Survey of deciduous tree and shrub biomass construction costs at Black Rock Forest demonstrates connection between ecological role and plant physiology

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Abstract

In the past century, normal patterns of ecological succession in northeastern U.S. forests have been complicated by rising temperatures, native forest recovery, and continued human disturbance. Under these conditions, secondary growth species may be pushed out more easily by invasive species that take command of disturbed areas. A plant's ability to take over new environments is tied to its growth rate, which in turn is tied to the efficiency at which the plant can mobilize its energy resources. Thus, one method of quantifying patterns of trophic disturbance is through the biomass construction cost (CC) metric, which measures the amount of stored glucose required to construct one unit of leaf material in deciduous species. CC has been used to compare native and invasive plants on a specific, individual comparison basis. This survey expands upon such application of CC by utilizing the metric in a survey of 33 deciduous tree and shrub species at Black Rock Forest, an ecology research site in the Hudson Highlands of upstate New York. We chose representative species of native, expanding invasive, and widespread invasive ecological positions at the Forest for sampling, and calculated CC of each species. Resulting CC values demonstrate that the energy investments of native-class deciduous tree species are significantly higher than the energy investments of widespread invasive tree species. Sub-surveys of shrub species and of red maple *(Acer rubrum)* in different Forest habitats add additional context to these results. These results support our hypothesis that a plant's ecological position is connected to its energy use; further research may investigate the chemical mechanisms for such connection and variation within individuals of the same species.

Introduction

Forests in the northeastern United States have changed drastically in the past century. Average temperatures in the region have increased by nearly 2˚ F from 1895 to 2011 (Horton et al. 2014). This warming climate is coupled with increasing precipitation (an average of 5 inches in overall rainfall), rising emissions in carbon dioxide and other heat-trapping gasses, and encroaching human industrial development. Changes to the region over the past century follow human development which has occurred in the northeastern U.S. for over 10,000 years, beginning with clearing along waterways and controlled burning by indigenous groups; while some forest recovery has taken place in areas developed several centuries ago, the region is still losing forest cover overall (Thompson et al. 2013).

A combination of rising temperatures, native forest recovery, and continued human disturbance has complicated natural patterns of ecological succession in northeastern forests. In a typical pattern of succession, an available patch of land is first filled by primary growth tree species which grow quickly in order to colonize the area. These fast-growing species are eventually replaced by secondary growth tree species, which grow more slowly yet survive longer, as they are more specifically adapted to their environment (Luken 1990). When a forest is subjected to more frequent change, however, secondary growth species are pushed out by natural disaster or human disturbance before they can fully adapt to the environment, and primary growth species remain dominant. It is important to qualify here that "primary growth" and "secondary growth" tree species are relative terms, into which particular species may be categorized differently depending on their ecological niche in a particular habitat. A widespread species such as red maple *(A. rubrum)* may be considered primary growth in one area and

secondary growth in another, or may even shift niches in the same area over an extended period of time.

In a review of northeastern U.S. forests over the past century, Thompson et al. (2013) found that late succession species, specifically trees in the oak, beech, and chestnut families, are much less abundant, and early succession species, specifically red maples *(A. rubrum*), are much more abundant in the region now than they were during the pre-colonial period. Climate change and human disturbance have additionally enabled invasive tree species to become increasingly prevalent in the northeast. Invasive species thrive in disturbed areas; when land is stripped of trees for human farmland, roads, or other developments, they are able to quickly take over (Didham et al. 2007). The Lower Hudson Partnership for Regional Invasive Species Management Working Group has identified over 250 invasive plant species that threaten their native counterparts in the lower Hudson region alone (encompassing Manhattan, Bronx, Westchester, Putnam, Dutchess, Rockland, Orange, and half of Ulster Counties) (Rohleder et al. 2018).

Patterns of ecological succession are tied to plant physiology. In order to grow (or, build new tissues), a plant utilizes the glucose it has stored from performing photosynthesis. The efficiency at which a plant is able to mobilize its energy resources is thus tied to its growth rate, which is in turn tied to the capacity of foreign plants to invade new environments (Wang et al. 2013). Broadly speaking, it is commonly hypothesized that invasive plant species use their stored glucose more efficiently in order to grow more quickly and colonize new areas. Meanwhile, native species use their stored glucose less efficiently in order to devote energy to specific adaptations for their ecosystem (such as a root system to draw nutrients out of rocky soils or strong bark to protect against pests), and thus grow more slowly.

Botanists have studied the efficiency at which plants harness their stored energy using a metric called the biomass construction cost (abbreviated here as CC). This metric was defined by Williams et al. (1987) as: "the amount of glucose required to provide carbon skeletons, reductant, and ATP for synthesizing the organic compounds in a tissue via standard biochemical pathways." More simply: a plant's biomass construction cost is the amount of glucose required to construct one unit of leaf material. A unit of leaf material may be expressed in terms of mass or area. The CC metric is an effective means of studying trophic dynamics, or the movement of energy within and throughout populations, communities, and ecosystems; it directly connects a plant's chemical structure (organic carbon and nitrogen content) with a calorimetric estimate of its conversion of stored energy to the active building of new biomass (heat of combustion) (Griffin 1994). Revised adaptations of the technique allow for the calculation of construction cost from only three analytical values: a plant's carbon content, nitrogen content, and ash content after combustion (Williams et al. 1987); this technique was further simplified by Poorter (1994).

Following Williams et al.'s adaptation of the technique, the construction cost has proven useful in comparing the physiology of native and invasive species as ecologists study the trophic shifts resulting from human impacts on plant communities. Wang et al. (2013) used the construction cost among other methods to compare the physiology of *Eupatorium adenophorum*, an invasive forb in the subtropics, with similar native species. These authors found that, surprisingly, *E. adenophorum* had a higher construction cost than comparable native species; still, separate energy use metrics demonstrated that this invasive forb was maximizing its carbon use by other means. Song et al. (2010) similarly used the construction cost to compare *Wedelia trilobata*, an invasive weed in South China, with related native species *W. chinensis.* These authors found that the increased CO_2 use by the invasive *W. trilobata* caused a significant

decrease in organic nitrogen and resulted in a lower construction cost compared to the native species.

The biomass construction cost has additionally been utilized for species comparisons at Black Rock Forest in Cornwall, New York. This forest has served as an ecological preserve and research field station in the lower Hudson Highlands since 1927; long-term plots have been maintained since 1931 (Schuster et al. 2008). Schuster et al.'s examination of changes to aboveground biomass in the forest since 1938 (when a tree survey was first conducted on this land) reveals the extirpation of three old-growth tree species and the invasion of seven tree species that are either invasive or have moved north from warmer climates. The forest's composition has additionally shifted to include a higher abundance of red maple. These changes are coupled with a mean annual temperature increase of about 1 ◦C during the past century (Warrach et al. 2006). These climatic and ecological shifts, demonstrated by a wealth of data on Black Rock Forest's flora over the past century, make this site an ideal model location to study the trophic dynamics of northeastern forests.

Similarly to scientists elsewhere in the world, botanists studying the Black Rock Forest plant community have utilized the biomass construction cost metric to compare specific invasive species to corresponding native species. Boyd et al. (2009) compared *Berberis thunbergii,* a highly invasive shrub, with two similar native shrubs, and Nagel and Griffin (2001) compared *Lythrum salicaria,* an invasive flowering plant, with five corresponding natives. Both studies found lower construction costs in the invasive species. CC has additionally been used at Black Rock Forest to compare native species (red oak, *Quercus rubra,* chestnut oak, *Quercus montana,* and red maple, *A. rubrum)* at two sites across an environmental gradient (Nagel et al. 2002). Nagel et al. found that *A. rubrum* had a significantly lower construction cost than the two oak

species at both sites, demonstrating that CC may be used to examine interspecific dynamics even between tree species in the same ecological position.

While the construction cost is demonstrably useful for comparing particular species, it has not been utilized to thoroughly survey plant communities since Merino (1987) conducted a survey of 46 native plants to central and southern Spain. This project aims to test the modern construction cost technique for such a broad comparison through a survey of deciduous tree and shrub species at Black Rock Forest.

We determined the construction costs of 22 deciduous tree species, placing a focus on comparing this forest's native (secondary growth) species with species that have expanded their ranges from the southern U.S. (primary growth) and foreign invasive species. Species included in the survey were specifically selected in order to test for a relationship between a plant's degree of invasiveness and its construction cost. This survey thus tested the hypothesis that native tree species will have higher CC, because they grow more slowly and devote more resources to environment-specific adaptations, while primary growth and invasive plant species will have lower CC, because they grow more quickly in order to colonize new areas. In addition to surveying tree species, we determined the construction costs of 11 deciduous shrub species, including both native shrubs and foreign invasive species; this sub-survey sought to identify whether the energy requirements of shrub species follow a similar pattern to those of trees. We additionally aimed to contextualize CC variation among species by examining variation within species through a sub-survey of the highly adaptable red maple *(A. rubrum)* in different parts of the forest; this sub-survey sought to identify whether environmental factors significantly impact a single species' patterns of energy use.

