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THE EFFECTS OF PRESCRIBED BURNING AND MICROHABITAT TYPE ON ANT (FORMICIDAE) FUNCTIONAL GROUPS AND A SURVEY OF ANTS IN THE DR. HOWARD REYNOLDS NATURE AREA (A MIXED GRASS PRAIRIE)

A Thesis Presented to the Graduate Faculty of the Fort Hays State University in Partial Fulfillment of the Requirements for the Degree of Master of Biology

by

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ABSTRACT

This project sought to understand how prescribed burning and microhabitat type impacts Kansas ant functional groups and also whether prescribed burning in different microhabitat types altered the burn's impact on those functional groups. The Dr. Howard Reynolds Nature Trail, located in Hays, Kansas, was burned in the spring of 2019. The area consists of 2 distinct habitat types: a dry, mixed-grass dominated uphill area and a moist, densely vegetated downhill area. Pitfall trapping was conducted during the summers of the year prior to the burn (2018) and the year following the burn (2019). 15 pitfall traps were spread across each microhabitat during each year. Ants collected were categorized into functional groups, which allowed a comparison of ants with certain ecologies in response to the treatments. It was found that prescribed burning did not significantly impact the population size or richness of any of the ant functional groups found in this project. What played more of a role in their population dynamics were the conditions of the habitat that ants were collected from, such as the environmental stress factors and the presence of ant competitors in each microhabitat type. The uphill area, which was an open area with less environmental stress in the form of vegetative shade, favored highly competitive functional groups. The downhill area, which had higher environmental stress, favored the stress-tolerant and hypogaeic functional groups.

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INTRODUCTION

THE IMPORTANCE OF ANTS IN THEIR ENVIRONMENT. Ants (Family Formicidae) are an integral part of the ecosystems that they inhabit. Though individually small in size, ants can constitute up to 20% of the total animal biomass in some areas (Schultz 2000). The combined impacts of multiple colonies of ants in an area can have significant effects on the health of that ecosystem.

In grasslands, ants perform important ecological roles that improve the productivity of their habitats (Johnson et al. 2015, Wills and Landis 2018). Ants serve as ecosystem engineers, meaning that they alter the habitats around them in ways that impact biotic and abiotic aspects of the ecosystem (Johnson et al. 2015, Subedi 2016). Ants aerate the soil with their tunneling, add organic matter into the soil with food and feces deposits, and rid the areas around them of detritus; all of which have indirect impacts on the plants and animals that they live amongst (Jouquet et al. 2006, Subedi 2016). They also have direct ecological impacts such as providing a food source for other animals, regulating the population sizes of some species through predation, dispersing seeds, and serving as pollinators (Folgarait 1998, Carson and Root 1999, Wills and Landis 2018).

Ants are also economically important. In particular, predaceous ants are common forms of biological pest control because of their affinity for preying upon other arthropods. Predatory ants have been used to protect mandarin orange trees since the 1700's, possibly making them one of the earliest forms of biological pest control (Way and Khoo 1992). Wood ants (*Formica* spp.) and carpenter ants (*Camponotus* spp.) have been shown to be effective in controlling gypsy moth populations by feeding on the moth's early larval instars (Way and Khoo 1992). More recently, their role in pest management has aided in organic farming practices by both protecting crops from pests by non-chemical means and increasing the health of the soil (Nielsen et al. 2018).

However, there are also many species of ants that are pests that can devastate crops and negatively impact the health of their habitats. Ants that cause the most damage are omnivorous or herbivorous (usually granivorous) (Reed and Landolt 2019). The red imported fire ant (*Solenopsis*

invicta) is both an urban and agricultural pest that is common in the southern United States. From an agricultural standpoint, these ants are major pests that have been reported to cause over \$90 million worth of crop damage annually in Texas alone (Lard et al. 2002). The tawny crazy ant (*Nylanderia fulva*) is a South American pest of homes and ecosystems that has recently encroached into the southern United States. *Nylanderia fulva* in residential areas have a tendency to be drawn to electrical currents which can lead to the short circuiting of electrical equipment (Wang et al. 2016). A common urban pest throughout the United States include little black ants (*Monomorium minimum*) which are very small ants that invade homes (Whitworth et al. 2009).

THE IMPORTANCE OF EVALUATING SPECIES RICHNESS AND POPULATION

SIZE. Analyzing species richness and population size is useful in evaluating the health of an ecosystem (Scott et al. 1987). Ants are useful in these evaluations because of their role as bioindicators (Dufrene and Legendre 1997, Stephens and Wagner 2006, Moranz et al. 2013). Studies often evaluate community structure elements such as species richness and abundance of ants to evaluate how environmental factors impact ant communities (Anderson et al. 1989, King et al. 1998, Panzer 2001, Farrenberg et al. 2006, Hartley et al. 2007, Houdeshell et al. 2011, González et al. 2018, Kaynaş et al. 2018, Triyogo et al. 2020). Evaluating species richness and population size can be used to detect changes in the environment (Murphy and Romanuk 2013) which can aid in the detection of ecological problems before major issues arise (Scott et al. 1987). Early detection of ecological problems can be more financially feasible than delaying management plans until after problems have already escalated (Scott et al. 1987).

IMPORTANCE OF USING ANT FUNCTIONAL GROUPS. Ant functional groups are species assemblages based on their phylogeny and ecology (Greenslade 1978, Andersen 1995). The ant functional group system (Figure 1) was proposed by Greenslade (1978) and was based on Grime's (1977) C-S-R model, which theorizes that there are 3 strategies that plants have developed to tolerate different levels of stress, disturbance, and competition. In Grime's model, species that tolerate high levels of stress, disturbance, and competition were referred to as the stress-tolerant, the ruderal, and the competitive species, respectively (Grime 1977). Similarly, ant species also respond differently to stress, disturbance,

and competition. Ant species that share close ancestry and have similar strategies for handling these 3 environmental factors form the basis of a functional group (Greenslade 1978, Andersen 1995, 1997). In the ant functional group system, climate specialists (CS) represent the stress-tolerant species, opportunists (OPP) represent the ruderal species, and dominant Dolichoderinae (DD) and (to a lesser extent) generalized Myrmicinae (GM) represent the competitive species (see Figure 1).

The use of functional groups has been used to better understand ant community composition patterns on a biogeographic scale (King et al. 1998). Classifying species into functional groups allows for more accurate predictions to be made based on how groups are known to respond to factors in their environment (Andersen 1995, Kind et al. 1998). The use of functional groups means that research findings from any given area can be used to make predictions in other areas around the globe (Andersen 1995). For example, one of the most widespread and abundant ant genera in Australia is *Iridomyrmex* (AntWiki 2020). Members of this genus are small ants that have generalized diets, are aggressive, and are often dominant over other ant species within their habitats (Andersen 1995, 1997, AntWiki 2020). *Forelius* spp. and *Iridomyrmex* spp. have ecological similarities, such as having generalized diets and being competitively dominant over other ants within their habitats, and are classified within the subfamily Dolichoderinae (Andersen 1997). It is because of these similarities that Andersen (1997) categorized these ants in the same functional group (=Dominant Dolichoderinae). This means that ecological studies on *Iridomyrmex* spp. in Australia can be used to inform decisions regarding *Forelius* spp. in North America and vice versa.

Placement of ants into functional groups is beneficial for recognizing ecological conditions within habitats (Greenslade 1978, Andersen 1995 and 1997, Stephens and Wagner 2006, Hoffman and James 2011, González et al. 2018, Triyogo et al. 2020). Ant species within functional groups will behave and respond similarly to those conditions because they have similar genetics, habitat requirements, and ecologies (Andersen 1995). This means that ant functional groups can be used as bioindicators to judge the health of ecosystems (Dufrene and Legendre 1997, Stephens and Wagner 2006, Moranz et al. 2013).

Many studies have used arthropods to better understand how fire affects ecosystems (Anderson et al. 1989, King et al. 1998, Panzer 2001, Farrenberg et al. 2006, Hartley et al. 2007, Houdeshell et al. 2011, Kaynaş et al. 2018). Studies that evaluate the effects of fire on ants usually focus on ants at the Family (Anderson et al. 1989, Hartley et al. 2007), genus (Kaynaş et al. 2018), or species (Houdeshell et al. 2011) level. Although there seems to be a rising interest in studying ant functional groups, there have been very few studies on ant functional groups in North America (Stephens and Wagner 2006, Fitzpatrick et al. 2011, Moranz et al. 2013). Each of these North American studies have struggled classifying North American ant species into functional groups. This is because North American ant species have been found to behave differently in different habitat types (Andersen 1997). More research needs to be done in North America in order to better our understanding of how North American ants respond to factors in their environments.

In this project, captured ant species were categorized into functional groups based on the North American ant functional group classification system developed by Alan N. Andersen (1997) (Table 1). This system was originally proposed by Greenslade (1978) for classifying Australian ant functional groups. Andersen expanded Greenslade's work (1995) and developed a North American ant functional group system based on comparisons of both the phylogenies and ecological behaviors of Australian ant taxa with those of the North American ant taxa (1997). Many studies have used this classification scheme (King et al. 1998, Stephens and Wagner 2006, Moranz et al. 2013, González et al. 2018, Triyogo et al. 2020). Ants found in this project followed Andersen's functional group classification system, with *Neivamyrmex* sp. as an exception. The reason for this deviation is described below. Ants belonging to 6 of the 9 original functional groups described by Greenslade were collected in this study (Table 1).

