Global change effects on ant-mediated seed dispersal

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Academic Abstract

Seed dispersal mutualisms, including seed dispersal by ants, are critical to the assembly of communities and the function of ecosystems. However, the consequences of human-caused global change factors, such as habitat fragmentation and climate change, for the future of these mutualisms are not fully understood. My dissertation consists of five chapters that investigated the effects of habitat fragmentation and climate change on ants and their seed dispersal mutualisms. Chapter 1 is an introduction that provides background information on habitat fragmentation and anthropogenic climate change and their impacts on biodiversity. In Chapter 1, I also introduce my study system of ant-mediated seed dispersal mutualisms, myrmecochory. My next two chapters (Chapters 2 and 3) explored the effects of reconnecting fragmented habitat patches with corridors in restored longleaf pine savanna systems in South Carolina. We used a landscape scale experiment to investigate how reducing isolation affects the assembly of ant communities over time (Chapter 2) and seed dispersal of the myrmecochorous forb, *Piriqueta cistoides* (Chapter 3). For Chapter 2, we found evidence that both habitat connectivity and edge effects underly the effects of corridors on ant communities over time. We found that connected patches accumulated ant species faster than isolated patches over time suggesting that corridors function by facilitating colonization. We also found evidence that edge effects play a role with greater ant functional group diversity in patches with higher edge than patches with lower edge amounts. For Chapter 3, we also found evidence of corridor and edge effects with ants dispersing seeds of *P. cistoides* longer

distances in patches connected via corridors than isolated patches, but only in the center of patches. In Chapter 4, we investigated the effects of predicted climate change scenarios for seed dispersal mutualisms in eastern deciduous forests. For this chapter, we conducted a mesocosm experiment in which we crossed temperature with altered precipitation magnitude and frequency. Our mesocosms contained a common spring ephemeral wildflower, *Sanguinaria canadensis*, and whole colonies of their mutualist seed disperser, *Aphaenogaster rudis*. This design allowed us to collect high-resolution data on how ants interacted with seeds under different climate change scenarios that incorporated warming temperatures and altered precipitation. We found that warming effects depended on the precipitation treatment with negative effects of warming on the collection of seeds by ants under historical precipitation regimes and positive effects of warming under simulated precipitation conditions altered under predicted climate change. Finally, Chapter 5 describes my general conclusions from this body of work. Taken together, the research making up my dissertation provides valuable insights into how changing environmental conditions under habitat fragmentation and climate change may alter ant seed dispersal mutualisms. Importantly, we often found that the impacts of global change were context dependent and that our experiments were important tools in disentangling that context dependency. Further, this work demonstrates the value of understanding the basic ecology of the interactions among organisms. Understanding the natural history of organisms across changing environmental conditions will benefit the ways in which we conserve and restore ecosystems in a fragmented and warmer world.

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General Audience Abstract

Most plants and animals engage in mutualisms, which are interactions between species in which both benefit from interacting with each other. The focus of this dissertation are the impacts of human-caused environmental change on the mutualism between ants and plants in which ants move a plant’s seeds. The dispersal of ant-dispersed plants is considered a mutualism because the ants receive a food reward in the form of a fat- and protein-rich appendage that grows on the seed while the seeds of the plant get moved to a better location for germination. The mutualisms between ants and the plants they disperse are critical to how plants are distributed in many ecosystems, yet the consequences of human-caused environmental change, such as habitat loss and climate change, for these mutualisms are not fully understood. My dissertation consists of five chapters that investigated the effects of habitat fragmentation (the breaking apart of larger habitats into smaller, more isolated patches as a result of habitat loss) and climate change on ants and their seed dispersal mutualisms. My first chapter introduces background on the consequences of habitat fragmentation and climate change on organisms, ant seed dispersal mutualisms, and the potential effects of altered environmental conditions on seed dispersal by ants. My second two chapters (Chapters 2 and 3) explored the effects of reconnecting isolated habitat patches with habitat corridors (strips of habitat restored between the isolated habitat patches). In restored longleaf pine savanna systems in South Carolina, we used a long-term, landscape scale experiment to study how increasing connectivity and changing the shape of habitats via corridors affects

