intercellular CO2 concentration). C_i equals C_c, assuming mesophyll conductance (g_m) is infinite. This might be an unfair assumption in many cases. See reviews on estimating mesophyll conductance and the effect on A/C_c versus A/C_i modeling (e.g., Niinemets *et al*., 2009; Flexas *et al.*, 2013).

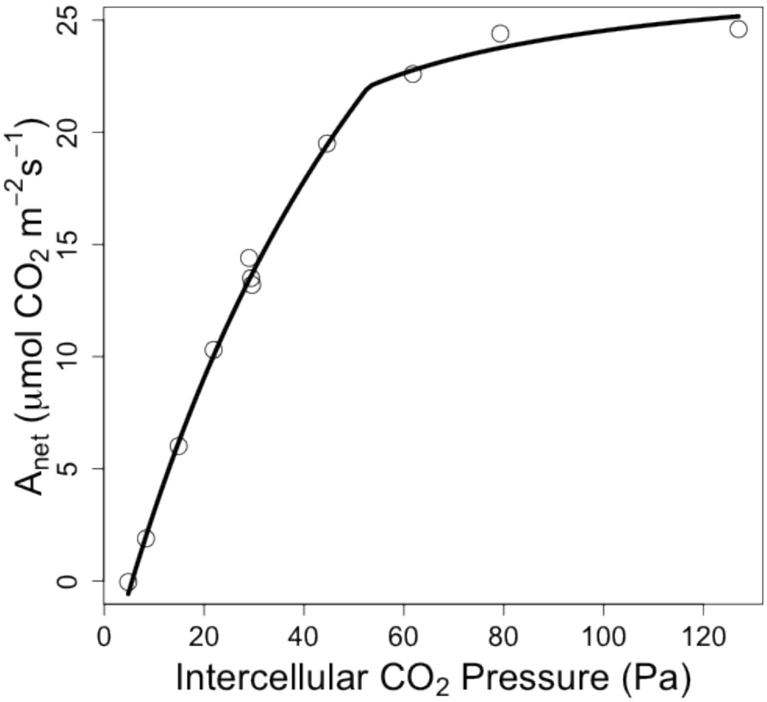

Figure A1.2. *Sample A/Ci data with fitted curve. CO2R varied with light held at constant at saturating light level. Fitted parameter values (± 1 se):*

R_d = 1.18 ± 0.33 (mitochondrial "day" respiration; μmol CO_2 m^{-2} s^{-1})
$V_{c,max}$ = 60.51 ± 1.55 (max carboxylation rate; μmol CO_2 m^{-2} s^{-1})
J_{max} = 115.73 ± 1.93 (electron transport rate; μmol electrons m^{-2} s^{-1})
TPU not fit here.

Other considerations: area- vs. mass-based values

Another aspect to consider is the interpretation of the photosynthetic data (and other traits) on the basis of leaf mass vs. leaf area. For example, $A_{net,area}$ (raw data from gas exchange measurement) can be converted to $A_{net,mass}$ through SLA (specific leaf area, protocol for SLA here). Mass based measures tend to be correlated more tightly in the leaf economics spectrum (e.g., Wright *et al*., 2004). There has recently been some discussion on the ecological significance of area vs. mass basis (e.g., Lloyd *et al*., 2013; Osnas *et al*., 2013; Westoby *et al*., 2013).