Cold Climate Specialists (CC). These ants occur in colder climates. They are commonly restricted to northern areas of North America or to higher elevations in the southern United States, such as the Rocky Mountains (Andersen 1997). These ants are usually poor competitors that have higher productivity in areas where stronger competitors are absent (Agosti et al. 2000). The foraging strategy of

these ants is mainly focused on avoiding competition with other ants by foraging at temperatures that are lower than most other ant species can tolerate (Andersen 1995).

Cryptic Species (CS). Cryptic ant species are a polyphyletic grouping of ants that are minute in size, usually have colonies with relatively few workers (approximately 100 or less), and have foraging behaviors that usually restrict them from open areas (Andersen 1995, 1997, Agosti et al. 2000). Workers are commonly found foraging in leaf litter or under rocks, which help them to avoid competition with other ant species (Andersen 1995).

Dominant Dolichoderinae (DD). These ants are members of subfamily Dolichoderinae. They are prevalent across North America and typically numerous within their habitats (Andersen 1995, Agosti et al. 2000). These ants thrive in open areas, are usually highly competitive, and defend large territories (Andersen 1995, 1997, Agosti et al. 2000, AntWiki 2020). These ants drive away other ant species and can clear away vegetation and other obstacles along their foraging paths (Middleton et al. 2019). Model members of this functional group *Iridomyrmex* species in Australia (Greenslade 1987, Andersen1995, 1997, Agosti et al. 2000).

Generalized Myrmicinae (GM). These ants all have nonspecific dietary and nesting requirements and have a nearly cosmopolitan distribution (Andersen 1995, Agosti et al. 2000). They also have lower competitive abilities, but can colonize areas quickly when more competitive species are removed (usually following disturbances) (Agosti et al. 2000). Many of these species become pests in homes due to their wide dietary ranges and their affinity for disturbed areas. These ants are much less aggressive than dominant Dolichoderinae (DD) species, but they can still dominate resource-rich areas by rapidly recruiting workers (Andersen 1995).

Opportunists (OP). Opportunist species are a polyphyletic group of ants that all have distributions dependent on the presence or absence of other, more competitive, species (Andersen 1995, Agosti et al. 2000). These ants are not strong competitors. Instead, they rely on colonizing areas of poor habitat quality in order to avoid competition (Andersen 1995, Agosti et al. 2000).

Specialist Predators (SP). These ants have predatory behaviors that are uncommon among the majority of other ant taxa. Specialist predators include slave-makers and brood raiders (Andersen 1995). These ants do not respond in predictable ways to environmental stress and disturbance, as opposed to many of the other functional groups (Andersen 1995, 1997, Agosti et al. 2000).

THE IMPORTANCE OF THIS RESEARCH. Despite the importance of ants in maintaining the health of their ecosystems, ants are not usually taken into consideration in habitat management practices (Dahms et al. 2005, Hartley et al. 2007). In prairie systems, fire is a natural form of disturbance that many species have adapted to tolerate (Hartley 2007). However, wildfires historically occurred in the summer, whereas prescribed burns in Western United States usually occur in the spring or fall (Ryan et al. 2013). This means that management practices, such as prescribed burning, that are expected to increase the health of an ecosystem may actually have negative impacts on the ant fauna that are necessary for healthy habitat functioning (Hartley 2007).

Many of the previous studies on ants' responses to fire have found that fire will initially decrease ant species richness and population size immediately after an area is burned, but will ultimately increase the species richness and will usually decrease the species population size of most ant taxa within two to three years after the burn has taken place (Anderson et al. 1989, Panzer 2001, Ferrenberg et al. 2006). The population size of ant taxa was also shown to rebound to their pre-burn numbers within the first year after burning (Anderson et al 1989). These studies were done on large plots of land that had minimal human interactions (Anderson et al. 1989, Panzer 2001, Ferrenberg et al. 2006).

In this study, the change in the population size and species richness of ant functional groups in the Dr. Howard Reynolds Nature Trail area, a 22 acre nature area in Hays, Kansas, was analyzed before and after prescribed burning took place. The purpose of this research was two-fold: 1) to better understand what species of ants were in the area, and 2) to better understand how different environmental factors might affect ant functional groups in this area. Environmental factors of interest included prescribed burning and different microhabitat types. I hypothesized that the population size of ants in each functional group would decrease and the species richness of ants in each functional group would increase after

prescribed burning took place, as previous studies have shown. I also hypothesized that the microhabitat type that ants were collected from would have a significant impact on the population size and richness of ants in each functional group collected, independent of the burn. This was expected because, in previous years of trapping in the area, there was a noticeable difference in the species of ants collected in each of the habitat types. This idea also follows what is known about the habitat preferences of many ant taxa, as different ant species are adapted to tolerate different environmental factors. Finally, I hypothesized that the interaction between the burn and the microhabitat would be significant in the population size and richness of ants in each functional group. This was inferred because of personal observations of the differences in flammable vegetative cover in each microhabitat. It has been shown in previous studies that species will be impacted differently depending on the vegetation within an area (Dufrene and Legendre 1997).

MATERIALS AND METHODS

STUDY AREA: Pitfall trapping was conducted at the Dr. Howard Reynolds Nature Trail area in Hays, Kansas (38°53'25" N, 99°18'06" W). The nature trail is a mixed grass prairie situated on 22 acres of land near the Sternberg Museum of Natural History (Dr. Howard Reynolds Nature Trail 2017). The nature trail area is situated south of Interstate 70 and southeast of Big Creek (Figure 2). Historically, the area was used as a grazing pasture for horses. The area was purchased by the Sternberg Museum of Natural History in 2010. Since then, management practices have taken place to restore the habitat's health after years of overgrazing.

The area is divided into two distinct microhabitat types by a slope near the center of the area (Figure 2). The uphill area had an elevation of 2,038 feet (Ogle 2016) and was characterized by short vegetation such as buffalograss (*Bouteloua dactyloides*) (Nutt.) J.T. Columbus, switchgrass (*Panicum virgatum*) L., sideoats gramma (*Bouteloua curtipendula*) (Michx.) Torr., blue gramma (*Bouteloua gracilis*) (Willd. Ex Kunth) Lag. Ex Griffiths, and sand dropseed (*Sporobolous cryptandrus*) (Torr.) A. Gray. The area also contained scarce shrubs and scattered cacti. The soil was armo loam (WebSoilSurvey) and rarely flooded. The downhill area had an elevation of 2,021 feet (Ogle 2016) and was characterized by numerous forbe species such as sunflowers (*Helianthus* spp.) L., bromes (*Bromus* spp.) L., big bluestem (*Andropogon gerardii*) Vitman, switchgrass (*Panicum virgatum*) L., and Indiangrass (*Sorghastrum nutans*) (L.) Nash. The soil in the downhill area was a roxbury silt loam (WebSoilSurvey) that was generally wetter and flooded more often than in the uphill area.

Prescribed burning has taken place in the area very few times since the land was purchased. Timing of prescribed burns has been based on the amount of fuel buildup and cedar encroachment in the nature area. A burn was conducted in April of 2017 to manage leaf litter. However, the vegetation was too green at that point in the year, which hindered the burning process. A more complete burn was conducted in the early spring of 2019.

TRAPPING PROCEDURE: Pitfall traps were chosen because, when compared to other trapping methods, pitfall trapping has been shown to collect a greater richness of ant species in open grassland habitats than other trapping types (Majer 1997, Steiner et al. 2005, Higgins and Lindgren 2011, Sheikh et al. 2018). Pitfall traps were purchased from BioQuip Products Inc. (Bioquip, catalog #2838A). Each trap consisted of two plastic containers (measuring 4.5" x 3"), a plastic plate, three nails, and three washers. Thirty pitfall traps were used. Fifteen traps were placed in the downhill area and fifteen were placed in the uphill area (Figure 2). Traps were spaced approximately 7.6 meters (25 feet) apart from each other and were spread out in a line from the northern to the southern part of the trail. At each trap location, plastic containers were placed one inside the other and buried so that the tops of the containers were level with the surface of the soil and the plate was placed over the top with the nails and washers holding it up. The plate was elevated over the containers by the nails to provide cover while still allowing the insects to easily enter the traps.

Trapping took place weekly from the end of May to early August in both 2018 and 2019, as well as in the preliminary year of trapping in 2017. One day each week, the traps were filled a fourth of the way with a 20% saltwater solution to preserve the specimens. Two days later, trap contents were individually strained and specimens were transferred to plastic vials with a 70% ethyl alcohol preservative solution for counting and identification.

Each week, two assistants and I identified the collected ants and recorded the ant counts for each of the 30 traps. Ants collected from pitfall traps were initially identified without magnification, and occasionally with a dissecting microscope. All ants were recorded to genus level. Representative specimens of morphologically distinct ant taxa were saved for species level identification.

