BIODIVERSITY OF LEAF LITTER AND ARBOREAL ANTS (HYMENOPTERA: FORMICIDAE) IN A TEMPERATE MID-ATLANTIC FOREST

By

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CHAPTER 1: The effect of leaf litter mass on the species richness and biodiversity of ants in the E.A. Vaughn Wildlife Management Area

Abstract

Bioindicator species, whose presence and/or abundance are likely to change with natural and man-made changes, can provide important information regarding the environmental health of a particular ecosystem. My research aimed to measure the diversity and species richness of leaf litter ants (Hymenoptera: Formicidae) in the E.A. Vaughn Wildlife Management Area (WMA), a temperate forest on the lower eastern shore of Maryland. Additionally, leaf litter depth and mass was investigated to determine if there was a relationship with ant abundance at this field site. Leaf litter was collected along twenty-four, 100m transects during May, July, and September of 2015 and placed into Berlese funnels for ant extraction. A total of 14 species were collected from 144 leaf litter samples. Estimates of species richness (Chao1: 14.0 species, ACE: 15.0 species) suggest there may be one additional species that was not sampled in my study; a species accumulation curve suggests similar findings. Diversity measures were relatively low with a Shannon exponential of 4.88 and Simpson reciprocal of 2.82. The leaf litter of E.A. Vaughn WMA was dominated by a single species, *Nylanderia faisonensis* (Forel 1922), which accounted for 57.3% of the total ant specimens collected. Neither leaf litter depth (R^2= 4E-5) nor mass (R^2= 9E-6) was found to have a substantial impact on ant abundance as a whole. When individual species were examined for relationships between ant abundance and leaf litter depth/mass, no significant values were found. Thus, we conclude that leaf litter does not predict ant abundance.

Key Words: bioindicator, Berlese funnel, North temperate forest, diversity, species richness, *Nylanderia faisonensis*

Environmental indicator taxa are sensitive to disturbance and can be used to indicate the health of a habitat. Indicator taxa provide an estimation of species variety, often in terms of the number of different species in a given area that can be monitored and used to gain an overview of changing ecosystem trends (Andersen 1997; McGeoch 1998; Longino et al. 2002). Indicator taxa can also be used to locate areas of high biodiversity (biodiversity indicators) or estimate the impact from a specific one time disturbance event (Caro and O'Doherty 1999). High levels of diversity in a given region act as a safeguard against the collapse of an ecosystem; the loss of a single species may be filled by another with a similar niche or by other organisms with similar lifestyles (Ribas et al. 2003). Biodiversity surveys rely on indicator taxa for conservation planning, developing management plans, studying the impact of agricultural practices on habitat, and providing justification for the existence of protected areas (Spector and Forsyth 1998; Caro and O'Doherty 1999).

Ants are often cited as excellent bioindicator taxa due to their great numbers, species variety, and ability to occupy the vast majority of terrestrial habitats (Andersen 1997; Bestelmeyer and Wiens 2001; Martelli et al. 2004; Underwood and Fisher 2006) which in turn allows researchers to study a number of different aspects of ecosystems and the species that inhabit them (Andersen 1997; Steiner et al. 2005). Ant are also known to react quickly to changes in their habitat, with decreased diversity levels after an ecosystem is disturbed and increasing as the habitat recovers (Longino et al. 2002; Martelli et al. 2004; McGlynn et al. 2009). Leaf litter ants, in particular, are useful in predicting plant diversity (Resende et al. 2013). While ants are not the only family of insects that can be used as a bioindicators, their large biomass make large scale changes to their numbers easy to observe (Ivanov and Keiper 2009).

Some authors suggest that minute changes may have an impact on populations utilizing microhabitats, thus the loss of ant species in a fragmented habitat could have a profound impact on the ecosystem as a whole (Crist 2009). The role of leaf litter mass in predicting ant abundance has been mixed, with some studies finding a correlation between increased amounts of leaf litter and ant abundance (Lopes and Vasconcelos 2008; McGlynn et al. 2009) while others found no support (Lynch et al. 1988; Wilkie et al. 2010) or only very weak support (Kaspari 1996a). Regardless of human interaction, approximately 60% of known ant species have been reported living in leaf litter, suggesting it is a critical habitat to examine (Silvestre et al. 2012).

The majority of ant studies take place in the tropics despite their widespread distribution (Ellison et al. 2007), leading to a lack in ant diversity data elsewhere (Lynch 1981, Lynch et al. 1988). One reason for this may be due to the difficulty in the collection and curation of the vast numbers required to survey a field site (Wilkie et al. 2010). Another complication is that ant collection methodology is varied with no one methodology being ideal in all situations (Agosti et al. 2000; Ivanov and Keiper 2009; Lopes and Vasconcelos 2008). Commonly used methods to examine ant biodiversity are often time intensive, and require knowledge of the terrain type to efficiently collect data (Frye et al. 2014). Thus, finding the ideal collection method for a particular habitat type is of utmost importance when undertaking a study (Vele et al. 2009). Many studies however use multiple sampling methods because of the lack of consensus about the best way to sample ants (Agosti et al. 2000; King and Porter 2005).

While disagreement regarding sampling techniques is common, there is agreement that more research needs to be performed, especially outside of the tropics. Within many regions of the United States, basic information such as species range is incomplete, even for

common ant species. Partial state inventories have been conducted in Florida (King and Porter 2005), Oklahoma (Albrecht and Gotelli 2001), Tennessee (Sanders et al. 2007b), New York (Ellison et al. 2007), Oregon (Ratchford et al. 2005; Sanders et al 2007a) and California (Ratchford et al. 2005; Sanders et al 2007a). The only state to have completed what is considered a full inventory of all ants in its borders is Ohio (Coovert 2005; Ellison et al. 2007).

In Maryland ant studies and investigation of ant ecology, have mostly focused on species richness and seasonality in habitats west of the Chesapeake Bay (Lynch et al. 1980, Lynch 1981, Lynch et al. 1988) or interactions between different ant species (Fellers 1987, Fellers 1989). On Maryland's Eastern Shore, only two biodiversity studies have been conducted. Frye and Frye (2012) hand collected ants from trees in the ridge woodlands and inland sand dune communities of Worcester County, MD. Thirty-five species of ants were sampled from 241 trees. Frye et al. (2014) on the other hand, used pitfall traps and leaf litter samples to examine ant species richness and diversity. They reported 67 species of ants from 25 genera. Both of these studies, however, were restricted to xeric habitats characterized by dry conditions with low moisture levels (Frye et al. 2014).

The objective of my research was to determine the ant species diversity and richness of leaf litter ants in the E.A. Vaughn Wildlife Management Area (WMA), a temperate forest in Worcester Co., Maryland. Additionally, leaf litter depth and dry leaf litter mass were measured to determine if there was a correlation with ant abundance. Seasonality was not a primary focus of this study though some natural history data is provided.

METHODS AND MATERIALS

Field site.

I collected ants in a six-hectare forest of the E.A. Vaughn Wildlife Management Area (38° 4' 49"N 75° 23' 38"W) located in lower Worcester County (Girdletree, Maryland). The E.A. Vaughn WMA consists of 1064 hectares of forest (Maryland Department of Natural Resources 2016b) surrounded by agriculture on all sides. This feature is often typical of forested areas in the Mid-Atlantic region (Crist 2009). The study site was composed of both dry uplands and low-lying wetlands in a 60,000m^2 area that is periodically flooded by the Maryland Department of Natural Resources during the fall as part of a "green tree reservoir" (Maryland Department of Natural Resources 2016a). The forest contained a variety of trees, including loblolly pine (*Pinus taeda*), Virginia pine (*Pinus virginiana*), southern red oak (*Quercus falcate*), American holly (*Ilex opaca*), swamp white oak (*Quercus bicolor*), white ash (*Fraxinus Americana*) and green ash (*F. pennsylvanica*.)

Figure 1.1 View of twenty-four, 100 m transect lines set in E.A. Vaughn Wildlife Management Area. The two halves of the sampling area are separated by the blue and yellow line.