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Annotated R code for fitting photosynthetic light response curves

```
# November 2013, JM Heberling (jmheberling@gmail.com)
# Modeling light response curves (aka A/Q or LRC)
# Model of Marshall & Biscoe (1980) J Exp Bot 31:29-39

# See Lachapelle & Shipley (2012) Ann Bot 109: 1149-1157 for an
 alternative
# Amax can alternatively be estimated as Amax (max gross photo) = Asat
 (Anet at saturating light) + Rd (Anet at PAR = 0)
#---

 # Read in text file from Licor 6400
lrc<- read.csv("/YOURDIRECTORYHERE/sample_lrc.txt",sep="",skip=16)

#lrc<na.omit(lrc) #remove lines with remarks (*check notebook/file for
 comments)


# ---Inspect and graph raw data (A vs. PPFD) ---

PARlrc<-lrc$PARi #PAR (aka PPFD or Q)
photolrc<-lrc$Photo #net photosynthetic rate (Anet)

curvelrc<-data.frame(PARlrc,photolrc)
curvelrc # *inspect raw data and check notebook (data reasonable or
 need edited/discarded?)

par(mar=c(3,3,0,0),oma=c(1.5,1.5,1,1))
plot(PARlrc,photolrc,xlab="", ylab="", ylim=c(-
 2,max(photolrc)+2),cex.lab=1.2,cex.axis=1.5,cex=2)
mtext(expression("PPFD ("*mu*"mol photons "*m^-2*s^-
 1*")"),side=1,line=3.3,cex=1.5)
mtext(expression(A[net]*" ("*mu*"mol "*CO[2]*" "*m^-2*s^-
 1*")"),side=2,line=2.5,cex=1.5)

# --- Nonlinear least squares regression (non-rectangular hyperbola).
 4 parameter model: Amax (max gross photosytnthetic rate), Rd (dark
 respiration), AQY (apparent quantum yield), Theta (curvature
 parameter, dimensionless) ---
# Another option is to fit AQY (initial slope) and Rd (y-intercept)
 separately using linear regression on data points that are not light-
 saturated, then use those model fits in the non-linear model to
 parameterize Amax and the curve parameter (theta). However, this
 requires user to subjectively decide which points are not light
 saturated (initial linear portion of curve).
# For more or Rd estimation see protocol text.
# Depending on data, quantile regression can be implemented through
 nlrq()
```

```
curve.nlslrc = nls(photolrc ~ (1/(2*theta))*(AQY*PARlrc+Am-
 sqrt((AQY*PARlrc+Am)^2-4*AQY*theta*Am*PARlrc))-
 Rd,start=list(Am=(max(photolrc)-min(photolrc)),AQY=0.05,Rd=-
 min(photolrc),theta=1))

summary(curve.nlslrc) #summary of model fit

# ---Graph raw data with modeled curve---

par(mar=c(3,3,0,0),oma=c(1.5,1.5,1,1))
plot(PARlrc,photolrc,xlab="", ylab="", ylim=c(-
 2,max(photolrc)+2),cex.lab=1.2,cex.axis=1.5,cex=2)
mtext(expression("PPFD ("*mu*"mol photons "*m^-2*s^-
 1*")"),side=1,line=3.3,cex=2)
mtext(expression(A[net]*" ("*mu*"mol "*CO[2]*" "*m^-2*s^-
 1*")"),side=2,line=2,cex=2)
curve((1/(2*summary(curve.nlslrc)$coef[4,1]))*(summary(curve.nlslrc)$c
 oef[2,1]*x+summary(curve.nlslrc)$coef[1,1]-
 sqrt((summary(curve.nlslrc)$coef[2,1]*x+summary(curve.nlslrc)$coef[1,1
 ])^2-
 4*summary(curve.nlslrc)$coef[2,1]*summary(curve.nlslrc)$coef[4,1]*summ
 ary(curve.nlslrc)$coef[1,1]*x))-
 summary(curve.nlslrc)$coef[3,1],lwd=2,col="blue",add=T)

# ---Solve for light compensation point (LCPT), PPFD where Anet=0 ---
x<-function(x)
 {(1/(2*summary(curve.nlslrc)$coef[4,1]))*(summary(curve.nlslrc)$coef[2
 ,1]*x+summary(curve.nlslrc)$coef[1,1]-
 sqrt((summary(curve.nlslrc)$coef[2,1]*x+summary(curve.nlslrc)$coef[1,1
 ])^2-
 4*summary(curve.nlslrc)$coef[2,1]*summary(curve.nlslrc)$coef[4,1]*summ
 ary(curve.nlslrc)$coef[1,1]*x))-summary(curve.nlslrc)$coef[3,1]}

uniroot(x,c(0,50))$root #LCPT

# ---Solve for light saturation point (LSP), PPFD where 75% of Amax is
 achieved (75% is arbitrary - cutoff could be changed)
x<-function(x)
 {(1/(2*summary(curve.nlslrc)$coef[4,1]))*(summary(curve.nlslrc)$coef[2
 ,1]*x+summary(curve.nlslrc)$coef[1,1]-
 sqrt((summary(curve.nlslrc)$coef[2,1]*x+summary(curve.nlslrc)$coef[1,1
 ])^2-
 4*summary(curve.nlslrc)$coef[2,1]*summary(curve.nlslrc)$coef[4,1]*summ
 ary(curve.nlslrc)$coef[1,1]*x))-summary(curve.nlslrc)$coef[3,1]-
 (0.75*summary(curve.nlslrc)$coef[1,1])+0.75*(summary(curve.nlslrc)$coe
 f[3,1])}

uniroot(x,c(0,1000))$root #LSP
```

