To Burn or Not to Burn Oriental Bittersweet: A Fire Manager's Conundrum

Third Report
September 30, 2011

Noel B. Pavlovic[1]
Stacey A. Leicht-Young[2]
Dan Morford[3]
Neal Mulconrey[3]

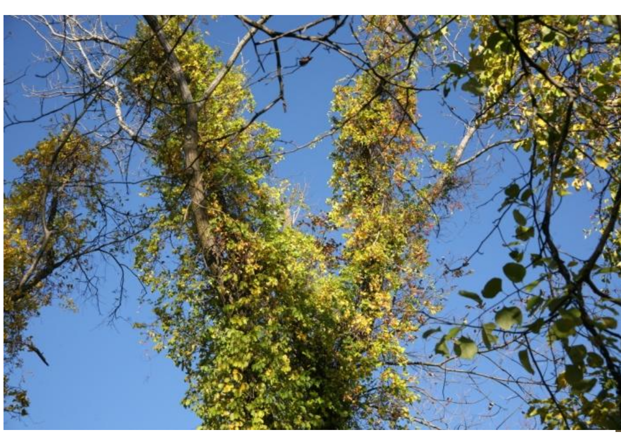
[1] Lake Michigan Ecological Research Station
Great Lakes Science Center
U.S. Geological Survey
1100 N. Mineral Springs Rd.
Porter, IN 4630

[2]Research Ecologist
Providence, Rhode Island

[3]Indiana Dunes National Lakeshore
National Park Service
1100 N. Mineral Springs Rd.
Porter, IN 46304

For

The Joint Fire Science Program



Introduction

This is the third progress report detailing the research about Oriental bittersweet (*Celastrus orbiculatus*) and fire which has been ongoing for three years. We highlight the further results from three components of the study: 1) Susceptibility of different habitats to invasion of Oriental bittersweet, 2) The impact of fire on established individuals of Oriental bittersweet, and 3) Modeling Oriental bittersweet invasion proliferation and presence on the basis of fire history and vegetation.

Project Justification

Oriental bittersweet (*Celastrus orbiculatus*) is a highly invasive liana (woody vine) that occurs throughout the Eastern United States. This twining plant can blanket and girdle adjacent vegetation, affecting succession and damaging trees. In areas where prescribed fire is a management tool, the response of Oriental bittersweet to fire needs to be quantified, rather than relying on anecdotal evidence. Currently, in areas already infested with this species, there are no strategies for prioritizing the efficacy of pre- or post-fire treatments on Oriental bittersweet. This largely results from a lack of understanding of the nature of post-fire resprouting by this species. Sprouting of bittersweet can at least double with fire and sprouts appear to respond to fire with an increase in growth rate (Pavlovic and Young pers. obs.). Beyond this basic need to understand the interaction between fire and Oriental bittersweet resprouting, we need to investigate how fire may interact with light, soil moisture, litter and other environmental factors to either increase or decrease abundance of this species. Finally, it is unknown how fire regimes influence the distribution of Oriental bittersweet on the landscape; thus we need to model the distribution of Oriental bittersweet in a fire impacted landscape. If we determine through our research that fire enhances the spread of this species, modification of fire suppression tactics and potential fire exclusion zones may be necessary. Thus we will be able to provide land managers throughout the Eastern US with data-driven decision support tools for more successful management of this species in fire dependent and invaded areas.

Background

Oriental bittersweet is an exotic woody vine, or liana, introduced from Asia as a horticultural plant into the northeastern United States in the 1860s. It is present in many habitats, including open dry foredunes, forest understories, oak savannas, "edge" habitats (Pavlovic and Young, pers. obs.), and eastern deciduous forests. This species continues to spread westward from the east coast of the US, and now can be found from Maine to Georgia and west to Minnesota, Iowa, Arkansas, and Missouri. Oriental bittersweet is of great concern to land managers because of its ability to completely blanket the vegetation that it uses as support for climbing, to overtop neighboring vegetation, girdle trees, weigh down tree limbs resulting in wind and ice damage, and to alter successional trajectories (Fike and Niering 1999). Vine diameters can reach as much as 18 cm after reaching canopy tree crowns (Leicht-Young et al. 2007). This species is particularly threatening because of its ability to invade high quality habitats and mature forests with low light levels at the ground layer (Leicht-Young et al. 2007, Leicht-Young and Pavlovic, pers. obs). Land managers of public lands such as Indiana Dunes National Lakeshore (INDU) wish to control Oriental bittersweet before it dominates as it has in much of the northeastern and mid-Atlantic regions of the United States, and to slow its westward expansion (McNab and Loftis 2002). The unquantified effect of fire on Oriental bittersweet adds another layer of complexity to a species that is already challenging to manage and is invading west into regions once covered by fire dominated prairies.

The spread of Oriental bittersweet even further west than its current distribution is likely because of its wide light and moisture tolerances, including an ability to grow in very dry conditions (Leicht-Young et al. 2007) and because of long-distance seed dispersal by birds. Finally, when one examines the native range of Oriental bittersweet in China, it has not yet reached the same latitudinal extent in the United States (Zheng et al. 2004).

There have been few published studies examining the effects of fire on invasive non-native plant species in the Eastern US (Dibble et al. 2007, Glasgow and Matlack 2007, Richburg et al. 2004). Most studies on fire in this region focus on the response of native species to fire (Elliott et al. 1999). Glasgow and Matlack (2007) made the observation that since many native species experience increased recruitment and growth in response to fire, there is no reason to believe that non-native invasive species would not respond in the same way.

Fire can promote recruitment of seedlings by removal of litter (Glasgow and Matlack 2007), enhancing nutrients and decreasing canopy cover (Elliott et al. 1999). Previous research has shown that fire increases germination of Japanese stiltgrass (*Microstegium vimineum)* and multiflora rose (*Rosa multiflora)* in Eastern forests (Glasgow and Matlack 2007). Oriental bittersweet seeds require cold stratification for germination, but little is known about fire temperature effects on these seeds. While Leicht-Young et al. (2007) demonstrated that Oriental bittersweet seedlings can survive and grow under a broad range of light levels, establishment from seeds across gradients in light and soil chemistry have not been examined. Whether fire creates ground layer light and soil conditions that increase the susceptibility of habitats to bittersweet establishment is unknown as well. Therefore we propose to examine burning and post fire effects on the germination, establishment and growth of Oriental bittersweet.

Fire may also enhance the spread of Oriental bittersweet by causing plants to sprout from root crowns, root fragments, and runners (Howard 2005). While prescribed fire is limited to sandy regions of the Northeast and mid-Atlantic region, it is a prevalent management tool for habitat restoration in the Midwest, Great Lakes region, and Southeast. This presents a potential conflict between using fire as a community restoration tool and promoting the spread of Oriental bittersweet. Howard (2005) states "there is no literature suggesting that fire can be used as a management tool to control Oriental bittersweet…However, wildfire that removes much of Oriental bittersweet's aboveground biomass may provide opportunities for other control measures." While sprouting in shrubs has been studied (Richburg et al. 2004, Gurvich et al. 2005), sprouting of vines such as Oriental bittersweet has been little documented except in tropical forests. Understanding how to manage vines in a more general sense is of increasing importance since researchers report that vines are increasing worldwide (Wright et al. 2004). Gerwing (2001) found that vines in the Amazon have significant effects on tree growth and that cutting was more effective in reducing vine impact than burning. Bebawi and Campbell (2002) determined that the season of fire had differing effects on mortality of the invasive rubber vine (*Cryptostegia grandiflora*) in Australia. Both studies showed that vines of different size classes experienced different levels of mortality when burned. Resource managers need critical information that quantifies resprouting ability of Oriental bittersweet at both the population and individual levels and determines which combinations of cutting and season of fire are most effective for its control.

Sprouting characteristics are ecologically and evolutionarily important trait that assist in understanding vegetation structure, function, and dynamics (Bond and Midgley 2001). After top killing, resprouting in plants occurs from the root crown, root caudex or roots. Research has shown that total nonstructural carbohydrates (TNC) in roots influences seasonal resprout growth rate and that burning when TNC reserves are low can increase mortality (Gurvich et al. 2005, Richburg et al. 2004). Thus, we will investigate the influence of fire on resprouting ability and on TNC by examining how these two factors may influence post-fire regeneration of Oriental bittersweet.

Landscape scale models have predicted Oriental bittersweet occurrence based on land use history and strength of association between correlated environmental and habitat variables. In the Appalachian Mountains, mesic forests tended to be invaded (McNab and Loftis 2002), whereas upland flats were more invaded in southern Illinois (Pande et al. 2007). Neither of these studies, however, examined how past and current fire regimes may influence Oriental bittersweet distribution on the landscape. Studies on other non-native invasive species have used fire regime as an explanatory variable. Floyd (2006) showed that depending on the habitat and soil characteristics, a location could be more prone to invasion after burning than if had not burned. We propose to model the relationship among fire regime, habitat and soil, and Oriental bittersweet distribution. As a result of the lack of systematically collected data on Oriental bittersweet, our research will make a major contribution to the problem of invasive non-native plant species in the Eastern US.

Project Objectives

We hypothesize that fire is an important facilitator of the spread and growth of Oriental bittersweet on the landscape. We will test this general hypothesis on Oriental bittersweet life history stages at scales up to the landscape level. More specifically, in our study we propose to:

1. Examine fire effects on the different life stages of Oriental bittersweet.Determine whether fire modifies the susceptibility of habitats to invasion by Oriental bittersweet.
2. Quantify the rate of Oriental bittersweet resprouting caused by fire compared to cutting to determine whether fire response is equivalent and whether both in combination can reduce TNC reserves.
3. Determine if growing season cutting or burning are more effective at controlling bittersweet compared to cutting or burning during the dormant season. We hypothesize that growing season burns (June) will have a greater negative effect on Oriental bittersweet than dormant season burns. Since soil productivity influences resprouting in woody plants (Iwasa and Kubo 1997), we will investigate how soil productivity influences Oriental bittersweet resprouting. In 2010 we added an ancillary experiment to determine the effects of herbicide and burning separately and in combination to better evaluate control strategies.
4. Model the presence and abundance of Oriental bittersweet in a fire mosaic landscape. From this project we will be able to identify the positive and negative interactions of fire with bittersweet life history and how that may translate into its distribution on the landscape.

The information derived from our proposed research will determine:

1. If fire affects recruitment of Oriental bittersweet from seed
2. If fire affects the growth rate of Oriental bittersweet
3. If pre-fire cutting or fire seasonality can reduce the positive response of Oriental bittersweet to fire
4. If past and current fire histories influence the current distribution of Oriental bittersweet on the landscape

In this third progress report, we present the remaining three studies below with the following headings:

I Fire and the susceptibility of invasion experiment
II Fire effects on established plants of Oriental bittersweet
III Predicting bittersweet presence and abundance in a fire mosaic landscape

Study Site

The Indiana Dunes National Lakeshore (INDU), a unit of the National Park Service (NPS), is a 6,000 hectare natural area at the southern tip of Lake Michigan, known for its high native biological diversity (Pavlovic and Bowles 1996). INDU is a mosaic of upland and wetland vegetation from dunes along the shore to wetlands and glacial moraine forests. Soils range from sands of low productivity to rich morainal clay soils. The dominant oak savanna-woodland complex is largely fire dependent, but large portions have experienced decades of fire suppression. This landscape is being invaded by Oriental bittersweet. Research burns commenced in 1986 and prescribed management burns have increased in frequency and coverage since 1992. Fire history is known for the 20th century derived from tree core analyses (Henderson and Long 1984). Historical fire maps for 28 years commencing in 1982 are available on GIS. While present in the landscape for at least 40 years (Pavlovic, pers. obs.), the highly invasive Oriental bittersweet is currently beyond the lag phase of invasion and is invading all units of the park. Therefore, the period of invasion matches the period for which detailed fire records are available permitting model development of fire effects history on Oriental bittersweet distribution.

I. Fire and the susceptibility of invasion experiment:
Methods

Experimental design

To test the extent to which fire makes different habitats susceptible to invasion by Oriental bittersweet, we sowed seeds in sand prairie or moraine prairie, oak savanna, oak hickory forest, beech maple forest, and sand oak forest in 12 randomized blocks in each community type (Table 1). A permit from INDU was obtained for this study with assurances that we would remove and kill the introduced plants at the termination of the study.

Blocks were selected in each area that lacked infestations of Oriental bittersweet. Most blocks were more than 30 m from each other when they occurred in the same habitat type and patch, except for one pair in Howes Prairie that were 8 m apart. Each block was 6 X 6 m with four 2 X 2 m nested treatment plots separated by 2 m buffer zones. Randomized treatments consisted of low intensity burn, high intensity burn, litter removal, and intact litter (6 habitats * 12 blocks * 4 plots = 288, Figure 1). See first paragraph in 'fire treatments' for explanation of treatments. Extreme winter weather and snow cover prevented us from establishing the plots until April 2009. The first snowfall arrived November 18, 2008 and the ground was frozen until late March. With the rapid onset of the fire season and the closure of burning on April 15 at the Heron Rookery and Mnoke Prairie we were unable to burn the oak hickory, sand oak forest, and beech maple plots in spring of 2009. The Heron Rookery and Mnoke Prairie were closed by the U.S. Fish and Wildlife Service to prevent fire impacts on the endangered Indiana Bat (*Myotis sodalis*). We conducted the burns in the sand savanna, sand prairie, and moraine prairie in spring of 2009. We conducted the burns in the oak hickory, beech maple, and oak forest in the fall of 2009, when the leaf litter was dry, undecomposed, and flammable. As in spring of 2009, we planted 25 seeds in each of the four subplots the week of April 26, 2010 within a randomly placed 25 cm x 25 cm plot. Within these plots we created a 5 x 5 grid with cells of 1 cm each to place each seed for easier relocation (Figure 2). Seedlings were monitored monthly through September. In 2010, an unusually wet spring and hot and dry summer probably influenced this set of plots (see results below). Rainfall in June 2010 was twice the average and the rest of the summer was hotter than average.