Collections.

I sampled leaf litter ants once a week during May, July, and September of 2015 (12 weeks total). One advantage of using leaf litter collections is that they may sample species of upper soil, subterranean ants that cannot be caught from other methods (Longino et al. 2002). I used twenty-four, 100 m transects (Figure 1.1), set 25 m apart, in order to reduce the likelihood of sampling from the same colonies more than once. To further prevent sampling from nearby transects during the same week, I divided the study site into two sections (Figure 1.1) and two transects, one from each side, were chosen using a random number generator. This allowed us to reduce interference from sampling adjacent transects during a sampling week and allowed for more random sampling of ants throughout the study site. Additionally, this random sampling method reduced the chance that data trends could be attributed to differences along the forest site as well as reduction in error due to tree density and moisture content (Delsinne and Arias-Penna 2012).

I conducted research between 1000 and 1600, when ants are known to be most active, using the methods of Frye et al. (2014). Using a 0.25 m^2 quadrat made of PVC piping, I sampled leaf litter every 20 m (6 samples per transect/ 12 total) along each transect. Leaf litter was collected into 14.1L (42.4 x 30.4 x 16.7 cm) Tupperware containers and taken back to the Salisbury University greenhouse. I then placed them into Berlese funnels (Figure 1.2) made of five gallon buckets and a tractor funnel (diameter = 25 cm) set inside each. Mesh screen with 0.64 x 0.64 cm holes were set inside the funnel to allow ants to travel through, while preventing leaf debris from moving down the funnel (Figure 1.2). I positioned 65-watt bulbs above the litter for 48 to 96 h until the leaf litter was dry. Whirlpak bags filled with 95% ethanol were attached to the bottom of the funnel to collect insects as they fell through the mesh.

Figure 1.2. Example of a Berlese funnel (n = 12) used to sort ants from leaf litter debris.

I sorted, curated, and identified worker ants using a variety of resources, including the Ants of Ohio (Hymenoptera: Formicidae) (Coovert 2005), and A Field Guide to the Ants of New England (Ellison et al. 2012). Queens and male ants were excluded from the analysis because species keys do not exist for most species. I sent species I was unable to identify to Jennifer Selfridge (Maryland Department of Natural Resources) and Tim Foard (Maryland Entomological Society). Reference specimens will be stored in the Salisbury University Price Entomological Collection (Salisbury, MD, USA), and the Towson University Biodiversity Center (Towson, MD, USA).

I determined the amount of leaf litter at each location using two methods: 1) In the field I measured the depth of leaf litter using a ruler at each corner of the 0.25 m^2 quadrat as well as in the center and then calculated the average of the five points, similar to Kaspari (1996a, 1996b); 2) In the lab I weighed the dry leaf litter taken from each Berlese sample after ants had been extracted.

Data analysis.

Using EstimateS 9.1 software, I calculated Chao1 and ACE (Abundance Based Coverage Estimator), two non-parametric species richness estimators for leaf litter ants (Chao 1984; Chao and Lee 1992). Chao1 uses a ratio of singletons (species captured once) over doubletons (species captured twice) to gain a measurement of species richness, while ACE takes into account species captured ten or fewer times (Hughes et al. 2001; Magurran 2004). I determined species diversity using the Shannon exponential mean ($e^{H'}$), derived from the Shannon's diversity index ($H' = - \Sigma p_i \ln(p_i)$) where p_i is the proportion individuals belonging to the ith species, which provides the number of species found in the sample had all species been equally common (Whittaker 1972). Additionally, I used the Simpson (inverse) diversity index ($1/D$), where $D = 1 – ((\Sigma n(n-1))/(N(N-1)))$ where n is the number of individuals of a particular species and N is the total number of individuals of all species, which captures the variance of the species abundance distribution (Magurran 2004). I created scatterplots of ant abundance compared to leaf litter mass and leaf litter depth in Excel to look for correlations between these variables.

RESULTS

I collected 14 species (4208 individuals) of leaf litter ants (Tables 1.1 and 1.2). All 14 species were collected during May (1398 individuals), 12 during July (1742 individuals), and

just ten during September (1068 individuals). Variation in abundance was observed for individual transects, with a range of 71 – 534 ants (with the exception of transect 16 where only 5 ants were collected).

Estimates of species richness suggest that only one additional species may be found in the leaf litter (Chao1 = 14 and ACE = 15). The species accumulation curve support these findings, suggesting that just one or two more species may be collected using this method (Figure 1.3). The majority of species were captured with the first 2000 specimens sampled (Figure 1.3). Diversity indices were found to be low, with a Shannon exponential (e^H) of 4.88 and Simpson's reciprocal (1/D) of 2.82 (Table 1.2). Fifty-seven percent of the individuals collected were *Nylanderia faisonensis* (Forel 1922), which was found within all twenty-four transects. The rank abundance curve corroborates this finding that *N. faisonensis* dominates the E.A. Vaughn ant community (Figure 1.4).

Table 1.1. Leaf litter ants collected from E.A. Vaughn WMA during May, July and September of 2015.

Species Name	May Transects								Total	July Transects								Total	September Transects								Total	Species Total
	3	6	7	9	13	17	18	22		1	8	10	12	15	20	21	24		2	4	5	11	14	16	19	23		
Aphaenogaster fulva Roger							3	2	5	17	27	9	8	8	18	37	30	154	6	10	6	18	23	1	9	12	85	244
Aphaenogaster rudis Enzmann	10	18	8	6	7	15	13	6	83	4		2				2	5	13	1	1	1	1	9	1	1	1	16	112
Crematogaster cerasi (Fitch)				1					1				1					1									0	2
Formica subsericea Say			1						1									0									0	1
Lasius alienus (Foerster)	3	54			25	114	19	26	241	17	25	8	7	10	48	1		116	1	7							8	365
Lasius umbratus (Nylander)							2		2	1			197	5				203					1				1	206
Myrmecina americana Emery	6	9	1	1		9	1	1	28	13	7	4	4	6	12	4	5	55	13		18	2	9	1	5	27	75	158
Nylanderia faisonensis (Forel)	80	104	39	245	15	155	28	118	784	79	48	48	127	55	414	8	98	877	135	137	11	146	149	1	136	35	750	2411
Ponera pennsylvanica Buckley	14	35	21	36	47	4	7	21	185	21	8	5	12	13	24	22	31	136	3	3	14	13	11		2	55	101	422
Stigmatomma pallipes (Haldeman)		2		1					3						5	3	5	13				1	2		2		5	21
Strumigenys dietrichi Smith, M.R.					1				1	15	40		2				8	65				4			1	7	12	78
Solenopsis carolinensis Forel		30						14	44									0									0	44
Solenopsis molesta (Say)	2			4		2	3		11	14	4	39	14	3	4	3	6	87									0	98
Temnothorax curvispinosus (Mayr)	2	4	1	2					9	1	10	1			8	1	1	22		1	8	3		1	2		15	46
TOTAL	117	256	71	295	94	300	77	188	1398	182	169	116	371	100	534	81	189	1742	159	159	58	188	204	5	158	137	1068	4208

Table 1.2. Species richness and abundance of leaf litter ants collected from E.A. Vaughn WMA during May, July, and September of 2015.