Methods

Field site: Black Rock Forest

Black Rock Forest (BRF) is a 1566-hectare forest preserve located in Cornwall, New York between the Hudson Highlands and the Hudson River Basin. Elevations range from 100 to 450 m above sea level. It has a climate typical of temperate forests in the Northeastern United States: mean annual precipitation is 1190 mm and mean air temperature ranges from -2.7 °C in January to 23.4 °C in July (Nagel et al. 2002). Soils are primarily loams, including soils of the Hollis series in upper elevation areas and soils of the Charlton series in lower elevation areas, and the Precambrian granite gneiss bedrock is often quite shallow. The most dominant trees in the forest are red oak *(Q. rubra)* and chestnut oak *(Q. montana)* in upper elevation areas, and red oak and red maple *(A. rubrum)* in lower elevation areas (Nagel et al. 2002).

The forest is home to over 700 vascular plant species, including over 60 tree species (Barringer and Clemants 2003). In the last comprehensive count of all tree species on the property done by forest staff in 2011, 65 tree species were documented. These include 10 species classified as "planted, non-native" (introduced to the forest by human intervention) and 4 species classified as "non-native" (invasive species that arrived in the forest due to other factors) (Munson et al. 2011).

Leaf sampling

Of the 65 tree species documented at Black Rock Forest, leaf samples from 22 were collected in this survey. Only deciduous trees were sampled, as reliable analytical methods of determining biomass construction costs of conifers have not yet been developed (Griffin 1994).

Trees were chosen for sampling in order to investigate a possible relationship between biomass construction cost and degree of invasiveness. To identify species that would best reflect taxa native to the forest, invasive species that reflect changes in the forest due to a warming climate, and foreign invasive species that pose significant threats to BRF biodiversity, I consulted the Munson et al. (2011) tree list, the Barringer and Clemants (2003) flora, past literature on the forest (Nagel et al. 2001, Nagel et al. 2002), and the expertise of Black Rock Forest staff.

Species included in the survey were classified into three categories reflecting their degree of invasiveness: native, emerging invasive/range expansion, and widespread invasive (Table 1). Native trees are trees demonstrated to be well-established at BRF (Schuster et al. 2008). Emerging invasive/range expansion trees are trees native to the southern United States that have been observed in small numbers at BRF in recent decades (Schuster et al. 2008). Widespread invasive trees are native to other regions of the world entirely; these trees have been introduced at BRF or in nearby disturbed areas by humans and pose threats to the native ecosystem through competition. To be classified as an widespread invasive species in this study, a species must be classified as a Tier 3 or Tier 4 Invasive Species in the Lower Hudson region by the Lower Hudson PRISM (Rohleder et al. 2018).

Sampling of trees in these three categories was conducted during two field work periods: June 2017 and June 2018. I sampled native trees primarily in less disturbed, high elevation areas. I sampled range expansion and widespread invasive trees primarily in low elevation areas and in disturbed areas: near roads, in forest clearings, and on the banks of ponds and reservoirs. Forest Director Dr. William Schuster, Forest Manager John Brady, Research Manager Katie Terlizzi, and other BRF staff members were consulted in order to locate and identify different species. The BRF herbarium additionally served as a tool for identifying species locations in the

forest, and the Peterson Eastern Trees field guide was utilized to identify species in the field (Peterson et al. 1998). A pole saw was utilized to collect leaf samples from branches approximately 3 to 5 meters above the ground. A shotgun was utilized to collect leaf samples from branches 6 or more meters above the ground.

All plants sampled in June 2018 were mapped using the Avenza Maps smartphone application. For each sample collected in 2018, the following data were logged in Avenza: date collected, time collected, species, latitude, longitude, northing, easting, photo(s) of plant, and notes on general location, age, and appearance. Trees sampled in 2017 were not mapped.

During the 2017 sampling period, 120 samples were collected from 15 tree species and 1 shrub species. During the 2018 sampling period, 220 samples were collected from 18 tree species and 10 shrub species (Table 1). There was some overlap in tree species collected over the two sampling periods; specifically, additional samples were collected in 2018 from species for which 2017 CC results showed errors and/or high variance (see CC calculation methods below).

In 2018, a sub-survey was conducted including 11 shrub species; I undertook this survey because I learned from forest staff and from consulting the Lower Hudson PRISM categorizations that several of the aggressively invasive species that pose the greatest threats to biodiversity at BRF are shrubs (Rohleder et al. 2018). These shrub species were classified similarly to the tree species as native or widespread invasive (Table 1). Because the shrubs of BRF have not been tracked over the years as closely as this forest's trees (they are only included in general flora surveys, not tree surveys), it was not possible to identify range expansion/nonaggressive invasive shrubs.

The project was additionally expanded in 2018 to include an intraspecies sub-project: samples of red maple *(A. rubrum)* were collected across different habitats in the forest. 44 samples of red maple leaves were collected from 8 different locations representing varying environmental conditions (Table 2). Red maple was chosen for this intraspecies study because the species is highly adaptable; it flourishes in all habitats within the forest (Nagel et al. 2002, Schuster et al. 2008). Additionally, 2017 CC results for red maple demonstrated high variance.

Over the two sampling seasons, a total of 340 leaf samples were collected from 33 tree and shrub species. This survey includes 13 native tree species, 6 emerging invasive/range expansive tree species, 3 widespread invasive tree species, 7 native shrub species, and 4 widespread invasive shrub species (Table 1).

Elemental data collection

The following constituted a leaf sample from one tree: a set of leaves cut off at their petioles from a single branch or concurrent branches with a combined wet weight of between 7 and 30 grams. The area of each sample was calculated using a leaf area meter. All samples were dried in a drying oven at 60 °C for at least 12 hours or until all moisture was removed, then the dry weight was measured. Leaf area and dry weight were used to calculate leaf-mass per area (LMA) of each sample. Samples were then transferred into 20 mL plastic vials and ground using a Spex Industries Mixer/Mill 8000 mixing machine.

Subsamples (each constituting 3-5 mg of a leaf sample deposited in a sealed tin capsule) and acetanilide standards (each constituting 2-6 mg of EA Consumables acetanilide OAS 1g deposited in a sealed tin capsule) were prepared using a microbalance. Percent Carbon (%C) and

Percent Nitrogen (%N) were determined from each subsample using a Thermo Scientific Flash 1112 Elemental Analyzer.

The mineral content of each leaf sample was determined by burning a subsample (0.1 g to to 1 g) of leaf powder in a muffle furnace at 400 °C for 8 hours to obtain ash, then dividing the ash weight by the subsample's dry weight.

Construction cost calculation

The following equation modified by Poorter 1994 was used to calculate the biomass construction costs of all leaf samples:

$$CC = (-1.041 + 5.077 * C_{om}) * (1 - M) * (5.325 * N_{org})$$

in which CC is the total stored energy cost to produce one gram of plant biomass (g glucose per g dry weight), C_{om} is the carbon content of the organic biomass (%C, g/g), M is the mineral content (derived from %Ash, g/g), and N_{org} is the nitrogen content (%N, g/g).

CC values derived in grams glucose per grams dry weight (g/g) were then converted to CC values in grams glucose per square meter (g/m^2) by multiplying CC in g/g by LMA for each sample.

Statistical analysis

R Studio and the Interactive Tree of Life program were utilized to build a phylogeny of all trees and shrubs sampled (Figure 1). QGIS mapping software was utilized to map the locations of all trees and shrubs sampled in 2018 (Figure 2)**.** An ANOVA was used to compare

CC of native, nonaggressive invasive, and aggressive invasive trees, and an ANOVA was also used to compare CC of *A. rubrum* trees sampled in different locations in the intraspecies sub-survey. Welch two-sample t-tests were performed to compare CC of all other groupings: native vs. invasive shrubs, trees vs. shrubs, all natives vs. all invasives. All statistical calculations were performed using both CC in g/g and CC in g/m^2 values.

Table 1: All species sampled in this survey in both sampling seasons (June 2017 and June 2018), organized by classification: native trees, emerging invasive/range expansion trees, widespread invasive trees, native shrubs, widespread invasive shrubs. Table shows the number of leaf samples collected for each species.

Classification		Species			Number of leaf samples collected		
		Common name	Scientific name	Abbreviation	2017	2018	Total
Trees	Native trees	Striped maple	*Acer pensylvanicum*	APE	6	0	6
		Red maple	*Acer rubrum*	AR	13	44	57
		Sugar maple	*Acer saccarhum*	AS	12	6	18
		American chestnut	*Castanea dentata*	CD	5	2	7
		American beech	*Fagus grandifolia*	FG	6	6	12
		White ash	*Fraxinus americana*	FA	0	6	6
		Tulip poplar	*Liriodendron tulipifera*	LT	5	0	5
		White oak	*Quercus alba*	QA	9	6	15
		Bear oak	*Quercus ilicifolia*	QI	7	6	13
		Chestnut oak	*Quercus montana*	QM	8	6	14
		Red oak	*Quercus rubra*	QR	13	6	19
		Black oak	*Quercus velutina*	QV	7	6	13
		Black gum	*Nyssa sylvatica*	NS	8	7	15
	Emerging invasive/ range expansion trees	Southern catalpa	*Catalpa bignonioides*	CB	6	0	6
		Red mulberry	*Morus rubra*	MR	6	0	6
		Eastern cottonwood	*Populus deltoides*	PD	6	6	12
		Willow hybrid	*Salix*	S?	0	6	6
		Slippery elm	*Ulmus rubra*	UR	6	6	12
		Winged elm	*Ulmus alata*	UA	0	6	6
	Widespread invasive trees	Norway maple	*Acer platanoides*	APL	0	6	6
		Tree-of-heaven	*Ailanthus altissima*	AA	6	6	12
		Black locust	*Robinia pseudoacacia*	RP	0	6	6
Shrubs	Native shrubs	Huckleberry	*Gaylussacia baccata*	GB	0	6	6
		American witch hazel	*Hamamelis virginiana*	HV	3	0	3
		Mountain laurel	*Kalmia latifolia*	KL	0	6	6
		Highbush blueberry	*Vaccinium corymbosum*	VC	0	6	6
		Late lowbush blueberry	*Vaccinium pallidum*	VP	0	6	6
		Deerberry	*Vaccinium stamineum*	VS	0	6	6
		Mapleleaf viburnum	*Viburnum acerifolium*	VAS	0	6	6
	Widespread invasive shrubs	Angelica tree	*Aralia elata*	AE	0	6	6
		Japanese barberry	*Barberis thunbergii*	BT	0	6	6
		Burning bush	*Euonymus alatus*	EA	0	6	6
		Multiflora rose	*Rosa multiflora*	RM	0	6	6

Table 2: All locations where red maple *(A. rubrum)* was sampled in the 2018 intraspecies survey, including the number of samples collected at each site, mean CC expressed in mass, and mean CC expressed in area.