Fire treatments

To create the different fuel treatments, litter from the litter removal plot was placed on the high intensity plot to increase its fuel load. In the low intensity plots the fuel load was unmodified from what litter was present. On the day of the low-intensity and high-intensity burns, a 0.25 by 0.25 m litter sample was taken to assess fuel loads and moisture content in 2009 and 2010. Ten centimeter deep soil samples were taken from the low and high intensity burn plots prior to the burning to assess soil composition prior to the burns. The two meter buffers were weed whipped and raked prior to the burns as well. Our second set of burns was in the fall of 2009. Burns at Chellberg Farm and Heron Rookery were on November 5, the oak forest plots at Howes Prairie and the oak hickory plots at Mnoke Prairie were on November 6 and the West Beach oak forests on November 9. Litter was removed from the random sampling location, placed in a pre-weighed plastic bag and then reweighed. We then transferred the litter to a pre-weighed paper bag for drying at 70 ˚C for 12 hours. Dry weight was measured and these data were used to calculate litter biomass and percentage litter moisture. In each, plot temperature sensitive paint tags were deployed on a stake at 0, 0.5, 1, 1.5, 2.5, 3.5, 4.5 feet above the ground (Cole et al. 1992). Temperature paints were 250 ºF (121 ºC) , 300 (149 ºC), 400 (204 ºC), 500 (260 ºC), 600 (316 ºC), 700 (371 ºC), 800 (427 ºC), 900 (482 ºC), 1000 (538 ºC), 1200 (649ºC). During the burns rate of fire spread, flame height (minimum, maximum, and average), and wind speed and direction were recorded.

Table 1. Summary of number of plots in each experimental block, when plots were burned and when oriental bittersweet seeds were added

	Sand Prairie	Moraine Prairie	Sand Oak Savanna	Moraine Oak Hickory	Moraine Beech Maple Forest	Sand Oak Forest	Date of Burn	Date of Planting
Howes Prairie	6		4				Spring 2009	April 2009
						6	Fall 2009	April 2010
Mnoke Prairie		12		12			Spring 2009	April 2009
Inland Marsh	6		4				Spring 2009	April 2009
Miller Woods			4				Spring 2009	April 2009
Chellberg Farm					6		Fall 2009	April 2010
Heron Rookery					6		Fall 2009	April 2010
West Beach Burn Unit						6	Fall2009	April 2010

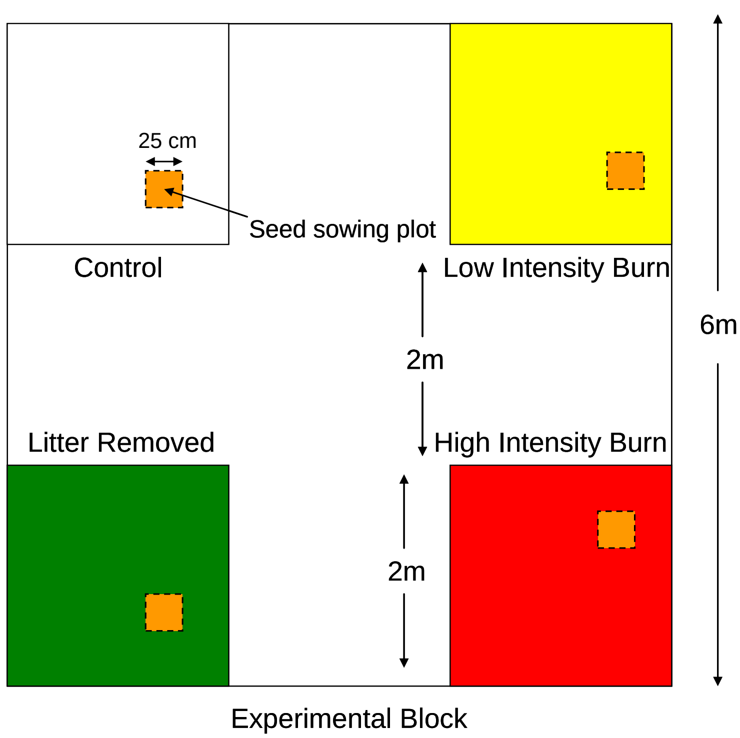


Figure 1. Layout of susceptibility of invasion experimental block.

Monitoring

In 2009, seedlings were monitored on May, 28, June 23 and 24, July 24 and 27, August, 19 and 20 and September 29 and 30 (Figure 8). In 2010, seedlings and second year plants were monitored on May 25-26, June 21 and 23, July 21-23, August 16-17, and September 14-16. In 2011, second year plants were monitored on May 26-27, June 21-22, July 25, August 22, 23, 26, and final harvest occurred during September 2011. At each sampling time, the number of live seedlings or second year plants and height of plant from the ground to the apical meristem were measured. At the end of September 2010, we took the final height measurements of the plants for the year and then harvested the second year plants (the Howes Prairie prairie and savanna sites, Mnoke Prairie prairie sites, Inland Marsh and Miller Woods sites). The same was done in 2011. During the course of the growing season, we collected additional environmental data on each of the four subplots. We took canopy photographs and photosynthetically active radiation (PAR) readings at the new locations. These variables will be used in our final analysis of the susceptibility of different habitats to invasion by oriental bittersweet.

Statistical analyses

For experimental fire parameters, we conducted three types of ANOVA analyses: 1) soil by canopy by liter type, 2) soil by habitat, and 3) plant community. Analyses were conducted using the maximum percent germination across all five months that we monitored seedlings and second year plants. The fire treatment effect on percent germination was conducted using an ANOVA followed by a post-hoc Tukey's b test.

Figure 2. Grid for monitoring germination of oriental bittersweet seedlings. Seedling is circled.

Results:

Research burns:

The main differences in experimental burns was between the prairie fuels and the forest fuels, with the moraine prairie having the hottest and quickest burns. The maximum height of tag with temperature above ambient was significantly greater in prairie (60 ± 5 cm) compared to oak forest and mesic forests (33 ± 3 and 37 ± 2 cm respectively) ($F_{2,138} = 19$, $P < 0.001$). This height was significantly greater in high intensity (55 ± 27cm) compared to low intensity treatments ( ) ($F_{1,136} = 43$, $P<0.001$) This height did not vary in forested communities between sand and moraine (34 ± 18 and 37 ± 17 cm), but in open sites was significantly greater on the moraine (69 ± 38 cm) compared to the sand dunes (43 ± 25 cm) ($F_{1,136} = 16$, $P < 0.001$).

Mesic forests had significantly lower maximums fire temperatures (746°F ± 34) compared to prairie (848°F ± 31) with oak forests being intermediate (794 °F ± 33) ($F_{2,138} = 4.4$, $P = 0.013$). Fire temperatures on the moraine were greater in the open habitats (933 ± 46°F) compared to the forested habitats (760 ± 39), but were lower on the sand dunes (752 ± 21°F) ($F_{1,136} = 4.4$, $P = 0.036$.

Maximum flame heights showed a significant interaction with general habitat and soil type ($F_{2,130} = 13$, $P < 0.001$). Mesic oak forests on sand dunes had higher flame heights (59 ± 6 cm) than those on the moraine (38 ± 3cm) and prairies on the moraine had higher flame heights (108 ± 8cm) compared to those on the sand dunes (70 ± 14cm). Flame heights in oak forests averaged 39 ± 8 cm.

Rate of fire spread was 75% greater in the prairies than in the mesic and oak woods ($F_{2,130} = 25.5$, $P < 0.001$): prairie = 0.75 ± 0.07 m/minute, oak forests = 0.45 ± 0.02 m/minute, and mesic forest = 0.43 ± 0.01 m/minute. The greater rate in spread of burns in the prairies contributed to the significantly greater rate of spread in the open habitats compared to the forests ($F_{1,128} = 17$, $P < 0.001$).

When examining the maximum number of seedlings per sampling period over the two years, the forested habitat plots (maple and oak forest) had significantly greater percent germination than the open plots (prairie, savanna; Figure 3); however this comparison is confounded with year of sowing and differences in weather between years. Plots on moraine soil vs. sandy soils had greater overall percent germination. In the oak and maple forest habitats, the germination percentage in the control treatment was significantly lower than the low and high fire treatments (Table 2). The litter removed treatment was not significantly different from the control or the two burn treatments.

We noticed some differences in how seedling mortality progressed throughout the growing season for 2010 vs. 2009 (Figure 3). In 2009, for the prairie and savanna plots, the maximum number of germinants peaked in August. Over the winter, it appears that mortality was high for the plants in the prairie plots (both the sand and moraine prairie). In 2010, however, for the new forest plots (oak and maple forests), germinants peaked in June and have steadily declined into August. The seedlings remaining in the prairie and savanna plots also showed some mortality throughout the 2010 growing season, but it was not nearly as steep a decline.

Summary of fire and the susceptibility of invasion experiment:

By manipulating the amount of litter in the plots, we were able to successfully create a high fire intensity treatment that was significantly different than the low intensity treatment. We also saw differing fire behaviors in the prairie vs. savanna habitats. In terms of germination, we saw an increase in the number of germinating seeds from June to August in the prairie and savannas during 2009, whereas in 2010 the peak in the oak forest and maple forest was in June. In our examination of the maximum percent germination, we found that sand prairies and sand savannas were similar in their germination percentages while the moraine prairie had much higher germination rates. This is most likely due to the richer soils and higher moistures of these moraine prairies vs. those habitats located on sand. It will be interesting to compare these values to our forested habitats results this coming season. We also determined that it is possible that fire may not necessarily make prairie and savanna habitats more susceptible to invasion from seed of oriental bittersweet. We found that the control plots had the highest maximum percentage of germination suggesting that the litter provided some shelter from drying out and excessive insolation in the early stages of germination. The two burned plots had intermediate percent germination compared to the control and litter removal plots, probably because fire did not remove all the litter whereas raking did. In the next growing season, we will be able to determine if growth rates and overall survival differ between burn treatments and habitats.

We have harvested the plants grown in the oak hickory forest, black oak forest, and beech-maple forest plots on sand and moraine, but results must wait until samples have been weighed.

Table 2. Mean ± SE maximum percent germination for the forested plots in the four treatments. Different letters indicate significant differences at the $\alpha = 0.05$ level.

	High Intensity	Low Intensity	Litter Removed	Control
Maximum % Germination	14.3 ± 1.7[a]	12.7 ± 1.3[a]	10.2 ± 1.3[ab]	8.4 ± 1.4[b]

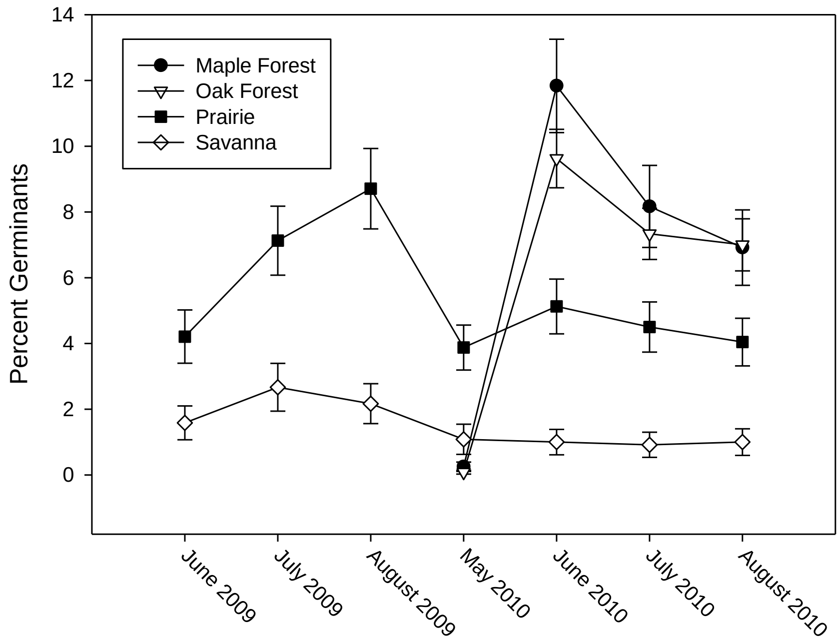


Figure 3. The mean percent germinants of oriental bittersweet (seedlings and second year plants) for each of the four main habitat types. Means (±SE) are across all treatmentsd error.

II Fire effects on established plants of Oriental bittersweet:

Methods:

Experimental design

Eight experimental blocks, split between sand and moraine soils, were located in major infestations of Oriental bittersweet to test effects of combinations of cut and burn treatments on vine resprouting in the spring of 2009. Our original intent was to balance these blocks between sand versus moraine soils and burned and unburned sites; however we were unable to find a second burned moraine sites (Table 3). We tried to maintain a 5 m buffer between treatment plots, but in some cases this was not possible given the spatial arrangement between the bittersweet patches. The minimum distance between plots was 1m. Treatments were allocated randomly among blocks: control, dormant season burned, growing season burned, dormant season cut, growing season cut, dormant season cut + burned, and growing season cut + burned (Figure 4). At five of the block sites we added two additional treatments at the request of the NPS: cut and herbicide, and burn and herbicide. The burn plus herbicide treatments will be conducted in the fall of 2010. Ultimately, we had to reassign some of the treatments due to complications described below in the fire treatment section. These treatments were compared to the responses of the controls. Each treatment plot is 10 x 10 m. and has a meter-wide buffer around the periphery. We placed four random 1 x 1 m subplots within each quadrant of the plot where the population response was quantified (Figure 5). We pre-sampled the subplots for abundance of Oriental bittersweet in July of 2009.

In the fall 2010 we instaledl a series of ancillary plots at West Beach to examine the impacts of management treatments on population and individual responses. These were installed to compliment the main study. These were located in the northwest dune portion of West Beach that had never been burned since the park was created. where a prescribed burn was conducted later that fall for the first time in this unit. Five blocks of six plots were installed in regions infested with oriental bittersweet. The six treatments included control, burn alone, cut alone, cut and burn, herbicide, and herbicide and burn. Each treatment plot was 1 m2. Herbicide application was conducted early in October 2010. The prescribed burn occurred on November 9, 2010. Control, cut, and herbicide plots were raked around the perimeter to prevent fire from burning through.