Ants identified from pitfall traps in 2017 were used to make a quick-reference identification guide used in 2018 (Appendix A) for the purpose of aiding identifications. More descriptive keys were made for 2019, including a guide for ant morphology (Appendix B). These keys included a dichotomous key to ant subfamilies (Appendix C) and lower taxonomic levels (Appendix D). However, these keys were replaced in favor of an updated quick-reference identification guide (Appendix E). Saved specimens were inspected at later dates using a stereo microscope. Guides to

morphological terms and species identifications were obtained from AntWiki (2019). Morphology terms were defined on the AntWiki website (Bolton 2018). The guides provided by AntWiki included the book "Ants of North America" (Fisher and Cover 2007) for subfamily and genus level identifications. Species level guides were used to identify saved ant specimens (Buren 1968, Ward 2005, Borowiec 2016). Additional species level guides were used from MacGown (2014). Species' descriptions were used from both AntWiki and AntWeb (AntWeb 2019).

Each species identified was checked with previous distribution records and photographs for accuracy. The most recent formicid checklist published by the Kansas School Naturalist (DuBois 1994) was used for a historical comparison to species found in this project. The distribution of species identified in this project were also compared to the data on AntMaps (Janicki et al. 2016, Guénard et al. 2017). Photographs of type specimens and species variants were found on AntWeb.

STATISTICS: Data collected from each week of trapping were pooled based on the trap of capture so that captures within each pitfall trap could be compared (Appendix F, G). This means that the 30 pitfall traps (from both microhabitat types) before the burn were compared to the 30 traps after the burn to access the impact the prescribed burn had on functional groups, and the 30 uphill traps (from both years of trapping) were compared to the 30 downhill traps to test the impact of the microhabitat types on functional groups. This was done because trapping was not done at the same time each year and there was also 1 extra week of trapping in 2018. Consolidating the temporal aspect of the data also reduced the impact of varying weather conditions on results.

Data was analyzed using the statistical program "R" (version 3.6.3). The data consisted of 2 independent variables (the burning treatment, the microhabitat types, and their interaction) and 3 dependent variables (ant counts within each functional group collected based on the burn, the microhabitat type, and the interaction between the 2 treatments). Using the Shapiro-Wilk test of normality, the data were found to not be normally distributed (p<0.001). Data were analyzed using the nonparametric version of a Two-Way ANOVA, the Scheirer Ray Hare test of variance, which allows for

the impacts of multiple factors on multiple responses to be analyzed. This test has been used in past formicid studies to analyze the impact of multiple treatments on different behavioral responses (Schafer et al. 2006, Trettin et al. 2014, Metzler et al. 2018). The Scheirer Ray Hare test provides an H statistic (H), degrees of freedom (df), and a p-value (p) as results. Though the Scheirer Ray Hare is a useful tool in judging significance between multiple independent and dependent variables, there is also some concern as to its statistical power related to the interaction between those variables (Mangiafico 2016). As such, significant results regarding the interaction between the burn and microhabitat should be viewed askance.

The Scheirer Ray Hare test was used to analyze whether the burn, the microhabitat type, or the interaction between the microhabitat type and the burn had significant impacts on the richness or population size of ants within each functional group collected. A significance level of 0.05 was used. To judge the directionality of the impact on richness or population size, median captures within traps relating to their corresponding treatments were compared. Medians were found using Microsoft Excel 2016.

RESULTS

<u>ANT SPECIES IN THE NATURE AREA.</u> There were 18,097 ants collected over the pre-burn (2018) and post-burn (2019) years. 15,920 ants were collected before the burn and 2,177 were collected after the burn. Combining the data from both years, there were 15,244 ants collected in the uphill microhabitat and 2,677 ants collected in the downhill microhabitat. The number of ants collected before the burn and in the uphill microhabitat were largely due to *Dorymyrmex insanus*. Before the burn, 12,060 *Dorymyrmex* were collected, whereas only 6 were collected after the burn. Excluding *Dorymyrmex* from the counts, there were 3,860 ants collected before the burn and 2,111 ants collected after the burn. There were 12,066 *Dorymyrmex* collected in the uphill microhabitat, where it was found exclusively. Excluding *Dorymyrmex*, there were 3,178 ants found in the uphill area.

There were 19 ant species identified, belonging to 16 different genera and 6 different subfamilies (Table 2). Myrmicinae was found to be the most diverse subfamily of ants in the nature area, comprising 7 of the 19 identified species. The subfamilies Amblyoponinae and Dorylinae were the least diverse in this project, each containing only 1 representative species. All but 1 of the species identified in this study have previous occurrence records in Kansas. This is the first record for *Aphaenogaster ashmeadi* in Kansas, although *A. ashmeadi* are known to occur in Missouri and Texas (Janicki et al. 2016, Guénard et al. 2017).

ANTS SPECIES WITHIN FUNCTIONAL GROUPS. Statistical analyses in this project were done at the genus level. However, it is important to note which species were captured in the nature area as Andersen's (1997) functional group classification system does make distinctions between certain ant species with distinct ecologies. This means that certain ant species belonging to the same genus may be classified in separate functional groups if their behaviors are distinct.

The 5 of the 7 Myrmicinae species collected (*Crematogaster lineolata, Monomorium minimum, Monomorium pharaonis, Pheidole bicarinata*, and *Solenopsis molesta*) were classified in the Generalized Myrmicinae (GM) functional group. *Crematogaster lineolata* (2,714 total collected) and *Monomorium*

minimum (1,060 total collected) were abundant species that were frequently collected in traps. *Pheidole bicarinata* (53 total collected) and *Solenopsis molesta* (14 total collected) were rarely collected, and only 1 *Monomorium pharaonis*, an alate, was collected over both years of trapping. Only minor *Pheidole bicarinata* workers were found in traps. Major workers were collected from a nest near the trapping site and were used to get a species level identification. This means that it is possible that the species of *Pheidole* found in the nearby nest was not the same species collected in traps. Of the other myrmicines, *Aphaenogaster ashmeadi* was classified as an Opportunist (OP) and *Temnothorax pergandei* was classified as a Cold Climate Specialist (CC). *Aphaenogaster ashmeadi* was collected in moderate numbers over both years (126 total collected), but only 1 *Temnothorax pergandei* worker was collected once over both years of trapping.

There were 4 species in Subfamily Formicinae identified (*Formica pallidefulva, Lasius neoniger, Lasius americanus, Nylanderia terricola*). The majority of Formicinae species were classified as Cold Climate Specialists (CC) (*F. pallidefulva, L. neoniger, L. americanus*), while *N. terricola* was classified as an Opportunist (OP). Both species of *Lasius* were frequently collected (238 total collected), *Formica* individuals were rarely collected (14 total collected), and only 1 individual *Nylanderia* was found in this project.

There were 4 Dolichoderinae species identified, 2 of which were classified as dominant Dolichoderinae (DD) (*Forelius mccooki, Forelius pruinosus*) and the other 2 were classified as Opportunists (OP) (*Dorymyrmex insanus, Tapinoma sessile*). Both *Forelius* species were frequently collected throughout the nature area (1,426 total collected) with *F. pruinosus* being much more abundant than *F. mccooki. Dormyrmex* sp. were only collected in the traps nearest to the interstate in the uphill area, but was found in great numbers in those traps (12,060 total collected). I had debated on classifying *Dorymyrmex* as a DD, but I decided to go with Andersen's decision to classify them as OP (Andersen 1977) for this project. *Tapinoma sessile* was a frequently misidentified species in this project. They were not collected during the pre-burn year, and they were not properly identified until halfway through the post-burn year. Before the quick reference guides were updated to include *Tapinoma*, they were

misidenfied as *Lasius* or *Forelius* depending on the participant identifying them. Exact distributions of *Tapinoma* within the area is unclear, since most specimens were discarded.

Ponera pennsylvanica and *Hypoponera opacior* were the only species of Subfamily Ponerinae identified. Both species were classified as cryptic species (CS). *Ponera pennsylvanica* and *Hypoponera opacior* were each only collected once throughout this project. Only 1 individual *H. opacior* specimen was collected and only 2 individual *P. pennsylvanica* specimens were collected in the same trap and on the same day in May, both in 2019.

Neivamyrmex nigrescens was the only member of Subfamily Dorylinae identified in this project. They were classified as specialist predators (SP) because they raid the nests of other ants and feed on their brood. This genus was originally classified as a tropical climate specialist by Andersen (1997). Andersen's classification was based on comparisons between Australian Old World army ants with the New World army ants in North America (Andersen 1997). New World and Old World army ants have similar ecologies, but are phylogenetically distant. The distribution of the species of *Neivamyrmex* found in this project, *Neivamyrmex nigrescens*, reaches into the northern temperate regions of North Dakota (United States Department of Agriculture 1967), suggesting that their distributions may not be as closely associated with tropical climates as other *Neivamyrmex* species. It was for this reason that the *Neivamymrex nigrescens* was classified as a specialist predator in this project (Table 1, 2). Subfamily Amblyoponinae also had 1 representative species in this project (*Stigmatomma pallipes*), and they were classified as cryptic species (CS). *S. pallipes* was only collected once during this project in 2018.

