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Seasonal Leaf Structure and Function Tradeoffs in Native and Invasive Shrubs

A Capstone Project Submitted in Partial Fulfillment of the
Requirements of the Renée Crown University Honors Program at
Syracuse University

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and Renée Crown University Honors
Spring 2018

Honors Capstone Project in Biology

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Abstract

The spread of invasive alien plants continues to raise the overall threat to biodiversity around the world. The Enemy Release Hypothesis indicates that invasive alien plants are less regulated by natural enemies when introduced to a region (Keane and Crawley, 2002). Due to this, I hypothesize that invasive plants have more flexibility to allocate to photosynthetic ability, while native plants are required to allocate to structural defense in order to defend against more enemy regulation. To test this, woody shrub species leaves were collected and tested for nitrogen/carbon content, chlorophyll content, photosystem quantum efficiency, and fiber analysis across three seasons. As expected, invasive shrubs did produce higher chlorophyll content and greater photosystem quantum efficiency compared to native shrubs, indicating that the invasive shrubs did allocate more to photosynthetic capacity. However, fiber analysis indicated that native shrubs did not produce more fiber and structural components like I expected. Although native shrubs did not allocate more to structural defense, it is possible that resources could have been used for chemical defense instead.

Executive Summary

Climate change continues to be a critical issue that researchers and the public have to face. However, climate change and habitat destruction are not the only threats to biodiversity. The spread of invasive plants also presents many problems to biodiversity and ecosystem function (Funk et al. 2013). Invasive refers to those plants that are dominant and spreading quickly through an area. Invasive alien species continue to outcompete natives all over the world, and these invasive species are changing ecosystem function due to this ability to outcompete native plants in an area. Invasive plants are an issue even here in Upstate New York. For example, the wild parsnip and the slender false brome are two invasive plants that grow in New York. Wild parsnip is an invasive plant from Europe and Asia that is now naturalized in New York. It's sap causes burning and blistering of skin ("Wild Parsnip"). This is an example of an invasive plant that directly causes harm to animals and humans. The slender false brome is an invasive grass. This specific species can tolerate a wide range of habitats, so the slender false brome can outcompete many native species ("Slender False Brome"). As the slender false brome spreads, that is when we see less biodiversity in the community. Research is needed in order to better understand the mechanisms and biological reasons why invasive alien species are able to outcompete native species. With more research and information, we can better combat the problem.

It is important to understand that nitrogen is essential to all plants, both native and invasive. Nitrogen is used to build essential proteins needed for photosynthesis and absorption of light. Therefore, a limited nitrogen supply would

hinder a plant's ability to fully function and conduct proper photosynthesis. Nitrogen is a limiting resource in plant growth and function, meaning that plants only have a finite supply of nitrogen that can be used. So, it would be interesting to determine how plants are allocating this supply throughout their leaves. If a species needs tough, durable leaves in order to thrive in an environment, then the species will most likely allocate more nitrogen to the cell wall structure and components that make the leaves more fibrous (Onoda et al. 2004). Or, on the other hand, plants can allocate their nitrogen supply to proteins for photosynthesis rather than cell wall structures. Overall, each part of the leaf is in competition for the carbon:nitrogen supply, and the plant has to decide how to allocate this supply in order to survive best in its environment. I am interested in these allocation mechanisms, because the differences in nitrogen allocation between native and invasive plants might offer some explanation to the invasive ability to outcompete native plants. It is unknown how native and invasive species differ in their allocation of nitrogen and carbon to leaf function and photosynthetic capacity compared to allocation to production of proteins and fiber in leaf structure.

In 2002, Keane & Crawley developed the Enemy Release Hypothesis that states that invasive alien species are less regulated by herbivores and enemies when introduced to a new environment, compared to the native species of that area. When invasive species spread to a new region, their abundance seems to increase, as the invasive species do not have to combat as many natural enemies as the native species. Therefore, since the invasive plants are less regulated, they do not require producing fibrous leaves for structural defense. Rather, the invasive plant can now allocate the nitrogen supply to proteins and structures for increased photosynthetic function. Invasive plants seem to have more flexibility with how they allocate their resources (less to leaf structure and more to leaf function), which helps the invasive plants to thrive and survive better than native species. So, we hypothesize that the invasive alien species allocate more of their carbon and nitrogen resources to photosynthetic function, while native species have to allocate to leaf defense and structure.

To test this hypothesis, leaf samples were collected from woody shrub species in Syracuse, NY across three seasons: spring, summer, fall. Then, we used a C:N autoanalyzer to determine the total carbon and nitrogen content in the leaves. This result tells us what the finite supply of carbon and nitrogen is within each leaf sample. Next, I conducted chlorophyll extractions and ran these samples through a spectrophotometer in order to determine the chlorophyll content within each leaf sample. Then, I used the Ankom fiber analyzer to determine the structural composition of each sample. The fiber analyzer determines how much cellulose, soluble materials, fiber, and other structural components are in each leaf sample. Lastly, I took fluorometer (Fv/Fm) readings for each plant species across all three seasons. These Fv/Fm readings indicate the optimal photosynthetic efficiency of that leaf sample, so higher Fv/Fm indicates that the leaf has higher photosynthetic capacity. So overall, the total carbon:nitrogen content determined is the pool of resources from which the plant can allocate to parts of the leaf. The Ankom fiber analyzer and the chlorophyll extractions tell us where these resources are being allocated, whether it’s to structural components or chlorophyll. And lastly, the fluorometer tells us if the nitrogen and carbon allocation allowed for optimal photosynthetic yield each season. If a plant produces more chlorophyll and has higher Fv/Fm readings, then I know that plant is allocating for higher photosynthetic ability, especially since chlorophyll is essential for photosynthesis. If a plant produces more fiber, then we assume that plant is allocating for more structural defense.