ant community diversity over time (Chapter 2) and seed dispersal of the ant-dispersed plant, pitted stripeseed (*Piriqueta cistoides*) (Chapter 3). For Chapter 2, we found both habitat connectivity and patch shape effects underly the effects of corridors on ant community over time. We found that connected patches accumulated ant species faster than isolated patch types which suggests that corridors may function by facilitating colonization into the patches they connect. We also found evidence that patch shape plays a role in supporting greater ant functional group diversity in patches with greater perimeter (more edge habitat) than patches with less perimeter (less edge habitat). We found that ants in patches with more edge habitat represented a greater number of functional groups, which are categories that describe the roles ants play in ecosystems. For Chapter 3, we also found evidence of corridor and patch shape effects with ants dispersing seeds of pitted stripeseed longer distances in patches connected via corridors than isolated patches, but only in the center of patches. In Chapter 4, we investigated the effects of predicted climate change scenarios on seed dispersal mutualisms in eastern deciduous forests. For this chapter, we conducted an experiment in which we crossed temperature with altered precipitation in mesocosms, which are small, simulated ecosystems that allowed us to investigate the effects of warming and altered precipitation in a controlled setting. Our mesocosms contained a common spring ephemeral wildflower, bloodroot (*Sanguinaria canadensis*), and whole colonies of their mutualist seed disperser, winnow ants (*Aphaenogaster rudis*). We found that the effects of warming temperatures depended on the precipitation treatment. Warming had a negative effect on the number of seeds collected by ants under historical precipitation regimes, but a positive effect under simulated precipitation conditions under predicted climate change (higher in magnitude and lower in frequency). Finally, Chapter 5 describes my general conclusions from this body of work. Taken together, the research making up my dissertation provides valuable insights into how changing environmental conditions under habitat fragmentation and climate change may alter ant seed dispersal mutualisms. Importantly, we often found that the impacts of global change were context dependent. Our experiments were important tools in disentangling that context dependency. Further, this work demonstrates the value of understanding the basic ecology of the interactions among organisms. Understanding the natural history of organisms, especially their responses to changing environmental conditions, will ultimately benefit the ways in which we conserve and restore ecosystems in a fragmented and warmer world.

Dedication

This dissertation is dedicated to my sons, Henry and Lucas

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Chapter 1 – Introduction

Human-caused global change and its consequences for biodiversity

Ecologists face myriad challenges in trying to make predictions about how ecological communities are responding and will continue to respond to human-caused factors of global change (Sutherland et al. 2013). Globally, not only have habitats been fragmented at alarming rates owing to human land use (Haddad et al. 2015), but at the same time habitats are undergoing unprecedented changes to climate (IPCC 2018, 2022). The multidimensional nature of human-caused global change combined with the multidimensional nature of ecological systems makes forecasting the consequences of this change a formidable challenge. However, a better understanding of how global environmental change factors affect ecological systems will provide much needed insights for how to conserve and restore habitats in the face of global change.

With an estimated 70% of forests worldwide within just a single kilometer of a habitat edge (Haddad et al. 2015), habitat fragmentation is often considered the top threat to global biodiversity (Wilcove et al. 1998, Haddad et al. 2015, Fletcher et al. 2018). Typically the result of habitat loss and degradation, habitat fragmentation results in the breaking apart of a large, contiguous piece of habitat into smaller fragments (Lindenmayer and Fischer 2006). In addition to decreasing habitat connectivity, an increase in the amount of edge within fragments can also play an important role in how habitat fragmentation affects biodiversity (Fletcher 2005, Pfeifer et al. 2017). Habitat edges frequently result in altered abiotic conditions owing to the difference in the human-changed matrix and intact habitat within remaining fragments (Tuff et al. 2016). In general, habitat fragmentation leads to increasing isolation and results in less habitable area for species (Fahrig 2003), which can lead to reduced movement from one isolated patch to another, reduced colonization rates, and increased extinction rates (Haddad et al. 2015, Damschen et al. 2019).