Species Richness	14
Abundance	4208
Estimated Species Richness	
Chao 1	14
ACE	15
Species Diversity	
Shannon exponential mean (e^H)	4.88
Simpson's index (1/D)	2.82
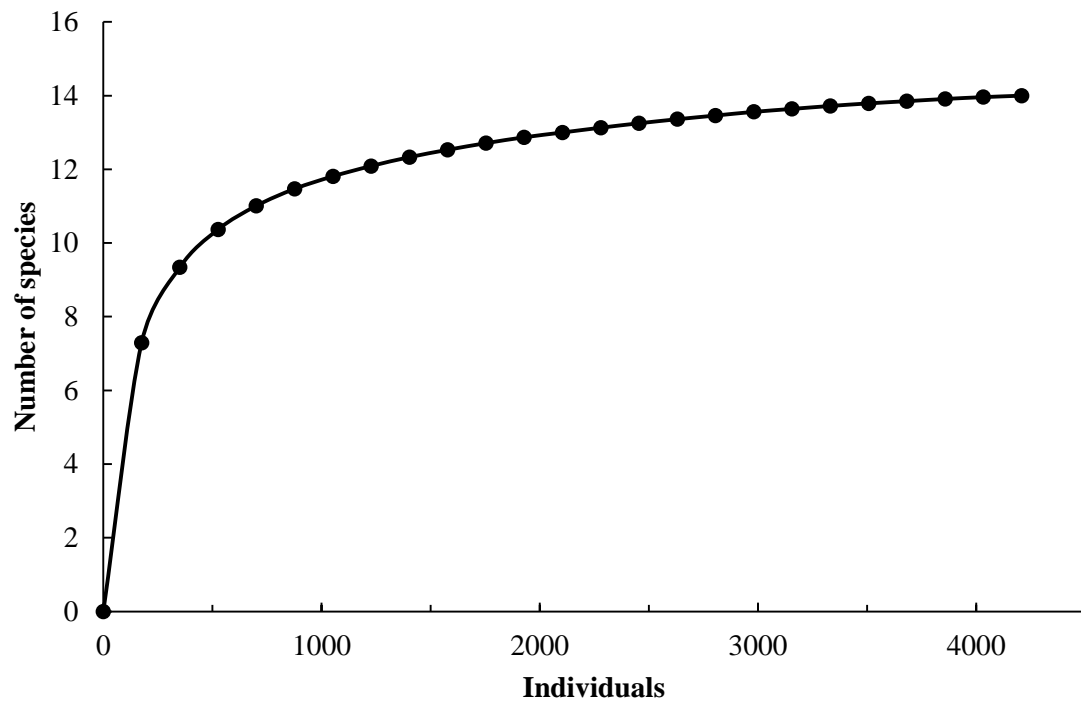
Figure 1.3. Species accumulation curve of leaf litter ants collected at the E.A. Vaughn WMA during May, July and September of 2015.
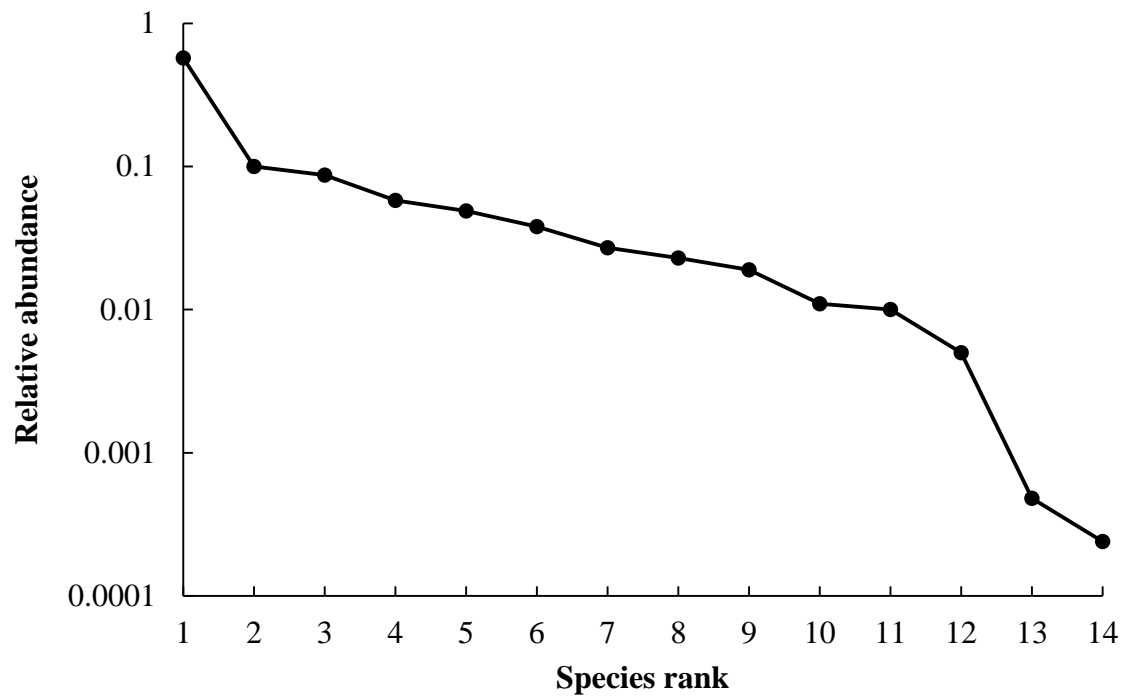
Figure 1.4. Rank abundance curve of leaf litter ants collected at the E.A. Vaughn WMA during May, July and September of 2015.

Leaf litter depth and mass.

Litter depth and dry leaf litter mass did not have an impact on the total abundance of ants in a given 0.25m^2 quadrant (R^2=9E-6 for mass: Figure 1.5, R^2=4E-5 for depth: Figure 1.6). The number of ants collected ranged from 0 to 202 specimens per quadrant with leaf litter mass centered around 237.38g (Figure 1.5) and leaf litter depth centered around 3.13cm (Figure 1.6).
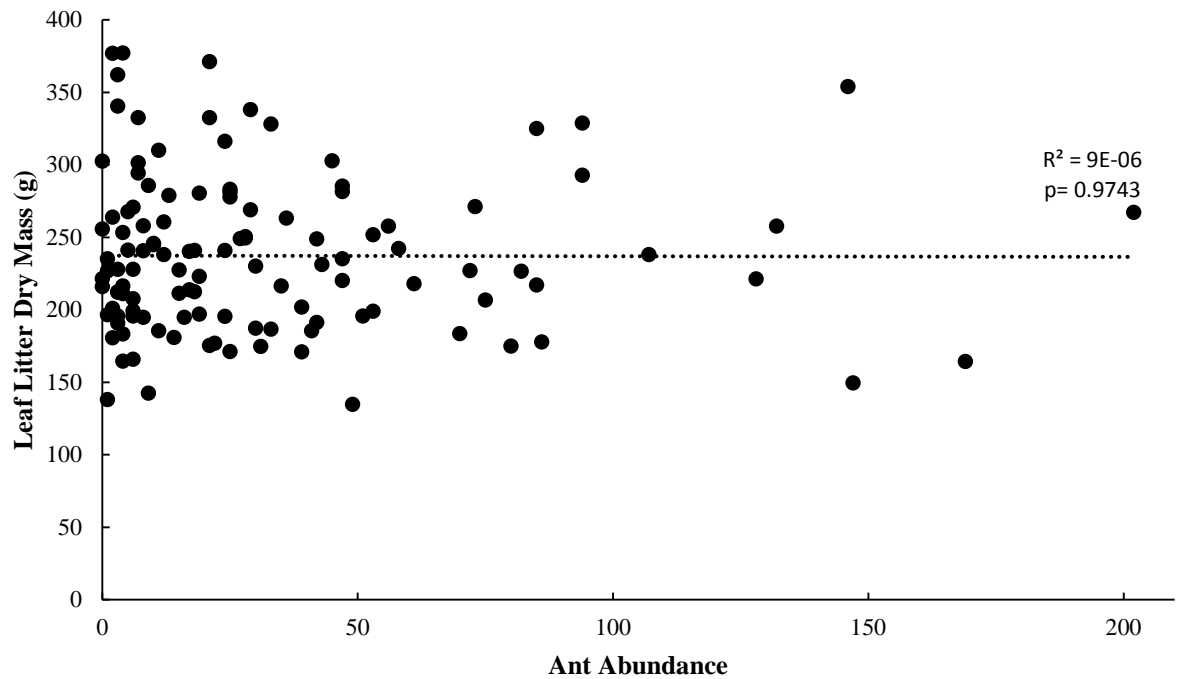
Figure 1.5. Change in total ant abundance with increasing leaf litter mass (grams) for 144 samples (R^2= 9E-6).
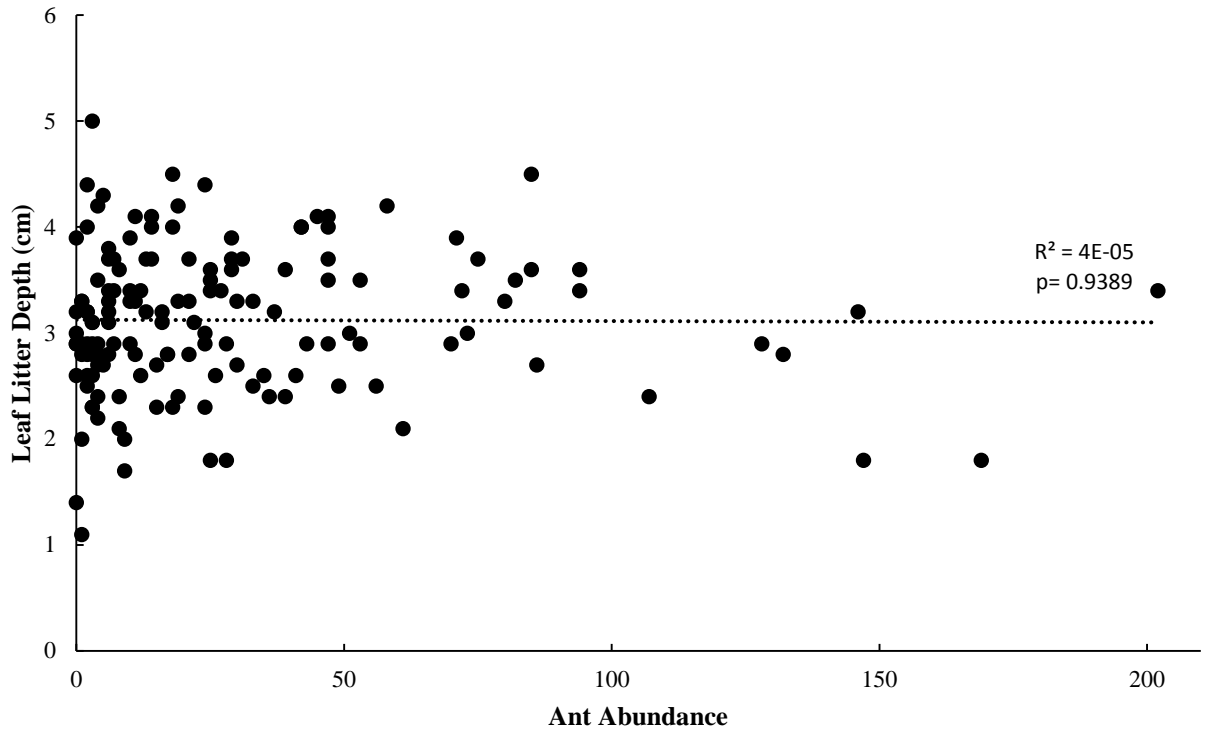
Figure 1.6. Change in total ant abundance with increasing leaf litter depth (cm) for 144 samples (R^2=4E-5).

