BIODIVERSITY OF ANTS (HYMENOPTERA: FORMICIDAE) IN RESTORED GRASSLANDS OF DIFFERENT AGES

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

BIODIVERSITY OF ANTS (HYMENOPTERA: FORMICIDAE) IN RESTORED GRASSLANDS OF DIFFERENT AGES

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ABSTRACT

Grasslands are an endangered ecosystem. Unfortunately, few studies monitoring the health of these natural resources have examined arthropods, thus leaving out a vital biodiversity component. Ants have been proven to be reliable indicators of restoration success in some habitats. Literature regarding the benefits of ants in ecosystems is abundant; however, studies examining ant grassland ecology are limited. The availability of Conservation Reserve Program (CRP) land of different ages allowed us to use the 'space-for-time substitution' approach as an alternative for a long-term study on ant succession. This provides an ample opportunity to examine succession of ants in disturbed grasslands. Ant diversity (richness and abundance), species composition, and functional groups were investigated utilizing four sampling techniques (pitfall traps, litter samples, hand collection, and soil core sampling) on four different ages (0, 3, 7-8, 14-16 yrs) of grassland in east-central Missouri. Efficacy of sampling methods was also examined. A total of 18,743 ants were collected, representing 28 species in 16 genera. Ants were most abundant in older ages of CRP land. Species richness peaked in 7-8 yr fields. Some species showed patterns of being either early colonizers, late colonizers, or present in all field ages. The functional groups Cold Climate Specialists, Opportunists, Cryptic species, and Generalized Myrmicines (in descending order) dominated CRP land. Pitfall traps were the most effective sampling method. The results of this study provide

baseline information on how ants establish on restored grassland in the CRP and provide information for future comparative studies.

Chapter 1
Literature Review

Ants are diverse organisms that have an impact on their surrounding environment (Hölldobler and Wilson 1990, Andersen and Sparling 1997, Majer and Nichols 1998, Peck et al. 1998, Lobry de Bruyn 1999, Agosti et al. 2000). They are one of the dominant organisms on land (Agosti et. 2000). If all the world's ants were combined, it is estimated that they would weigh about as much as all human beings (Hölldobler and Wilson 1994). They also participate in every part of the trophic system (Carroll and Janzen 1973, Trager 1998). They play a major role in dispersing seeds for many plant species (Berg 1975, Beattie, 1985, Willson et al. 1990), are the chief predators of insects and other arthropods (Mirenda et al. 1980, Youngs 1983, Porter and Savignano 1990), and other invertebrates (Whitcomb et al. 1973, Jackson et. 1998), and vertebrates prey on them for food (Milne and Milne 1950, Taigen and Pough 1983, Reiss 2001). Ants circulate and aerate more soil in the tropics than do earthworms, thus moving nutrients throughout the landscape (Lobry de Bruyn and Conacher 1990, Hölldobler and Wilson 1994). In a study of *Formica cinerea montana* Emery, (Baxter and Hole 1967) found that mineral soil in the upper half to two-thirds of a representative mound consists of about 85% B horizon material. Ants also fill diverse niches including soil (Tschinkel 2003), rotting logs (Chen et al. 2002), trees (Djieto-Lordon and Dejean 1999), leaf litter (Leponce et al. 2004), acorns (Pratt and Pierce 2001) and twigs (Armbrecht and Ivette Perfecto 2003).

Ants have mutualistic relationships with many plant and animal species. The carnivorous pitcher plant, *Nepenthes bicalcarata* Hook houses *Camponotus* sp. worker in

its tendrils and feeds the ants captured prey that has fallen into the pitcher (Clark and Kitching 1995). The ants easily run up and down the slick walls and swim in the pitcher's digestive juices retrieving prey. In return, the ant prevents accumulation of organic matter, which would lead to the pitcher rotting (Clark and Kitching 1995). The oak-feeding aphid *Stomaphis quercus* Linnaeus only occupies trees that are within 17 m of the nests of the ant *Lasius fulignosus* Latreille and is strongly associated with trees that housed these ants (Hopkins and Thacker 1999). The ants receive a nutritious food award of honeydew and in return, the ants protect the aphids from potential predators (Buckley 1987, Hopkins and Thacker 1999).

Ants have adapted to both cold and hot conditions (Cerdá et al. 1998). Hölldobler and Wilson (1994) observed the active foraging of ants on their visits to Finland in mid-May, north of the Artic Circle, in 12 °C . In contrast, the Saharan silver ant, *Cataglyphis bombycina* Smith initiates foraging at surface temperatures exceeding 45 °C when most desert ants discontinue foraging as surface temperatures exceed 35-45 °C (Wehner et al. 1992).

Adult Morphology

Ants are ecologically diverse organisms (Hölldobler and Wilson 1990, Bourke and Franks 1995) which have a similar body shape. Variations in morphological structures have evolved enabling different species to fill appropriate niches (Ettershank 1966, Mayhe and Caetano 1994). Ants range in size from a few millimeters or less (e.g., one of the smallest ants in the world is in the genus *Brachymyrmex* at only 1.5 mm in length) to over a centimeter (e.g., one of the largest ants in the world is the Malaysian

giant forest ant, *Camponotus gigas* Latreille where the mean body length of the major worker is 28.1 mm (Pfeiffer 1996).

Ants, as other Aculeata Hymenoptera, have a unique condition in that a constriction exists posterior to where the abdomen is fused to the thorax. Since the first abdominal segment is attached to the thorax, the two posterior body segments are known as the mesosoma, and metasoma (often referred to as the gaster). The mesosoma is made up of the pronotum, mesonotum, and propodeum. The mesosoma is attached to the metasoma by one or two nodes, termed the petiole and post-petiole, respectively.

Ants have long, distinctly elbowed antennae consisting of a long scape and a series of antennal segments referred to as the funicle. In some species, the terminal segments of the funicle are enlarged and are referred to as the antennal club. Antennae typically have from six to twelve segments. The typical mandibular shape of ants is triangular with a smooth external margin and an internal masticatory margin with a variable number of teeth and denticles. In some species, mandibular shape has been modified to fit their proposed function (Wheeler 1910, Bolton 1994). Species of the genus *Polyergus*, an obligatory slave-making ant, has sickle-shaped mandibles to efficiently kill the opposing *Formica* in pupal brood raids (Trager and Johnson 1985). The tribe Dacetini has evolved a mandibular trap mechanism referred to as trap-jaws (Gronenberg 1996). The mandibles which are long, thin, and linear; with an apical fork of 2 or 3 spine-like teeth are held open awaiting prey (typically collembola); when hairs are triggered the mandibles abruptly close and catch the prey (Gronenberg 1996). Eyes can be vestigial, absent (i.e., subfamily Dorylinae), or well developed with several hundred ommatidia (i.e., subfamily Pseudomyrminae) (Creighton 1950).

The alates of ants have four membranous wings, the front wings being larger than the hind wings. The mesosoma has long gressorial/cursorial legs. The tip of the abdomen can have a stinger (Myrmicinae subfamily) or an orifice, which releases formic acid (Formicinae subfamily). While most ants are dark or have earth tone colors ranging from brown, black, reds, and golden browns some species are more colorful with green, blue, and purple (i.e., Green-head ants from Australia, *Rhytidoponera metallica* Smith) (Ward 1986).

Life Cycle of Colony

The life cycle of an ant colony was compared to a perennial flowering plant by Hölldobler and Wilson (1990) because the colony periodically offers a crop of seeds (i.e., reproductives), then returns to a solely vegetative (i.e., worker) growth. This comparison is described by the progression of the ant colony through three stages: founding, growth, and reproduction (Oster and Wilson 1978).

The founding stage begins when reproductives depart from their nest of origin (Hölldobler and Wilson 1990). The emergence of alates follows one of two distinct mating patterns (Hölldobler and Bartz 1985). The male-aggregation syndrome is the predominant mode of dispersal (Hölldobler and Bartz 1985). In this mode, males swarm to a prominent feature in the landscape. Female alates then begin their nuptial flight by flying into the male swarm and mating in flight. Less common in ants is the female-calling syndrome (Hölldobler and Bartz 1985). In contrast to the male-aggregation syndrome, the queen is wingless and releases pheromones from the ground or low-lying vegetation. She then waits for the male to find and mate with her. After mating, the queen sheds her wings (if applicable), establishes a nest gallery, and initiates egg laying.

Males typically die within 24 hours of mating. Most species are claustral in that the queen remains enclosed in her gallery and feeds her first brood solely with her body's reserves (Keller and Passera 1989, Wheeler and Buck 1996). Some species are partially claustral in that they leave their nesting gallery to forage during the founding stage (Keller and Passera 1989). This behavior was thought to occur in only primitive ants (i.e., *Amblyopone* and *Myrmecia*) (Haskins and Haskins 1951) but recent studies have shown this behavior in higher ants also (Lenoir and Dejean 1994, Johnson 2002)

The founding stage is dangerous for queens. Many die during the nuptial flight, after alighting on the ground, or in the founding gallery (Whitcomb et. al. (1973), Hölldobler and Wilson 1990, Nichols and Sites 1991). Queens have to evade many enemies to establish a successful colony. Whitcomb et al. (1973) studied queens of the red imported fire ant, *Solenopsis invicta* Buren, and observed dragonflies and birds feeding on queens in mid-air and ants, earwigs, tiger beetles, and wolf spiders feeding on queens on the ground. Nichols and Sites (1991) found predation on *S. invicta* occurred inside enclosed nesting galleries by both conspecifics and 17 other ant species. Many queens also die from abiotic factors (e.g., desiccation) (Hadley 1994, Johnson 2000).

Some species have multiple queens (polygyny) within a single colony whereas other species only have a single queen (monogyny). When one queen founds a nest it is called haplometrosis. If two queens found the nest, it is called pleometrosis. Pleometrosis is advantageous since the founding period is dangerous (Bernasconi and Strassman 1999, Johnson 2004) and it increases the chances for colony survival since twice as many workers are produced (Bartz and Hölldobler 1982 ,Nonacs 1990). Depending on the species, as colony size increases, the dominant queen may kill

supernumerary queens or additional queens may be added (Hölldobler and Wilson 1977, Forsyth 1980).

Some ant queens are wingless or not capable of extended flights. In such cases the dispersal method used is termed budding (Peeters and Ito 2001). Budding is a process where queens leave the main nest with a group of worker ants and brood to establish a new colony (Peeters and Ito 2001). Budding is unpredictable in time and more research is needed to determine the cues for budding (Harris 2002). Buczkowski et al. (2005) found that applying cypermethrin, a repellent pyrethroid insecticide, resulted in colony budding in Pharaoh ant, *Monomorium pharaonis* (Linnaeus). The Argentine ant, *Linepithema humile* (Mayr) is a highly invasive ant species that uses budding as its mode of dispersal (Rust et al. 2003). Although this mode limits physical distance of dispersal, it is advantageous in that the workers aid the queen in establishing a nest, rearing the brood, and foraging (Peeters and Ito 2001). New queens typically establish their nest near the original nest and both remain connected, often sharing workers (Harris 2002). Over time, this web of interconnecting nests grows into an infestation that may cover many hectares, (Harris 2002) but the rate of budding is relatively slow averaging 150m/year (Suarez et al. 2001).

Species-specific diel rhythm and weather conditions influence the timing of nuptial flights (Hölldobler and Wilson 1990, Kipyatkov 1993). Whitcomb et al. (1973) found in their study of *S. invicta* that nuptial flights usually occurred during periods of high humidity following rainfall after weeks of drought. Boomsma and Leusink (1981) found in their study of four European ant species that light intensity and temperature (varied among species) triggered initiation of nuptial flights.

During the growth stage, a queen's role becomes specialized in that she only lays eggs while workers provision for her and the colony (Hölldobler and Wilson 1990). The first workers, nanitics or minims, are usually timid and smaller than minor workers in older colonies of conspecifics (Porter and Tschinkel 1986, Tschinkel 1993a). Nanitics are small due to lack of nutrients provided by the queen, and their reduced size allows the queen to raise more workers with limited body reserves. Subsequent generations of workers are larger. Worker ants forage for food, enlarge the colony, tend to young, and defend the nest. If the colony survives through development of the first and second broods, known as the precarious period, the colony will enter a period of exponential growth (Hölldobler and Wilson 1990). As the colony grows, a division of labor occurs.

The reproductive stage begins when the colony produces alates. This stage may begin after a single warm season for some species, while others take up to five or more years for reproductives to be produced (Hölldobler and Wilson 1990). Factors such as time of year and food availability controls when the colony produces alates (Rockwood 1975, Tschinkel 1993b). Nuptial flights usually occur in summer and fall (Talbot 1945, Lofgren et al. 1975). Some colonies produce only males in a season, some only females, and some both (Nonacs 1986).

Life Cycle of Ants

Ants go through a complete metamorphosis that includes four stages of development: egg, larva, pupa, and adult. Larvae emerge from minute, oval shaped eggs with a sticky surface that adheres them together for easy transport by workers. The larvae, termed vermiform (Triplehorn and Johnson 2005), are white, grub-like, legless, and shaped as a crook-necked squash (Wheeler and Wheeler 1963) with the posterior

portion swollen and straight. First-instar larvae feed via trophallaxis from workers and/or trophic eggs (Masuko 2003, Perry and Roitberg 1996, Cassill and Tschinkel, 1999). Trophallaxis continues throughout larval development but they are also fed pieces of insects or seeds depending on the natural food source of the particular species (Handel and Beattie 1990, Hölldobler and Wilson 1990). Larvae go through several instars before reaching the pupal stage. The prepupae are larvae that have ceased feeding in preparation for pupation (Wagner 1993, Fraser et al. 2001). When the prepupal exuvium is shed, the pupal stage is initiated (Wheeler and Wheeler 1963). The pupal type of ants is termed exarate because appendages are free and not glued to the body (Triplehorn and Johnson 2005). All Myrmicinae and Dolichoderinae have naked pupae whereas Ponerinae and most Formicinae have cocoons (Wheeler and Wheeler 1963). Wheeler and Wheeler (1963) state that the genus *Formica* have both naked pupae and pupae in cocoons in a single nest. Emerged adults are initially pale in color and take several days to fully sclerotize and darken their exoskeleton.

The developmental cycle from egg to adult varies depending on species, available food, and temperature. It takes an average of 35-89 days to complete (Newell and Barber 1913, Peacock et al. 1959, Bruder and Gupta 1972, Mallis 1990). The average worker ant egg incubation period is 12 to 30 days (Newell and Barber 1913, Peacock et al. 1959, Bruder and Gupta 1972, Mallis, 1990). Several larval instars follow this for a period of 10 to 34 days (Newell and Barber 1913, Peacock et al. 1959, Bruder and Gupta 1972, Mallis 1990). The prepupal stage then takes 2 to 3 days followed by an 11 to 23 day pupal stage after which the young ant emerges as an adult (Newell and Barber 1913, Peacock et al. 1959,Bruder and Gupta 1972, Mallis 1990).

Division of Labor

A conspicuous biological trait of ants is a defined caste system that divides labor between different adult forms (Oster and Wilson 1978). Ant colonies typically have three distinct castes: workers (sterile females), reproductive females (queens), and reproductive males. Queens lay eggs and workers care for the colony. The male's only function is mating.

Labor is divided amongst worker castes by age polytheism and physical polymorphism (Bourke and Franks 1995). Age polyethism is the changing of labor roles by colony members as they age (Hölldobler and Wilson 1990) whereas; physical polymorphism indicates that workers have distinct anatomical sizes and shapes which enable them to do specialized tasks (Oster and Wilson 1978, Bourke and Franks 1995). In age polytheism, newly emerged workers tend to the developing young while the older ants forage for and defend the colony (Bourke and Franks 1995). There can be flexibility in age castes. A lab study of the minor workers of *Pheidole dentata* Mayr showed that when young minor workers were excluded from the colony, older minor workers could successfully complete the work of both old and young workers (Calabi and Traniello 1989).

Typical ant colonies are monomorphic in that they only have a single worker size. Conversely, physical polymorphism occurs in about 20% of ant genera (Bourke and Franks 1995). When there is either a major or minor worker size, the colony is referred to as being dimorphic whereas if there are three or more worker sizes it is considered polymorphic. Most colonies have only two or three different worker castes, although

four castes have been documented in *Atta sexdens* Linnaeus (Wilson 1980). The different worker castes have specialized functions. Major workers undertake more defense and heavy work. Colonies of *Messor* sp. deploy major workers when bigger or preferred seeds are available (Heredia and Detrain 2005). Helms (1995) found major workers of *Pheidole desertorum* Wheeler foraged when food is abundant and clumped. Army ant majors serve as guards for the colony with pincher-like mandibles held open for the raiding column and queen when they vacate the nest during their nomadic stage (Schneirla 1956).

Communication

Ants live in eusocial communities (Bourke 1988). Eusociality is characterized by adults caring for immatures, overlapping of generations in the same nest, and a division of labor into reproductive and nonreproductive castes (Hölldobler and Wilson 1994). In order for the colony to flourish, ants must be able to communicate effectively with each other (Hölldobler and Wilson 1990). Communication is necessary in all aspects of colony life from foraging (Cross et al. 1979, Hölldobler et al. 1982), defending the nest (Kugler 1979), sending out alarms (Markl 1965, Kugler 1979, Droual and Topoff 1981), mating (Cherix et al. 1993, Ayasse et al. 2001), and recruitment of nest mates (Hölldobler et al. 1982, Roces 1993).

Ants communicate using both mechanical and chemical methods (Dumpert 1978). The drumming of body parts against a substrate and stridulation are two mechanical signals used by ants (Fuchs 1976, Markl et al. 1977). Ants living in wooden substrates usually rock back and forth drumming their mandibles and gaster on the surface creating vibrations in the wood (Hölldobler and Wilson 1990). In *Camponotus*, Fuchs (1976)

found that vibrations made by the gaster and mandibles on wood could be perceived by nestmates at distances of 20 cm or more. Stimuli such as air currents, sound, touch, or chemical contaminants may cause ants to begin drumming (Markl and Fuchs 1972). Stridulation of one body part against another occurs using a file and scraper (Dumpert 1978). The file and scraper are located on the fourth (first segment of gaster) and third abdominal segments respectively. When rubbed together, they produce sounds that the human ear can hear (Schilliger and Baroni Urbani 1985, Hölldobler and Wilson 1990). In a study of *Atta cephalotes* Linnaeus, Markl (1965) reported that workers would dig out a buried worker who was stridulating. The subfamilies Ponerinae, Nothomyrmeciinae, Pseudomyrmecinae, and Myrmicinae all exhibit this sound production capability (Markl 1973).

Chemical communication relies on the presence of various exocrine glands that release pheromones. The six most important are Dufour's gland, the poison gland, the pygidial gland, sternal glands, mandibular glands, and metapleural glands (Hölldobler and Wilson 1990). These six exocrine glands occur widely in Formicidae which serve various functions and house various chemicals across species (Hölldobler 1995). Some glands are lacking in certain subfamilies (i.e., Formicinae lacks the pygidial gland with the exception of the genus *Polyergus* (Hölldobler and Wilson 1990). The Dufour's gland mediates alarm, recruitment, and sexual attraction; poison gland produces formic acid or venom used in predation and defense; pygidial gland releases alarm pheromone, defensive substances, or both; sternal glands secrete trail, orientation, and recruitment pheromones; and mandibular glands mediate defense and alarm communication (Hölldobler and Wilson 1990). The metapleural gland function is uncertain at this time.

Studies of this gland have reported that its pheromones are used for recognition and identification of nestmates and alien species (Brown 1968), territorial markers (Jaffe and Puche 1984), and as an antiseptic substance (Maschwitz 1974). Ants receive pheromone information from olfactory receptors located in antennal sensilla (Kleineidam et al. 2005). Pheromones are also exchanged by compounds mixed with food and exchanged via trophallaxis, which establishes colony odor (Dahbi et al. 1999).

Taxonomy

Ants are classified in a single family (Formicidae) in the order Hymenoptera and are closely related to wasps and bees. Currently, ants are classified in 283 genera in 21 subfamilies (Bolton 2003). In total, 11,914 ant species are currently described (Agosti and Johnson 2006). The number of undescribed species is immense, consequently, constant taxonomic revisions are necessary in all higher taxa, and as many as 20,000 ant species may exist (Hölldobler and Wilson 1990). About 580 of the described species are found in the Nearctic region (North America north of Mexico) (Smith 1979, Hölldobler and Wilson 1990).

Seven ant subfamilies (Cerapachyinae, Dolichoderinae, Dorylinae, Formicinae, Myrmicinae, Ponerinae, and Pseudomyrminae) were recognized by Wheeler (1922), which continued for 50 years, until three additional subfamilies were proposed (Wheeler & Wheeler 1972). As the importance of additional characters has been considered, new subfamilies have been described to the point that currently, 21 subfamilies are recognized (Bolton 2003).

The Ants of North America by Creighton (1950) is still the most complete and most used key for Nearctic ants. Supplemental keys are necessary for correct

identification of some ant species (Wheeler and Wheeler 1986). Revision is necessary in some of the larger groups including *Pheidole, Camponotus, Crematogaster, Iridomyrmex,* and *Solenopsis* (Hölldobler and Wilson 1990). *Myrmica* is a taxonomically difficult genus and is currently under revision by Franceour, and many species of *Myrmica* have not yet been formally described (Wheeler and Wheeler 1986).

Although ant taxonomy is unresolved, there is a fairly good taxonomic base when compared to other organisms that are often used in biodiversity studies (i.e., Collembola and mites). Many advancements in ant taxonomy have occurred recently with the publications of Bolton (1994, 1995, 2003) and Hölldobler and Wilson (1990). Many websites also exist with excellent illustrations and keys, and databases of primary taxonomic publications, such as *Antbase* (Agosti and Johnson 2006) and *AntWeb* (California Academy of Sciences 2002-2006).

Functional Groups

Ants are excellent candidates for biodiversity studies because of their high richness, numerical dominance, a good taxonomic base, ease of collection, stationary nesting habits that allow them to be resampled over time, sensitivity to environmental change, and interactions with other organisms (Agosti et al. 2000). Ants are often categorized into functional groups. A functional group allows predictability in the ant's reaction to stress and disturbance (Phillips 2006). Seven functional groups have been identified on restored mine sites in Australia (Andersen 1995, Andersen 2000, Andersen and Majer 2004) and parallel studies have been conducted in Arizona (Andersen 1997a). The seven functional groups these authors identified are: 1) Dominant Dolichoderinae,

2) Subordinate Camponotini, 3) Climate Specialists, 4) Cryptic species 5) Opportunists, 6) Generalized Myrmicinae, and 7) Specialist Predators.

Dominant Dolichoderinae (i.e., *Iridomyrmex*) inhabit hot, open areas of low disturbance. *Iridomyrmex* dominates hot, open areas in Australia (Andersen 1997a, Andersen 2000). In cool-temperate North America, dominant Dolichoderinae are absent in most habitats and cold climate specialist Formicinae dominate (Andersen 1997a, Andersen 2000). In Australia where *Iridomyrmex* are present, dominant Formicinae are absent (Andersen 1997a, Andersen 2000). Subordinate Camponotini (i.e., *Camponotus*) are usually behaviorally submissive to dominant Dolichoderinae. Cryptic species are small ants that nest and forage primarily in soil, litter, and rotting logs. The group is typically comprised of Myrmicinae and Ponerinae (Andersen 1997a, Andersen 2000). Opportunists are poorly competitive and predominate at sites that are highly disturbed and where behavioral dominance by other ants is low (Andersen 1997a, Andersen 2000). Generalized Myrmicinae are among the most abundant ants (i.e., *Crematogaster, Monomorium, and Pheidole*) in warm areas and predominate in environments experiencing moderate levels of stress or disturbance (Andersen & Majer 2004). They are often competitive with dominant Dolichoderinae (Andersen 1997a, Andersen 2000). Specialist predators (i.e., *Pachycondyla* and *Leptogenys*) have little interaction with other ants except for direct predation (Agosti et al. 2000).

