2019 RESEARCH REPORT Principle Investigator: Carmela M. Buono, PhD Student Dr. Kirsten Prior, Faculty Advisor

Department of Biological Sciences Binghamton University, State University of New York PO Box 6000, Vestal, New York 13902 Phone (cell): (732) 425-2666 Email: cbuono1@binghamton.edu

Project Title: Historical land use impacts on ant-mediated seed dispersal in northeastern deciduous forests

# Location: Cornell Botanical Gardens (Bald Hill, Carter Creek, Fall Creek Valley North and South, and Slaterville 600)

### I. STUDY PURPOSE AND OBJECTIVES

As humans increasingly develop and change the surface of the earth, large impacts are being made to natural communities such as habitat loss and a reduction in biodiversity. One of the largest anthropogenic contributors to the disruption of natural ecosystems is *land use change* [1]. Historical changes in land use can leave legacy effects such as, habitat fragmentation, changes in soil chemistry, and altered species composition [2–4]. With the additional threat of anthropogenic climate change, it is becoming increasingly important to understand how multiple anthropogenic stressors are impacting our ecosystems so that we may better conserve and restore them. In order to implement effective management and restoration practices, we first must understand if ecological systems can recover from the impacts of land use change. By understanding if and in what contexts systems can recover from land use change, then we can more appropriately apply management and restoration practices. This project focuses on one form of anthropogenic impacts, *land use change history*, and how they are impacting ecosystem biodiversity and function. This project addresses the research questions below and sections II.-III. will detail preliminary data from summer 2018-19 as well as future and continued work.

Human alteration of landscapes is the major cause of biodiversity loss and of the disruption of ecosystem functions [5]. The majority of forests in northeastern North America have at one time been cleared for agricultural or timber use but recently these fragments of previously cleared land have been abandoned and allowed to passively regenerate [6]. As a result, contemporary forests are composed largely of "secondary" or previously cleared forest, with less than 1% of "primary" forests remaining [6–9]. Given that much of our natural lands have already been converted for food production and living space, with continuing pressure [5], a key question for ecologists tasked with conserving and restoring natural systems is whether these systems can recover from large-scale land use change disturbances.

In northeastern forests, entire forest compositions were altered when forests were cleared. In secondary forests, it has been observed the tree and shrub layers have successfully recovered passively [6], while understory plant communities, which represent the greatest plant diversity in forests, seem to be greatly reduced in richness and abundance [6,10]. Particularly absent in these understory communities are myrmecochorous plants [11], which are plants specialized for seed dispersal by ants (i.e., their seeds have a lipid-rich appendage, "elaiosome") [8]. Myrmecochores are particularly rich in eastern deciduous forests, making up 35-40% of understory species, and including many well-known species, such as Asarum canadense, Sanguinaria canadensis, Uvularia perfoliata, and Anemone acutiloba [12–14]. Ants are attracted to and use the elaiosome to carry the seeds to their nests where they remove the elaiosome and feed it to their larvae, after which they "plant" the intact seed outside of their nests. This is a mutualistic interaction, in which ants receive a food reward while myrmecochores benefit by having their seeds dispersed away from conspecifics and protected from seed predators [15,16]. In eastern forests, ants in the genus Aphaenogaster are responsible for the majority of seed dispersal and are often referred to as "keystone dispersers" [17]. Seed dispersal by ants is an essential ecosystem function for understory plants in deciduous forests, yet few studies have examined how land use change affects this interaction, specifically across different regions in the northeast [17–19]. In order to conserve and work to restore the understory forest plant community in secondary forests, we need to better understand how seed dispersal is impacted, and if and how we may need to also restore or enhance this key function in secondary forests [17]. In my research, I am investigating if ant-mediated seed dispersal is intact in secondary forests by conducting a large-scale natural experiment, comparing myrmecochores, ants, ant habitat, and dispersal rates in primary and secondary forests in across the northeast.