Fire treatments

Our original plan was to conduct burns in the spring of 2010 for the dormant season burn and summer 2010 for the growing season burn. However, during a prescribed burn at Kintzele Dunes on November 9, 2009, after fire mop-up, a single tree fell across the fire line into the block, burning five of the nine treatment subplots. Similarly, at the Marquette site after mop-up from a prescribed burn on March 28, 2010, an ember must have blown into the unburned block and burned four of the treatment plots. At Kintzele the treatments were rearranged so that the spring burn treatments were those four plots that were not burned and the five burned plots were included in the study as described below. At Marquette, the five unburned plots included those that would be burned in the spring. Thus, our dormant season spring burns were conducted as planned. However, twice the normal rainfall in June 2010 created conditions unfavorable for the planned growing season treatments (growing season cut, growing season burn, and growing season cut-and-burn). The impacts of extreme precipitation varied with community. In the savannas the high spring rainfall created rampant growth of Oriental bittersweet which covered most of the ground preventing light reaching the litter layer. Thus lack of light at the litter layer made combustion highly unlikely. In the moraine sites, conditions were warm enough with the abundant rainfall to completely decompose the litter layer. Thus there was nothing to burn. So the growing season treatments have been delayed until the fall of 2010. Since the accidentally burned plots at Kintzele and Marquette would now have one full season of growth before the late season treatment application, it was decided to retain them in the study. In the analysis we will be attentive to aberrant or divergent response values that might reflect effects of the unplanned burns. One of the herbicide treatment plots was cut in early July to show the impacts of a growing season cut on TNC and plant response, next year (summer 2011). The complete set of reassigned treatments can be seen in Fig. 5.

We conducted the spring 2010 dormant season burns from March 24 to April 15. Before the burns took place, we cut all the oriental bittersweet in the 10 x 10 m treatment plot area in both the cut and cut-and-burn treatments. On the day of the burn, in each, plot temperature

Table 3. Sites and number of subplots) by substrate type and fire history for conducting fire effects experiments at Indiana Dunes National Lakeshore.

	Fire history	
Substrate type	**Previously burned**	**Previously Unburned**
Sand	Kintzele Dunes - 9	Kemil Rd. (Furnessville) - 9
	Marquette Trail - 9	Mineral Springs Rd - 7
Moraine	Mnoke Prairie - 7	Bailly Picnic Area - 7
		Learning Center - 9
		Chellberg Farm - 9

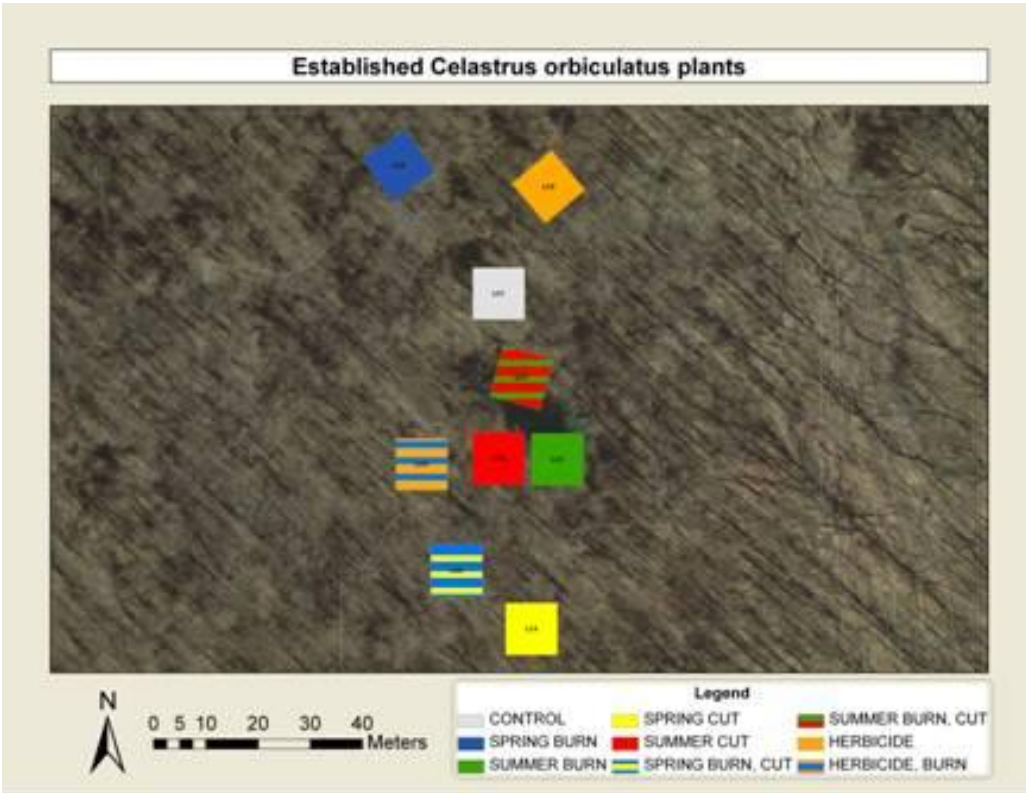


Figure 4. Example of layout of treatment plots at the Learning Center.

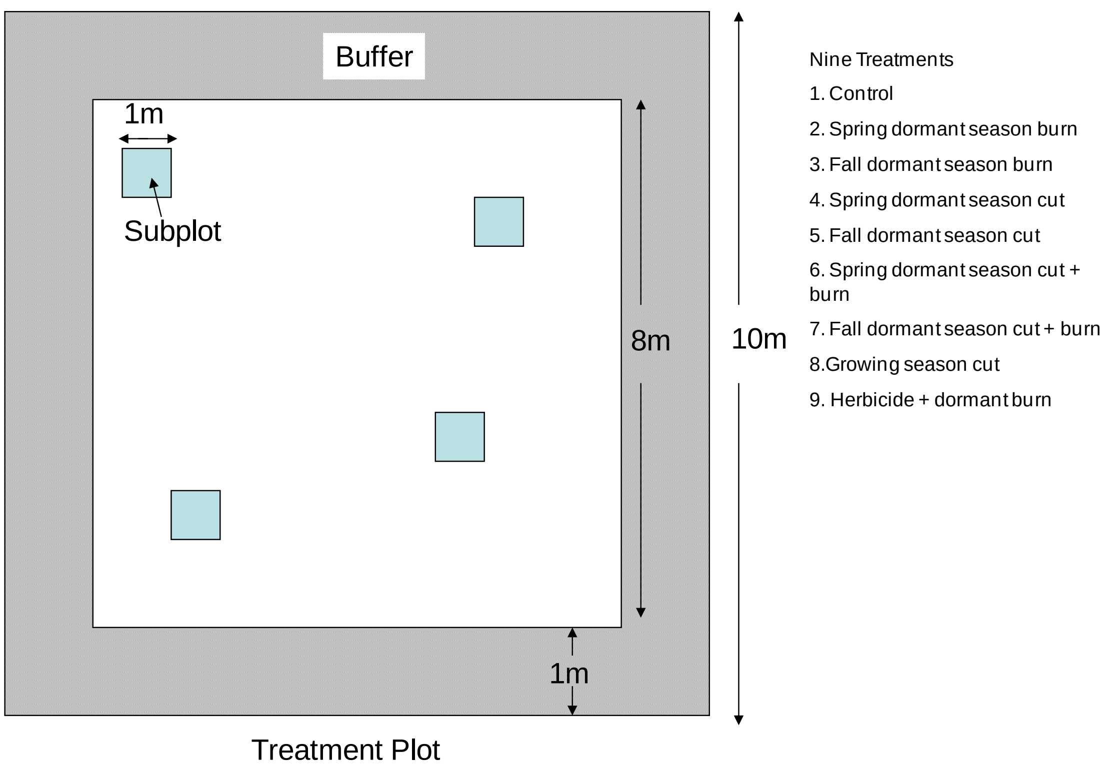


Figure 5. Treatment plot layout with sampling subplots. Not indicated are the randomly located individuals within each quadrat.

Figure 6. Thermocouples and temperature sensitive paint-tag stakes in treatment plot.

sensitive paint tags were deployed on a stake at 0, 0.5, 1, 1.5, 2.5, 3.5, 4.5 feet above the ground (Cole et al. 1992). Temperature paints were 250 ºF (121 ºC) , 300 (149 ºC), 400 (204 ºC), 500 (260 ºC), 600 (316 ºC), 700 (371 ºC), 800 (427 ºC), 900 (482 ºC), 1000 (538 ºC), 1200 (649ºC). We also deployed thermocouples to record the actual temperatures of the fires at the litter layer (K type thermocouple) and at 6 inches off the ground (J type; Figure 6). During the burns, the rate of fire spread, flame height (minimum, maximum, and average), and wind speed and direction were recorded. Fires were primarily backing fires, although in some instances dramatic wind shifts produced head fires. At the Chellberg Farm site, leaf litter was so sparse that we had to add litter from the adjacent mesic oak forest to burn the plots. The other moraine sites had just enough litter to sustain the burn. We employed the same techniques in the West Beach management experiment.

Oriental bittersweet survey methods

a) Population response – In each 1 m subplot, we used ocular estimates of total cover of Oriental bittersweet. We followed this by counting the number of stems in each of six size categories: seedlings, < 2.5 mm in diameter, 2.5 – 5.0 mm, 5.1 – 10 mm, 10.1 – 15 mm and > 15 mm. We measured the diameter of one randomly selected plant per plot in 2009 and following treatment in 2010. For one randomly selected subplot in each treatment plot (see Fig. 5) we identified and determined the percent cover of all plant species less than 1 m in height, so that we could determine if the plant community associated with oriental bittersweet changed after application of the treatments. We also took environmental measurements in each plot: PAR, soil moisture (2009 only: Dynamax HH2 moisture meter) and canopy cover. Canopy cover was quantified using Gap Light Analyzer from a hemispherical photograph taken at the plot center in August and September of 2009, 2010, and 2011. For the West Beach management experiment, cover and stem counts were donducted prior to the treatments in September 2010 and repeated one year later.

b) Individual response – To examine the response of individual Oriental bittersweet plants, in the fall of 2009, we marked 25 individuals in each control, spring burn, spring cut, and spring cut/burn treatment. We marked five plants with individually numbered tags in each of five size categories: seedlings, < 5.0 mm, 5 – 10 mm, 10 – 15 mm and > 15 mm. There were a few sites where we could not get the full complement of 25 in each treatment plot but we sampled as many as possible. On each of these plants we measured the basal diameter both pre-treatment (2009) and post treatment (2010). This data was not collected from the West Beach management experiment.

c) Total nonstructural carbohydrate study – In each treatment plot we harvested three separate root samples (1 to 5 mm in diameter) in March, May, July, October of 2010 and Early April, May, and July 2011 to quantify carbohydrate reserves over the time course of the treatments. Root samples were collected as widely spaced as possible to ensure they were from separate individuals. Samples were collected between 8 and 11 am so that diurnal variation in TNC was minimized. Each root was placed in a labeled plastic bag and stored on ice in an ice chest until returned to the lab late in the morning. Samples were washed to remove soil, heated at 100° C for an hour to kill the tissue, dried to constant weight at 70° C, and then ground in a Wiley mill at the University of Notre Dame and at LMERS in 2011. The sample was passed through a 40 mesh screen in the mill. Dried and ground root samples were stored in scintillation vials in a freezer until analysis. We used the extraction and analysis methods described in Kobe (1997) and (Kobe Richard K. et al. 2010) (See appendix of detailed protocol modified from that provided by Richard Kobe and annotated by Sarah Strobl). TNC was not sampled from the West Beach management experiment.

Statistical analyses

a) Population response – We used the differences in oriental bittersweet cover, stem counts and diameters as our response variable for the preliminary comparisons between 2009 and 2010. We only examined those plots that had treatments applied as of this growing season – control, spring burn, spring cut and spring cut-and-burn. We analyzed the cover and stem data using ANOVA with treatment and soil type as fixed effects and followed this analysis with a Tukey's-b post-hoc test. When the experiment is completed in 2011, we will examine multivariate responses using MANOVA. Plant community data were analyzed in PC-ORD using a nonmetric multidimensional scaling (NMS) ordination.

b) Individual response – Using ANOVA we examined the response of the number of resprouts to both the burn treatment and the size class of the plant as factors. We excluded the control treatment because there were very few resprouts (almost 0) which resulted in highly skewed data . We examined the percentage of plants killed to see if the treatment and size class affected whether or not a given bittersweet plant was killed.

c) Total nonstructural carbohydrates – We used analysis of variance to compare % TNC by treatment and soil type for each month individually. Significant differences among treatments were assessed using Tukey's b multiple mean comparison statistic.

Results:

For temperature sensitive paints, the maximum temperatures, the lowest maximum temperature among subplots, maximum height of elevated temperatures were not significantly different between treatments (burn and cut-and-burn), soil type and their interaction. The lowest maximum height of elevated temperatures among subplots was significantly different among soil types, with elevated temperature reaching on average 9.8 ± 1.7 inches above the ground on sand and 3.0 ± 1.8 inches on moraine soil ($F_{1,12} = 7.4$, $P = 0.019$).

Temperature data from temperature sensitive paints were uncorrelated with thermocouple temperatures, suggesting that temperature sensitive paints are too crude to estimate fire temperatures. In our experience vertical arrays of temperature sensitive fire tags are useful for obtaining an index of flame height and fire intensity (height of elevated temperatures). Typically head fires and high intensity fires melt paints to greater heights than flanking or backing fires.