Location	Location abbreviation	Number of leaf samples collected	CC average (g/g)	CC STDEV (g/g)	CC average (g/m^2)	CC STDEV (g/m^2)
Glycerine Hollow Upper Watershed	GHU	6	1.531	0.072	75.108	9.066
Honey Hill Peak	HH	5	1.486	0.253	76.495	13.753
Stone House	SH	6	1.635	0.102	75.813	12.485
Continental Road	CR	6	1.539	0.332	84.407	21.179
Glycerine Hollow Lower Watershed	GHL	6	1.286	0.307	45.684	11.399
Off-road by Upper Reservoir	UR	5	1.583	0.262	65.027	25.660
Off-road by White Oak	WO	5	1.472	0.069	109.575	20.355
Sphagnum Pond	SP	5	1.502	0.017	98.695	13.382

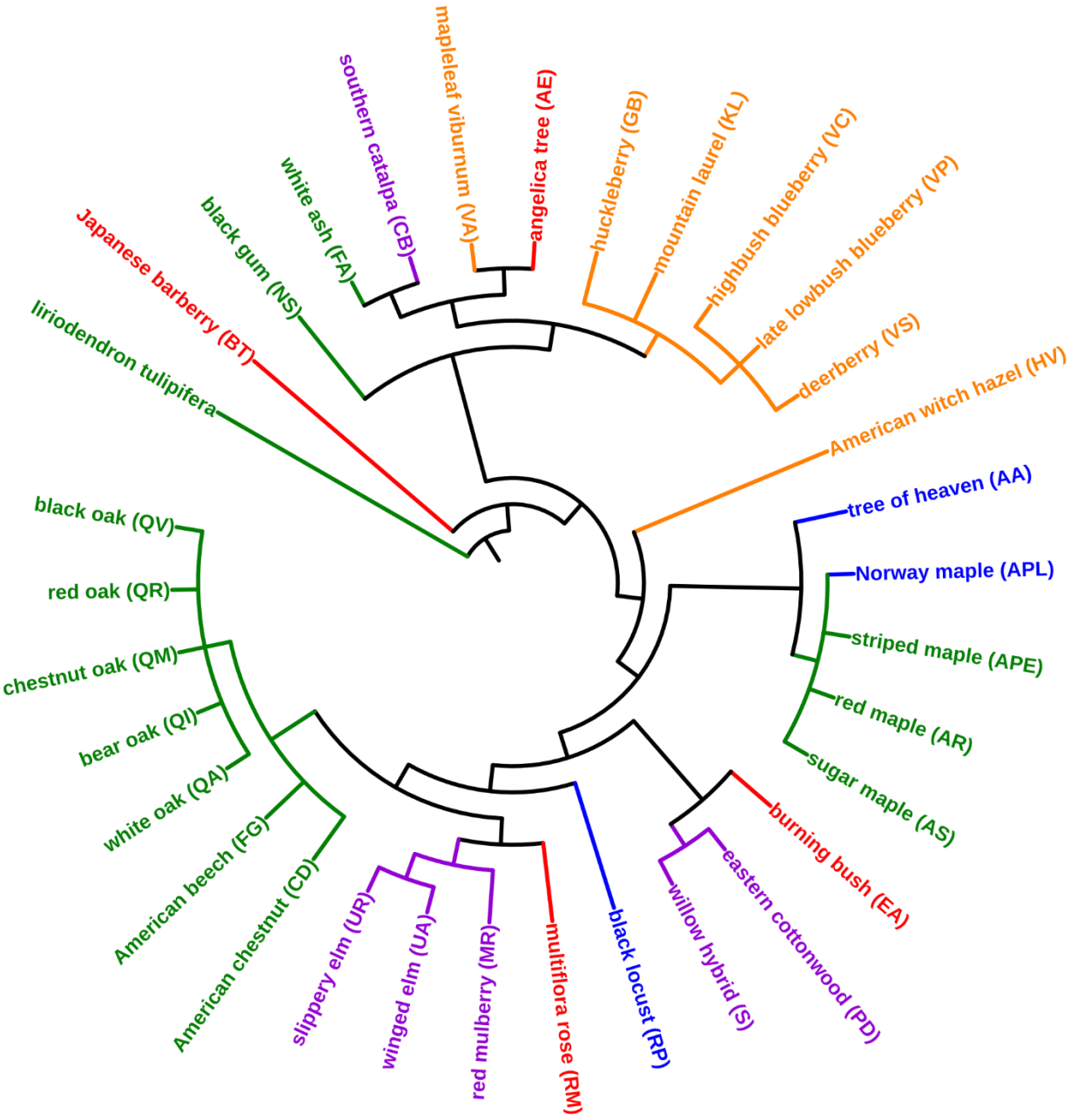


Figure 1: A phylogeny of all species sampled in 2017 and 2018. Species are color coded by classification:

Native trees
Expanding invasive/range expansion trees
Widespread invasive trees
Native shrubs
Widespread invasive shrubs

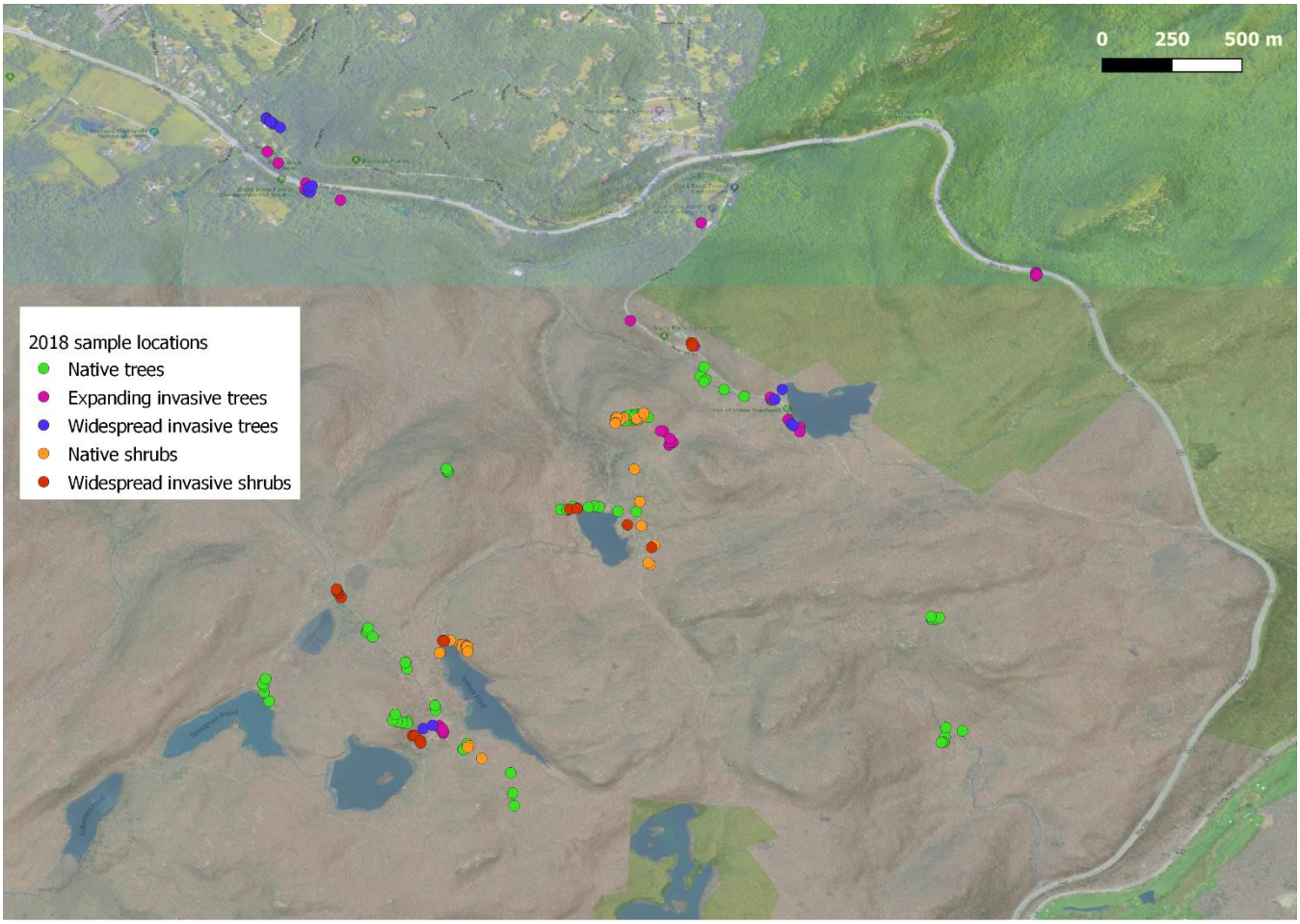


Figure 2: Locations of all individual trees and shrubs sampled in June 2018; location data was collected using the Avenza Maps GIS application and mapped using QGIS software.