Another major threat to global biodiversity is human-caused climate change, a threat increasing faster in magnitude though time than ever before (IPCC 2018, 2022). Increasing temperatures, changes in precipitation regimes, and changes to freeze-thaw dynamics are already impacting ecological systems (Walther et al. 2002, Araújo and Rahbek 2006, Parmesan 2006, Damschen et al. 2010, IPCC 2018, 2022) and are expected to continue to have impacts across all biological scales from organisms to communities (Parmesan and Yohe 2003, Araújo and Rahbek 2006, Parmesan 2006, Jamieson et al. 2015, Scheffers et al. 2016). Organisms and populations may respond in three ways to changing climates: 1) track changing conditions by moving up in latitude or elevation, 2) adapt to changing climates, or 3) perish (i.e. go extinct). The ability to forecast how ecosystems will respond to changing climates will require being able to scale up from the effects of climate change on individual organisms to the cascading effects on entire communities.

To accurately forecast the impacts of habitat fragmentation and climate change on communities, it is imperative that we understand not only the direct effects of these global change factors on individual organisms but also their effects mediated through interactions with other species. Mutualisms, interactions between species in which both benefit, such as pollination and seed dispersal, play a critical role in the functioning of ecosystems ranging from shaping plant communities to maintaining biodiversity (Howe and Smallwood 1982, Jordano 1995, Fenner 2000, Strauss and Irwin 2004, Bascompte and Jordano 2007, Ollerton et al. 2011, Bronstein 2015). However, these interactions are increasingly threatened by habitat fragmentation and climate change (Kiers et al. 2010, McConkey et al. 2012, Aslan et al. 2013, Teixido et al. 2022). Habitat fragmentation disrupts seed dispersal by reducing the availability of suitable habitats and increasing isolation between patches, limiting the movement of seed dispersers (Cordeiro and Howe 2003, Rodríguez-Cabal et al. 2007, Magrach et al. 2014). Overall, limiting movement of seed dispersers can lead to decreased seed dispersal distances, altered seed deposition patterns, and ultimately, reduced plant recruitment and genetic connectivity. Furthermore, climate change exacerbates these challenges by influencing the behavior and distribution of seed dispersers, altering fruiting and flowering phenology, and potentially disrupting the synchronization between plant reproduction and biotic disperser activity (Rafferty et al. 2015). As a result, seed dispersal mutualisms are increasingly vulnerable to breakdown, with the possibility of cascading effects for plant population dynamics and community composition (Aslan et al. 2013). Preserving and restoring functional seed dispersal networks in fragmented landscapes under changing climatic conditions is crucial for maintaining ecosystem resilience and biodiversity (Rogers et al. 2021, Fricke et al. 2022), yet most seed dispersal mutualisms are difficult to assess with experiments because of the large scale at which these mutualisms function.

Study system: ants and their seed dispersal mutualisms.

The dispersal of seeds by ants (also known as myrmecochory) is a critical mutualism shaping plant populations and community dynamics (Zelikova et al. 2008, 2011, Lengyel et al. 2009, 2010, Prior et al. 2014, 2020, Warren II and Giladi 2014). In many ecosystems, ants provide an important ecosystem service as seed dispersers, with an estimated 11,000 plant species worldwide primarily dispersed by ants (Lengyel et al. 2010). Ant seed dispersal mutualisms are especially common in eastern deciduous forests of North America, in sclerophyllous shrublands of Australia, in the fynbos of South Africa, in the Mediterranean, and in the semi-arid caatinga ecosystem of northeast Brazil with as many as 30% of plant species in these regions dispersed by ants (Beattie and Culver 1981, Rice and Westoby 1981, Bond and Slingsby 1982, Beattie 1985, Leal et al. 2007). The seeds of most myrmecochorous plants have a lipid-rich appendage on their seed coat called an elaiosome, which attracts ants to pick up seeds and carry them back to their nest (Beattie 1985). Once at the nest, ants remove the elaiosome and feed it to their larvae. Typically, the seeds are later re-dispersed out of the nest and into their trash middens. Engaging in this mutualism provides the adaptive benefit of dispersal for the plant, while the ant gets a food reward (Beattie 1985). Dispersal by ants can benefit the plant in a variety of ways including: 1) movement away from the parent plant, which typically reduces intraspecific competition (Handel 1978), 2) avoidance of potentially destructive disturbance events such as fire (Berg 1975, Bond and Slingsby 1983), 3) avoidance of seed predators such as small mammals (Bond and Breytenbach 1985, Smith et al. 1989), and 4) dispersal to microsites enriched with nutrients, such as ant nests or trash middens (Culver and Beattie 1980, Davidson and Morton 1981, Rice and Westoby 1986, Giladi 2006).