Maximum thermocouple temperature, minimum of maximum temperatures among subplots, maximum temperature duration, above lethal temperature duration, and total lethal degree seconds did not differ significantly among treatment and soil types. Fires among treatments and soil did not differ in temperatures, lethal temperature duration, but fires on sandy soils had elevated temperatures to greater height compared to those on moraines. This difference may be attributable to differences in litter type: oak versus mixed oak and mesophytic leaf litter.

a) **Population response –** Oriental bittersweet cover significantly decreased from 2009 to 2010 in the spring burn and spring cut-and-burn treatments compared to the control and the cut treatments ($F_{7,3}$ = 6.8, P < 0.001) . Soil type was also significant with plants on sandy soil having adecreased greater reduction in cover than those on the moraine soils ($F_{7,1}$ = 4.3, P = 0.04; Fig 7A). The interaction between treatment and soil type was not significant ($F_{7,3}$ = 1.4, P = 0.23). Fire treatment did not have a significant effect on the number of seedlings present from 2009 to 2010 ($F_{7,3}$ = 2.1, P = 0. 1), while there was a significantly positive change in seedling numbers on sandy soils compared to the moraine ($F_{7,1}$ = 6.6, P = 0.01; Fig 7B). There was a significant increase in the number of stems less than 2.5 mm in diameter in the spring burn and spring cut-and-burn treatments compared to the control and cut treatments from 2009 to 2010 ($F_{7,3}$ = 17.2, P < 0.001). There was no difference in stems on the two soil types ($F_{7,1}$ = 2.9, P = 0.09; Fig 7B). The increase in small stems was not surprising since with the destruction of the larger stems by fire, resprouting stems will have a smaller diameter. For the 2.5 – 5 mm class from 2009 to 2010, the fire treatment itself was again significant ($F_{7,3}$ = 3.0, P = 0.03), with the increase in spring cut-and-burn being significantly greater than the control, but not different from the spring burn and spring cut treatments. These treatments were not different from the control. For this size class, soil type was not a significant factor in changing plant density ($F_{7,1}$ = 0.02, P = 0.89; Fig 7C). Interestingly, the increase in stems in this size class for the spring cut-and-burn is mostly on the moraine soils. These were probably resprouts that, because of the richer soils of the moraine, reached the next size class. For the remaining larger size classes (5.1 -10 mm, 10.1 – 15 mm and greater than 15mm), there was no significant treatment or soil effect on the number of stems from 2009 to 2010. Although the graphs (Figs 7D, E, and F) indicate a negative change in stems in these larger size classes as we would expect, the results were not statistically significant. Another possibility is that between the 2.5 – 5 and 5 – 10 mm size classes, oriental bittersweet is better able to survive fire, and plants that are cut in this size class often resprout from the main stem. Finally, the difference in total stems was significantly influenced by fire treatment ($F_{7,3}$ = 14.3, P < 0.001) but not soil ($F_{7,1}$ = 2.0, P = 0.16; Fig 7F), with the number of stems increasing in the spring burn and spring cut-and-burn treatments from 2009 to 2010.

In 2011, we noticed that some plots on the moraine showed decreased cover while others increased independent of treatment. We suspect that this is a function of local topography that affects soil saturation. When we have wet years, we suspect the lowest and wettest plots have reduced cover because oriental bittersweet does poorly in wet conditions. During the winter of 2011, we plan to survey the plots at each site using a level and staff to obtain relative elevations. This information will also be useful in understanding the plant community trends in time.

A total of 121 species were sampled in the plant community plots from the initial treatments (control, spring burn, spring cut, and spring cut-and-burn). Sixty-six were herbaceous, 16 were graminoids, seven were vines, 21 were shrubs, and 21 were tree seedlings. The plant communities within sites were dissimilar, especially at Bailly, Kintzele Ditch, and Marquette Trail (Fig. 8) (complete species by community data are presented in Table 8 in the Appendix). Vegetation richness was on average 9 ± 4 species per plot and did not vary with treatment ($F_{3,56}$ = 1.2, P >0.05), but was two species greater on the moraine compared to the sand dunes ($F_{1,60}$ = 5.7, P = 0.02: 10 ± 1 > 8 ± 1). Total vegetation cover including *C. orbiculatus* was 66 ± 35%. Four vegetation communities were identified using Flexible beta agglomeration algorithm (Fig 8). We called the four vegetation communities disturbed woods, depauperate woods, oak savanna, and shrub savanna. The four communities differed significantly in native shrub cover ($F_{3,56}$ = 9.5, P < 0.001), native forb cover ($F_{3,56}$ = 11.0, P <0.0001), native vine cover (excluding Oriental bittersweet, $F_{3,56}$ = 7.1, P < 0.0001) , evenness ($F_{3,56}$ = 6.2, P = 0.001). None of the other life forms were significantly different in cover among communities: exotic shrub – $F_{3,56}$ = 0.6, P =

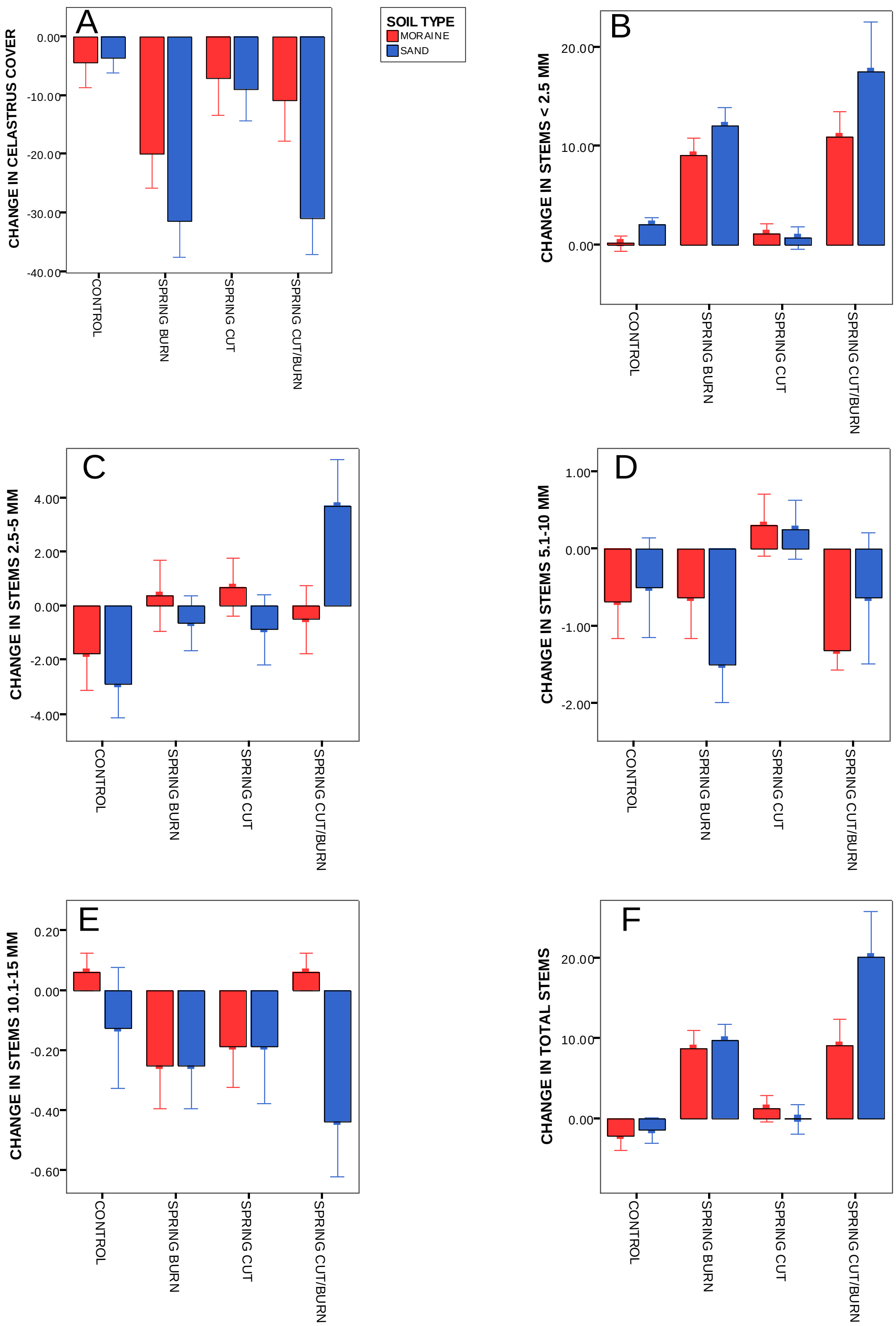


Figure 7. Change in cover and number of stems of Oriental bittersweet from 2009-2010. Values are mean ± SE. Red bars are the moraine soil type and blue are the sand.

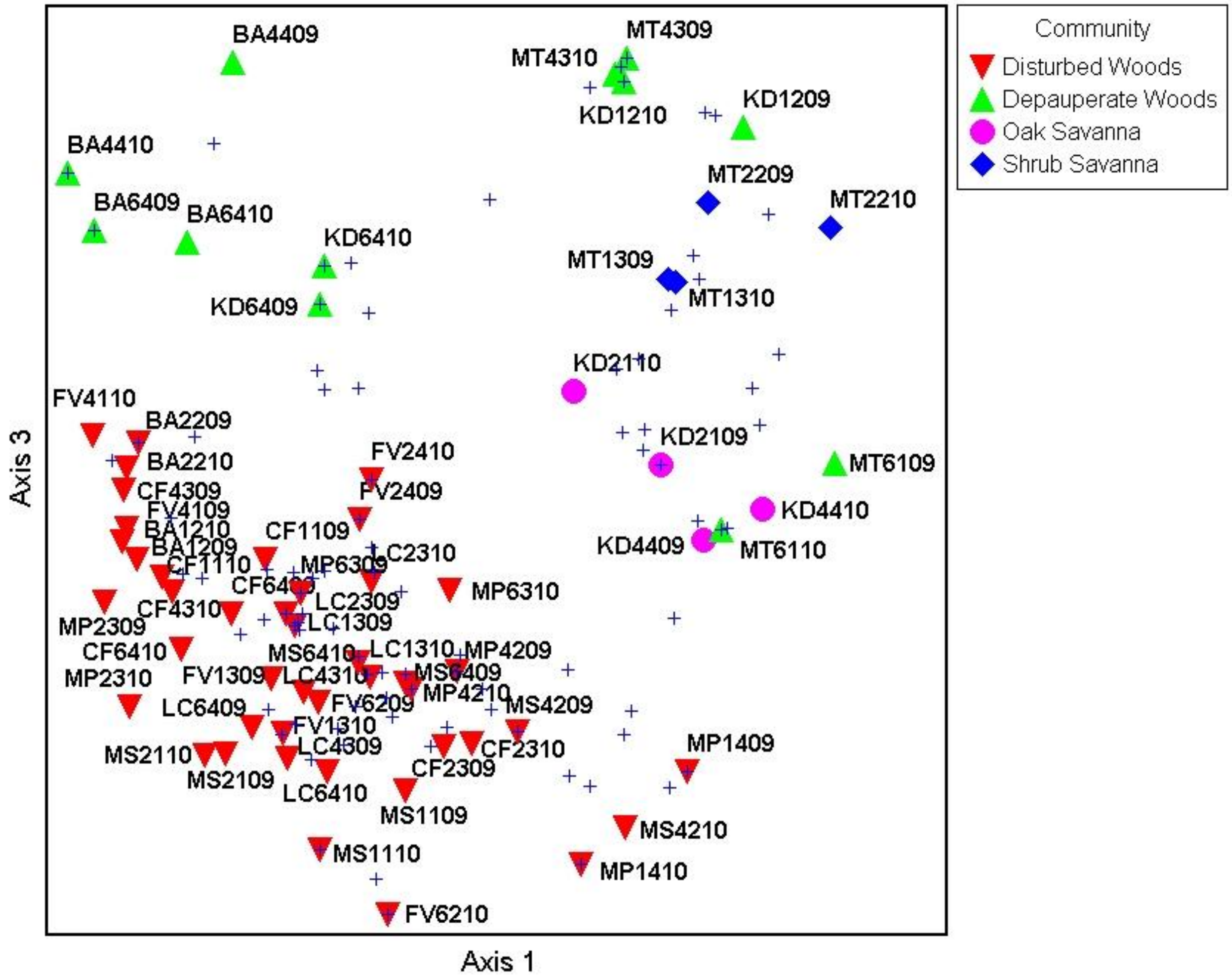


Figure 8. NMS ordination of bittersweet establishment plots from 2009 to 2010. Sites are BA = Bailly, CF = Chelburg Farm, FV = Furnessville, KD = Kintzele Ditch, LC = Learning Center, MP = Mnoke Prairie, MS = Mineral Springs, and MT = Marquette Trail. The '+' symbols represent unlabeled species centroids. Plot identification codes that end in 09 were sampled in 2009 and those ending in 10 were sampled in 2010.

Table 4. Mean percent cover ± SE of species (bold values are the ten most abundant species) within the four community types for the 2009 and 2010 surveys. Invasive species are in bold italics.