Of the 16 different genera captured in this project, 13 genera only had 1 representative species. The genera *Forelius, Lasius*, and *Monomorium* each had 2 different species. Within each genus, members shared very similar ecologies. It is because of this that ants were categorized in their respective functional groups based on their genera (Table 1, 2).

POPULATION SIZES OF THE ANTS WITHIN FUNCTIONAL GROUPS. The overall community structure of the nature area was noticeably altered by the burn. There was a noticeable decline in the number of ants in many functional groups after the burn (Table 3, Figure 3, 4). Functional groups

that were more abundant before the burn (opportunists) were subordinate to other functional groups after the burn (generalized myrmicinae and dominant dolicoderinae) (Figure 5, 6). There was also a noticeable difference in the number of ants collected over the summers of each year. There were more ants collected each week in the summer before the burn than in the year after the burn (Figure 7). The Schreirer Ray Hare (SRH) test of variance was used to compare the counts of ants within each functional group before the burn with the counts in the year after the burn (Appendix F). The population sizes of ants within each functional group was not significantly impacted by the burn (Table 4). This was the case for all 6 functional groups (Table 4). The burn may have had some impact on dominant dolichoderinae (DD) (H= 3.559, df= 1, 56, p= 0.059) and, to a lesser extent, on specialist predator (SP) (H= 3.104, df= 1, 56, p= 0.078) species. The median number of captures of DD in the 30 traps before the burn (36) was greater than the median number of captures after burn (14). Due to the scarcity of SP collections, the median number of captures of SP before and after burn were zero. However, there were 117 SP collected in the pre-burn year and only 10 SP collected post-burn.

Overall, the number of captures of most ant taxa decreased after the burn (Table 3, Figure 3, 4). Functional groups that had more captures after the burn included cold climate specialists (CC) and cryptic species (CS), although 2 CS were captured before the burn and only 3 were captured after the burn. Genera that had more captures after the burn compared to their captures before burn included *Monomorium* (GM), *Pheidole* (GM), *Solenopsis* (GM), *Lasius* (CC), and *Formica* (CC). Genera that were not found before the burn but were captured after the burn included *Hypoponera* (CS), *Ponera* (CS), *Tapinoma* (OP), and *Temnothorax* (CC).

The microhabitat type that ants were collected from seemed to have more of an impact on the number of ants captured than the burning treatment did. There were noticeable differences in the number of ants in each functional group that were collected in each microhabitat type (Table 3, Figure 8, 9). The microhabitat type did not significantly affect the amount of generalized Myrmicinae (GM) and specialist predators (SP) collected (Table 5). Though a significant impact was not detected, the median number of GM captures was slightly greater in the uphill microhabitat (uphill = 52, downhill = 43). The median

number of SP was zero in each microhabitat, but there were 123 SP individuals collected in the uphill microhabitat and only 4 collected in the downhill microhabitat over both years of trapping. The microhabitat that ants were collected from was found to have a significant impact on the number of captures of cold climate species (CC), cryptic species (CS), dominant Dolichoderinae (DD), and opportunist species (OP) (Table 5). The median number of captures of cold climate species (CC) (uphill = 0, downhill = 3) and opportunist species (OP) (uphill = 0, downhill = 3) were both greater in the downhill microhabitat, although the differences were small and may have been by chance. The median number of captures of dominant Dolichoderinae (DD) were found to be greater in the uphill microhabitat (uphill = 27, downhill = 8). The population size of cryptic species (CS) was found to be impacted by the microhabitat type, but the medians of captures in each area were zero. However, all 5 CS individuals were collected from the downhill microhabitat.

Of the 6 functional groups, DD, OP, SP, and to a lesser extent GM all had greater population sizes in the uphill microhabitat while CC and CS had greater population sizes in the downhill microhabitat. However, not all genera within their functional groups showed the same trends. Of the CC functional group, *Formica* and *Lasius* were both more abundant in the downhill microhabitat. While the other CC member, *Temnothorax* was more abundant uphill, it was also never captured downhill. All CS members (*Hypoponera, Ponera,* and *Stigmatomma*) were exclusively captured in the downhill microhabitat. All GM members (*Crematogaster, Monomirum, Pheidole,* and *Solenopsis*) were more abundant in the uphill microhabitat types were very slight for all GM genera. Of the members of the OP functional group, *Aphaenogaster* was more abundant in the downhill microhabitat as well as *Tapinoma*, which was only found downhill. The other OP members (*Dorymyrmex* and *Nylanderia*) were more abundant in the uphill microhabitat, although *Nylanderia* was also never captured downhill. The only SP member, *Neivamyrmex*, was more abundant uphill.

The interaction between the burn and the microhabitat type did not significantly affect the number of captures in any of the 6 functional groups (Table 6). The interaction between the treatments may have had some impact on Generalized Myrmicinae (GM) species (H= 2.867, df= 1, 56, p= 0.090). There seemed to be an inverse relationship to how burning impacted GM in each microhabitat type. The median number of GM in the downhill microhabitat was greater before the burn (65 before, 32 after), whereas median captures were greater after the burn in the uphill microhabitat (52 before, 62 after). However, the total number of GM collected in either microhabitat type decreased after the burn (Table 3). This was also the case for DD and SP. There were far more CC collected in the downhill microhabitat and the number of individuals collected doubled after the burn in the downhill microhabitat (Table 3). The number of OP collected in the uphill microhabitat more than doubled after the burn, whereas the number of OP collected in the downhill microhabitat more than doubled after the burn (Table 3). There were not enough CS collected to notice any trends between the interaction of the burn and the microhabitat type.

RICHNESS OF GENERA WITHIN FUNCTIONAL GROUPS. There was a noticeable difference in the genera captured in many functional groups before and after burning had taken place (Table 7). In the uphill microhabitat, *Formica* and *Temnothorax* were not present before the burn but they were found there after the burn. In the downhill microhabitat, this was also the case for *Ponera*, *Hypoponera*, *Solenopsis*, and *Tapinoma*. There were also some genera that were not found after the burn such as *Nylanderia* in the uphill microhabitat as well as *Stigmatomma* in the downhill microhabitat.

The SRH test was used to compare the number of different genera in each functional group before and after prescribed burning took place in the nature area. The number of different genera collected in any of the 6 functional groups was not significantly impacted by the burn (Table 8, Figure 10, 11). The richness of specialist predator (SP) genera may have had some impact from the burn (H= 3.214, df= 1, 56, p= 0.073). Though the median number of SP genera captured was zero in both years, there was only 1 SP representative, *Neivamyrmex nigrescens*, which was captured in relatively few traps (Appendix G). *N. nigrescens* was captured in the uphill area both before and after the burn, but they were absent from the downhill area after the burn (Table 7). The microhabitat type did not have a significant impact on the number of genera collected for cryptic species (CS), dominant Dolichoderinae (DD), generalized Myrmicinae (GM), or specialist predators (SP) (Table 9). Although CS were rarely captured (Appendix G), all CS genera were only found in the downhill microhabitat (Table 7). The median richness of DD (1 genus uphill, 1 genus downhill) and GM (medians = 2 uphill, 2 downhill) were equal in both microhabitats and all genera from both functional groups were found in each microhabitat type (Table 7). There was only 1 representative of the SP functional group, *N. nigrescens*, which was found in 7 of the uphill traps and only 2 of the downhill traps (Appendix G). Due to the scarcity of their captures, the median number of SP in each microhabitat was found to be zero. The microhabitat type was found to have a significant impact on the number of genera collected in CC and OP (Table 9). However, the difference in their richness was too small to make strong conclusions (CC = median of 0 genera uphill and 1 downhill, OP = median of 1 genus uphill and 0 downhill).

The interaction between the burn and the microhabitat type was not found to have a significant impact on the number of genera collected in any of the functional groups (Table 10). The median number of genera in all functional groups show that each treatment, independently, seemed to have their own separate impacts on genera richness. There were no obvious trends in genus richness in response to the interaction between the 2 treatments (Table 7, 10).

DISCUSSION

The purpose of this study was twofold: 1) to better our understanding of how ant functional groups respond to fire and different microhabitat types and 2) to better understand what ant species are present at the Dr. Howard Reynolds Nature Trail. Hypotheses were based on what was already known about ant responses to fire (Anderson et al. 1989, King et al. 1998, Panzer 2001, Farrenberg et al. 2006, Hartley et al. 2007, Houdeshell et al. 2011, Kaynaş et al. 2018) and habitat preferences (González et al. 2018, Triyogo et al. 2020). Although results in this project didn't align with the results of these previous studies, many results did align with what is known about ant functional group responses to the environmental factors of interest (Andersen 1995, 1996, Agosti et al. 2000).