Annotated R code for fitting photosynthetic CO_2 response curves

```
# November 2013, JM Heberling (jmheberling@gmail.com)
# Modeling CO2 response curves (A/Ci)
# Farquhar-von Caemmerer-Berry (FvCB) model (1980) Planta 149:78-90
# Simultaneous estimation detailed in Dubois et al. (2007) New Phyt
 176:402-414
#---

 # Read in text file from Licor 6400
aci<- read.csv("/YOURDIRECTORYHERE/sample_aci.txt", sep="",skip=16)

aci<-na.omit(aci) #remove lines with remarks (*check notebook/file for
 comments)

# ---Inspect and graph raw data (A vs. Ci) ---

Ci<-aci$Ci # Ci (ppm)
Ci_Pa<-aci$Ci_Pa # Ci (Pa)
Photo<-aci$Photo #Anet - net photosynthetic rate
CO2R<-aci$CO2R

TotalCurveData<-data.frame(Ci, CO2R, Photo, Ci_Pa)
TotalCurveData # *inspect raw data and check notebook (data reasonable
 or need edited/discarded?)

par(mar=c(3,3,0,0),oma=c(1.5,1.5,1,1))
plot(Ci_Pa,Photo,ylab="", xlab="",cex.lab=1.2,cex.axis=1.5,cex=2)
mtext(expression("Intercellular "*CO[2]*" Pressure
 (Pa)"),side=1,line=3.3,cex=1.5)
mtext(expression(A[net]*" ("*mu*"mol "*CO[2]*" "*m^-2*s^-
 1*")"),side=2,line=2.5,cex=1.5)

# ---Temperature adjusted coefficients:

# Constants published in Sharkey et al (2007) Plant Cell Env 30: 1035-
 1040
# Measured using transgenic tobacco (ASSUMED to be similar across
 higher plants)
# Ci units in Pa; Sharkey et al (2007) recommend partial pressures
# **Be sure units are correct for your input data** (Ci is in Pa or
 ppm?)

R=0.008314 #(kJ mol^-1 K^-1)
aci$Kc=exp(35.9774-80.99/(R*(aci$Tleaf+273.15))) #Michaelis-Menten
 constant for Rubisco for O2 (Pa)
aci$Ko=exp(12.3772-23.72/(R*(aci$Tleaf+273.15))) #Michaelis-Menten
 constant for Rubisco for CO2 (kPa)
aci$GammaStar=exp(11.187-24.46/(R*(aci$Tleaf+273.15)))
 #Photorespiration compensation point (Pa)
O=21 #oxygen (O2) partial pressure (kPa)
```

```
# Alternative constants from Bernacchi et al. (2001) Plant Cell Env
 24: 253-259
# Ci units in ppm (but can be converted to Pa by atmospheric pressure)
#R=0.008314 #(kJ mol^-1 K^-1)
#aci$Kc=exp(38.05-79.43/(R*(aci$Tleaf+273.15))) #umol mol-1
#aci$Ko=exp(20.30-36.38/(R*(aci$Tleaf+273.15))) #mmol mol-1
#aci$GammaStar=exp(19.02-37.83/(R*(aci$Tleaf+273.15))) #umol mol-1
#O=210

# ---RuBisCO limited portion---
#(Vcmax*(Ci_Pa-GammaStar))/(Ci_Pa+(Kc*(1+(O/Ko)))))-Rd

# ---RUBP limited portion---
#((J*(Ci_Pa-GammaStar))/((4*Ci_Pa)+(8*GammaStar)))-Rd

# ---TPU.limited portion
#3*TPU-Rd
# Few studies find triose phosphate limitation (under natural
 conditions), but could easily be added to model below to test (but
 with loss of statistical power); it is often not considered, but
 depends on the dataset
# Miao et al (2009) Plant, Cell, Env 32:109-122 recommend fitting when
 possible or removing TPU limited points from dataset before fitting



# Simultaneous estimation method described by Dubois et al. 2007 New
 Phyt 176:402-414
# Could change optimization algorithm (default here is Gauss-Newton)
# Could also do a "grid search" if estimates are sensitive to starting
 values

aci.fit<-nls(Photo~ifelse(((Vcmax*(Ci_Pa-
 GammaStar))/(Ci_Pa+(Kc*(1+(O/Ko)))))<((J*(Ci_Pa-
 GammaStar))/((4*Ci_Pa)+(8*GammaStar))),((Vcmax*(Ci_Pa-
 GammaStar))/(Ci_Pa+(Kc*(1+(O/Ko))))),((J*(Ci_Pa-
 GammaStar))/((4*Ci_Pa)+(8*GammaStar))))-
 Rd,start=list(Vcmax=50,J=100,Rd=0.5),data=aci) #if error: reconsider
 starting values, bad dataset? (too few points or response curve not
 clear)

summary(aci.fit)

Vcmax<-summary(aci.fit)$coef[1,1]
