EFFECTS OF MYRMECOCHORE SPECIES ABUNDANCE, DIVERSITY, AND
FRUITING PHENOLOGY ON *APHAENOGASTER* (HYMENOPTERA:
FORMICIDAE) NESTING AND FORAGING IN SOUTHERN APPALACHIAN RICH
COVE FORESTS

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ABSTRACT

EFFECTS OF MYRMECOCHORE SPECIES ABUNDANCE, DIVERSITY, AND FRUITING PHENOLOGY ON *APHAENOGASTER* (HYMENOPTERA: FORMICIDAE) NESTING AND FORAGING IN SOUTHERN APPALACHIAN RICH COVE FORESTS

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In forests of the southern Appalachians, myrmecochory, or ant-mediated seed dispersal, is a diffuse mutualism between more than 42 species of plants and ants of the genus *Aphaenogaster*. Myrmecochores produce lipid-rich appendages on their seeds called elaiosomes, a reward that attracts *Aphaenogaster* ants and facilitates dispersal. Since myrmecochores set seed from March to September, forests with higher myrmecochore diversity may provide a more continuous source of elaiosomes for *Aphaenogaster* ants, likely affecting foraging and nesting behavior. I compared myrmecochore abundance and diversity among three forest ages (young, middle-aged and mature) and tested for correlations between herb community dynamics and *Aphaenogaster* foraging behavior, nest colonization, and elaiosome availability. I paired artificial ant nests with bait stations that I provisioned with tuna in temporal patterns to mimic patterns of theoretical elaiosome availability in communities with low myrmecochore diversity and high myrmecochore diversity.

Higher myrmecochore abundance and higher soil temperature marginally correlated with increased *Aphaenogaster* foraging. Myrmecochore diversity measures among forest ages were not significantly different, and varying availability of resource

did not affect foraging or nesting behavior. Higher soil moisture in mature forests may explain why the majority of colonized nests appeared in mature forests. Fruit phenology showed elaiosomes were more consistently available in middle-aged forests; however, this greater availability was not correlated with myrmecochore species diversity or abundance, and did not affect *Aphaenogaster* nesting or foraging. My results suggest *Aphaenogaster* dynamics are influenced more by moisture and temperature than by resource availability. The lack of significant differences in diversity among forest ages precludes measuring the effects of diversity on either elaiosome availability or on *Aphaenogaster* dynamics. However, my results support current research showing elaiosomes provide a supplement to resources preferred by *Aphaenogaster* ants.

INTRODUCTION

Myrmecochory (seed dispersal by ants) is a global phenomenon that has evolved independently more than 100 times (Lengyel et al., 2009). Worldwide, it occurs in more than 10,000 plant species in 80 families, involves members from four of the seven subfamilies of ants, and is an important driver of plant diversity (Beattie, 1985; Lengyel et al., 2009). In the rich coves of eastern North American deciduous forests, 30-40% of forest herbs are myrmecochores (Beattie & Culver, 1981; Rico-Gray & Oliveira, 2007). More than 42 species of plants in 20 different genera native to eastern deciduous forests belong to this guild (Beattie & Culver, 1981; Beattie, 1985; Gaddy, 1986; Mitchell, 2002, and references therein; Gorb & Gorb, 2003; Rico-Gray & Oliveira, 2007, and references therein). Myrmecochores begin blooming earlier than most other forest herbs, and continue through late summer (Warren et al., 2014). Seed-set can occur as early as March (*Claytonia virginica*, *Anemone acutiloba, A. americana, Epigaea repens, Viola rotundifolia*) and as late as August and September (*Prosartes lanuginosum*, *Uvularia grandiflora*) (Radford, 1968; Spira, 2011; Weakley, 2011).

Myrmecochores have special anatomical, biochemical, and phenological adaptations that increase the effectiveness of seed dispersal by enhancing attraction to ants (Hughes et al., 1994; Gorb & Gorb, 2003; Warren et al., 2014). Myrmecochores produce a lipid-rich appendage (elaiosome) on their seeds that specifically attracts carnivorous and omnivorous ant species but does not attract granivorous ant species (Gammans et al., 2005, 2006; Sheridan et al., 1996). The elaiosome contains proteins, sugars, and an abundance of oleic and linoleic acids that stimulate ants to carry the

diaspore (seed with elaiosome) back to the nest (Sheridan et al., 1996; Marshall et al., 1979; Gordon, 1983; Skidmore & Heithaus, 1988; Brew et al., 1989; Lanza et al., 1992). The ants remove the elaiosome from the seed, feed it to larvae, and deposit the undamaged seed in a refuse pile or abandon it near the nest entrance (Beattie 1985).

Studies on elaiosome content show remarkable similarity between the fatty acids they contain (palmitic, palmitoleic, stearic and oleic acids) and those in insects, another major food source for carnivorous and omnivorous ants (Skidmore & Heithaus, 1988; Brew et al., 1989; Hughes, 1993). Elaiosome consumption can enhance ant colony fitness by increasing larval weight and number, pupal weight and number, and worker fitness (Gammans et al., 2005), and influence colony dynamics by shifting member ratios in reproductive output toward female bias without affecting the number of workers or the size of the queen (Morales & Heithaus, 1998). Larvae that accumulated more lipids from elaisomes tended to develop into virgin queens, whereas other female larvae developed into workers (Bono & Heithaus, 2002). However, more recent work found no difference in *Aphaenogaster* colony productivity, fat content, or mean larval production in colonies fed solely on elaiosomes versus insect protein (Clark & King, 2012). These findings and the documented *Aphaenogaster* preference for insect carcasses over elaiosomes suggest that elaiosome consumption only benefits ants when other food resources are limited.

Benefits for the plant, however, may outweigh benefits for the ant in this mutualism. Dispersal services provided to the myrmecochore guild by *Aphaenogaster* ants may have profound ecological and evolutionary consequences for those plant populations and assemblages. Ecologically, ant-mediated seed dispersal affects plant population structure, influences population dynamics and persistence, and affects species

distribution and abundance (Ness et al., 2009; Warren et al., 2010, Sorrels & Warren, 2011; Zelikova et al., 2011). Evolutionarily, myrmecochory influences the level of gene flow between myrmecochore guild populations and affects local adaptation, speciation, and the evolution of life-history traits (Rico-Grey & Oliveira, 2007). Traits that affect seed dispersal mutualisms, such as myrmecochory, are likely to be under strong selection pressure (Giladi, 2006; Warren et al., 2014).

There are three generally accepted hypotheses on the selective advantages that myrmecochory confers to plants in temperate forest communities (Giladi, 2006). The predator-avoidance hypothesis suggests that rapid collection by highly effective ant dispersal agents like *Aphaenogaster* removes prized elaiosome-bearing diaspores from the threat of consumption by granivores, including birds, small mammals, and other insects (Heithaus et al.,1980; Beattie, 1985). The competition-avoidance hypothesis suggests that seed dispersal removes vulnerability to interspecific competition for resources between sympatric congeners (Handel, 1978; Beattie 1985). A third hypothesis is an extension of the competition avoidance hypothesis, where dispersal reduces intraspecific competition between parents and their other progeny and decreases juvenile plant mortality (Luond & Luond, 1981; Beattie, 1985). The relative importance of these benefits varies by region or habitat, and are seldom exclusive (Rico-Grey & Oliveira, 2007).

Research shows that ants of the genus *Aphaenogaster* (subfamily Myrmicinae) are the most common and effective dispersal agents for ant-dispersed plant species (Ness et al., 2009). There are roughly 40 species of the genus *Aphaenogaster* in North America that occupy diverse habitats, from grasslands to pine barrens and sandhills(Fisher &

Cover, 2007). In temperat forest of the eastern US, *Aphaenogaster* are ubiquitous and cosmopolitan woodland ants (King et al., 2013) that are behaviorally subordinate to more aggressive ant species (Fisher & Cover, 2007). *Aphaenogaster* ants specialize in fast discovery of food resources (Umphrey, 1996; Warren et al., 2011b; Warren et al., 2012), and collect between 50 and 70% of myrmecochore seeds in eastern forests (Giladi, 2006; Ness et al., 2009, Warren et al., 2010). Ness et al. (2009) found myrmecochore species density and species richness correlated positively with *Aphaenogaster* density among patches within an intermediate-aged (150 yrs) eastern deciduous forest and identify *Aphaenogaster* as a keystone mutualist in these habitats, given the benefit conferred to the plants and the pervasiveness of the disperser.

The highly specialized and dominant role of *Aphaenogaster* ants as disperser increases the vulnerability of the mutualism to disruption with significant consequences for plant populations. A potential source of disruption is the temporal unavailability of nutrient-rich elaiosomes that attract the ants to the seeds. Because plants in the myrmecochore guild bloom and set seed from March to September, high myrmecochore species diversity within a forest setting could provide temporal overlap and consistent elaiosome availability for *Aphaenogaster* ants. Because myrmecochores are limited in their dispersal range and have difficulty re-colonizing disturbed areas (Giladi, 2004; Ness & Morin, 2008; Sorrels & Warren, 2011; Warren et al., 2010), a change in myrmecochore diversity, either by a shift in species abundance, distribution, or dominance, may threaten the integrity of this mutualism. Changes in environmental drivers of myrmechcore fruit and flowering phenology may introduce a shift in synchrony with *Aphenogaster* foragers (Warren et al., 2011a; Cook et al., 2012; Ellwood

et al., 2013). A greater diversity of myrmecochores may translate into a more continual availability of ealiaosomes and may buffer consequences of phenological asynchrony between elaiasome production and *Aphaenogaster* dispersal services.

Evidence of higher herb diversity in mature forests (Duffy & Meier, 1992; Pearson et al., 1998; McLachlan & Bazely, 2001; Mitchell et al., 2002; Harrelson & Matlack, 2006; Wyatt & Silman, 2010), suggests that the temporal range of elaiosome production by myrmecochores in mature forests potentially provides an uninterrupted source of elaiosomes that may affect *Aphaenogaster* foraging behavior and colony location. *Aphaenogaster* are highly mobile and average nest emigration occurs every 30 days (Smallwood & Culver, 1979; Smallwood, 1982a,b). Warren et al. (unpublished data) found that *Aphaenogaster* spp. show a marked preference for nests nearest food and colonize a majority of artificial nests adjacent to bait stations, but colonize significantly fewer located just one meter away. This pattern suggests that proximity of resource availability plays an important role in nesting dynamics, and plant and ant distributions.

The baseline diversity and abundance of myrmecochores in mature, intact, forested ecosystems is largely absent from the body of myrmecochory research. Most, if not all, research on this mutualism was conducted in disturbed, second-growth forests, and little comparison has been made between myrmecochore populations in older, mature forests and younger, disturbed forests of the southern Appalachians. Studies on the effects of patch size and land use history on myrmecochores have shown reduced abundance of myrmecochores in disturbed landscapes (Pearson et al., 1998; Mitchell et al., 2000). Myrmecochory in younger, disturbed forests has direct relevance to plant reestablishment following disturbance (Beattie & Culver, 1981; Andersen, 1988, 1991;

Rico-Gray & Oliveira, 2007; Ness & Morin, 2008; Sorrels & Warren, 2011). However,

no analysis has been conducted on whether reduced myrmecochore abundance or

diversity in disturbed forests affects temporal availability of elaiosomes as a resource for

Aphaenogaster.

Because ants are responsive to changes occurring at small spatial and temporal

scales (Talbot, 1934; Puntilla et al., 1991; Kremen et al., 1993; Crist & Wiens, 1994;

Warren et al., 2011a), disturbance can potentially disrupt ant-facilitated seed dispersal

with profound consequences for plant population dynamics (Andersen & Morrison, 1998;

Ness & Morin, 2008; Mitchell et al., 2002; Warren et al., 2011b). A comparison of

myrmecochore diversity, abundance, and fruiting phenology among different aged forests

with varying times since disturbance coupled with an analysis of *Aphaenogaster* nesting

and foraging behavior in those communities, allows us to investigate and better assess the

effects of disturbance on this important mutualism and could provide a greater

understanding of the conditions within which myrmecochory may have evolved.

In this study, I compare herb and myrmecochore abundance and diversity, and

Aphaenogaster foraging behavior and nesting dynamics, among three forest ages in rich

cove forests of western North Carolina. Rich cove forests are characterized by a mixed

mesophytic tree community and species-rich herb layer, and generally occur in low-to

middle elevations between 2000 and 4500 feet. They typically occur on concave,

sheltered land surfaces with high soil pH and fertility (Busing, 1998; Schafale, 2012). I

define young forest stands as those having experienced severe disturbance such as clear-

cut or similar logging within the last 70 years; middle-aged stands as forests aged

between 100 and 200 years; and mature forest as stands aged 200 years or more without

having been significantly disturbed. I established three sites in three different rich cove forests of each age category. I tested for correlations among forest ages, diversity measures, and *Aphaenogaster* dynamics, and for differences in soil moisture and temperature among forests.

I established treatment plots in each of my nine sites with artificial ant nests and bait stations using tuna for bait to investigate whether diversity, forest age, or temporal interruption of resource availability affects *Aphaenogaster* colonization frequency and persistence. I used three treatment categories for bait station provisioning to mimic theoretical elaiosome availability among different aged forests with different disturbance levels and different myrmecochore abundance: (1) a continuous treatment where bait stations were provisioned weekly to mimic mature forests with intact herb communities; (2) interrupted treatments (weekly at three week intervals) to mimic disturbed forests with low myrmecochorous diversity; and (3) no tuna provisioned (control). I mapped fruiting phenology of myrmecochores in my sites to determine whether differences exist among forest ages or abundance and diversity levels, and whether *Aphaenogaster* foraging behavior or nesting dynamics are affected by temporal availability of elaiosomes as a food resource.