Conservation Reserve Program Land

United States grasslands have declined by more than 98% since European settlement listing them as a critically endangered ecosystem (Noss et al. 1995). The increasing need to protect biodiversity is becoming more apparent. Natural ecosystems

in which plant and animal species live are experiencing increased degradation, destruction, and fragmentation. Tallgrass prairies once covered 15 million acres of Missouri (Missouri Department of Conservation 1999). Of those millions, only 90,000 acres of native prairie remain (Missouri Department of Conservation 1999). Humans have dramatically modified grassland ecosystems by converting the majority of natural grasslands to agriculture land (Iverson 1988, Warner 1994). Since the introduction of the steel blade plow in 1837 and the high yield mechanical and chemical cultivation practices that followed (Warner 1994, Hurt 2002), the prairies' nutrients have been depleted and erosion is higher (National Research Council 1992, Compton and Boone 2000). This land-use change has given us crops for food but anthropogenic effects have increased fragmentation of land and erosion of soil. Urbanization is also a key component in the decline of grasslands. As cities proliferate, asphalt, cement and other impermeable surfaces are replacing floodplains. Thus, forcing rain to runoff into our water systems instead of being soaked into the environment (National Research Council 1992, Naiman et al. 1995).

Many conservation programs now exist to help conserve natural resources, one of which is the Conservation Reserve Program (CRP). CRP is the federal government's largest private land retirement program (Johnson 2005). The primary objectives of CRP are to take erodible or eroding lands from agricultural production by establishing perennial vegetation, and to enhance habitat for fish and wildlife populations. It is also hoped that the reduction in soil erosion will reduce sedimentation of streams, and thus improve water quality (Johnson 2005). The CRP program began in 1985 and is administered by the Farm Service Agency of the U. S. Department of Agriculture

(USDA), with the Natural Resources Conservation Service (NRCS) providing technical land eligibility determinations, Environmental Benefit Index scoring, and conservation planning (Johnson 2005). This voluntary program uses financial incentives to encourage farmers and ranchers to enroll in CRP contracts of 10 to 15 years.

Succession of Land

Ecological succession is the change in species composition and community structure after fire, heavy grazing, flooding, or other natural or human-related disturbance (Smith 1996). After disturbance, land goes through numerous stages where a distinctive flora and fauna are present for one year to several decades along a successional continuum. Eventually, the land will reach a climax community (Phillips 1935). Development from fallow agriculture land to grassland on CRP fields is an example of secondary succession (Horn 1974), since plants were previously present. Fallow agricultural land is prone to erosion and settling (Lal 2001) and plants that first colonize these sites are adapted to anthropogenic disturbance. These plants typically establish during what is termed the pioneer stage (Collins and Adams 1983). Some pioneer grassland plants are foxtail, mare's tail, ragweed, tall fescue, and Eurasian creeping clovers (*Trifolium*) (Trager 2005). Plants that colonize disturbed areas must also have a well established seed bed in the soil that is viable for many years (Phillips 1935). Pioneer plants typically have a short life cycle and produce many seeds (Phillips 1935). After a few years plants with longer life cycles eventually take over and push out pioneer plants (Phillips 1935).

Plant composition is not the only thing that changes through succession. Soil composition also changes (Lal 2001). There is twice as much biomass in prairie soil than

an agricultural field due to the larger aggregates of soil which provide a larger surface area for organisms to live (ants, mychorrhizal fungi, mites, springtails, etc.) (Blumberg 1999). Prairie soils contain more nutrients than agricultural soils because prairie soils have a bigger nutrient load from the break-down of organic matter by bacteria and fungi (Blumberg 1999).

CRP land is aided in succession because a seed mix of dominant warm season grasses (i.e., Big bluestem, *Andropogon gerardii* Vitman; Little bluestem *Andropogon scoparius* Michx; Indian grass *Sorghastrum nutans* (L.) Nash) or cool season grasses (i.e., Tall fescue, *Festuca arundinacea* Schreb.; Orchard grass, *Dactylis glomerata* L.; Kentucky blue grass, *Poa pratensis* L.) are initially planted on the land. CRP farmers manage their land with mowing and fire (Kansas Department of Wildlife and Parks 2005). Mowing helps prevent other plants from establishing until plants from this seed mix are established. Fire keeps out invading trees and aids in the removal of thatch (Kansas Department of Wildlife and Parks 2005).

Summary

Ants are diverse organisms that dominate the land. They live in a variety of habitats, eat many foods, and have various life history traits. Ants fill many important roles in ecosystems including harvesting seeds, predating and scavenging organisms, tilling soil, and tending honey-dew producing insects. Ants' highly developed eusocial systems and their ability to efficiently communicate with each other combine to form the key components to their success. Ants are well studied providing a good taxonomic base. The combination of taxonomic knowledge and the roles ants play in ecosystems make them great candidates for terrestrial bioindicators. With most of our nation's land

converted to agriculture, indicators are necessary to assess the quality of land. Studies on Australian mine sites have shown, that ants can indicate restoration success. In fact, seven function groups of ants have been looked at addressing the reestablishment of land. The Conservation Reserve Program (CRP) promotes reestablishment of grasslands. CRP takes highly erodible agriculture land out of production and restores native grasses on the land. These native grasses provide grassland habitats for animals and allows us to examine the usefulness of ants as bioindicators of CRP land.

Chapter 2
Comparison of Ant Communities across Four Ages of Grasslands in the Conservation Reserve Program

Introduction

Within landscapes, natural habitats play a major role in the recycling of nutrients, breaking down of wastes, and maintaining clean air, water, and soil (Turner et al. 1998). Recovery time from disturbances on natural habitats depends directly on communities of plants and animals (Tilman and Downing 1994, Turner et al. 1998, De Deyn et al. 2003).

Land-use change has drastically affected the environment but much is still to be learned about the effects of degradation and fragmentation of land on plant and animal populations. Fragmentation of a habitat into smaller units due to urbanization or agriculture can result in species richness decline (Tilman et al. 1994, Stone 1995). In a study of carrion and dung-feeding beetle communities, Klein (1989) showed that one- and ten-hectare rain forest fragments, isolated from contiguous forests by at least 350 meters for two to six years, had fewer species, sparser populations and smaller individuals than beetle communities in undisturbed rain forests. Suarez et al. (1998) studying the effects of fragmentation on native ant communities in coastal southern California found that increased fragmentation promoted the spread of the invasive Argentine ant (*L. humile*) and displaced native ant species. Grassland birds are experiencing extensive population decline because of fragmentation (Herkert 1994, Vickery et al. 1994). Helzer and Jelinski (1999) tested different sizes of patch-area and perimeter-area on grassland breeding birds and found that although large patch size is important, patch shape had more influence on the presence and richness of bird species.

In extreme cases, some animals become extinct, partly due to fragmentation of land, such as the Carolina Parakeet, *Conuropsis carolinensis* Linnaeus (Dickson 1991), and the Xerces Blue butterfly, *Glaucopsyche xerces* (Boisduval); the first butterfly known to become extinct in North America (Emmel & Emmel 1993).

Many conservation programs exist in Missouri to help conserve Missouri's natural resources, one of which is the Conservation Reserve Program (CRP). The CRP's primary objectives are to remove erodible or eroding lands from agricultural production by establishing perennial grassland vegetation, and to enhance habitat for fish and wildlife populations (Johnson 2005). It is hoped that a reduction in soil erosion will also reduce sedimentation of streams, and improve water quality.

CRP land is typically fragmented into various sizes and surrounded by farmland. Studies have shown that CRP patch size influences which animal species can colonize the land. Specifically, Johnson and Igl (2001) found that the northern harrier, sedge wren, clay-colored sparrow, grasshopper sparrow, Baird's sparrow, Le Conte's sparrow, and bobolink favor larger grassland patches, whereas two edge species, mourning dove and brown-headed cowbird, tended to favor smaller grassland patches. However, little is known of the effects of fragmentation on prairie ant communities.

Ants form an essential component of grasslands by participating in most levels of the trophic system (Carroll and Janzen 1973, Trager 1998). Ants disperse seeds for many plant species (Berg 1975, Beattie 1985, Willson et al. 1990), are the chief predators of insects and other arthropods (Mirenda et al. 1980, Youngs 1983, Porter and Savignano 1990), and both invertebrates (Whitcomb et al. 1973, Jackson et. 1998) and vertebrates prey on them for food (Milne and Milne 1950, Taigen and Pough 1983, Reiss 2001).

Ants also circulate and aerate vast quantities of soil (Baxter and Hole 1967, Lobry de Bruyn and Conacher 1990) providing nutrient rich microhabitats for grassland seeds to establish (Dunn 2005).

Ants are a useful group to be used in diversity studies because they show high diversity and have numerical and biomass dominance in almost every habitat (Hölldobler and Wilson 1990, Agosti et al. 2000). There is also a good taxonomic knowledge base (Creighton 1950, Bolton 2003), ants are easily collected (Romero and Jaffe 1989, Olson 1991, Wang et al. 2001), they have stationary nesting habits that allow them to be resampled over time (Brian et al. 1966, Bristow et al. 1992), and they are sensitive to environmental change (Andersen 1990, 1995, Peck et al. 1998, Agosti et al. 2000).

Although ants have a great impact on their surrounding environment, few studies have examined the ant communities of restored and recreated grasslands (Trager 1990, Whiles and Charlton 2006). Previous studies (Trager 1990, Petersen et al. 1998) have shown that native prairie ants naturally colonize restored prairies once appropriate vegetation has been reestablished. A typical prairie remnant is able to support 25-35 ant species (Trager 1998). Trager (1990) found that in two artificial prairies, isolated from other prairie remnants, and planted where no prairie occurred for at least 50 years prior to their establishment; each had approximately 20 ant species. In a study of a reconstructed tallgrass prairie plot, (Petersen et al. 1998) found that the dominant grass species were Big bluestem (*A. gerardii*), Indian grass (*S. nutans*) and prairie dropseed (*Sporobolus heterolepsis* Gray), and 11 species of ants were collected. Of these, nine were in native tallgrass prairie and the remaining two were in open fields.

Little is known on the rate of ant succession onto reestablished grasslands. Dauber and Wolters (2004) found ant species composition on five phases of land (3 to >46 years) was highly variable during early successional phases and quite constant in later phases shifting from anthropogenic tolerant and opportunistic ant species to an increasing dominance of ants interacting with biotic factors (i.e., tending aphids).

This study investigated ant community changes across four ages of grasslands in the Conservation Reserve Program. Ant species richness and relative abundance on each age was documented in an effort to understand how ant communities recolonize previously disturbed sites such as fallow farmland in mid-Missouri. I predict that ants will naturally colonize fields in the Conservation Reserve Program as previous similar studies have shown (Trager 1990, Petersen et al. 1998). I hypothesize that ant abundance and species richness will increase the longer the land is in the Conservation Reserve Program.

Materials and Methods

Study Sites

Ant communities were surveyed on 12 Conservation Reserve Program (CRP) grasslands located in Audrain, Monroe, and Boone counties, in east-central Missouri during the summer and fall of 2004 (Fig. 1). The availability of CRP land of different ages allowed us to use the space-for-time substitution approach as an alternative for a long-term study on ant succession (Pickett 1989). The 12 fields comprised four age classes (0, 3, 7-8, and 14-16 yrs) with three replications per age class. Fields enrolled during 2004 were called 0-yr, fields enrolled during 2001 were called 3-yr, fields enrolled during 1996 and 1997 were called 7-8-yr, and fields enrolled during 1988, 1989, and 1990 were called 14-16-yr. Acreage of these fields ranged from 9.1 to 31.3, mean = 17.24 (Table 1). Fields had similar maintenance practices that included either mowing only or no burning within three years prior to the study.

All fields had been planted with a seed mix of warm season grasses (e.g., Big bluestem, *A. gerardii*; Little bluestem, *A. scoparius*; Indian grass, *S. nutans*) of varying proportions (Table 1). Some fields also had various forbs (e.g., lespedeza) as food plots for quail (Table 1). The soils were similar, consisting of Leonard silty clay loam, Armstrong loam, Mexico silt loam, Keswick silt loam, Mexico silt loam, Chariton silt loam, and Gifford silt loam (Table 1) (R. Hagedorn, T. Hill, A. King, and M. Krueger, pers. comm.).

Mean annual rainfall in the region is approximately 1016 mm of which about 635 mm, or about 65 percent usually falls in April through September (USDA-NRCS1995).

Temperatures are high in summer where the average temperature is about 23.8 °C and the average daily maximum temperature is about 30.5 °C (USDA-NRCS 1995). The summer of 2004 was unusually mild with well-below average temperatures and above normal precipitation (Guinan 2004).

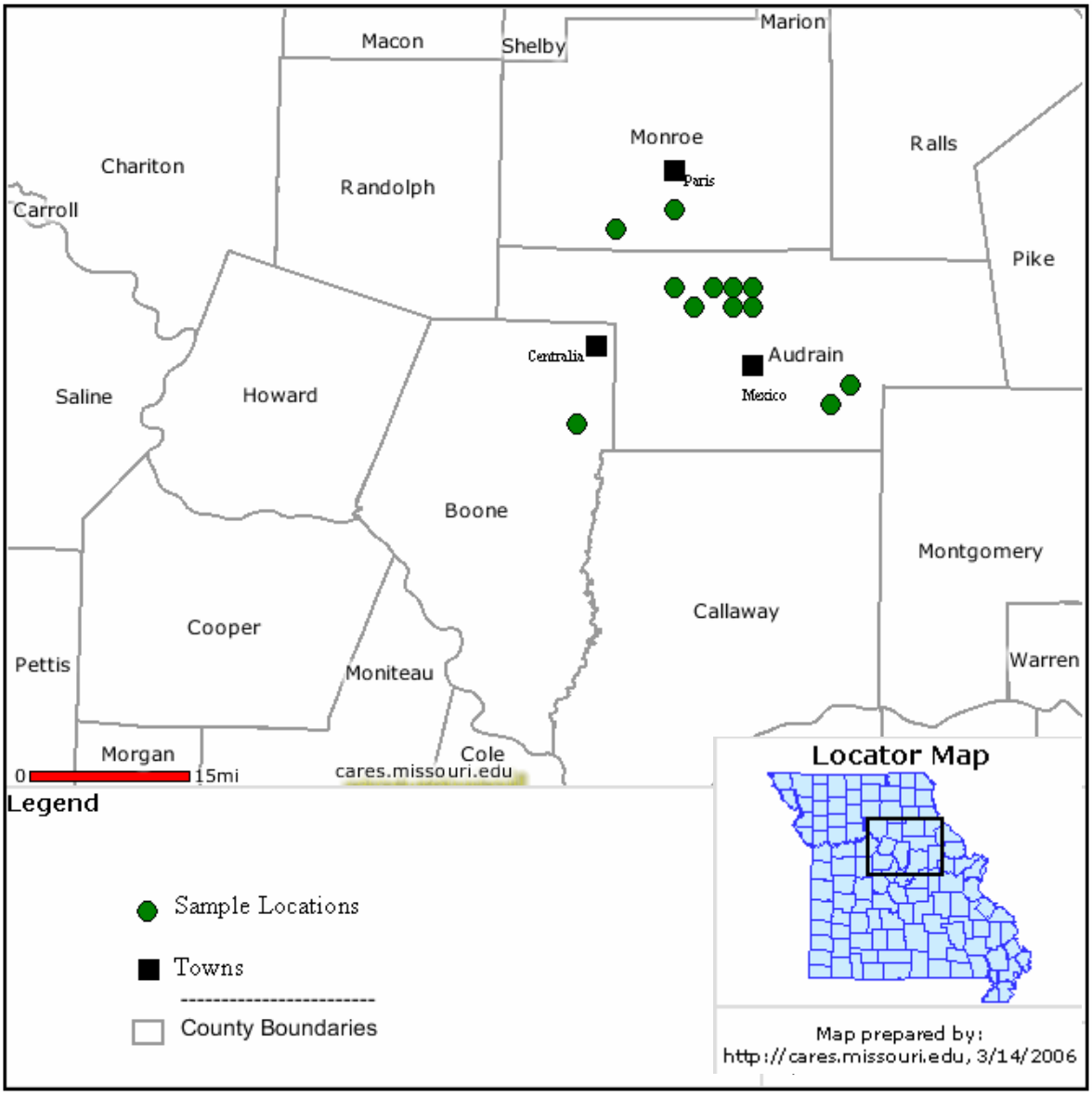


Figure 1. Locations of Conservation Reserve Program land sampled in east-central Missouri.

Table 1. Location of CRP fields with corresponding age, acreage, soil type and seed mix.

CRP field	Location GPS Waypoint	Entered Program	Acreage	Soil Types	Seed Rate Pounds/acre	By Vegetation
1	Section 19, T52N,R8W 39.27791 -91.834147	2004	10.3	Leonard silty clay loam Armstrong loam	3.2 2.1 1.0 0.7 .25	Little Bluestem Side-oats grama Alfalfa Indian grass Native Forbs
2	Section 4, T50N, R11W 39.14348 -92.157352	2004	15.2	Keswick silt loam Mexico silt loam	3.5 1.0 0.25 0.25 0.5	Little Bluestem Side-oats grama Indian grass Big Bluestem Native Forbs
3	Sections 13&14, T53N, R 10W 39.38232 -91.983646	2004	9.1	Leonard silty clay loam Mexico silt loam	1.7 1.7 1.6 0.8 0.25 1.0	Big Bluestem Indian grass Eastern Gamagrass Switch grass Native Forbs OR Alfalfa
4	Section 7, T52N, R8W 39.29491 -91.838153	2001	11.2	Leonard silty clay loam	3.3 1.1 1.4 1.0	Big Bluestem/Indian grass (sum of two grasses) Little Bluestem Side-oats Grama Annual Lespedeza
5	Section 10, T52N, R8W 39.29594 -91.79123	2001	31.3	Leonard silty clay loam Chariton silt loam Gifford silt loam	3.3 1.1 1.4 1.0	Big Bluestem/Indian grass (sum of two grasses) Little Bluestem Side-oats Grama Annual Lespedeza
6	Section 6, T50N, R7W 39.14634 -91.728137	2001	13.6	Leonard silty clay loam Mexico silt loam	3.3 1.1 1.4 1.0	Big Bluestem/Indian grass (sum of two grasses) Little Bluestem Side-oats Grama Annual Lespedeza 5 species native warm-season grasses 10 species Native Forbs
7	Section 25, T53N, R11W 39.34649 -92.094261	1997	11.2	Leonard silty clay loam Mexico silt loam	7.0	Big Bluestem
8	Section 11, T52N, R9W 39.2949 -91.891359	1996	18.4	Leonard silty clay loam Armstrong loam	4.0	Switch grass
9	Section 7, T52N, R8W 39.30593 -91.846859	1996	24.4	Leonard silty clay loam Mexico silt loam	4.0	Switch grass
10	Section 36, T52N, R9W 39.24813 -91.868503	1990	22.4	Leonard silty clay loam Armstrong loam	3.0 4.0	Big Bluestem Indian grass
11	Section 16, T50N, R8W 39.10688 -91.80355	1989	19.8	Leonard silty clay loam Mexico silt loam	2.0 2.0 1.0 1.0	Big Bluestem Indian grass Little Bluestem Side-oats grama
12	Section 24, T52N, R9W 39.27368 -91.862968	1988	20	Leonard silty clay loam Armstrong loam	7.0	Big Bluestem

Study Design

Three sampling techniques were utilized to survey ant communities in this study. I followed a modified Ants of the Leaf Litter (ALL) protocol (Agosti et al. 2000), which uses the techniques of pitfall traps, litter samples, and hand collecting. Six 100 m long parallel transects were placed on each plot of land; two pitfall transects and four litter/hand collecting transects. Each transect had 10 samples taken spaced 10 m apart (Fig. 2).

Since the shape and size of fields in this study were variable, transects were placed so that two edges of field were equidistant from the transects. The shortest length and width of all twelve fields were determined and the approximate midpoint of the smallest field was chosen, then applied to all other fields. These distances were 130.9 m (429.5 feet) lengthwise and 49.8 m (163.5 feet) widthwise. Based on these length and width measurements, a center point was marked (Fig. 2, Step 1). Pitfall transects were then established 10 m on opposite sides from this central point (Fig. 2, Step 2). Individual pitfall sampling points along these transects were marked at 10 m intervals with 2 m tall PVC pipes and fluorescent flagging. Litter/hand collecting transects were established 1 m on both sides of pitfall transects (Fig. 2, Step 2). The July litter/hand collecting transect was 1 m on the inside of the transect, closer to the center point and the September litter/hand collecting transect was 1 m on the outside of the transect.

Pitfall trap sampling

Pitfall traps were used to sample surface-dwelling ants. A total of 20 pitfall traps (n=19 in field 5 during fall) was placed in each field. Ten pitfall traps were placed along each of the 100 m transects at 10 m intervals. A 30 cm per side equilateral wooden

triangle with a 15 cm nail placed in each corner served as a cover to prevent rain from falling into pitfall traps (Fig. 3). A Pro II Hole Cutter (10.8 cm (4 ¼")) diameter and (13.97 cm (5.5")) depth) used for cutting holes in golf course greens, was used to make pitfall trap holes and to minimize disturbance of the soil surface around each trap. Each trap consisted of a 16 oz. Pro-Kal deli container from Fabri-Kal Corporation, Kalamazoo, MI [with a diameter of 11cm (4.5")] placed inside a 32 oz. V-32 deli container from Plastic Packaging Corporation, West Springfield, MA. The 32 oz. V-32 deli container was placed in the hole, making sure it was flush with the soil surface. Pitfall traps were closed using a tightly fitting lid for at least a week after placement to reduce any digging-in effect (Greenslade 1973) which could bias sampling by attracting ants to the disturbed habitat (Agosti et al. 2000). Pitfall traps were activated for 72 h, over a two-day period on June 22-23, 2004 and September 4-5, 2004. Activation of traps was executed a day apart from each other to keep temporal variation to a minimum. When a trap was not activated, the lid for the 16 oz. Pro-Kal deli container was placed securely on to keep arthropods from falling in. When activated, the lid was removed and approximately 150 ml (5 oz.) of 50% propylene glycol was added to the container. After 72 h lids were placed on the samples and taken to the lab for processing. Empty containers with lids were placed back in the 32 oz. containers in the field so invertebrates would not fall in.

In the laboratory, propylene glycol was strained from the contents of the pitfall traps with a 250 μm sieve. Contents were rinsed with distilled water and 80% ethanol to remove any remaining propylene glycol. When soil was abundant in the sample after propylene glycol was decanted, contents were placed in a 100 ml container where a hypertonic salt solution was utilized to float invertebrates out of the soil (Agosti et al.

2000). A saturated Morton® salt (plain and iodized) solution was poured in the 100 ml container, contents stirred, aqueous portion strained through 250 μm sieve, and the process repeated one to two times. Contents remaining in the sieve were rinsed with distilled water to remove any remaining salt. All invertebrates were sorted from the remaining debris using a dissecting microscope at 7x power. Ants were separated to morphospecies then identified to species and stored in 95% ethanol. Adult and immature beetles were separated into a separate vial, stored in 80% ethanol, and labeled. All other invertebrates were also stored in 80% ethanol and labeled with collection data.