It is important to note that *Dorymrymex* was wrongfully classified as an opportunist (OP) in this project. Although Andersen (1997) had classified them as an OP, *Dorymyrmex* spp. are known to be much more aggressive and competitively dominant in grassland habitats (Andersen 1997). This also was supported by our findings. *Dorymyrmex insanus* was the most abundant ant species found in the nature area in both the preliminary and pre-burn years of trapping. They were found in only a few, closely approximated traps in the uphill area (thousands were found in the 2 most northern traps and only a couple hundred were found in the 5 traps south of those northern traps). Other ant species were noticably absent, or in stark numbers, in traps that *Dorymyrmex* were found in. This suggests that those traps were placed inside their territory. Establishing and defending territories is a common behavior of dominant Dolichoderinae (DD) (Andersen 1995, 1997, Agosti et al. 2000). These observations suggest that it would have been more appropriate to classify *Dorymyrmex* as a DD in this project. Results of the SRH test were based on *Dorymrymex* being classified as an OP. However, *Dorymyrmex* responses will be compared to the responses of the other DD species (*Forelius* spp.).

Neither the population size nor the richness of ants within functional groups were significantly impacted by the prescribed burn in either microhabitat (Table 4, 6, 8). This evidence does not support the hypotheses that prescribed burning would decrease the population size and increase the richness of ants

within each functional group. The burn had some impact on the population size of dominant Dolichoderinae (DD) as the median of their population size before the burn was greater than their population size after (medians = 24 before, 12 after). Although the impact did not meet the significance level for this project, it was close (Table 4).

The results of this experiment do not align with what is already known about how ants respond to fire (Anderson et al. 1989, Panzer 2001, Ferrenberg et al. 2006). This was because previous studies that this project was based on focused on ants as a Family (Anderson et al. 1989, Hartley et al 2007), as genera (Kaynaş et al. 2018), as species (Houdeshell et al. 2011), or as other ecological groupings (Panzer 2001, Farrenberg et al. 2006). Based on what is already known about ant functional groups, all functional groups should not have been expected to respond to environmental factors in similar ways (Grime 1977, Andersen 1995, 1997) (Figure 1). This could also be because the other studies had taken place over longer time frames, had been done on much larger areas, and had much greater capture numbers.

Ant functional groups in this project did respond to fire in similar ways to what is already known about functional group responses to disturbance (Grime 1977, Andersen 1995, 1997). Dominant Dolichoderinae are very sensitive to disturbance, and are abundant in areas where disturbance is low (Andersen 1995, 1997, Agosti et al. 2000). In this project, *Forelius* spp. were the only members classified as dominant Dolichoderiae (DD). However, *Dorymyrmex* sp. was found to respond to fire much more like a DD than it did as an opportunist (OP). Before the burn, these 2 dolichoderines were found in much greater numbers than they were after the burn (Dorymyrmex = 12,060 before, 6 after, Forelius = 735 before, 324 after). Generalized Myrmicinae (GM) are also strong competitors that tolerate disturbance more efficiently than DD, but less efficiently than other functional groups (Andersen 1995, 1997, Agosti et al. 2000). In this project, GM became the most abundantly collected ants in the post-burn year (Figure 6). OP are known take advantage of moderately disturbed areas, as these areas are seen as unfavorable for more competitive ants like DD and GM (Andersen 1995, 1997, Agosti et al.

2000). Excluding *Dorymyrmex* from the OP group, OP in this project were found in greater numbers in the post-burn year than in the pre-burn year (pre-burn = 76, post-burn = 133).

For many functional groups, the microhabitat in which the collection took place had a significant impact on their population size (CC, CS, DD, OP) (Table 5, Figure 8, 9) and richness of genera (CC, OP) (Table 9). For other functional groups, the microhabitat did not play a significant role in their population size (GM, SP) or richness (CS, DD, GM, SP). These results reject the original hypotheses that the population size and the richness of each of the functional groups would be significantly impacted by the microhabitat type. This is because the unique resource preferences of each functional group were not considered beforehand. Functional groups that are known to thrive in areas with higher environmental stress (such as the cold climate specialists) should not have been expected to respond similarly to groups that avoid high stress areas (such as dominant Dolichoderinae) (Grime 1977, Andersen 1995, 1997) (Figure 1).

Trends relating to how functional groups respond to different forms of environmental stress were also found in this project. The population size of dominant Dolichoderinae (DD) species (*Forelius* and *Dorymyrmex*) was greater in the uphill microhabitat (*Forelius* = 1,059 uphill, 367 downhill, *Dorymyrmex* = 12,060 uphill, 0 downhill). This matches with what is known about DD preference for foraging in unshaded areas (Andersen 1997). Previous studies have shown that cold climate specialists (CC) have a negative impact with the presence of DD, and they are also better adapted to tolerate environmental stress than other functional groups (Grime 1977, Andersen 1995, 1997, King et al. 1998, Agosti et al. 2000) (Figure 1). This impact was also found in this study as the population size of cold climate species (CC) was greater in the downhill microhabitat where DD population sizes were lower than in the uphill area (Table 3). The presence of opportunist species (OP) has a strong negative association with the presence of other ants, as opportunists are not strong competitors (Andersen 1995, 1997, Agosti et al. 2000). Excluding Dorymyrmex from the OP counts, OP were found in greater numbers in the downhill area, where there were fewer highly competitive DD (OP = 33 uphill, 175 downhill). The results align with what is known about generalized Myrmicinae (GM), as their resource requirements are generalized.

meaning that they are likely to persist similarly in each microhabitat type. In this project, all of the GM species found in the uphill microhabitat were also found in the downhill microhabitat (Table 7) and the population size of GM in each microhabitat was similar (Table 3).

Results that did not align with what is known about ant functional group responses to environmental stress and habitat preferences may have been due to issues with our data. The richness of dominant Dolichoderinae (DD) did not show any variation between microhabitats because there was only 1 genus (*Forelius*) in this functional group found during this project (Table 1, 7). This was also the case for the one specialist predator (SP) member, *Neivamyrmex* (Table 1, 7). The population size of cryptic species (CS) in past studies has been found to be positively associated with the amount of leaf litter available for them to reside amongst (Andersen 1995, 1997, Agosti et al. 2000). In this study, the significance of the microhabitat on CS was not great enough to find a relationship between their population size or richness with either microhabitat type (Table 3). Due to the scarcity of their collections, the medians of CS captured in the 30 traps in each microhabitat type were zero. However, all CS captured in this project were collected in the downhill microhabitat (Table 3, 7), which was observed to have much denser vegetation that provided much more cover than the uphill microhabitat. However, with so few CS collected (Appendix F, G, Table 3), their preference for the downhill microhabitat may be due to chance.

The data also rejects the hypotheses that the interaction between the burn and the microhabitat would have a significant impact on the population size and richness of ants collected in each functional group. The interaction between the burn and the microhabitat type may have had some impact on the population size of the generalized Myrmicinae (Table 6). After the burn, the median number of GM collected increased in the uphill area (52 before, 62 after) and decreased in the downhill area (65 before, 32 after). This may have been because many GM species have behaviors that tie them closely with vegetation, such as the fact that *Monomorium* spp. and *Crematogaster* spp. are known to tend aphids (DuBois 1985). This may have directly put more workers at risk when the area was burned, or it may have indirectly impacted these species by reducing their food and aphid supply (Agosti et al. 2000).

The prescribed burn preformed at the Dr. Howard Reynolds Nature Trail was intended to increase the health of the area by burning away encroaching woody plants. Our results suggest that the burn did not have a significant impact, positive or negative, on the ant fauna in the area. Even though the generalists, many of which we recognize as pest species, became dominant in the area after the burn, this is not uncommon for areas in the Nearctic Region, where both dominant Dolichoderinae (DD) and generalized Myrmicinae (GM) frequently compete for dominance (Agosti et al. 2000). Although the burn may have favored GM dominance, this does not mean that the DD will not recover. Ant populations frequently show negative responses in the year directly following a burn, but their numbers usually return to their pre-burn states within an approximately two years (Anderson et al. 1989, Panzer 2001, Ferrenberg et al. 2006). Had this study taken place for one more summer, this same pattern may have also been seen. Ants' roles as bioindicators allow us to make judgements on the health of a habitat in relation to the environmentally sensitive species that reside in those habitats (Dufrene and Legendre 1997, Stephens and Wagner 2006, Moranz et al. 2013). From these data, we can conclude that the prescribed burn that took place at the Dr. Howard Reynolds Nature Trail did not negatively impact the health of the habitat.

Errors in this project were due, in part, to specimen misidentification. Identifying ants is challenging even with the use of a microscope. There are color and morphological variations within ant species that are not always accounted for in identification keys. The challenge of identifying species correctly despite these variations was only amplified based on the way that we had identified specimens in this study. Specimens were identified the day they were taken from the traps and very few were kept to confirm identities afterwards. This led to some questionable reports that could not be checked for accuracy. Ants were also identified without looking for key characteristics under a microscope. Species that have similar morphology were likely to be identified differently based on the person identify them. This was the case for many of the records of *Forelius, Lasius,* and *Tapinoma*. It is also possible that there were more species present in our traps than were recorded in this project, as only the preserved specimens were identified to species level. Some of the identification material I had made also contained errors such as how an abnormally small *Pheidole* morph was classified as "*Stennama*" (Appendix E). However, these

errors were corrected. In future studies, this problem could be avoided by assigning a single person with knowledge and experience identifying ants to this task. Damage or other complications with pitfall traps also may have affected captures. The plates covering the traps would occasionally be damaged by hail, animal, or human interactions which may have impacted the likelihood of capturing species that preferred the shade. There were instances, in both years, where rain had flooded traps, which led to specimens being washed out of the containers. This was particularly the case for traps in the downhill area, meaning that there may be fewer recorded ant collections in the downhill area than were actually collected in the traps. Though steps could be taken to reduce these environmental complications, they are not completely avoidable.