I hypothesized that mature forests would have higher herb diversity and abundance, and higher myrmecochore diversity and abundance, resulting in temporally consistent availability of elaiosomes for ant consumption. Further, I predicted that *Aphaenogaster* foraging occurrence would be higher in areas with greater myrmecochore abundance and diversity and with more consistently available elaiosomes. Finally, I

hypothesized that *Aphaenogaster* nest colonization frequency would be higher in nests

located adjacent to consistently provisioned bait stations in all forest age categories.

MATERIALS AND METHODS

SITE DESCRIPTIONS

I established sites in three different forest-age stands: (1) young forest stands, (severe disturbance such as clear-cut or similar logging within the last 100 years); (2) middle-aged stands (aged between 100 and 200 years); (3) and mature forest stands, (aged 200 years or more and without evidence of significant disturbance). Three sites were identified in each age category to total nine research sites in the western North Carolina counties of Buncombe, Jackson, Graham, and Macon. Geographic coordinates for sites are given in degrees-minites-seconds format using the 1983 North American Datum (NAD83).

Mature Forests

1. Walker Cove (N 35 45' 08.0" W 82 21' 28.1") is an undisturbed, exceptionally high-quality, rich, broad cove located in Walker Falls Research Natural Area on the Toecane District of Pisgah National Forest near Barnardsville, NC. The site is a gentle north-northeast-facing slope (24%) at 3810 feet (1160 m) elevation. It has elements of a northern hardwood forest community dominated by large sugar maple (*Acer saccharum*), yellow buckeye (*Aesculus flava*), American beech (*Fagus grandifolia*), basswood (*Tilia americana),* and white ash (*Fraxinus americana*). The community layers are well stratified and the herb layer is rich and dense. Ages for many trees exceed 250-300 years (Smith, 1999). This site is designated a significant natural heritage area by the NC Department of Environment and Natural Resources (NCDENR) Natural Heritage Program.

2. Big Ivy (N 35 47' 59.8" W 82 21' 51.3") is part of the Big Ivy Watershed near Barnardsville, NC in the Pisgah National Forest Grandfather Ranger District near flat Spring Knob and Strait Creek/Forest Rd 3542. It is a small section of high quality, rich cove forest in a broad swale with north, north-east, and north-west facing slopes that average a moderate 38.8% located at 3540 feet (1080 m) elevation on the north and north-west face of Big Butt Mountain near Flat Spring Knob and Strait Creek. This site is a registered North Carolina Natural Heritage site and an exemplary remnant of old growth forest that contains some very large canopy trees dominated by sugar maple (*Acer saccharum*), yellow buckeye (*Aesculus flava*), American beech (*Fagus grandifolia*), basswood (*Tilia americana),*white ash (*Fraxinus americana*), and yellow poplar (*Liriodendron tulipifera*) that range in DBH from 36-48." Tree cores age the larger trees at 250-300 years (Smith 1999).

3. Wright Creek (N 35 19' 44.6" W 83 57' 35.5") in the Cheoah Ranger District, Graham County, NC is part of the Santeetlah Creek headwaters area in the Unicoi Mountains of western North Carolina. It lies at 3470 feet (1060 m) elevation southeast of Huckleberry Knob, the tallest peak in the Unicoi range (5545 ft). The Art Stewart ridge to the west and Seven Springs Gap to the east form the perimeter of the Wright Creek Watershed. The site is a small, remote patch (~15 acres) of undisturbed, primary forest surrounded by boulder fields to the north and east, and clear cuts from 1967-1968 to the south and west (Josh Kelly, pers.comm) on an acutely steep southeast-facing slope (70%). The community is dominated by yellow poplar (*Liriodendron tulipifera*), red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), yellow buckeye (*Aesculus flava*), and white ash (*Fraxinus americana*) ranging from 28-36" DBH, and punctuated by an

enormous tulip poplar (*L. tulipifera*) standing 137 feet tall with a 6.79 foot (207cm) DBH. This site is designated a significant natural heritage area by the NCDENR Natural Heritage Program.

<u>Middle Aged Forests</u>

1. Bent Creek (N 35 27' 46.3" W 82 39' 49.1") is a 6,000 acre experimental forest established in 1925 within the Pisgah National Forest by the USDA Forest Service (USFS), administered by the USFS Southern Research Station. The site is a moderately steep (47%) north-north east-facing slope at 2930 feet (890 m) elevation on the south side of Rich Cove Branch. Prior to the purchase of the property in 1902 by George W. Vanderbilt, the banks of Rich Branch were cleared and kept under cultivation and in pasture (Nesbitt, 1941). Since then, the south bank has remained intact with no FS silvicultural or other human-induced disturbance, placing the age of the forest stand at approximately 100 years (Henry McNabb, pers. comm.). The stand is characterized by a relatively sparse herb layer, but numerous small trees and saplings (*Lindera benzoin, Acer pensylvanicum, Acer rubrum, Sassafras albidum*). Dominant canopy tree species include yellow poplar (*Liriodendron tulipifera*), white ash (*Fraxinus americana*), Fraser magnolia (*Magnolia fraseri*), bitternut hickory (*Carya cordiformis*), and basswood (*Tilia americana*).

2. Coweeta, Dryman Fork (N 35 01' 20.2" W 83 26' 17.6") is a moderately-sloping site (50.4%) that lies at 3300 feet (1010 m) elevation and is part of the Coweeta Hydrologic Laboratory, a Long Term Ecological Research site in Otto, NC that is cooperatively managed by the USDA forest service and the University of Georgia. The 5400-acre property situated in the Nantahala Mountain Range consists of two adjacent,

bowl-shaped basins that contain several well-defined watersheds and over 45 miles of stream. The Cherokee Indian Nation and later, white settlers, routinely logged and burned the land for farming and grazing until the early 1900s, when it was bought from the Nantahala Company by the W. M. Ritter lumber company. Logging continued in the basin until 1934, when the USFS acquired the property, established Coweeta as an Experimental Forest, and later, in 1948, designated the basin as a hydrological laboratory (Douglass & Hoover, 1988). No research activity or human disturbance has occurred in the Dryman fork watershed since the Forest Service took ownership, placing the forest stand age at approximately 80 -100 years (Jason Love, pers. comm.). Dominant canopy tree species in this site include sugar maple (*Acer saccharum*), yellow buckeye (*Aesculus flava*), and white ash (*Fraxinus americana*). This site is designated a significant natural heritage area by the NCDENR Natural Heritage Program.

3. Walker Cove 2 (N 35 45' 10.3" W 82 21' 38.8") is a selectively logged site located just west of and across Forest Service Rd 74 from the mature forest site, Walker Cove 1 (see above), in the Toecane District of Pisgah National Forest near Barnardsville, NC. This second-growth stand also lies on the north-facing rim of the Walker Branch watershed between Big Andy Ridge on the north, Brush Fence Ridge on the east, and Walker ridge to the south. The site is moderately steep (50.7% slope) and occurs at 3770 feet (1150 m) elevation and dominated by yellow poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), and American beech (*Fagus grandiflora*). This site is designated a significant natural heritage area by the NCDENR Natural Heritage Program.

<u>Young Forests</u>

All young forest sites are located at Balsam Mountain Preserve, a 4400-acre private community in Jackson County NC, formerly owned and logged by Champion International Paper Company to provision their mill in Canton, NC. Logging records show stands in these sites aged roughly 30-40 years at the time of data collection, having been harvested in the late 1970's and early 1980's. They are all early-successional rich cove forests (typic montane type), as assessed by Dr. Peter Bates, Western Carolina University Department of Geosciences and Natural Resources, Associate Professor and Program Director of the Natural Resource Conservation and Management Department, using the Classification system of the Natural Communities of North Carolina developed by the conservation non-profit organization, NatureServe.

1.Cashie Branch (N 35 23' 35.7" W 83 07' 33.8") is situated in the northwest corner of the BMP property at 2880 feet (878 m) elevation, the lowest of the nine study sites. It is bordered to the north by a state-owned road on the BMP property boundary and to the east by a private gravel access road. The site is gently sloped toward Cashie Branch creek to the east, but includes steeply cut banks where an old logging road traverses the site. It is characterized by a dense mix of small, how about sparsely brached, small diameter (<25 cm dbh), young trees, including yellow buckeye (*Aesculus flava*), sweet birch (*Betula lenta*), red maple (*Acer rubrum*), and tulip poplar (*Liriodendron tulipifera*) with small patches of open canopy. This site is designated a significant natural heritage area by the NCDENR Natural Heritage Program.

2. East Reach (N 35 23' 34.6" W 83 05' 40.7") is a gentle to moderate-sloping site (42%) situated at 3950 feet (1200 m) elevation; the highest of the nine study sites, it

exhibits some canopy tree species assemblages characteristic of a northern hardwood forest community, such as white ash (*Fraxinus americana*), basswood (*Tilia americana var. heterophylla*), and black cherry (*Prunus serotina*). The 2500 sq m site lies between a gravel road and a small, seasonal drainage creek.

    3. Cook House (N 35 23' 51.1" W 83 06' 4.0") lies between East Reach road (gravel) to the north and Cook House road (gravel) to the south at 3630 feet (1100 m) elevation. This site has a steep-to moderate slope (49.50%) and a relatively open canopy, dominated by white ash (*Fraxinus Americana*), and basswood (*Tilia americana var. heterophylla*). This site also has numerous shrub species, including *Pyrularia pubera, Lindera benzoin* and *Calycanthus floridus*, as well as large, coarse herbs, such as *Aruncus dioicus*, and *Arnoglossum reniforme*.

DATA COLLECTION

<u>Sites</u>

    I established a 50 x 50 meter study area in each of the nine forested sites; three in each forest age category (n=9). In each 2500 m^2 area, I installed 50 m transects at 10 m intervals and randomly established nine 1 m^2 treatment plots (n=81) along the transects, ensuring each was at least 10 m apart. Treatment plots were used for both floristic inventories and for installation of artificial nests and bait stations.

    Soil samples to a depth of 0-20 cm were collected from study sites between May and June 2012. Soil pH was measured on moist samples using a hand-held Oakton pH Testr 3 meter with 0.01 pH resolution and $\pm$ 0.02 accuracy. Volumetric soil moisture percentage was measured each week with a hand-held Hydrosense Soil Water Content Measurement System (Campbell Scientific, Inc.), and ambient soil temperature was

measured weekly in treatment plots with a digital soil thermometer inserted 10 cm. Site data were collected at each of the nine 1x 1 m nest/herb plots and averaged. Slope was measured using a Suunto PM-5/360 PC clinometer; aspect with a Suunto MCA-D compact mirror compass; and geographical coordinates and elevation were recorded using a hand-held Garmin eTrex Vista GPS Navigator unit with North American Datum 83.

<u>Herb and Myrmecochore Species Abundance and Diversity</u>

I conducted herb and myrmecochore inventories between April 2012 (Bent Creek, Walker Cove 1, Walker Cove 2, and Wright Creek), May 2012 (Coweeta), and June 2013 (Cashie Branch, Cook House, and East Reach). I recorded species' presence and abundance in each of the nine treatment plots (n=9 per site) to determine diversity and abundance of myrmecochores within the 2500 m^2 study area and within treatment plots. One-square-meter inventory quadrats constructed of PVC were centered on each artificial nest, herb species rooted within the quadrat were recorded, and stems counted. Surveys were performed by the author, accompanied by Dr. H. David Clarke, botanist, Associate Professor of Biology, and curator of the herbarium at the University of North Carolina at Asheville. Species area curves were developed and used to determine representative accuracy of the sample size (9 m^2) for the site (2500 m^2). Vegetation was identified using Wofford (1989), and Weakley (2011), with verification by Clarke and Jay Kranyik, Botanical Gardens of Asheville Manager, and Horticulture committee chair. Nomenclature follows Weakley (2011).

Aphaenogaster foraging and nesting

To test for correlation between *Aphaenogaster* foraging behavior, nest colonization, and resource availability, I paired artificial ant nests with tuna bait stations in each treatment plot (n=9 per site). I provisioned bait stations with roughly 1.0 g of tuna in different temporal patterns to mimic theoretical elaiosome availability in conditions of low myrmecochore diversity (interrupted availability) and high myrmecochore diversity (continuous availability). Tuna is a standard bait used to sample ant communities because it contains many of the same diglycerides as elaiosomes (Bestelmeyer et al., 2000). Bait stations consisted of small 3" x 3" or 1" x 1" plastic weigh boats to hold tuna, and 3" roofing nails to anchor them to the forest floor. Artificial ant nests were constructed from 5" x 5" x 1" untreated white pine pieces routed with tunnels and chambers, and covered with 1.5 mm Plexiglas. Routed tunnels and chambers were cut 1.5 cm deep with access to the nest via a 10×4 mm entrance between the wood and Plexiglas. Nests were installed such that the wood bottom was in contact with soil, below litter, and the nest was covered with 6" x 6" ceramic tile to block sunlight and allow easy access by observers.

For continuous treatments, I provisioned bait stations with tuna on a weekly schedule to mimic uninterrupted availability of elaiosomes found in mature forests with intact herb communities. Interrupted treatments mimicking disturbed forests with low myrmecochorous diversity had bait offered for a one-week period at three-week intervals; and no tuna was provided for the control treatments. Three of the nine treatment plots in each study area received continuous treatments, three received interrupted treatments and three received control treatments. This way, each forest age category (young, middle

aged, and mature) had nine continuous treatments, nine interrupted treatments, and nine control treatments.

I began provisioning in March 2012, and monitored bait stations weekly for a 60-minute period for 12 weeks. I identified ants visiting bait stations to genus to determine frequency and abundance within the study areas. I randomly placed an additional foraging survey tray in the site during each visit and observed at roughly 20, 40, and 60-minute intervals to assess forest age effects on ant resident communities independent of treatments associated with nest colonization, I checked for occupation and presence/absence of colonies in artificial nests during weekly visits by lifting the ceramic tile cover and observing nest contents through the Plexiglas top without disturbing the nest box.