Hand Collection and Litter Sampling

Hand and litter sampling were conducted July 13 - August 5, 2004 and September 19 – October 9, 2004. Two transects, (each 100 m in length) were placed 1 m away from each pitfall trap transect. Every 10 m along these transects, a 0.25 m^2 quadrat was placed on the ground. Litter depth was recorded within the quadrat by placing a dowel rod marked off in 0.5 cm increments in the center of each quadrat. Other measurements taken within the 0.25 m^2 quadrat were percentage live forbs and dominant plant(s) species. Grass litter samples were collected by gathering all the grass litter in the 0.25 m^2 quadrat and placing it in a white plastic bag. Grass litter samples were taken to the lab and placed in Berlese funnels for 48-72 h. Hand sampling occurred in the same quadrat. Hand collection consisted of thoroughly searching vegetation above litter level immediately before litter was collected and on the bare ground for two minutes immediately after litter was collected. There were times when all observed ants could not be collected due to the abundance and/or speed of some species. In most cases, at least one representative of each species was collected. Flowering plants were also examined in

the field for ants. All hand collected ants were aspirated and stored in containers of 95% ethanol.

Processing

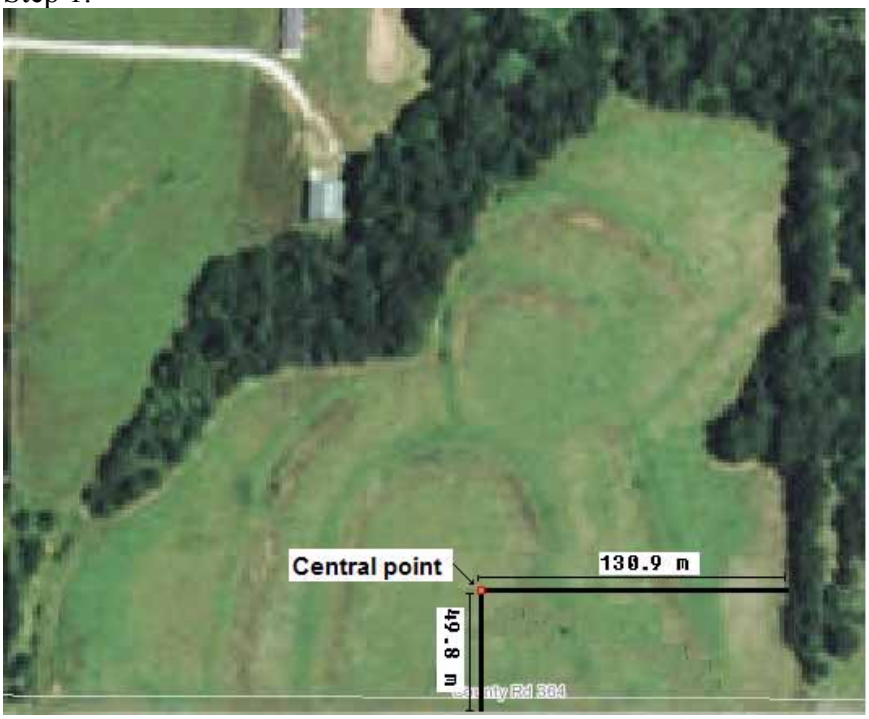
The contents from all samples were taken to the laboratory, processed, sorted, identified to species, and counted. The functional group of each ant species was documented (Andersen 1997a). Identification was performed using a dissecting microscope (7-30x) with a 2x objective. Identification of specimens to the level of genus was obtained using Creighton (1950) and Bolton (1994) and to the species level using Creighton (1950), Trager (1984), Wheeler and Wheeler (1986), Bolton (1994), Francoeur (2005), and Trager et al. (in press). Species identifications were confirmed by Dr. James Trager (Shaw Nature Reserve). Samples were stored in 95% ethanol. Voucher specimens from the study were deposited at the Enns Entomology Museum at the University of Missouri-Columbia.

Data Analysis

Workers were the only caste examined for this study as their presence provides evidence of an established colony (Longino et al. 2002). Pitfall traps, hand collection, and litter data were combined to determine total species richness and abundance. The relationship between total abundance (seasons and methods pooled) and how many years the field had been in CRP was analyzed using linear regression ($P \leq 0.05$). I also regressed ant abundance and species richness against habitat measurements for acreage, mean percentage live forb, mean litter depth (cm), and dominant plant species for each field ($P \leq 0.05$). Vegetation measurements were only collected in fall and therefore only analyzed against fall data. Mean litter depth was regressed against ant abundance and

species richness in litter samples and in all methods combined (0-yr data were not included in the analysis because

Step 1.



Step 2.

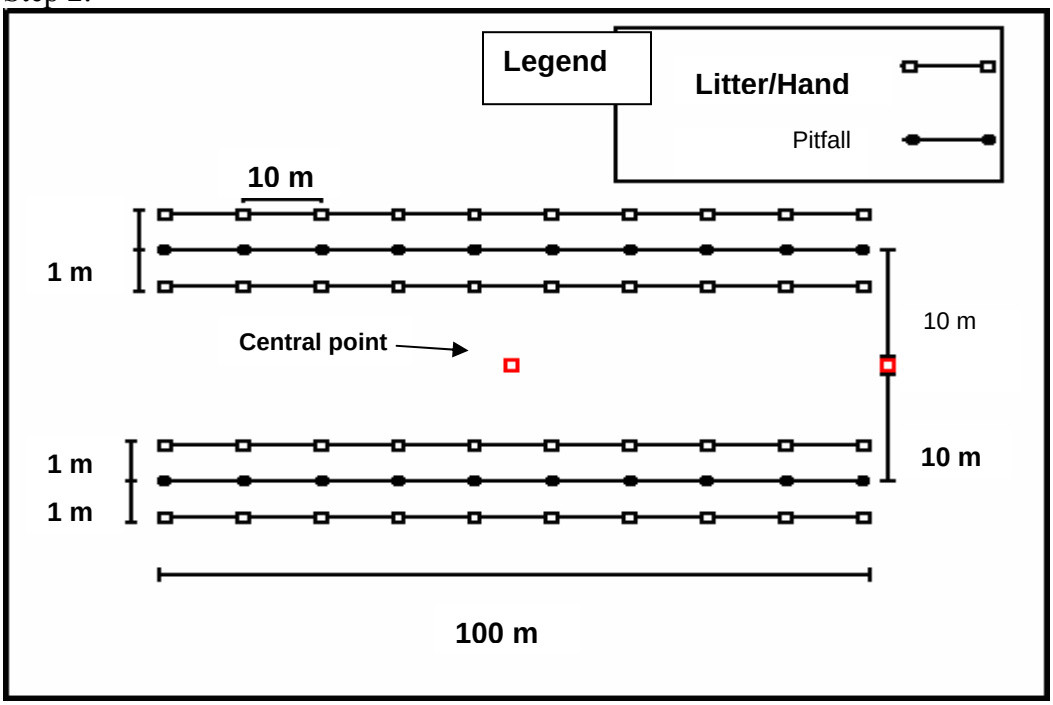


Figure 2. Sampling design used on each Conservation Reserve Program field


Figure 3. Rainhood suspended over pitfall traps using a 15cm nail placed in each corner.

little to no litter was found on these fields). Dominant plant data were collected for each field and reported as the proportion of 20 quadrats on each field containing a particular dominant species. One-way analysis of variance (ANOVA) was performed using ant abundance and species richness (in separate analyses) as dependent variables on land age in summer, fall, and seasons combined. To isolate which group(s) differ from the others, a Tukey's Test ($P \leq 0.05$) was used. Brillouin's Diversity Index was calculated for all fields, $H = \frac{1}{N}\log\left(\frac{N!}{n_1!n_2!n_3!\cdots}\right)$ where N = total number of individuals in entire collection, n_1 = number of individuals belonging to species one, n_2 = number of individuals belonging to species two. This index treats the samples as collections rather than as random samples from a large biological community, which addresses the clumped nature

of ants (Pielou 1966). All statistical analyses were performed using SigmaStat 2.0 (SPSS Inc. 1997) unless otherwise stated.

Species accumulation curves were created for individual fields to determine if sufficient samples were taken. Curves were generated by randomizing the order of all traps within each sampling unit 100 times. Before randomization, species data from all methods were combined for each trap location. For instance, Trap A data contained all species collected from that location using pitfall, litter, and hand collecting. In most fields, there were 20 of each trap per method.

To quantify ant species composition for comparison between sites, dendrograms were produced by SPSS using cluster analysis (nearest neighbor hierarchical algorithm and squared Euclidean distance interval measure) performed on Jaccard's similarity index scores (SPSS 2005). An outgroup was included in analyses to determine how similar the ant composition on CRP fields are to a native prairie system. Trager's (1990) study of Tucker prairie provided the outgroup species list for this study, due to Tucker prairie's proximity to the field sites.

Each ant species was assigned to one of five exclusive functional groups: Cold Climate Specialists, Cryptic Species, Opportunists, Generalized Myrmicinae, and Specialist Predators. Assignments were based upon functional group assignments by Andersen (1997a) for North American ant communities. Percentage of total ant species within each functional group was compared among all four ages of CRP land during summer, fall, and seasons combined.

Ants are social and therefore strongly aggregated. When sampling methods capture a portion of or an entire colony, a particular species may outweigh other species

numerically making them look more abundant then they actually are on the land. Frequency data are preferred for analyses to prevent this potential bias. Collection frequency of each species was determined as the proportion of traps containing each species. A comparison of total abundance (both seasons) was made with frequency data (both seasons) to determine if similar results were found. Ant species composition was examined to determine if predictable changes occur as the land ages by examining the frequency of ant species in pitfall traps.

Results

In total, I observed 17,927 ants representing 28 species in 16 genera across all Conservation Reserve Program fields in summer and fall of 2004 (Table 2). The 28 species sampled included three uniques (known from only one sample) and three duplicates (known from only two samples). In general, total ant abundance increased the longer land has been in the CRP program. However, species richness was consistent throughout, with a peak in 7-8 yr fields.

Number of samples

Species accumulation curves from the 12 study sites show that a sufficient number of samples were taken as indicated by the flattening lines. Figure 4 shows the results of combining the species collected from the same trap number from all three trapping methods (pitfall traps, hand collection, and litter sampling). These curves also show that, on average, 7-8-yr fields were the most species rich, 3-yr and 14-16 yr were next, and 0-yr fields had the least number of species.

Abundance

Ant abundance increased the longer the land had been in CRP use ($P \leq 0.001$) (Fig. 5). Total abundance notably increased between the 0-yr and 3-yr fields. An appreciable increase was also noted between the 3-yr and 7-8-yr fields. Although total abundance continued to increase from the 7-8-yr to the 14-16-yr fields, the extent of the increase was markedly reduced (Table 3 and 4, Appendix A).

In total, 14-16-yr fields yielded 11.9% more individuals than 7-8-yr fields, 113.4% more individuals than 3-yr fields, and 1582.5% more individuals than 0-yr fields.

Abundance of ants showed a significant difference among ages in summer (F= 5.264; df= 3, 8; P= 0.027) (Fig. 6A), fall (F= 4.896; df= 3, 8; P= 0.032) (Fig. 6B), and when seasons were combined (F= 13.980; df= 3, 8; P= 0.002) (Fig. 6C). Variation was observed on individual plots within the same age group. In the summer, 7-8 yr fields had the most variation ranging from 587 ants to 2389 ants. In the fall, 14-16 yr fields had the most variation ranging from 355 to 1246 ants. A Brillouin's diversity index did not reveal any trends with land age.

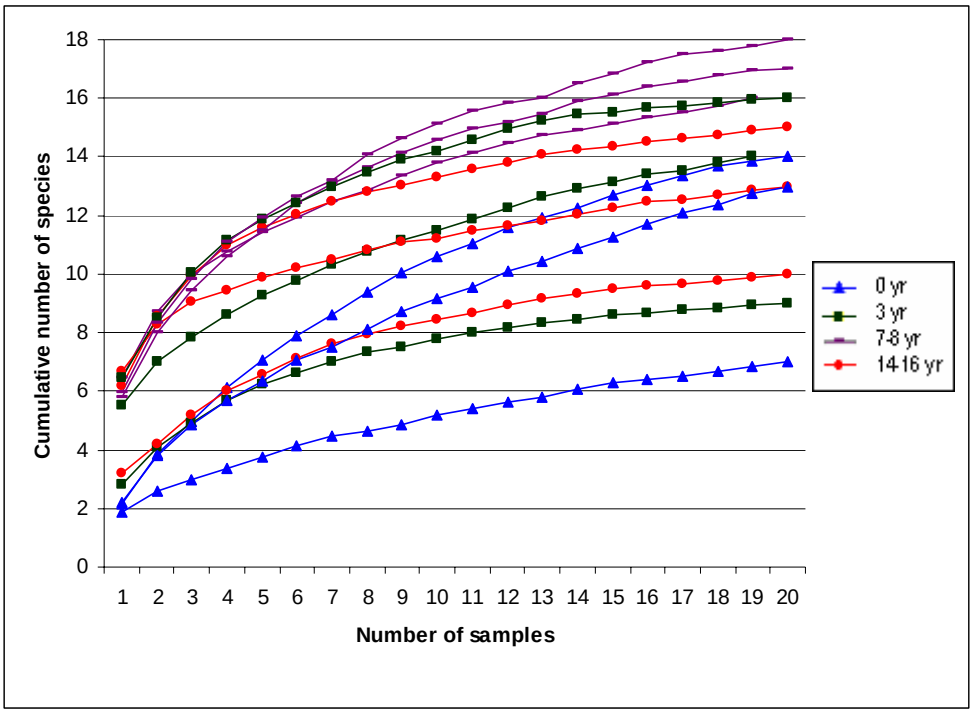


Figure 4. Species accumulation curves for all methods and seasons on 12 CRP fields.

The ant species *Tapinoma sessile* (Say), *Lasius neoniger* Emery, *Temnothorax ambiguus* Emery, *Solenopsis molesta* Say, *and Myrmica americana* Weber (in descending order) dominated CRP land (Table 5). Among the four subfamilies of Formicidae, Myrmicinae dominated all ages of CRP in terms of total abundance (8196), total genera (9), total species (15) and capture events (383). Examining ages separately, using all methods, revealed that 3-yr and 7-8-yr fields had the same top five most abundant species as when ages were pooled. Zero-yr fields had four of the top five most abundant species and 14-16-yr fields, with the addition of *Lasius alienus* Foerster, had all five most abundant species represented in the top six (Table 5).

Table 2. Taxa of Formicidae and functional group (Andersen 1997a) to which they belong with field and season from which each was collected. Fields 1 to 3 = 0-yr, 4 to 6 = 3-yr, 7 to 9 = 7-8-yr, and 10 to 12 = 14-16-yr. Season "S" refers to summer,"F" refers to fall.

Subfamily	Genus	Species	Field(s)**	Season	Functional Group
Dolichoderinae	*Tapinoma*	*Tapinoma sessile* (Say)	1,3,4,5,6,7,8,9,10,11,12	S,F	Opportunist
Formicinae	*Formica*	*Formica incerta Emery/*sp. *	1,3,4,5,7,8,9,10,11,12	S,F	Opportunist
		Formica dolosa Wheeler	8,12	S,F	Opportunist
		Formica pallidefulva Latreille	1,2, 3,4,5,7,8,9,10,12	S,F	Opportunist
		Formica subintegra Emery	7	F	Opportunist
		Formica subsericea Say	1,2,4,6,7,10,12	S,F	Opportunist
	Lasius	*Lasius alienus* Foerster	1,3,7,9,10,11,12	S,F	Cold Climate Specialist
		Lasius neoniger Emery	2,3,4,5,6,7,8,9,10,11,12	S,F	Cold Climate Specialist
	Paratrechina	*Paratrechina faisonensis* (Forel)	1, 8, 9	S,F	Opportunist
		Paratrechina terricola (Buckley)	2,8	S,F	Opportunist
	Polyergus	*Polyergus lucidus* Mayr	8	F	Specialist Predator
Myrmicinae	*Aphaenogaster*	*Aphaenogaster carolinensis* Wheeler	4, 7,8,9,10,12	S,F	Opportunist
		Aphaenogaster fulva Roger	3,10	S	Opportunist
		Aphaenogaster mariae Forel	7	S	Opportunist
	Crematogaster	*Crematogaster cerasi* Emery	4,6,8,9,11	S,F	Generalized Myrmicinae
		Crematogaster lineolata (Say)	4,5,7,8,9,10,11	S,F	Generalized Myrmicinae
	Monomorium	*Monomorium minimum* (Buckley)	1,3,4,5,7,8,9	S,F	Generalized Myrmicinae
	Myrmecina	*Myrmecina americana* Emery	12	F	Cold Climate Specialist
	Myrmica	*Myrmica americana* Weber	2,3,4,5,6,7,8,9,10,11,12	S,F	Opportunist
		Myrmica emeryana Forel	1,2,3,5,7,9,12	S,F	Opportunist
		Myrmica spatulata M. R. Smith	1,7,12	S,F	Opportunist

	Pheidole	*Pheidole pilifera* (Roger)	1,3,4,5,8	S,F	Generalized Myrmicinae
	Solenopsis	*Solenopsis molesta* (Say)	1,3,4,5,6,7,8,9,10,11,12	S,F	Cryptic
	Stenamma	*Stenamma brevicorne* (Mayr)	6	S,F	Cold Climate Specialist
	Temnothorax	*Temnothorax ambiguus* Emery	1,2,3,4,5,6,7,8,9,10,11,12	S,F	Cold Climate Specialist
		Temnothorax pergandei Emery	4,5,8,9	S,F	Cold Climate Specialist
Ponerinae	*Hypoponera*	*Hypoponera opacior* (Forel)	1,4,5	F	Cryptic
	Ponera	*Ponera pennsylvanica* Buckley	1,3,4,5,6,7,8,9,10,11,12	S,F	Cryptic

*Species are morphologically indistinguishable in samples where few specimens are present. ** Refer to Table 1 for additional field information.

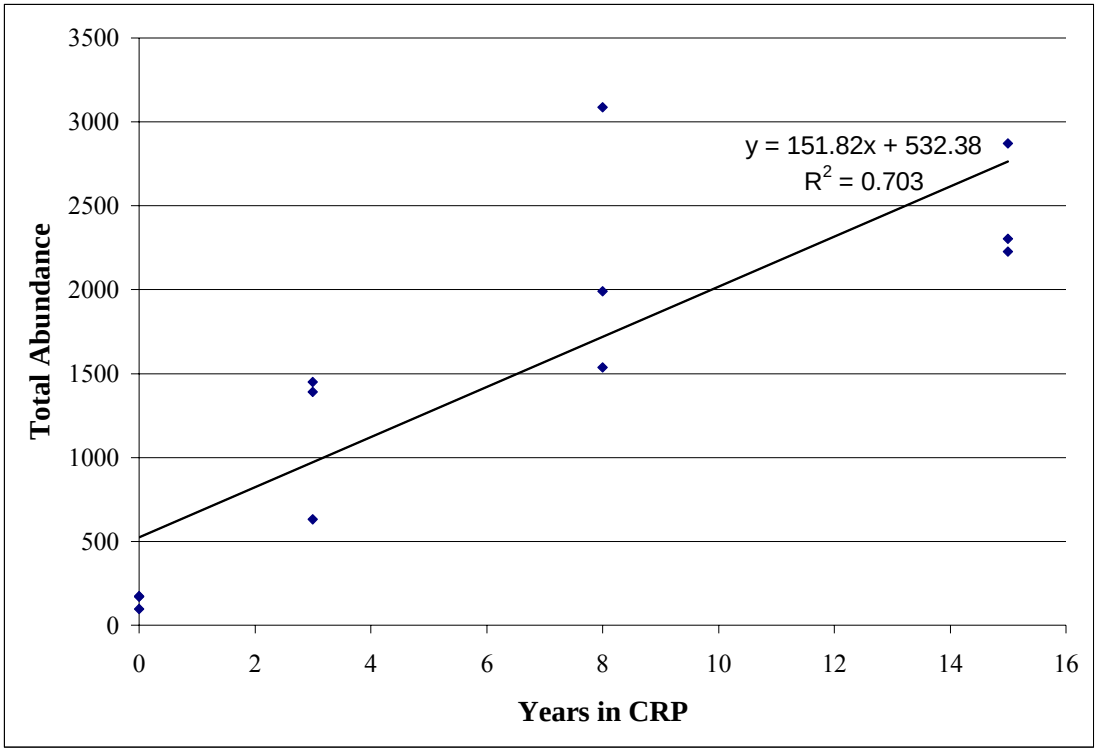


Figure 5. Total ant abundance plotted against number of years in CRP ($P \leq 0.001$). (NOTE: Because of overlapping values for 0-yr, only 2 of 3 observations are plotted).

Table 3. Ant abundance for each age group of CRP land for summer, fall, and seasons pooled.

Season	Age of CRP land			
	0-yr	3-yr	7-8-yr	14-16-yr
Summer	300	1712	4442	4637
Fall	140	1757	2173	2766
Total	440	3469	6615	7403

Table 4. Ant abundance, species richness, and diversity index value (Brillouin's) for each field and method (i.e., pitfall traps, hand collection, and litter sampling) for summer and fall.

			Pitfall			Hand			Litter		
Field**	Age	Season	Abundance	Richness	Diversity	Abundance	Richness	Diversity	Abundance	Richness	Diversity
1	0	Summer	96	9	2.048	0	0	-	2	2	0.500
		Fall	66	10	1.816	5	4	1.181	0	0	-
2	0	Summer	69	5	1.132	0	0	-	0	0	-
		Fall	22	6	1.798	7	2	0.401	0	0	-
3	0	Summer	132	11	2.140	0	0	-	1	1	*
		Fall	34	8	1.902	6	2	0.431	0	0	-
4	3	Summer	549	13	2.653	66	8	2.121	10	1	*
		Fall	674	12	1.492	17	9	2.002	133	3	0.230
5	3	Summer	618	11	1.754	156	8	1.944	36	4	1.244
		Fall	240	8	1.877	73	6	1.235	267	3	0.735
6	3	Summer	163	8	1.724	4	1	n/a	110		0.964
		Fall	143	6	1.119	12	2	0.299	198	4	0.919
7	7	Summer	1161	16	1.842	9	3	0.997	296	7	1.336
		Fall	130	11	2.418	14	2	0.272	381	7	1.206
8	8	Summer	388	16	2.930	107	6	0.994	92	3	0.261
		Fall	251	15	1.997	37	3	1.028	663	4	1.213
9	8	Summer	2033	14	1.336	120	9	2.450	236	3	0.693
		Fall	305	11	2.089	57	7	1.470	335	2	0.981
10	14	Summer	892	12	2.486	52	5	2.005	763	3	1.342
		Fall	221	10	2.539	28	4	1.263	916	6	1.243
11	15	Summer	400	10	2.249	1	1	*	580	3	0.384
		Fall	727	7	0.335	3	1	*	516	3	0.857
12	16	Summer	1832	12	2.130	68	4	0.995	49	1	*
		Fall	254	11	2.804	26	6	1.624	75	2	0.270

* Only one species was found, so diversity index could not be calculated.