Will ant seed dispersal mutualisms be resilient in the face of ongoing global change? Both habitat fragmentation and climate change alter abiotic conditions within habitat which may have cascading consequences for seed dispersal by ants. For example, both habitat fragmentation and climate change can alter the thermal conditions of habitats where myrmecochorous plants and their ant seed disperser mutualists coexist (Magrach et al. 2011, Christianini and Oliveira 2013, Tuff et al. 2016, Parr and Bishop 2022). Although ants and plants engaged in myrmecochory mutualisms may have coevolved under similar environmental conditions, these partners may differ in how they respond to the rapidness in which environmental conditions are altered under human-caused global change (Lengyel et al. 2010, Vidal et al. 2021). Indeed, plants and their seed dispersing ants may diverge in how each respond, disrupting their mutualism (Vidal et al. 2021).

My dissertation has focused on ants and their seed dispersal interactions not only because both ants and their mutualisms are prevalent and important across ecosystems, but because the scale of this mutualism makes doing experiments at large and small scales both feasible yet meaningful. In my field-based research, we were able to follow ants as they dispersed seeds in real-time which allowed for getting accurate seed dispersal measurements and as well as locations where seeds ended up. Other seed dispersers, such as bats, birds, or other seed dispersing mammals, disperse seeds at scales not so easily measurable, making their seed dispersal distances difficult to estimate with accuracy, if at all. In addition to being a great field system, ant seed dispersal mutualisms occur at such a small scale that one can also construct small mesocosms to conduct controlled experiments that follow seed dispersal (e.g., Prior et al. 2014, 2020, Meadley-Dunphy et al. 2019). Working in the context of a mesocosm allows for tracking seeds during the whole process of seed dispersal including up to seed fate. A thorough understanding of ant seed dispersal mutualisms in this way is important for disentangling the mechanisms driving the outcomes of this seed dispersal mutualism under ongoing human-caused global change.

Chapter Summaries

The overall goal of my dissertation research was to investigate the impacts of human-caused global change factors on ant communities and ant-mediated seed dispersal mutualisms. For my first two data chapters (Chapters 2 and 3), I used a long-term, landscape-scale habitat connectivity experiment to understand the effects of connectivity and edge amount on ant community dynamics over time (Chapter 2) and on ant seed dispersal mutualisms (Chapter 3;

Burt et al. 2022). This research took place at the Savannah River Site near New Ellenton, SC in restored longleaf pine savannah ecosystems. In this experiment, we used habitat corridors to alter habitat connectivity. For Chapter 2, we investigated the effects of habitat connectivity on several axes of ant community diversity as ant communities assembled over time. Notably, we found that habitat patches with corridors accumulated ant species at a faster rate than isolated patch types. Additionally, ant beta-diversity (diversity among patches) was also greater for patches with corridors than those that were isolated. For Chapter 3, we found that ants dispersed seeds further in the center of patches with corridors than isolated patches. Although we did not find simultaneous changes to the taxonomic diversity of ants moving seeds.