Myrmecochore Phenology

Reproductive phenology data were collected for all herb species in the treatment plots and across the site by recording species in bloom, species in fruit, and evidence of fruit dehiscence during each weekly visit. Continuous presence/absence data of reproductive structures on myrmecochores were analyzed to determine temporal variation, overlap, and interruption in resource (elaiosome) availability within each $2500m^2$ study site.

DATA ANALYSIS

All statistical analyses were performed using R statistical software, (R Development Core Team, 2013).

Diversity: Herb and Myrmecochore Abundance

Abundance, or stem counts, were conducted and averaged to compare density of

herb and myrmecochore populations among sites and among sites grouped by forest age.

Pearson's Product-Moment Correlation analysis with pair-wise comparisons was

performed for forest age, total stems, and myrmecochore stems. I conducted Analysis of

Variance (ANOVA) to compare means among forest ages for total herb species

abundance, myrmecochore species abundance, and perecent myrmecochore species

stems. Post-hoc Tukeys' test for multiple comparisons of means was used to determine

location of variance identified by ANOVAs.

Diversity: Species Richness

Species richness, or number of species, was recorded for all sites and was

averaged among sites grouped into forest age categories. Pearson's Product-Moment

Correlation analysis with pair-wise comparisons was performed for forest age, total herb

species richness, myrmecochore species richness, and percent myrmecochore species. I

conducted ANOVAs to compare means among forest ages for total herb species richness,

myrmecochore species richness, and perecent myrmecochore species. Post-hoc Tukeys'

test for multiple comparisons of means was used to determine location of variance

identified by ANOVAs for overall species richness.

Herb Diversity and Myrmecochore species Diversity

Herb and myrmecochore species diversity were calculated using Unbiased

Simpson Diversity Index: , where   is the proportion of species i, and S is

the number of species so that   = 1. The Simpson's diversity index incorporates the

measure of abundance in its calculation, and is simply a calculation of the probability that

any two individuals selected randomly and independently from a population will belong to a particular species (Magurran, 1988; Oksanen, 2013). Diversity indices' means were compared by one-way Analysis of Variance (ANOVA) among sites grouped by forest age (n= 3 replicates of each age category). Post-hoc Tukey's HSD was performed to identify location of differences in means resulting from the ANOVA.

Fruiting Phenology

One-way ANOVA was conducted to compare mean number of myrmecochore species in fruit among forest ages and for mean number of species with dehiscent fruit among forest ages.

Aphaenogaster Nesting and Foraging

Foraging analysis was conducted using a subset of the total data set that included plots with continuously provisioned bait stations. Number of *Aphaenogaster* foragers visiting bait stations were recorded and analyzed for correlation between forest age, herb diversity and abundance, and myrmecochore diversity and abundance. One-way ANOVA was conducted to compare means of *Aphaenogaster* foragers with respect to forest age and to myrmecochore abundance. *Aphaenogaster* forager data were corrected for co-linearity and over-dispersion, and analyzed over time as a function of forest age, average soil moisture, average soil temperature, myrmecochore species richness, myrmecochore species abundance, and total herb abundance, with a logistic regression using a poisson distribution of generalized linear mixed models (GLMMs) in the 'lme4' package (Bates et al., 2012), MASS package (Venables & Ripley, 2002), rms (Harrell, 2013) and vegan package (Oksanen et al., 2013) for the 'R' statistical program. GLMMs were analyzed for best fit using Akaika Information Criterion (AIC).

Nesting behavior was analyzed for correlation between number of nests colonized and average soil moisture and temperature, forest age, myrmecochore abundance, myrmecochore richness and total herb abundance. One-way ANOVAs were conducted to compare means of nests colonized with bait present or absent and bait treatments associated with colonized nests. Colony sizes were estimated and mean sizes were compared between nests colonized with bait present and with bait absent.

DIVERSITY

INTRODUCTION

      The ecological benefits of diversity are well established and research on the relationship between ecosystem diversity and stability is plentiful. Ecosystems with high species richness have greater retention of resources, which are better acquired and maximized at every trophic level (Chapin et al., 1996). High species diversity also reduces the risk of changes in ecosystem processes resulting from invasions of pathogens or invasive species (Chapin et al., 1996). Ives and Carpenter (2007) reviewed empirical ecosystem stability-diversity research and found it overwhelmingly demonstrated positive diversity-stability relationships. They assert that species interactions are imperative to understanding the mechanisms that drive the relationship between ecosystem diversity and stability. Those mechanisms include the strength of species interactions, the mode of interaction (i.e. competitors, predators, mutualists, etc) that defines food web topology, and the ways species assemblages respond to disturbance. Alternate levels of diversity not only result from perturbations external to the ecosystem, but also from internal fluctuations that occur locally on a smaller scale (Hurlbert, 1971).

      The importance of herb species diversity in temperate forested ecosystems is well established. The herb layer provides habitats for many animal species, plays an important role in nutrient cycling, and is a sensitive indicator of forest site ecology (Collins et al., 1985; Thomas et al., 1999; Gilliam & Roberts, 2003; Wyatt & Silman, 2010). Herb community dynamics provide essential ecological information regarding forest ecosystem characteristics such as past land use (Gilliam, 2007). Species

interactions in the herb layer of a forest ecosystem can preserve and perpetuate ecological function and community processes, influence regeneration success of communities in higher strata, and play a crucial role in resilience to disturbance across broad temporal and spatial scales (Chapin et al., 1996; McCann, 2002; Ives & Carpenter, 2007). Forest biodiversity is largely a function of herb diversity because species diversity is typically highest in the herb layer (Gilliam & Roberts, 2003).

Rich cove forests of the southern Appalachians are typically species rich, with moderate to high soil fertility and a moderate disturbance regime characterized by ongoing gap-dynamic regeneration of mixed mesophytic forest communities (Pittillo et al., 1998; Ulrey 2002; Gilliam, 2007 Wyatt & Silman, 2010). High species richness is further supported by complex topography, high rainfall, and microsite variation characteristic of southern Appalachians rich coves. Additionally, rich coves are thought to have acted as a refuge for many plant species during the advance and retreat of previous ice ages (Whittaker 1956, Pittillo et al., 1998). Species richness influences the degree to which species interactions define community structure, relative to availability of new sites for colonization and rates of population growth (Horn, 1974; Runkle, 1982; Honnay et al.,, 2005).

The sensitivity of this rich herbaceous community to disturbance, variations in resource availability, and changes in spatial heterogeneity is well-established (Duffey & Meir, 1992; Pearson, et al., 1998; Gilliam, 2002; Mitchell et al., 2002). Low frequency of disturbance that reduces new colonization sites and mortality within populations increases the importance of inter-specific interactions. Herb distribution patterns in low disturbance sites may also represent highly suitable microhabitat, which promotes

vegetative reproduction among clonal plants. Investment in reproductive structures and seed production may be considered as a waste of energy in communities with low-level disturbance regimes and established herb populations (Honnay, 2005). Aggregations of suitable microhabitats across the landscape increases with site heterogeneity, which, for long-lived clonal perennials like myrmecochores, likely comes in the form of canopy gaps (Horn, 1974; Runkle, 1982).

Myrmecochore species presence in the herb layer contributes significantly to herb community diversity, particularly in rich cove temperate forests, where myrmecochores can comprise up to 45 % of plants and 30 % of species (Beattie and Culver, 1981; Rico-Gray and Oliveira, 2007; Ness et al., 2009). Given the temporal range of elaiosome production by the myrmecochore guild, higher myrmecochore herb diversity in the herb community potentially provides an uninterrupted source of elaiosomes that would likely affect *Aphaenogaster* colonizing behavior and colony persistence. Diversity in the myrmecochore guild of the herb layer may have profound implications for the fate of those populations. Higher herb diversity in undisturbed intact herb communities in mature forests, which may be lacking in disturbed forests (Duffy & Meier, 1992; Pearson et al. 1998; McLachlan & Bazely, 2001; Mitchell et al., 2002; Harrelson and Matlack, 2006; Wyatt & Silman, 2010), likely translate into higher myrmecochore diversity in those forests.

Until now, the baseline diversity data of myrmecochores from mature, intact, forested ecosystems has been absent from the body of myrmecochory research. Most research on this mutualism has been conducted in disturbed, second-growth forests where it has direct relevance to plant reestablishment following disturbance (Andersen, 1988,

1991; Mitchell et al., 2002; Ness & Morin, 2008; Sorrels & Warren, 2011;). Little comparison has been made between myrmecochore diversity in older, mature forests and younger, disturbed forests of the southern Appalachians.

Threats to this mutualism may have serious consequences for myrmecochore population dynamics (Andersen &Morrison 1998; Mitchell et al. 2002; Ness & Morin, 2008; Warren et al., 2011a). Habitat disturbance can severely disrupt ant-facilitated seed dispersal because ants respond to changes that occur at small spatial and temporal scales (Talbot, 1934; Puntilla et al., 1991; Kremen et al., 1993; Crist & Wiens, 1994; Warren et al., 2011b). Habitat disturbance can also lower myrmecochore diversity, by a shift in myrmecochore species abundance, distribution, or a change in dominance. Additionally, myrmecochores are limited in their dispersal range and have difficulty re-colonizing disturbed areas (Mitchell et al., 2002; Giladi, 2004; Ness & Morin, 2008; Warren et al., 2010; Sorrels & Warren, 2011). Further, no analysis has been conducted on whether myrmecochore diversity in these forests affects temporal availability of elaiosomes. Because plants in the myrmecochore guild bloom and set seed from March to September, high myrmecochore diversity could provide temporal overlap and consistent elaiosome availability for *Aphaenogaster* ants. I hypothesized that mature forests will have higher herb diversity and abundance, and, more specifically, higher myrmecochore diversity and abundance, resulting in temporally consistent availability of elaiosomes for ant consumption.

RESULTS

Abundance by Site

Total herb abundance among research sites ranged from 1,094 stems in one of the three mature sites, Big Ivy, to 210 stems in one of the three middle-aged sites, Bent Creek (Table 1). The second-highest herb abundance also occurred in a mature site, Walker1, which had 701 total stems. The highest percent of myrmecochore species occurred in Walker1, a mature site, where 61.5% of stems recorded were myrmecochores. The site with the highest stem count, Big Ivy, ranked 5th out of nine for myrmecochore abundance, with only 43% myrmecochore stems. The lowest percent myrmecochores occurred in Bent Creek, a middle-aged site, where only 28.6 percent of stems were myrmecochores. Of the top four sites with greatest number of myrmecochore stems, three were mature sites (Big Ivy, Walker1, and Wright Creek); a middle-aged site, Walker2, ranked third in number of myrmecochore stems. The lowest myrmecochore abundance occurred in the other two middle-aged sites, Bent Creek, with only 60 stems, and Coweeta, with 124 stems (Table 1). Overall, myrmecochores constituted 44 percent of total stems across all sites (2069 myrmecochore stems/4645 total stems).

Abundance by Forest Age

The greatest average abundance among forest age categories for total herbs and for myrmecochores occurred in mature forests. Average total herb abundance was remarkably similar for middle aged and young forests, both averaging 391 stems. Average myrmecochore abundance for middle and young forests was also similar. Young forests ranked second with an average of 181 stems, and middle-aged forests averaged lowest, with 136 stems (Table 2). Correlation analysis with pair-wise

comparisons among forest age, total stems, and myrmecochore stems expectedly showed

a strong correlation between herb abundance and myrmecochore abundance ($r = 0.92$, $t =$

6.336, df = 7, p-value = 0.0003904), and showed a moderate correlation between forest

age and herb abundance ($r = 0.62$; $t = 2.1148$, df = 7, p-value = 0.07227), and between

forest age and myrmecochore abundance ($r = 0.62$, $t = 2.0666$, df = 7, p-value = 0.07761;

Table 3). Neither mean number of total stems (Table 4) or percent myrmecochore stems

differed among forest age categories (Table 5). However, means of myrmecochore stem

counts differed significantly ($F_{2,6} = 5.57$; $P = 0.043$) among forest ages (Table 6).

Tukey's test for multiple comparisons of means showed statistical significance ($F_{2,6} =$

5.57; $P = 0.046$; Table 7) in mean (+/-SD) species richness between old forests ($m =$

373+/- 135.6) and middle aged forests ($m = 136$+/- 82.7; Table 7).

Table 1. Summary statistics for herb abundance variables from greatest to least in nine
myrmecochore research sites across three forest age categories in western North Carolina.
Sites measured 2500 m^2 located in stands of rich cove forest; 1 m^2 herb plots (n = 9) were
randomly placed along transects crossing the site at 10 m intervals.

MYRMECOCHORE STEMS			TOTAL HERB STEMS			% MYRMECOCHORES		
SITE	AGE	#	SITE	AGE	#	SITE	AGE	%
Bigivy	old	470	Bigivy	old	1094	Walk1	old	61.48
Walk1	old	431	Walk1	old	701	Cook	young	53.40
Walk2	mid	224	Walk2	mid	569	Cash	young	52.17
Wright	old	218	Wright	old	501	Wright	old	43.51
Cook	young	204	East	young	471	Bigivy	old	42.96
East	young	170	Cwt	mid	395	Walk2	mid	39.37
Cash	young	168	Cook	young	382	East	young	36.09
Cwt	mid	124	Cash	young	322	Cwt	mid	31.39
Bent	mid	60	Bent	mid	210	Bent	mid	28.57
Total		2069			4645			43.22

Table 2. Average number of herbaceous stems in each of three age categories from nine myrmecochore research sites in western North Carolina. Sites measured 2500 m^2 located in stands of rich cove forest; 1 m^2 herb plots (n = 9) were randomly placed along transects crossing the site at 10 m intervals.