J<-summary(aci.fit)$coef[2,1]
Rd<-summary(aci.fit)$coef[3,1]

# ---Graph raw data with modeled curve---

par(mar=c(3,3,0,0),oma=c(1.5,1.5,1,1))
plot(Ci_Pa,Photo,ylab="", xlab="",cex.lab=1.2,cex.axis=1.5,cex=2)
```

```
mtext(expression("Intercellular "*CO[2]*" Pressure
 (Pa)"),side=1,line=3.3,cex=1.5)
mtext(expression("Net photosynthetic rate  (umol  "* CO[2]*   m^-2*
 s^-1*")"),side=2,line=2.5,cex=1.5)
curve(ifelse(((Vcmax*(x-
 mean(aci$GammaStar)))/(x+(mean(aci$Kc)*(1+(O/mean(aci$Ko))))))<((J*(x-
 mean(aci$GammaStar)))/((4*x)+(8*mean(aci$GammaStar)))),((Vcmax*(x-
 mean(aci$GammaStar)))/(x+(mean(aci$Kc)*(1+(O/mean(aci$Ko)))))),((J*(x-
 mean(aci$GammaStar)))/((4*x)+(8*mean(aci$GammaStar)))))-Rd,add=T)
 #Reasonable fit? Could check goodness of fit, model assumptions

# ---Frequently used alternative: "disjunct segment estimation" (sensu
 Dubois et al 2007)---
# See Sharkey et al (2007) Plant Cell Env 30: 1035-1040
# This method entails selecting which points on the curve are limited
 by rubisco, RUBP, or TPU a priori (or subjectively). The data is
 subsetted by these cutoff points and each segment modeled separately.


# ---Considering mesophyll conductance (gm) ---

# The original Farquhar-von Caemmerer-Berry (1980) model was developed
 for Cc (chlorplastic CO2 concentration) not Ci (intercellular CO2
 concentration).  Ci equals Cc, assuming mesophyll conductance (gm) is
 infinite, which might be an unfair assumption in many cases.  See
 reviews on estimating mesophyll conductance and the effect on A/Cc
 versus A/Ci modeling (eg.,  Niinemets et al, 2009 J Exp Bot 60:2271-
 2282; Flexas et al., 2013 J Exp Bot 64:3965-3981)

# Cc = Ci - A/gm

# ---Revised model with gm as a fitted parameter (modified "Ethier
 method") ---
#  See Ethier & Livingston (2004) Plant, Cell, Env 27:137-153
#  Some authors highlight the need to fit gm (e.g., Niinemets et al,
 2009 J Exp Bot 60:2271-2282) while others suggest gm estimation based
 on gas exchange data curve fitting alone is not preferred (Pons et al.
 2009 J Exp Bot 60:2217-2234)
#  Techniques for measuring gm are still under development (see Flexas
 et al., 2013 J Exp Bot 64:3965-3981)
#  Ideally, gm would be independently measured and Cc would be known

aci.fit<-nls(Photo~ifelse(((Vcmax*((Ci_Pa-(Photo*gmInv))-
 GammaStar))/((Ci_Pa-(Photo*gmInv))+(Kc*(1+(O/Ko)))))<((J*((Ci_Pa-
 (Photo*gmInv))-GammaStar))/((4*(Ci_Pa-
 (Photo*gmInv)))+(8*GammaStar))),((Vcmax*((Ci_Pa-(Photo*gmInv))-
 GammaStar))/((Ci_Pa-(Photo*gmInv))+(Kc*(1+(O/Ko))))),((J*((Ci_Pa-
 (Photo*gmInv))-GammaStar))/((4*(Ci_Pa-
 (Photo*gmInv)))+(8*GammaStar))))-
 Rd,start=list(Vcmax=50,J=100,Rd=0.5,gmInv=0),data=aci) #gm cannot be
 negative; gm=1/gmInv; fitting gm can be statistically difficult for
 many datasets
```

```
# ---Other notes: ---
# Miao et al (2009) Plant, Cell, Env 32:109-122 review A/Cc fitting
 methods and recommend fitting full model (including gm and TPU)
 combining grid search and two stage nonlinear least square regression
# Dubois et al (2007) do not fit gm and warn it may not be reliable.
 However, if decided to model, they suggest fitting 1/gm if you do
 since gm can approach zero; They also suggest using a grid search to
 avoid local minima (sensitivity to initial starting values).  I have
 not found this to be an issue, but a grid search could be incorporated
 in this code.

#see Appendix 2 for code for Bayesian implementation of C3
 photosythesis model
```