### Main Question:

**Does** *land use history* **alter ecological function in secondary forests?** I will examine how land use history affects an important ecosystem function, ant-mediated seed dispersal in eastern deciduous forests. In particular, I will examine if land use affects the presence and strength of seed dispersal function by conducting observational surveys of vegetative communities, ant mutualist presence and abundance, the presence of seed dispersal function, and the quantity of ant habitat in paired primary and secondary forests in the northeast.

**A1. PATTERNS IN MYRMECOCHOUS PLANT COMMUNITIES** by surveying the abundance and diversity of forest understory plants, including myrmecochores.

**A2. PATTERNS IN NATIVE MUTUALIST ANT SPECIES ABUNDANCE** by surveying the identity and abundance of seed-dispersing ants with pitfall traps.

**A3. PATTERNS IN ANT HABITAT QUANTITY** by surveying and quantifying the type and abundance of potential nesting sites for the key mutualist species (logs, rocks, and leaf litter).

# **A4. PATTERNS IN ANT-MEDIATED SEED DISPERSAL** by measuring mutualist function (seed dispersal rates) with seed depot trials.

To this end, I have identified 20 primary and secondary forest sites in three regions (A: New Jersey, eastern New York; B: northern PA; C: central NY). In 17 sites, I conducted vegetative surveys (**including Bald Hill, Carter Creek, Fall Creek Valley North and South, and Slaterville 600**), and I am still analyzing this data. In 20 sites, I conducted seed dispersal trials and pitfall traps (**including Bald Hill, Carter Creek, Fall Creek Valley North and South, and Slaterville 600**). Overall, I am finding some exciting preliminary results (Fig. 1 & 2) that are detailed below in addition to the protocols I have utilized for my surveys.

# **II. RESEACH DESIGN AND LOGISTICS**

I plan to observe the interactions of ant mutualists on the native herbaceous community. I chose to work at **Bald Hill, Carter Creek, Fall Creek Valley North and South, and Slaterville 600** due to it containing secondary and old-growth forest habitat where the key mutualism for this project is found. To do this, I conducted i) vegetative surveys, ii) ant community surveys, iii) ant habitat surveys, and iv) seed dispersal rate measurements. These surveys consisted of three 50-meter semi-permanent belt transects that will be surveyed for another summer (Fig. 3). The transects are located in the center of the forest (50 meters or more away from an edge). Each transect includes 20 5\*5m survey plots, occurring on either side of the transect (5 as "plant" plots, and 5 as "invertebrate" plots, 15 of each plot per site). The surveys are observational and did *not* include any damaging of specimens.

To mark the plots for semi-permanent use, 1-foot 1-inch-wide PVC pipe were installed at each end of the transects. These posts are blue in color and look like (Fig. 4). Each PVC post has contact and identification information laminated and attached to the post.

i). The vegetative surveys of the herbaceous layer compare diversity, cover, and composition of vegetative species in the 5x5 "plant" plots. I measured the presence and cover of understory plants (including myrmecochores) in 4, 1 m<sup>2</sup> quadrats within plant plots. In each plot, I also measured shrub and tree composition and cover, along with potential correlates that could influence understory plants, such as soil temperature, soil moisture, soil pH, patch size, and light availability, and ground cover. The vegetative surveys are observational and did not include any damaging of specimens. Observations from the vegetative plots can be found in Table 1.

ii). In a subset of plots, I sampled the ant community by placing pitfall traps (7 cm deep, 9 cm wide, and 9 oz) out for 24 hours. Two pitfall traps were in the "invertebrate" plots (total site n = 30) and placed flush with ground level. A combination of biodegradable soap and water were placed into these cups and left out for 24 hours. A wire mesh filter was placed on top of each of these containers to eliminate the chances of small rodents or salamanders accidentally being caught. After 24 hours, the cups were collected, and the contents of the containers were emptied and later identified. These pitfall traps were left out for the 24 hours on a dry day and collected (cups and all other materials) and removed from the ground immediately (leaving no trace).

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iii). For ant habitat, we set up 2 1m wide belt transects along the edge of the plots starting from the transect line (Fig. 7). In each belt transect, we measured the length, width, and height of all 'potential nests' which include 1) leaf piles, 2) downed wood, and 3) rocks within the belt transects. We also noted the presence of ant colonies in each 'potential nest' material.