Results

Over the course of two sampling seasons, 340 leaf samples were collected from 33 tree and shrub species, including representatives of both native and invasive species in each group. The results of this leaf survey demonstrate that average CC of native species is significantly higher than average CC of invasive species, with a more significant difference when CC is calculated in g/m^2.

Biomass construction costs of BRF tree species

22 deciduous tree species were sampled, including 13 native tree species, 6 nonaggressive invasive/range expansion tree species, and 3 widespread invasive tree species. For each species, 5 to 10 representative leaf samples were collected in a given season; some trees were sampled only in 2017, some only in 2018, and some both years (Table 1). Notable exceptions to this sampling method are American chestnut and red maple. Only 7 samples total were collected of American chestnut *(Castanea dentata),* as only 7 trees of this species could be found at Black Rock Forest; this species is almost extinct in North American forests (Thompson et al. 2013). Over 50 samples were collected of red maple *(A. rubrum)* as this species was surveyed for intraspecies analysis (see subsection 3).

Average biomass construction costs of the 22 tree species sampled ranged from 1.82 ± 0.07 g/g (willow hybrid, *Salix)* to 1.24 ± 0.09 g/g (tulip poplar, *Liriodendron tulipifera*) (Table 3, Figure 3). When converted to g/m^2, average construction costs ranged from 175.30 ± 26.40 g/m^2 (bear oak, *Quercus ilcifolia)* to 38.67 ± 9.56 g/m^2 (Norway maple, *Acer platanoides)* (Figure 4). The average biomass construction cost of the trees classified as native was 1.53 ± 0.19 g/g or

81.18 ± 40.47 g/m^2; the average CC of the trees classified as nonaggressive invasive/range expansion was 1.52 ± 0.20 g/g or 79.37 ± 26.12 g/m^2; and the average CC of the trees classified as widespread invasive was 1.47 ± 0.16 g/g or 54.04 ± 13.32 g/m^2 (Figure 5).

When CC was calculated in terms of mass, there was no significant difference between the three groups (ANOVA: F=1.589, classification df=2, residual df=258, p=0.206). However, when CC was calculated in terms of area, there were differences between the groups (ANOVA: F=6.389, classification df=2, residual df=258, p=0.00196): Tukey's HSD test found that both the native trees and the range expansion trees had significantly higher CCs than the widespread invasive trees (native-widespread invasive: p=0.0012; range expansion-expanding invasive: p=0.0254).

Biomass construction costs of BRF shrub species

11 shrub species were sampled, including 7 native species and 4 widespread invasive species (Table 1). 3 representative leaf samples of American witch hazel *(Hamamelis virginiana)* were collected in June 2017. All other shrub species were sampled in June 2018; 6 representative leaf samples were collected of each species.

Average biomass construction costs of the 11 shrub species sampled ranged from 1.96 ± 0.25 g/g (huckleberry, *Gaylussacia baccata)* to 1.34 ± 0.11 g/g (maple-leaf viburnum, *Viburnum acerifolium)* (Table 3). When converted to g/m^2, average construction costs ranged from 228.18 ± 75.9 g/m^2 (mountain laurel, *Kalmia latifolia)* to 35.99 ± 5.92 g/m^2 (mapleleaf viburnum, *V. acerifolium)* (Figure 6). The average CC of the shrubs classified as native was 1.61 ± 0.23 g/g or

95.35 ± 27.51 g/m^2; the average CC of the shrubs classified as widespread invasive was 1.55 ± 0.18 g/g or 61.20 ± 16.79 g/m^2 (Figure 7).

When CC was calculated in terms of mass, there was no significant difference between groups (Welch Two Sample t-test: t=0.83, df=56.39, p=0.41). But when CC was calculated in terms of area, the CC of the native shrub species was found to be significantly higher than the CC of the invasive shrub species (Welch Two Sample t-test: t=2.506, df=39.5, p=0.016.) However, when the significant outlier *K. latifolia* was excluded (see Figure 6), the average native shrub CC dropped to 68.78 ± 18.50 g/m^2; this mean was not significantly different from the mean of the widespread invasive shrubs (Welch Two Sample t-test: t=0.69, df = 50.08, p= 0.49).

Biomass construction costs compared within species: red maple *(A. rubrum)* across habitats

An intraspecies survey was conducted in 2018: 44 samples of red maple *(A. rubrum)* were collected from 8 different locations at Black Rock Forest (Table 2). When calculated in terms of mass, average biomass construction costs of the *A. rubrum* trees at these 8 locations ranged from 1.64 ± 0.10 g/g (Stone House) to 1.29 ± 0.31 (Glycerine Hollow lower watershed) (Figure 8). When calculated in terms of area, average CC ranged from 109.57 ± 20.36 g/m^2 (White Oak) to 45.68 ± 11.40 g/m^2 (Glycerine Hollow lower watershed) (Figure 9). There was significant variation among CC (calculated in g/m^2) for *A. rubrum* trees sampled at these different locations (ANOVA: F=5.515, location df=7, residual df=31, p=0.00035).

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Table 3: Means and standard deviations of biomass construction costs (CC) for all tree and shrub species sampled at Black Rock Forest. CC is defined as the amount of stored glucose required to construct one unit of leaf material, and is calculated in both mass (g/g) and area (g/m^2). Species are sorted by classification.

Classification	Common name	Abbreviation	CC average (g/g)	CC STDEV (g/g)	CC average (g/m^2)	CC STDEV (g/m^2)
Native trees	Bear oak	QI	1.790	0.258	175.303	26.398
	Red oak	QR	1.706	0.132	132.965	26.991
	Black oak	QV	1.784	0.189	114.328	13.471
	Chestnut oak	QM	1.620	0.063	103.486	17.069
	White oak	QA	1.550	0.090	92.548	12.245
	Red maple	AR	1.525	0.234	73.712	20.587
	American chestnut	CD	1.468	0.096	58.998	16.324
	White ash	FA	1.261	0.146	57.639	12.001
	American beech	FG	1.452	0.129	57.496	27.324
	Sugar maple	AS	1.719	0.306	55.790	13.995
	Tulip poplar	LT	1.244	0.085	43.553	8.725
	Striped maple	APE	1.379	0.058	42.437	6.792
	Black gum	NS	1.369	0.073	47.061	24.650
Expanding invasive trees	Willow hybrid	S?	1.820	0.066	108.077	14.313
	Eastern cottonwood	PD	1.659	0.184	99.159	27.460
	Winged elm	UA	1.548	0.271	98.813	37.301
	Slippery elm	UR	1.465	0.315	64.644	20.174
	Southern catalpa	CB	1.315	0.031	62.314	11.485
	Red mulberry	MR	1.322	0.105	43.187	5.745
Widespread invasive trees	Black locust	RP	1.629	0.074	61.889	16.057
	Tree of heaven	AA	1.474	0.190	61.570	10.420
	Norway maple	APL	1.314	0.045	38.660	9.559
Native shrubs	Mountain laurel	KL	1.690	0.043	228.184	75.903
	Huckleberry	GB	1.958	0.245	78.861	10.908
	Highbush blueberry	VC	1.736	0.602	78.413	32.003
	Late lowbush blueberry	VP	1.422	0.031	77.911	6.678
	Deerberry	VS	1.524	0.045	72.742	8.826
	Mapleleaf viburnum	VAS	1.336	0.111	35.989	5.919
Widespread invasive shrubs	Japanese barberry	BT	1.623	0.275	71.978	14.708
	Angelica tree	AE	1.765	0.318	69.523	14.433
	Burning bush	EA	1.417	0.058	67.099	12.227
	Multiflora rose	RM	1.386	0.091	36.194	11.454