APPENDIX 2.

Annotated R Code for Bayesian implementation of FvCB C_3 photosynthesis model

The core script below was used for photosynthetic models presented in Chapter 3 (see Methods and references therein) and represents an alternative to maximum likelihood approaches fitting curve-by-curve (Appendix 1). It was based on Feng & Dietze (2013) with motivating elements from Patrick *et al*. (2009) and Peltier & Ibáñez (2015). The model structure can be altered to add in effects of different traits or site environment, modify the random effects structure, update the prior distributions, treat particular parameters as constants derived from the plant physiology literature (*e.g.*, CO_2 compensation point), and/or model new parameters (*e.g.*, mesophyll conductance, Rubisco kinetic parameters; Patrick *et al*., 2009).

```
mod <- "model
{
#Classic FvCB C3 photo model (1980), excluding TPU limitation

#Priors
Vcmax.int ~ dnorm(25,0.001)  #Peltier&Ibanez (Table 1)but bounded by
 0: dnorm(25,0.001) I(0, ); values currently allowed to be negative
Jmax.int ~ dnorm(55,0.001)  #Peltier&Ibanzez (Table 1)but bounded by
 zero: dnorm(55,0.001) I(0, )
Rd.int ~ dnorm(0,0.001) #Peltier&Ibanzez (Table 1) dnorm(0,1) I(0, )
GammaStar.int ~ dnorm(4.275,10) #informative, from Patrick et al; or
 treat as constant (comment out) or dnorm(3.74,10) or dnorm(3.86,10)
 depending upon treatment of M-M kinetic coefficients
alpha.int ~ dnorm(0.24,100) #strong prior, low variation across
 species - Feng & Dietze 2013: dnorm(0.24,100)

#fixed effect flat priors (non-informative)
beta.N ~ dnorm(0,0.0001) #leaf N effect on Vcmax
beta.SLA ~ dnorm(0,0.0001) #SLA effect on alpha
#beta.chl ~ dnorm(0,0.0001) #chl effect on alpha

tau <- sigma^-2 #convert SD to precision (1/variance)
sigma ~ dunif(0, 100) #uniform prior for normal SD

# ind.tau.Gstar <- ind.sigma.Gstar^-2 #if including individual RE for
```

```
 Gstar
# ind.sigma.Gstar ~ dunif(0, 100)
spp.tau.Gstar <- spp.sigma.Gstar^-2
spp.sigma.Gstar ~ dunif(0, 100)

# ind.tau.Rd <- ind.sigma.Rd^-2 #if including REs for Rd
# ind.sigma.Rd ~ dunif(0, 100)
# spp.tau.Rd <- spp.sigma.Rd^-2
# spp.sigma.Rd ~ dunif(0, 100)

ind.tau.Vcmax <- ind.sigma.Vcmax^-2
ind.sigma.Vcmax ~ dunif(0, 100)
spp.tau.Vcmax <- spp.sigma.Vcmax^-2
spp.sigma.Vcmax ~ dunif(0, 100)

ind.tau.Jmax <- ind.sigma.Jmax^-2
ind.sigma.Jmax ~ dunif(0, 100)
spp.tau.Jmax <- spp.sigma.Jmax^-2
spp.sigma.Jmax ~ dunif(0, 100)

# ind.tau.alpha <- ind.sigma.alpha^-2 #if including REs for Rd
# ind.sigma.alpha ~ dunif(0, 100)
# spp.tau.alpha <- spp.sigma.alpha^-2
# spp.sigma.alpha ~ dunif(0, 100)


for(i in 1:N) { #loop through observations (A/Ci and A/q data)

 Anet[i] ~ dnorm(An[i],tau) #Anet is the observed response, which takes
 on the predicted value An plus normal error

 An[i] <- min(Av[i],Aj[i]) - Rd[i] #An = min of two functions