For my final data chapter (Chapter 4), we used a multifactor climate change experiment to investigate how different axes of climate may affect the mutualism between a native understory wildflower, *Sanguinaria canadensis* (bloodroot) and its seed dispersing ants, *Aphaenogaster rudis*. In this experiment we manipulated temperature and precipitation magnitude and frequency in mesocosms that contained field-collected *A. rudis* colonies and *S. canadensis*. With this experiment, we found that the effect of warming on the number of seeds initially moved by ants to their nests depended on the amount and frequency of precipitation. Under historical precipitation regimes, ants moved more seeds initially under ambient conditions, but the pattern with warming flips for increased magnitude, decreased frequency precipitation regimes where ants moved more seeds under warmed than ambient conditions. Overall, these initial differences in ant-mediated seed dispersal did not translate into changes in the number of seeds that ants re-dispersed out of their nests and into the mesocosms nor the overall distance seeds were moved away from the nest box or the *S. canadensis* plants. Taken together, these results suggest that for eastern deciduous forests where climates are expected to be warmer but more wet, the predicted increase in precipitation with climate change may buffer the added stress of warming temperatures on ant activity. Thus, ant seed dispersal mutualism may be tolerant to forecasted future climate change.

Overall, my dissertation research has used long-term field- and greenhouse-based experiments with the aim to gain a better understanding of the mechanisms driving the responses of ants and their seed dispersal mutualisms under the expected consequences of human-mediated global change. Through this set of controlled experiments, my collaborators and I aimed to gain insights into how these important relationships may fare under ongoing human-caused global change. Ultimately, I hope this dissertation sets a foundation for a career in which I can continue to contribute to advance our fundamental understanding of the ecological dynamics of biodiversity under global change.

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Chapter 2 - The effects of experimental corridors on ant community dynamics over time

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Abstract

Globally, habitat loss and fragmentation pose significant threats to biodiversity. Habitat corridors are often used in fragmented landscapes to reconnect isolated patches. However, understanding how corridors function can be challenging, as their effects may be confounded with simultaneous changes to habitat area, edge amount, and connectivity among patches. Here, we used a long-term, landscape scale experiment to investigate two potential mechanisms by which corridors may function - via increasing habitat connectivity or through increasing the amount of edge. With pitfall trap sampling conducted over 5 years (9 to 15 years since site establishment), we assessed the relative effects of connectivity and edge effects by measuring ant biodiversity in four ways: (1) taxonomic diversity, (2) functional diversity, (3) community composition, and (4) site-to-site variance in community composition (i.e., beta diversity). We found evidence of both connectivity and patch shape effects on ant diversity and community composition. Taxonomic diversity increased linearly in all patch types over time yet accumulated more quickly in connected than isolated patches. The faster accumulation of taxonomic diversity in connected patches was associated with greater site-to-site variance in community composition, but we found no differences in mean taxonomic diversity within patches (alpha diversity). With comparisons among patches that vary in edge amount relative to area, we found no difference in how fast taxonomic diversity accumulated over time, however patches with less edge habitat relative to interior habitat had greater within patch diversity. Taken together, these results add to a body of literature that shows the potential for habitat corridors to have positive effects on biodiversity by increasing connectivity and that these effects may continue to accrue over time.

Introduction

Habitat loss and fragmentation are often considered the biggest threats to biodiversity globally (Wilcove et al. 1998, Haddad et al. 2015, Fletcher et al. 2018). In many ecosystems, what were once large contiguous habitats have been broken into smaller habitat patches that contain less area, are more isolated from each other, and have a greater amount of habitat edge (Fahrig 2003, Fletcher 2005, Pfeifer et al. 2017). These changes can have wide-ranging consequences for biodiversity. Less habitat area, increased isolation of patches, and more edge as a consequence of fragmentation has been associated with reduced movement of animals, reduced species establishment, increased rates of extinction, and changes to community assembly dynamics (Debinski and Holt 2000, Damschen et al. 2008, 2019, Haddad et al. 2015, Brocardo et al. 2018, Resasco 2019, Griffin and Haddad 2021, Resasco and Fletcher 2021). However, studies are often unable to disentangle the relative effects of these usually simultaneously occurring factors (Ewers and Didham 2006). A better understanding of their relative effects may improve conservation aimed at mitigating negative effects of fragmentation on biodiversity.