My results showed that the invasive shrubs were able to take in more nitrogen for a greater nitrogen supply than the native plants. This already presents an advantage that the invasive shrubs had over native. Further, invasive shrubs did allocate more to photosynthetic capacity, seen through their higher chlorophyll content and Fv/Fm values compared to the native shrubs. However, contrary to my hypothesis, native shrubs did not produce more fibrous leaves or invest more in structural defense. So, I found that invasive shrubs do have the flexibility to allocate more to photosynthetic function than native shrubs, but it does not seem that the native shrubs are allocating to structural defense either. More research is needed to determine just how native species are allocating their supply, but it is possible that native shrubs were allocating to chemical defense rather than structural defense. I am able to conclude though that invasive shrubs do have the advantage of being able to allocate to photosynthetic function more than the natives. With further research, ecologists will better understand how invasive species obtain these advantages.

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Introduction

Invasive plants are those that are dominant and spread quickly through an area. Invasive alien species have continued to outcompete native species in many areas, including Upstate New York. Ecologists have been working to better understand the physiology of invasive alien species and how these invasive non-native plants differ from natives. As invasive aliens outcompete natives and continue to spread, there is a decrease in biodiversity and ecosystem function (Funk et al. 2013). With the environmental damage and economic loss that comes from invasive species, these invasive aliens are seen as almost as much of a threat to native biodiversity as habitat destruction (Feng et al. 2008). It is important to understand how invasive alien species are able to outcompete natives in order to control this rising problem.

As nitrogen is an important limiting resource in plant growth and function, researchers have studied how plants allocate nitrogen supply to their cell walls or photosynthetic capacity. Non-native invaders deploy strategies for more efficient photosynthetic nitrogen partitioning (Feng et al. 2008). It is the invaders' ability to allocate to Rubisco, bioenergetics pools, or light-harvesting complex proteins that may be providing the functional advantage over natives (Katahata et al. 2007). Onoda et al. (2004) found that plants sometimes allocate more nitrogen to cell walls at the cost of Rubisco, which led to a lower photosynthetic nitrogen-use efficiency. Plants that need tough, durable leaves will most likely allocate more nitrogen to cell wall structure in order to obtain a longer leaf life span, rather than allocating to Rubisco for stronger photosynthetic capacity (Onoda et al. 2004). In 2002, Keane &

Crawley developed the Enemy Release Hypothesis that states that invasive alien species are less regulated by herbivores and enemies when introduced to a new region, compared to the native species of that region. Native species regularly combat pathogens and specialist herbivores in their environment (Keane and Crawley, 2002). When invasive species spread to a new region, their abundance seems to increase, as the invasive species do not have to combat the same specialist herbivores, pathogens, or generalist herbivores as much as the native species. It is not an absence of enemies that necessarily provides the advantage to non-natives; rather, it is the reduced impact on alien species compared to natives (Keane and Crawley, 2002). An empirical model by Keane and Crawley in 2002 found that under conditions of full enemy exclusion, the abundance of invasive non-natives greatly decreased. When enemies are excluded, natives gain the competitive advantage and are able to competitively exclude non-natives (Keane and Crawley, 2002). Therefore, the model shows that the greater impact of natural enemies on natives is providing opportunities for non-natives to outcompete invaders. Through acknowledging herbivore and natural enemy impact on native plants with the Enemy Release Hypothesis, one could assume from these results that native plants would need to allocate more resources to cell wall structure compared to non-native plants. Whether allocating to Rubisco or light harvesting proteins, this flexibility in allocation and resource use efficiency creates the non-natives' ability to out compete native species.

As this enemy release reduces ecological restrictions on non-native plants, there is capability for evolutionary changes. Preliminary tests conducted by Blossey and

Notzold in 1995 support the two predictions of the Evolution of Increased Competitive Ability (EICA) hypothesis: a) non-natives will produce more biomass than natives in the native range; and b) plants with lower herbivore pressure will have increased growth. The competitive abilities of these non-native plants come from maximizing vegetative growth or reproductive activity depending on what is most important in a given area (Blossey and Notzold, 1995). For example, EICA suggests that invasive plants achieve their competitiveness by evolving to allocate more to growth rather than structural or chemical defense (Feng et al. 2009). As most leaf nitrogen is dedicated to photosynthesis, it is reasonable that a plant seeking to grow faster would adjust allocation in order to provide more nitrogen to photosynthetic function. Feng et al. (2009) demonstrated that introduced non-native *A. adenophora* selected for nitrogen allocation to photosynthesis and growth with reduced allocation to cell wall defenses through selecting for high specific leaf area and nitrogen-use efficiency.

Funk et al. 2013 hypothesized that invasive species can utilize a variety of nitrogen allocation mechanisms in order to thrive in a new region. For example, some species might allocate more stored nitrogen to proteins and amino acids, while other species allocate to growth with less carbon assimilation (Funk et al. 2013). Invasive species from Hawaii were found to preferentially allocate nitrogen to metabolism and amino acids rather than membrane-bound protein for photosynthetic light reactions, when compared to the native species studied (Funk et al. 2013). However, the native species studied showed more allocation to membrane-bound proteins. Compared to natives, invasive species have higher nitrogen concentration and photosynthetic rate with lower leaf mass per unit area, which represents the “high-return” section of the leaf economics spectrum (Funk et al. 2013). Nitrogen can be allocated to soluble proteins for carbon assimilation or membrane-bound proteins for light harvesting. Or, plants can also allocate some of the nitrogen resource to non-photosynthetic compounds like amino acids, cell wall proteins, and defense compounds (Funk et al. 2013). This high-return end of the leaf economics spectrum dictates that an invasive species should allocate more nitrogen to photosynthetic enzymes and amino acids that are required for protein synthesis and growth. It has been hypothesized that nitrogen allocation to amino acids and nucleic acids could actually create more positive performance for a plant than just allocation to soluble proteins (Funk et al. 2013). Funk et al. (2013) proposed that invasive species may thrive due to engaging in multiple nitrogen allocation mechanisms.