	Disturbed Woods		**Depauperate Woods**		**Oak savanna**		**Savanna Shrub**	
	2009	2010	2009	2010	2009	2010	2009	2010
Sample size	44	44	12	12	4	4	4	4
Vines								
Celastrus orbiculatus	44.4 ± 6.1	28.4 ± 5.5	28.7 ± 12.6	23.5 ± 8.7	35.0 ± 5.0	34.0 ± 1.0	21.0 ± 9.0	13.5 ± 3.5
Parthenocissus quinquefolia (L.) Planch.	**3.1 ± 0.7**	2.3 ± 0.6	**1.2 ± 1.2**	1.7 ± 1.7	1.0 ± 1.0	2.0 ± 2.0	0 ± 0	0 ± 0
Toxicodendron radicans (L.) Kuntze ssp. *radicans*	0.6 ± 0.4	0.8 ± 0.5	<0.1 ± <0.1	0 ± 0	**3.0 ± 3.0**	4.0 ± 4.0	0 ± 0	0 ± 0
Vitis riparia Michx.	0 ± 0	0.1 ± 0.1	<0.1 ± <0.1	<0.1 ± <0.1	**12.0 ± 12.0**	5.0 ± 5.0	0 ± 0	0.4 ± 0.3
Herbaceous Species								
Agrimonia gryposepala Wallr.	0.2 ± 0.1	0.5 ± 0.3	0.1 ± 0.1	0 ± 0	**1.2 ± 1.2**	1.0 ± 1.0	0 ± 0	0 ± 0
Alliaria petiolata (Bieb.) Cavara & Grande	**1.2 ± 0.5**	0.3 ± 0.1	**0.9 ± 0.8**	0.3 ± 0.2	0 ± 0	0 ± 0	0.8 ± 0.3	0.5 ± 0.5
Boehmeria cylindrica (L.) Sw.	0.4 ± 0.4	2.3 ± 2.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Carex pensylvanica Lam.	0 ± 0	0.1 ± 0.1	0.5 ± 0.3	0.2 ± 0.1	**4.0 ± 4.0**	4.0 ± 4.0	0 ± 0	0 ± 0
Circaea lutetiana L. ssp. *canadensis* (L.) Asch. & Magnus	**1.1 ± 0.3**	1.1 ± 0.4	**0.7 ± 0.7**	1.2 ± 0.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Geum canadense Jacq.	**2.4 ± 0.4**	2.6 ± 0.6	<0.1 ± <0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Helianthus divaricatus L.	0.1 ± 0.1	0.2 ± 0.2	0.7 ± 0.4	0.6 ± 0.5	0 ± 0	0 ± 0	**3.5 ± 3.5**	5 ± 5
Maianthemum racemosum (L.) Link ssp. *racemosum*	0.1 ± 0.1	<0.1 ± <0.1	0.3 ± 0.3	0.3 ± 0.3	0 ± 0	0 ± 0	**1.0 ± 1.0**	0.4 ± 0.4
Maianthemum stellatum (L.) Link	0 ± 0	0 ± 0	**2.7 ± 1.9**	0.3 ± 0.2	0 ± 0	0 ± 0	**0.5 ± 0.5**	0.5 ± 0.5
Polygonum virginianum L.	**5.7 ± 1.6**	5.9 ± 1.4	0 ± 0	0 ± 0	0 ± 0	0.25 ± 0.25	0 ± 0	0 ± 0
Pteridium aquilinum latiusculum (Desv.) Underw.	0 ± 0	0 ± 0	**0.7 ± 0.7**	0.3 ± 0.3	**23.5 ± 6.5**	27.5 ± 12.5	0 ± 0	4.0 ± 4.0
Sanicula canadensis L.	**2.7 ± 2.5**	3.8 ± 2.6	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Solidago caesia L.	0 ± 0	0 ± 0	**1.0 ± 0.6**	1.2 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Shrubs								
Euonymus alatus (Thunb.) Siebold	<0.1 ± <0.1	0 ± 0	**2.2 ± 1.8**	0.5 ± 0.3	**4.5 ± 4.5**	1.0 ± 1.0	0 ± 0	0 ± 0
Rosa carolina L.	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	2.5 ± 2.5	**0.8 ± 0.8**	0.5 ± 0.5
Rosa multiflora Thunb.	**3.7 ± 1.6**	2.5 ± 0.8	0.2 ± 0.2	0 ± 0	1.0 ± 1.0	0 ± 0	0 ± 0	0 ± 0
Rubus flagellaris Willd.	**2.3 ± 0.9**	5.1 ± 3.4	0.3 ± 0.3	0.1 ± 0.1	**17.5 ± 2.5**	14.0 ± 6.0	0 ± 0	0 ± 0
Rubus idaeus L. ssp. *strigosus* (Michx.) Focke	**1.4 ± 0.7**	1.2 ± 0.5	0 ± 0	0 ± 0	1.0 ± 1.0	0 ± 0	0 ± 0	0 ± 0
Vaccinium pallidum Aiton	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	**26.0 ± 4.0**	20.5 ± 12.5
Viburnum lentago L.	0 ± 0	0 ± 0	**2.5 ± 2.5**	1.2 ± 1.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Viburnum rafinesquianum Schultes	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**12.5 ± 12.5**	12.5 ± 12.5
Vinca minor L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**14.0 ± 14.0**	3.5 ± 3.5	0 ± 0	0 ± 0
Tree seedlings								
Fraxinus americana L.	**1.5 ± 0.6**	0.4 ± 0.2	0.1 ± 0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Juglans nigra L.	0.1 ± 0.1	0 ± 0	**0.9 ± 0.9**	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Prunus serotina Ehrh.	0.2 ± 0.16	0.1 ± 0.1	0.3 ± 0.3	0.3 ± 0.2	0 ± 0	0 ± 0	**2.0 ± 2.0**	1.2 ± 1.2
Quercus velutina Lam.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**5.0 ± 5.0**	4.0 ± 4.0	0 ± 0	0 ± 0
Sassafras albidum (Nutt.) Nees	0.1 ± 0.1	0.3 ± 0.2	**2.7 ± 1.9**	4.9 ± 4.4	0 ± 0	3.0 ± 2.0	0 ± 0	0 ± 0
Status/Life Form								
ExoticShrub	3.9 ± 1.8	2.6 ± 0.9	2.3 ± 1.8	0.5 ± 0.3	5.9 ± 5.9	1.3 ± 1.3	0 ± 0	0 ± 0
NativeShrub	4.6 ± 1.5^{A}	7.9 ± 3.6^{A}	3.7 ± 2.4^{A}	3.2 ± 1.7^{A}	18.5 ± 3.5^{A}	16.5 ± 8.5^{A}	39.2 ± 9.3^{B}	33.5 ± 0.5^{B}
NativeVine	3.9 ± 0.8^{A}	3.4 ± 0.8^{A}	1.5 ± 1.4^{A}	2.1 ± 2.1^{A}	16.0 ± 14.0^{B}	11.0 ± 7.0^{B}	0 ± 0^{A}	0.4 ± 0.3^{A}
NativeTree	2.3 ± 0.6	1.4 ± 0.3	5.1 ± 1.9	5.6 ± 4.3	5.0 ± 5.0	7.0 ± 6.0	2 ± 2	1.3 ± 1.3
ExoticTree	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1
ExoticGraminoid	0.02 ± 0.02	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
NativeForb (ln)	16.0 ± 3.1^{A}	19.7 ± 3.8^{A}	6.8 ± 1.9^{AB}	4.6 ± 1.6^{AB}	2.2 ± 2.0^{B}	1.2 ± 0.8^{B}	5.0 ± 4.0^{AB}	6.1 ± 5.1^{AB}
ExoticForb	1.4 ± 0.5	0.4 ± 0.1	0.9 ± 0.8	0.3 ± 0.2	14.3 ± 14.2	3.5 ± 3.5	0.8 ± 0.3	0.5 ± 0.5
NativeGraminoid	0.4 ± 0.1	0.3 ± 0.1	0.6 ± 0.5	0.2 ± 0.1	4.0 ± 4.0	4.0 ± 4.0	0 ± 0	0 ± 0
Total cover (Sqrt)	32.4 ± 4.0^{AB}	35.5 ± 5.3^{AB}	20.9 ± 6.9^{A}	16.4 ± 7.9^{A}	65.9 ± 1.9^{B}	44.5 ± 2.5^{B}	47.0 ± 15.0^{B}	41.9 ± 6.8^{B}

0.61; native trees – $F_{3,56}$ = 3.1, P = 0.031(nonsignificant Tukey's b test), species richness – $F_{3,56}$ = 1.5, P = 0.22, total vegetation cover including Oriental bittersweet – $F_{3,56}$ = 2.4, P = 0.081. The largest cluster, disturbed woods, consisted of moraine (Bailly, Chelburg Farm, Learning Center, Mnoke) or late successional dune plots from Furnessville and Mineral Springs. Furnessville was formerly a homesite and horse pasture, and a tornado passed through the site in the late 1970's when the old field was in the tree sapling stage. Some of the plots at Bailly, Kintzele Ditch and Marquette clustered together because of shared tree seedling species while being depauperate in herbs (Depauperate Woods). One small cluster consisted of two plots having high cover of savanna shrubs (Marquette 1 & 2). The other small group, Kintzele Ditch 2 & 4, were similar to savanna sites, but having high cover of native vines compared to the other communities.

b) **Individual response** – We found that the cut-and-burn treatment had significantly greater numbers of resprouts compared to the cut treatment ($F_{2,1}$ = 2.1, P = 0.1; Fig). In addition, the number of resprouts increased with size class ($F_{4,1}$ = 9.8, P < 0.001; Table 5). When we examined the percentage of plants that were killed in each size class we found, as expected, that the largest size class had the most survival overall. The spring cut-and-burn had the most killed stems, but also had the highest number of resprouts (Table 5). It is interesting that in the population response, the cut-and-burn had the lowest cover. We would predict that next year, that these cut-and-burn plots with their high number of resprouts, will have much greater cover than was exhibited this year.

c) Total nonstructural carbohydrates

Percent total non-structural carbohydrate did not differ between treatments in March ($F_{4,51}$ = 0.16, P = 0.96) and May ($F_{4,51}$ = 0.62, P = 0.65) (Fig. 10). However, in July the bittersweet plots that were cut in early July had significantly lower TNC than all other treatments ($F_{4,56}$ = 4.46, P = 0.003). No other treatments were significantly different for the cut plots in July.

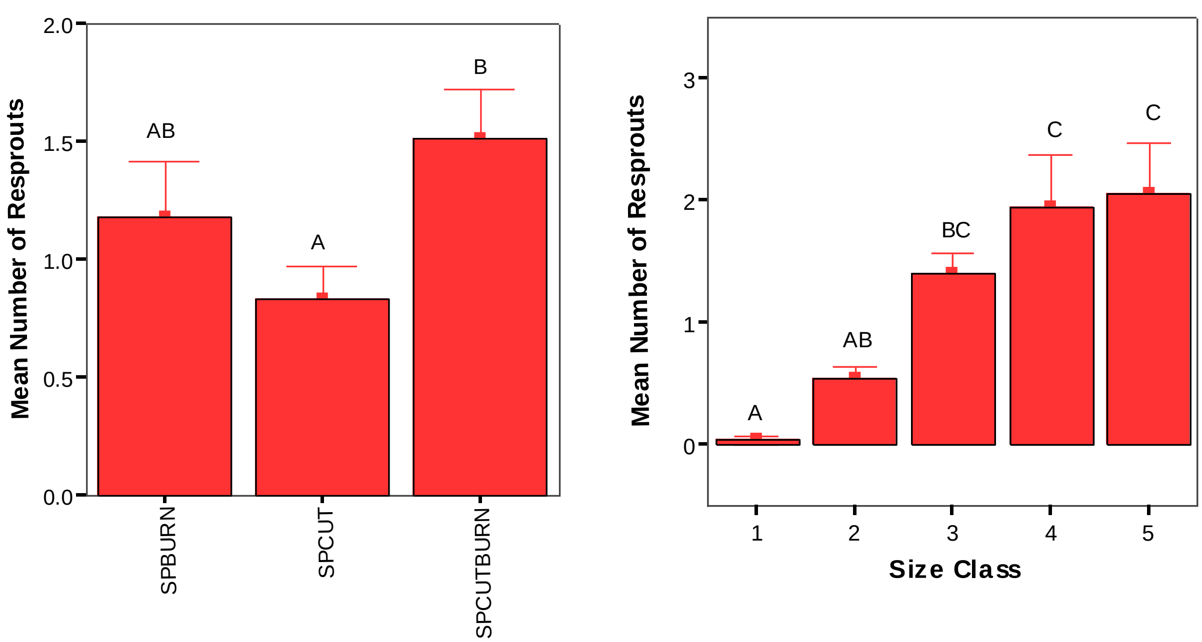


Figure 9. Mean number of resprouts ± SE of: a) the burn treatments and b) the size classes. SPBURN = spring burn, SPCUT = spring cut and SPCUTBURN = spring cut-and-burn. Size class 1 = seedling, 2 = < 5.0 mm, 3 = 5.1-10 mm, 4 = 10.1-15 mm and 5 = > 15 mm. Different letters indicate statistically significant differences at the α = 0.05 level.

Table 5. Percentage of bittersweet plants killed in each size class and treatment.

Size Class	% Killed
Seedling	64.5
< 5.0 mm	34.4
5.1-10 mm	34.8
10.1-15 mm	30.1
> 15 mm	17.4
Treatment	
Control	7.5
Spring Burn	36.5
Spring Cut	36.1
Spring Cut-and-burn	62.3

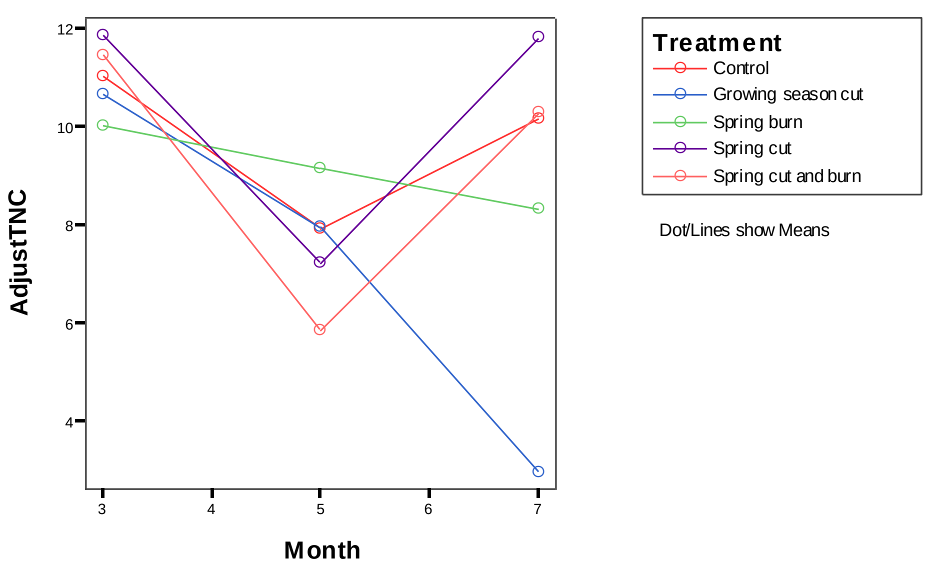


Figure 10. Changes in percent total non-structural carbohydrate in roots of oriental bittersweet by month and treatment.

Why spring burn plots trend was linear rather than v shaped is not clear. TNC on average is 10 to 12 % in the dormant season, decreases to 6 to 9% in the early growing season and increase in mid summer to 9-12%. Early July cutting reduced percent TNC to 3%, a 75% reduction from dormant season values. Clearly, percent TNC is reduced, from a dormant season peak, early in the growing season as new growth is initiated. Later in the season where photosynthesis exceeds loss to growth, TNC accumulates and increases (Fig. 10). Average prevent TNC was 11.0 ± 4.5% in March, 7.7 ± 4.6 % in May and 10.2 ± 4.3 % (control only) in July. There was a slightly higher (non-significant) percent TNC on moraine soils compared to sandy soils.