A tool that land managers often use to reconnect isolated fragments is habitat corridors, which are strips of habitat constructed between isolated patches (Hilty et al.

2006). Although habitat corridors are intended to support biodiversity by reducing isolation and increasing the movement of species among patches, how they function is often not fully understood. As with habitat fragmentation, the addition of corridors to a landscape may confound effects of increasing connectivity, habitat area (e.g., habitat gained from the corridor itself), and altered edge effects (e.g., altered environmental conditions at the boundary between habitat types). Long-term, field-based experiments replicated at the scale of landscapes are important for predicting the consequences of restoring habitat connectivity with corridors, as they may enhance biodiversity through various simultaneously acting mechanisms.

Another aspect obscuring our understanding of corridor function is a bias toward vertebrates in long-term and large-scale studies (Proença et al. 2017). In the face of growing concerns about insect declines, it is imperative that we also understand the impacts of fragmentation and corridors on insect biodiversity (Didham et al. 2020, Wagner et al. 2021, Weisser et al. 2023). Often used as indicator species because of their diversity and their importance for ecosystem functioning (Andersen et al. 2002, Tiede et al. 2017), ants are ideal taxa for investigating questions about the consequences of human-caused changes to landscapes. Ants play key roles in predation, seed dispersal, soil movement, trophic dynamics, and nutrient cycling within ecosystems (Folgarait 1998, Del Toro et al. 2012). Additionally, the ecology and behavior of ants suggests that they could be affected by both connectivity and altered edge dynamics.

Previous research has shown that fragmentation and edge effects are important drivers of ant community assembly (Leal et al. 2012, Resasco and Fletcher 2021). Colony establishment in most ant species begins with the dispersal of alates, which are winged reproductive queens and male ants. The fragmentation of habitats by human land use may create inhospitable areas between fragments that disrupts the dispersal of alates out of fragments (King and Tschinkel 2016, Helms 2018). For example, Noordijk et al. found that alates of *Myrmica rubra*, *Myrmica ruginodis* and *Stenamma debile* were most abundant at the edges of fragments suggesting that the dispersal of these species was impeded by the surrounding human-modified matrix (2008). Here, corridors may mediate isolation effects by providing a pathway for the dispersal of alates between isolated fragments potentially leading to an increase in ant diversity over time. Alternatively, if corridors give competitively dominant invasive ant species a colonization advantage, native ant diversity may decrease. In restored longleaf pine savannas dominated by *Solenopsis invicta* (the red imported fire ant), positive effects of corridors on the dispersal of *S. invicta* resulted in an overall negative effect of connectivity on native ant species diversity (Resasco et al. 2014). Effects of fragmentation mediated by edges may also result in an increase in ant diversity. This may occur when fragment edges contain ant species representative of both the remnant habitat type as well as the neighboring human-modified matrix (Risser 1995). Alternatively, edge effects on ant diversity can be negative if altered abiotic factors such as higher temperatures and lower soil moisture typical at edges are limiting ant community assembly (Fletcher 2005, Tuff et al. 2016). Since connectivity and edge effects can result in similar diversity patterns, experiments that control for both factors independently are necessary to disentangle their contributions to fragmentation effects on ant communities.

Here, we investigated the effects of corridors on ant communities through time with a long-term landscape experiment that manipulated both connectivity and edge amount among patches of restored long-leaf pine savanna habitat. We used pitfall traps to sample ant community composition over five consecutive years beginning 9-15 years after patch establishment. Our experiment investigated two key hypotheses explaining how corridors function: (1) by enhancing habitat connectivity and (2) by altering edge effects. Our present study follows up on previous research in this same experiment that found a positive, but transient, effect of corridors on polygyne *S. invicta* that was associated with fewer native ant species in connected than isolated patches (Resasco et al. 2014, 2023). Given the transient nature of connectivity effects on *S. invicta*, we predicted that corridors may affect the assembly of ant communities by facilitating greater colonization rates over time. This may result in higher taxonomic and functional group diversity over time in patches with corridors. Greater ant biodiversity in connected patches could also lead to differences in community composition both within (alpha-diversity) and among patches (beta-diversity). To determine the independent effects of connectivity and edge amount, we measured ant biodiversity in four ways: (1) taxonomic diversity, (2) functional group diversity, (3) community composition, and (4) site-to-site variance in community composition (i.e., beta diversity).