Although nitrogen allocation has been studied for native and invasive species, it has not yet been determined how carbon allocation mechanisms provide possible advantages to alien species. According to Leishman et al. (2010), the carbon capture strategies of alien invasive and native species have been found to be similar unless an environmental disturbance causes a species to adjust for faster growth. Leishman et al. (2010) suggested the position of a species on the leaf economics spectrum is affected by resource availability in an area, so non-native and native species existing in the same region should develop similar leaf traits for similar carbon capture strategies. However, in 2013, Heberling & Fridley found that invaders in eastern North American deciduous forests were both more productive and more efficient with their carbon-capture strategies. The invaders exhibited greater daily carbon gain and greater carbon gain per unit of carbon or nitrogen invested, compared to the native species (Heberling & Fridley, 2013). My study focuses on non-native and native plants that are co-existing in an area, so we can determine the amount of carbon within the samples and the differences in methods of allocation in plants that exist in the same environment. Thus, I studied how invasive alien woody shrub species allocate nitrogen and carbon supply in leaf structure and function to create a competitive advantage over native shrubs. Particularly, I looked at how native and invasive non-native shrub species differ in their allocation of nitrogen and carbon to leaf function compared to allocation to proteins and fiber in leaf structure. There is competition between processes in the leaf for the carbon and nitrogen supply, and the allocation of these resources changes the plant's survival and production in an environment.

It is not yet known how native and invasive species differ in their allocation of nitrogen and carbon to leaf function and photosynthetic capacity compared to allocation to production of proteins and fiber in leaf structure. It is important to determine these allocation mechanisms in order to understand how invasive species are obtaining advantages for leaf survival and overall abundance. I hypothesize that the invasive alien species have the flexibility to allocate carbon:nitrogen resources to chlorophyll and other photosynthetic compounds rather than leaf defense and structure, due to the Enemy Release Hypothesis. It is likely that native shrubs are more restrained in allocation due to their need for more defenses. Since invasive alien shrubs would not have to use defense processes to combat natural enemies in their new region, the invasive alien shrubs can allocate their resource supply to more productive leaf function and photosynthetic components.

Materials & Methods

Leaves from 43 woody shrub species (21 native and 22 non-native species) were collected in the spring, summer, and fall of 2017 from a common garden at Syracuse University (Table A). One to four replicates were chosen of each species for a total of 103 woody shrub plants collected. Each species was chosen based on which shrubs had viable, fully developed leaves that were undamaged. About 20 healthy leaves were collected from each plant across each season. During the fall collection, some species either lost their leaves already or only had leaves that were in bad condition. Leaves were collected on June 9th and 15th, July 26th, and November 8th of 2017. These dates were chosen to accommodate the light changes of the seasons and the shade tolerance of the shrubs. For example, prior to the summer collection (mid-June), a shade cloth with 20% neutral light transmittance was placed over the plants to decrease the amount of light received by the shrubs (Fridley et al. 2012). I chose to wait several weeks after the shade cloth was put up in order to allow the leaves to adjust before the summer collection. Likewise, the shade cloth was removed in October, so the fall leaves were not collected until a few weeks after the shade cloth was removed.

Prior to drying the samples, fresh leaves from each plant were used to conduct a chlorophyll extraction. This chlorophyll extraction provides the amount of chlorophyll a and b contained in each leaf sample. A 2 cm diameter disk was punched out from each fresh leaf avoiding large veins, and a razor was used to scratch the surface of the leaf disk. The leaf disk was then placed in a labeled test tube with 2 mL of dimethylformamide (DMF). Test tubes sat in the fridge for 2-6 days. After the 2-6 days, a single read spectrophotometer was used to determine the actual chlorophyll concentration from each sample. For our data collection, a microplate reader was used instead of a single read spectrophotometer for some samples. However, in order to keep measurements consistent, some samples were run on both machines and a regression equation was used to convert the microplate results to match the spectrophotometer. Each sample was run through three different wavelengths 646, 663, and 750. Using these results and the equations in the Porra, R.J. et al. (1989) table, the amount of Chlorophyll a and chlorophyll b in each sample was determined [See Table B]. These wavelengths were chosen because 646 correlates with a wavelength where chlorophyll b can absorb light, and 663 correlates with chlorophyll a (Martinez, 2017). The wavelength of 750 is used as a reference; one must subtract the absorbance at 750 from the absorbance at 646 or 663 to get the corrected result.

Remaining leaves were placed in a drying oven until needed. Using a mortar and pestle, the leaves were ground into a fine powder. Additionally, to speed up the grinding process, a Wiley Mill with a 40-mesh screen was used.

For fiber analysis and determination of the structural composition of each sample, I performed a sequential digestion using the Ankom fiber analyzer. Ground leaf material of 0.5-0.6 grams was placed into each of the fiber filter bags, and the bag was labeled with a permanent marker. Then, each bag was individually heat-sealed. After preparing the sample bags, each set of 24 bags (23 different samples and 1 blank) had to go through 4 different extractions. First, the bags were placed in the tree within the cylinder of the Ankom machine and 1800-1900 mL of ambient

NDF (neutral detergent solution) was used as a neutral detergent to extract out carbohydrates, lipids, and soluble proteins. Then, the second round used 1800-1900 mL of ADF (acid detergent solution) to wash off hemicellulose and membrane-bound proteins like chlorophyll. The third/ADL round used 300 mL of 72% sulfuric acid to wash off any cellulose in the sample (Ritchie, Ankom Protocol). Finally, each bag was placed in a crucible and left in an ashing furnace to burn off lignin. At the end, only recalcitrant materials are left over. After each round, the bags were dried at 105 C for at least 24 hrs until the next round of extraction. Then, prior to the next step, each of the bags were weighed straight out of the oven. The amount lost after each step determines how much of that compound was in the leaf sample.