** Refer to Table 1 for additional information about fields.

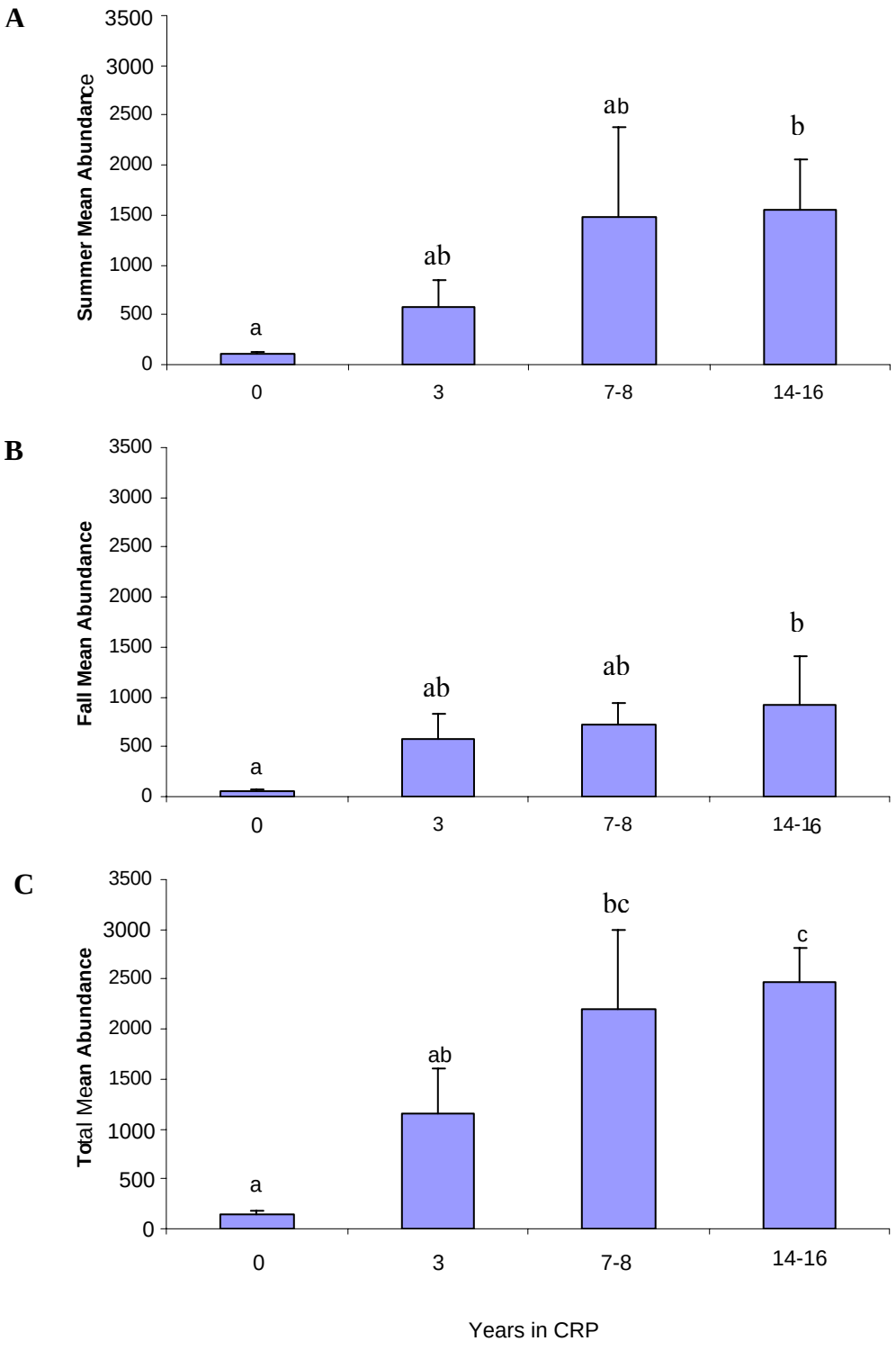


Figure 6. Comparison of mean ant abundance (± SD) on four different ages of CRP land for summer (A), fall (B), and both seasons combined (C). Histograms with different letters are significantly different (Tukey Test, $P \leq 0.05$).

Table 5. Species list of worker ant abundance (all methods and seasons pooled) for each age on Conservation Reserve Program land in east-central Missouri. Listed from most to the least encountered.

	Age of CRP Land				
Species	0	3	7-8	14-16	Total
Tapinoma sessile	61	791	1341	2042	4235
Lasius neoniger	39	317	2311	1007	3674
Temnothorax ambiguus	3	244	1144	1723	3114
Solenopsis molesta	145	1193	506	625	2469
Myrmica americana	58	623	897	327	1905
Lasius alienus	13	0	49	1000	1062
Formica incerta/sp.*	35	71	15	228	349
Formica subsericea	4	3	8	190	205
Aphaenogaster carolinensis	0	14	92	93	199
Pheidole pilifera	24	83	16	0	123
Formica pallidefulva	5	10	43	35	93
Crematogaster lineolata	0	12	41	35	88
Crematogaster cerasi	0	21	63	2	86
Monomorium minimum	22	40	13	0	75
Ponera pennsylvanica	6	17	24	25	72
Myrmica spatulata	1	0	4	58	63
Temnothorax pergandei	0	18	14	0	32
Myrmica emeryana	5	2	13	10	30
Hypoponera opacior	10	2	0	0	12
Formica subintegra	0	0	11	0	11
Stenamma brevicorne	0	8	0	0	8
Paratrechina faisonensis	4	0	3	0	7
Paratrechina terricola	4	0	2	0	6
Formica dolosa	0	0	3	1	4
Aphaenogaster fulva	1	0	0	1	2
Aphaenogaster mariae	0	0	1	0	1
Myrmecina americana	0	0	0	1	1
Polyergus lucidus	0	0	1	0	1

*Species are morphologically indistinguishable in samples where few specimens are present.

Richness

A total of 28 species was collected from all CRP fields sampled. A total of 18 species was sampled on 0, 3, and 14-16 yr fields. A peak of 24 species was observed on the 7-8-yr age. In summer, 24 species in 13 genera were observed and in fall, 26 species in 16 genera were observed. The most species were sampled in both summer and fall in the 7-8-yr fields, followed by 3-yr fields, 14-16-yr fields, and lastly the 0-yr fields (Table 6). A range of 7-14 ant species were found on 0-yr fields, 9-16 species on 3-yr fields, 16-17 species on 7-8-yr fields, and 10-15 species on 14-16-yr fields (Table 7, Appendix A).

A peak in species richness in 7-8-yr fields was consistent for all fields during both seasons. In total, 7-8-yr fields yielded 34.2% more species than 14-16-yr fields, 30.8% more species than 3-yr fields, and 50% more species than 0-yr fields. Although a peak of richness was present on these fields, only three species were collected on the 7-8-yr fields that were not collected on other age fields (*Formica subintegra* Emery, *Polyergus lucidus* Mayr, and *Aphaenogaster mariae* Forel (Table 7), and these species were not abundant. The intermediate disturbance hypothesis, (Wilson 1994, Collins et al. 1995) which states that land is able to support the highest diversity when disturbance is neither frequent nor rare, may explain the abundance of ant species present on mid-aged fields.

Stenamma brevicorne (Mayr) was collected on one 3-yr field with pitfall traps in both summer and fall (Table 7). The fact that it was collected is unusual. The genus *Stenamma*, although common in forest habitats, is rarely encountered due to its cryptic nature and its well-concealed nests in leaf litter, soil and rotting wood (Smith 1957). *Myrmecina americana* Emery was collected only from the 14-16 yr fields (Table 7). The 7-8-yr fields showed a significant difference in species richness from the 0-yr fields in summer ($F = 4.446$; $df = 3, 8$; $P = 0.041$) (Fig. 6 A). No significant interactions were observed in species richness for other field ages during summer, fall, or both seasons combined (Fig. 6 A-C).

Table 6. Ant species richness for summer and fall for each age of CRP land.

Age of CRP Land	Summer	Fall
0-yr	16	15
3-yr	17	17
7-8-yr	22	19
14-16-yr	17	16

Table 7. Comparison of ant species richness and abundance of 12 CRP fields.

Functional group	Species	Age of CRP Land / Field #											
		0 yr			3 yr			7-8 yr			14-16 yr		
**		1	2	3	4	5	6	7	8	9	10	11	12
	Dolichoderinae												
OP	*Tapinoma sessile*	12	-	49	281	421	89	487	586	268	1103	935	4
	Formicinae												
OP	*Formica incerta* /sp. *	2	-	33	48	23	-	1	8	6	16	1	211
OP	*Formica dolosa*	-	-	-	-	-	-	-	3	-	-	-	1
OP	*Formica pallidefulva*	1	3	1	9	1	-	4	32	7	2	-	33
OP	*Formica subintegra*	-	-	-	-	-	-	11	-	-	-	-	-
OP	*Formica subsericea*	2	2	-	2	-	1	8	-	-	111	-	79
CCS	*Lasius alienus*	4	-	9	-	-	-	41	-	8	2	61	937
CCS	*Lasius neoniger*	-	37	2	160	153	4	654	14	1643	263	155	589
OP	*Paratrechina faisonensis*	4	-	-	-	-	-	-	1	2	-	-	-
OP	*Paratrechina terricola*	-	4	-	-	-	-	-	2	-	-	-	-
SP	*Polyergus lucidus*	-	-	-	-	-	-	-	1	-	-	-	-
	Myrmicinae												
OP	*Aphaenogaster carolinensis*	-	-	-	14	-	-	26	24	42	89	-	4
OP	*Aphaenogaster fulva*	-	-	1	-	-	-	-	-	-	1	-	-
OP	*Aphaenogaster mariae*	-	-	-	-	-	-	1	-	-	-	-	-
GM	*Crematogaster cerasi*	-	-	-	14	-	7	-	14	49	-	2	-
GM	*Crematogaster lineolata*	-	-	-	9	3	-	4	35	2	1	34	-
GM	*Monomorium minimum*	20	-	2	38	2	-	1	11	1	-	-	-
CCS	*Myrmecina americana*	-	-	-	-	-	-	-	-	-	-	-	1
OP	*Myrmica americana*	-	50	8	58	521	44	396	121	380	144	15	168
OP	*Myrmica emeryana*	3	1	1	-	2	-	12	-	1	-	-	10
OP	*Myrmica spatulata*	1	-	-	-	-	-	4	-	-	-	-	58
GM	*Pheidole pilifera*	23	-	1	81	2	-	-	16	-	-	-	-
CRY	*Solenopsis molesta*	84	-	61	715	183	295	37	215	254	520	27	78
CCS	*Stenamma brevicorne*	-	-	-	-	-	8	-	-	-	-	-	-
CCS	*Temnothorax ambiguus*	1	1	1	4	71	169	292	439	413	611	992	120
CCS	*Temnothorax pergandei*	-	-	-	13	5	-	-	12	2	-	-	-
	Ponerinae												
CRY	*Hypoponera opacior*	10	-	-	1	1	-	-	-	-	-	-	-
CRY	*Ponera pennsylvanica*	2	-	4	2	2	13	12	4	8	9	5	11
Total Species Richness		14	7	13	16	14	9	17	18	16	13	10	15
Abundance		169	98	173	1449	1390	630	1991	1538	3086	2872	2227	2304

*Species are morphologically indistinguishable in samples where few specimens are present. ** Functional Groups: OP = Opportunist, CCS = Cold Climate Specialist, SP = Specialist Predator, GM = Generalized Myrmicinae, CRY = Cryptic species.

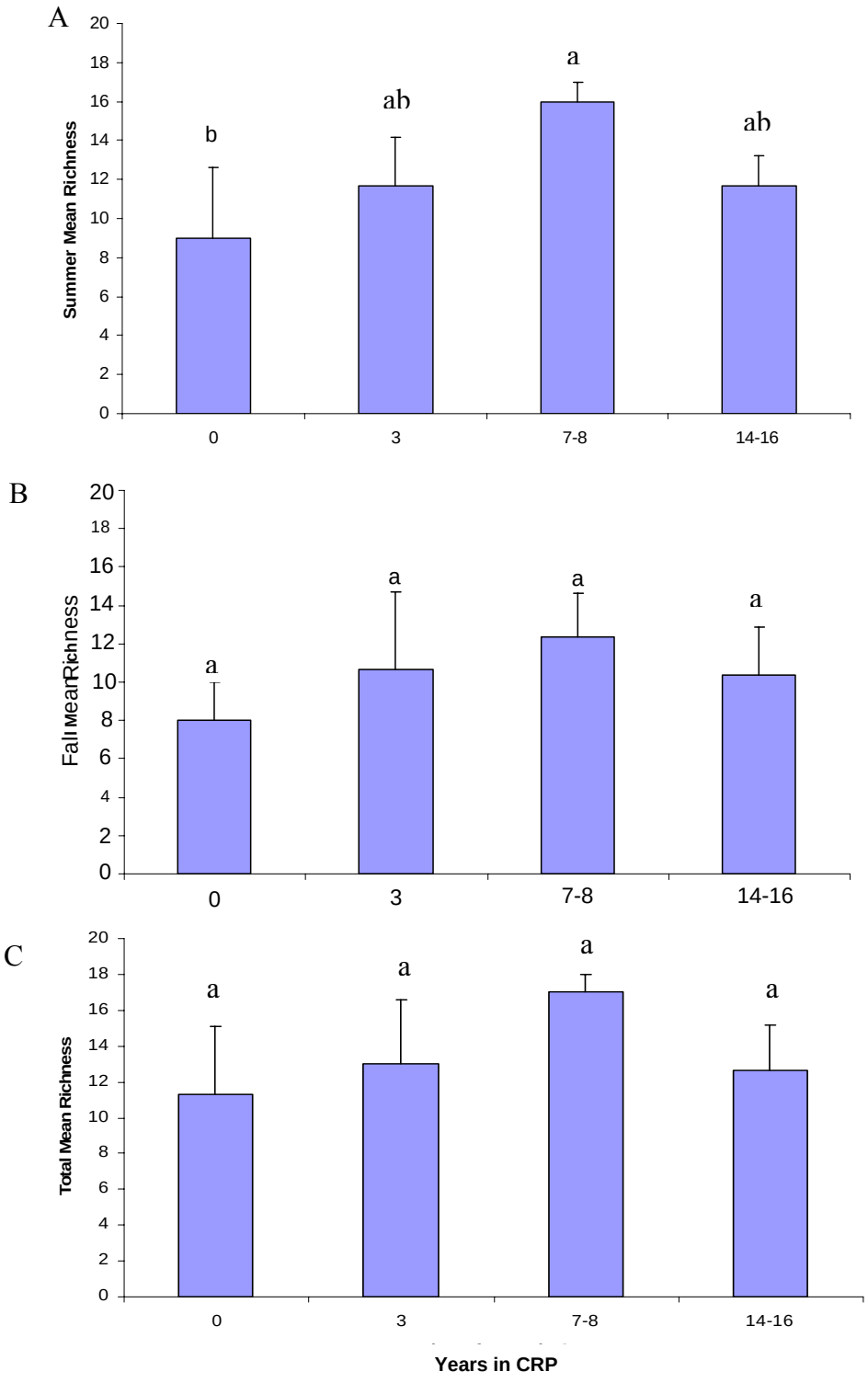


Figure 7. Comparison of mean species richness (± SD) on four ages of CRP land for summer (A), fall (B), and both seasons combined (C). Histograms with different letters are significantly different (Tukey Test, $P \leq 0.05$).

Species Composition

Dendrograms were produced by SPSS using cluster analysis (nearest neighbor algorithm) performed on Jaccard's similarity scores of all ant species on each age of CRP field (Figs. 8-11). Figure 8 shows fields of all ages (0-yr, 3-yr, 7-8-yr, and 14-16-yr) (1, 2, 3, 4 respectively) clustered together and were separate from the native prairie outgroup.

When the 12 CRP fields were individually analyzed by season with all methods combined (Fig. 9, 10) and seasons pooled with all methods combined (Fig. 11), most combinations resulted in no relevant groupings. In many instances, fields of different ages grouped together because species composition was similar across age groups. For instance, the summer season dendrogram (Fig. 9) grouped fields 3, 5, &10 together, (0-yr, 3-yr, and 14-16-yr respectively). A more relevant grouping occurred in the fall season and pooled season dendrograms (Figs. 10 & 11 respectively) where at least two of the fields were of the same age (fields 10 & 11). In all analyses the outgroup source, Tucker prairie, (field 13) (Trager 1990) failed to group with any of the CRP fields.

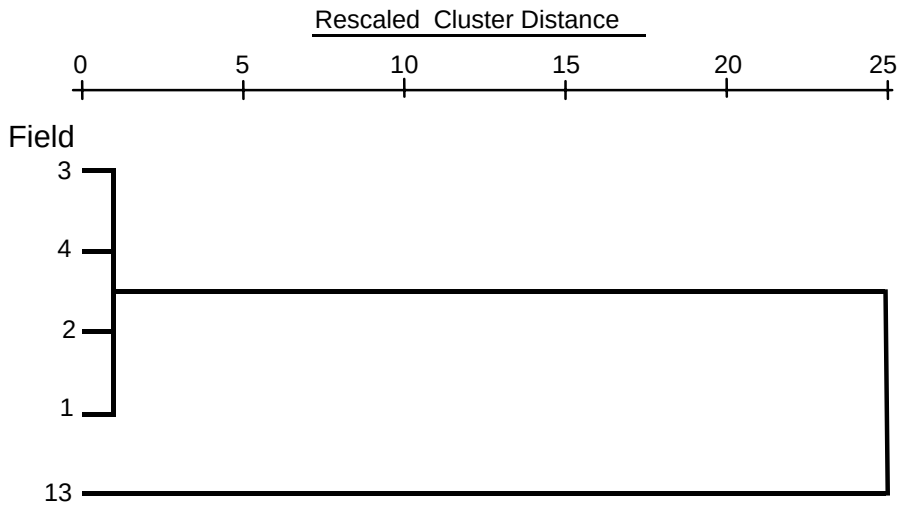


Figure 8. Ant communities classified by cluster analysis using Jaccard's Similarity Index scores on fields of same age (seasons and collection methods pooled). Field 1, 2, 3, 4, represents the ages of land in CRP 0-yr, 3-yr, 7-8-yr, and 14-16-yr, respectively. Field 13 represents the outgroup ant composition from Tucker prairie (Trager 1990).

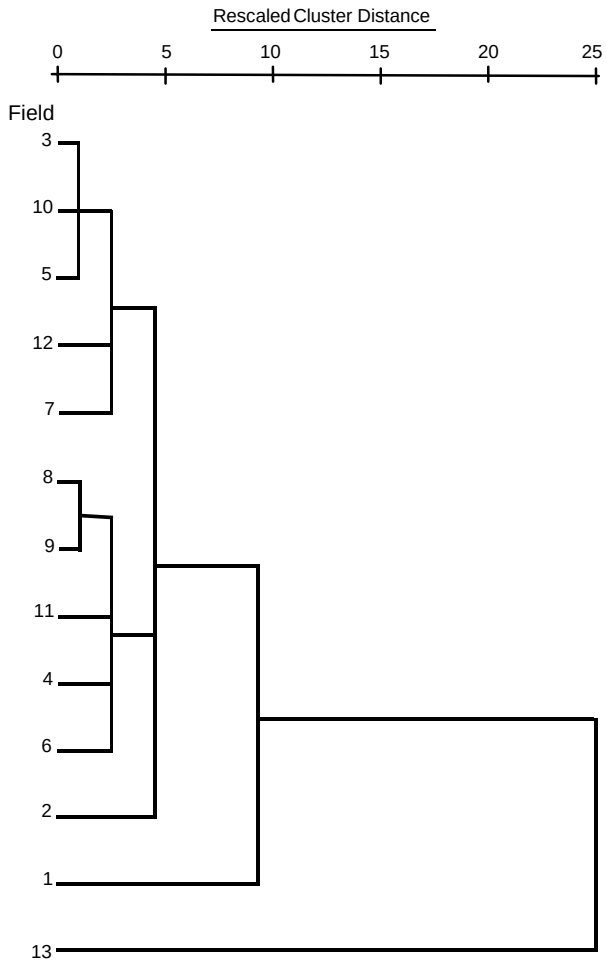


Figure 9. Ant communities classified by cluster analysis using Jaccard's Similarity Index scores on each field (summer only; methods pooled). Refer to Table 1 for additional field information. Refer to Table 2 for corresponding list of ant species in each field plot assemblage. Field 13 represents the outgroup ant composition from Tucker prairie (Trager 1990).

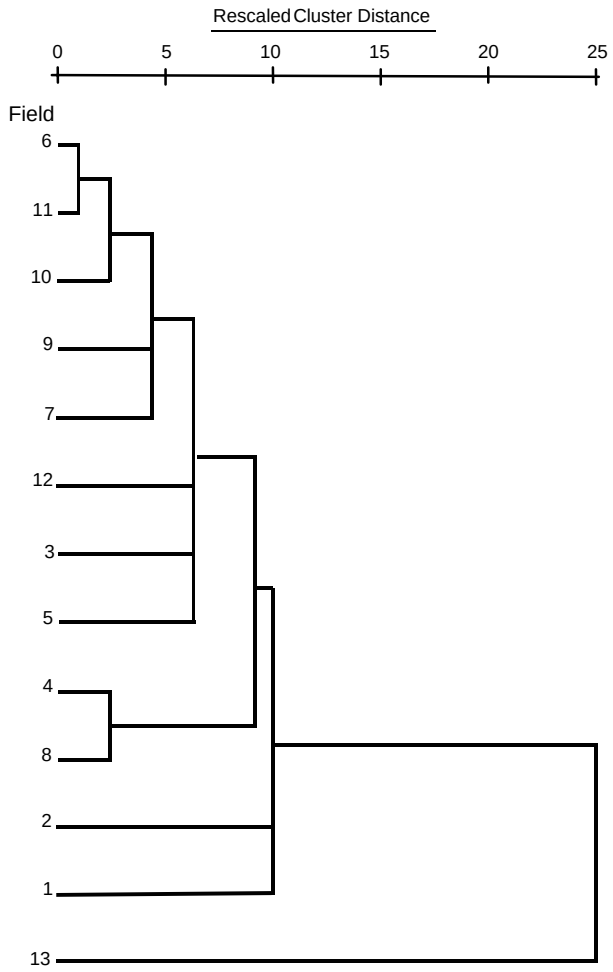


Figure 10. Ant communities classified by cluster analysis using Jaccard's Similarity Index scores on each field (fall only; methods pooled). Refer to Table 1 for additional field information. Refer to Table 2 for corresponding list of ant species in each field plot assemblage. Field 13 represents the outgroup ant composition from Tucker prairie (Trager 1990).

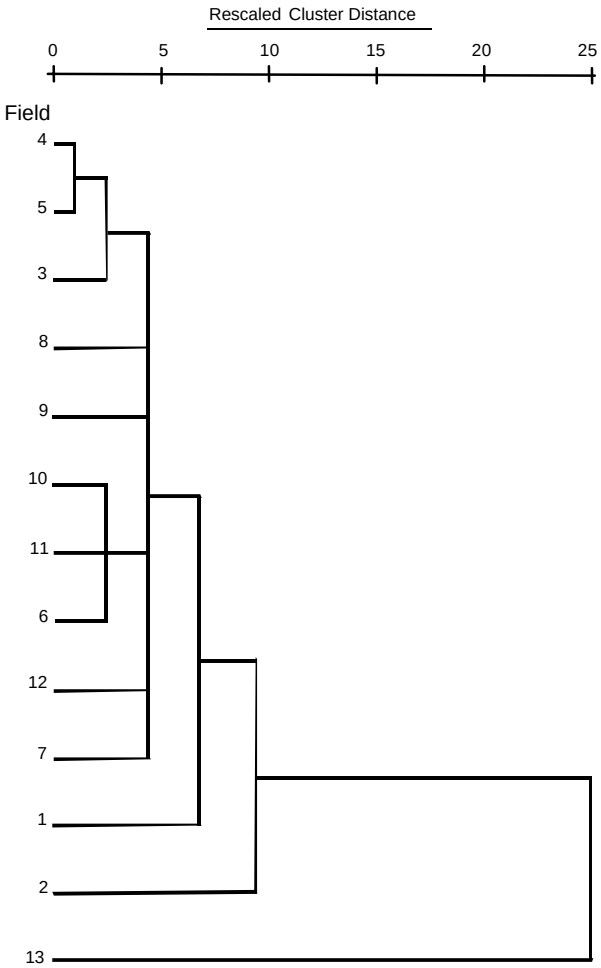


Figure 11. Ant communities classified by cluster anlaysis using Jaccard's Similarity Index scores on each field (seasons and collection methods pooled). Refer to Table 1 for additional field information. Refer to Table 2 for corresponding list of ant species in each field assemblage. Field 13 represents the outgroup ant composition from Tucker prairie (Trager 1990).