 Rd[i]<- Rd.int#+ b.spp.Rd[spnumber[i]]+ b.ind.Rd[indiv[i]]#could add
 in random effects terms here

 Av[i] <- ((Vcmax[i]*(Ci_Pa[i]-
 GammaStar[i]))/(Ci_Pa[i]+(Kc[i]*(1+(O/Ko[i]))))) #Rubisco limited
 photosynthesis (early part of curve)

 Vcmax[i] <-
 Vcmax.int+b0.spp.Vcmax1[spnumber[i]]+b0.ind.Vcmax1[indiv[i]]+beta.N*(l
 eafNarea[plot_sp[i]])

#Electron transport limited (latter part of curve)
 Aj[i] <- ((J[i]*(Ci_Pa[i]-
 GammaStar[i]))/((4*Ci_Pa[i])+(8*GammaStar[i])))

#J light dependency according to Tenhunen et al 1976

 J[i]<-
 (alpha[i]*q[i]/(sqrt(1+(alpha[i]*alpha[i]*q[i]*q[i])/(Jmax[i]*Jmax[i])
```

```
)))

#could include Jmax-trait submodel; Feng&Dietze (2013) do alpha-trait
submodel

Jmax[i]<-
Jmax.int+b0.ind.Jmax[indiv[i]]+b0.spp.Jmax[spnumber[i]]#+beta.SLA*(SLA
[i])

alpha[i]<-
alpha.int+beta.SLA*(SLA[i])#+b0.ind.alpha[indiv[i]]+b0.spp.alpha[spnum
ber[i]]#+beta.chl*(chl[i])#
##+beta.Thick*(Thick[i]) #could include other leaf structural traits
or leaf chlorophyll

GammaStar[i]<-GammaStar.int+b.spp.Gstar[spnumber[i]] #with species RE

#get posteriors for Amax, defined at ambient Ci, saturating light
Amax[i] <- min(((Jmax[i]*(40-
GammaStar[i]))/((4*40)+(8*GammaStar[i]))),Vcmax[i]*(40-
GammaStar[i])/(40+(40.49*(1+(O/27.84)))))#at 40 Ci_Pa (ambient)
    } #end  loop

    # #random intercept for individual effect on Rd
    # for(i in 1:N.indiv) {
    # b.ind.Rd[i] ~ dnorm(0,ind.tau.Rd)
    # }
    # #random intercept for sp effect on Rd
    # for(i in 1:N.spp) {
    # b.spp.Rd[i] ~ dnorm(0,spp.tau.Rd)
    # }

    # #random intercept for individual effect on Gstar
    # for(i in 1:N.indiv) {
    # b.ind.Gstar[i] ~ dnorm(0,ind.tau.Gstar)
    # }

    #random intercept for sp effect on Gstar
    for(i in 1:N.spp) {
    b.spp.Gstar[i] ~ dnorm(0,spp.tau.Gstar)
    }


#random intercept for plot (individual effect on Vcmax)
  for(i in 1:N.indiv) {
    b0.ind.Vcmax1[i] ~ dnorm(0,ind.tau.Vcmax)
    }
#random intercept (spp effect on Vcmax)
  for(i in 1:N.spp) {
    b0.spp.Vcmax1[i] ~ dnorm(0,spp.tau.Vcmax)
    }
    #random intercept for plot (individual effect on Jmax)
```

```
  for(i in 1:N.indiv) {
    b0.ind.Jmax[i] ~ dnorm(0,ind.tau.Jmax)
   }
      #random intercept (spp effect on Jmax)
  for(i in 1:N.spp) {
    b0.spp.Jmax[i] ~ dnorm(0,spp.tau.Jmax)
   }
    #random intercept for plot (individual effect on alpha)
  # for(i in 1:N.indiv) {
    # b0.ind.alpha[i] ~ dnorm(0,ind.tau.alpha)
   # }
     # # #random intercept (spp effect on alpha)
  # for(i in 1:N.spp) {
    # b0.spp.alpha[i] ~ dnorm(0,spp.tau.alpha)
   # }

#output will include overall Amax posterior based on fitted intercepts
 at ambient Ci
Amax.int <-min(((Jmax.int*(40-
 4.275))/((4*40)+(8*4.275))),Vcmax.int*(40-
 4.275)/(40+(40.49*(1+(O/27.84)))))


}" #end model
```