A C:N NC2100 autoanalyzer was used to determine the total mass-based carbon and nitrogen concentration in each sample (Fox et al. 2017). I used 2-3 mg of ground leaf powder for each sample and followed the protocol of A. Fox et al. (2017). Fluorometer readings were taken in the Spring, Summer, and Fall of 2017. Dark adaptation clips were placed on one leaf of each plant, and the leaves were dark adapted for 30 min following Murchie and Lawson (2013). After 30 minutes, the fluorometer probe was placed in the clip and the Fv/Fm reading was taken. Leaves are dark adapted because it allows for measurement of minimum fluorescence with all reaction centers open, then max fluorescence after the intense light of the fluorometer saturates and closes reaction centers. Light energy can take three paths when going into the leaf: it can turn into chemical energy as it is used during photosynthesis, it can be released as heat energy, or the light can be fluoresced back out (Murchie and Lawson, 2013). All three of these paths are in competition with one another, and the fluorescence emission indicates the quantum efficiency of photochemistry (Murchie and Lawson, 2013). When the leaf is in the dark, all reaction centers in the thylakoid membrane are open and waiting for light. So, this is when minimum fluorescence is measured, nothing is fluoresced back out. When the fluorometer shines a bright light on the leaf, this light then saturates the reaction centers in that part of the leaf and the reaction centers close, not allowing anymore light in. Therefore, the rest of the light hitting the leaf is fluoresced back out, and that is when maximum fluorescence is measured, which is an estimate of the efficiency of the leaf if all reaction centers were open. The fluorometer readings provide information about the quantum efficiency of photochemistry. Fv/Fm values were recorded for each sample, and it has been found that Fv/Fm values are an indicator of the maximum quantum yield of Photosystem II chemistry (Murchie and Lawson, 2013). Unstressed leaves express consistent Fv/Fm values of ~0.83, and this indicates the maximum quantum yield of photosynthesis (Murchie and Lawson, 2013). Through dark-adapting the leaves for 30 minutes before measuring Fv/Fm, we are measuring the stress in the leaves and quantum yield of photosynthesis. Fv/Fm values less than the 0.83 standard indicate that there is a stressor or condition affecting the quantum yield of photosynthesis.

Results

First, when analyzing the percent carbon in each season, there were no significant trends or differences found. Comparing native and invasive species, both had relatively the same percent carbon content with no significant difference in all three seasons. However, significant trends were found in the nitrogen content. On average, the invasive plants had higher nitrogen content than the natives in all three seasons. In the spring, the invasive plants had about 0.5% more nitrogen than native plants (Figure 1, t-value= -2.685, $p<0.05$). Similarly, in the fall, invasive plants had about 0.75% more nitrogen than the natives (Figure 1, t-value= -3.45, $p<0.001$). The invasive plants showed higher nitrogen content in the summer, but with a p-value of 0.19, the difference was not significant (Figure 1). Also, the overall amount of nitrogen decreased over time, which is common as nitrogen decreases with leaf age.

Further, invasive plants also contained more total chlorophyll than natives in each season (Figure 2). All three comparisons in spring, summer, and fall were significantly different (Figure 2; t-value= -2.408, $p<0.05$; t-value= -2.174, $p<0.05$; t-value= -3.85, $p<0.001$, respectively). Similarly, invasive plants also had higher Fv/Fm values than natives in every single season. Spring, summer, and fall comparisons of Fv/Fm values were significantly different (Figure 3, 4, 5; t-value= -2.747, $p<0.05$; t-value= -5.093, $p<0.001$; t-value= -4.06, $p<0.001$, respectively).

When analyzing the fiber analysis, there is no significant difference in fiber levels between invasive and native plants ($p>0.05$ across seasons). Hemicellulose, lignin, and recalcitrant minerals were not statistically different between natives and invasive plants each season (Figure 6; $p>0.05$ for hemicellulose, lignin, and recalcitrant minerals across seasons). Invasive plants had significantly more cellulose than native plants in spring, but then this difference disappeared in the summer and fall (Figure 6; t-value= -3.477, $p<0.001$). Also, native plants had more soluble content in the spring, but then this difference also disappeared in the following seasons (Figure 6; t-value= 2.032, $p<0.05$). Overall, invasive and native plants had similar fiber analysis profiles throughout each season.

Discussion

In this study, I have shown that invasive aliens have the ability to take in more nitrogen than native species, which is similar to a study by Jo, Fridley, & Frank in 2017. Invaders were able to take up more nitrogen from soil through the production of fine roots with higher specific root length (Jo et al. 2017). Increased nitrogen content will provide a competitive advantage for invasive plants, as the invasive plants will have more resources to allocate towards photosynthetic function. Liu et al. 2018 demonstrated that an elevated nitrogen environment can allow for an initially weak invasive to become more aggressive over native plants in the area. This further points to the idea that invasive species have a mechanistic advantage over native species to take in and make better use of nitrogen resources. Further research is needed to determine the mechanisms in how invasive plants are able to take up more nitrogen. Currently, it is hypothesized that below ground processes allow for this. Greater invader productivity above ground has been found to correlate with productivity of fine roots and the ability of invaders to take up soil resources more efficiently (Jo et al. 2015). Although invasive dominance threatens ecosystem biodiversity and function, it has been demonstrated that the increased nitrogen uptake by invasive plants can actually enhance ecosystem nitrogen cycling (Jo et al. 2017). Invasive above- and below-ground processes allow for an increased flow of nitrogen to soil, which allows for increased forest ecosystem functioning (Jo et al. 2017). Further research is needed to better understand the overall impact of invasive species on the environment.