Most species were collected during each sampling month, with some exceptions. *Lasius alienus* (Foerster) and *Solenopsis molesta* (Say) are most prevalent during May and July. Similar to Coovert (2005) findings, *Aphaenogaster rudis* Enzmann is most abundant during May while *Aphaenogaster fulva* Roger is most abundant during July and September. *Myrmecina americana* Emery, *Nylanderia faisonensis* (Forel), and *Ponera pennsylvanica* Buckley were collected from all or nearly all transects. Although more individual specimens

were collected overall during July when temperatures were highest; there was no correlation seen with temperature and ant abundance $R^2 = 0.00243$.

DISCUSSION

In the E.A. Vaughn Wildlife Management Area, I collected 14 species of leaf litter ants (4,208 individuals) from a 60,000m^2 area during May, July, and September of 2015. Compared to other temperate leaf litter ant studies conducted in the Mid-Atlantic, my results were similar in species richness and abundance (Lynch 1981; Lynch et al. 1988; Ellison et al. 2007). In Edgewater, Maryland, a coastal plain region, Lynch (1981) examined the seasonal, successional, and vertical segregation of ants among three types of habitat (old field, young forest, and mature forest). Although he collected throughout the entire year (July 1975 to June 1976) and used 450 leaf litter samples, he reported the maximum number of species per 10 litter samples was the same (n = 14) from all three habitats. Lynch et al. (1988) collected 4124 ant specimens represented by 22 species in a mature deciduous forest of the Maryland coastal plains. Ellison et al. (2007) reported 21 species in Black Rock Forest (Cornwall, NY) when using sieved litter samples.

More recent studies conducted in inland dune habitats of Worcester County reported 52 species (16,873 individuals) from 60 samples (3 liters of material per sample) of leaf litter during 2008 (Frye et al. 2014). This high species richness may be attributed to a number of species that are restricted to xeric habitats, as well as those that are often found in anthropogenic environments, such as road shoulders, pastures, lawns, and agricultural fields, as long as the soil is sandy (Frye et al. 2014).

Altered or fragmented habitats with low diversity tend to be dominated by a small number of extremely abundant generalist species (Resende et al. 2013). At the E.A. Vaughn

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WMA the most notable of these species appeared to be *N. faisonensis.* Lynch et al. (1988) also reported *N. faisonensis* (reported as *Paratrechina faisonensis*) to be their most abundant species, making up 61.2% of the total individuals collected. This species is found throughout the eastern U.S., from New Jersey to Florida, west to Ohio and throughout the southeast (Trager 1984; Coovert 2005) and have been reported as one of the most common ant species in the Mid-Atlantic (Coovert 2005; Kjar 2009), especially within deciduous forests or mixed deciduous/pine forests (Trager 1984).

Similar to the findings of Lynch et al. (1988), *Ponera pennsylvanica* Buckley was the second most abundant species found in my study. This species was present in small numbers in over half of my collection points (78 out of 144) and found within 23 of 24 transects. This species forms small colonies under rocks, within rotting wood and is rarely seen on the surface (Coovert 2005). Additional species that were sampled in high abundance (>100 specimens) include, *L. alienus, A. fulva* Roger, and *A. rudis*, and *Lasius umbratus* (Nylander), where the latter was only abundant within one collection point (196 individuals of the 206 collected). *Aphaenogaster fulva* has been found to be one of the most common ant species in the region along with *N. faisonensis* (Kjar 2009).

Competition on both a broad and local level plays a large role in the species diversity of ants within a given location (Fellers 1987, Fellers 1989; Sanders et al. 2007a). In habitats where biotic and abiotic factors vary greatly, such as edge habitats between forest and agricultural field, invasive species are more likely to lower the diversity of native species through competition (Crist 2009). In fragmented forests where much of the land has been converted to agricultural fields, like that of the Mid-Atlantic region (Crist 2009), both ant species richness and overall abundance have declined (Fayle et al. 2010). This decline can often have an effect on other taxa due to their importance in soil modification and mutualist

behaviors with other species (Crist 2009). In cases such as these, an introduced species or habitat disturbance may upset this delicate balance, causing a cascade of species loss, ultimately leading to ecosystem collapse (Gullan and Cranston 2014). These habitats are more likely to survive ecological upset due to human expansion and climate effects (Resende et al. 2013).

Although seasonality and temporal patterns were not a goal of this research, some general patterns were observed both here and in other Maryland studies west of the Chesapeake Bay. The two closely related species, *A. rudis*, and *A. fulva* appear to be separated temporally with *A. rudis* collected most during May while most *A. fulva* were collected during July and September. According to Fellers (1987), *A. rudis* was considered a "subordinate ant" when in the presence of many other species, including *L. alienus*; *A. rudis* reduced their feeding time when it encountered *L. alienus*. Both species of *Aphaenogaster* are common throughout the eastern United States and are found within similar habitats, mainly forests and forest edge zones (Coovert 2005).