III Predicting bittersweet presence and abundance in a fire mosaic landscape:

Methods:

We have developed the following criteria for the sample site selection:

a) There will be equal representation among fire histories to benefit subsequent data analysis.
b) We will also sample near and far from roads to understand the interaction between fire and disturbance along roads on the invasion of Oriental bittersweet.
c) We will use a simple vegetation classification derived from the INDU vegetation map.

We have acquired the most recent fire maps from the NPS and Indiana Dunes State Park, thus we have a complete fire history from 1982 to June 2010. We developed a fire history map for the park over this period and used vegetation classes, distance from road and fire frequency to identify sampling classes. The Indiana Dunes vegetation map developed from photographs taken in 2005 was used to develop the vegetation classes (Hop et al. 2009). We agglomerated the vegetation associations into 10 classes: general land use, herbaceous, savanna, woodland, hydric forest, mesic forest, xeric forest, non-vegetated, sparse vegetation and shrubland.

A 50 m buffer around roads was created to compare invasion near and away from roads. A 50 m buffer was selected because this is the distance that edge effects on average penetrate from edges into forests (Matlack 1993). In the data analysis we have the option to use distance from road, a continuous variable rather than the discrete variatble of near road versus away from road. This 50 meter buffer was used to ensure that random samples fell near roads as well as sway from them.

Fire frequencies were classified into 4 year frequency classes: unburned, low burn (1-5 fires), moderate burn (6-10 fires), high burn (11-15 fires). We then randomly selected at most ten locations to sample for each combination of fire frequency (4), road distance (2), and vegetation type (10). Once classified, these were randomly sampled from the GIS layer (Figure 1).

We have uploaded the waypoints into the GPS, and last fall, we sampled about 40 plots to ensure that the methods are giving us the data we need. We completed the random point sampling to generate data for creation and validation of the landscape models of bittersweet presence and abundance as a function of fire history. We have selected more than 400 points to be sampled using either ArcMap. A total of 400 plots (5 m radius) will be sampled with 300 used to model development and 100 for model validation. We developed a data dictionary modified from the protocol used in the Invasive Plant Atlas of New England (IPANE) (Table 6).

Results:

We collected field data from the West Unit of the park in the fall of 2010 and East Unit of the park in 2011 We have sampled a total of 151 plots as of September 30, 2011. We will complete the sampling in October of 2011 or spring of 2012. Data processing and clean-up will begin in November 2011.

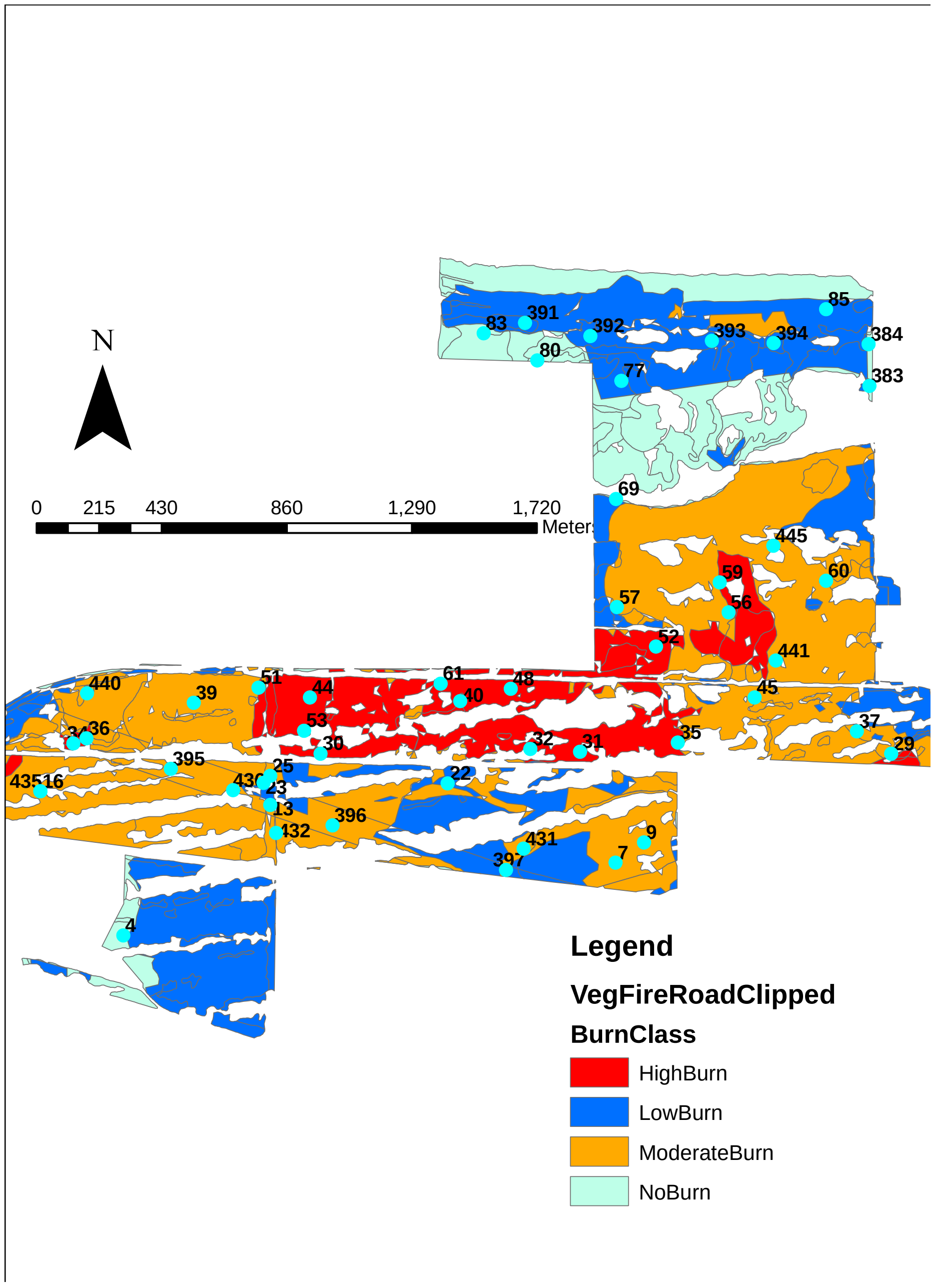


Figure 11. ArcMap display of random sampling points in Miller Woods with fire regimes displayed as back ground colors.

Table 6. Data fields for landscape scale sampling

Variable	Options
Plot number	
Canopy closure with densiometer	
Slope (degrees)	
Aspect (compass)	N, NE, E, SE, S, SW, W, NW, Flat
Soil type	Dry, moist, saturated, inundated
Habitat	Upland/wetland, field/forest, lake edge, roadside, floodplain forest, wet woods, mixed hardwood, beech/maple, oak/hickory, oak savanna, oak woodland, foredune, secondary dune, dune forest, open field, old field, prairie opening, stream bank, old home site
Celastrus abundance	Single stems, <20, 20-99, 100-999, >1000
Celastrus distribution	Single plant, Evenly sparse, Single patch, Multiple patches, Dense throughout
Celastrus strata	Seedlings, groundlayer, subcanopy, tree canopy.
Celastrus cover (%)	<1%, 1-5%, 6-25%, 26-50%, 51-75%, 76-100%
Abundance, distribution and cover of the woody exotic species present within the plot	Species include: *Ailanthus altissima*, *Berberis thunbergii*, *Elaeagnus umbellata*, *Euonymus alatus*, *Lonicera* spp., *Rosa multiflora*

Summary:

Fire and the susceptibility of invasion experiment:

The removal of litter from burning did not improve the germination and establishment of Oriental bittersweet compared to controls which had higher germination. Habitat has a stronger influence on Oriental bittersweet establishment. A higher percentage of Oriental bittersweet seeds germinated in forested sites compared to savanna and prairie.

Fire effects on established plants of Oriental bittersweet:

On a plot basis, burn alone and cut-and-burn significantly reduced Oriental bittersweet cover compared to the control and cut alone plots in the growing season after the treatment application. Density of stems < 2.5 mm in diameter and total stems were significantly greater in burn and cut-and-burn plots compared to the control and cut alone plots. Changes in numbers of stems > 2.5 mm were mixed and inconclusive. During the winter we will survey in all plots at all sites to examine whether micortopography is influencing cover and community responses.

On an individual basis, we found that Oriental bittersweet mortality was negatively correlated with stem size, and was greater in cut-and-burn compared to controls and cut or burn alone. There were significantly greater numbers of resprouts in cut-and-burn treatments compared to cut only whereas numbers of resprouts in burned plots were intermediate between the two treatments. The number of resprouts significantly increased with plant size.

Total nonstructural carbohydrates declined from March to May, and then increased again in July; however cutting of Oriental bittersweet in early July significantly reduced TNC by mid month. Since most of the growth of Oriental bittersweet occurs in late May and June, cutting in July might be an effective strategy to weaken the individuals in preparation for subsequent foliar herbicide in early September.

These results suggest that spring burning or cutting and burning can reduce the cover of Oriental bittersweet. The degree of post fire resprouting may be dependent on the size class distribution and density of the Oriental bittersweet at the site. Cutting of plants in late June and early July significantly lowers root carbohydrate reserves in Oriental bittersweet. Cutting at this time might be useful in conjunction with other treatments in controlling this invasive species.

Predicting bittersweet presence and abundance in a fire mosaic landscape: We continued the sampling in 2011, but have yet to initiate the data analysis.

Project Duration, Timeline, and Progress

This project started on September, 2008 and will be completed on September 30 of 2012. The progress per the proposal is indicated below and in Table 7 by the red text.

Post-Award/Prefundng Period (September 2008)

- Temperature effects on seed viability: we will collect seed and fruits of Oriental bittersweet for this and the susceptibility experiment. **Completed**
- Susceptibility Experiment: layout of plots in park. **Completed**
- Resprout Experiment: setup 8 blocks. **Completed**

First Funding Year (2009)

- Temperature effects on seed viability: conduct lab experiment during winter. **Completed**
- Susceptibility Experiment: conduct burns, rake litter, sow seeds and fruit in late winter (March/April), and assess emergence and response during the growing season. **Completed**
- Resprout Experiment: presample population, individual, and environmental variables. **Completed**
- Fire Regime Study: identify paired sites in proposed prescribed burns for late fall and late winter burn seasons. **In progress**
- Progress Summary due in September. **Completed**

Second Funding Year (2010)

- Susceptibility Experiment: follow second year growth rates and disassemble experiment, commence analysis and paper writing. **Completed**
- Resprout Experiment: apply burn and cut treatments and sample population, individual, and environmental variables. **Completed.**
- Predicting Bittersweet Abundance/Growth in fire mosaic: Analyze fire mosaic, locate random sampling points, and commence sampling. **Completed.**
- Progress Summary due in September. **Completed**.

Third Funding Year (2011)

- Resprout Experiment: sample population, individual, and environmental variables. **Completed**
- Resprout Experiment: apply burn treatments and sample population, individual, and environmental variables. **Completed**
- Predicting Bittersweet Abundance/Growth in fire mosaic: Complete sampling and develop models. **In progress**
- Progress Summary due in September. **Completed**

Fourth Funding Year (2012)

- Susceptibility Experiment: disassemble experiment, commence analysis and paper writing **In progress**
- Resprout Experiment: sample population and environmental variables. Analyze and prepare paper. **In progress**
- Predicting Bittersweet Abundance/Growth in fire mosaic: Complete sampling and develop models. **In progress**
- Final report due September 30, 2012. **In progress**

Table 7. Deliverable, Description and Delivery Dates

Deliverable Type (See Format Overview, Section VIII)	**Description**	**Delivery Dates**
Refereed Publication	Paper titled: Roles of fire intensity, disturbance, habitat, and environmental variables in the susceptibility of Oriental bittersweet establishment	July 2010
Refereed Publication	Paper titled: Experimental and prescribed fires: their effect on the growth and spread of Oriental bittersweet	Dec 2011
Refereed Publication	Paper titled "Burning questions in managing Oriental bittersweet"	
Field Demonstration/Tour	Public display at Bailly experimental plots. Tours will be given to the public twice per year with the National Park Service.	Starting 2009 **In progress**
Workshop	Midwest Manager workshop to be held at the Indiana Dunes Environmental Learning Center	2012
Conference Symposia	Symposium on Fire and Exotic Plant Management in the Eastern US at Ecological Society of America/Natural Areas Conference	August/October 2010 **Postponed to 2011 when we have results to discuss.**
Non-refereed publication	USGS Fact Sheet Concerning Fire and Oriental Bittersweet: paper and web based.	2011
Non-refereed publication	Progress Summaries	Sept. 30, 2009-2010
Non-refereed publication	Final Report and educational multimedia DVD	Sept. 30, 2012

Acknowledgments

We thank the Dan Morford, FMO at Indiana Dunes for supporting this project and conducting the research burns in 2009 and 2010. Neal Mulconrey, NPS, for writing the burn plans that made this project possible. Laura Cremin, Katie Kangas, and Kelly McAvoy assisted with the 2009 field work and Katie Kangas, Sarah Strobl, Cathy Martin and Danny Ferry assisted in the 2010 field and lab work. Katie Kangas, Caleigh Hoiland, Mark Fortelka, Brian Van Asdall, and Seth

Bostian were instrumental in completing the work in summer of 2011. Betzi Poole and Tammy Patterson provided excellent GIS expertise to calculate fire regime statistics and develop GIS maps fire frequency, proximity to road, and vegetation! Use of trade, product, or firm names does not imply endorsement by the U.S. government.

Literature Cited

Bebawi FF, Campbell SD. 2002. Effects of fire on germination and viability of bellyache bush (Jatropha gossypiifolia) seeds. Australian Journal of Experimental Agriculture **42**: 1063-1069.

Bond WJ, Midgley JJ. 2001. Ecology of sprouting in woody plants: the persistence niche. Trends in Ecology and Evolution **16**: 45-51.

Cole KL, Klick KF, Pavlovic NB. 1992. Fire temperature monitoring during experimental burns at Indiana Dunes National Lakeshore. Natural Areas Journal **12**: 177-183.