Further, I hypothesized that invasive plants would allocate their resources to photosynthetic capacity more than the native plants. Across each season, I found that the invasive plants produced more chlorophyll for light absorption than native plants. This combined with the finding that invasive plants also had significantly higher Fv/Fm values across each season allows me to conclude that the invasive population did invest in photosynthetic capacity more than the native plants. In 2013, Heberling & Fridley also found that invaders exhibited greater photosynthetic abilities, specifically finding that invaders had greater photosynthetic nitrogen-use efficiency.

Additionally, due to the need to combat natural enemies, I hypothesized that the native plants, compared to invasive, would collectively invest more to structural defense. Therefore, I predicted that the native plants' leaves would develop more cellulose, lignin, and structural components in order to make fibrous and tough leaves. However, I did not find a significant difference in fiber levels between invasive and native leaves. The increase in cellulose content that was seen in spring invasive leaves is possibly due to the fact that invasive plants leaf out earlier, so those leaves were likely farther along in their thickening process than the native leaves (Xu et al. 2007). Also, both native and invasive leaves showed a decrease in soluble content from the spring to the summer. It is possible that this loss of soluble content correlates with a decrease in photosynthetic capacity from spring to summer (Martinez & Fridley, 2018).

Overall, similar to findings compiled in a review from 2008, we found that the invasive species were better able to take in nitrogen compared to native species

(Funk et al. 2008). This difference in nitrogen provides an advantage to invasive plants as nitrogen drives a plant's metabolic rate through the ability to use nitrogen to produce more proteins for biological activity. With higher chlorophyll content and higher photosystem quantum efficiency, invasive plants did seem to invest in their photosynthetic capacity more than native plants, as we predicted. Lastly, since the structural analysis showed similar fiber profiles between native and invasive plants, it does not seem that the natives allocated to structural defense preferentially. We know that invasive aliens gain an advantage through their ability to take in more nitrogen, but this study also shows that invasive aliens are able to preferentially invest more in photosynthetic capacity than the native plants, which provides advantages to the invasive aliens. Further, native plants are not preferentially allocating their carbon and nitrogen resources to structural defense. Even though I did not find native investment in structural defense, it is possible that the native leaves are investing in a form of chemical defense rather than structural in order to combat enemies. Phenolics and secondary metabolites that are synthesized within plants can deter enemies through toxicity and antifeedant activity (Rehman et al. 2012). It is possible that the native species in this study invested in these secondary metabolites more than structural defense compounds.

Future studies are needed to determine if allocation to chemical defense is a possible key difference between native and invasive plants. This study provides more information to uncover the mystery of invasive ability to outcompete native plants. An invasive investment in photosynthetic function is evident from my results, and I am left wondering whether a need for chemical defense is limiting native plants' ability to freely allocate to photosynthetic capacity instead.

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Table A

Woody Shrub Species Collected + Replicates

Species	Replicates
Acer pensylvanicum	2
Berberis canadensis	1
Berberis thunbergii	3
Berberis vulgaris	2
Calycanthus floridus	2
Celastrus orbiculatus	4
Celastrus scandens	5
Cornus amomum	2
Elaeagnus multiflora	2
Elaeagnus umbellata	3
Euonymus alatus	3
Euonymus atropurpureus	3
Euonymus bungeanus	1
Euonymus europaeus	2
Euonymus hamiltonianus ssp. Sieboldianus	2
Euonymus obovatus	3
Euonymus phellomanus	2
Frangula alnus	3
Frangula caroliniana	1
Hamamelis virginiana	3
Hydrangea aborescens	3
Lindera benzoin	4
Lonicera fragrantissima	2
Lonicera hirsuta	3
Lonicera japonica	3
Lonicera maackii	4
Lonicera periclymenum	1
Lonicera reticulata	3
Lonicera sempervirens	2
Lonicera standishii	2
Lonicera tatarica	3
Lonicera xylosteum	2
Rhamnus alnifolia	2
Rhamnus cathartica	3
Sambucus racemosa	2

Viburnum acerifolium	2
Viburnum dentatum	3
Viburnum dilatatum	2
Viburnum lantana	2
Viburnum lentago	1
Viburnum prunifolium	2
Viburnum rafinesquianum	2
Viburnum setigerum	1

Table B

Equations for chlorophyll concentrations in μg/ml

$$Chl\ a = 12.00A^{663.8} - 3.11A^{646.8}$$
$$Chl\ b = 20.78A^{646.8} - 4.88A^{663.8}$$
$$Chls\ a + b = 17.67A^{646.8} + 7.12A^{663.8}$$

(Taken from Porra et al. 1989)