Forest Age	Myrm. Stems	Total Stems	% Myrms
Young	181	391.7	46
Mid	136	391.3	35
Old	373	765.3	49

Table 3. Pearson's Product Moment correlation with pair-wise analysis between three forest age categories, total stems, and myrmecochore stems from nine myrmecochore research sites (three of each age) in rich cove forest stands of western North Carolina. Herbs were inventoried from 1 m^2 plots (n=9) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 site.

Correlation

Source	Forest Age	Myrm. stems	Total stems
Forest Age	1.00		
Myrm. stems	0.62	1.00	
Total stems	0.62	0.92	1.00
Percent Myrms.	0.08	0.60	0.27

P-Values from pairwise correlation analysis:

	Forest Age	Myrm. stems	Percent Myrms.
Forest Age	1.00		
Myrm. stems	0.08	1.00	
Percent Myrms.	0.83	0.09	1.00
Total Stems	0.07	0.01	0.48

Table 4. ANOVA and summary statistics for herb abundance (total stem count) in nine myrmecochore research sites in rich cove forests of western NC. Herbs were inventoried from 1 m^2 plots (n=9) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 site.

ANOVA: Total stems

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Forest age	2	279503	139751	3.253	0.110
Residuals	6	257734	42956		

Summary: Total stems

Source	mean	sd	data:n
Young	391.67	74.969	3
Mid	391.33	179.528	3
Old	765.33	301.689	3

Table 5. ANOVA and summary statistics for percent myrmecochore stems in nine herb community research sites in rich cove forests of western NC. Herbs were inventoried from 1 m^2 qm plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 site.

ANOVA: Percent Myrmecochore Stems

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Forest age	2	407.9	203.94	2.788	0.139
Residuals	6	439.0	73.16		

Summary: Percent Myrmecochore Stems

Source	mean	sd	data:n	
Young	43.90	8.774	3	
Mid	33.13	5.605	3	
Old	49.33	10.540	3	

Table 6. ANOVA and summary statistics for myrmecochore abundance in nine herb community research sites grouped by age category in rich cove forests of western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 site.

ANOVA: Myrmecochore Abundance

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Forest age	2	95156	47578	5.567	0.043
Residuals	6	51281	8547		

Summary

Age	mean	sd	data:n		
Young	180.67	20.23	3		
Mid	136.00	82.66	3		
Old	373.00	135.64	3		

Table 7. Tukey's post-hoc comparison of means to identify variance in myrmecochore abundance across three forest age categories from nine herb community research sites in rich cove forests of western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 site.

Multiple Comparisons of Myrmecochore Abundance Means: Tukey Contrasts

Source	Estimate	Std. Error	t value	Pr(>|t|)
mid - young	-44.67	75.48	-0.592	0.829
old - young	192.33	75.48	2.548	0.096
old - mid	237.00	75.48	3.140	0.046

Species Richness by Site

The total number of species across research sites was 116, with 31 (27%) total

myrmecochore species. The young sites had highest species richness. The lowest

species richness (37) was in a middle-aged site, and the next lowest (42 species) was

shared by Walker1 (mature), Big Ivy (mature) and Walker2 (middle). The percentage of

myrmecochore species was weakly correlated with species richness; the young site with the highest species richness also had the lowest percent myrmecochore species; 28 percent. The site with the greatest percent of myrmecochore species, a mature site, Walker 1, with 43 percent, had relatively low overall species richness (42; Table 8). Twenty species that occurred in the 2500 m^2 forest sites were not present in 1 m^2 inventory plots, and were not captured for species richness analysis.

Species Richness by Forest Age

The distribution of species across the different age classes was fairly even, with 43% of all species occurring in all three age classes. Of the 92 species surveyed in the herb plots, 16 of 67 only occurred in young sites, seven of 59 were unique to middle-age sites, and 12 of 65 only occurred in mature sites. Sixteen of the 92 species surveyed occurred in two of the three age classes. Six species were limited to young and middle-aged forests, six species were limited to middle-aged and old forests, and four species occurred in only young and old forests. Of the 29 myrmecochore species inventoried, 62 % (18) occurred in all three forest age classes. Only two of those were specific to young sites and two were specific to mature sites; only one was unique to middle aged sites. Six myrmecochore species occurred in two of the three age classes: two in young/middle-aged, two in middle-aged/old, and two in young/mature forests.

Correlation analysis between forest age, total species richness, myrmecochore species richness, and percent myrmecochore species using Pearson's product moment in R statistical software showed moderately strong negative correlation between species richness and percent myrmecochore species (r = -0.74, t = -2.9154, df = 7, p-value = 0.02249;), and a moderately strong negative correlation between forest age and total

species richness (r = -0.70, t = -2.564, df = 7, p-value = 0.03733). Correlation analysis

showed marginal positive correlation between forest age and myrmecochore species

richness (r = 0.61, t = -0.6295, df = 7, p-value = 0.549), total species richness and

myrmecochore species richness (r = 0.52, t = 1.6305, df = 7, p-value = 0.147), and forest

age and percent myrmecochore species (r = 0.61, t = 2.0183, df = 7, p-value = 0.08334;

Table 9).

One-way ANOVA shows no statistically significant difference among means for

myrmecochore richness (Table 10), percent myrmecochore species (Table 11), and total

species richness (Table 12) among forest age categories. However, a marginally

significant difference did occur between means of total species richness among forest

ages ($F_{2,6}$ = 4.71, P = 0.059). Tukey's test for multiple comparisons of means shows less

statistical difference (P = 0.99) of mean richness between old forests (m = 42.6, sd =

1.15) and middle-aged forests (m = 43.3, sd = 7.1) than between young (m = 54.33, sd =

5.51) and old (P = 0.076) and young and middle-aged (P = 0.092; Table 13).

Table 8. Species richness, myrmecochore species richness, and % myrmecochore species ranked in order of greatest to least, for nine myrmecochore research sites across three forest age categories in western North Carolina. Sites measured 2500 m^2 located in stands of rich cove forest; 1 m^2 herb plots (n = 81) were randomly placed along transects crossing the site at 10 m intervals.

TOTAL SPECIES			MYRMECOCHORE SPECIES			PERCENT MYRMECOCHORES		
Site	Forest Age	#	Site	Forest Age	#	Site	Forest Age	#
Cook	young	58	Cash	young	21	Walk1	old	43
Cash	young	57	Walk1	old	18	Bent	mid	41
Cwt	mid	51	Cwt	mid	18	Walk2	mid	40
East	young	48	Walk2	mid	17	Wright	old	38
Wright	old	44	Wright	old	17	Cash	young	37
Bigivy	old	42	East	young	16	Bigivy	old	36
Walk1	old	42	Cook	young	16	Cwt	mid	35
Walk2	mid	42	Bent	mid	15	East	young	33
Bent	mid	37	Bigivy	old	15	Cook	young	28

Table 9. Pearson product-moment correlation matrix comparing overall herb species richness, myrmecochore species richness, and percent myrmecochore species among three forest age classes in nine rich cove forest research sites in Western NC. Herb inventory was conducted in 1 m^2 plots (n = 81) randomly placed along transects located at 10 m intervals in each 2500 m^2 site.

Source	Forest age	Myrm. species	Percent myrms.
Forest age	1.00		
Myrm. species	-0.23	1.00	
Percent myrms	0.61	0.16	1.00
Total richness	-0.70	0.52	-0.74

Table 10. ANOVA and summary statistics for analysis of myrmecochore species richness across three forest age categories in nine rich cove forest sites in western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

ANOVA: Myrmecochore Species Richness

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Forest age	2	2	1	0.231	0.801
Residuals	6	26	4.333		
SUMMARY					
Source	mean	sd	data:n		
Young	17.67	2.89	3		
Mid	16.67	1.53	3		
Old	16.67	1.53	3		

Table 11. ANOVA and summary statistics for analysis of percent myrmecochore species across three forest ages in nine rich cove forest sites in western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

ANOVA: Percent Myrmecochores

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Forest age	2	76.22	38.11	2.618	0.152
Residuals	6	87.33	14.56		
SUMMARY					
Source	mean	sd	data:n		
Young	32.67	4.51	3		
Mid	38.67	3.21	3		
Old	39.00	3.61	3		

Table 12. ANOVA and summary statistics for analysis of total species richness across three forest ages in nine rich cove forest sites in western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

ANOVA: Total Species richness

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Forest age	2	257.6	128.78	4.711	0.0589
Residuals	6	164	27.33		

SUMMARY

Source	mean	sd	data:n
Young	54.33	5.51	3
Mid	43.33	7.09	3
Old	42.67	1.15	3

Table 13. Tukey's post-hoc comparison of means to identify variance in overall herb species richness across three forest age categories from nine herb community research sites in rich cove forests of western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

Multiple Comparisons of Total Herb Species Richness Means: Tukey Contrasts

Source	Estimate	Std. Error	t value	Pr(>|t|)
mid - young	11.0000	4.268	-2.577	0.092
old - young	11.6667	4.268	-2.733	0.076
old - mid	0.6667	4.268	-0.156	0.987

Herb Diversity

Young forest sites unexpectedly had highest total herb diversity and mature sites were two of the three least diverse. Simpson's diversity indices ranged from the lowest (21.54) at Walker1, a mature forest site, to the highest (40.40) at Cook House, a young forest site (Table 14). The average diversity index was 34.03 for young forest sites,

27.62 for middle-aged sites, and 24.37 for mature sites (Table 15). The average overall herb diversity across sites measured with Simpson's index was 28.68 (Table 14).

The one-way ANOVA shows a marginally significant statistical variance ($F_{2,6}$ = 4.05, P = 0.077; Table 15). A post-hoc Tukey's pair-wise comparisons of means (+/- SD) revealed a greater difference in mean diversity indexes (P = 0.070) between mature forests (m = 24.37+/- 2.47) and young forests (m = 34.03+/- 6.02) than between other age categories (Table 16).

Myrmecochore Diversity

Diversity within the myrmecochore guild was strikingly different from overall herb diversity. Simpson's indices ranged from 7.92 for the least diverse (Walker1) to 13.47 for most diverse, which occurred in the middle-aged site, Walker2. The average Simpson's diversity index for myrmecochores in all nine sites was 10.71, with middle-aged forests average index ranking highest (12.04), young forests ranking a close second (11.46) and mature forests ranking lowest at 8.65 (Table 17).

The one-way ANOVA performed to test for differences in mean Simpson's diversity index for myrmecochores across three forest age categories shows a marginally significant statistical difference ($F_{2,6}$ = 4.23, P = 0.072; Table 18). Tukey's pair-wise comparisons of means revealed a greater difference between mean (+/- SD) diversity indexes (P = 0.077) between mature forests (m = 8.65+/- 1.09) and middle-aged forests (m = 12.04+/- 1.81) than between other age categories (Table 19).

Table 14. Simpson's Diversity index coefficients, ranked in order of greatest to least, for overall herb diversity in nine myrmecochore research sites across three forest age categories in western North Carolina. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

Simpson Diversity Index

SITE	AGE	DIV. COEF.
Cook	young	40.40
Cash	young	33.27
Cwt	mid	31.49
East	young	28.42
Wright	old	26.01
Bent	mid	26.00
Bigivy	old	25.58
Walk2	mid	25.38
Walk1	old	21.54
Avg.		28.68

Table 15. ANOVA and summary statistics for analysis of Simpson's Diversity Indices (SDI) for overall herb species diversity across three forest ages in nine rich cove forest sites of western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

ANOVA: Simpson's Overall Herb Diversity

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Forest Age	2	144.8	72.41	4.046	0.0772
SDI	6	107.4	17.90		

Summary

Forest Age	mean	sd	data:n
young	34.03	6.02	3
mid	27.62	3.37	3
old	24.37	2.47	3

Table 16. Tukey's post-hoc comparison of means to identify variance in Simpson's diversity indices for total herb diversity across three forest age categories from nine rich cove forested research sites of western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

Multiple Comparisons of Total Herb Diversity: Tukey Contrasts

	Estimate	Std. Error	t value	Pr(>|t|)
mid - young	-6.41	3.454	-1.854	0.2315
old - young	-9.66	3.454	-2.795	0.0703
old - mid	-3.25	3.454	-0.941	0.6367

Table 17. Simpson's Diversity index coefficients, ranked in order of greatest to least, for myrmecochore herb diversity in nine myrmecochore research sites across three forest age categories in western North Carolina. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

Simpsons Diversity Index of Myrmecochores (Ranked)

SITE	FOREST AGE	DIVERSITY COEFFICIENT
Walk2	mid	13.47
Cook	young	12.74
Cwt	mid	12.66
Cash	young	11.95
Bent	mid	10.00
Bigivy	old	9.90
East	young	9.68
Wright	old	8.14
Walk1	old	7.92
AVG.		10.72

Table 18. ANOVA and summary statistics for analysis of Simpson's Diversity Indices (SDI) for myrmecochore species diversity in nine rich cove forest sites of western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

ANOVA: Simpson's Myrmecochore Diversity Indices

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Forest Age	2	19.68	9.841	4.228	0.0715
SDI	6	13.96	2.327		

SUMMARY

Age	mean	sd	data:n
young	11.46	1.58	3
mid	12.04	1.81	3
old	8.65	1.09	3

Table 19. Tukey's post-hoc comparison of means to identify variance in Simpson's diversity indices for myrmecochore diversity across three forest age categories from nine rich cove forested research sites of western NC. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site.