Functional groups

Cold Climate Specialists, Opportunists, Cryptic species, and Generalized Myrmicines (in declining order) dominated CRP plots when looking at total abundance (Fig. 14A). When comparing total abundance with collection frequency, similar results were found. However, Cold Climate Specialists decreased and Opportunists increased (Fig. 14B). A single specialist predator *P. lucidus* was collected in one 7-8-yr site but

because this sample was small, it does not appear on the graphs (Figs. 13 and 14). In both seasons, the proportion of Cold Climate Specialists increased as CRP land aged, while Generalized Myrmicines and Cryptic species decreased. Opportunists decreased with CRP age in summer but increased in fall (Figs. 12 and 13). The functional group profiles for 0-yr and 3-yr were closely matched in both summer and fall (Figs. 12 - 14). The same occurred with 7-8 yr and 14-16 yr (Figs. 12 - 14).

Collection Frequency

A trend was found when looking at how many pitfall traps a species was collected in, out of all pitfall traps in a field. The trend shows that certain species are early colonizers (i.e., *Pheidole pilifera* (Roger)), late colonizers (i.e., *Aphaenogaster carolinensis* Wheeler), or present at all ages (i.e., *T. sessile, Myrmica. americana*, and *S. molesta)* (Table 8).

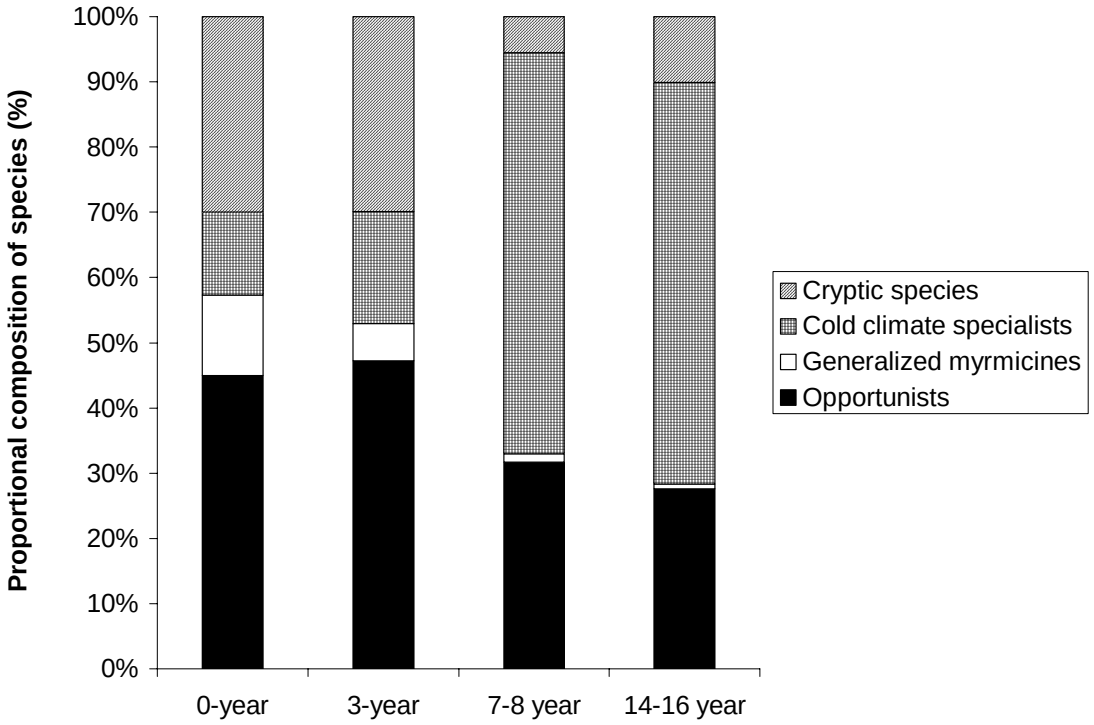


Figure 12. Percentage of total ant species in each functional group from four ages of CRP land in summer. Refer to Table 2 for species functional group assignments.

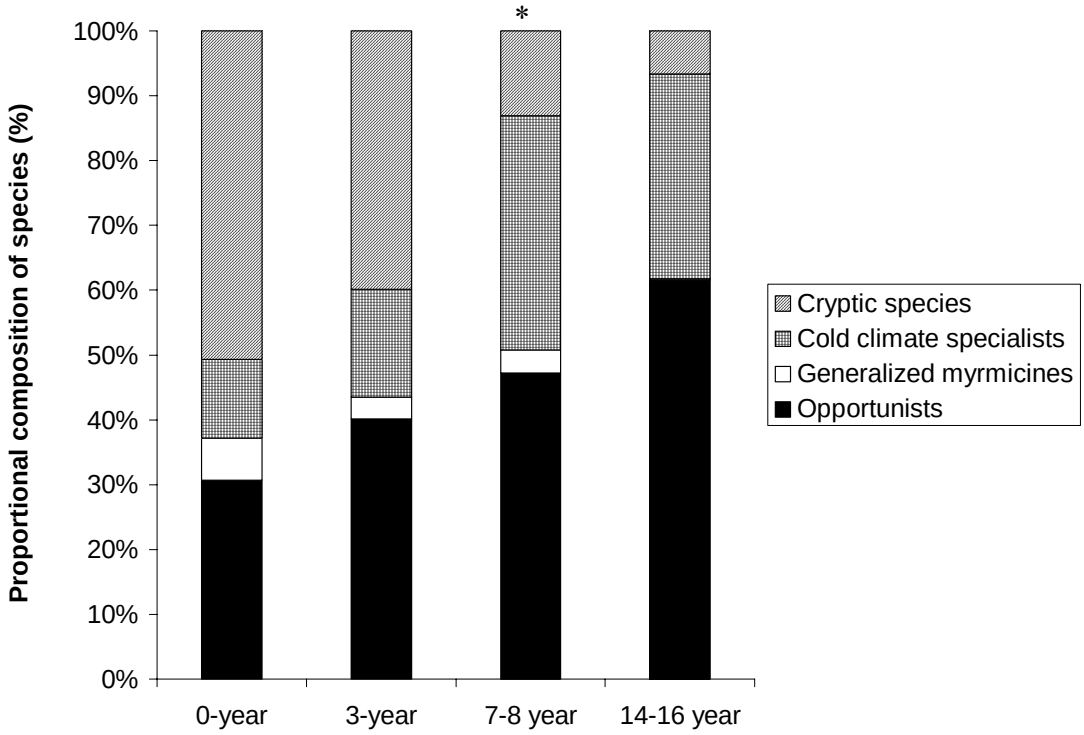


Figure 13. Percentage of total ant species in each functional group from four ages of CRP land in fall. Refer to Table 2 for species functional group assignments. *A single specialist predator *Polyergus lucidus* Mayr was collected in a 7-8-yr site.

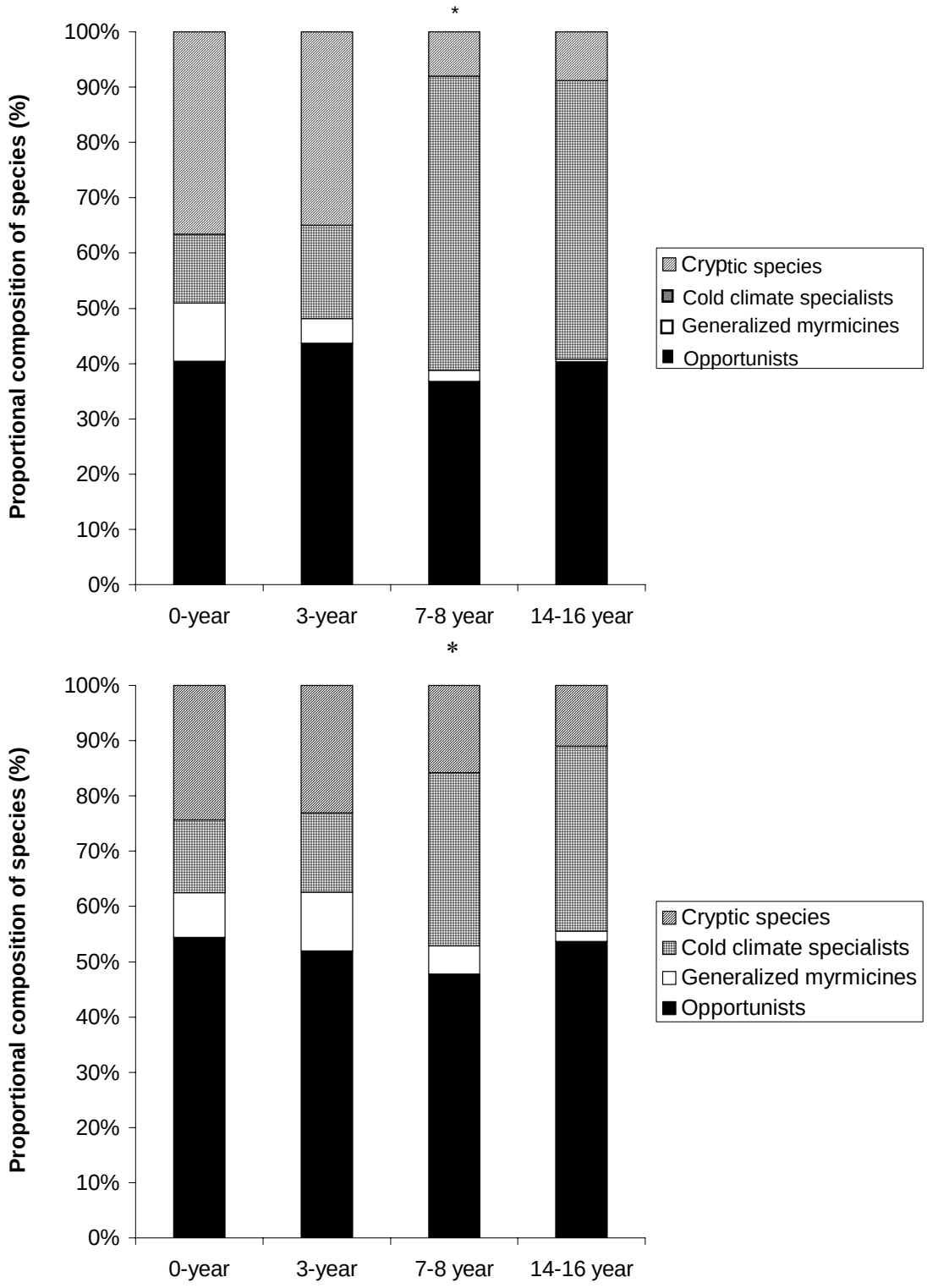


Figure 14. Percentage of ant species in each functional group from four ages of CRP land, seasons pooled, examining total abundance (A) and total occurrence (B). Refer to Table 2 for species functional group assignments. *A single specialist predator *Polyergus lucidus* Mayr was collected in a 7-8-yr site.

Table 8. Mean percentage (± SD) of traps in which each species was collected from different aged plots of CRP land.

	Age of CRP Land			
	n=120	n=119	n=120	n=120
Taxa	0 yr	3 yr	7-8 yr	14-16 yr
Dolichoderinae				
Tapinoma sessile (Say)	0.21 ± 0.04	0.68 ± 0.03	0.58 ± 0.04	0.51 ± 0.07
Formicinae				
Formica incerta Emery/sp. *	0.13 ± 0.03	0.24 ± 0.05	0.08 ± 0.01	0.38 ± 0.06
Formica dolosa Wheeler	-	-	0.02 ± 0.00	0.01 ± 0.00
Formica pallidefulva Latreille	0.04 ± 0.01	0.04 ± 0.01	0.18 ± 0.02	0.11 ± 0.03
Formica subintegra Emery	-	-	0.02 ± 0.01	-
Formica subsericea Say	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.33 ± 0.05
Lasius alienus Foerster	0.03 ± 0.01	-	0.08 ± 0.02	0.27 ± 0.06
Lasius neoniger Emery	0.12 ± 0.03	0.37 ± 0.05	0.53 ± 0.07	0.37 ± 0.05
Paratrechina faisonensis (Forel)	0.02 ± 0.00	-	0.01 ± 0.00	-
Paratrechina terricola (Buckley)	0.01 ± 0.00	-	0.01 ± 0.00	-
Polyergus lucidus Mayr	-	-	0.01 ± 0.00	-
Myrmicinae				
Aphaenogaster carolinensis Wheeler	-	0.04 ± 0.01	0.21 ± 0.03	0.21 ± 0.05
Aphaenogaster fulva Roger	0.01 ± 0.00	-	-	0.01 ± 0.00
Aphaenogaster mariae Forel	-	-	0.01 ± 0.00	-
Crematogaster cerasi Emery	-	0.08 ± 0.01	0.08 ± 0.01	0.02 ± 0.00
Crematogaster lineolata (Say)	-	0.03 ± 0.01	0.07 ± 0.01	0.08 ± 0.02
Monomorium minimum (Buckley)	0.05 ± 0.01	0.10 ± 0.02	0.05 ± 0.01	-
Myrmecina americana Emery	-	-	-	0.01 ± 0.00
Myrmica americana	0.20 ± 0.05	0.58 ± 0.05	0.68 ± 0.03	0.43 ± 0.05
Myrmica emeryana Forel	0.04 ± 0.01	0.02 ± 0.00	0.08 ± 0.02	0.03 ± 0.01
Myrmica spatulata M. R. Smith	0.01 ± 0.00	-	0.01 ± 0.00	0.11 ± 0.03
Pheidole pilifera (Roger)	0.05 ± 0.01	0.21 ± 0.05	0.02 ± 0.00	-
Solenopsis molesta (Say)	0.25 ± 0.04	0.74 ± 0.02	0.55 ± 0.06	0.28 ± 0.03
Stenamma brevicorne (Mayr)	-	0.03 ± 0.01	-	-
Temnothorax ambiguus Emery	0.01 ± 0.00	0.01 ± 0.00	0.23 ± 0.03	0.31 ± 0.06
Temnothorax pergandei Emery	-	0.03 ± 0.01	0.06 ± 0.02	-
Ponerinae				
Hypoponera opacior (Forel)	0.02 ± 0.01	0.01 ± 0.00	-	-
Ponera pennsylvanica Buckley	0.03 ± 0.01	0.04 ± 0.01	0.11 ± 0.00	0.13 ± 0.01

*Species are morphologically indistinguishable in samples where few specimens are present.

Habitat Effects

Dominant plant species varied across fields (Table 9). Early goldenrod, *Solidago juncea* Ait. was the most widespread dominant plant species on all CRP fields. Plot frames (.25m^2) from the 14-16-yr fields had 76% less forbs than 7-8-yr fields, 60% less than 3-yr fields, and 62% less than 0-yr fields (Table 9). *Formica subsericea* Say and *T. sessile* were collected on Partridge pea, *Cassia fasciculata* Michx and were observed feeding on extrafloral nectaries. Vegetation factors (percentage mean live forbs, mean

litter depth (cm), and frequency of dominant plant species) showed no relationship with ant species richness or abundance on CRP fields (Table 10). The only habitat measurement that showed a relationship with total ant abundance was acreage of CRP field ($P = 0.05$) (Fig. 15).

Table 9. Frequency of dominant plant species and mean percent forb per plot frame found on CRP fields.

	Field*												
Plant Species	1	2	3	4	5	6	7	8	9	10	11	12	Total
Sorghastrum nutans	0	0	0	0.45	0.05	0.8	0	0	0	0.4	0	0	1.7
Andropogon gerardii	0	0	0	0.1	0	0	0	0	0	0.35	0.25	0.95	1.65
Setaria sp.	0.3	0.15	0	0	0	0	0	0	0	0	0	0	0.45
Leersia oryzoides	0	0	0	0.05	0	0	0	0	0	0	0	0	0.05
Panicum capillare	0.05	0	0.65	0	0	0	0	0	0	0	0	0	0.7
Panicum virgatum	0	0.1	0	0	0	0	0	0	0.1	0	0	0	0.2
Bouteloua curtipendula	0	0.45	0	0	0	0	0	0	0	0	0	0	0.45
Zea mays	0.05	0	0	0	0	0	0	0	0	0	0	0	0.05
Cassia fasciculata	0.05	0	0	0	0	0	0	0	0	0	0	0	0.05
Erigeron annus	0	0	0	0	0	0	0	0.1	0.05	0	0	0	0.15
Solidago juncea	0	0	0	0	0.1	0.1	0.7	0.8	0.65	0.15	0.1	0.05	2.65
Aster pilosus	0	0	0	0.05	0.7	0	0	0.05	0	0	0	0	0.8
Ambrosia artemisiifolia	0.05	0	0	0	0	0	0	0	0	0	0	0	0.05
Euthamia graminifolia	0	0	0	0	0	0.05	0.3	0	0	0	0	0	0.35
Trifolium sp.	0.45	0	0	0	0	0	0	0	0	0	0	0	0.45
Kummerowia sp.	0	0	0.35	0	0	0	0	0	0	0	0	0	0.35
Euphorbia serpens	0.05	0	0	0	0	0	0	0	0	0	0	0	0.05
Unknown grass	0	0.3	0	0.35	0.15	0.05	0	0.05	0.1	0.1	0.65	0	1.75
Unknown forbs	0	0	0	0	0	0	0	0	0.1	0	0	0	0.1
Mean percent forb per plot frame	35.25	11.75	30.25	11	52.25	9.85	33.75	35	53.75	10.25	9.75	9.5	

* Refer to Table 1 for additional information about fields.

Table 10. Relationship of ant species richness and abundance against frequency of dominant plant species on 12 CRP fields ($\alpha = 0.05$).

	Species Richness			Abundance		
Habitat Effect	R^2	F	P	R^2	F	P
Sorghastrum nutans	0.007	0.07	0.79	0.003	0.03	0.87
Andropogon gerardii	0.07	0.7	0.42	0.2	2.42	0.15
Setaria sp.	0.06	0.68	0.43	0.32	4.68	0.06
Leersia oryzoides	0.26	3.45	0.09	0.0002	0.002	0.97
Panicum capillare	0.07	0.71	0.42	0.18	2.26	0.16
Panicum virgatum	0.087	0.95	0.35	0.002	0.02	0.90
Bouteloua curtipendula	0.22	2.84	0.12	0.18	2.14	0.18
Zea mays	0.001	0.01	0.91	0.16	1.88	0.20
Cassia fasciculata	0.001	0.01	0.91	0.16	1.88	0.20
Erigeron annus	0.25	3.4	0.1	0.06	0.58	0.46
Solidago juncea	0.18	2.18	0.17	0.22	2.8	0.13
Aster pilosus	0.001	0.01	0.09	0.0009	0.009	0.92
Ambrosia artemisiifolia	0.001	0.01	0.91	0.16	1.88	0.20
Euthamia graminifolia	0.0001	0.001	0.97	0.01	0.11	0.74
Trifolium sp.	0.001	0.01	0.91	0.16	1.88	0.20
Kummerowia sp.	0.06	0.69	0.43	0.16	1.87	0.20
Euphorbia serpens	0.001	0.01	0.91	0.16	1.88	0.20
Unknown grass	0.03	0.34	0.58	0.02	0.16	0.70
Unknown forbs	0.005	0.05	0.82	0.23	2.97	0.12
Mean Percent Forb Cover	0.03	0.28	0.61	0.01	0.15	0.71
Mean Litter Depth vs. All methods	0.28	2.71	0.14	0.09	0.66	0.45
Mean Litter Depth vs. Litter only	0.0005	0.08	0.78	0.02	2.97	0.09

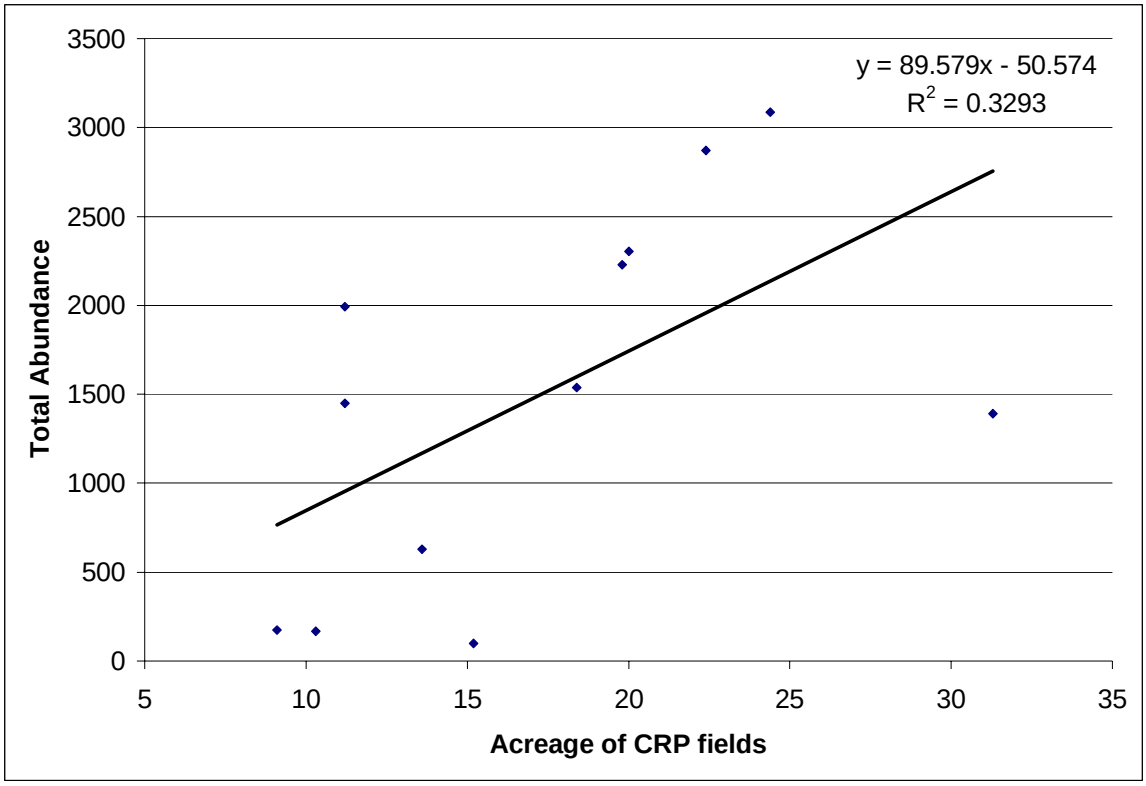


Figure 15. Total ant abundance plotted against acreage of CRP fields (P =0.05).

Discussion

This study provides important data about which ants recolonize restored grassland in the Conservation Reserve Program in an effort to determine their potential as bioindicators of restoration success. Ants have proved to be efficient bioindicators in Australian reclamation sites, by colonizing mines in predictable succession (Majer 1983, Andersen 1997a). Ants have also been proposed as bioindicators elsewhere in the world (Agosti et al. 2000). Unfortunately, few studies have looked at ants as bioindicators in the United States. Fewer still have looked at how ants reestablish grasslands in the process of restoration.

The availability of CRP land of different ages allowed us to use a space-for-time substitution approach as an alternative to a long-term study on ant succession (Pickett 1989). Using this substitution, some assumptions were made about the ant communities sampled on the different ages of land. Although there was some variation in abundance and richness in fields of the same age group, an assumption is being made that, as the younger fields age, ant abundance and species richness will increase to approximately the same abundance and species richness found in the older fields. I am also assuming the converse, such that when the older-aged fields were in earlier successional stages, their abundance and richness data were comparable to data I found on younger fields.

Sixty species of ants are typically found in prairies, and a good-sized prairie remnant is able to support 25-35 of these species (Trager 1998). Fields in this study ranged from 9.1 to 31.3 acres and had 7 – 18 species present. I collected 11 of the 22 species that Trager (1990) sampled from a nearby native prairie system (Tucker prairie).