Figure 1. Average percent nitrogen of native and invasive plants across seasons

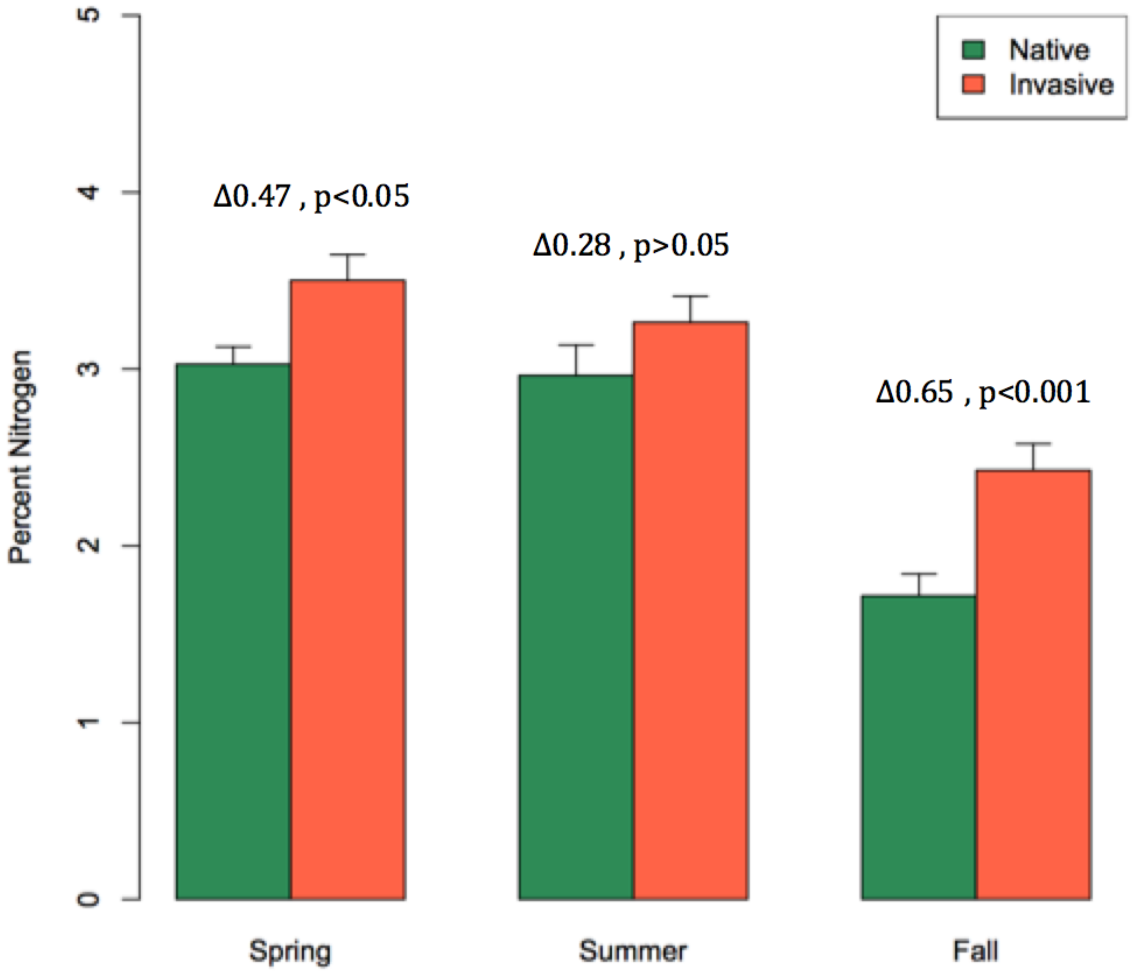

Figure 2. Average total chlorophyll of native and invasive plants across seasons