Though the greatest abundance of ants was found in July (1,742 individuals), there is no evidence to suggest that ants prefer warmer temperatures according to my data ($R^2 =$ 0.0024). It is possible that seasonal variation and collection period could have affected my sampling and led to biases in collecting certain species over others. The abundance of ants collected during July was influenced by two species, *N. faisonensis* and *L. umbratus,* which inflated the overall count of ants for that month. It is likely that a large number of ants collected during July came from nests and not from general foraging habits. While higher July temperatures did not correspond to more ants, neither did it cause a large drop in ant abundance as may have been predicted for common generalist species (Lynch et al. 1980, Lynch et al. 1988).

The effect of the weather (precipitation) on ant activity is another factor to consider when examining my data. Though no collections were made during a period of precipitation, according to U.S. Climate Data via NOAA's 1981-2010 climate normal, the average annual rainfall at the site was 46.1 inches which is only slightly greater than Maryland's 44.5 inches per year. In the tropics, Levings and Windsor (1984) reported increased arthropod activity during periods of precipitation, which may have ramifications for ant species richness and abundance. In my research, I randomized the transects to minimize the effect that a particularly wet or dry week may have on the overall collection data.

One downside to leaf litter sampling is the difficulty in capturing ants active outside the collection time frame. Some temperate studies have found greater overall species richness during warmer periods of the year (Sanders et al. 2007b) such as during my July sampling period. However, I did not detect *Prenolepis impairis* (Say 1836) despite having been reported from a number of Maryland studies (Lynch et al. 1980, Lynch 1981, Lynch et al 1988; Fellers 1987, Fellers 1989; Frye and Frye 2012, Frye et al. 2014). Lynch (1981) suggests that this species is less active during warmer time periods when temperatures were high (Lynch 1981).

Research conducted by Lopes and Vasconcelos (2008) in the Brazilian Cerrado and McGlynn et al. (2009) in a Costa Rica forest suggest that leaf litter mass plays a key role in determining ant abundance and species richness. These authors found that ant abundances increased with increasing litter mass. Studies of this type however are not unanimous in their findings. Kaspari (1996a) and Wilkie et al. (2010) reported either no association between ant abundance and leaf litter mass or only a very weak positive connection (in the form of nest site abundance).

My findings suggest that neither leaf litter depth nor mass had an effect on the abundance of leaf litter ants in E.A. Vaughn WMA. This was true both for the collection as a

whole and for individual abundant species such as *N. faisonensis*, *L. alienus*, *P. pennsylvanica*, and *A. fulva* where leaf litter's relationship to abundance was not seen (highest R = 0.14 for *A. fulva* involving leaf litter depth). In general, the composition of trees in terms of density, size, and type only varied slightly throughout my study site. Furthermore, the overall leaf litter mass and depth did not change a great deal throughout, ranging from 134.7 to 377.1 grams in mass and 1.1 to 5.0 cm in thickness. Much of the leaf litter studies dealing with ants have been conducted in the tropics (Ellison et al. 2007), and thus it is possible that a relationship between leaf litter and ant abundance exists in those habitats, but is absent in cooler climates where ants are less abundant overall. Furthermore, quadrants dominated by deciduous leaves may have been different than those dominated by conifer needles, yet assigning a quantitative value to the composition of the leaf litter would be difficult to do and was not performed during this study due to time limits and resources. The amount of leaf litter taken at each point is also a consideration. Many leaf litter studies use the A.L.L. protocol (Ants of the Leaf Litter), which suggests taking 1 m^2 leaf litter samples and using Winkler bags to process (Kaspari 1996b; Agosti et al. 2000; Fayle et al. 2010; McGlynn et al. 2009). These methods have been recommended for studies in tropical environments due to feasibility and resources. Here, I chose to take 0.25 m^2 samples of leaf litter and use Berlese Funnels for processing following methods of Levings and Windsor (1984). Although patchiness in distributions is inherit in many organisms, ants included, (Kaspari 1996a, Kaspari 1996b), smaller sampling sizes allowed us to take more samples in a small temperate forest. Feasibility of processing ants was a prominent concern in my design plan.

One downfall to using leaf litter collections is the likelihood of collecting a portion of a colony, rather an even distribution of nearby ants (Gotelli et al. 2010). This often makes it difficult to gain a true representation of the ant species diversity as some ant colonies can be

quite large while the number of reproductives present are the same as small colonies (Schlick-Steiner et al 2006). For this reason, I used transects of 100m and sampled from as many as would fit into my forest area. Another disadvantage to leaf litter sampling is a tendency to miss large bodied ants (Agosti et al. 2000) or those that are primarily nocturnal (Martelli et al. 2004). This was seen in my project as no *Camponotus* individuals were collected from the leaf litter.

One of the most difficult hurdles to overcome when considering ants as biodiversity indicators is cohesion in collection methods (Wilkie et al. 2010). Many have followed the ALL (Ants of the Leaf Litter) protocol using one or two methods that have been modified for a particular region, terrain and ecosystem, and most importantly available resources (Lopes and Vasconcelos 2008; Wilkie et al. 2010; Frye et al. 2014). Data collected however, sometimes provides little information to determine estimated species diversity due to individual workers collected being less indicative of true diversity than the ant colonies collected from as a whole (Gotelli et al. 2010). Low species richness, available taxonomic keys, and local myrmecologists allowed us to thoroughly sample the leaf litter in a 60,000 square meter area. This is not to say that these methods could not be employed in the tropics, yet the increase in species richness and abundance would considerably increase time spent in the lab with ant curation and identification as biodiversity sampling can be time-consuming and labor intensive (Gotelli et al. 2010).

A large number of factors play a role in determining ant diversity in any given environment. Disturbance events (Resende et al. 2013), edge habitats (Crist 2009), location (Ellison et al. 2007), interactions with other species (Feller 1987, Feller 1989), and the composition of the habitat, be it an old growth forest, a young forest, a field, or somewhere else (Lynch 1981). Information regarding ant diversity and distribution in many areas is

lacking, however, generally due to the sampling effort required to fully inventory a given area, and the high diversity of ant species, rare species in particular (Gotelli et al 2010). Because most states lack a full inventory, range distribution, presence/absence data, leaf litter preference, temperature ranges, and more is lacking for many species (Ellison et al 2007). This research is intended to serve as baseline data for monitoring the stability and health of a temperate deciduous-pine forest in the Mid-Atlantic region. Future research using additional methods according to the ALL protocol (Agosti et al. 2000) would be suggested in order to gain a better understanding of ant communities in the Mid-Atlantic.

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CHAPTER 2: Ant foraging preference in a mixed hardwood and coniferous forest

Chapter Abstract

The foraging preference of ants on deciduous and coniferous trees was examined in the E.A. Vaughn Wildlife Management Area (WMA), a coastal temperate forest of Worcester Co., Maryland. Ants were sampled using aspirators and hand collection methods from 261 trees during May, July and September of 2015. The aim of my research was to determine if ants are preferentially associated with deciduous (oaks and maples) or coniferous trees. I collected a total of 679 ants comprising 21 species, 19 on deciduous trees and 17 on conifers. Estimates of species richness (Chao1: 21.25, ACE: 22.38) suggest there may be one or two additional species that were not sampled. Using a two-tailed t-test, I found a significantly higher abundance of ants on deciduous than coniferous trees, 446 and 233 respectively (p < 0.05) though species richness was not found to vary significantly. A second goal was to examine whether ants have a preference for foraging on small, medium or large trees for both types, deciduous and coniferous. Large deciduous trees were found to have higher abundances of ants than medium or small. *Aphaenogaster rudis* (Emery 1895) and *Aphaenogaster fulva* (Roger1863a) were the most abundant ants collected. This research contributes to the ongoing bioinventory of E.A. Vaughn WMA.