Subterranean root-aphid tending species are commonly found in prairie ecosystems (Trager 1990), which I confirmed in this study as *L. neoniger* was the second most abundant ant sampled. Mound-building *Formica* is also abundant in prairie ecosystems, which was shown in this study as *F. incerta* was frequently collected in the CRP fields. Although most ants that I sampled are characteristic to a prairie ecosystem, I also sampled species typical of a woodland ant fauna (*A. mariae*, *L. alienus*, and *P. faisonensis*) (Trager pers comm. 2006).

Species richness on our sites is less than the amount typically found on prairie plots but the reasons for this may be due to the fact that prairie remnants are relatively untouched versus CRP land that recently has undergone disturbance. Many of these fields were annually plowed and devoid of native vegetation for many years prior entering the CRP program. It is also possible however, that even though my sampling was intensive, some ants may not have been sampled on some fields. The appearance of three uniques (known from only one sample) and three duplicates (known from two samples) shows that sampling of some ant species was episodic. This may be due to their cryptic nature, small colony size, or that few colonies have established, all of which does not allow many foragers to be available to collect.

Fewer ant species found on restored grasslands in this study may also be partly due to the smaller size of CRP fields relative to remnant prairies. The fields in this study were relatively similar in size and did not show any trends with the number of species sampled per site. However, other studies have shown that fragmentation of land can decrease diversity. A small-scale study by Robinson et al. (1992) looking at arthropod diversity, found in pasture grasslands that although total diversity remained static across

fragment sizes; species richness decreased in smaller fragments. A pasture of 5,000 m^2 or 1.24 acres had 367 species, medium-sized patches (288 m^2; 0.07 acres) had 315 species and small patches (32 m^2; 0.01 acres) had 303 species (Robinson et al. 1992).

In my study abundance generally increased as the time in the CRP program increased. Whether it was due to increased numbers of colonies or to an increase in the number of a few dominantly established colonies was not determined. Comparable studies are also absent on this topic. Most ant community successional studies looked at species richness and composition, not abundance.

In my study species richness was similar for all ages except for a peak at the 7-8-yrs. The peak in species richness in the 7-8-yr fields is best explained by the intermediate-disturbance hypothesis (IDH). This hypothesis states that land is able to support the highest diversity when disturbance is neither frequent nor rare (Wilson 1994; Collins et al. 1995). Based on my study, 7-8-yrs seems to be a sufficient amount of time for the effects of disturbance to decrease on CRP fields. It appears that when land is in the program less than 3-yrs however, disturbance is still recent enough that the ant communities have not fully recovered. When land has been in CRP greater than 14-yrs, the vegetation is established and disturbance is rare, thus not being able to support as many species as 7-8-yr fields. Andersen (1997b), found when looking at ant communities following mining in Australia, that species richness increased rapidly for the first five years of reclamation before beginning to stabilize.

Andersen (1997a) studying different habitats along the elevation gradient in Chiricahua Mountains, Arizona, found that the numbers of genera varied greatly along a gradient. Although my study was in terms of a temporal gradient, I did observe that

particular ants are early colonizers (i.e., *Pheidole pilifera* (Roger)), late colonizers (i.e., *Aphaenogaster carolinensis* Wheeler), or present in all ages (i.e., *T. sessile, Myrmica. americana*, *S. molesta).* Ant species that were collected infrequently cannot be described as early or late colonizers, since collecting them could be episodic. I expect that since some ants, such as *T. sessile*, were abundant in all ages of land, that when older-aged fields were younger, those species were also abundantly present on that land.

My study found some trends that both support and refute Andersen's (1997a, 2000) findings of responses of ant functional groups to disturbance. Andersen (1997a, 2000) found that Generalized Myrmicinae was correlated with disturbance. I found the same in my study because Generalized Myrmicinae decreased with age which may be attributed to decreased disturbance as CRP fields aged. Andersen also noted that Cold Climate Specialist in the subfamily Formicinae would dominate the land. I found this was true as *Lasius* sp. were the most dominant ants found on CRP land. Further, I propose an additional aspect, that Cold Climate Specialists are the most abundant on fields with little disturbance. For instance, in summer and fall seasons, the proportion of Cold Climate Specialists increased on the 7-8-yr and 14-16-yr fields. Although it seems there is less disturbance as CRP land ages, Cold Climate Specialists may increase because increased litter depth allows *T. ambiguus* to make inroads into these environments.

Cold Climate Specialists and Opportunists were the dominant functional groups in our grassland study. A relatively high abundance of Opportunists is indicative of moderate disturbance where behavioral dominance by other ants is low (Andersen 1997a, 2000). I refute this claim and propose that abundance of Opportunists stays fairly

consistent during high and low levels of disturbance. Cryptic species typically decline with disturbance (Andersen 1997a, 2000) and I propose from my study that abundance of cryptic species actually increases with disturbance of land.

In order to effectively recognize the impact of ant functional groups on land, an understanding of its community dynamics must be known (Andersen and Majer 2004). Numerous studies have been conducted on minesites in Australia and they have shown patterns of ant response to disturbance. Some groups increase in relation to disturbance while other groups decrease (Hoffman and Andersen 2003).

We have determined the functional groups used by Andersen (1997a, 2000) may not perfectly fit the ants collected in this study and therefore propose a new set of functional groups should be outlined for ant communities in North American grasslands. Some proposed thoughts of functional group is groups based upon their response to vegetations changes. Australian studies have also shown that ants have a strong correlation to structural diversity of vegetation. Vegetation provides nesting and foraging sites, and where vegetation is missing, there is increased insolation of the ground (New 2000). In my study, *T. ambiguus* was commonly collected in litter samples, so a correlation might be made that litter-dwelling species will decrease with disturbance because there will not be enough litter for the species to inhabit.

Further studies examining ant responses to disturbance would be required to group the ants more appropriately into functional groups for North American prairie systems. Ants can be more affected by changes in habitat complexity than by the composition of plants in their environment (New 2000). In examining ant assemblages on grasslands in southeastern Australia, (New 2000) stated that small scale heterogeneity

is sufficiently high that the predictive indicator values of ants are limited. Ants have also been positively correlated with soil microbial biomass (Andresen 1997b), which increases as CRP lands age.

Conclusion

With the decline in our natural resources, people are increasingly concerned with preserving and restoring natural habitats. This study is a baseline study examining how ants colonize restored grassland in the Conservation Reserve Program in an effort to establish information for comparative studies. In general, ant abundance increased the longer land had been in the CRP program. Thus, as age increased, ant abundance increased. This may be due to ant colonies becoming more established in older fields and more colonies being present. Species richness was constant among the four ages, except for a peak in richness in 7-8 yr fields. The intermediate disturbance hypothesis may explain this (Wilson 1994; Collins et al. 1995), indicating that 7-8-yrs, is an intermediate time since disturbance on CRP fields.

Future studies could include follow-up on earlier-successional fields, to determine if they have similar species richness and abundance when reaching the ages examined in this study. Another study could compare this study to ant communities on undisturbed prairie remnants. Other taxa collected from this study should be examined to determine if any similarities in total abundance and species richness exist with ants. Majer (1983) has shown a correlation of ant richness with the richness of other invertebrate groups. The relationship between ants and beetles are not well understood, although both are used as bioindicators in studies (Majer 1983, Oliver and Beattie 1996). Functional group studies should also be examined, to build a knowledge base of ant's response to disturbance in prairie habitats and other ecosystems.

Chapter 3
A Comparison of Four Methods for Sampling Ant Communities in Grasslands

Introduction

Many methods are used to sample ants including baiting, pitfall trapping, quadrat sampling, direct sampling, surface digging, and litter techniques (Agosti et al. 2000). Ideally, the relative abundance of species in a sample should reflect relative abundance in the community from which the sample was taken (Agosti et al. 2000). Several studies have examined sampling techniques for utility, bias, and efficacy (Adis 1979, Delabie et al. (1994), Abensperg-Traun and Steven 1995, Ward et al. 2001).

Ants have many behaviors that affect which method best samples a particular species. Pitfall traps are a commonly used method for sampling ground-surface active ants (Bestelmeyer and Wiens 1996, Andersen 1997a, Majer 1997, James 2004). Pitfall traps are relatively simple to use, inexpensive, yield high numbers from a wide range of species (Ward et al. 2001) and are capable of operation day and night over an extended period of time (Majer 1997). Criteria used to examine the efficacy of pitfall traps include trap diameter (Abensperg-Traun and Steven 1995, Borgelt and New 2005), digging-in effects (Greenslade 1973) preservative (Adis 1979, Abensperg-Traun and Steven 1995) and pitfall trap space (inter-trap spacing) (Ward et al. 2001). Borgelt and New (2005) found that although larger-diameter pitfall traps captured more ant species, smaller traps collected many species also and would be more effective if a broadly viewed study is being conducted. Pitfall traps show bias toward ants that have increased locomotor movement (e.g., an ant that moves quicker and foragers further distances may be sampled

more frequently then ants that move slower and forage near the colony) (Andersen 1991a).

Winkler extraction and Berlese funnels are commonly used methods of sampling litter-dwelling ants (Agosti et al. 2000). Although litter collecting is relatively labor intensive and costly, Fisher (1998) found in Madagascar rainforest localities that litter sifted in the Winkler technique collected the majority of ant species in the area. Delabie et al. (1994) found in litter sampling that although ant abundance increased dramatically in larger quadrat sizes (i.e., 0.25 m^2 to 1 m^2), the mean number of species found was not as pronounced, therefore it is more efficient to take a greater number of smaller litter samples than a lesser number of larger litter samples (Agosti et al. 2000). Litter samples typically collect small and specialist ant species and tend to under-sample large, active species (Olson 1991, Agosti et al. 2000).

Quadrat sampling is used to sample surface-active ants in a quadrat-delimited sample area (Andersen 1991a). Quadrat sampling is inexpensive but can be labor intensive if repeated ant activity data are collected. Quadrat sampling is conducted within a fixed, transportable quadrat where ant species are counted and/or collected within a given time interval (Agosti et al. 2000). Ants are collected with either forceps or an aspirator. Agosti et al. (2000) states that quadrats sample epigaeic ant forager densities more accurately than pitfall traps. When sampling ants in an Australian tropical savanna, Andersen (1991b), found that commonly recorded ant species in quadrats were also collected in pitfall traps and their relative abundances were highly correlated from both trapping methods. While quadrat sampling is capable of collecting diurnal ants, nocturnal ants are difficult to sample with this method.

A single sampling method is unlikely to collect a full ant assemblage (Agosti et al. 2000). King and Porter (2005) evaluated the efficacy of four sampling methods (baiting, pitfall traps, leaf litter extraction with Berlese funnels, and hand collecting) and concluded that individual methods were complementary, and sampled only part of the entire ant community. Romero and Jaffe (1989) found from examining various sampling methods that a combination of hand collecting and pitfall traps obtained the best results. Wang et al. (2001) compared the efficacy of bait traps and pitfall traps and found bait traps sampled one species not found in pitfall traps, but missed three of the species collected with pitfall traps. To decrease biases of sampling methods and to increase species richness, a complementary set of methods is recommended for ant surveys (Majer 1997).

To further promote the use of ants in biodiversity studies Ants of the Leaf Litter (ALL) protocol was established (Agosti et al. 2000). The ALL protocol states that a sample of 20- 1 m^2 leaf litter plots and 20 pitfall traps is sufficient to sample at least 70% of the ant fauna (Agosti et al. 2000). The ALL program has two objectives: 1) create an easily followed sampling protocol which would encourage field biologists and other researchers to include ants into their biodiversity studies and 2) standardize the methodology of ant collection techniques so results can be compared between studies and researchers (Agosti et al. 2000). This study provided an opportunity to use the ALL protocol in a grassland community and examine grass litter as a substitute for leaf litter.

This study examined the efficacy of four sampling methods (pitfall traps, grass litter extraction with Berlese funnel, hand collection from quadrats, and soil digging) utilizing a modified ALL protocol for ant communities of grasslands in the Conservation

Reserve Program. Ant abundance was recorded for each sampling method. The dominant species collected in each sampling method was also noted. I wanted to determine which sampling methods are most effective for collecting the highest diversity of ants on CRP land. I hypothesize that more than one collecting method is necessary to sample the full ant assemblage present on CRP land.

Materials and Methods

Study Sites

Ant communities were surveyed on 12 Conservation Reserve Program (CRP) grasslands located in Audrain, Monroe, and Boone counties, in east-central Missouri during the summer and fall of 2004 (Fig. 16). The availability of CRP land of different ages allowed us to use the space-for-time substitution approach as an alternative for a long-term study on ant succession (Pickett 1989). The 12 fields comprised four age classes (0, 3, 7-8, and 14-16 yrs) with three replications per age class. Fields enrolled during 2004 were called 0-yr, fields enrolled during 2001 were called 3-yr, fields enrolled during 1996 and 1997 were called 7-8-yr, and fields enrolled during 1988, 1989, and 1990 were called 14-16-yr. Acreage of these fields ranged from 9.1 to 31.3, mean = 17.24 (Table 11). Fields had similar maintenance practices that included either mowing only or no burning within three years prior to the study.

All fields had been planted with a seed mix of warm season grasses (e.g., Big bluestem, *A. gerardii*; Little bluestem, *A. scoparius*; Indian grass, *S. nutans*) of varying proportions (Table 11). Some fields also had various forbs (e.g., lespedeza) as food plots for quail (Table 11). The soils were similar, consisting of Leonard silty clay loam, Armstrong loam, Mexico silt loam, Keswick silt loam, Mexico silt loam, Chariton silt loam, and Gifford silt loam (Table 11) (R. Hagedorn, T. Hill, A. King, and M. Krueger, pers. comm.).

Mean annual rainfall in the region is approximately 1016 mm of which about 635 mm, or about 65 percent usually falls in April through September (USDA-NRCS1995).

Temperatures are high in summer where the average temperature is about 23.8 °C and the average daily maximum temperature is about 30.5 °C (USDA-NRCS 1995). The summer of 2004 was unusually mild with well-below average temperatures and above normal precipitation (Guinan 2004).

Study Design

Four sampling techniques were utilized to survey ant communities in this study. I followed a modified Ants of the Leaf Litter (ALL) protocol (Agosti et al. 2000), which uses the techniques of pitfall traps, litter samples, hand collecting, and surface digging. Six 100 m long parallel transects were placed on each plot of land; two pitfall/soil transects and four litter/hand collecting transects. Each transect had 10 samples taken spaced 10 m apart (Fig. 17).

Since the shape and size of fields in this study were variable, transects were placed so that two edges of field were equidistant from the transects. The shortest length and width of all twelve fields were determined and the approximate midpoint of the smallest field was chosen, then applied to all other fields. These distances were 130.9 m (429.5 feet) lengthwise and 49.8 m (163.5 feet) widthwise. Based on these length and width measurements, a center point was marked (Fig. 17, Step 1). Pitfall transects were then established 10 m on opposite sides from this central point (Fig. 17, Step 2). Individual pitfall sampling points along these transects were marked at 10 m intervals with 2 m tall PVC pipes and fluorescent flagging. Litter/hand collecting transects were established 1 m on both sides of pitfall transects (Fig. 17, Step 2). The July litter/hand collecting transect was 1 m on the inside of the transect, closer to the center point and the September litter/hand collecting transect was 1 m on the outside of the transect.

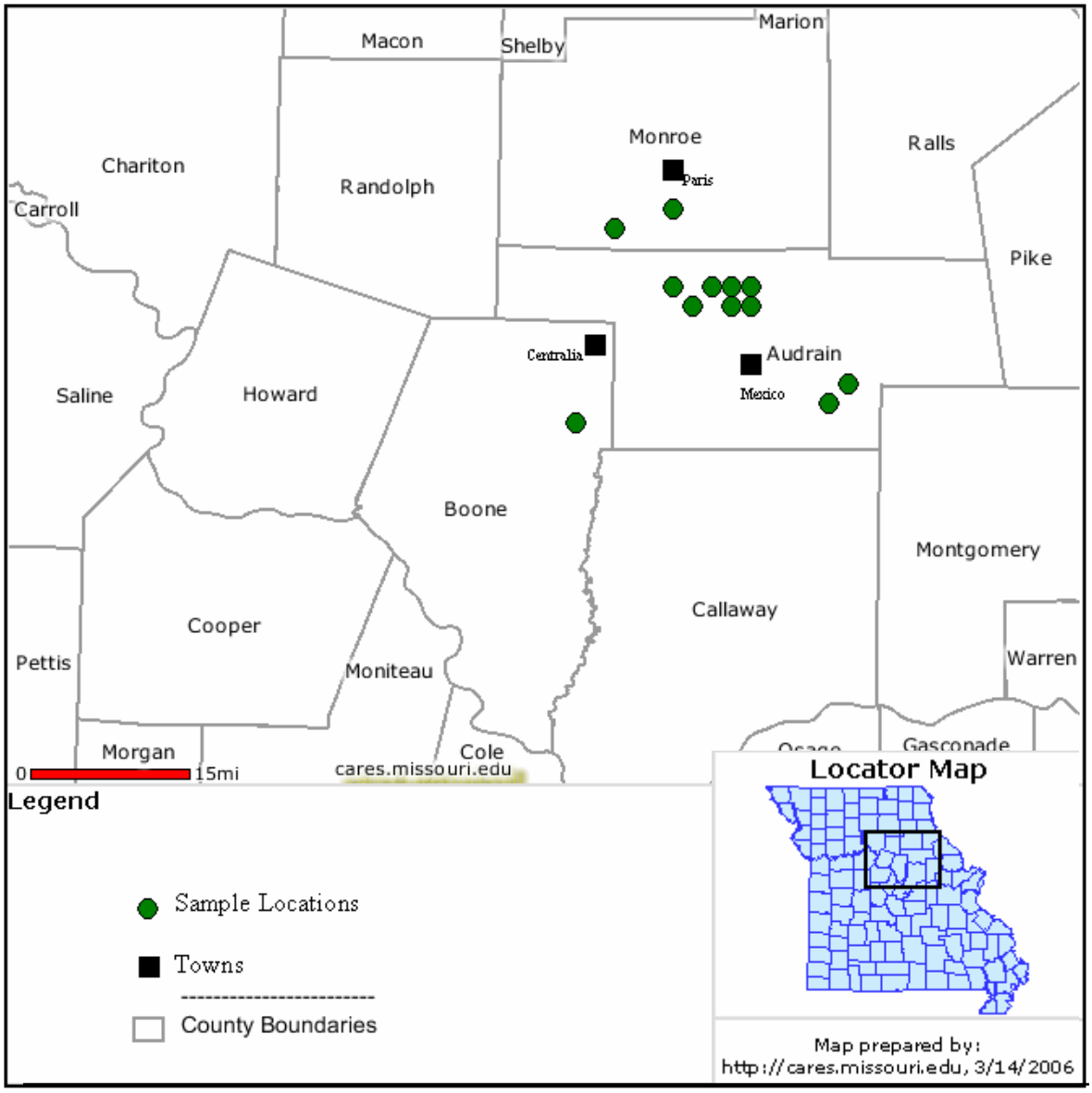


Figure 16. Locations of Conservation Reserve Program land sampled in east-central Missouri.

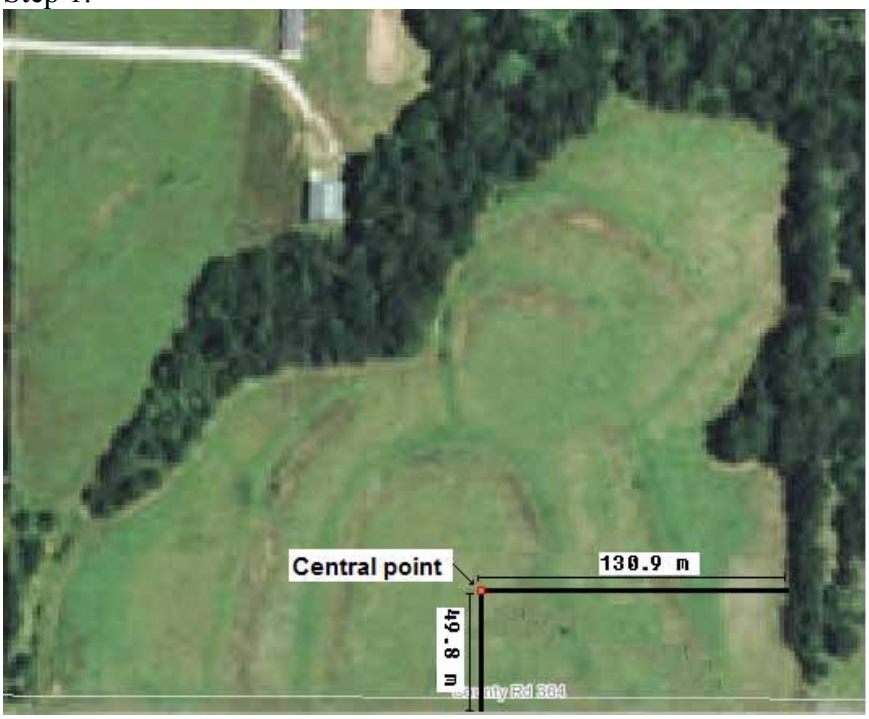
Table 11. Location of CRP fields with corresponding age, acreage, soil type and seed mix.

CRP field	Location GPS Waypoint	Entered Program	Acreage	Soil Types	Seed Rate Pounds/acre	By Vegetation
1	Section 19, T52N,R8W 39.27791 -91.834147	2004	10.3	Leonard silty clay loam Armstrong loam	3.2 2.1 1.0 0.7 .25	Little Bluestem Side-oats grama Alfalfa Indian grass Native Forbs
2	Section 4, T50N, R11W 39.14348 -92.157352	2004	15.2	Keswick silt loam Mexico silt loam	3.5 1.0 0.25 0.25 0.5	Little Bluestem Side-oats grama Indian grass Big Bluestem Native Forbs
3	Sections 13&14, T53N, R 10W 39.38232 -91.983646	2004	9.1	Leonard silty clay loam Mexico silt loam	1.7 1.7 1.6 0.8 0.25 1.0	Big Bluestem Indian grass Eastern Gamagrass Switch grass Native Forbs OR Alfalfa
4	Section 7, T52N, R8W 39.29491 -91.838153	2001	11.2	Leonard silty clay loam	3.3 1.1 1.4 1.0	Big Bluestem/Indian grass (sum of two grasses) Little Bluestem Side-oats Grama Annual Lespedeza
5	Section 10, T52N, R8W 39.29594 -91.79123	2001	31.3	Leonard silty clay loam Chariton silt loam Gifford silt loam	3.3 1.1 1.4 1.0	Big Bluestem/Indian grass (sum of two grasses) Little Bluestem Side-oats Grama Annual Lespedeza
6	Section 6, T50N, R7W 39.14634 -91.728137	2001	13.6	Leonard silty clay loam Mexico silt loam	3.3 1.1 1.4 1.0	Big Bluestem/Indian grass (sum of two grasses) Little Bluestem Side-oats Grama Annual Lespedeza 5 species native warm-season grasses 10 species Native Forbs
7	Section 25, T53N, R11W 39.34649 -92.094261	1997	11.2	Leonard silty clay loam Mexico silt loam	7.0	Big Bluestem
8	Section 11, T52N, R9W 39.2949 -91.891359	1996	18.4	Leonard silty clay loam Armstrong loam	4.0	Switch grass
9	Section 7, T52N, R8W 39.30593 -91.846859	1996	24.4	Leonard silty clay loam Mexico silt loam	4.0	Switch grass
10	Section 36, T52N, R9W 39.24813 -91.868503	1990	22.4	Leonard silty clay loam Armstrong loam	3.0 4.0	Big Bluestem Indian grass
11	Section 16, T50N, R8W 39.10688 -91.80355	1989	19.8	Leonard silty clay loam Mexico silt loam	2.0 2.0 1.0 1.0	Big Bluestem Indian grass Little Bluestem Side-oats grama
12	Section 24, T52N, R9W 39.27368 -91.862968	1988	20	Leonard silty clay loam Armstrong loam	7.0	Big Bluestem

Pitfall trap sampling

Pitfall traps were used to sample surface-dwelling ants. A total of 20 pitfall traps (n=19 in field 5 during fall) was placed in each field. Ten pitfall traps were placed along each of the 100 m transects at 10 m intervals. A 30 cm per side equilateral wooden triangle with a 15 cm nail placed in each corner served as a cover to prevent rain from falling into pitfall traps (Fig. 18). A Pro II Hole Cutter (10.8 cm (4 ¼")) diameter and (13.97 cm (5.5")) depth used for cutting holes in golf course greens, was used to make pitfall trap holes and to minimize disturbance of the soil surface around each trap. Each trap consisted of a 16 oz. Pro-Kal deli container from Fabri-Kal Corporation, Kalamazoo, MI [with a diameter of 11 cm (4.5")] placed inside a 32 oz. V-32 deli container from Plastic Packaging Corporation, West Springfield, MA. The 32 oz. V-32 deli container was placed in the hole, making sure it was flush with the soil surface. Pitfall traps were closed using a tightly fitting lid for at least a week after placement to reduce any digging-in effect (Greenslade 1973) which could bias sampling by attracting ants to the disturbed habitat (Agosti et al. 2000). Pitfall traps were activated for 72 h, over a two-day period on June 22-23, 2004 and September 4-5, 2004. Activation of traps was executed a day apart from each other to keep temporal variation to a minimum. When a trap was not activated, the lid for the 16 oz. Pro-Kal deli container was placed securely on to keep arthropods from falling in. When activated, the lid was removed and approximately 150 ml of 50% propylene glycol was added to the container. After 72 h lids were placed on the samples and taken to the lab for processing. Empty containers with lids were placed back in the 32 oz. containers in the field so invertebrates would not fall in.