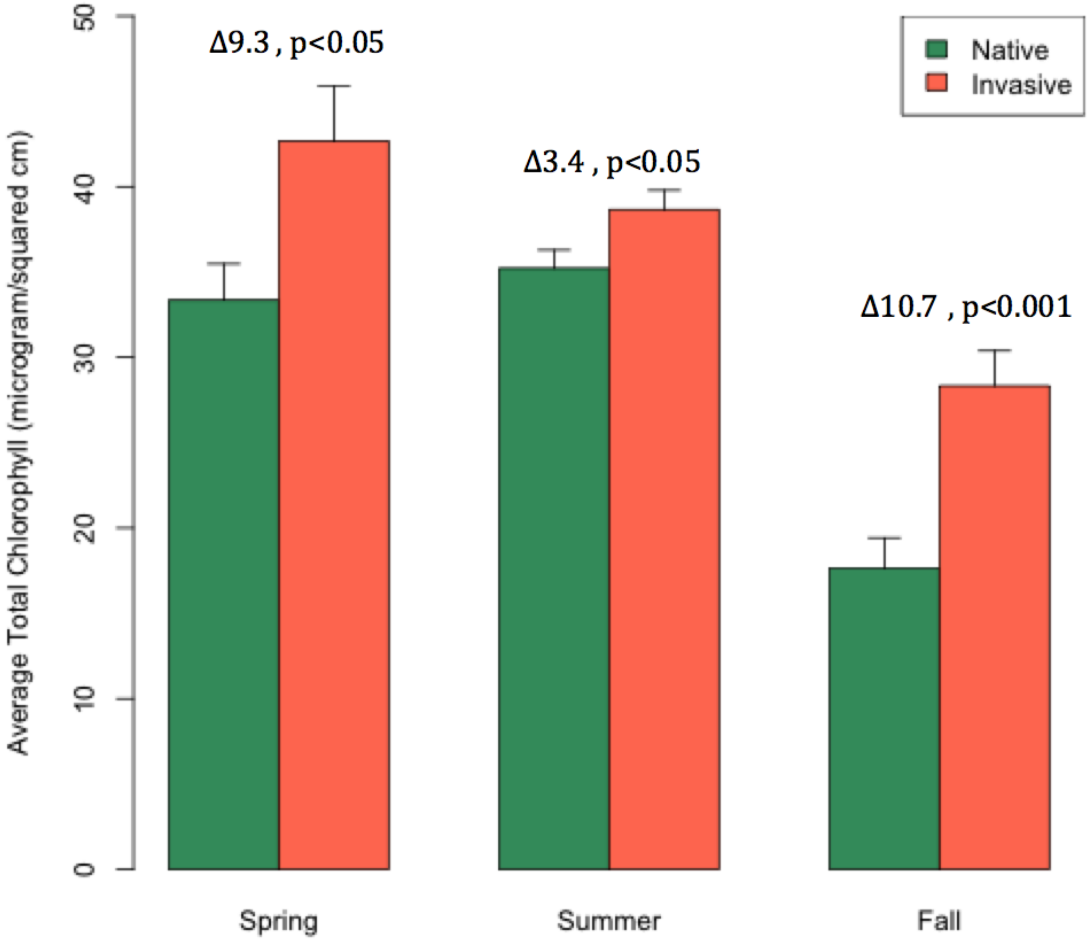

Figure 3. Spring Fv/Fm values of native and invasive plants

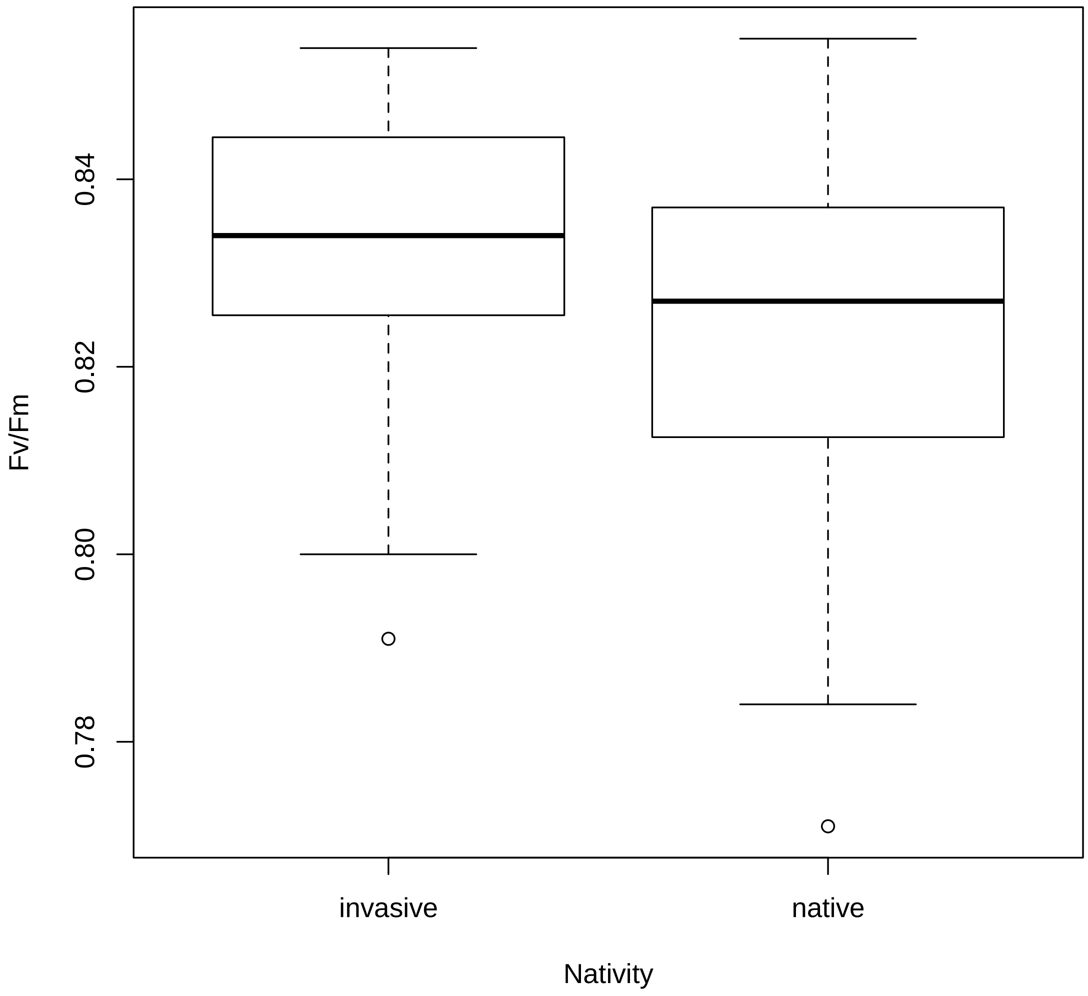

Figure 4. Summer Fv/Fm values of native and invasive plants