Key Words: Foraging behavior, arboreal, Formicidae, tree preference

Ants use trees and plants for multiple purposes, including nest sites (dying trees or rotting wood), foraging grounds, and in the case of some species, farming of other insects (Gullan and Cranston 2014). Ants are one of the most common arthropod groups utilizing tree trunk surfaces (Hanula and Franzreb 1998), suggesting these habitats are important for examination of their ecology and foraging behavior. In Ulyshen's (2011) review of arthropod vertical stratification in temperate deciduous forests, he suggested several factors that may contribute to the stratification of arthropods, including forest age, season, forest structure, plant community composition, resource availability, and inter-specific interactions.

Ants also have been shown to demonstrate preference for different types of trees as well as tree size. In an examination of the arboreal ants of pine forests in northern Florida, baits were used to determine species richness and abundance of ants attracted to different sized trees, including pines and hardwood (Tschinkel and Hess 1999). These authors reported that although the total species richness found among the different sized trees (small, medium, and large) was similar, the actual species present, and their abundance, did change with increasing tree diameters. Other studies reported similar findings in the black carpenter ant, with higher abundances on larger diameter trees than smaller ones (Verble and Stephen 2009a, b).

Disturbance and fragmentation also play a role in ant abundance and distribution. Tschinkel and Hess (1999) reported on the foraging preference of arboreal ants on longleaf pine verses slash pine with the effect of vegetation composition. On sites subjected to frequent prescribed fires, ground cover consisted mainly of wiregrass. On sites with less frequent burns, various woody shrubs dominated the ground cover. In general, their findings were mixed and dependent upon the presence of wiregrass; arboreal ants were more likely to occur on longleaf than slash pine, yet the proportion of arboreal ants increased with frequency of

wiregrass. It was suggested that the factors that favor wiregrass also favor arboreal species (Tschinkel and Hess 1999).

In temperate regions, deciduous trees tend to have smoother bark than coniferous trees such as pines (Nicolai 1993) and this has been proposed as a potential factor in arboreal arthropod preference. Several studies have found that trees with smooth bark contained a greater variety of invertebrates than trees with rough bark (Nicolai 1993; Nicolai 1995; Majer et al. 2003). Among different species of conifers, Nicolai (1993) noted that the smoother barked species had higher arthropod species richness than those with rougher bark (Nicolai 1993). This trend of species richness however is sometimes reversed in European forests (Nicolai 1993). For example, Menzel et al. (2004) reported rough bark trees to have a higher species richness of arthropods, namely beetles. Differences in bark structure between deciduous and coniferous trees are vast, and thus may play a role in both ant foraging behavior directly or indirectly through interaction with other insect species that use bark as a conduit from the forest floor to the canopy (Majer et al. 2003).

In a more recent study, Frye and Frye (2012) conducted research in 30 dune and ridge woodland sites of Worcester County, MD, approximately 15-20 kilometers from the E.A. Vaughn WMA. These authors compared the species composition of ants on shortleaf pine, loblolly pine, and various species of oaks to determine if there was a foraging preference for a certain type of tree. Of the 35 species collected, their findings suggest a significant difference in foraging preference of oaks over pines, yet no difference was found when comparing individual shortleaf and loblolly pines.

The primary objective of my research was to investigate the foraging preference of temperate arboreal ants in the E.A. Vaughn Wildlife Management Area (WMA). This area consists of a mixed deciduous/coniferous forest community, owned by the Maryland

Department of Natural Resources since 1943 and expanded to its current size with later acquisitions (Maryland Department of Natural Resources 2016a). Here I tested whether ants have a preference for foraging on deciduous or coniferous trees. I also measured the size of the tree to determine whether ant abundance increased with tree diameter (cm). This research was intended to provide baseline data regarding the foraging behavior of arboreal ants in this region of Maryland.

METHODS AND MATERIALS

Field site and collections.

My study site was a mixed deciduous/coniferous forest located in the E.A. Vaughn Wildlife Management Area, Worchester County, Maryland (38° 4' 49"N, 75° 23' 38"W). Using the 24-100m transects described in Chapter 1 (Figure 1.1), I collected arboreal foraging ants along each transect, every 20 m (6 points total) using aspirators (Figure 2.1) and hand collection methods. Hand collection or hand counting has been noted as an effective method of gathering ants (Ellison et al. 2007; Gotelli et al. 2010), due to its use in a wide variety of environments, the ease of use, and the low material cost (Agosti et al. 2000).

At each 20 m location point, the nearest coniferous and deciduous tree ranging in size from 50 to 250 cm in diameter was marked (with the exception of one 45 cm tree). Using methods of Frye and Frye (2012), I collected ants between 1000 and 1600 hours from each marked tree. I recorded tree diameters and observed each tree from the base of the trunk up to a height of 2 meters for fifteen minutes (Frye and Frye 2012).

Collected specimens were placed in vials with 95% ethyl alcohol for later identification. If ants were observed in a foraging line, only ten ants were collected in order to not over sample a single species. If a tree of appropriate size or type (deciduous/conifer) was

not present, it was noted as an absence. Thus collections were made from 144 deciduous trees and 123 coniferous trees.

Figure 2.1 Aspirator used to collect ants from tree surface.
Image courtesy of BioQuip Products.

Data analysis.

I used a Generalized Linear Model (GLM, SPSS 21.0.0) to compare ant abundances with tree type with tree circumference as a co-variate to determine if ant abundance varied between tree sizes and the interaction between tree type and tree size. If the interaction had a p > 0.25, I removed it from the model (Sokal and Rohlf 1995, pp. 654–662). Because we sampled both a conifer and a deciduous tree at each location, they were not completely spatially independent. So I also analyzed data using a paired *t*-test (SPSS 21.0.0) to determine if ant abundance differed on deciduous trees versus coniferous trees. I log-transformed (natural log) the data to achieve normality. I then investigated abundance of the most common species. Due to non-normal of this abundance data, Mann-Whitney U tests (Mann and Whitney, 1947) were performed on the most abundant species to investigate tree type preference.

Species richness estimators Chao1 (Chao 1984) and ACE (Chao and Lee 1992) were calculated in EstimateS 9.1 to estimate the number of ant species present on the tree trunks.

Diversity statistics (Shannon exponential mean and Simpson reciprocal index) were used to gauge overall ant diversity.

RESULTS

Collections.

I collected a total of 21 species of arboreal foraging ants, comprising 679 individuals, from 267 trees (144 deciduous and 123 coniferous) at E.A. Vaughn WMA (Table 2.1). Nineteen species (446 individuals) were found on deciduous trees and 17 species (233 individuals) were found on coniferous trees (Table 2.1). The interaction between tree size and tree type was removed from the model ($F_{1,(261)} = 0.588$, $p = 0.556$). Tree type and tree size both significantly affected ant abundance (Figure 2.2, Figure 2.3, Table 2.2). In the paired *t*-test, overall foraging ant abundance was significantly higher on deciduous than coniferous trees ($p = 0.016$).

Species richness estimates indicated 21.25 (Chao1) and 22.38 (ACE) species were present on the trees based on my collection methods (Table 2.3). Species evenness was higher on the trees than in the leaf litter at the E.A. Vaughn WMA with a Shannon exponential (e^H) of 10.49 and Simpson's reciprocal (1/D) of 7.57 (Table 2.3). Among the most common species collected, Mann-Whitney U tests showed a significant preference for deciduous trees in both *A. fulva* (p=0.005) and *A. rudis* (p=0.005) but not in *Lasius alienus* (p=0.319). A species rank index showing ant species evenness indicates that no one species dominates the trees of the E.A. Vaughn WMA (Figure 2.2).

Table 2.1 Ants collected from deciduous and coniferous trees in the E.A. Vaughn WMA, during May, July, and August.