Step 1.



Step 2.

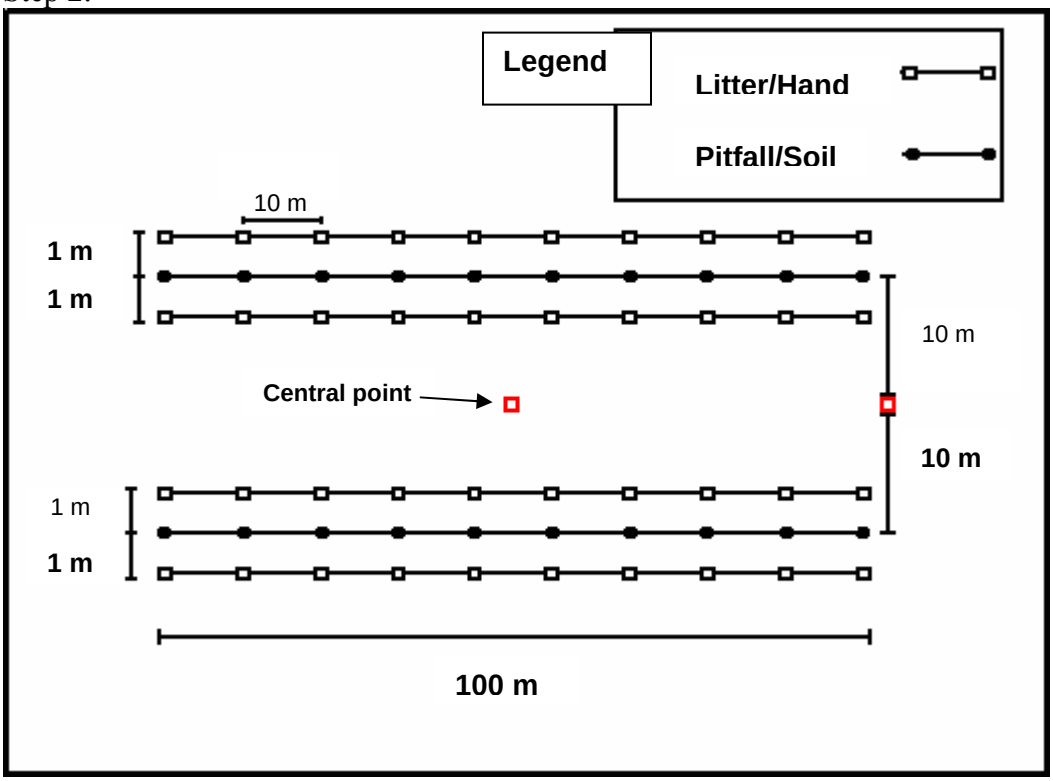


Figure 17. Sampling design used on each Conservation Reserve Program field.

Figure 18. Rainhood suspended over pitfall traps using a 15cm nail placed in each corner.

In the laboratory, propylene glycol was strained from the contents of the pitfall traps with a 250 µm sieve. Contents were rinsed with distilled water and 80% ethanol to remove any remaining propylene glycol. When soil was abundant in the sample after propylene glycol was decanted, contents were placed in a 100 ml container where a hypertonic salt solution was utilized to float invertebrates out of the soil (Agosti et al. 2000). A saturated Morton® salt (plain and iodized) solution was poured in the 100 ml container, contents stirred, aqueous portion strained through 250 µm sieve, and the process repeated one to two times. Contents remaining in the sieve were rinsed with distilled water to remove any remaining salt. All invertebrates were sorted from the remaining debris using a dissecting microscope at 7x power. Ants were separated to morphospecies then identified to species and stored in 95% ethanol. Adult and immature

beetles were separated into a separate vial, stored in 80% ethanol, and labeled. All other invertebrates were also stored in 80% ethanol and labeled with collection data.

Soil Samples

Soil core samples were taken to survey subterranean ants. Soil excavated when establishing each pitfall trap with the Pro II Hole Cutter [10.8 cm (4 ¼")] diameter and [13.97 cm (5.5")] depth was placed in plastic bags in the field. Soil core samples were collected only in the summer sampling season. Samples were transported to the laboratory where the top 5 cm of the soil core was broken apart by hand and the ants aspirated and stored in 95% ethanol.

Hand Collection and Litter Sampling

Hand and litter sampling were conducted July 13 - August 5, 2004 and September 19 – October 9, 2004. Two transects, (each 100 m in length) were placed 1 m away from each pitfall trap transect. Every 10 m along these transects, a 0.25 m^2 quadrat was placed on the ground. Grass litter samples were collected by gathering all the grass litter in the 0.25 m^2 quadrat and placing it in a white plastic bag. Grass litter samples were taken to the lab and placed in Berlese funnels for 48-72 h. Hand sampling occurred in the same quadrat. Hand collection consisted of thoroughly searching vegetation above litter level immediately before litter was collected and on the bare ground for two minutes immediately after litter was collected. There were times when all observed ants could not be collected due to the abundance and/or speed of some species. In most cases, at least one representative of each species was collected. All hand collected ants were aspirated and stored in containers of 95% ethanol.

Processing

The contents from all samples were taken to the laboratory, processed, sorted, identified to species, and counted. The functional group of each ant species was documented. Identification was performed using a dissecting microscope (7-30x) with a 2x objective. Identification of specimens to the level of genus was obtained using Creighton (1950) and Bolton (1994) and to the species level using Creighton (1950), Trager (1984), Wheeler and Wheeler (1986), Bolton (1994), Francoeur (2000-2005), and Trager et al. (in press). Species identifications were confirmed by Dr. James Trager (Missouri Botanical Garden - Shaw Nature Reserve). Samples were stored in 95% ethanol. Voucher specimens from the study were deposited at the Enns Entomology Museum at the University of Missouri-Columbia.

Data Analysis

Workers were the only caste examined for this study as their presence provides evidence of an established colony (Longino et al. 2002). Pitfall traps, hand collection, litter collecting, and soil core sampling were examined for efficacy according to total mean richness and mean richness per sample. Soil cores were only employed in the summer and therefore were only analyzed in the comparison of efficacy for methods used during the summer season.

One-way analysis of variance (ANOVA) was performed using species richness as the dependent variable and collecting method as the independent variable. To isolate which method(s) differed from the others, a Tukey's Test ($P \leq 0.05$) was used. One-way analysis was also performed using richness per individual sample as the dependent variable and method as the independent variable. This analysis failed the normality test

($P < 0.001$), due to many zeros in the data so ANOVA on ranks was used. To isolate which method(s) differed from the others, Dunn's Method ($P \leq 0.05$) was used. Zero-yr data were excluded from these analyses due to the lack of hand collection and litter sampling data which could not be directly compared to older fields.

A three-way ANOVA was used to examine the dependent variables of method, age, and season to the independent variable of richness. Soil core data were not included in the analysis due to unequal sample size. A Tukey's test ($P \leq 0.05$) was used to isolate which group(s) were significantly different from each other for each analysis. All statistical analyses were performed using SigmaStat 2.0 (SPSS Inc. 1997) unless otherwise stated.

Species accumulation curves were created by combining seasons and fields of the same age to determine if enough samples were taken using each sampling technique. Curves were generated by randomizing the order of all traps within each sampling unit 100 times. Before randomization, traps from different fields of the same age were pooled as one (e.g., Trap A from Field 1, Field 2, and Field 3).

From the species accumulation curves I could determine how many traps were needed on average to collect 90% of the ant species. I determined this value for each field and method (e.g., Field 3 sampled 12 species with pitfall traps so I calculated how many traps were needed to sample 90% or 10.8 species). Using this information, the mean richness and standard deviation of each age group for each method was calculated.

I also predicted how many traps of each method it would take to collect 90% of the total ant fauna (28 spp.). Species accumulation curves were generated as in the previous description except all fields and ages were pooled. Since I had to project a

number >20 traps I assumed that the curve would continue to increase past trap 20 at a similar rate as the slope between trap 11-20. A line was fit to the points along the species accumulation curve between traps 11-20 using linear regression. The regression equations are as follows y = 0.3021(x – 10) + 25.035 for pitfall traps, y = 0.182(x – 10) + 17.372 for hand collection, y = 0.1702(x – 10) +8.3287 for litter samples, and y = 0.0662(x – 10) + 6.4027 for soil cores (Fig. 19). By inputting the value equal to 90% of the total ant fauna (25.2) for y, I was able to calculate how many additional samples would be necessary to sample 90% of the total ant fauna using each method.

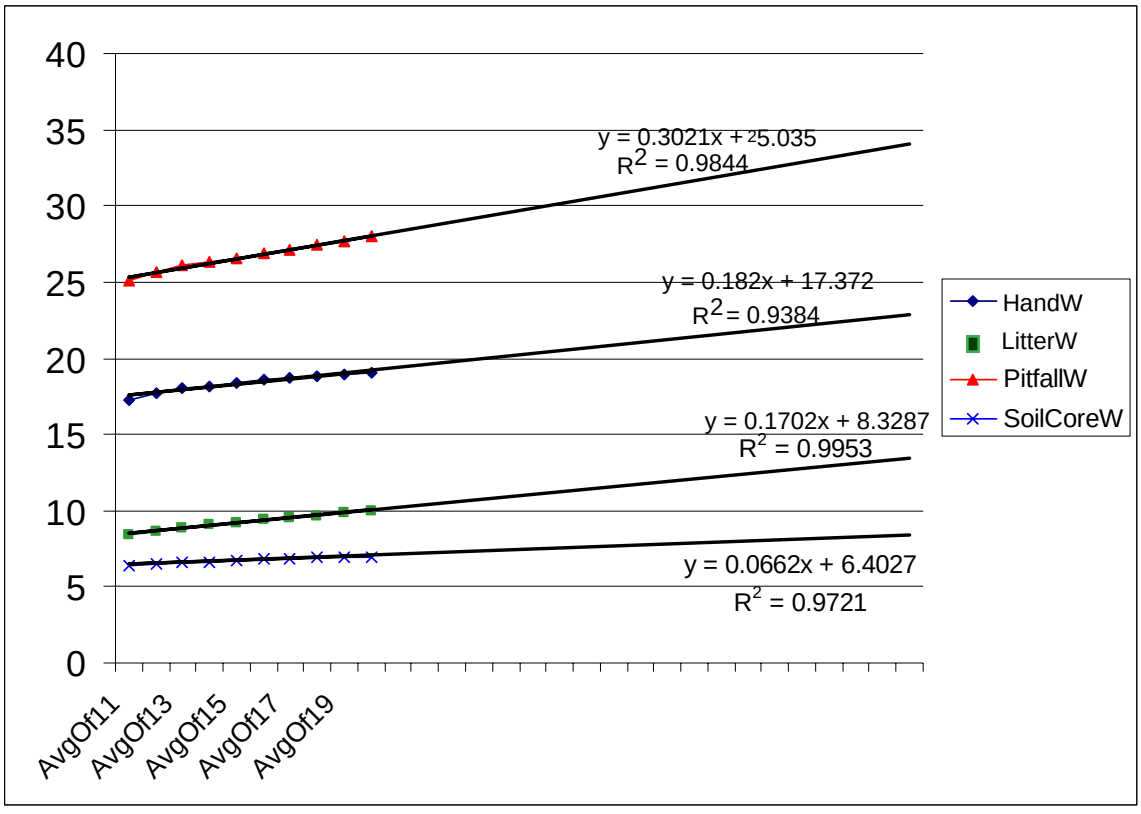


Figure 19. Linear slope of species accumulation curves based on traps 11-20 and used to project the number of samples needed to collect ninety percent of the total ant fauna using each method.

Results

Ant Species

In total, I observed 18,743 ants representing 28 species in 16 genera across all Conservation Reserve Program fields in summer and fall of 2004. The checklist of the ant species found and the abundance of each species collected by each sampling method (from pooled seasonal data) is shown in Table 12. A total of 11,400 ants were collected from pitfall traps, 5,659 from litter sampling, 868 from hand collection, and 816 from soil core samples (Table 12). Five ant species were dominant in this study. Pitfall traps and hand collection sampled *L. neoniger, T. sessile*, *Myrmica. americana*, and *S. molesta*. These four species made up 78% and 73%, respectively of total ant abundance for each method (Table 12). Litter sampling collected three dominant species: *T. ambiguus, T. sessile*, and *S. molesta*, which made up 99% of the total ants sampled with this method (Table 12). The dominant species collected in soil cores *were S. molesta L. neoniger*, and *T. sessile*, which made up 86% of the total for this method (Table 12).

Method Efficiency

In general, the most effective sampling methods in decreasing order were pitfall traps, hand collecting, litter sampling, and soil cores. Pitfall traps were the most effective in terms of the total number of species sampled per field and the total number of species sampled per individual trap. For all fields and seasons, 28 species were sampled by pitfall traps, 19 by hand collection, 10 by litter samples, and seven by soil cores (Table 12, Fig. 20). Pitfall traps showed a significant difference in the total number of species collected in summer and fall ($F = 32.307$; $df = 2, 33$, $P < 0.001$) (Fig. 20 A, B, and C).

Pitfall traps yielded 135% more species than hand collection and 264% more than litter samples. July pitfall traps yielded 204% more species than hand, 343% more than litter, and 408% more than soil cores. No significant differences were observed for species richness between litter, soil core, and hand samples (Fig. 20, A, B, and C).

The mean number of ant species collected per trap was significantly greater for pitfalls than hand, litter, or soil samples (Fig. 21). When looking at species richness per individual trap (sampling effort) for both seasons - pitfall traps sampled 3.5 species per trap, litter 0.81 species, and hand 0.70 species (Fig. 21). Pitfall traps yielded 400% more species than hand collecting and 338% more species than litter samples for summer and fall combined. During summer only, the average pitfall sampled 4.2 species, litter 0.68, hand 0.81, and soil cores 0.43 species. Pitfall traps yielded 419% more species than hand collecting, 518% more than litter samples, and 877% more than soil cores.

Comparing method effectiveness on different ages and seasons

Table 13 shows the results of a three-way ANOVA used to examine the relationship of method, age, and season to species richness. Mean species richness was calculated by pooling fields of the same age and season. Species richness was significantly different between methods ($F= 85.482$; $df = 2, 48, 71$; $P < 0.001$) and ages ($F = 11.940$; $df = 3, 48, 71$; $P < 0.001$). Species richness in pitfall traps was significantly different from all other methods and species richness in 0-yr fields was significantly different from 3-yr and 7-8-yr fields. Pitfall traps yielded 19% more species during summer than fall. No differences were found between seasons for each method ($F= 1.751$; $df= 2, 48, 71$; $P= 0.185$). More ant species were collected by all methods on 7-8-yr field plots with the exception of 3-yr hand collecting. Each method sampled the

fewest species in 0-yr fields. Pitfall traps in 7-8-yr fields, yielded 34% more species than 14-16-yr fields, 43% more than 3-yr fields, and 69% more than 0-yr fields. Litter sampling in the 7-8-yr fields yielded 44% more species than 14-16-yr and 3-yr fields and 766% more than 0-yr fields. Soil cores from 7-8-yr fields yielded 67% more species than 14-16-yr fields, 11% more than 3-yr fields, and 399% more than 0-yr fields. Hand sampling on 3-yr fields yielded 13% more species than 7-8-yr fields, 43% more than 14-16-yr fields, and 87% more than 0-yr fields.

Species Accumulation Curves

Species accumulation curves from the 12 study plots show in most cases that a sufficient amount of samples was collected by each method (Fig.22). Pitfall traps consistently collected more ant species than the other methods. Hand collecting sampled more species than litter samples. Fields belonging to the 0-yr age group were poor for comparing sampling methods due to few ants collected with any method besides pitfall traps (Fig. 22A). Although litter and hand collecting did not collect as many species as pitfall traps, the flattened line indicated that the sampling method collected the maximum amount of ant species.

When looking at seasons combined for each field individually, the species accumulation curves predict that 90% of the ant species collected in this study by each method could have been captured using only 13.83 ± 2.12 (pitfall traps), 15.75 ± 2.9 (hand collecting), and 12.22 ± 2.82 (litter) (Table 14). When compared to hand collecting, pitfall traps would require fewer sampling units to collect 90% of the species because each trap would remain open day and night for 72 h whereas hand collection would be completed in two minutes. For 0-yr fields, it would take more pitfall traps and

hand collecting to sample 90% of the ant species present as compared to other methods (Table 14). For hand collecting and pitfall traps the 14-16-yr age group would require the fewest samples to collect 90% (Table 14).

Projections from species accumulation curves of the number of samples needed to collect 90% of the total ant fauna using each method (ages and seasons) are found in Table 15. Pitfall traps would require only 10.5 samples, whereas hand collection would require 53, litter 109, and soil cores would need 294 samples in order to collect 90% of the total ant fauna found in this study.

Table 12. Comparison of ant species richness and abundance between four sampling methods (pitfall traps, hand collecting, litter sampling, and soil cores).

Species	Pitfall	Hand collecting	Litter	Soil core	Grand Total
Lasius neoniger	3589	85	0	186	3860
Tapinoma sessile	1827	351	2057	145	4380
Myrmica americana	1776	126	3	27	1932
Solenopsis molesta	1735	79	655	369	2838
Lasius alienus	989	68	5	1	1063
Formica incerta/sp.*	330	19	0	0	349
Formica subsericea	193	11	1	0	205
Aphaenogaster carolinensis	191	8	0	0	199
Temnothorax ambiguus	159	63	2892	2	3116
Pheidole pilifera	119	4	0	0	123
Formica pallidefulva	91	2	0	0	93
Monomorium minimum	72	2	1	0	75
Crematogaster cerasi	68	18	0	0	86
Myrmica spatulata	62	0	1	0	63
Crematogaster lineolata	60	7	21	0	88
Ponera pennsylvanica	46	3	23	86	158
Myrmica emeryana	29	1	0	0	30
Temnothorax pergandei	16	16	0	0	32
Formica subintegra	11	0	0	0	11
Hypoponera opacior	9	3	0	0	12
Stenamma brevicorne	8	0	0	0	8
Paratrechina terricola	6	0	0	0	6
Paratrechina faisonensis	5	2	0	0	7
Formica dolosa	4	0	0	0	4
Aphaenogaster fulva	2	0	0	0	2
Polyergus lucidus	1	0	0	0	1
Aphaenogaster mariae	1	0	0	0	1
Myrmecina americana	1	0	0	0	1
Total	11400	868	5659	816	18743

*Species are morphologically indistinguishable in samples where few specimens are present.

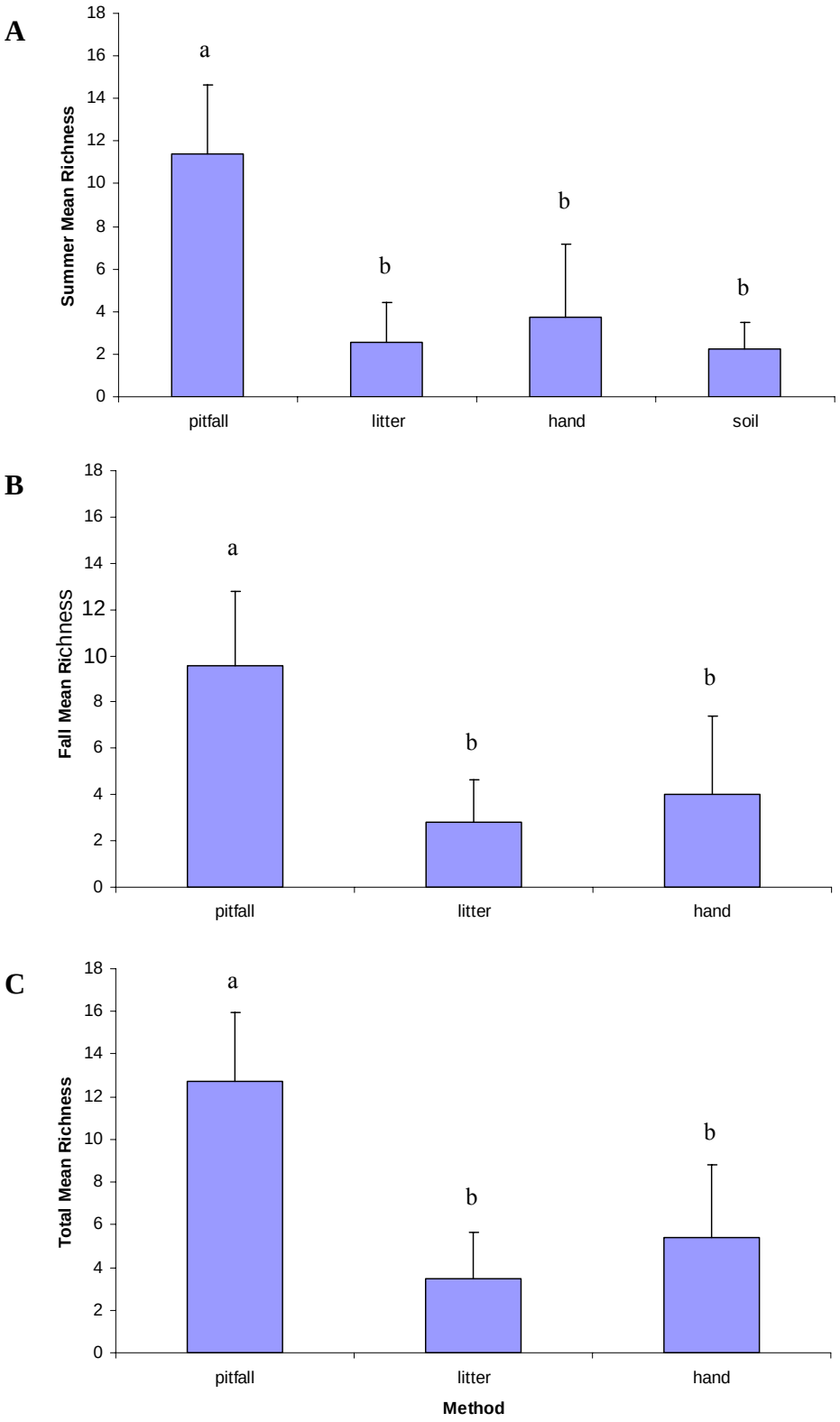


Figure 20. Comparison of mean species richness (± SD) from different sampling methods for summer (A), fall (B), and both seasons combined (C). Histograms with different letters are significantly different (Tukey test, $P \leq 0.05$).