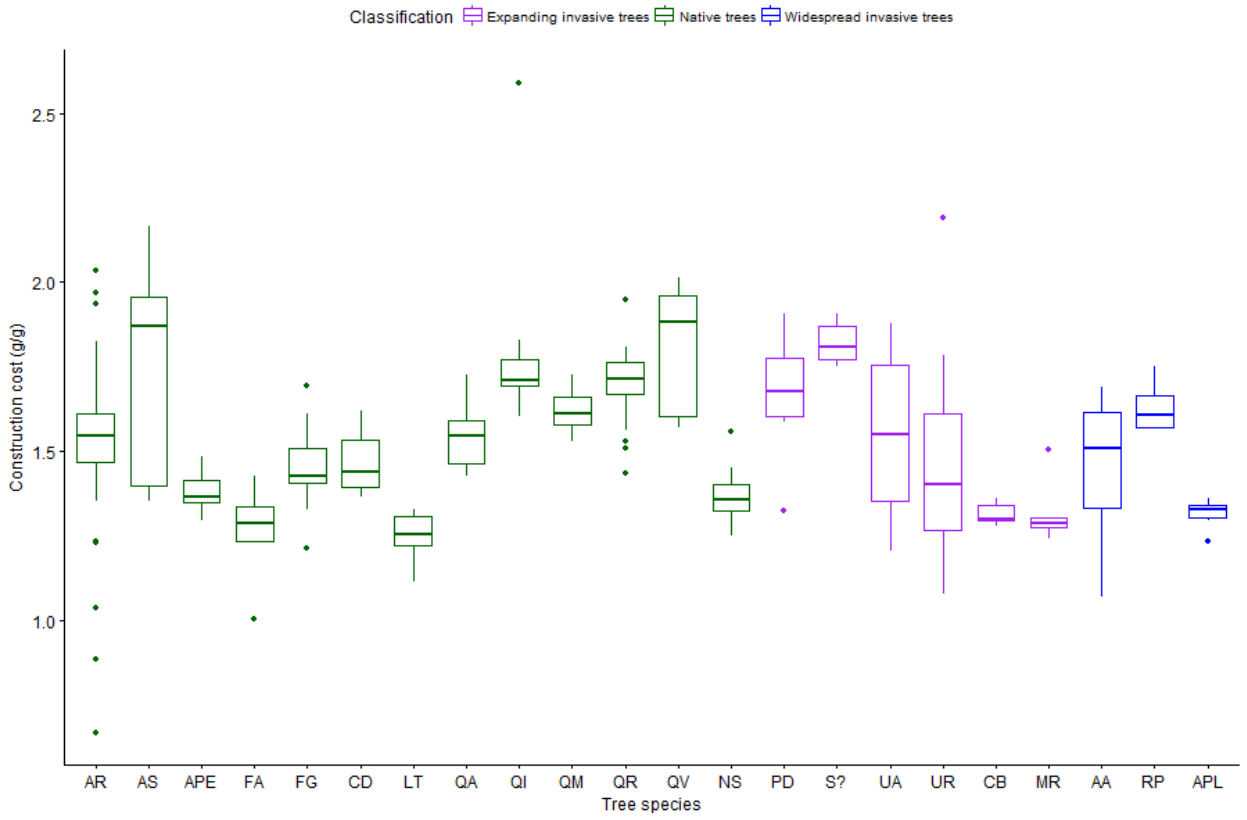


Figure 3: Box and whisker plot showing the biomass construction costs of deciduous tree species sampled at Black Rock Forest in June 2017 and June 2018, reported in terms of mass (g glucose required to produce g leaf material). Species codes on the *x* axis correspond to the initials of these species' scientific names, which are listed in Table 1 (eg. AR = *A. rubrum*, red maple). Species are categorized by classification: native trees (green), expanding invasive trees (purple), and widespread invasive trees (blue). Horizontal bars within boxes represent species means, boxes represent standard deviations, and points represent outliers.

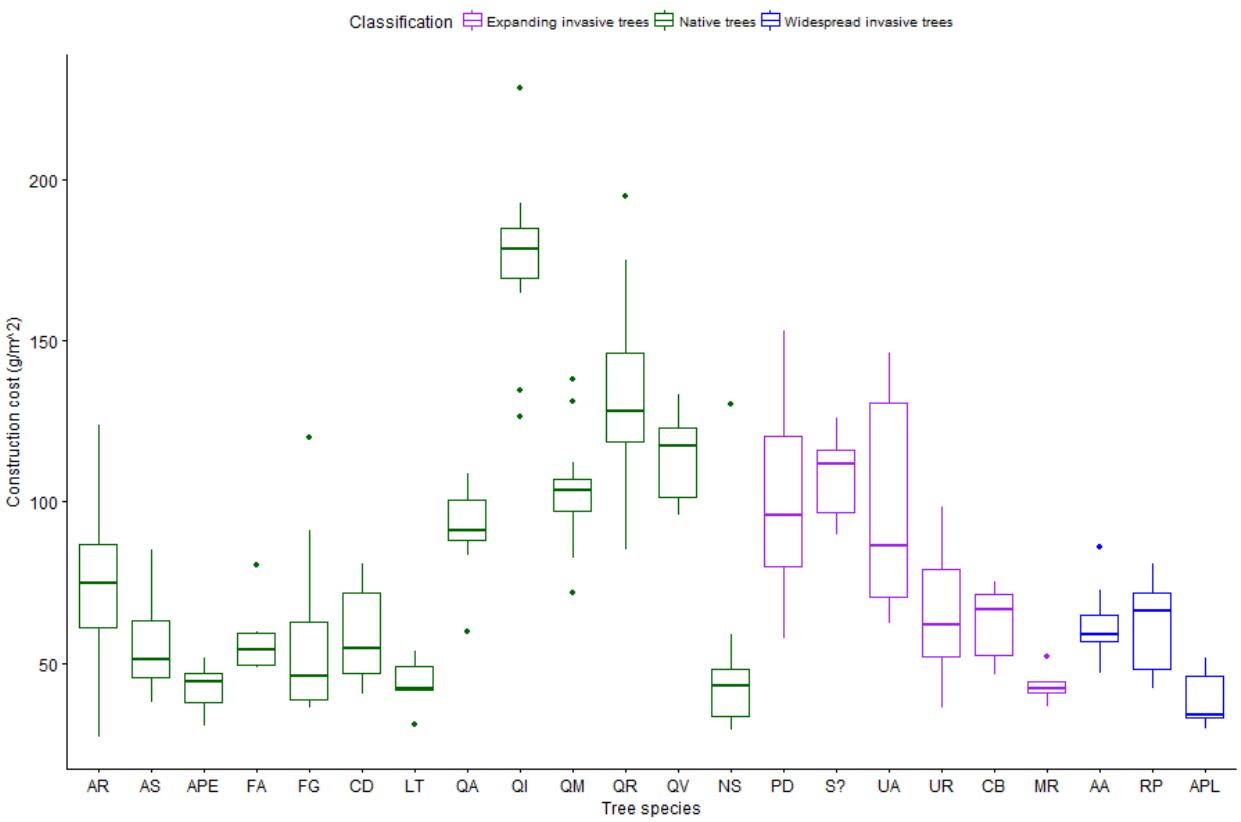


Figure 4: Box and whisker plot showing the biomass construction costs of deciduous tree species sampled at Black Rock Forest in June 2017 and June 2018, reported in terms of area (g glucose required to produce m^2 leaf material). Species codes on the *x* axis correspond to the initials of these species' scientific names (found in Table 1). Species are categorized by classification: native trees (green), expanding invasive trees (purple), and widespread invasive trees (blue). Horizontal bars within boxes represent species means, boxes represent standard deviations, and points represent outliers.

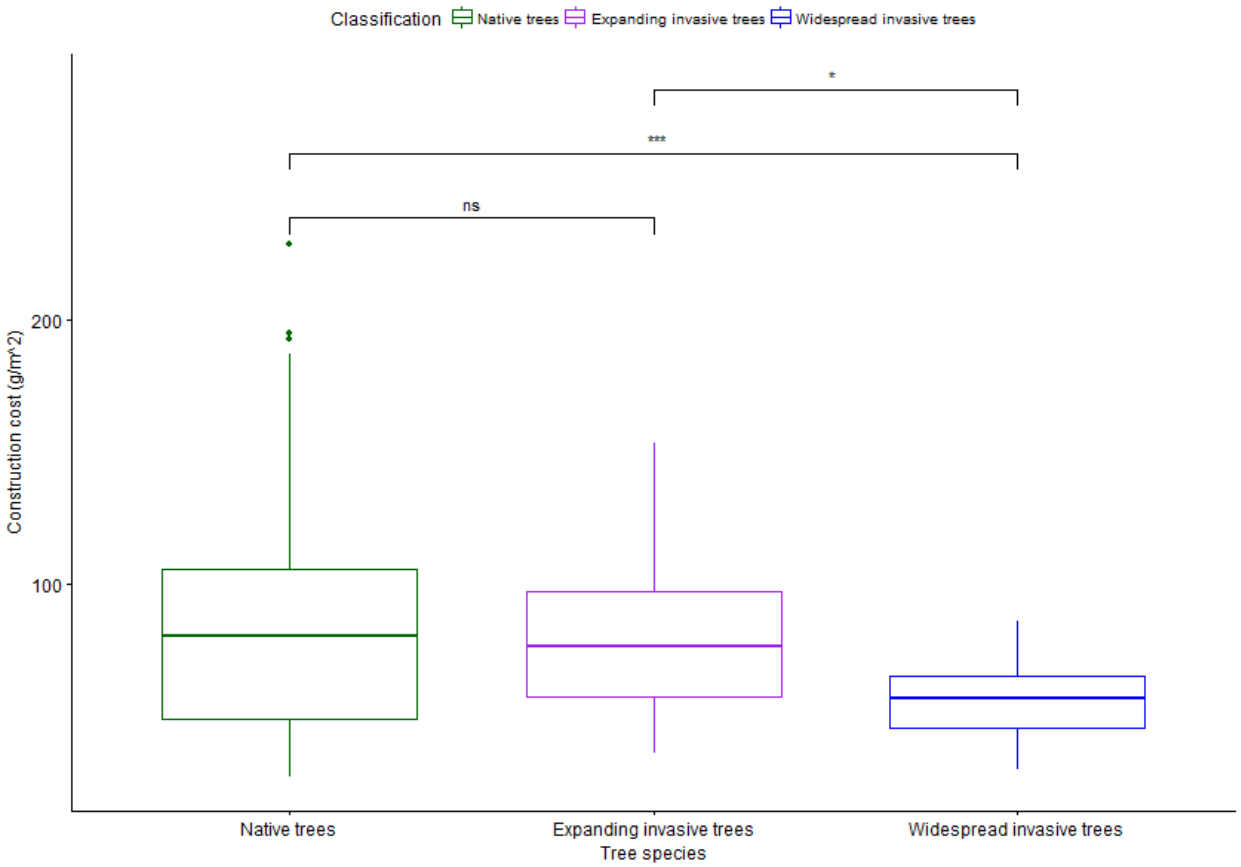


Figure 5: Box and whisker plot showing the biomass construction costs of three main categories of deciduous trees sampled at Black Rock Forest in June 2017 and June 2018, reported in terms of area (g glucose required to produce m^2 leaf material). Horizontal bars within the boxes represent category means, boxes represent standard deviations, and points represent outliers. Asterisks represent significant p values: * signifies that p is in the 0.05 range, and *** signifies that p is in the 0.001 range.