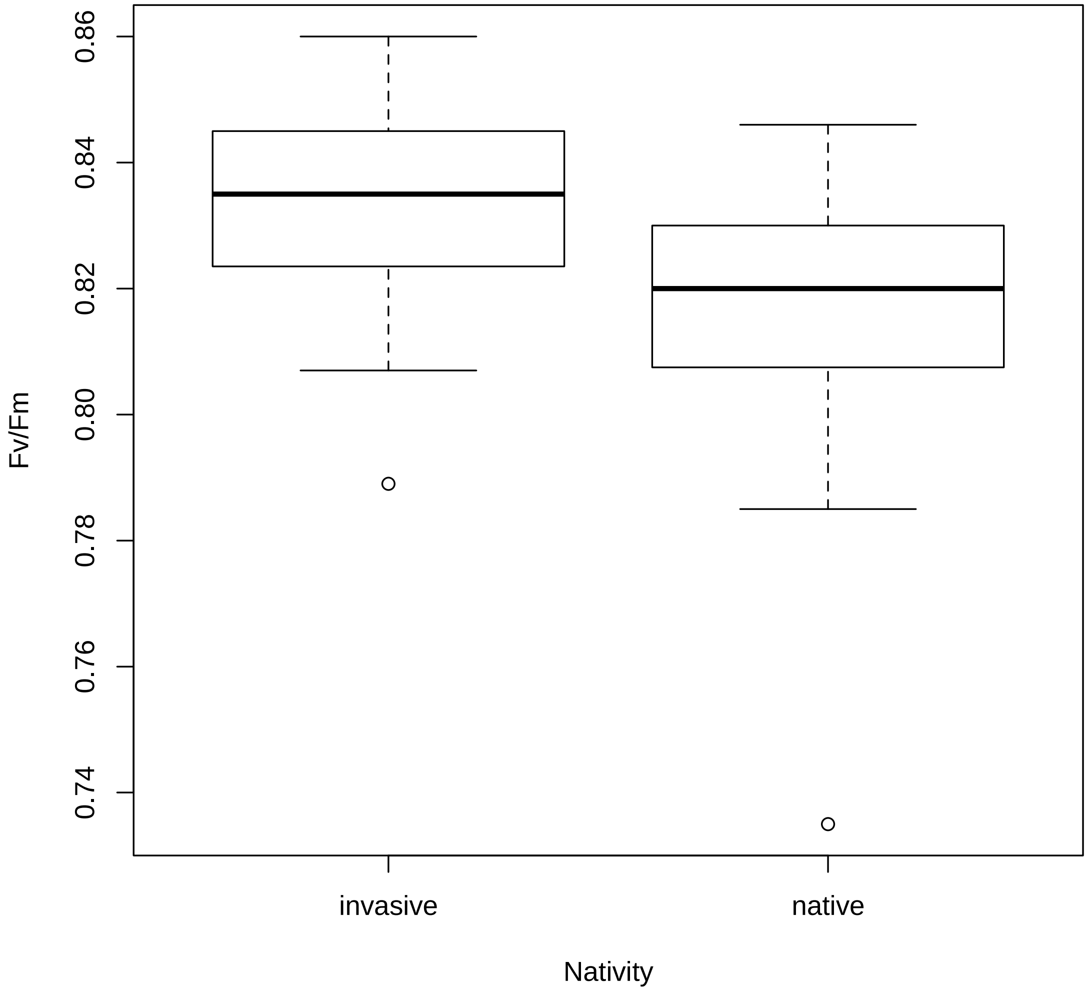

Figure 5. Fall Fv/Fm values of native and invasive plants

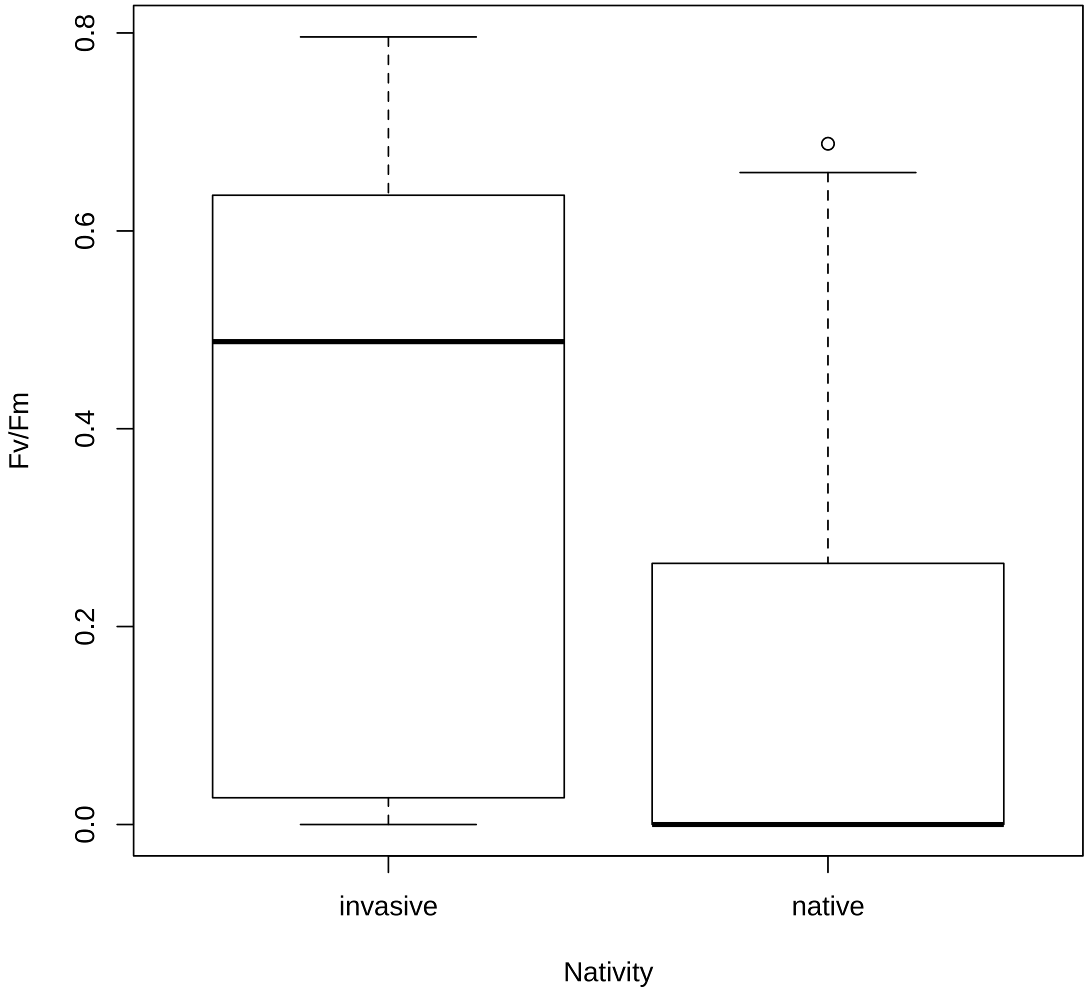

Figure 6. Seasonal fiber analysis of native and invasive plants