VITA

JACOB MASON HEBERLING

EDUCATION

2010-2015	PhD in Biology, Syracuse University, Syracuse, NY Advisor: Dr. Jason D. Fridley
2006-2010	BS in Biology, Pennsylvania State University

AWARDS AND FELLOWSHIPS

2012-2015	National Science Foundation Graduate Research Program Fellowship (NSF GRFP)
2010-2012	Syracuse University Graduate Fellowship
2009	NSF Research Experience for Undergraduates (REU)

RESEARCH EXPERIENCE

2015-present	Postdoctoral researcher, Dept. of Biological Sciences, University of Pittsburgh, PA; Mentor: Dr. Susan Kalisz
2014-2015	Visiting Scholar, University of Pittsburgh, Pittsburgh, PA
Dec 2013	Visiting scientist (invited), Landcare Research, Lincoln, New Zealand; Host: Dr. Norman Mason
June 2013	Visiting researcher, Université de Picardie Jules Verne, Amiens, France; Host: Dr. Guillaume Decocq
2008-2010	Research Assistant, Penn State University, University Park, PA Mentor: Dr. Katriona Shea

PUBLICATIONS

Heberling, J.M. & Fridley, J.D. (2013) Resource-use strategies of native and invasive plants in Eastern North American forests. *New Phytologist* **200**: 523-533.

Heberling, J.M. & Fridley, J.D. (2012) Biogeographic constraints on the worldwide leaf economics spectrum. *Global Ecology and Biogeography* **21**: 1137-1146.

Siefert, A., Ravenscroft, C., Althoff, D., Alvarez-Yapiz, J., Carter, E., Glennon, K., Heberling, J.M., Jo, I., Pontes, A., Sauer, A., Willis, A. & Fridley, J.D. (2012) Scale dependence of vegetation-environment relationships: a meta-analysis of multivariate data. *Journal of Vegetation Science* **23**: 942-951.

Russo, L., Stehouwer, R., Heberling, J.M. & Shea, K. (2011) The composite insect trap: an innovative combination trap for biologically diverse sampling. *PLoS ONE* **6(6)**: e21079.

Zhang R., Heberling, J.M., Haner, E. & Shea, K. (2011) Tolerance of two invasive thistles to repeated disturbance. *Ecological Research* **26**: 575-581.

SELECTED PRESENTATIONS

Heberling, J.M. (2014) Invited seminar: "Functional trait perspectives on forest invasions in the Eastern United States." Ecology and Evolution Seminar Series, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA.

Heberling, J.M., Kichey, T., Decocq, G., Fridley, J.D. (2014) Oral presentation: "Pre-adapted to invade? Resource-use strategies of reciprocal woody invaders in NE USA and France." Northeast Natural History Conference, Springfield, MA, USA.

Heberling, J.M. (2013) Invited seminar: "Plant resource-use efficiency and forest invasions in the Northeastern United States." Ecology and Dynamics of Human Influenced Systems (EDYSAN), Université de Picardie Jules Verne, Amiens, France.

Heberling, J.M. & Fridley, J.D. (2013) Oral presentation: "Functional comparisons of native and invasive plants in Eastern North American forests: do they differ in resource-use strategies?" 56th International Association of Vegetation Science Annual Symposium, Tartu, Estonia.

Heberling, J.M. & Fridley, J.D. (2012) Oral presentation: "East Asian plants in Eastern US forests: are invaders pre-adapted for more efficient resource use?" International symposium: The East Asian flora and its role in the formation of the world's vegetation. Botanical-Garden Institute, Russian Academy of Sciences, Vladivostok, Russia.

SERVICE

2013-2014	Graduate Representative, Undergraduate Curriculum Committee, Department of Biology, Syracuse University
2012-2013	Vice President, Biology Graduate Student Organization, Syracuse University
2012	Library Committee Member and Biology Department Senator, Graduate Student Organization, Syracuse University

Peer reviewer (*ad hoc*) for the following journals:

Nature Plants, Ecology Letters, Biological Invasions, Global Ecology and Biogeography, Journal of Ecology, Diversity and Distributions, Journal of Vegetation Science (x2), Botanica Pacifica, International Journal of Plant Biology, Botanical Studies, Northeastern Naturalist (x3)