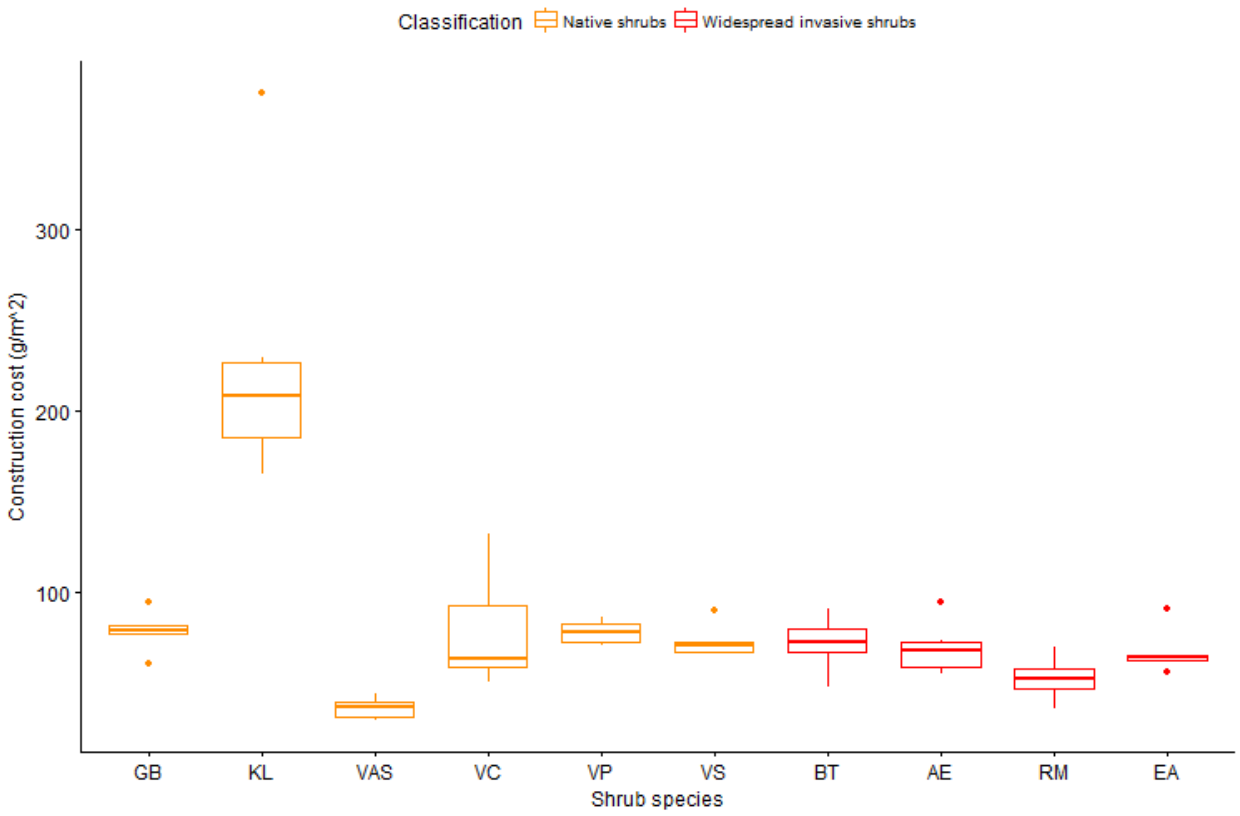


Figure 6: Box and whisker plot showing the biomass construction costs of deciduous shrub species sampled at Black Rock Forest in June 2017 and June 2018, reported in terms of area (g glucose required to produce m^2 leaf material). Species codes on the *x* axis correspond to the initials of these species' scientific names found in Table 1 (eg. GB = *G. baccata*, huckleberry). Species are categorized by classification: native shrubs (orange) and widespread invasive shrubs (red). Horizontal bars within boxes represent species means, boxes represent standard deviations, and points represent outliers. KL (*K. latifolia*, mountain laurel) is a prominent outlier.

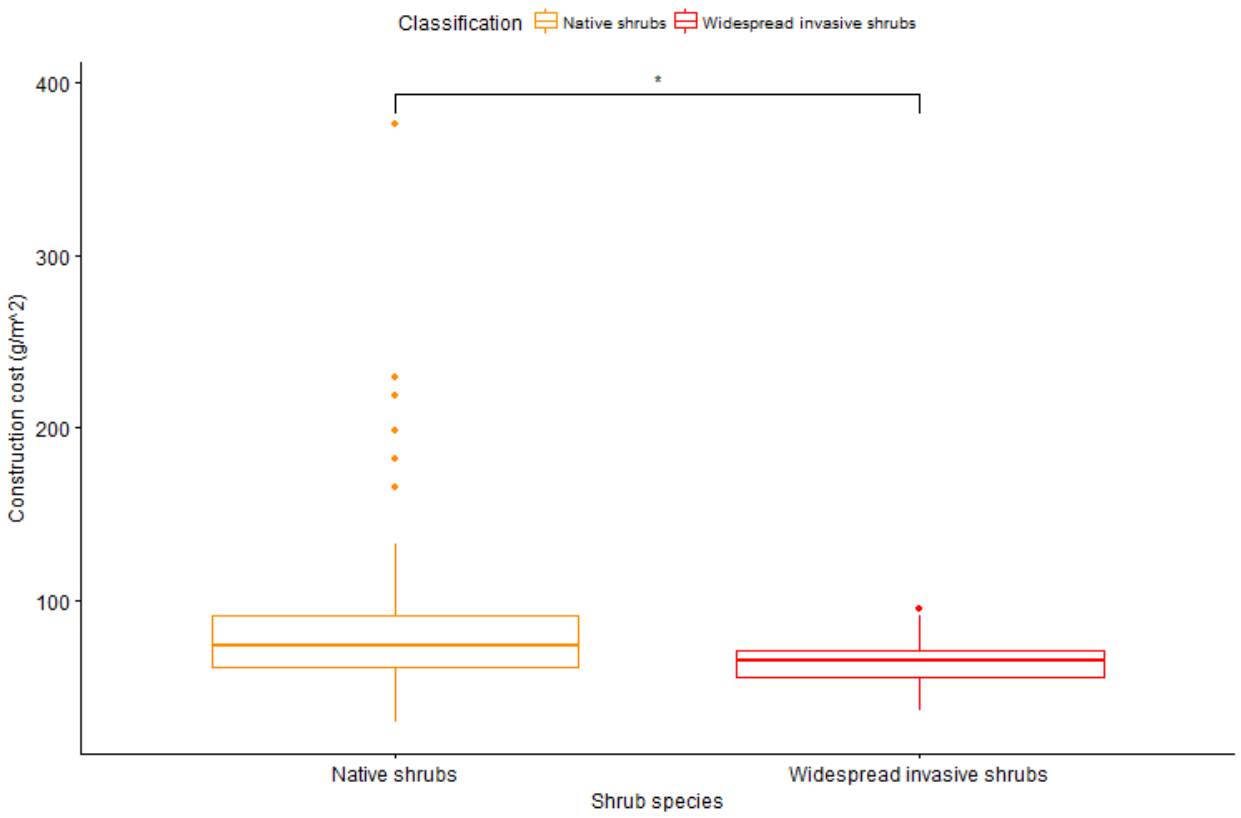


Figure 7: Box and whisker plot showing the biomass construction costs of two main categories of deciduous shrubs sampled at Black Rock Forest in June 2017 and June 2018, reported in terms of area (g glucose required to produce m^2 leaf material). Horizontal bars within the boxes represent category means, boxes represent standard deviations, and points represent outliers. The asterisk (*) represents $p<0.05$.