Species Name	Deciduous				Coniferous			
	May	July	Sept.	Total	May	July	Sept.	Total
Aphaenogaster fulva Roger	34	66	16	116	7	34	0	41
Aphaenogaster rudis Enzmann	12	28	63	103	5	7	16	28
Camponotus castaneus (Latreille)	1	5	2	8	0	3	0	3
Camponotus chromaiodes Bolton	5	4	1	10	6	2	0	8
Camponotus nearcticus Emery	8	13	2	23	3	0	2	5
Camponotus pennsylvanicus (De Geer)	2	10	2	14	5	1	4	10
Camponotus subbarbatus Emery	0	1	0	1	0	1	0	1
Crematogaster ashmeadi Mayr	1	10	0	11	12	2	10	24
Crematogaster cerasi (Fitch)	0	1	0	1	2	1	1	4
Crematogaster pilosa Emery	16	0	0	16	0	0	2	2
Formica neogagates Viereck	0	0	0	0	0	2	0	2
Formica subsericea Say	22	10	0	32	0	0	0	0
Lasius alienus (Foerster)	32	20	2	54	1	35	13	49
Lasius umbratus (Nylander)	0	2	0	2	6	17	10	33
Myrmecina americana Emery	3	3	2	8	1	0	0	1
Nylanderia faisonensis (Forel)	5	12	15	32	1	17	2	20
Stigmatomma pallipes (Haldeman)	0	0	1	1	0	0	0	0
Temnothorax curvispinosus (Mayr)	3	0	1	4	1	0	0	1
Temnothorax longispinosus (Roger)	7	0	1	8	0	0	0	0
Temnothorax morpho spp.	0	2	0	2	0	0	0	0
Temnothorax schaumii (Roger)	0	0	0	0	1	0	0	1
Total	**151**	**187**	**108**	**446**	**50**	**122**	**60**	**233**

Table 2.2 Generalized Linear Model of ant abundance from deciduous and coniferous trees with size as a covariate.

Tests of Between-Subjects Effects

Dependent Variable: Abundance

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	173.808[a]	2	86.904	5.533	.004
Intercept	42.970	1	42.970	2.736	.099
Size	78.923	1	78.923	5.025	.026
Tree type	133.192	1	133.192	8.480	.004
Error	4146.529	264	15.707		
Total	6042.000	267			
Corrected Total	4320.337	266			

a. R Squared = .040 (Adjusted R Squared = .033)

Table 2.3 Species richness and abundance of arboreal ants collected during May, July, and September of 2015 from E.A. Vaughn WMA.

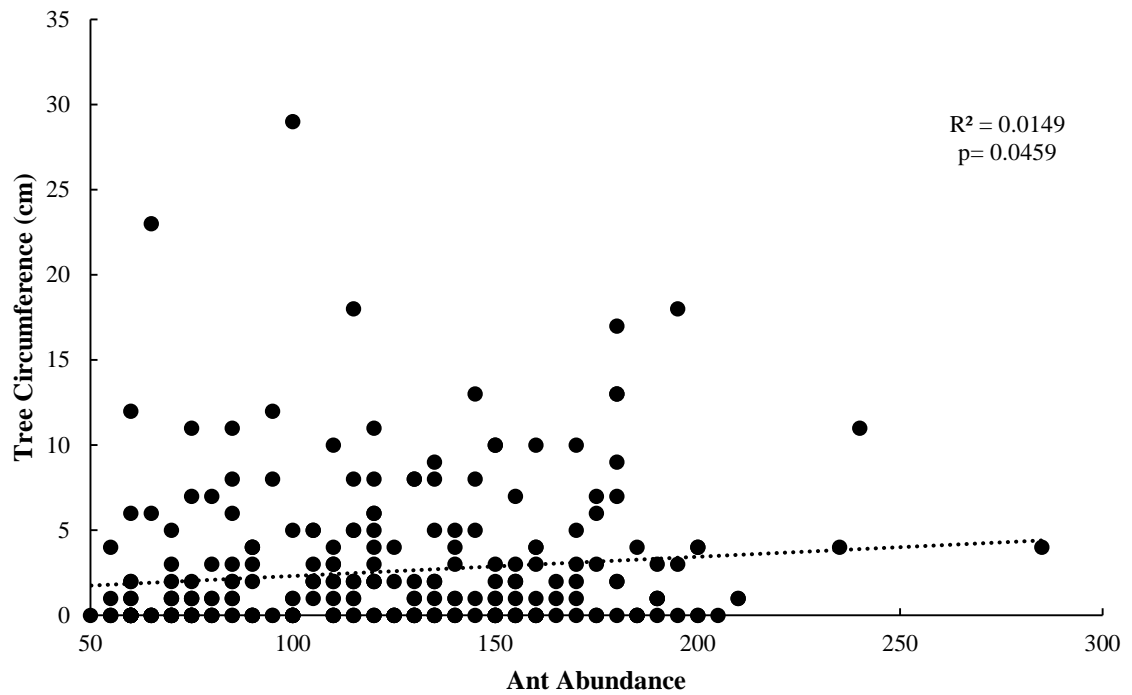|	
Species Richness	21
Abundance	679
Estimated Species Richness	
Chao1	21.25
ACE	22.38
Species Diversity	
Shannon exponential mean (e^H)	10.49
Simpson's reciprocal index 1/D	7.57

Figure 2.2 Overall abundance of ants collected from all trees as a function of tree size (R^2= 0.0149).
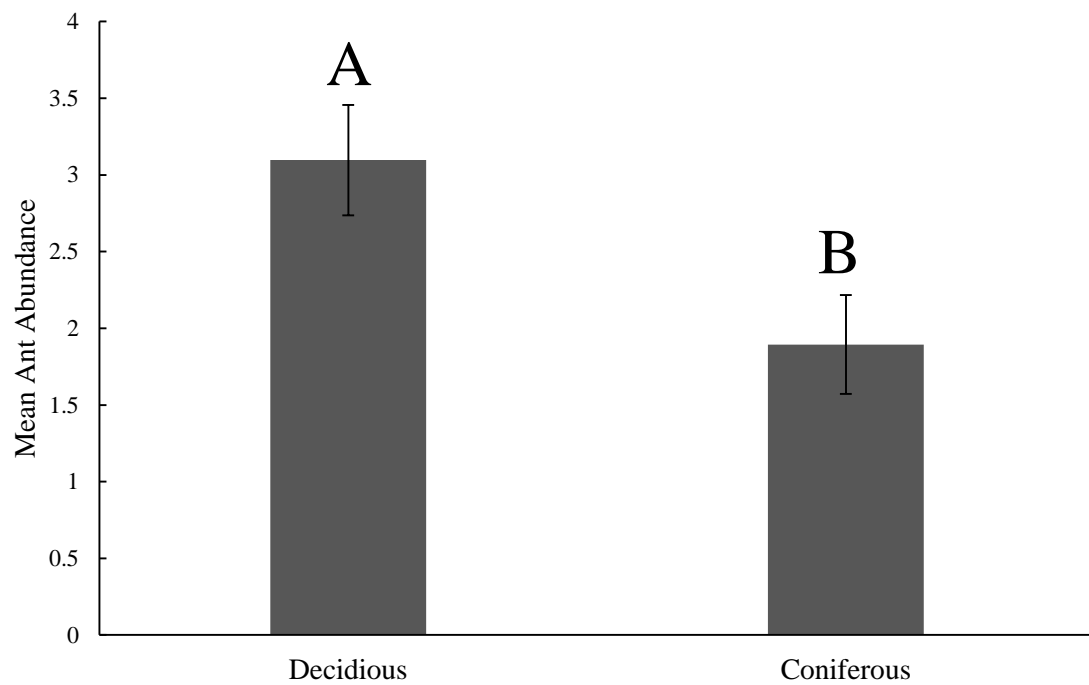