Multiple Comparisons of Myrmecochore Diversity: Tukey Contrasts

	Estimate	Std. Error	t value	Pr(>|t|)
mid - young	0.588	1.246	0.472	0.887
old - young	-2.801	1.246	-2.249	0.141
old - mid	-3.390	1.246	-2.721	0.077
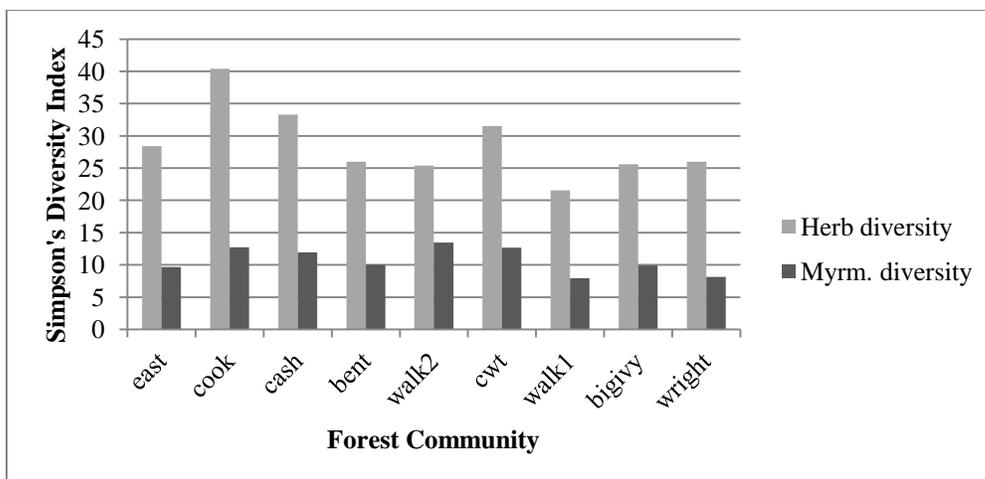
Figure 1. Simpson's Diversity indices for nine forest herb communities and myrmecochore communities across three forest age categories in western North Carolina. Herbs were inventoried from 1 m^2 plots (n=81) randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research site. The first three sites (East, Cook, Cash) are young sites, aged <100 years; Bent Walk2 and CWT are middle-aged, 100-200 years; Walk1, BigIvy, and Wright are mature, >200 years.
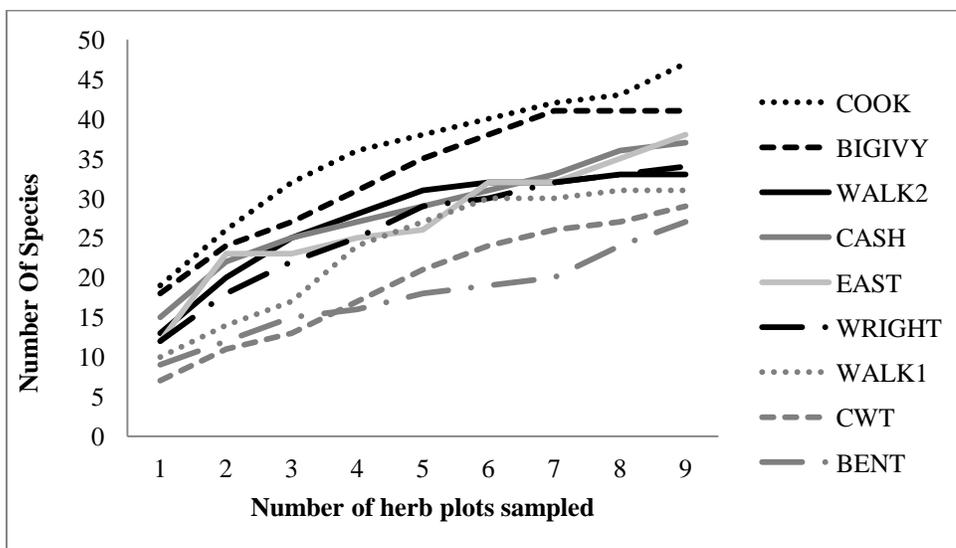
Figure 2. Species accumulation curves for 1 m^2 herb inventory plots (n=81) that were randomly placed along transects spaced at 10 m intervals in each 2500 m^2 research sites in rich cove forest communities of Western North Carolina. Twenty species occurred in 2500 m^2 research sites that did not occur in inventory plots.

DISCUSSION

My hypothesis that mature forests have higher herb diversity than younger, more recently disturbed forests was not supported by my data. Contrary to established research (Duffy & Meier, 1992; Pearson et al., 1998; McLachlan & Bazely, 2001; Mitchell et al., 2002; Harrelson & Matlack, 2006; Wyatt & Silman, 2010), there was a trend toward higher herb diversity in the younger forests (disturbance within the last 100 years) among my sites. The data only partially supported my hypothesis that high diversity in the herb community would translate to high diversity in myrmecochore populations. The site with the highest myrmecochore diversity ranked second to last in overall herb diversity; however, the two sites with highest herb diversity also had relatively high myrmecochore diversity (Fig. 1).

Diversity indices correlated with species richness rather than abundance among the most diverse sites, where the greatest number of species present provided the basis for highest diversity. Higher diversity did not appear correlated with abundance, however. The most densely populated sites for both overall herb abundance and myrmecochore abundance were two of the three least diverse. Although mature forests did not exhibit higher herb or myrmecochore diversity, they did house the greatest abundance of myrmecochore species. The lack of statistically significant differences in herb species abundance among forest ages was surprising, given that mature forests contained nearly twice the average number of stems (791) than the other age classes (365 for both mid and young). The lack of statistical significance is particularly baffling because the measures of myrmecochore species abundance did show statistically significant difference

(P=0.047) between average number of stems in mature forests (375) and other age classes (young, 180; mid, 136).

Given that all my sites are categorically rich cove forests (Schafale, 2012), myriad factors may explain the discrepancy between my results and those found in previous research. Younger, more recently disturbed communities can often exhibit higher species diversity because of increased availability of space and resources, which decline over time through competitive exclusion as locally superior competitors eliminate opportunistic species. Because results from Pearson-product moment tests for correlations among site characteristics show no correlation among sites' Simpson's diversity indices, forest age, elevation, slope, or soil pH, perhaps the most important factor affecting diversity in my sites would be scale, both spatial and temporal. Small-scale diversity is driven by localized species interactions, whereas large-scale diversity is predominantly driven by ecosystem processes (Chandy et al., 2006).

Positive correlation between mature forests and herb abundance may be attributed to higher average soil moisture in those sites ($p < 0.005$). Although a correlation does exist between soil moisture and both herb and myrmecochore abundance, my samples are fairly limited in both size and scope and likely do not reflect ecological processes that affect richness, abundance, or diversity on a larger scale. Pearson et al. (1998) established a positive correlation between presence and abundance of many rich cove forest herb species and larger patches (>200ha) of forest that have escaped intense disturbance. Mitchell et al. (2002) found plant diversity and myrmecochore abundance markedly affected by forest patch size and distance from a forest edge. Ecological processes, such as degree and frequency of smaller local disturbances and effects of

shorter-term succession, may also have been masked by my designation of forest age classes. Local disturbance and short-term succession influence species interactions and environmental variables such as site heterogeneity, canopy density, litter characteristics, and propagule sources—all factors contributing to herb community diversity.

Species accumulation curves conducted for each study sites suggest that increased sampling would lead to increased species richness in five of the nine sites (Cook, East, Wright, CWT, Bent). Rates of increase on plotted curves slowed or reached plateaus after nine 1sqm herb survey plots in two mature sites (Big Ivy, Walker1), a middle-aged site (Walker 2) and a young site, (Cashie) (Fig. 2), suggesting a more accurate account of diversity was captured with nine 1 sq. meter herb inventory plots in each of those sites.

Studies on the relationship between diversity and abundance of seed-dispersing ants and the abundance and diversity of myrmecochores have yielded conflicting results. Wolff and Debussche (1999) found no correlation, while Mitchell et al. (2002) found a negative correlation along an environmental gradient. However, Mitchell et al. (2002) also found that the diversity and abundance of myrmecochorous plants in general, and of young stages in particular, increased with an increase in the abundance of ants from the genus *Aphaenogaster*, which suggests that the presence of this particular genus in the ant community has a greater effect on myrmecochore population dynamics.

RESULTS

Of the 35 myrmecochorous species in my sites, 25 were observed fruiting, and 15 of those were observed dehiscing. Fruiting began in late March and dehiscence was first observed in the second week of April. Duration of fruit set and dehiscence varied by species (Fig. 3). Number of species with dehiscent fruits increased over time and ranged from one species during the second week of April, to 11 dehiscent species during the third week of May. The total number of myrmecochore species in fruit across sites peaked in the first week of April, and the total number of species with dehiscent fruits across sites peaked in the third week of May (Fig. 4). The number of species in fruit and with dehiscent fruit across sites appears to have a bimodal distribution, with peaks in mid April and in mid March (Fig. 5). The bimodal pattern also appears in fruit phenology of young and middle-aged forests, whereas mature forests show a uniform distribution over time (Fig. 6). No significant differences occurred among forest ages for number or duration of species in fruit or dehiscent. Variance in the number of species in fruit among forest ages was greatest in middle-aged forest, which ranged from one species to five species (Fig. 7).

	4.2	4.3	4.4	5.1	5.2	5.3	5.4	6.1
CLCA	▬	▬	▬	▬				
ANAC		▬	▬	▬	▬			
VIHA		▬	▬	▬	▬			
ANQU		▬	▬	▬				
VISO		▬	▬	▬				
DICU		▬	▬					
VICA				▬	▬	▬	▬	▬
ERUM					▬	▬	▬	
ASCA						▬	▬	▬
PROSA						▬	▬	▬
SANGCA						▬	▬	▬
TICO						▬	▬	▬
TRER						▬	▬	▬
UVGR						▬	▬	▬
VIOLA						▬	▬	▬
Total	1	6	5	5	4	11	8	8

Figure 3. Onset and duration of fruit dehiscence for 15 myrmecochorous species (Month.week) in nine rich cove forests in western North Carolina. CLCA = *Claytonia caroliniana*, ANAC = *Anemone acutiloba*, VIHA= *Viola hastata*, ANQU = *Anemone quinquefolia*, VISO = *Viola sororia*, DICU = *Dicentra cucullaria*, VICA = *Viola canadensis*, ERUM = *Erythronium americanum*, ASCA = *Asarum canadense*, PROSA = *Prosartes* spp, SANGCA = *Sanguinaria canadensis*, TICO = *Tiarella cordifolia*, TRER = *Trillium erectum*, UVGR = *Uvularia grandiflora*, VIOLA = *Viola* spp.
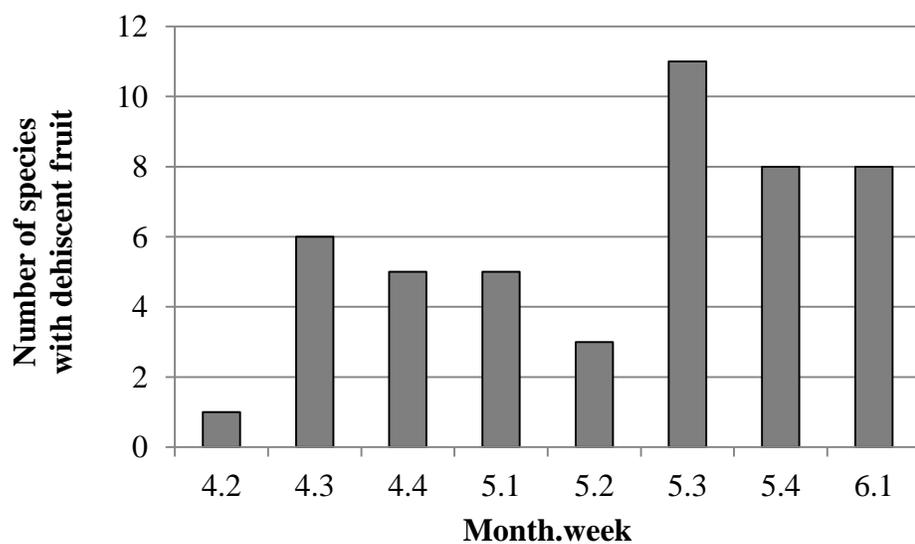
Figure 4. Number of myrmecochorous species (N = 29) observed with dehiscent fruits by date (Month.week) from April 1, 2012 to June 10, 2012 in nine rich cove forests of western North Carolina.
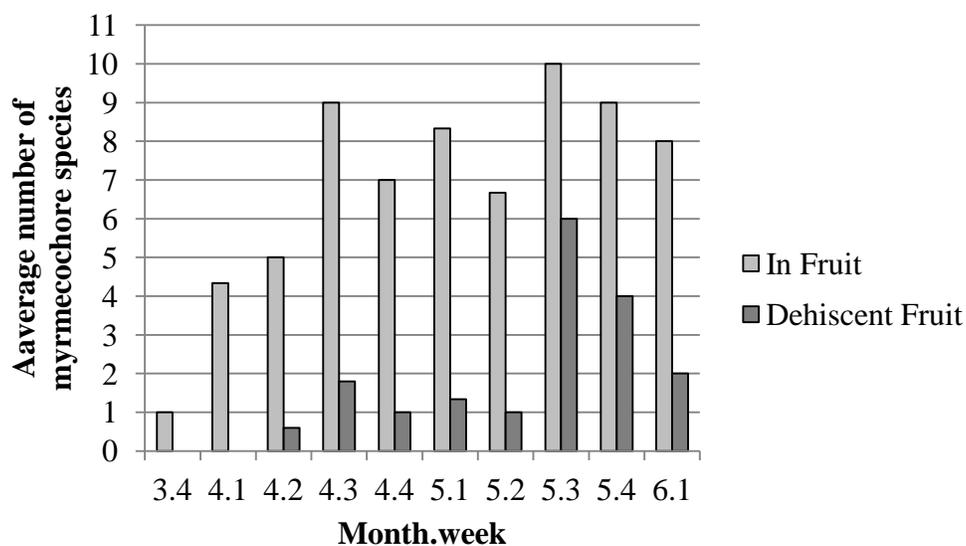
Figure 5. Average number of myrmecochore species in fruit and with dehiscent fruits over time (Month.week) in nine rich cove forests of western North Carolina during spring and summer of 2012.
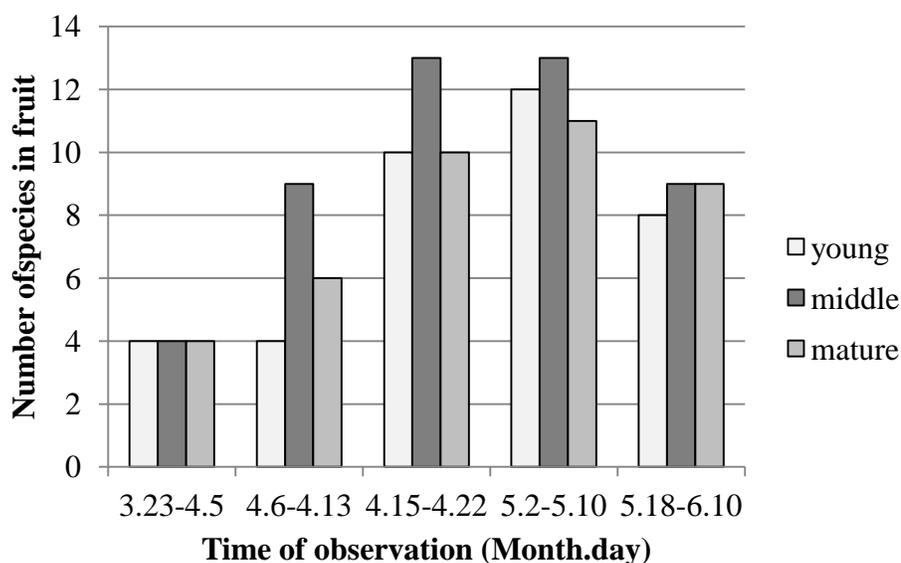
Figure 6. Average number of myrmecochore species in fruit in each forest age category over time (Month.week) in nine rich cove forests of western North Carolina during spring and summer of 2012.