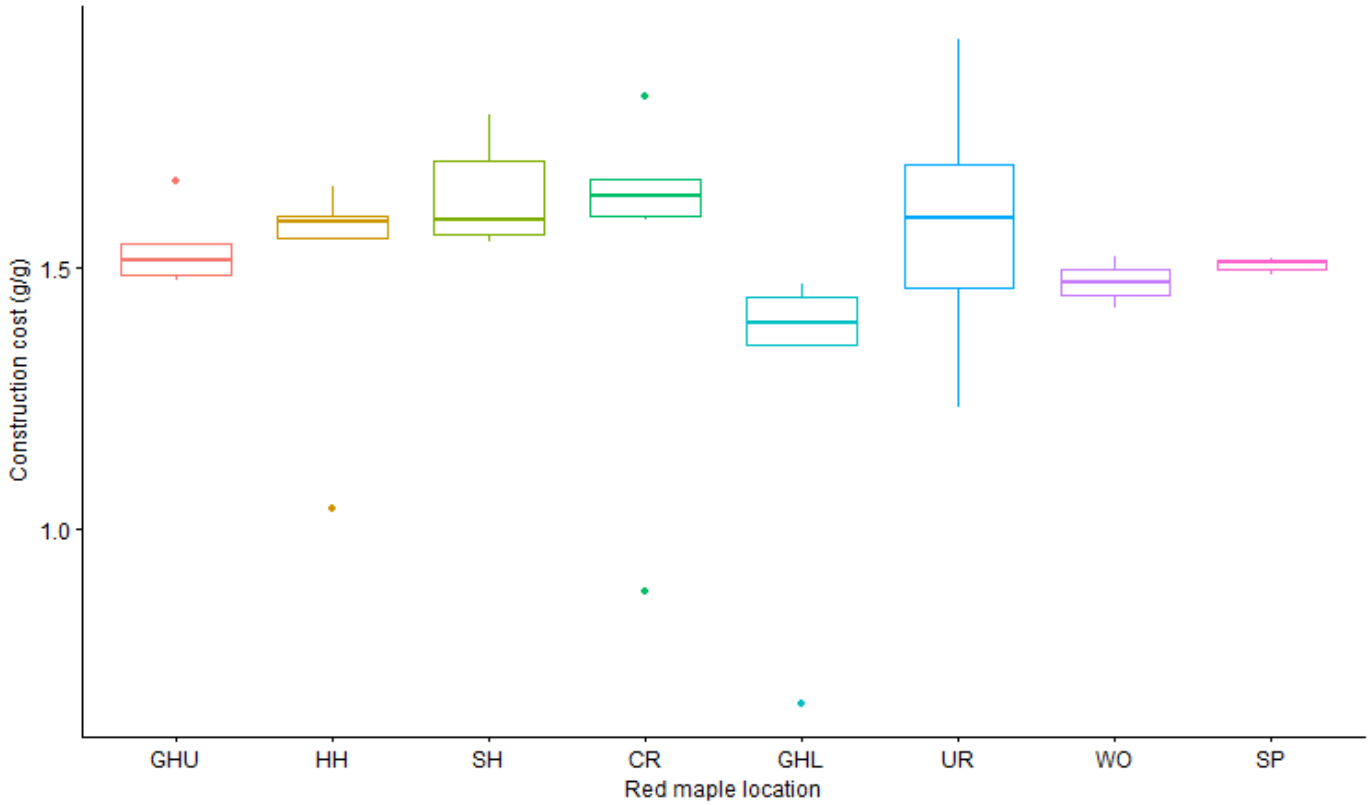


Figure 8: Box and whisker plot showing the biomass construction costs of red maple *(A. rubrum)* trees sampled in 8 different locations across Black Rock Forest in June 2018, reported in terms of mass (g glucose required to produce g leaf material). Codes on the *x* axis correspond to locations (see Table 2). Horizontal bars within the boxes represent location means, boxes represent standard deviations, and points represent outliers.

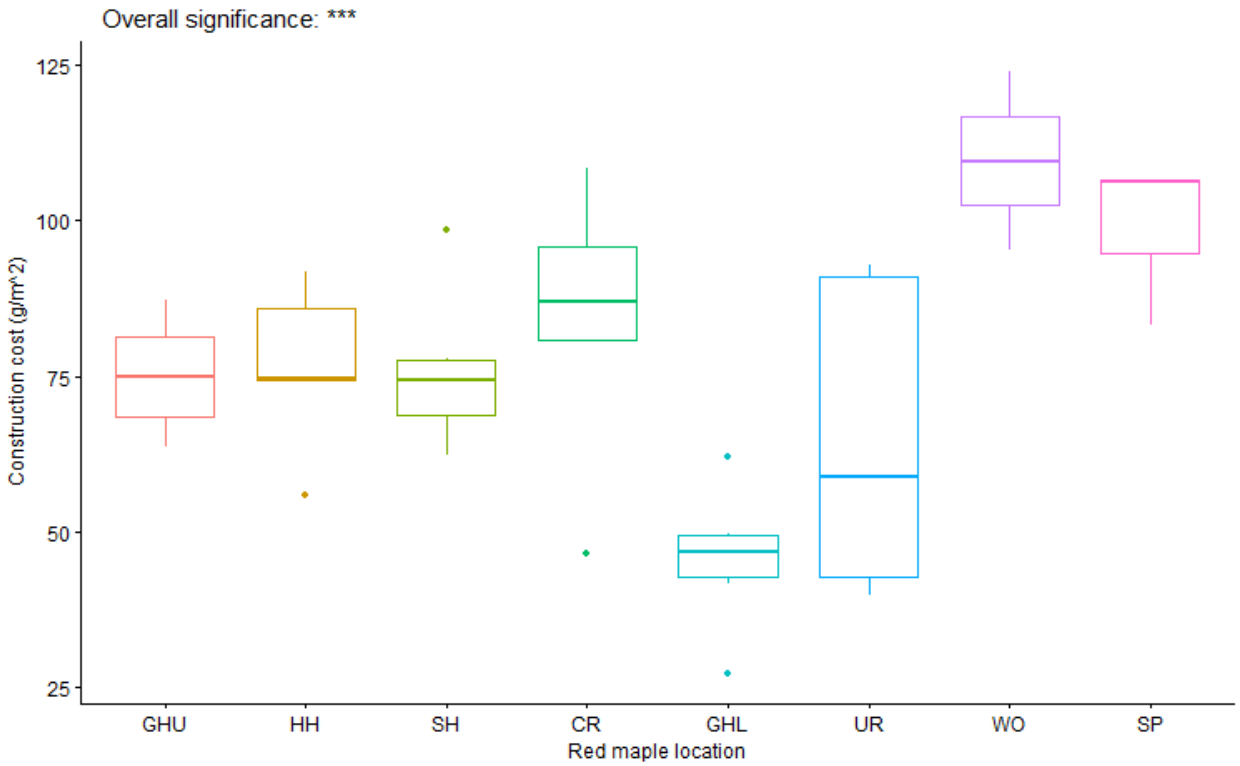


Figure 9: Box and whisker plot showing the biomass construction costs of red maple *(A. rubrum)* trees sampled in 8 different locations across Black Rock Forest in June 2018, reported in terms of area (g glucose required to produce m^2 leaf material). Codes on the *x* axis correspond to locations (see Table 2). Horizontal bars within boxes represent location means, boxes represent standard deviations, and points represent outliers. The three asterisks (***) represent $p<0.001$.

Discussion

This study set out to test the viability of the biomass construction cost metric as a method for examining trophic dynamics across a temperate forest ecosystem. Using the CC metric, the energy investment requirements of 33 deciduous tree and shrub species at Black Rock Forest were surveyed, with a focus on comparing plants of different ecological positions. The results of this survey demonstrate that, overall, the CCs of foreign invasive species are lower than those of both well-established native species and less well-established range expansion species. The results of this survey also demonstrate that plant species may significantly alter their energy investment strategy depending on environmental factors, as is shown by the differences in CC for *A. rubrum* at different locations. These differences in energy investment support our hypothesis: tree physiology is connected to ecological position.

The overall tree survey done in this study demonstrates a relationship between a tree's ecological role in a specific ecosystem (Black Rock Forest) and the means by which it utilizes stored energy resources to construct leaf material. This survey, similarly to the more specific energy investment studies described in the Introduction, suggests that native tree species that are well-adapted to a particular area appear to invest energy more carefully and grow more slowly in order to better compensate for environmental conditions, such as soil contents or access to sunlight. For example, bear oak (*Q. ilicifolia*), which had the highest average CC of the tree species (175.30 ± 26.40 g/m^2), occupies the highest, rockiest parts of the Forest where soils are thin; this high construction requirement suggests that this small tree needs to invest more stored energy to grow and prosper. On the other hand, aggressive species that have invaded Black Rock Forest from other environments have a lower energy barrier to construct leaf compounds, and seem to grow quickly in order to colonize new areas. For example, Norway maple *(A.*

platanoides), which had the lowest average CC of the tree species (38.67 ± 9.56 g/m^2), is found at the very edge of Black Rock Forest, along the highway; this tree appears to maximize the use of its large leaves in order to grow quickly.

Other tree survey results demonstrate a potential relationship between a tree's position in ecological succession and its growth strategy, while also underscoring the need for future investigation into the mechanisms behind such growth strategies. For example, all trees in the oak *(Quercus)* taxon had high CC (Figure 4); these species have been dominant at BRF and in other northeastern forests for decades, and hold secondary growth positions in these communities (Schuster et al. 2008, Thompson et al. 2013). Conversely, black gum (*Nyssa sylvatica),* has been found at BRF since the 1930s, but this species' low average CC (47.06 ± 24.65 g/m^2) suggests that it may still be a fast-growing primary-growth tree in this ecosystem (Schuster 2008). Also, interestingly, winged elm *(Ulmus alata)* had relatively high CC compared to other invasive species (98.81 ± 37.30 g/m^2). *U. alata* is native to the southeast U.S., has not been prominently introduced in the northeast, and was found at BRF for the first time during this survey, yet its CC is higher than that of maples and other native trees (Figure 4). This comparison suggests that *U. alata* may be a secondary growth species in its native environment, and may slowly move into inner forest locations at BRF ("Plant profile for *U. alata* (winged elm)" 2019). To better investigate this species' capacity for range expansion, it would be informative to directly compare the CC of *U. alata* individuals at a northeastern site such as BRF with the CC of *U. alata* individuals in this tree's native habitat in the south.