Dibble AC, White RH, Lebow PK. 2007. Combustion characteristics of north-eastern USA vegetation tested in the cone calorimeter: Invasive versus non-invasive plants. International Journal of Wildland Fire **16**: 426-443.

Elliott KJ, Vose JM, Swank WT, Bolstad PV. 1999. Long-term patterns in vegetation-site relationships in a southern Appalachian forest. Journal of the Torrey Botanical Society **126**: 320-334.

Fike J, Niering WA. 1999. Four decades of old field vegetation development and the role of *Celastrus orbiculatus* in the northeastern United States. Journal of Vegetation Science **10**: 483-492.

Floyd ML, Hanna D, Romme WH, Crews TE. 2006. Predicting and mitigating weed invasions to restore natural post-fire succession in Mesa Verde National Park, Colorado, USA. International Journal of Wildland Fire **15**: 247-259.

Gerwing JJ. 2001. Testing liana cutting and controlled burning as silvicultural treatments for a logged forest in the eastern Amazon. Journal of Applied Ecology **38**: 1264-1276.

Glasgow LS, Matlack GR. 2007. The effects of prescribed burning and canopy openness on establishment of two non-native plant species in a deciduous forest, southeast Ohio, USA. Forest Ecology and Management **238**: 319-329.

Gurvich DE, Enrico L, Cingolani AM. 2005. Linking plant functional traits with post-fire sprouting vigour in woody species in central Argentina. Austral Ecology **30**: 868-875.

Henderson NR, Long JN. 1984. A comparison of stand structure and fire history in two black oak woodlands in northwestern Indiana. Botanical Gazette **145**: 222-228.

Hop K, Lubinski S, Dieck J, Drake J, Menard S. 2009. National Park Service Vegetation Inventory Program, Indiana Dunes National Lakeshore, Indiana. La Crosse, WI and St. Paul, MN: Department of the Interior, U. S. Geological Survey, Upper Midwest Environmental Science Center and NatureServe. Report no.

Howard JL. 2005. Celastrus orbiculatus. (November 2, 2005 2005; http://www.fs.fed.us/database/feis/plants/vine/celorb/all.html)

Iwasa Y, Kubo T. 1997. Optimal size of storage for recovery after unpredictable disturbances. Evolutionary Ecology **11**: 41-65.

Kobe RK. 1997. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. Oikos **80**: 226-233.

Kobe RK, Iyer M, Walters MB. 2010. Optimal partitioning theory revisited: Nonstructural carbohydrates dominate root mass responses to nitrogen. Ecology **91**: 166-179.

Leicht-Young SA, Pavlovic NB, Grundel R, Frohnapple K. 2007. Distinguishing native (Celastrus scandens L.) and invasive (C. orbiculatus Thunb.) bittersweet species using morphological characteristics. Journal of the Torrey Botanical Society **134**: 441-450.

Matlack GR. 1993. Microenvironment variation within and among forest edge sites in the eastern United States. Biological Conservation 66: 185-194.

McNab WH, Loftis DL. 2002. Probability of occurrence and habitat features for oriental bittersweet in an oak forest in the southern Appalachian mountains, USA. Forest Ecology and Management **155**: 45-54.

Pande A, Williams CL, Lant CL, Gibson DJ. 2007. Using map algebra to determine the mesoscale distribution of invasive plants: The case of Celastrus orbiculatus in Southern Illinois, USA. Biological Invasions **9**: 419-431.

Pavlovic NB, Bowles ML. 1996. Rare plant monitoring at Indiana Dunes National Lakeshore. Pages 253-280 in Halvorson WL, Davis GE, eds. Science and Ecosystem Management in the National Parks. Tucson: University of Arizona Press.

Richburg JA, Patterson III WA, Ohman M. 2004. FIre managment options for controlling woody invasive plants in the Northeastern and Mid-Atlantic US. Amherst, MA: University of Massechusetts, Department of Natural Resources Conservation. Report no. Joint Fire Science Program, Project 00-1-2-06.

Wright SJ, CalderÃ³n O, HernandÃ©z A, Paton S. 2004. Are lianas increasing in importance in tropical forests? A 17-year record from panama. Ecology **85**: 484-489.

Zheng H, Wu Y, Ding J, Binion D, Fu W, Reardon R. 2004. Invasive plants of Asian origin established in the United States and their natural enemies. Morgantown, WV: USDA, Forest Service, Forest Health Technology Enterprise Team. Report no. FHTET 2004-05.

Noel B. Pavlovic, Stacey L. Young
U. S. Geological Survey, Lake Michigan Ecological Research Station
Analysis of soluble carbohydrates in roots of Oriental Bittersweet, modified by Sarah Strobl from protocol developed in Kobe laboratory.

Chemicals
95% alcohol
Phenol
Sulfuric acid
Dextrose anhydrous powder
NaOH pellets
Acetic acid
Amyloglucosidase
Glucose oxidase reagent

Materials (for one round of 32 samples and blanks, *excludes* standards)
32 15-ml conical centrifuge tubes
33 16x125mm glass tubes
32 disposable pipettes
85 13x100mm glass tubes
85 1-ml cuvettes
41 1000-µl tips
36 200-µl tips

Solutions Preparations
1. 80% ethanol
   Add 150 ml DI water to 800 ml 95% alcohol. Mix.
   This is sufficient for ~145 samples.
2. 5% phenol. **Caution: phenol is a known carcinogen and a health hazard. WORK IN HOOD WITH APPROPRIATE SAFETY EQUIPMENT.**
   Dissolve 25 g of phenol crystal in a beaker with a little DI water.
   Transfer to a 500 ml volumetric flask and dilute with DI to line.
   Store in an amber bottle or cover with foil to protect from light.
   This is sufficient for ~740 samples.
3. 10 mg/ml glucose standard
   Dissolve 250 mg of dextrose anhydrous powder in a beaker with ~10 ml DI.
   Transfer to a 25 ml volumetric flask and dilute with DI to line.
   This is sufficient for ~725 samples.
4. Acetate buffer, 0.1 M, pH 5.0
   If no 1-2 M NaOH solution is available, make by dissolving 4-8 g of NaOH pellets in 100 ml DI.
   Add 6 ml acetic acid to a beaker containing ~750 ml DI.
   Add NaOH solution drop-wise to the acetic acid solution as it is being stirred on a stir plate, measuring pH as you add. Stop when pH is 5.00.
   Transfer to a 1000 ml volumetric flask and dilute with DI to line. Store in fridge.
   This is sufficient for ~475 samples.

5. Amyloglucosidase, 1 mg/sample = 100 µl/sample
    For 32 samples, make enough for 40 by dissolving 2 ml enzyme in 2 ml cold acetate buffer.
    Shake *gently* to dissolve. *Shaking too vigorously may degrade enzyme.*
6. Glucose oxidase reagent
    Dissolve contents of one reagent bottle in 100 ml DI water.
    This is sufficient for 100 samples. Store in fridge between rounds.

Extraction:

1. Dry the samples in a plant oven for 2-3 hours in open scintillation vials. Let cool in desiccator overnight.
2. Turn on water bath and set to 75°C (167°F).
3. Label 32 16x125mm glass tubes.
4. Weigh out ~20 mg of sample into the labeled 16x125mm tubes. Record the weights (this is dry mass).
5. Add 2 ml 80% alcohol to each sample. Vortex at medium speed on "touch" setting.
6. Place in water bath for 30 minutes, keeping the lid open.
7. While samples are in water bath, label 32 15-ml conical centrifuge tubes. Record the weights of these tubes empty and without caps.
8. To each centrifuge tube, add ~6 ml DI. Cover all tubes with a sheet of saran wrap and set aside.
9. Also while samples sit in water bath, prepare standards for phenol assay. These can be covered with parafilm and refrigerated for 2-3 weeks.
    Add 1 ml 10 mg/ml glucose standard to a 16x125mm glass tube.
    Add 1 ml 80% alcohol to the same tube. **Vortex**. This makes a 5 mg/ml standard.
    Prepare 100 ml of 12.5% alcohol by diluting 15 ml 80% alcohol with 85 ml DI water.
    Label 10 16x125mm glass tubes as 0, 5, 10, 20, 30, 40, 60, 80, 100, and 120 µg/ml.
    Add 5 ml 12.5% alcohol to each of these tubes.
    Pipette 0, 5, 10, 20, 30, 40, 60, 80, 100 and 120 µl of the 5 mg/ml standard into the appropriate tube. **Vortex**.
    These are now approximately the concentrations as labeled. See table for exact concentrations.
10. Centrifuge samples for 5 minutes at 3500 rpm (1917 g). The radius should already be set to 14 cm.
11. Decant/transfer the supernatant into the appropriate centrifuge tube from step 7.
12. Re-extract the pellet by adding 2 ml 80% alcohol to each 16x125mm tube. Vortex. Set in 75°C water bath for 15 minutes. Centrifuge for 5 minutes as before. Decant supernatant into same centrifuge tube.
13. Extract the pellet a third time by again adding 2 ml 80% alcohol. Vortex. Set in 75°C water bath for 15 minutes. Centrifuge for 5 minutes as before. Decant supernatant into same centrifuge tube.
14. These 16x125mm tubes with the pellet should now be placed back in the 75°C water bath for about 0.5 hour. Then transfer to the plant oven to dry. This should take about 2 hours. When dry, cover the tubes with parafilm and store in the dessicator

until starch analysis is possible. If the dessicator is unavailable, samples can be stored in the fridge.

14. Label 43 13x100mm glass tubes. (32 for the samples, 10 for the standards made in step 9, and 1 for a blank).
15. Weigh the centrifuge tubes with no caps and record weights.
16. Cap all tubes and shake well. Add 0.5 ml of sample extract to its appropriate previously labeled 13x100mm glass tube. In order to not waste pipette tips, at the same time take the mass for density. Do this by placing a beaker on the scale and tare. Add 0.5 ml of sample extract and record weight. Tare. Add 0.5 ml of the next sample and record weight. Continue for all samples. Discard when finished.
17. Add 0.5 ml of the standards (made in step 12) to the appropriate labeled 13x100mm glass tubes.
18. Add 0.5 ml of DI water to the labeled 13x100mm glass tube for the blank.

Analysis: phenol-sulfuric acid assay

1. WORKING IN THE HOOD WITH DOUBLE GLOVES, carefully pipette 0.5 ml 5% phenol into the sample, standard, and blank test tubes from steps 16-18. **Wipe the entire pipette down with alcohol.**
2. Using the dispenser, carefully add 2.5 ml concentrated sulfuric acid to the test tubes. Make sure to add it directly to the liquid surface rather than the side of the test tube to ensure proper mixing.
3. Let sit in the hood for 10 minutes.
4. Vortex at top speed for a count of 4 (also in the hood).
5. Let sit in the hood for 30 minutes. (After about 20 minutes, turn on the spec to warm up).
6. Label 43 cuvettes (32 for samples, 10 for standards, 1 for blank). Pour ~1 ml of liquid into cuvette.
7. **Instructions for spec:**
   Push the button under "Set nm" on screen. Type in 490 and hit "Enter". Kimwipe each cuvette before putting it in spec. Blank goes in spot labeled "B" and stays in until all samples are read. The arrow on the front of the cuvette should face the front side of the opening (where the silver bit is). Close the lid and hit "Measure Blank". Once it is done, hit "B" and make sure it reads 0.000. If it does not, hit "Measure Blank" again. If it reads 0.000, continue to read absorbance of samples in the same order in which the reagents were added (hit "1", then "2", etc.). Set the blank each time a new set of samples is put in.
8. For clean up, pour remaining contents of each cuvette and test tube into "phenol waste" jar. Rinse each cuvette and test tube twice with DI water. Discard cuvettes and tubes.

Standards (μg/ml)	**Exact Concentrations (μg/ml)**	**Optical Density @ 490nm**
0	0.000	
5	4.995	
10	9.980	

20	19.920	
30	29.821	
40	39.683	
60	59.289	
80	78.740	
100	98.039	
120	117.188	

Calculations for soluble sugar concentration

1. To develop standard curves, plot optical density (dependent variable) on concentration (independent variable). Optical density is the dependent variable because it is measured with some error, consistent with assumptions of regression. We assume that we get the concentrations exact (or nearly so) and thus it has less error and is the dependent variable.
2. You will get a linear (y = mx + b) equation as follows:
   Optical Density (absorbance) = M (slope) x Concentration (µg/ml) + b (y-intercept)
   To calculate the concentration in your sample, rearrange to solve for concentration:
   Concentration = [Optical Density – b] / M
3. At this point, we know the concentration in the sample, but this is not very meaningful unless it is normalized by the total mass of plant tissue and the dilutions (in this case, the entire solution is analyzed, so we just need to know the volume of the sample). Calculate % mass that is soluble carbohydrate as:

The sample volume can be calculated as follows (should come out to ~12 ml):

Analysis: starch in pellet

1. Dissolve dried starch pellet in 2 ml cold acetate buffer. Vortex. Make a blank solution of 1 ml DI water and 1 ml acetate buffer in a 16x125mm tube. Tubes should be capped with aluminum foil. Autoclave all 33 tubes at 125°C for 10 minutes. (This will actually take much longer than 10 minutes; the water bath should be turned on and set to 57°C at some point during autoclaving).
2. Cool samples for 10 minutes.
3. Add 100 µl Amyloglucosidase solution to all tubes. Shake **gently**. *Shaking too vigorously may degrade enzyme.* Re-cap and place in 57°C water bath for 3 hours.
4. While samples are in water bath, prepare standards for starch analysis. These can also be covered with parafilm and refrigerated for 2-3 weeks.