For future projects, I recommend trapping in larger areas that are likely to have less human impact, similar to those in previous studies (Anderson et al. 1989, Panzer 2001, Ferrenberg et al. 2006). Doing so would likely lessen damages to pitfall traps, yield greater ant capture numbers, and prevent the possibilities of pseudoreplication. I recommend that all formicid specimens be collected and preserved rather than being discarded after their initial identification to lessen the concerns of misidentifications that were present in this study. I also recommend making hypotheses that are tailored towards the unique ecologies of each functional group instead of making broad hypotheses like those made in this project. My assumption that all functional groups would respond similarly to the burn did not account for what is already known about each functional groups' response to environmental stress and disturbance.

If this study were to be performed again, there are some changes that I would implement. Capture numbers were not great enough for each functional group to make meaningful conclusions. I suspect that trapping in multiple different ways would produce data that would better represent each functional group. Previous studies have shown that ant species that are arboreal, cryptic, and hypogaeic are underrepresented in studies that only use pitfall traps (Majer 1997). Using Winkler traps, sweep netting, and hand collecting would yield more accurate accounts of these species (Majer 1997). Another change I would implement would be to lengthen the time frame of the study. It would be interesting to see if the
ant communities in the nature area would rebound after 2 or more years after the burn, which has been found to be a trend in other studies (Anderson et al. 1989, Panzer 2001, Ferrenberg et al. 2006).

The results of this project are useful in furthering what is known about ants, their functional groups, and how prescribed burning and microhabitat preferences affect their richness and population size in grasslands in Kansas. It is important that studies focus on ants as functional groups, as functional group patterns can be extrapolated in areas around the globe (Andersen 1995). It is because of the vital roles that ants play in their ecosystem that special attention be made towards preserving their populations.

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FIGURES AND TABLES

Table 1. The genera of ants found during this project and their associated functional groups.

Classifications were based on the North American ant functional group system developed by Andersen

(1997).

Functional Group	Members
Cold Climate Specialists	Formica spp., Lasius spp., Temnothorax spp.
Cryptic Species	Hypoponera spp., Ponera spp., Stigmatomma spp.
Dominant Dolichoderinae	Forelius spp.
Generalized Myrmicinae	Crematogaster spp., Monomorium spp., Pheidole spp., Solenopsis spp.
Opportunists	Aphaenogaster spp., Dorymyrmex spp., Nylanderia spp., Tapinoma spp.
Specialist Predators	Neivamyrmex spp.

Table 2. Ant species identified in the Dr. Howard Reynolds Nature Trail in Hays, Kansas the coinciding subfamily and functional group for each species.

Species/Probable Species	Subfamily	Functional Group
Aphaenogaster ashmeadi (Emery) 1895	Myrmicinae	Opportunist
Crematogaster lineolata (Say) 1836	Myrmicinae	Generalized Myrmicinae
Dorymyrmex insanus (Buckley) 1866	Dolichoderinae	Opportunist
Forelius mccooki (McCook) 1879	Dolichoderinae	Dominant Dolichoderinae
Forelius pruinosus (Roger) 1863	Dolichoderinae	Dominant Dolichoderinae
Formica pallidefulva Latreille 1802	Formicinae	Cold Climate Specialists
Hypoponera opacior (Forel) 1893	Ponerinae	Cryptic Species
Lasius neoniger Emery 1893	Formicinae	Cold Climate Specialists
Lasius americanus Emery 1893	Formicinae	Cold Climate Specialists
Monomorium minimum (Buckley) 1867	Myrmicinae	Generalized Myrmicinae
Monomorium pharaonis (Linnaeus) 1758	Myrmicinae	Generalized Myrmicinae
Neivamyrmex nigrescens (Cresson) 1872	Dorylinae	Specialist Predators
Near Nylanderia terricola (Buckley) 1866	Formicinae	Opportunist
Pheidole bicarinata Mayr 1870	Myrmicinae	Generalized Myrmicinae
Ponera pennsylvanica Buckley 1866	Ponerinae	Cryptic Species
Near Solenopsis molesta Say 1836	Myrmicinae	Generalized Myrmicinae
Stigmatomma pallipes (Haldeman) 1844	Amblyoponinae	Cryptic Species
Tapinoma sessile (Say) 1836	Dolichoderinae	Opportunists
Temnothorax pergandei (Emery) 1895	Myrmicinae	Cold Climate Specialists

Table 3. The sum of all ants captured in each functional group in the uphill and downhill microhabitats before and after prescribed burning. These data are a compressed version of the data used in the Scheirer Ray Hare test of variance (Appendix A). Data in this table is the sum of all ants captured in all 30 pitfall traps in either burn treatment or microhabitat type.

Functional Group	Uphill Population Size Before	Uphill Population Size After	Downhill Population Size Before	Downhill Population Size After
Cold Climate Specialists	2	2	84	161
Cryptic Species	0	0	2	3
Dominant Dolichoderinae	735	325	212	154
Generalized Myrmicinae	1180	765	1273	615
Opportunists	12086	7	50	125
Specialist Predators	113	10	4	0

Table 4. The results of the Scheirer Ray Hare test of variance in relevance to the impact of prescribed burning on the population size of ants within each functional group (H = H statistic, df = degrees of freedom, p = p-value).

Functional Group	Н	df	р
Cold Climate Specialists	0.935	1, 56	0.335
Cryptic Species	0.001	1, 56	0.973
Dominant Dolichoderinae	3.559	1, 56	0.059
Generalized Myrmicinae	2.743	1, 56	0.098
Opportunists	1.722	1, 56	0.189
Specialist Predators	3.104	1, 56	0.078

Table 5. The results of the Scheirer Ray Hare test of variance in relevance to the impact of microhabitat type on the population size of ants within each functional group (H = H statistic, df = degrees of freedom, p = p-value).

Functional Group	Н	df	p
Cold Climate Specialists	14.073	1, 56	<0.001
Cryptic Species	4.210	1, 56	0.040
Dominant Dolichoderinae	16.080	1, 56	< 0.001
Generalized Myrmicinae	0.001	1, 56	0.971
Opportunists	6.768	1, 56	0.009
Specialist Predators	0.354	1, 56	0.552

Table 6. The results of the Scheirer Ray Hare test of variance in relevance to the interaction between prescribed burning and microhabitat type had on the population size of ants within each functional group

Functional Group	Н	df	р
Cold Climate Specialists	0.015	1, 56	0.901
Cryptic Species	0.001	1, 56	0.973
Dominant Dolichoderinae	2.256	1, 56	0.133
Generalized Myrmicinae	2.867	1, 56	0.090
Opportunists	2.710	1, 56	0.100
Specialist Predators	0.735	1, 56	0.391

(H = H statistic, df = degrees of freedom, p = p-value).

Table 7. The presence or absence of ant genera captured in each functional group that were lost or gained in the uphill and downhill microhabitats before and after prescribed burning (based on data in Appendix B).

Functional Group	Uphill Genera Before	Uphill Genera After	Downhill Genera Before	Downhill Genera After
Cold Climate Specialists	Lasius	Formica, Lasius, Temnothorax	Formica, Lasius	Formica, Lasius
Cryptic Species			Stigmatomma	Hypoponera, Ponera
Dominant Dolichoderinae	Forelius	Forelius	Forelius	Forelius
Generalized Myrmicinae	Crematogaster, Monomorium, Pheidole, Solenopsis	Crematogaster, Monomorium, Pheidole, Solenopsis	Crematogaster, Monomorium, Pheidole	Crematogaster, Monomorium, Pheidole, Solenopsis
Opportunists	Aphaenogaster, Dorymyrmex, Nylanderia	Aphaenogaster, Dorymyrmex	Aphaenogaster	Aphaenogaster, Tapinoma
Specialist Predators	Neivamyrmex	Neivamyrmex	Neivamyrmex	

Table 8. The results of the Scheirer Ray Hare test of variance in relevance to the impact of prescribed burning on the richness of ant genera within each functional group (H = H statistic, df = degrees of freedom, p = p-value).

Functional Group	Н	df	р
Cold Climate Specialists	0.854	1, 56	0.354
Cryptic Species	0.345	1, 56	0.557
Dominant Dolichoderinae	< 0.001	1, 56	1.000
Generalized Myrmicinae	2.658	1, 56	0.103
Opportunists	1.490	1, 56	0.222
Specialist Predators	3.214	1, 56	0.073

Table 9. The results of the Scheirer Ray Hare test of variance in relevance to the impact of microhabitat type on the richness of ant genera within each functional group (H = H statistic, df = degrees of freedom,

Functional Group	Н	df	р
Cold Climate Specialists	9.834	1, 56	0.002
Cryptic Species	0.345	1, 56	0.078
Dominant Dolichoderinae	2.035	1, 56	0.154
Generalized Myrmicinae	1.600	1, 56	0.207
Opportunists	10.072	1, 56	0.002
Specialist Predators	0.129	1, 56	0.720

p = p-value).