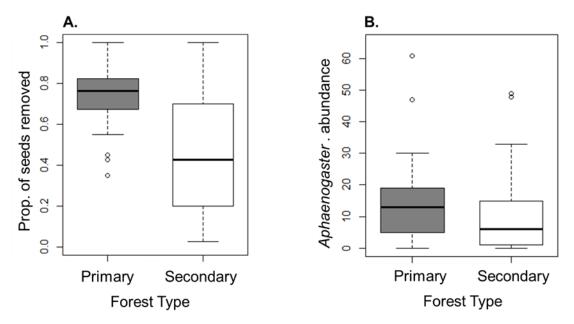
iv). Finally, to test the ability of the ants to disperse seeds, seed depots with 8 native plant seeds were placed out with a mesh covering (to exclude rodents). These depots were left out for a total of 24 hours on a dry day and the presence or absence of seeds were recorded the next day. On the final day, all materials were removed and from the ground (leaving no trace). Seeds were from native *Asarum canadense* and are not viable.

The primary goal of these two summers was to gather baseline data of the biotic interactions that I will be measuring between primary and secondary forests. In addition, I used this opportunity to indicate the locations for my vegetative and ant surveys so that I can return to them for the next summer to collect additional data. This work is part of a PhD dissertation, in which I will pair this natural experiment with a manipulative experiment.

#### **III. PRELIMINARY RESULTS**

I have completed 17 sites of (A1), 16 sites of (A3) and 20 of (A2 & A4). This upcoming field season, I plan to finish plant and ant habitat surveys in the summer of 2020.

I performed linear and generalized linear models to test if region, land use history, and other measured variables affects plant, ant, and habitat response variables. From completed A4, I am already finding interesting results - consistent seed removal in primary forests and low and variable removal in secondary forests (Fig. 2). In addition, from A2 I am finding lower abundance of the keystone seed-dispersing ants (ants in the genus *Aphaenogaster*) (Fig. 3). **This work is part of a PhD dissertation**, in which I will pair this natural experiment with a manipulative experiment.



**Figure 2.** A) Proportion of seeds removed from depots and (B) Aphaenogaster sp. abundance between primary and secondary forest sites at the transect level (n = 30). Forest type has a significant effect in both cases, with region retained as a random effect in models. Thick lines in box plots represent medians, boxes represent 1st and 3rd quartiles, whiskers represent minimums and maximums, and points represent outliers.

Genus	Species	Genus	Species			
<b>Canopy Layer</b>	r Bald Hill	Shrub Layer Bald I	Shrub Layer Bald Hill			
Acer	rubrum	Acer	pensylvanicum			
Betula	lenta	Hamamelis	virginiana			
Carpinus	caroliniana	Kalmia	latifolia			
Crataegus	pruinosa	Lindera	benzoin			
Fagus	grandifolia	Lonicera sp.				
Fraxinus	americana	Vacinnium sp.				
Hamamelis	virginiana	Shrub Layer Carte	Shrub Layer Carter Creek			
Nyssa	sylvatica	Acer	pensylvanicum			
Ostrya	virginiana	Berberis	thunbergii			
Quercus	alba	Rosa	multiflora			
Quercus	motana	Shrub Layer Fall V	Shrub Layer Fall Valley Creek			
Quercus	rubra	Berberis	thunbergii			
Tsuga	canadensis	Lindera	benzoin			
Canopy Layer Carter Creek		Lonicera	morrowii			
Acer	rubrum	Rubus	occidentalis			
Acer	saccharum	Shrub Layer Slater	ville 600			
Betula	lenta	Acer	pensylvanicum			
Carpinus	caroliniana					
Fagus	grandifolia					
Fraxinus	americana					
Ostrya	virginiana					
Tilia	americana					
Tsuga	canadensis					
<b>Canopy Layer</b>	r Fall Creek Valley					
Fagus	grandifolia					
Acer	saccharum					
Fraxinus	americana					
Ostrya	virginiana					
Prunus	serotina					
Tsuga	canadensis					
Tilia	americana					
Acer	rubrum					
Carya	glabra					
Canopy Layer	r Slaterville 600					
Acer	saccharum					
Carpinus	caroliniana					
Fagus	grandifolia					
Fraxinus	americana					
Ostrya	virginiana					
Tilia	americana					