Figure 2.3 Difference in mean ant abundance per tree type (p= 0.003)
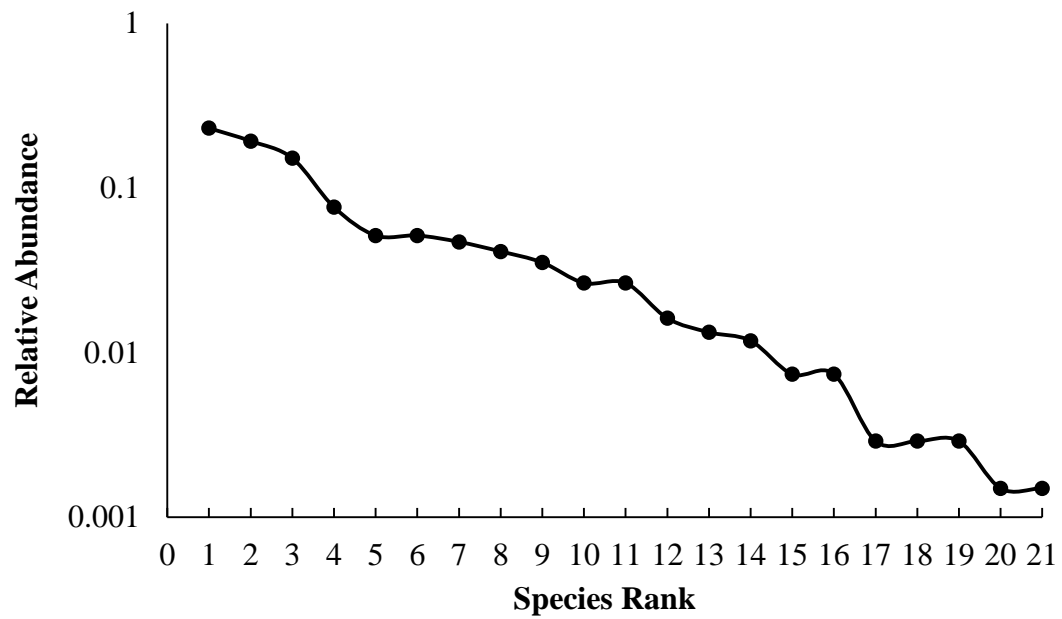
Figure 2.4 Rank abundance curve of arboreal ants collected at the E.A. Vaughn WMA during May, July and September of 2015.

DISCUSSION

Tree preference

In the E.A. Vaughn WMA, arboreal ants significantly preferred foraging on deciduous trees over coniferous trees. Reasons for this preference are likely multifaceted and cover a number of factors relating to various evolutionary history traits, though an overall arthropod preference for deciduous trees has been noted in other studies of mixed forests (Nicolai 1993). Although I was unable to determine individual species preference, Frye and Frye (2012) reported *Camponotus pennsylvanicus* significantly more frequently on oaks while *Crematogaster ashmeadi* occurred more frequently on pine trees.

The most abundant species sampled, include *A. fulva*, *A. rudis*, and *L. alienus*. Both *A. fulva* and *A. rudis* have wide ranges encompassing most of the eastern continental United States, and are frequently observed in forest and forest edge habitats. *Lasius alienus* is extremely common throughout most of North America as well as Europe (Coovert 2005). While all three species are common in this region, there are some notable differences between their life histories and interactions with each other. For example, Fellers (1987) found that *A. rudis* was a "subordinate" species of ant that reduced feeding time when in the presence of more dominant ants such as *L. alienus*. *Aphaenogaster fulva* is noted as being more common in old growth forests (Kjar 2009), so its relative abundance in the fractured E.A. Vaughn WMA with nearby agriculture and roads is surprising. Conversely, the presence of more generalist species such as *L. alienus* that are more likely to be found near human habitation is less surprising (Kjar 2009).

Tree size.

Tree size was considered as a potential variable in the abundance of arboreal foraging ants. It has been suggested that older trees with larger diameters provide an important niche for arthropods, including species that are not restricted to that habitat which are not found as often on younger, smaller trees (Ulyshen 2011). On deciduous trees, increased sculpturing of the bark seen in older, larger trees has contributed to a greater abundance of arthropods when compared to younger trees, though this was not seen in coniferous trees (Nicolai 1993). There was some evidence to suggest this was the case with my own data, where the difference in abundance depended on size.

Frye and Frye (2012) conducted a similar study to examine arboreal foraging ants in an inland dune and ridge woodland habitat just 20km away from my study site. These authors found no relationship between tree size and ant abundance. In an examination of arboreal ants in a Northern Florida pine forest, Tschinkel and Hess (1999) found that while species richness stayed the same within different tree size groups, the composition of the species changed from ground ants to a higher abundance of arboreal species as tree size increased (Tschinkel and Hess 1999). Also noted was an increase in the proportion of trees that contained ants among larger size categories as well as higher ant abundance as tree diameters increased even as species richness remained unchanged (Tschinkel and Hess 1999). Other studies have noted that older (and thus larger) trees are of greater importance to bark dwelling arthropods (Ulyshen 2011).

One factor that needs to be considered with this type of research is potential for rare or difficult to detect species to be overlooked. Difficulties in collecting very small ants and/or cryptic ant species are more prevalent in disturbed or fragmented habitats (Underwood and Fisher 2006). Hand collections can also be biased if the researcher encounters a food source such as sap on a tree that might draw ants from a distance (Frye and Frye 2012). Furthermore

the skill of researchers varies with experience and perception may very; thus expertise plays a large role in the efficiency of hand collection (Gotelli et al. 2010). In order to avoid this drawback, the same four people collected each week, and fifteen minutes was employed to allow ample time for sight of small and/or cryptic species.

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CHAPTER 3: Conclusions

Comparison to leaf litter study

The behavioral ecology of arboreal foraging ants is interesting when compared to the leaf litter studies. Overall, I found greater species richness via hand collection from trees than from leaf litter. Samples for both leaf litter and tree foraging ants were collected on the same dates, at the same time of day, and from the same transects of the site location, eliminating these factors as potential explanations for the differences seen. Of the five species of *Camponotus,* which I collected only from trees and not leaf litter, all are reported to live in forested habitats, and nest in either decaying wood, rotting logs, stumps, or in living trees (Coovert 2005; Ellison et al. 2012). Although they were relatively uncommon, they reportedly play an important role in the breakdown of wood in forested ecosystems (Fisher and Cover 2007).

Crematogaster ashmeadi was another species collected from only trees, yet this is not surprising as the genus is reported to contain many arboreal species (Holldobler and Wilson 1990). Furthermore, Tschinkel and Hess (1999) reported this species to be the most abundant arboreal ant sampled among a pine forest community; individuals were collected from 50% or more of all trees sampled. Frye and Frye (2012) reported similar findings; *C. ashmeadi* was the most abundant species sampled and it had a preference for loblolly pine, though it was also found on shortleaf pine and oak. In my study, *Crematogaster pilosa*, *Formica neogagates*, and several *Temnothorax* species were also found only on trees. Of these species, *Temnothorax curvispinosus* in Maryland has been noted to occur in large numbers from sweep samples of the lower arboreal canopy, suggesting that it frequently forages on trees (Lynch 1981). Lynch (1981) also noted that *L. alienus* was frequently found in both leaf litter and tree samples, similar to my data, with this species being the third most abundant sampled in each study.

Significance and Future Directions.

       This aspect of the overall temperate ant project at the E.A. Vaughn WMA shed light on tree preference and tree size among ants in a fractured temperate forest, reinforcing the idea that ants generally forage more often on deciduous trees than conifers. Thus if deciduous trees are preferred by many ant species, the increased planting and spread of coniferous trees may have implications for ant species richness in the region. Carabid beetle research suggests that mixed forests deciduous/conifers were important for the wellbeing of some species, and monocultures of conifers had a negative impact on diversity (Koivula et al. 1999). Studies in Europe suggest that replacing conifer monocultures with a polyculture of different tree species increases diversity for species that are not already on the brink (Felton et al. 2010). Maintenance of mixed forests or at the very least prevention of conifer monocultures is likely important for sustaining healthy ant populations.

       Different sections of the E.A. Vaughn WMA have their own habitat management units that focus on different goals in terms of promoting specific species in need of protection, like the Delmarva Fox Squirrel. This species requires a certain landscape and ecosystem for survival (Maryland Department of Natural Resources 2012). Investigation of the management practices at E.A. Vaughn WMA may provide valuable information regarding knowledge of the ant ecology in this region. The habitat variation of this site, combined with an overall mission of conservation of sensitive species, along with recreational use of their grounds, suggests there may be different ant communities in different areas of the land (Maryland Department of Natural Resources 2012).

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