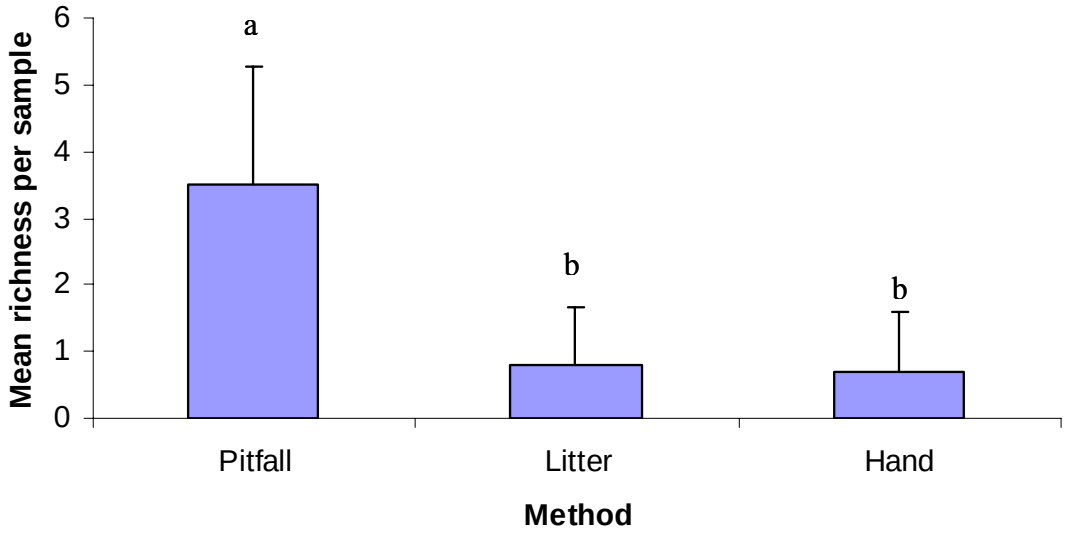


Figure 21. Mean species richness per trap (± SD) for different sampling methods (pooled seasons). Histogram with different letters are significantly different (Dunn's Method, $P \leq 0.05$).

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Table 13. Mean ± SD of species richness on CRP land of varying ages by method and season.

Age	Season	Pitfall	Hand	Litter	Soil Core	Total*
0 yr	Summer	8.33 ± 3.06	0 ± 0	1 ± 1	0.67 ± 0.58	3.33 ± 3.88 **A**
	Fall	8 ± 2	2.67 ± 1.15	0 ± 0	N/A	
3 yr	Summer	10.67 ± 2.52	5.67 ± 4.04	2.67 ± 1.53	3 ± 0	6.11 ± 3.72 **BC**
	Fall	8.67 ± 3.06	5.67 ± 3.51	3.33 ± 0.58	N/A	
7-8 yr	Summer	15.33 ± 1.15	6 ± 3	4.33 ± 2.31	3.33 ± 0.58	7.72 ± 5.00 **C**
	Fall	12.33 ± 2.31	4 ± 2.65	4.33 ± 2.52	N/A	
14-16 yr	Summer	11.33 ± 1.15	3.33 ± 2.08	2.33 ± 1.15	2 ± 1.00	5.61 ± 3.87 **B**
	Fall	9.33 ± 2.08	3.67 ± 2.52	3.67 ± 2.08	N/A	
Total**		10.50 ± 3.04 **a**	3.88 ± 2.92 **b**	2.71 ± 2.01 **b**	2.25 ± 1.22 **b**	

*Total column with different capitalized letters are significantly different (Tukey Test, $P \leq 0.05$) (Soil core data were not included in the totals). **Total row with different lower case letters are significantly different (Tukey Test, $P \leq 0.05$).

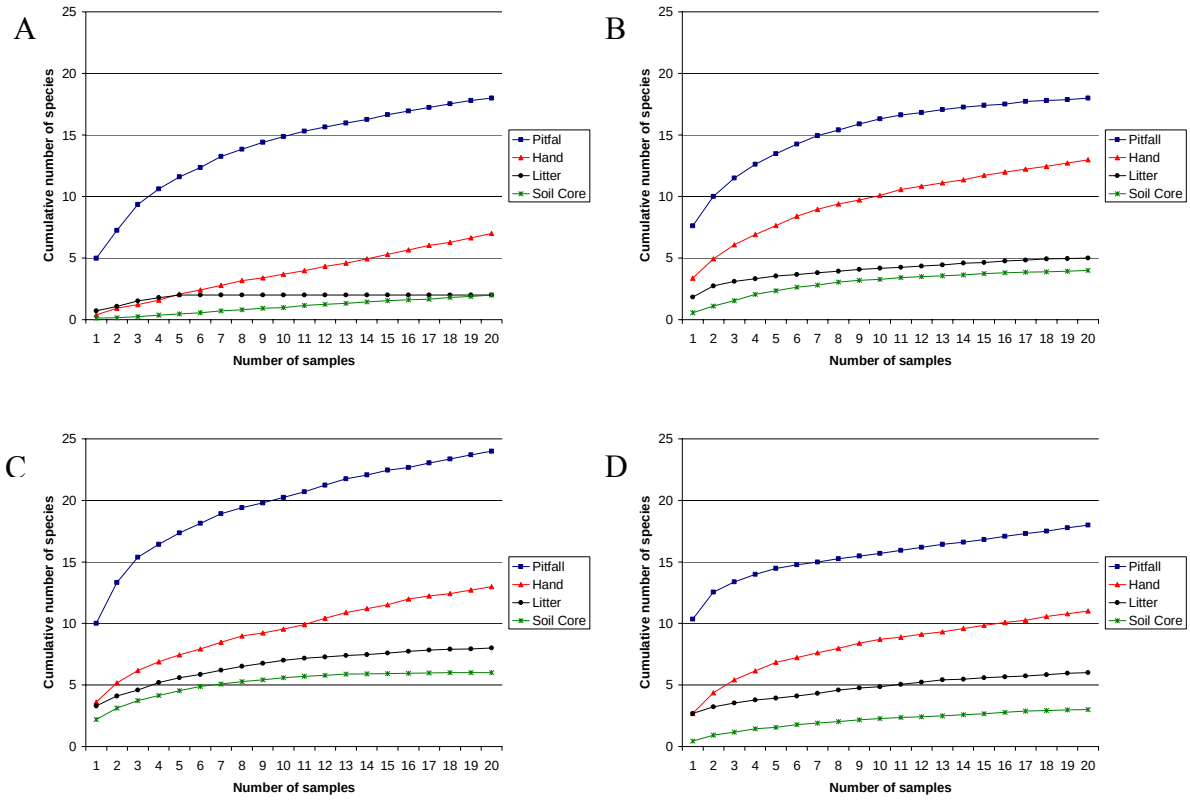

Figure 22. Species accumulation curves for all age groups (0-yr (A), 3-yr (B), 7-8-yr (C), 14-16-yr (D) and sampling methods (pitfall traps, hand collecting, and litter) for CRP fields.

Table 14. Comparison of mean number of samples needed to collect 90% of species from each age (± SD) of CRP land using each sample method.

Sampling Method	**Mean Richness ± SD**
Pitfall	
0-yr	16.00 ± 1.00
3-yr	13.33 ± 2.31
7-8yr	14.00 ± 1.73
14-16yr	12.00 ± 1.73
Hand	
0-yr	18.33 ± 0.58
3-yr	16.33 ± 0.58
7-8yr	16.00 ± 1.00
14-16yr	12.33 ± 4.04
Litter	
0-yr	N/A*
3-yr	13.00 ± 2.00
7-8yr	10.33 ± 3.21
14-16yr	13.33 ± 3.06

* No results for litter data due to no litter present on 0-yr fields.

Table 15. Estimated number of samples needed to collect 90% of all ant species on CRP fields.

Method	Slope	# of samples needed to collect 90% of ant species
Pitfall	0.3021	10.5
Hand collection	0.182	53
Litter sample	0.1702	109
Soil Core	0.0662	294

Discussion

This study found that pitfall traps are the most effective sampling method for restored grasslands in the Conservation Reserve Program. Pitfall traps collected the most species overall and per trap. Other sampling methods did not add any additional ant species to my survey. Few studies are available for comparing and contrasting collection methods in similar habitats in the same region. Peterson et al. (1998) although not comparing efficacy of sampling methods (pitfall traps, surface digging, and hand collection) stated that since most species were rarely observed more than a single method was necessary. In my study, many species were readily observed, allowing a single sampling method to collect all ant species observed from other sampling methods.

Peterson et al. (1998) found in reconstructed tallgrass prairie in Illinois that *L. alienus* was the most widely distributed species in the plot. In my study *T. ambiguus* was the most widely distributed, being present in all 12 fields. Five ant species *(T. sessile, L. neoniger, Myrmica. americana, S. molesta, and Ponera pennsylvanica* Buckley*)* were found in 11 out of 12 fields. Peterson et al. (1998) found six species that my study found*: T. sessile, F. subsericea, L. alienus, Crematogaster lineolata* (Say), *S. molesta, and P. pennsylvanica*. Trager (1998) stated that the ant genus *Formica* has the highest species richness in the tallgrass region. This was confirmed in my study as I collected a total of five species from this genus, more than all other ant genera.

Pitfall traps were the most effective method in my study for assessing richness. When sampling ants in disturbed sites on the Fall-Line Sandhills at Fort Benning, Georgia, Graham et al. 2004, found that pitfall traps collected the most species (44),

followed by sweep samples (31 species), and hand sampling on trees (15 species). Wang et al. (2001) sampled ant communities in national forests in Virginia and West Virginia and also concluded that pitfall trapping collected more ant species.

In most studies, a complementary set of methods is recommended for ant surveys (Majer 1997). Studies conducted by King and Porter (2005) in forests of northern and central Florida found that hand collecting was the best method for capturing species richness but that a variety of individual methods were complementary to one another. Romero and Jaffe (1989) found from examining various sampling methods on mainland savannas in the Venezuelan Llanos that a combination of hand collecting and pitfall traps obtained the best results.

The efficacy of sampling methods may vary with the type of habitat being sampled. Pitfall traps are effective in open habitats where litter does not interfere with locomotor activity and cryptic species are not abundant (Andersen 1991b). Forested sites, however, support a wider array of cryptic ants than grasslands and many more microhabitats are available in forests (i.e., leaf-litter, twigs, acorns) (Trager 1998), thus requiring a wider selection of methods to sample the ant fauna there. Litter extraction methods can also reveal cryptic species that are under-sampled in pitfall traps (Majer 1997), although I did not observe this in the study using grass litter.

While the species accumulation curves began to level off, indicating that enough samples were taken, there is no doubt that some species were missed in the samples, either because of their cryptic nature or because of their low abundance. My examination of the number of traps needed to collect 90% of species versus the age of CRP land was noteworthy. For 0-yr fields, it took more traps to sample 90% of the ant species present

as compared to other methods. This is likely due to the high disturbance of the land and that a lower population of ants are on these fields. The 14-16-yr age group required fewer hand collecting and pitfall traps to collect 90% of the ant species. This is likely due to larger and/or more colonies being present on these fields making the foragers more uniform across the sampling area.

When the pooled species composition of hand collecting was compared to the pooled species composition of pitfall traps, it appeared hand collecting might be a more effective sampling method for CRP fields than pitfall traps (Table 12). Hand collection caught the same common species as pitfall traps and it was more time efficient. With the exception of *Paratrechina faisonensis* (Forel) and *Hypoponera opacior* (Forel) the only species that hand collecting did not sample were the same species that pitfall traps sampled uncommonly (fewer than 11 individuals). The only commonly-sampled species that hand collection missed was *Myrmica spatulata* M. R. Smith. However, when fields were analyzed individually, hand collecting on average sampled less than half of the species collected with pitfall traps in the same field and during the same season (Appendix A). Hand collection and litter samples were also ineffective methods for 0-yr CRP fields. Ants were almost absent in samples from these methods due to the absence of litter and fewer established colonies. Majer (1997) noted that pitfall traps may under-sample cryptic species of ants but this was not evident in my study as pitfall traps frequently sampled more cryptic species then the other sampling methods in my survey.

Although particular species can be more commonly sampled with certain method(s); the choice of sampling method usually does not determine which ant species are found. Three species dominated my litter samples: *T. ambiguus, T. sessile, and S.*

molesta. It was evident that they were nesting in litter due to the presence of larvae, pupae, and queens in the samples. Although *T. sessile* is not classified as a cryptic species, it is very adaptable and capable of nesting in many diverse areas (Creighton 1950). *Temnothorax ambiguus* also is not classified as a cryptic species but this ant was the most commonly found in litter samples. Creighton (1950) stated *T. ambiguus* will nest in plant cavities or nest in hollow stems at the base of grass tufts. *Solenopsis molesta* is a cryptic species, yet it was common in my pitfall samples. Creighton (1950) stated that some small *Solenopsis* will nest near larger nests and pilfer food, and only occasionally forage above ground, whereas other species prefer preformed cavities in plant tissue.

Conclusion

This study is a baseline guide for considering the efficacy of four sampling methods in restored grasslands of the Conservation Reserve Program in east-central Missouri. A recommendation of using strictly pitfall traps for 72 h in similar habitats within this region will effectively census ant communities. Utilizing this single sampling method would decrease labor and time and could encourage more land managers to include ant surveys in their studies. I recommend that if a complete census is not necessary, a sample size of approximately 14 pitfall traps would collect approximately 90% of the ant species in a field of approximately 17 acres. However, it is important to remember that every habitat will be different, so a small pilot study should be conducted. Also, remember that pitfall traps of a smaller diameter may sample fewer ants than the results shown here.

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APPENDIX A

Total abundance and richness of species on 0-yr fields for pitfall traps, hand collection, and litter sampling for summer (S) and fall (F).

	0-yr Fields																	
	1						2						3					
	Pitfall		Hand		Litter		Pitfall		Hand		Litter		Pitfall		Hand		Litter	
	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F
Tapinoma sessile	7	1	0	1	0	0	0	0	0	0	0	0	13	4	0	1	0	0
Formica incerta/sp. *	1	1	0	0	0	0	0	0	0	0	0	0	7	6	0	0	0	0
Formica dolosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Formica pallidefulva	0	1	0	0	0	0	1	2	0	0	0	0	1	0	0	0	0	0
Formica subintegra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Formica subsericea	2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Lasius alienus	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Lasius neoniger	0	0	0	0	0	0	8	4	0	1	0	0	1	1	0	0	0	0
Paratrechina faisonensis	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratrechina terricola	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Polyergus lucidus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphaenogaster carolinensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphaenogaster fulva	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Aphaenogaster mariae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crematogaster cerasi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crematogaster lineolata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monomorium minimum	3	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Myrmecina americana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrmica americana	0	0	0	0	0	0	15	6	0	1	0	0	2	1	0	0	0	0
Myrmica emeryana	3	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Myrmica spatulata	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pheidole pilifera	3	2	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Solenopsis molesta	10	9	0	0	1	0	0	0	0	0	0	0	7	4	0	0	0	0
Stenamma brevicorne	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Temnothorax ambiguus	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0
Temnothorax pergandei	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypoponera opacior	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Ponera pennsylvanica		0	1	0	0	0	0	0	0	0	0	0	0	1	2	0	1	0	0
TOTAL	**Sp. Richness**	**9**	**10**	**0**	**4**	**2**	**0**	**5**	**6**	**0**	**2**	**0**	**0**	**11**	**8**	**0**	**2**	**1**	**0**
	Abundance	**31**	**21**	**0**	**4**	**2**	**0**	**26**	**15**	**0**	**2**	**0**	**0**	**36**	**20**	**0**	**2**	**1**	**0**

*Species are morphologically indistinguishable in samples where few specimens are present.

Total abundance and richness of species on 3-yr fields for pitfall traps, hand collection, and litter sampling for summer (S) and fall (F).

		3-yr Fields																	
		4						5						6					
		Pitfall		Hand		Litter		Pitfall		Hand		Litter		Pitfall		Hand		Litter	
		S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F
Tapinoma sessile		15	11	6	6	0	6	18	15	5	12	2	7	12	10	1	2	2	4
Formica incerta/sp.*		14	4	4	0	0	0	10	1	2	1	0	0	0	0	0	0	0	0
Formica dolosa		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Formica pallidefulva		4	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Formica subintegra		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Formica subsericea		2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Lasius alienus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lasius neoniger		9	7	2	1	0	0	15	11	1	1	0	0	2	0	0	0	0	0
Paratrechina faisonensis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratrechina terricola		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polyergus lucidus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphaenogaster carolinensis		3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphaenogaster fulva		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphaenogaster mariae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crematogaster cerasi		2	2	0	1	0	0	0	0	0	0	0	0	3	2	0	0	0	0
Crematogaster lineolata		2	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Monomorium minimum		5	6	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Myrmecina americana		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrmica americana		13	5	2	1	0	0	20	18	9	11	0	0	7	6	0	1	0	0
Myrmica emeryana		0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Myrmica spatulata		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pheidole pilifera		12	12	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Solenopsis molesta		17	19	2	2	5	0	13	14	2	1	3	1	14	11	0	0	6	3
Stenamma brevicorne		0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0
Temnothorax ambiguus		0	0	0	0	0	2	1	0	1	0	2	5	0	0	0	0	1	2
Temnothorax pergandei		1	0	4	1	0	0	2	0	3	0	0	0	0	0	0	0	0	0
Hypoponera opacior		0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Ponera pennsylvanica		0	1	1	0	0	0	1	0	0	0	1	0	1	2	0	0	0	2
TOTAL	**Sp. Richness**	**13**	**12**	**8**	**9**	**1**	**3**	**11**	**8**	**8**	**6**	**4**	**3**	**8**	**6**	**1**	**2**	**3**	**4**
	Abundance	**99**	**71**	**22**	**16**	**5**	**9**	**83**	**62**	**24**	**27**	**8**	**13**	**42**	**32**	**1**	**3**	**9**	**11**

*Species are morphologically indistinguishable in samples where few specimens are present.

Total abundance and richness of species on 7-8-yr fields for pitfall traps, hand collection, and litter sampling for summer (S) and fall (F).

		7-8-yr Fields																	
		7						8						9					
		Pitfall		Hand		Litter		Pitfall		Hand		Litter		Pitfall		Hand		Litter	
		S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F
Tapinoma sessile		17	13	2	6	7	10	16	9	11	5	2	12	8	6	2	3	3	5
Formica incerta/sp.*		1	0	0	0	0	0	4	2	0	0	0	0	3	0	1	0	0	0
Formica dolosa		0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Formica pallidefulva		3	0	0	0	0	0	8	4	0	0	0	0	5	2	0	0	0	0
Formica subintegra		0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Formica subsericea		3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lasius alienus		6	2	0	0	1	1	0	0	0	0	0	0	0	2	1	0	0	0
Lasius neoniger		15	7	2	0	0	0	2	1	0	0	0	0	19	20	12	5	0	0
Paratrechina faisonensis		0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Paratrechina terricola		0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Polyergus lucidus		0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Aphaenogaster carolinensis		2	0	0	0	0	0	8	3	0	0	0	0	10	2	1	1	0	0
Aphaenogaster fulva		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphaenogaster mariae		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crematogaster cerasi		0	0	0	0	0	0	2	3	1	0	0	0	2	3	2	0	0	0
Crematogaster lineolata		1	1	0	0	0	1	4	1	0	2	0	2	1	0	0	0	0	0
Monomorium minimum		1	0	0	0	0	0	2	2	0	0	0	0	1	0	0	0	0	0
Myrmecina americana		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrmica americana		20	13	0	1	1	1	13	10	3	0	0	0	15	10	5	5	0	0
Myrmica emeryana		3	5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Myrmica spatulata		1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pheidole pilifera		0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Solenopsis molesta		1	3	0	0	2	3	13	15	0	0	1	3	16	18	5	2	3	0
Stenamma brevicorne		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Temnothorax ambiguus		10	2	1	0	9	11	6	1	4	2	9	12	8	1	7	2	11	9
Temnothorax pergandei		0	0	0	0	0	0	6	0	1	0	0	0	1	0	0	0	0	0
Hypoponera opacior		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ponera pennsylvanica		2	2	0	0	1	5	2	2	0	0	0	0	3	2	0	1	0	0
TOTAL	**Sp. Richness**	**16**	**11**	**3**	**2**	**7**	**7**	**16**	**15**	**6**	**3**	**3**	**4**	**14**	**11**	**9**	**7**	**3**	**2**
	Abundance	**87**	**52**	**5**	**7**	**22**	**32**	**89**	**56**	**21**	**9**	**12**	**29**	**93**	**67**	**36**	**19**	**17**	**14**

*Species are morphologically indistinguishable in samples where few specimens are present.

Total abundance and richness of species on 14-16-yr fields for pitfall traps, hand collection, and litter sampling for summer (S) and fall (F).

		14-16-yr Fields																	
		10						11						12					
		Pitfall		Hand		Litter		Pitfall		Hand		Litter		Pitfall		Hand		Litter	
		S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F
Tapinoma sessile		19	11	7	7	5	8	19	12	1	2	5	8	0	0	0	0	0	2
Formica incerta/sp.*		7	4	0	0	0	0	1	0	0	0	0	0	18	16	0	1	0	0
Formica dolosa		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Formica pallidefulva		2	0	0	0	0	0	0	0	0	0	0	0	9	2	0	1	0	0
Formica subintegra		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Formica subsericea		13	11	5	0	0	1	0	0	0	0	0	0	9	6	3	0	0	0
Lasius alienus		1	0	0	0	0	0	2	0	0	0	0	0	16	13	9	5	0	0
Lasius neoniger		6	5	0	0	0	0	4	1	0	0	0	0	16	12	3	3	0	0
Paratrechina faisonensis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratrechina terricola		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polyergus lucidus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphaenogaster carolinensis		16	7	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Aphaenogaster fulva		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphaenogaster mariae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crematogaster cerasi		0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Crematogaster lineolata		0	1	0	0	0	0	6	2	0	0	0	0	0	0	0	0	0	0
Monomorium minimum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrmecina americana		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Myrmica americana		12	7	2	1	0	1	3	1	0	0	0	0	16	13	2	2	0	0
Myrmica emeryana		0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	1	0	0
Myrmica spatulata		0	0	0	0	0	0	0	0	0	0	0	0	6	7	0	0	0	0
Pheidole pilifera		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Solenopsis molesta		11	9	0	1	4	7	3	0	0	0	0	3	4	6	0	0	0	0
Stenamma brevicorne		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Temnothorax ambiguus		18	5	3	2	15	10	8	6	0	0	17	13	0	0	0	0	2	5
Temnothorax pergandei		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypoponera opacior		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ponera pennsylvanica		3	2	0	0	0	1	2	1	0	0	1	0	6	2	0	0	0	0
TOTAL	**Sp. Richness**	**12**	**10**	**5**	**4**	**3**	**6**	**10**	**7**	**1**	**1**	**3**	**3**	**12**	**11**	**4**	**6**	**1**	**2**
	Abundance	**109**	**62**	**19**	**11**	**24**	**28**	**49**	**24**	**1**	**2**	**23**	**24**	**105**	**80**	**17**	**13**	**2**	**7**

*Species are morphologically indistinguishable in samples where few specimens are present.