DISCUSSION

Many herbs in the temperate forest community, and almost all species in the myrmecochore guild, are long-lived perennials that are typically slow to establish. Adaptation for seed dispersal by animals or insects is frequently associated with specific regenerative strategies (Grime, 1979). Myrmecochores are overwhelmingly clonal, and often reproduce vegetatively from rhizomes, a life history trait that allows for less frequent production of reproductive structures and allocation of resources to growth and survival (van Kleunen et al., 2002, and references therein). As a regenerative strategy, vegetative, or clonal reproduction is most common in relatively undisturbed habitats, whereas all other strategies are adapted to exploit disturbance and vary according to the habitat, and the nature and frequency of the disturbance (Pudlo, 1980; Grime, 1979). Asexual, or vegetative reproduction, allows rapid response to habitat conditions, whereas

sexual reproduction is a more conservative response to natural selection that allows retention of genotypic variety and improves chances of long-term survival in variable habitat conditions (Williams, 1966, 1975; Grime, 1979; Pudlo, 1980).

Life history transitions among clonal herbs from vegetative reproduction to flowering stages can be affected by abiotic factors correlated with frequency and magnitudes of disturbance, such as light, moisture and resource availability, often from the previous season; and by factors such as herbivory, which can decrease leaf area and limit reproductive output (Small, 2002; Levine & Feller 2004; Patsias & Bruelheide 2011; Moore et al., 2012). Reproductive output and clonal expansion are also affected by competition for resources and can be density-dependent (Levine & Feller, 2004; Herben & Goldberg, 2014). Tradeoffs between resource allocation for reproductive structures and clonal expansion can be mitigated by habitat quality, whereas sexual reproduction offers relocation from poor habitats, vegetative reproduction is beneficial in high quality environments (Williams, 1975; Abrahamson, 1980). Moreover, sexual reproduction may be selected at the metapopulation level to support colonization of new sites and vegetative reproduction at the population level to support population persistence (Olivieri et al. 1995; Patsias & Bruelheide, 2011).

Low frequency of flowering in the literature among clonal plants, and myrmecochores in particular, support my own observations from my research sites. Cain et al. (1997) followed flowering phenology of four populations of *Asarum canadense* for seven years in both early and late successional forests in the northern extent of its range. They recorded an average of only 12% of individuals producing flowers in those populations that varied in size from 25 to 132 individuals over time. Moore et al. (2012)

followed *Trillium recurvatum* populations over 17 years and, in data collected from nine seasons, found juvenile and non-flowing plants comprised 82% of the populations and recorded only 11% seed-generated stems. Kudoh et al. (1999) elucidated the "waiting strategy" of *Uvularia perfoliata* populations, which persist vegetatively under closed canopies and only produce seeds under more optimal conditions associated with canopy gaps. Walker et al. (2009) recorded 66-67% of non-flowering plants in a *Trillium maculatum* population over a two-year period.

Lacking data on age structure and population reproductive effort in my sites, assumptions about effects of seasonal and cyclical dynamics on reproductive capacity and colonization in these communities are suspect, at best. However, long-term demographic data from seminal studies on clonal myrmecochores provide insight to the life histories of these herbs and their reproductive output, and support my observations and anecdotal evidence of severely limited flower and fruit production under certain environmental conditions.

APHAENOGASTER FORAGING AND NESTING

INTRODUCTION

Myrmecochory has long been considered a diffuse mutualism, where specialization between ant and plant species was rare. However, current research shows that ants of the genus *Aphaenogaster* are the most common and effective dispersal agents for ant-dispersed plants in eastern North American deciduous forests, (Anderson, 1988; Gorb & Gorb, 2003; Giladi, 2006; Rico-Gray & Oliveira, 2007; Ness et al., 2009). Difficulty in indentifying morphological differences among species of *Aphaenogaster* native to eastern North America has led to the aggregation of several species, including *A. rudis, A picea,* and *A. fulva,* into a group called the "rudis complex" (Umphry, 1996; Crozier, 1977). The general use of the name *Aphaenogaster* refers to this collection of species in this study.

Because *Aphaenogaster* are the most common and effective dispersal agents for ant-dispersed plant species, elucidating the mechanisms that control *Aphaenogaster* distribution is fundamental to understanding myrmecochore population ecology and to discovering the conditions under which this mutualism may have co-evolved. Studies on the relationship between diversity and abundance of seed-dispersing ants and the abundance and diversity of myrmecochores have yielded conflicting results. Wolff and Debussche (1999) found no correlation between abundance of myrmecochores and abundance of ant species, whereas Mitchell et al. (2002) found a negative correlation between ant species abundance and diversity relative to myrmecochore abundance and diversity along an environmental gradient. However, Mitchell et al. (2002) also found

that the diversity and abundance of myrmecochorous plants in general, and of young stages in particular, increased with an increase in the abundance of ants from the genus *Aphaenogaster*, which suggests that the presence of this particular genus in the ant community has a greater effect on myrmecochore population dynamics than other ant genera. Additionally, Ness et al. (2009) found myrmecochore species density and species richness correlated positively with *Aphaenogaster* density among patches within an intermediate-aged (150 yrs) eastern deciduous forest and qualify *Aphaenogaster* as a keystone mutualist in these habitats, given the apparent benefit conferred to the seeds, the increased fitness of ant populations, and the pervasiveness of the disperser.

To investigate the effects of myrmecochore diversity, forest age, and temporal interruption of resource availability on *Aphaenogaster* nest location and persistence, I established sites in three different forest-age stands: (1) young forest stands, (severe disturbance such as clear-cut or similar logging within the last 100 years); (2) middle-aged stands (aged between 100 and 200 years); (3) mature forest stands, (aged 200 years or more and without evidence of significant disturbance). Three sites were identified in each age category to total nine research sites in the western North Carolina counties of Buncombe, Jackson, Graham, and Macon. I installed nine treatment plots (n = 81) in each of my nine sites, and I paired artificial ant nests with bait stations (tuna bait) in each treatment plot. I used three treatment categories for provisioning bait to mimic theoretical elaiosome availability among different aged forests with different disturbance levels: a continuous treatment where bait stations were provisioned weekly to mimic mature forests with intact herb communities; interrupted treatments mimicking disturbed

forests with low myrmecochorous diversity had bait offered weekly at three-week intervals; and control treatments, where no bait was ever provided.

I predicted that *Aphaenogaster* foraging occurrence would be higher in areas with greater myrmecochore abundance and diversity and with more consistently available elaiosomes. Further, I hypothesize that *Aphaenogaster* nest colonization frequency would be higher in nests located adjacent to consistently provisioned bait stations in all forest age categories.

RESULTS

Foraging

Aphaenogaster foragers were observed at all nine sites, and were observed visiting bait trays on 53/ 356 observations occurring between March and June 2012. On 16 of those 53 occasions, *Aphaenogaster* foragers were observed visiting trays that were not provisioned with bait. The total number of foragers was 179, and the mean (+/-SE) number of foragers per observation was 3.3 (+/-0.3). Mean individuals per visit in young forests were 4 (+/- 0.8) individuals, 3.1 (+/- 0.3) in middle-aged forests, and 3.2 (+/- 0.4) in mature forests (Fig. 7). The greatest numbers of individuals observed in a single visit was 12 and occurred at East Reach, a young forest site. *Aphaenogaster* individuals were observed foraging on 13% of visits to young forests (12/94), on 16% of visits to middle-aged sites (20/126) and on 15% of visits to mature sites (21/136).

Environmental variables (soil moisture, soil temperature) and forest age were non-significantly, moderately correlated with *Aphaenogaster* foragers (Table 20). *Aphaenogaster* foraging data from treatment plots with continuous availability of tuna (three per site, n = 9) were analyzed for correlation among forest ages, herb diversity and

abundance, and myrmecochore diversity and abundance. One-way ANOVA showed no

difference among means in foraging behavior with respect to forest age ($F_{2, 24} = 0.446$; P

$= 0.645$) or between *Aphaenogaster* foraging behavior and myrmecochore abundance ($F_{2, 24} = 0.594$; P $= 0.829$). Poisson distribution of the general linear mixed effects model,

where *Aphaenogaster* forager data were corrected for co-linearity and over-dispersion,

shows that foraging behavior appears somewhat, but not significantly, affected by

myrmecochore abundance, soil temperature, and the interaction between those two

variables [R packages MASS (Venables & Ripley, 2002), lme4 (Bates et al., 2012), and

rms (Harell, 2013)]. Akaike information criterion (AIC) analysis suggests

Aphaenogaster foraging was higher in sites where myrmecochore abundance and soil

temperature was highest (Fig. 8).
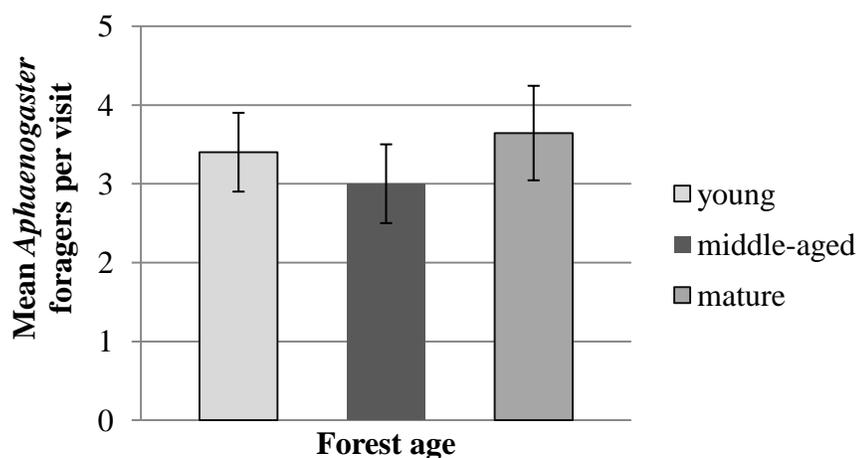
 Figure7. Mean number of *Aphaenogaster* foragers visiting bait stations from March, 2012 to June, 2012, in western North Carolina rich cove forests (n = 9) grouped by forest age (young = < 100 yrs, middle = < 150 years, and mature = > 200 years).

Table 20. Pearson's Product-Moment correlation coefficients for relationships among environmental variables, *Aphaenogaster* dynamics, and herb community characteristics from nine rich cove forests in western North Carolina. Coefficient value of +/- 1 represents total correlation and 0 represents no correlation. Values between +/-0.3 and +/-0.7 represent a moderately strong association between variables.

	Avg. Soil Moist	Avg. Soil Temp	Forest Age	Myrm. Abundance	Myrm. Richness	Aph. Forage
Avg. Soil Moisture	1.00					
Avg. Soil Temperature	-0.48	1.00				
Forest Age	0.46	-0.31	1.00			
Myrm. Abundance	0.66	-0.19	0.43	1.00		
Myrm. Richness	0.30	-0.36	0.06	0.43	1.00	
Aph. Forage	0.06	0.05	0.11	0.20	-0.16	1.00
Total Herb Abundance	0.45	-0.21	0.38	0.74	0.45	0.11
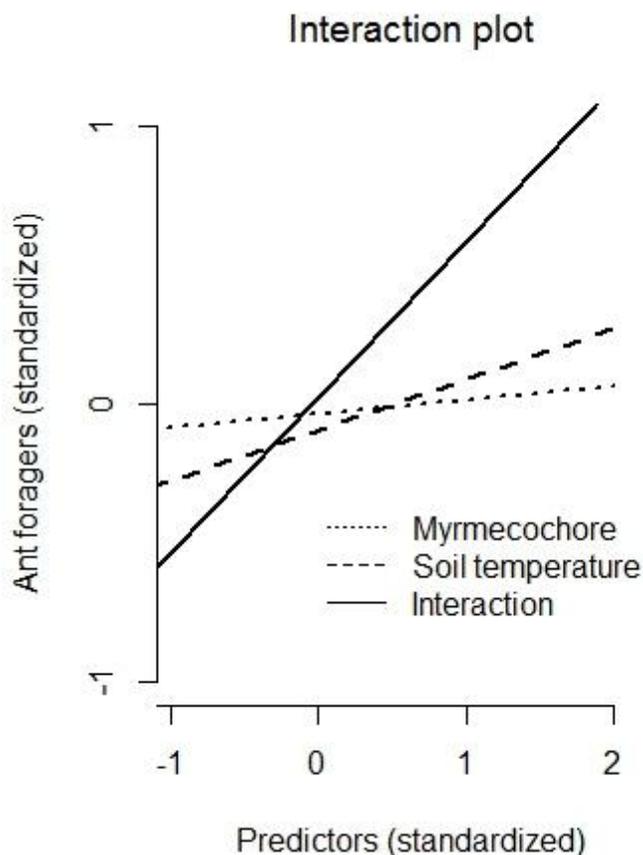
Interaction plot

Figure 8. Interaction plot of *Aphaenogaster* foraging as a function of myrmecochore abundance and soil temperature. *Aphaenogaster* foraging increases somewhat with greater myrmecochore abundance and soil temperature, but increases significantly more where abundance and temperature are highest.