Table 10. The results of the Scheirer Ray Hare test of variance in relevance to the interaction between prescribed burning and microhabitat type had on the richness of ant genera within each functional group. Each treatment has 15 pitfall traps associated with them (H = H statistic, df = degrees of freedom, p = p-value).

Functional Group	Н	df	р
Cold Climate Specialists	0.192	1, 56	0.661
Cryptic Species	0.345	1, 56	0.557
Dominant Dolichoderinae	<0.001	1, 56	1.000
Generalized Myrmicinae	0.010	1, 56	0.922
Opportunists	1.020	1, 56	0.312
Specialist Predators	1.157	1, 56	0.282

Figure 1. The ant functional group model based on Grime's C-S-R model (Grime 1977, Andersen 1997). The ant functional group model shows the relative pervasiveness of dominant Dolichoderinae (DD), generalized myrmicinae (GM), opportunists (OPP), and climate specialists (CS) functional groups in areas with different levels of stress, disturbance, and competition (Andersen 1997).



Stress

Figure 2. Google Earth image of the Dr. Howard Reynolds Nature Trail in Hays, Kansas. Locations of pitfall traps are shown for both the uphill (Up) and downhill (Down) microhabitats.



Figure 3. The population size of ants in each functional group in the uphill microhabitat before and after prescribed burning occurred in the Dr. Howard Reynolds Nature Trail. There were no CS (Cryptic Species) collected in this microhabitat in either year of trapping (CC = Cold Climate Specialists, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators).



Figure 4. The population size of ants in each functional group in the downhill microhabitat before and after prescribed burning occurred in the Dr. Howard Reynolds Nature Trail (CC = Cold Climate Specialists, CS = Cryptic Species, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators).



Figure 5. Pie chart showing the percentage of each functional group collected across the nature area in the summer of 2018, before burning took place (CC = Cold Climate Specialists, CS = Cryptic Species, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators). The percent of captures is as follows: CC = 0.55%, CS = 0.01%, DD = 6.02%, GM = 15.58%, OP = 77.10%, SP = 0.74%.



Figure 6. Pie chart showing the percentage of each functional group collected across the nature area in the summer of 2019, after burning had taken place (CC = Cold Climate Specialists, CS = Cryptic Species, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators). The percent of captures are as follows: CC = 7.52%, CS = 0.14%, DD = 22.10%, GM= 63.68%, OP = 6.09%, SP = 0.46%.





Figure 7. Line graph showing the number of ants collected each day over the summer of the pre-burn year and the post-burn year (after the burn had taken place).

Figure 8. Pie chart showing the percentage of each ant functional group collected across the nature area in the uphill microhabitat type over both years of trapping (CC = Cold Climate Specialists, CS = Cryptic Species, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators). The percent of captures are as follows: CC = 0.03%, CS = 0.00%, DD = 6.96%, GM = 12.78%, OP = 79.43%, SP = 0.15%.



Figure 9. Pie chart showing the percentage of each ant functional group collected across the nature area in the downhill microhabitat type over both years of trapping (CC = Cold Climate Specialists, CS = Cryptic Species, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators). The percent of captures are as follows: CC = 9.13%, CS = 0.19%, DD = 13.64%, GM = 70.37%, OP = 6.52%, SP = 0.15%.



Figure 10. The number of different genera within each ant functional group found in the uphill microhabitat before and after prescribed burning occurred in the Dr. Howard Reynolds Nature Trail. No CS (Cryptic Species) were found in this microhabitat in either year (CC = Cold Climate Specialists, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators).



Figure 11. The number of different genera within each ant functional group found in the downhill microhabitat before and after prescribed burning occurred in the Dr. Howard Reynolds Nature Trail (CC = Cold Climate Specialists, CS = Cryptic Species, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators).



APPENDIX

Appendix A. Quick-reference guide to common ant genera collected from the Dr. Howard Reynolds Nature Trail area used during pitfall trapping in 2018. Ants included in this guide were based on ants collected from pitfall trapping in 2017.

Ants with 2 petiole segments (Myrmicinae or Dorylinae):

Genus Neivamyrmex





- Appears to have no eyes
- Antennae situated close together in the

middle of the head

Genus Crematogaster



- petiole connects to the top of the gaster ("abdomen")

- distinct antennal club and oval head

Ants with one sharp, distinct petiole node (Formicinae):





- large, long legged

- front of "thorax" is much higher than the back

of the "thorax"

Genus Monomorium





- petiole nodes both look like tall, rounded

mountains

Genus Formica



- bright orange and shiny
- have 3 ocelli on forehead and large eyes

Genus Lasius



- back of the "thorax" slants down sharply

like a slide

- orange, compact bodies

Ants with one small, slanting/flat petiole node (Dolichoderinae):

Genus *Forelius*



- gaster ("abdomens") may have 2 colors
- rounded segments of the "thorax"

Genus Dorymyrmex



- has a cone on the back of the "thorax"
- long, stringy legs

Appendix B. Guide to the morphology of ants developed in 2018 for use in 2019 pitfall trapping.



Formicidae Anatomy: Body

Formica sp. showing the basic body anatomy.



Formica sp. showing basic head anatomy (left). Neivamyrmex sp. (Dorylinae) showing reduced eyes consisting of one ocular facet and antennal sockets closely approximated and centered (right).

Formicidae Anatomy: Petiole and Gaster



Formicinae gaster (left). Dolichoderinae gaster (left).



The sharp and distinct petiole of Formicinae (left). The small and hidden petiole of Dolichoderinae

(right).



Petiole and gaster segments of Amblyoponinae. Note that the petiole is similar in size and shape to that of the gaster segments. There is also a small stinger at the tip of the gaster.



Petiole and postpetiole with peduncles of Myrmicinae (left). The rounded petiole and postpetiole of

Dorylinae (right).

Appendix C. Dichotomous key to ant subfamilies used to identify ants in 2019. The key was based on ants collected from pitfall traps in 2018.

Key to Formicidae of the Dr. Howard Reynolds Nature Trail

Key to Subfamilies

1a – Petiole with only 1 segment	go to 2
1b – Petiole with 2 segments (petiole and postpetiole)	go to 4





2a – Petiole segment similar in shape to gaster. No eyes. Sting present _____

	Subfamily Amblyoponinae
2b – Petiole segment distinct	go to 3





3a – Tip of gaster with an acidopore (projecting tube possibly with ring of hairs). Petiole segment large

and peduncle sharp _____

____Subfamily Formicinae

3b – Tip of gaster with a slit and no acidopore. Petiole segment small and peduncle fairly flattened _____

_Subfamily Dolichoderinae





4a – Eyes well developed, antennal sockets spaced apart _____Subfamily Myrmicinae

4b – Eyes not well developed. Antennal sockets closely approximated ______

_Subfamily Dorylinae





Appendix D. Dichotomous key to ants in the three most prevalent subfamilies of ants at the Dr. Howard Reynolds Nature Trail area. This key was based off of ants collected in 2017 and 2018 for use in 2019 pitfall trapping.

Key to Formicinae genera

1a – Large in size, yellow to orange in color, 3 well developed ocelli on forehead ______ *Formica* sp.
1b – Medium to small in size, brownish to yellow in color, ocelli either absent or pale colored and not obvious ______ go to 2



2a – Yellow to brown-orange in color, body compact, posterior of mesosoma (propodeum) steeply slanted like a slide, head wider at the dorsum than near the mouth ______ *Lasius* sp.
2b – Variant of brown in color, propodeum quadrate to round but not slanted like a slide, head just as wide at dorsum as near mouth ______ NOT Formicinae. See Subfamily

Dolichoderinae





☆ If the ant has an acidopore but doesn't match the descriptions of *Formica* or *Lasius* spp., label it as "Unknown Formicinae" (UKNF) and save it.
Key to Dolichoderinae genera

- 1a Propodeum with an upright cone _____ *Dorymyrmex* sp.
- 1b Propodeum without an upright cone _____ go to 2





NOT Dolichoderinae. See Subfamily Formicinae

2a – Variant of brown in color, head rectangular, eyes close to antennae _____ Forelius sp.

2b - Yellow to brown-orange in color, head shield shaped, eyes near the dorsum of the head



☆ If the ant doesn't match the descriptions of *Dorymyrmex* or *Forelius* spp., label it as "Unknown Dolichoderinae" (UKND) and save it.

Key to Myrmicinae genera

 1a – Gaster heart-shaped, postpetiole is short and round compared to petiole and connects to the dorsum

 of the gaster, head round _______ Crematogaster sp.





2a – Large in size, long-legged, reddish in color, head oval in shape, mesonotum steeply slanted so that pronotum is much higher than the propodeum, petiole pick-shaped, postpetiole round, antennal scape with a cleaver-shaped lobe hear socket ______ *Aphaenogaster ashmeadi*2b – Not matching above description ______ go to 3



3a - Very small in size, black in color, antennal clubs present, petiole and postpetiole tall with rounded

apexes like mountains	Monomorium minimum
3b – Not matching above description	go to 4



4a – Yellow to orange in color, eyes small, head long ______ Solenopsis

molesta

4b – Orange to brown in color, pronotum raised like a hump, antennal club present _____ *Pheidole* sp.