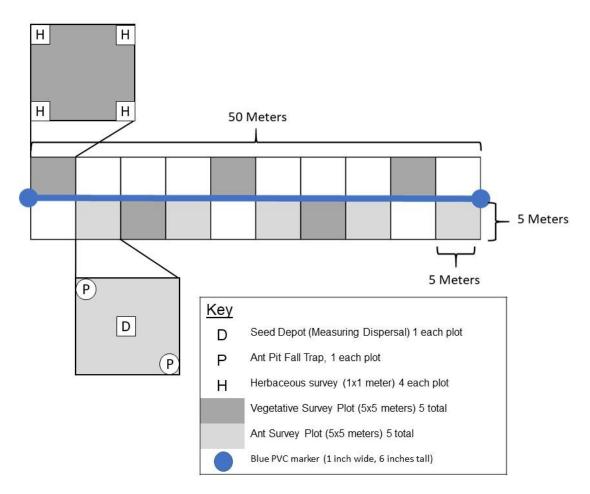
**Table 1.** Vegetative species identified (to lowest taxonomic group possible) during vegetativesurveys Cornell Botanic Gardens Property, summer 2019.

**Table 2.** Insect Orders identified from the pitfall traps implemented for the ant abundance surveys at Cornell Botanic Gardens Property, Summer 2019. We are currently identifying the ants to species. We are still processing the insects from Carter Creek and Fall Creek Valley.

Code	Order				
Slaterville 600					
ANA	ANNELIDA				
ARA	ARANEAE				
СНІ	CHILOPODA				
COLE	COLEOPTERA				
COLL	COLLEMBOLA				
DIPT	DIPTERA				
ISOP	ISOPODA				
LEP	LEPIDOPTERA				
OPI	OPILIONES				
Bald Hill					
ACA	ACARI				
ANA	ANNELIDA				
ARA	ARANEAE				
СНІ	CHILOPODA				
COLE	COLEOPTERA				
COLL	COLLEMBOLA				
DIPL	DIPLOPODA				
DIPT	DIPTERA				
GAST	STYLOMMATOPHORA				
HYM	HYMENOPTERA				
ISOP	ISOPODA				
LEP	LEPIDOPTERA				
OPI	OPILIONES				

Marker Code	Lat	Long	Marker Code	Lat	Long			
Sla	aterville 600		Bald Hill					
CN1PAL	N 42.422233	W 76.326033	CN1SAL	N 42.355283	W 76.382133			
CN1PAR	N 42.421917	W 76.325517	<b>CN1SAR</b>	N 42.354883	W 76.381683			
CN1PBL	N 42.421817	W 76.327200	CN1SBL	N 42.354700	W 76.381383			
CN1PBR	N 42.421350	W 76.326683	CN1SBR	N 42.354283	W 76.380983			
CN1PCL	N 42.421033	W 76.328033	CN1SCL	N 42.353850	W 76.381050			
CN1PCR	N 42.420700	W 76.327583	CN1SCR	N 42.353400	W 76.381000			
Carter Creek								
CN2PAL	N 42.333683	W 76.664433	CN2SAL	N 42.337883	W 76.662567			
CN2PAR	N 42.333733	W 76.665100	CN2SAR	N 42.337950	W 76.661967			
CN2PBL	N 42.334550	W 76.665367	CN2SBL	N 42.338450	W 76.662367			
CN2PBR	N 42.334483	W 76.665950	CN2SBR	N 42.338167	W 76.662883			
CN2PCL	N 42.335000	W 76.665567	CN2SCL	N 42.337050	W 76.661583			
CN2PCR	N 42.334933	W 76.666167	CN2SCR	N 42.337000	W 76.662183			
Fall Creek Valley								
CN3PAL	N 42.455083	W 76.451233	CN3SAL	N 42.458333	W 76.449417			
CN3PAR	N 42.455033	W 76.450700	CN3SAR	N 42.457933	W 76.449083			
CN3PBL	N 42.455361	W 76.450528	CN3SBL	N 42.458783	W 76.449100			
CN3PBR	N 42.454867	W 76.450367	CN3SBR	N 42.458417	W 76.448667			
CN3PCL	N 42.455100	W 76.450367	CN3SCL	N 42.458600	W 76.449200			
CN3PCR	N 42.455467	W 76.450067	CN3SCR	N 42.458222	W 76.448778			