Nesting

   Only 12 colonies were observed inhabiting artificial nests (n = 81) in all nine

sites. Nest colonization was not significantly correlated with forest age. Seven of those

12 were located in mature sites, three were in middle-aged sites, and two were in young

sites (Table 21). Of the colonized nests in mature forests, six of the seven colonies were

located in Wright's Creek (Table 21), and one was located in Big Ivy. Middle-aged

forests had three colonized nests, one in Walker2, and two in Bent Creek. The two young

sites with colonized nests were Cook House and Cashie Branch. No nests were colonized

at Walker Cove (mature), CWT (middle aged), or at East Reach (young). Notably, the

herb community at Walker Cove had greatest percentage of myrmecochores, but did not

host any colonized nests. The mature site that hosted the greatest number of colonized

nests, Wrights Creek, fell in the middle of the ranks in myrmecochore abundance, and

ranked second to last in myrmecochore diversity. Although no significant correlation

existed between forest age and nest occupancy, there was significant correlation between

occupied nests and forest sites (n = 9), where Wright's hosted 50 % of the total nests

colonized. Significant correlation also occurred between average soil moisture and forest

age (P< 0.001; Table 22) and between forest age and soil temperature (P = <0.001). Lack

of significance between nesting and forest age is perplexing, given nest occupancy in

mature forests was more than 50% greater than in young or middle aged forests.

Among the sites with colonized nests, the effect of tuna availability on nest

occupancy was only marginally statically significant ($F_{(1, 10)}$ = 4.5, p = 0.059), which is

further weakened by the low percentage of total nests occupied (14.8%). Of the 12 nests

occupied, six occurred when bait was present, and six occurred when bait was absent.

Those that were occupied in the absence of bait were located in control plots where no

bait was ever provisioned (Table 21).

No correlation existed between nest colonization and forest age or myrmecochore

abundance, however, one-way ANOVA showed that colonies where bait was present

were larger than colonies where bait was absent ($F_{1,10}$ = 4.5, P = 0.059; fig 9), and a

moderate correlation existed between colony size and the presence of bait (Pearson =

0.56, P= 0.059;). No nests remained colonized from one site visit to the next.

Table 21. Summary of artificial ant nests colonized by *Aphaenogaster* ants in nine myrmecochore research sites across three forest age categories (young = < 100 yrs, middle = < 150 years, and mature = > 200 years) in western North Carolina. Nests were situated in nine 1sq meter plots randomly placed along transects located at 10m intervals in each 2500sqm site. Treatments consisted of bait stations with tuna provisioned weekly (continuous), weekly at three-week intervals (interrupted), and no tuna (control).

Site	Forest Age	Treatment	Bait Present	Approximate Colony Size
Cashie	Young	Control	NO	50
Cook	Young	Continuous	YES	100
Bent	Middle	Control	NO	50
Bent	Middle	Intermediate	YES	100
Walk2	Middle	Control	NO	50
Wright	Mature	Intermediate	YES	100
Wright	Mature	Continuous	YES	50
Wright	Mature	Continuous	YES	100
Wright	Mature	Control	NO	75
Wright	Mature	Control	NO	75
Wright	Mature	Control	NO	50
Bigivy	Mature	Intermediate	YES	50

Table 22. One-way ANOVA and summary statistics for comparison of average soil moisture among forest age categories in nine rich cove forests of western North Carolina.

Forest Age and Soil Moisture

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Forest Age	2	502.5	251.23	6.42	0.006
Residuals	24	939.1	39.13		

Forest Age	Mean soil moisture	Sd	Data:N
Young	23.65	4.51	9
Mid	21.91	3.99	9
Old	31.80	9.00	9
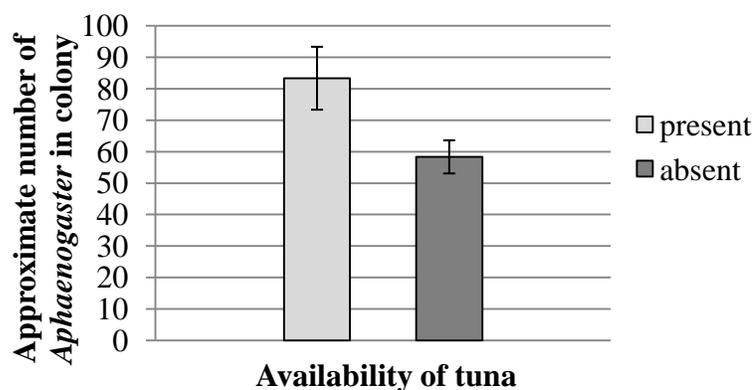
Figure 9. Mean *Aphaenogaster* colony size in artificial nests with adjacent bait stations provisioned and not provisioned with tuna ($F_{1, 10} = 4.5$, P = 0.059).

DISCUSSION

The effects of herb diversity and myrmecochore diversity on *Aphaenogaster* foraging behavior and nesting dynamics remains unresolved because no statistical differences in diversity were observed among my sites, which may also reflect the lack of correlation between forest age and foraging dynamics. The apparent influence of greater myrmecochore abundance combined with lower soil temperature in mature forests on *Aphaenogaster* foraging behavior in my study supports previous research. Fore example, Beattie and Culver (1981) documented a marked increase in ant activity, particularly *Aphaenogaster* ants, in areas with high myrmecochore abundance in their comprehensive study of West Virginia populations. Mitchell et al. (2002) found the diversity and abundance of myrmecochores, particularly younger plants, increased with an increase in *Aphaenogaster* ants compared with other ant genera. Ness et al. (2009) found myrmecochore species density and species richness correlated positively with

Aphaenogaster density among patches within an intermediate-aged (150 yrs) eastern

deciduous forest and go so far as to qualify *Aphaenogaster* as a keystone mutualist in

these habitats.

Despite no statistically significant correlation between forest age and

Aphaenogaster foraging or nesting in my sites, the significantly higher myrmecochore

abundance, higher soil moisture, and lower temperatures exhibited in mature forests has

implications for *Aphaenogaster* foraging and nesting dynamics. Analysis of individual

sites (n = 9) outside of forest age category revealed highly significant correlations among

average soil temperature and moisture and Wright's Cove, where 50% of colonized nests

occurred. Correlation between temperature and *Aphaenogaster* foraging dynamics is

well-established (Lynch, 1980; Smallwood, 1982; Southerland, 1988; Sanders et al.,

2007; Dunn et al., 2007; Warren et al. 2011b). However, Warren et al. (2010) showed

that soil moisture may have greater influence on *Aphaenogaster* foraging and dispersal

services compared with other environmental variables, such as temperature and light

conditions, resulting in failed seed dispersal in wetter habitats. Whereas soil moisture

was not the sole driver of *Aphaenogaster* distribution and habitat selection, it did

influence niche partitioning and species segregation with other co-occurring competitive

ant species (Warren et al., 2010; 2011a, 2014). Notably, Warren et al. (2011b) also

elucidated the potential phenological asynchrony between optimal foraging conditions for

Aphaenogaster and myrmecochore species fruit set resulting from changing climate

conditions.

Lynch et al. (1980) documented distinct daily and seasonal variation in

Aphaenogaster activity, where *Aphaenogaster* foraged mostly at night during cooler

months of spring and fall, but shifted to a strong diurnal pattern during warmer months

from June to September. They also noted that a shift in *Aphaenogaster* behavior

corresponded with increased abundance of other common, more aggressive ant species,

such as *Prenolepis impairs* and *Camponotus ferrugineus*, suggesting that *Aphaenogaster*

foraging dynamics, and therefore, dispersal services, may be affected by competitive

exclusion. These findings suggest that my results may have been affected by my inability

to observe and record foraging behavior at the same time for all my sites and by the

omission of potential displacement by aggressive co-occurring species.

Competition among myrmecochores is also a factor that structures this mutualism.

Warren et al. (2014) show through comparative literature and field trials that smaller

myrmecochorous diaspores are predominantly available early in the foraging season and

larger diaspores typically release later in the season. Because ants show preference for

larger diaspores, their findings suggest avoidance of competition for dispersers among

myrmecochores with staggered seed release by species with different sized seeds.

Temporal variation in diet preferences and in foraging behavior among

Aphaenogaster ants according to colony dynamics has also been well-documented

(Culver & Beattie, 1978; Lynch et al., 1980; Beattie, 1985; Fellers, 1989), where varying

nutritional needs dictate foraging preferences. Developmental phenology appears to be

the driver behind resource selection in some cases, in addition to the type of resource

available. Heithaus et al. (2005) found that *Aphaenogaster* show a marked preference for

insect carcasses when offered along with elaiosome-bearing diaspores. In cafeteria

experiments, they found that ants collected diaspores mainly when insect larvae were not

available, and collection decreased sharply after the alternative sources had been

exhausted. Satiation recorded for diaspore collection by these authors did not occur for insect carcasses in this study. These results conflicted with the frequency-dependent preferences showed by Smith et al. (1989), who found that foragers' preferences between two different myrmecochore diaspore offerings were affected by the density and species composition of the seed environment, where lower seed densities increased the probability of removal. Stuble et al. (2013) documented temporal segregation in foraging behavior among competing species within ant assemblages, where dominant ants generally foraged at night and subordinate species, such as *Aphaenogaster*, tended to recruit to baits more commonly during the day. These results support the competitive displacement analysis elucidated in Lynch et al. (1980).

Additionally, a significant correlation exists between forest age and soil moisture among my treatment plots used in foraging dynamics analyses (those with continuous bait treatments). Soils in mature forests exhibit higher moisture than both middle-aged and younger forests ($F_{2, 24} = 6.42$; $P = 0.00584$). Warren et al. (2012) found moisture, and not temperature, to be a primary driver of *Aphaenogaster* nesting dynamics, where *Aphaenogaster* occupied microhabitats significantly drier than the more aggressive and less-effective seed disperser, *Lasius alienus* . The moderate correlation between soil temperature and soil moisture (Pearson's = 0.48) in my sites suggests that if moisture and/or temperature drive *Aphaenogaster* foraging or nesting dynamics, the lack of correlation between those variables and *Aphaenogaster* foraging among my sites may be an anomaly, the result of a single season snapshot, or of an unanalyzed variable, such as leaf litter or coarse woody debris that may uniquely characterize the sites. The positive correlation between forest age and soil moisture and soil temperature, and between soil

moisture and soil temperature, also may contribute to the apparent influence of myrmecochore abundance combined with soil temperature on *Aphaenogaster* foraging behavior, as well as the disproportionate occurrence of colonized nests in the mature sites.

CONCLUSION

Rich cove forests of the southern Appalachians are typically species rich, with moderate to high soil fertility and a moderate disturbance regime characterized by ongoing gap-dynamic regeneration of mixed mesophytic forest communities (Pittillo et al., 1998; Ulrey 2002; Gilliam, 2007; Wyatt et al., 2010). This high species richness is further supported by complex topography, high rainfall, and microsite variation characteristic of southern Appalachian rich coves. Additionally, these coves acted as a refuge for many plant species during the advance and retreat of previous ice ages (Whittaker 1956, Pittillo et al. 1998). The sensitivity of this rich herbaceous community to disturbance, variations in resource availability, and changes in spatial heterogeneity in cove forests is well-established (Pearson, et al., 1998; Duffey & Meir, 1992; Gilliam, 2002; Mitchell et al., 2002). Species richness influences the degree to which species interactions define community structure, relative to availability of new sites for colonization and rates of population growth (Horn, 1974; Runkle, 1982; Honnay, 2005). Without frequent disturbance to create new colonization sites and cause mortality within populations, the importance of inter-specific interactions increases. Herb distribution patterns in low disturbance sites may also signify highly suitable microhabitat, which promotes vegetative reproduction among clonal plants. Investments in reproductive structures and seed production may be considered a waste of energy in communities with low-level disturbance regimes and established herb populations (Honnay, 2005). Aggregations of suitable microhabitats across the landscape increases with site

heterogeneity, which, for long-lived clonal perennials like myrmecochores, likely comes in the form of canopy gaps (Horn, 1974; Runkle, 1982).

Whereas my sites were certainly species rich, lack of significant statistical difference in diversity among herb communities and among the myrmecochore guild in my sites precluded any analysis strictly based on diversity. Although threshold for significance was the standard 0.05, P-values for my analysis were 0.07, which is generally accepted as marginally significant. Species accumulation curves plateaued only for the three least diverse sites, and were in sharp incline for three others. A larger sample size for the herb inventory would certainly provide a more accurate representation of the actual diversity and may have resulted in differences in diversity that met a 0.05 significance threshold. My results do imply that myrmecochore diversity within a community does not necessarily follow overall herb diversity. Likewise, fruiting phenology for myrmecochores does not follow flowering phenology, nor is the presence of a fruit necessarily an indicator of elaiosome availability.