☆ If the ant doesn't match any of these ants, label it as "Unknown Myrmicinae" (UKNM) and save it.

Appendix E. Quick-reference guide to common ants found at the Dr. Howard Reynolds Nature Trail area used during pitfall trapping in 2019. Ants in this key were based off of ants collected in pitfall traps in both 2017 and 2018. "*Stenamma*" was a misidentified small morph of a *Pheidole* minor worker.

Ants with 2 petiole segments: *Monomorium minimum*



- very small and always black
- mountain-like petiole and postpetiole

Crematogaster sp.



- heart-shaped gaster and round head
- postpetiole connects to the top of the gaster

<u>Aphaenogaster ashmeadi</u>



- large with long legs
- steeply slanted mesosoma

Pheidole sp. (minor worker)



- very small, propodeum armed with spines
- 3 segmented antennal club

<u>Solenopsis molesta</u>



- very small and yellow, head long, eyes small
- propodeum unarmed

<u>Stenamma sp.</u>



- very small and yellow
- propodeal spines small and upright
- 3 segmented antennal club

Ants with 2 petiole segments (continued): *Neivamyrmex* sp.

<u>Stigmatomma pallipes</u>



- reduced eyes
- petiole and postpetiole similar in shape

Ants with 1, small petiole segment:

Dorymyrmex sp.



- upright cone on propodeum
- varies in color

Forelius pruinosus



- propodeum quadrate and unarmed
- size and color varies
- body covered in flat pubescence
- similar to Lasius but eyes are low on head



- no eyes, petiole indistinct

<u>Forelius mccooki</u>



- similar to F. pruinosus but body covered in

erect hairs, also smaller

Ants with 1, large petiole segment:

Formica sp.



- large and normally shiny
- 3 well-developed ocelli

Lasius sp.



- propodeum steeply slanted like a slide
- color varies
- similar to Forelius but eyes are higher on head

<u>Nylanderia sp.</u>



- body and head covered in erect hairs
- scapes long

Appendix F. The number of individual ants in each functional group captured in each of the 30 pitfall traps. Data were organized based on the year that trapping was done (before or after prescribed burning), and also whether pitfall traps were in the uphill or downhill microhabitat. Once organized, these data were analyzed using the Scheirer Ray Hare test of variance (CC = Cold Climate Specialists, CS = Cryptic Species, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators).

Burn Treatment	Micro- habitat	Counts CC	Counts CS	Counts DD	Counts GM	Counts OP	Counts SP
Before	Uphill	1	0	28	516	2414	0
Before	Uphill	0	0	11	2	9215	11
Before	Uphill	0	0	24	86	294	0
Before	Uphill	0	0	56	38	0	0
Before	Uphill	0	0	23	38	0	0
Before	Uphill	1	0	62	13	1	0
Before	Uphill	0	0	33	29	0	0
Before	Uphill	0	0	40	52	136	35
Before	Uphill	0	0	80	33	0	0
Before	Uphill	0	0	92	59	0	0
Before	Uphill	0	0	16	28	24	67
Before	Uphill	0	0	131	54	0	0

Before	Uphill	0	0	27	53	2	0
Before	Uphill	0	0	36	52	0	0
Before	Uphill	0	0	76	141	0	0
Before	Downhill	0	0	6	29	4	0
Before	Downhill	0	1	48	47	2	0
Before	Downhill	0	0	16	27	1	0
Before	Downhill	0	0	8	26	2	1
Before	Downhill	0	0	0	51	1	0
Before	Downhill	0	0	8	78	3	1
Before	Downhill	0	0	6	25	0	0
Before	Downhill	14	0	10	44	1	0
Before	Downhill	3	0	40	65	0	0
Before	Downhill	21	0	40	96	0	0
Before	Downhill	6	0	11	198	2	0
Before	Downhill	5	0	3	128	3	1
Before	Downhill	13	0	8	181	16	1
Before	Downhill	5	1	6	156	10	0
Before	Downhill	17	0	2	110	5	0

After	Uphill	0	0	14	19	0	0
After	Uphill	0	0	23	35	0	0
After	Uphill	0	0	12	36	1	0
After	Uphill	1	0	11	98	5	0
After	Uphill	1	0	11	62	1	0
After	Uphill	1	0	41	68	0	0
After	Uphill	2	0	26	68	0	0
After	Uphill	0	0	77	66	0	0
After	Uphill	0	0	4	19	0	0
After	Uphill	0	0	5	13	0	0
After	Uphill	0	0	28	37	0	0
After	Uphill	0	0	31	63	0	2
After	Uphill	0	0	9	26	0	8
After	Uphill	0	0	25	81	0	0
After	Uphill	1	0	7	76	0	0
After	Downhill	0	0	11	25	4	0
After	Downhill	0	0	7	32	11	0
After	Downhill	0	0	10	23	4	0

After	Downhill	0	0	14	24	2	0
After	Downhill	2	0	12	25	3	0
After	Downhill	6	1	12	15	1	0
After	Downhill	82	0	24	30	79	0
After	Downhill	2	2	15	56	0	0
After	Downhill	0	0	5	33	3	0
After	Downhill	19	0	3	23	3	0
After	Downhill	4	0	28	38	0	0
After	Downhill	3	0	0	102	0	0
After	Downhill	33	0	3	54	0	0
After	Downhill	3	0	6	98	8	0
After	Downhill	7	0	5	41	7	0

Appendix G. The number of different ant genera in each functional group captured in each of the 30 pitfall traps. Data were organized based on the year that trapping was done (before or after prescribed burning) and also whether pitfall traps were in the uphill or downhill microhabitat. Once organized, these data were analyzed using the Scheirer Ray Hare test of variance (CC = Cold Climate Specialists, CS = Cryptic Species, DD = Dominant Dolichoderinae, GM = Generalized Myrmicinae, OP = Opportunists, SP = Specialist Predators).

Burn Treatment	Microhabitat	Richness CC	Richness CS	Richness DD	Richness GM	Richness OP	Richness SP
Before	Uphill	1	0	1	2	1	0
Before	Uphill	0	0	1	1	1	0
Before	Uphill	0	0	1	3	1	1
Before	Uphill	0	0	1	2	0	0
Before	Uphill	0	0	1	2	0	0
Before	Uphill	1	0	1	2	1	0
Before	Uphill	0	0	1	3	0	0
Before	Uphill	0	0	1	2	1	1
Before	Uphill	0	0	1	3	0	0
Before	Uphill	0	0	1	3	0	0
Before	Uphill	0	0	1	2	1	0
Before	Uphill	0	0	1	2	0	0

Before	Uphill	0	0	1	3	2	0
Before	Uphill	0	0	1	2	0	1
Before	Uphill	0	0	1	2	0	0
Before	Downhill	0	0	1	2	1	0
Before	Downhill	0	1	1	2	1	0
Before	Downhill	0	0	1	2	1	0
Before	Downhill	0	0	1	3	1	1
Before	Downhill	0	0	0	2	1	1
Before	Downhill	0	0	1	2	1	0
Before	Downhill	0	0	1	2	0	0
Before	Downhill	1	0	1	3	1	0
Before	Downhill	2	0	1	2	0	0
Before	Downhill	1	0	1	2	0	1
Before	Downhill	1	0	1	2	1	0
Before	Downhill	1	0	1	2	1	1
Before	Downhill	2	0	1	2	1	0
Before	Downhill	2	1	1	2	1	0
Before	Downhill	2	0	1	2	1	0

After	Uphill	0	0	1	2	0	0
After	Uphill	0	0	1	2	0	0
After	Uphill	0	0	1	2	0	0
After	Uphill	1	0	1	2	0	1
After	Uphill	1	0	1	2	0	1
After	Uphill	1	0	1	2	0	0
After	Uphill	1	0	1	3	0	0
After	Uphill	0	0	1	4	0	0
After	Uphill	0	0	1	2	1	0
After	Uphill	0	0	1	3	1	0
After	Uphill	0	0	1	2	1	0
After	Uphill	0	0	1	3	0	0
After	Uphill	0	0	1	4	0	0
After	Uphill	0	0	1	3	0	0
After	Uphill	1	0	1	3	0	0
After	Downhill	0	0	1	2	1	0
After	Downhill	0	0	1	2	1	0
After	Downhill	0	0	1	2	0	0

After	Downhill	0	0	1	2	0	0
After	Downhill	1	0	1	2	0	0
After	Downhill	2	0	1	3	1	0
After	Downhill	1	0	1	3	1	0
After	Downhill	1	1	1	3	1	0
After	Downhill	0	0	1	3	1	0
After	Downhill	2	0	1	2	1	0
After	Downhill	1	0	1	2	1	0
After	Downhill	1	0	0	2	1	0
After	Downhill	1	0	1	2	2	0
After	Downhill	1	0	1	3	0	0
After	Downhill	1	0	1	2	1	0

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