Dilute 10 mg/ml to 1 mg/ml by taking 0.1 ml of 10 mg/ml and adding 0.9 ml of DI.
Label 9 13x100mm tubes as 0, 50, 100, 200, 300, 400, 600, 800, and 1000 µg/ml.
Pipette 0, 5, 10, 20, 30, 40, 60, 80, and 100 µl of the 1 mg/ml into the corresponding tube.
Then add 100, 95, 90, 80, 70, 60, 40, 20, and 0 µl of DI water to the tubes, respectively.
Each tube should now contain a total of 100 µl of liquid. **Vortex.**

5. Also while samples sit in bath, label 42 13x100mm tubes and 42 cuvettes (32 samples, 9 standards, and 1 blank).
6. After 3 hours are up, turn on the spec to warm up, and set the water bath down to 37°C. (You may need to take out some water, and replace with cold DI water to help it cool faster).
7. Centrifuge sample tubes at 1504g for 5 minutes. RPM should be set to 3100. The radius should already be set at 14 cm.
8. Add 5 µl of each sample, standard, and blank to the appropriately labeled 13x100mm tube.
9. Add 1 ml glucose oxidase reagent to each tube.
10. Place all tubes in the 37°C water bath for at least 5 minutes.
11. Pour entire content from each tube into its corresponding cuvette in the order in which glucose oxidase was added.
12. Set wavelength to 500 nm (**note that this is different wavelength than phenol assay!**), and read the absorbance of each, again in order. Measure the blank each time a new set of samples is put in. ***The color is stable for at least 15 minutes, so be sure to spec the samples within 15 minutes of coming out of the bath.*

Calculations for starch concentration

1. Just as for the soluble sugar concentration, plot optical density (dependent variable) on concentration (independent variable) to develop the standard curve.
2. Percentage of total nonstructural carbohydrates can be calculated as follows:

****Note:** The 0.9 at the end of the equation is a correction factor. This correction factor is necessary when calculating percent starch in the sample because the repeating unit in starch is glucose minus one water molecule. When starch is broken down into glucose, it gains one water molecule.

Glucose has a molecular mass of 180. In starch, polymerized glucose has the molecular mass of 162. Thus it gains one molecule of water during our procedure. To figure out the mass of starch as a percentage of root mass, for example, it is necessary to correct for this increase in mass of the glucose monomer. So, 162 / 180 = 0.9.

However, in calculating the number of glucose equivalents contained in the mass of roots, the correction factor would not be necessary to apply (e.g. in an

APPENDIX

Table 8. Summary of vegetation composition of meter squared plots sampled for the established bittersweet study. Means ± SE/.

	Disturbed Woods		**Depauperate Woods**		**Oak savanna**		**Savanna Shrub**	
	2009	2010	2009	2010	2009	2010	2009	2010
	44	44	12	12	4	4	4	4
Celastrus orbiculatus	44.4 ± 6.1	28.4 ± 5.5	28.7 ± 12.6	23.5 ± 8.7	35 ± 5	34 ± 1	21 ± 9	13.5 ± 3.5
Achillea millefolium L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Actaea pachypoda Ell.	0.3 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Acalypha rhomboidea Raf.	0 ± 0	0 ± 0	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Acer rubrum L.	<0.1 ± <0.1	0.2 ± 0.1	<0.1 ± <0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1
Ageratina altissima (L.) King & H. Rob. var. altissima	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Agrimonia gryposepala Wallr.	0.2 ± 0.1	0.5 ± 0.3	0.1 ± 0.1	0 ± 0	1.3 ± 1.3	1 ± 1	0 ± 0	0 ± 0
Ailanthus altissima (Mill.) Swingle	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1
Alliaria petiolata (Bieb.) Cavara & Grande	1.2 ± 0.5	0.3 ± 0.1	0.9 ± 0.8	0.3 ± 0.2	0 ± 0	0 ± 0	0.8 ± 0.3	0.5 ± 0.5
Amphicarpa bracteata (L.) Fern.	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Amelanchier Medik.	0 ± 0	0 ± 0	1 ± 1	0.3 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Antennaria plantaginifolia (L.) Hook.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Anemone virginiana L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Arabis lyrata L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Arctium minus (Hill) Bernh.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Arisaema triphyllum (L.) Schott ssp. triphyllum	0.3 ± 0.2	0.5 ± 0.3	0.5 ± 0.5	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Aster spp.	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Berberis thunbergii DC.	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Bidens bipinnata L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Boehmeria cylindrica (L.) Sw.	0.5 ± 0.5	2.3 ± 2.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Botrychium matricariaefolium A.Br.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0
Bromus inermis Leyss.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Carya cordiformis (Wang.) K.Koch	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Carex pensylvanica Lam.	0 ± 0	<0.1 ± <0.1	0.5 ± 0.3	0.2 ± 0.1	4 ± 4	4 ± 4	0 ± 0	0 ± 0
CarexA	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
CarexB	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Carex swanii (Fern.) Mackenz.	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Caulophyllum thalictroides (L.) Michx.	0 ± 0	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Chenopodium leptophyllum Nutt.	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2
Cinna arundinaceae L.	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Cirsium arvense (L.) Scop.	<0.1 ± <0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Circaea lutetiana L. ssp. canadensis (L.) Asch. & Magnus	1.1 ± 0.3	1.1 ± 0.4	0.7 ± 0.7	1.2 ± 0.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Commelina communis L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Cornus florida L.	<0.1 ± <0.1	0.2 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Crataegus spp.	0.1 ± 0.1	<0.1 ± <0.1	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Cryptotaenia canadensis (L.) DC.	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Crataegus crus-galli L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Crataegus mollis (T. & G.) Scheele	0.1 ± 0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Desmodium canadense (L.) DC.	0 ± 0	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Diervilla lonicera Mill.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dioscorea villosa L.	0.1 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dichanthelium villosissimum (Nash) Freckmann var. villosissimum	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Elymus canadensis L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Elymus hystrix L. var. hystrix	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Elymus villosus Muhl.	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Euonymus alatus (Thunb.) Siebold	<0.1 ± <0.1	0 ± 0	2.2 ± 1.8	0.5 ± 0.3	4.5 ± 4.5	1 ± 1	0 ± 0	0 ± 0
Frangula alnus Mill.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.4	0.3 ± 0.3	0 ± 0	0 ± 0
Fraxinus americana L.	1.5 ± 0.6	0.4 ± 0.2	0.1 ± 0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Fragaria virginiana Duchesne	<0.1 ± <0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Galium aparine L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Galium asprellum Michx.	0.4 ± 0.2	0.5 ± 0.3	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0
Galium circaezans hypomalacum Fern.	<0.1 ± <0.1	<0.1 ± <0.1	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Galium pilosum Ait.	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0
Geum canadense Jacq.	2.4 ± 0.4	2.6 ± 0.6	<0.1 ± <0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Geranium maculatum L.	<0.1 ± <0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0.8 ± 0.8	0 ± 0	0 ± 0	0 ± 0
Glechoma hederacea L.	0.1 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0
PoaceaeA	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Hackelia virginiana (L.) I.M.Johnston	0.2 ± 0.1	0.4 ± 0.2	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Hamamelis virginiana L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Helianthus divaricatus L.	0.1 ± 0.1	0.2 ± 0.2	0.7 ± 0.4	0.6 ± 0.5	0 ± 0	0 ± 0	3.5 ± 3.5	5 ± 5
Hypericum perforatum L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Impatiens capensis Meerb.	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ionactis linariifolius (L.) Greene	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Iris L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Juglans nigra L.	0.1 ± 0.1	0 ± 0	0.9 ± 0.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Koeleria macrantha (Ledeb.) Schult.	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Krigia biflora (Walt.) Blake	0 ± 0	0 ± 0	0 ± 0	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Lactuca canadensis L.	<0.1 ± <0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Leersia virginica Willd.	0.1 ± 0.1	0.2 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Lindera benzoin (L.) Blume	0.3 ± 0.2	0.7 ± 0.4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Liriodendron tulipifera L.	0 ± 0	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Lonicera maackii (Rupr.) Herder	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Lonicera L.	0.3 ± 0.2	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Lysimachia ciliata L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Maianthemum canadense Desf.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Maianthemum racemosum (L.) Link ssp. racemosum	0.1 ± 0.1	<0.1 ± <0.1	0.3 ± 0.3	0.3 ± 0.3	0 ± 0	0 ± 0	1 ± 1	0.4 ± 0.4
Maianthemum stellatum (L.) Link	0 ± 0	0 ± 0	2.7 ± 1.9	0.3 ± 0.2	0 ± 0	0 ± 0	0.5 ± 0.5	0.5 ± 0.5
Osmorhiza claytoni (Michx.) C.B.Clarke	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Oxalis spp.	<0.1 ± <0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Parthenocissus quinquefolia (L.) Planch.	3.1 ± 0.7	2.3 ± 0.6	1.2 ± 1.2	1.7 ± 1.7	1 ± 1	2 ± 2	0 ± 0	0 ± 0
Pilea pumila (L.) Gray	1 ± 0.7	0.5 ± 0.4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Polygonatum biflorum (Walter) Elliot var. commutatum (Schult. & Schult. f.) Morong	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Poa compressa L.	<0.1 ± <0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Populus deltoides	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Podophyllum peltatum L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Polygonatum pubescens (Willd.) Pursh	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Potentilla simplex Michx.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Polygonum virginianum L.	5.7 ± 1.6	5.9 ± 1.4	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0
Prenanthes L.	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Prunus serotina Ehrh.	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0 ± 0	0 ± 0	2 ± 2	1.3 ± 1.3
Pteridium aquilinum latiusculum (Desv.) Underw.	0 ± 0	0 ± 0	0.7 ± 0.7	0.3 ± 0.3	23.5 ± 6.5	27.5 ± 12.5	0 ± 0	4 ± 4
Quercus rubra L.	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Quercus velutina Lam.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	5 ± 5	4 ± 4	0 ± 0	0 ± 0
Rhus copallina latifolia Engler	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Rosa carolina L.	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	2.5 ± 2.5	0.8 ± 0.8	0.5 ± 0.5
Rosa multiflora Thunb.	3.7 ± 1.6	2.5 ± 0.8	0.2 ± 0.2	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0
Rubus allegheniensis Porter	0.2 ± 0.2	0.4 ± 0.4	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Rubus flagellaris Willd.	2.3 ± 0.9	5.1 ± 3.4	0.3 ± 0.3	0.1 ± 0.1	17.5 ± 2.5	14 ± 6	0 ± 0	0 ± 0
Rubus idaeus L. ssp. strigosus (Michx.) Focke	1.4 ± 0.7	1.2 ± 0.5	0 ± 0	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0
Rubus pensylvanicus Poir.	0.5 ± 0.3	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sassafras albidum (Nutt.) Nees	0.1 ± 0.1	0.3 ± 0.2	2.7 ± 1.9	4.9 ± 4.4	0 ± 0	3 ± 2	0 ± 0	0 ± 0
Sanicula canadensis L.	2.7 ± 2.5	3.8 ± 2.6	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Smilax rotundifolia L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Smilax tamnoides hispida (Muhl.) Fern.	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Solidago altissima L.	0.2 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Solidago caesia L.	0 ± 0	0 ± 0	1 ± 0.6	1.2 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Solidago gigantea Ait.	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Solidago rugosa Ait.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Stellaria pubera Michx.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Symphyotrichum dumosum (L.) G.L. Nesom var. dumosum	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Symphyotrichum lateriflorum (L.) A. Löve & D. Löve var. lateriflorum	0.6 ± 0.2	0.9 ± 0.3	<0.1 ± <0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Symphyotrichum oolentangiense (Riddell) G.L. Nesom var. oolentangiense	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Tilia americana L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Toxicodendron radicans (L.) Kuntze ssp. radicans	0.6 ± 0.4	0.8 ± 0.5	<0.1 ± <0.1	0 ± 0	3 ± 3	4 ± 4	0 ± 0	0 ± 0
Tradescantia ohiensis Raf.	0 ± 0	0 ± 0	0.2 ± 0.2	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ulmus americana L.	0.1 ± 0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ulmus spp.	<0.1 ± <0.1	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ulmus rubra Muhl.	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Unk1	0 ± 0	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Unk209	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
PaceaeB	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Vaccinium pallidum Aiton	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	26 ± 4	20.5 ± 12.5
Veronica officinalis L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Viburnum acerifolium L.	0 ± 0	<0.1 ± <0.1	0.7 ± 0.7	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Vitis aestivalis Michx.	0.1 ± 0.1	0 ± 0	0.3 ± 0.3	0.4 ± 0.4	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Viburnum lentago L.	0 ± 0	0 ± 0	2.5 ± 2.5	1.2 ± 1.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Vinca minor L.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	14 ± 14	3.5 ± 3.5	0 ± 0	0 ± 0
Viola L.	0.1 ± 0	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Viburnum prunifolium L.	0 ± 0	0 ± 0	0 ± 0	1.2 ± 1.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Viburnum rafinesquianum Schultes	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	12.5 ± 12.5	12.5 ± 12.5
Vitis riparia Michx.	0 ± 0	0.1 ± 0.1	<0.1 ± <0.1	<0.1 ± <0.1	12 ± 12	5 ± 5	0 ± 0	0.4 ± 0.3
ExoticShrub	3.9 ± 1.8	2.6 ± 0.8	2.3 ± 1.8	0.5 ± 0.3	5.9 ± 5.9	1.3 ± 1.3	0 ± 0	0 ± 0
NativeShrub	4.6 ± 1.5	7.9 ± 3.6	3.7 ± 2.4	3.2 ± 1.7	18.5 ± 3.5	16.5 ± 8.5	39.3 ± 9.3	33.5 ± 0.5
NativeVine	3.9 ± 0.8	3.4 ± 0.8	1.5 ± 1.4	2.1 ± 2.1	16 ± 14	11 ± 7	0 ± 0	0.4 ± 0.3
NativeTree	2.3 ± 0.6	1.3 ± 0.3	5.1 ± 1.9	5.6 ± 4.3	5 ± 5	7 ± 6	2 ± 2	1.3 ± 1.3
ExoticTree	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1
ExoticGramin	<0.1 ± <0.1	<0.1 ± <0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
NativeForb	15.9 ± 3.1	19.7 ± 3.8	6.8 ± 1.9	4.6 ± 1.6	2.3 ± 2	1.3 ± 0.8	5 ± 4	6.1 ± 5.1
ExoticForb	1.4 ± 0.5	0.4 ± 0.1	0.9 ± 0.8	0.3 ± 0.2	14.3 ± 14.3	3.5 ± 3.5	0.8 ± 0.3	0.5 ± 0.5
NativeGramin	0.4 ± 0.1	0.3 ± 0.1	0.6 ± 0.5	0.2 ± 0.1	4 ± 4	4 ± 4	0 ± 0	0 ± 0