Table 3. GPS locations of transect plots at Cornell Botanic Gardens Property, Summer 2019



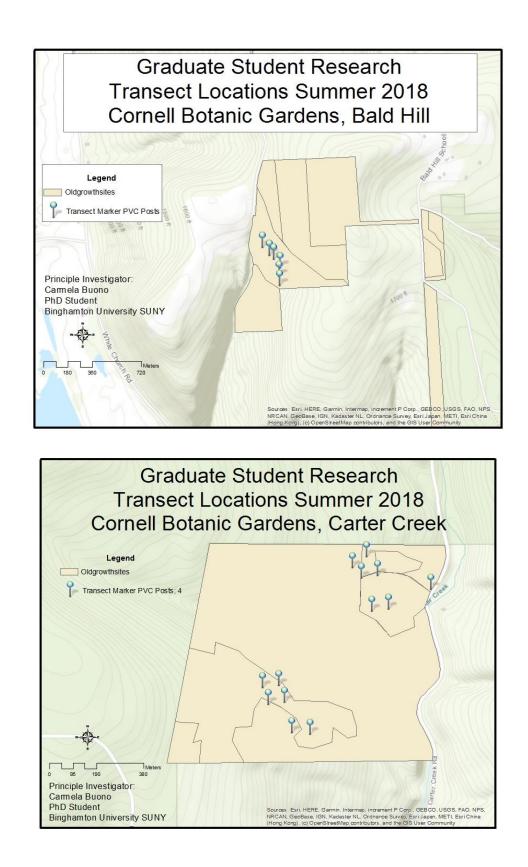
**Figure 3.** The illustration above is the complete design and layout of the survey transects that will be conducted for this research. Note that the only observable portion of these surveys that will remain in the forest are the two PVC posts that mark the two ends of the 50 meters transect. These posts will have contact information and identification attached. Please refer to the key for meanings of survey types and locations.

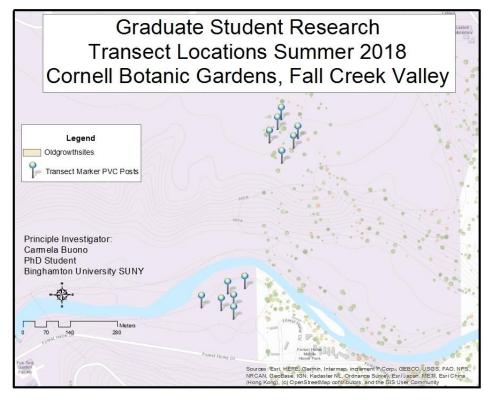


**Figure 4**. An image of the .5-feet-tall and 1-inch wide PVC posts that will be used to identify the ends of the study transects. In addition, these PVC posts are blue and have contact and identification information attached to them.

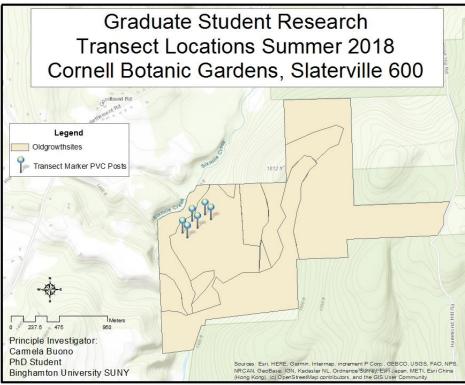


B.





D.



**Figure 5.** Map of survey regions on Cornell Botanic Gardens Property, Summer 2018 A. Bald Hill, B. Carter Creek, C. Fall Creek Valley, and D. Slaterville 600

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