The differences between tree energy investments become more evident when CC is calculated in terms of leaf area as opposed to mass. This trend suggests that, while a gram of leaf material has similar costs across ecological positions, leaf morphology differs significantly

across species and impacts construction methods. Aerts and Chapin (1999) posit that a tradeoff exists between tissue size and lifespan: species that grow faster may acquire resources more quickly through large leaves or long root systems, but need to constantly expend energy building back lost biomass. In contrast, species that grow more slowly will retain their tissues for longer, thus allowing them to expend a comparable amount of energy in building as their more excitable cousins. Our results support such a tradeoff, through the similarities in CC range between native and invasive species and between species with different leaf sizes when this metric is calculated in terms of mass (Figure 3).

Our overall results are complicated by the results of this project's two smaller surveys: the investigation of shrub species and the intraspecies survey. The shrub species have a range of CC values in the same range as the tree CC values, leading us to wonder if a unit of biomass has a comparable cost for plants across taxonomic positions, not just for different trees. Also, even though the shrub class includes BRF's most aggressively invasive species (Japanese barberry, Angelica tree), there is only a significant difference between native and invasive shrubs when the outlier mountain laurel *(Kalmia latifolia)* is included in the ranks. *K. latifolia*'s high CC may be explained by properties of its leaves: these leaves are extremely thick and well known to contain toxic alkaloids, and are thus likely to be very costly for the plant to produce (Forbes and Bechdel 1931). Still, the overall similarity between native and invasive shrub CC leads us to ask, are BRF's native shrubs simply too small to have adapted morphological mechanisms for better utilizing this environment's resources? Or is there some other broader similarity at play?

The results of the *A. rubrum* intraspecies survey additionally complicate ecological patterns, as these results demonstrate that a single species may significantly change its method of energy investment depending on where it is living. Our intraspecies survey results support the

results of Nagel et al. (2002): these authors found differences in CC between *A. rubrum* populations in upper and lower parts of BRF's Glycerine Hollow (two of the same sampling locations utilized in our survey), and connected these CC differences to differences in light availability and soil quality between the sites. True, *A. rubrum* is more flexible than most species at BRF, but these results still caution us from making general assumptions about the resource deployment of particular species. Resource use, then, may not be tied just to taxonomy, but also to environmental pressures. Oak species that live in rockier areas may be hardier because they have adapted to those areas; light-loving species like *Ailanthus altissima* may hug the road in order to reach to the open sunlight; and species like *A. rubrum* have the plasticity to adapt to these spaces and a variety of environments in between.

Future studies could use the construction cost metric to further investigate the connection between ecological niches and physiological traits. One may more closely examine the mechanisms for energy investment in species with notably high or low CC: what specific compounds, defense mechanisms, resource acquisition strategies, etc. are they employing? Such physiological understanding would enhance understanding of the ecological positions set up by this study. Future studies may also conduct further intraspecies comparisons. This study collected leaves primarily from adult, productive parts of trees during the peak of the summer season; other research may compare energy investment during this season to investment during other seasons, in other parts of the tree, or across other life stages of a single species. Future environmental studies would additionally be useful in connecting CC measurements to abiotic habitat characteristics such as elevation and soil composition.

This study was not only successful in its results, but also in its mere scope; over 300 leaf samples were collected and analytically processed, with the majority of work taking place over

the course of two field seasons. The most recent survey which examined plant energy requirements on a similar scale was Merino (1987); Merino determined leaf growth and maintenance costs for 46 tree, shrub, and vine species in central and southern Spain, using much older analytical techniques than those used in our BRF survey. With such a large quantity of samples, error is unfortunately a likely hazard. This project was hindered by the challenge of identifying species in the field at BRF, particularly range expansion species such as *U. rubra*, which were neither common through the forest nor located in specific disturbed areas. Analytical data acquisition brought challenges as well, as the Elemental Analyzer used to measure carbon and nitrogen content of leaf samples frequently required maintenance during the time this study took place and often became unreliable towards the end of a long sample run. However, we hope to verify our current results through additional elemental analysis of all samples.

In order to protect our forests from oncoming natural and man-made disasters, we must understand the relationships between the different organisms within these forests, and how those relationships change over time. Through painting a snapshot of Black Rock Forest via its tree energy investments, this survey examines how the trees of this forest engage with their surroundings and with each other. If studies such as these continue, trees may be increasingly considered as active responders to their environments, and the constant upheaval of ecological succession may become a better understood phenomenon.

References

Aerts, R., & Chapin III, F. S. 1999. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. In Advances in Ecological Research (Vol. 30, pp. 1-67). Academic Press.

Barringer, K., and S. E. Clemants. 2003. The vascular flora of Black Rock Forest, Cornwall, New York. Journal of the Torrey Botanical Society 130:292-308.

Boyd, J. N., C.-Y. Xu, and K. L. Griffin. 2009. Cost-effectiveness of leaf energy and resource investment of invasive *Berberis thunbergii* and co-occurring native shrubs. Canadian Journal of Forest Research 39:2109–2118.

Didham, R. K., J. M. Tylianakis, N. J. Gemmell, T. A. Rand, and R. M. Ewers. 2007. Interactive effects of habitat modification and species invasion on native species decline. Trends in Ecology & Evolution 22:489–496.

Forbes, E. B., and S. I. Bechdel. 1931. Mountain laurel and rhododendron as foods for the white tailed deer. Ecology 12:323–333.

Griffin, K. L. 1994. Calorimetric estimates of construction cost and their use in ecological studies. Functional Ecology 8:551–562.

Horton, R., G. Yohe, W. Easterling, R. Kates, M. Ruth, E. Sussman, A. Whelchel, D. Wolfe, and F. Lipschultz. 2014. Ch. 16: Northeast. Pages 371–395 Climate Change Impacts in the United States: The Third National Climate Assessment. U.S. Global Change Research Program.

Luken, J. O. 1990. Directing Ecological Succession. Springer Science & Business Media.

Merino, J. 1987. The costs of growing and maintaining leaves of mediterranean plants. Pages 553–564 *in* J. D. Tenhunen, F. M. Catarino, O. L. Lange, and W. C. Oechel, editors. Plant Response to Stress. Springer Berlin Heidelberg.

Munson, M., A. Kimple, and K. Barringer. 2011. Trees of Black Rock Forest. Black Rock Forest Consortium.

Nagel, J. M., and K. L. Griffin. 2001. Construction cost and invasive potential: comparing *Lythrum salicaria (Lythraceae)* with co-occurring native species along pond banks. American Journal of Botany 88:2252–2258.

Nagel, J. M., K. L. Griffin, W. S. F. Schuster, D. T. Tissue, M. H. Turnbull, K. J. Brown, and D. Whitehead. 2002. Energy investment in leaves of red maple and co-occurring oaks within a forested watershed. Tree Physiology 22:859–867.

Peterson, R. T., G. A. Petrides, and J. Wehn. 1998. A field guide to Eastern trees: Eastern United States and Canada, including the Midwest. Houghton Mifflin.

Plant profile for *Ulmus alata* (winged elm). 2019. . https://plants.usda.gov/core/profile?symbol=ULAL.

Poorter, H. (n.d.). Construction costs and payback time of biomass: a whole plant perspective:17.

Rohleder, L., D. Atha, D. Decker, T. Johansson, T. Lewis, T. Wenskus, M. Young, C. Mangels, J. Michelson, M. Marquand, and T. Rosenstock. 2018, February 12. Invasive species categorization for the Lower Hudson. Lower Hudson PRISM.

Schuster, W. S. F., K. L. Griffin, H. Roth, M. H. Turnbull, D. Whitehead, and D. T. Tissue. 2008. Changes in composition, structure and aboveground biomass over seventy-six years (1930-2006) in the Black Rock Forest, Hudson Highlands, southeastern New York State. Tree Physiology 28:537–549.

Song, L.-Y., C.-H. Li, and S.-L. Peng. 2010. Elevated CO2 increases energy-use efficiency of invasive *Wedelia trilobata* over its indigenous congener. Biological Invasions 12:1221–1230.

Thompson, J. R., D. N. Carpenter, C. V. Cogbill, and D. R. Foster. 2013. Four centuries of change in northeastern United States forests. PLoS ONE 8(9): e72540.

Wang, W.-B., R.-F. Wang, Y.-B. Lei, C. Liu, L.-H. Han, X.-D. Shi, and Y.-L. Feng. 2013. High resource capture and use efficiency and prolonged growth season contribute to invasiveness of *Eupatorium adenophorum*. Plant Ecology 214:857–868.

Warrach, K., M. Stieglitz, J. Shaman, V. C. Engel, and K. L. Griffin. 2006. Twentieth century climate in the New York Hudson Highlands and the potential impacts on eco-hydrological processes. Climatic Change 75:455–493.

Williams, K., F. Percival, J. Merino, and H. A. Mooney. 1987. Estimation of tissue construction cost from heat of combustion and organic nitrogen content. Plant, Cell & Environment 10:725–734.