Even in the absence of statistically significant differences in average number of species dehiscent among forest ages, an interesting phenological pattern emerged in my sites. Ten myrmecochore species were never observed producing fruit, and of the 25 myrmecochore species observed in fruit, only 15, or less than 43% of myrmecochore species, were observed dehiscent or mature by the conclusion of data collection on June 10, 2012. The number of myrmecochore species in fruit over time resembled a uniform distribution, but the number dehiscent presented a bimodal pattern in middle-aged and mature forests. The bimodal distribution suggests that elaiosome availability may fluctuate throughout the growing season in areas of lower myrmecochore diversity.

Comparison among sites with distinctly diverse myrmecochore populations would better elucidate whether myrmecochore diversity translates into a more consistent supply of elaiosomes. Quantification of individuals within populations producing reproductive structures or reaching fruit maturity would better elucidate the actual level of elaiosome availability for ant consumption.

Lack of significant correlation between *Aphaenogaster* foraging and environmental variables (soil moisture, soil temperature) or forest age contradicts previous published research. However, foraging did increase somewhat in sites with greater abundance in sites with higher soil moisture, and increased significantly more where both abundance and temperature were highest. This pattern suggests that whereas effects of a single variable are negligible, interactions among multiple variables may produce significant affect. The colonization of only 12 of 81 artificial nests by *Aphaenogaster* in my sites makes for a small sample size and limits statistical inference from any analysis.

My results support mounting evidence in current research for environmental conditions as drivers of *Aphaenogaster* nesting and foraging and their apparent indifference to elaiosomes as a food resource. A dispersal strategy where the dispersal agent receives minimai, if any, benefit, is limited in distance, and is less attracted to the diaspore than to other resources, suggests an ineffectual mechanism. *Aphaenogaster's* preference for other food sources, such as insect carcasses, and for novel seeds, coupled with temporally changing dietery needs and questionable benefits of elaiosome consumption supports the uncertainty of effective dispersal. Satiation documented by Heithaus et al. (2005) and preference for novel seeds suggest ants are more effective

dispersal agents in communities with low myrmecochore density and diversity, as documented in Pudlo (1980). This echoes the low resource dynamics and frequent disturbance regimes under which most myrmecochore systems operate outside temperate eastern US forests.

Additionally, myrmecochores are long-lived perennials that reproduce clonally and can persist indefinitely without producing flowers or seeds (Honnay, 2005, and references therein). Seedling establishment among forest herbs can be severely limited by densely populated community and successful regeneration by seed is typically restricted by the ability to exploit gaps (Grime, 1979; Kudoh et al., 1999; Honnay, 2005). Within low-distubance and high abundance conditions, short-distance dispersal services provided by *Aphaenogaster* may only be important where colonization opportunity exists. The importance of the myrmecochory mutualism for the plants also may be restricted to occasions of resource limitations, as in the case of most other ant-mediated dispersal systems.

Conditions within which myrmecochory evolved likely no longer exist, and myrmecochory may well be on the decline. The production of elaiosomes by myrmecochores would persist regardless of its dispersal effectiveness (or lack of) until genetic drift resulted in eventual reallocation of expensive resources from elaiosome production. Granted, climate change will likely cause asynchrony between fruiting phenology and optimal foraging conditions for *Aphaenogaster*, given the ants' ability to quickly relocate colonies and their tolerance thresholds for changing temperatures and moisture levels (Warren et al., 2011b; Warren & Chick, 2013). However, the doom and gloom predicted for the survival of myrmecochorous species in current myrmecochory

literature may be a bit overstated, and the future of myrmecochore populations in the eastern US may not be as dependent on a single genus of ant as most current research suggests. In the event that current conditions of the mutualism are disrupted, or in the face of losing *Aphaenogaster* as dispersal agent to phenological asynchrony, a shift to an alternate dispersal agent(s) that are common in myrmecochory communities in other regions, such as gastropods or wasps, may be one of many imaginable salvations for these long-lived clonal perennials that are largely diplochorous and employ multiple dispersal strategies.

The overwhelmingly ant-centricity of the scientific literature concerning this mutualism is a bit disconcerting. The abundance of current research revealing the lack of benefits conferred to the ants may also serve as evidence of limited benefits and ineffective dispersal services for the plants. In consideration of myrmecochore species demographics and life histories, their bet-hedging strategy of multiple dispersal mechanisms, their ability to persist vegetatively, and the apparent indifference toward elaiosomes by *Aphaenogaster*, I can only conclude that the dispersal services provided for the myrmecochore plant guild by *Aphaenogaster* ants are merely supplemental, just as elaiosomes are a supplemental resource of the ants. It is arguable that dispersal services by *Aphaenogaster* may be important under conditions of frequent disturbance or low herb abundance, but also may not be entirely necessary. This mutualism appears facultative, not obligate, for both *Aphaenogaster* and myrmecochores.

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APPENDIX

APPENDIX 1

List of herbs present in nine myrmecochore research sites across three forest age categories (young = < 100 yrs, middle = < 150 years, and mature = > 200 years) in rich cove forests of western North Carolina. Myrmecochorous species in bold.

Species	# sites occurred (n = 9)	Abbreviation
Actaea pachypoda	7	ACPA
Actaea racemosa	7	ACRA
Adiantum pedatum	4	ADPE
Aegopodium podagraria	5	AEPO
Allium tricoccum	1	ALTR
Anemone acutiloba	**4**	**ANAC**
Anemone quinquefolia	2	ANQU
Aplectrum hyemale	4	APHY
Aralia nudicaulis	5	ARNU
Arisaema triphyllum	5	ARTR
Arnoglossum reniforme	3	ARRE
Aruncus dioicus	4	ARDI
Asarum canadense	**8**	**ASCA**
Aster cordifolia	7	ASCO
Aster sp	9	ASTER
Botrypus virginianus	1	BOVI
Cardamine concatenata	4	CACO
Cardamine diphylla	6	CADI
Carex plantaginea	7	CAPL
Carex sp	7	CAREX
Caulophyllum thalictroides	8	CATH
Chimaphila maculata	1	CHMA
Claytonia caroliniana	**4**	**CLVI**
Claytonia virginica	**3**	**CLCA**
Clematis sp	7	CLEMA
Clintonia sp	1	CLINT
Clintonia umbellulata	5	CLUM
Cryptotaenia canadensis	8	CRCA
Delphinium tricorne	8	DETR

Desmodium nudiflorum	9	DENU
Dicentra canadensis	**3**	**DICU**
Dicentra cucullaria	**1**	**DICEN**
Dicentra sp	**6**	**DICA**
Dioscorea villosa	5	DIVI
Dryopteris goldiana	3	DRMA
Dryopteris intermedia	2	DRIN
Dryopteris marginalis	1	DRGO
Erigeron sp	4	ERIG
Erythronum umbilicatum	**1**	**ERUM**
Euonymous americana	8	EUAM
Fern sp	5	FERN
Galearis spectabilis	3	GASP
Galium sp	**1**	**GALIU**
Geranium carolinianum	3	GECA
Geranium maculatum	2	GERAN
Geranium sp	3	GEMA
Goodyera pubescens	8	GOPU
Heuchera americana	1	JEAM
Homalosorus pycnocarpos	3	HOPY
Houstonia caerulea	9	HOCA
Houstonia purpurea	1	HOPU
Houstonia sp	1	HOUS
Hydrastis canadensis	1	HYDRAS
Hydrophyllum canadense	4	HYDROVI
Hydrophyllum virginianum	1	HYDROC
Impatiens capensis	3	IMCA
Laportea canadensis	2	LACA
Lilium superbum	3	LISU
Luzula multiflora	3	LUMU
Lysimachia sp	5	LYSIM
Maianthemum racemosum	1	MARA
Medeola virginiana	1	MEVI
Mitella diphylla	1	MIDI
Monarda clinopodia	2	MOCL
Monarda sp	6	MONAR
Osmorhiza claytonii	1	OSCL
Panax quinquefolius	1	PANQU
Parthenocissus quinquefolia	2	PARQU
Phryma leptostachya	2	PHLE
Pilea pumula	2	PIPU

Poa sp	1	POA
Podophyllum peltatum	1	POPE
Polygonatum biflorum	2	POPU
Polygonatum pubescens	1	POBI
Polystichum acrostichoides	1	POAC
Prenanthes sp	2	PRENA
Prosartes lanuginosa	**6**	**PROSA**
Prosartes maculata	**4**	**PRMA**
Prosartes sp	**5**	**PRLA**
Ranunculus recurvatus	4	RARE
Ranunculus sp	1	RANUN
Sanguinaria canadensis	**1**	**SANGCA**
Sanicula canadensis	6	SANICA
Sanicula sp	3	SANICU
Sedum ternatum	2	SETE
Smilax racemosa	1	SMRA
Smilax sp	3	SMILA
Solidago flexicaulis	8	SOFL
Solidago sp	3	SOLID
Stellaria pubera	2	STPU
Thalictrum dioecum	4	THTH
Thalictrum thalictroides	4	THDI
Tiarella cordifolia	**6**	**TICO**
Tipularia discolor	7	TIDI
Tradescantia virginiana	4	TRVI
Trillium cuneatum	**8**	**TRCU**
Trillium erectum	**1**	**TRVA**
Trillium grandiflorum	**6**	**TRGR**
Trillium rugelii	**2**	**TRRU**
Trillium sp	**4**	**TRILL**
Trillium vaseyi	**9**	**TRER**
Uvularia grandiflora	**1**	**UVULA**
Uvularia perfoliata	**1**	**UVSE**
Uvularia sessilifolia	**1**	**UVPE**
Uvularia sp	**4**	**UVGR**
Veratrum viride	1	VEVI
Viola blanda	**3**	**VIBL**
Viola canadensis	**3**	**VIHA**
Viola cucularia	**2**	**VICA**
Viola hastata	**9**	**VIPU**
Viola pubescens	**4**	**VISO**

Viola sororia	**1**	**VICU**
Viola sp	**1**	**VIOLA**
Vitis sp	1	VITIS
Zizia aurea	8	ZIAU

APPENDIX 2

Species distribution among forest ages

Species	Young	Middle	Old
ACRA	x	x	x
ADPE			x
ANAC	x	x	x
ANQU	x	x	x
APHY		x	x
ARNU	x		
ARTR	x		
ARRE	x		
ARDI	x		
ASCA	x	x	x
ASTER	x	x	x
BOVI	x	x	x
CACO			x
CADI	x	x	x
CAPL			x
CAREX	x	x	x
CATH	x	x	x
CLCA	x	x	x
CLINT		x	
CLUM		x	
CRCA	x		
DETR			x
DENU	x	x	
DICA	x		x
DICU			x
DICEN	x		x
DIVI	x		
DRIN	x	x	x
DRMA		x	x
ERUM	x	x	

Species	Young	Middle	Old
FERN	x	x	
GASP	x		
GALIU	x	x	x
GERAN	x		
GOPU		x	
HEAM			x
HOUS	x	x	x
HYDRAS	x		
HYDROC		x	
HYDROVI	x		x
IMCA	x	x	x
LACA	x	x	x
LISU	x	x	x
LUMU			x
LYSIM	x		
MARA	x		
MEVI	x	x	x
MIDI			x
MONAR	x		x
OSCL	x	x	x
PANQU	x		x
PARQU	x	x	
PIPU	x		x
POA	x	x	x
POPE	x	x	x
POBI	x	x	x
POPU			x
POAC	x	x	x
PRENA	x	x	x
PRLA	x	x	x
PRMA		x	
PROSA	x	x	x
RANUN	x	x	x
SANGCA	x	x	
SANICU	x	x	x
SETE			x
SMILA		x	
SOLID		x	x
STPU	x	x	x
THDI	x	x	x

Species	Young	Middle	Old
THTH	x	x	
TICO	x	x	x
TIDI			x
TRVI	x		
TRCU			x
TRER	x	x	x
TRGR		x	x
TRRU	x		x
TRILL	x	x	x
TRVA	x	x	x
UVGR	x	x	x
UVPE	x		
UVSE		x	x
UVULA	x	x	x
VEVI		x	
VICA	x	x	x
VIHA	x	x	x
VIPU	x	x	x
VISO	x	x	x
VIOLA	x	x	x
VITIS	x		
ZIAU		x	x
Total	68	59	65

APPENDIX 3

Month and day for onset of fruiting and dehiscing for myrmecochorous species from nine rich cove forests in western North Carolina.

Month.day	Fruiting	Month.day	Dehiscing
3.25	*Viola hastata*	4.10	*Claytonia caroliniana*
3.26	*Anemone acutiloba*	4.16	*Anemone acutiloba*
3.26	*Claytonia caroliniana*	4.20	*Viola hastata*
3.30	*Sanguinaria canadensis*	4.21	*Anemone quinquefolia*
4.01	*Dicentra cucullaria*	4.21	*Dicentra cucullaria*
4.05	*Erythronium umbilicatum*	4.21	*Viola sororia*
4.06	*Viola canadensis*	5.03	*Viola canadensis*
4.06	*Viola pubescens*	5.07	*Erythronium umbilicatum*
4.09	*Tiarella cordifolia*	5.18	*Tiarella cordifolia*
4.09	*Trillium grandiflorum*	5.18	*Asarum canadense*
4.13	*Anemone quinquefolia*	5.18	*Prosartes maculata*
4.13	*Prosartes maculata*	5.18	*Trillium erectum*
4.13	*Uvularia grandiflora*	5.18	*Uvularia grandiflorum*
4.13	*Viola sororia*	5.18	*Viola spp*
4.16	*Prosartes spp*	6.10	*Sanguinaria canadensis*
4.16	*Viola spp*		
4.17	*Uvularia spp*		
4.20	*Prosartes laguniosa*		
4.20	*Trillium cuneatum*		
4.20	*Trillium erectum*		
5.03	*Uvularia perfoliata*		
5.07	*Galium spp*		
5.07	*Trillium rugellei*		
5.10	*Trillium vasyi*		
5.18	*Asarum canadense*		