Methods

Study Site: Savannah River Site (SRS) Corridor Experiment

Sites within the Corridor Experiment (e.g., Damschen et al. 2006, 2019, Resasco et al. 2014, Brudvig et al. 2015) were established at the Savannah River Site (SRS) in South Carolina in the winter of 1999-2000 and 2006. Each experimental landscape (N = 7) consists of a center square patch (1 ha) surrounded on each side by four peripheral patches that are each equal in area (~1.4 ha, Fig. 1) and located 150 m away. For each experimental landscape, one peripheral patch is connected to the center patch by a corridor (150×25 m;

hereafter patch type referred to as "connected"). The three remaining peripheral patches are isolated and are one of two types. They either have 75×25 m projections equal in area to the corridor on opposite sides (hereafter "winged") or are rectangular in shape. Three of our experimental landscapes have two of the rectangular isolated patches and one winged isolated patch and four of our experimental landscapes have two of the winged patches and one rectangular patch. Comparisons among these treatments allow us to disentangle the effects of connectivity and patch shape (edge amount) that typically confound the interpretation of habitat corridor effects. Thus, to isolate effects of connectivity, we compare winged and connected patches, and to isolate effects of patch shape we compare the winged patches with the rectangular patches.

Habitat patches are located within a planted pine (*Pinus* spp.) matrix and have undergone restoration to longleaf pine savanna. A biodiversity hotspot, longleaf pine (*Pinus palustris)* savanna ecosystems of the North American coastal plain are home to more than 6200 native plant species (Noss 2013, Noss et al. 2015) and to a wide diversity of ant species with greater than 332 species (MacGown 2003, Lubertazzi and Tschinkel 2003). Owing to considerable habitat loss and fragmentation, less than three percent of its original extent remains today, making it one of the most threatened and fragmented ecosystems in North America (Frost 2006). As a result of fire, longleaf pine ecosystems are open with few trees (Miller 2006). In the Corridor Experiment, we maintain the openness of the restored longleaf pine savanna patches with frequent prescribed burns every 2-3 years and periodical removal of resprouting hardwoods with brush saws and targeted herbicide. Within each patch we have also planted longleaf pine (*Pinus palustris*) at densities within the range of densities historically found in the southeastern United States (Noss 2013).

Annual Ant Community Survey

Between 2015 and 2019, we used pitfall traps to characterize the ant community in each patch type within each experimental landscape. In each year we deployed pitfall traps in the experimental landscapes between July and August, the time of peak ant activity in our study system. We placed 22 pitfall traps within a grid in each of the peripheral patch types (Fig. 1; 66 traps per experimental landscape × 7 experimental landscapes = 462 traps/year). We randomly chose one of the duplicated isolated peripheral patch types at the start of sampling. Each pitfall trap consisted of a 50-mL centrifuge tube filled with 25 mL of a 50/50 solution of propylene glycol and 70% ethanol. We added a couple of drops of clear, unscented dish soap to each gallon of trapping solution to help break surface tension when arthropods fell into the trap. To account for digging-in effects, we dug traps at least 48 hours prior to opening (Greenslade 1973). To dig in traps, we removed a core of soil the same size as the 50 mL centrifuge tube with a copper pipe and mallet. We opened traps for 48 hours when the chance of rain was low. Additionally, to prevent unanticipated rain from entering traps, we placed a pizza box stack over the open trap to decrease water flowing into the open trap during rain events (Fig. S1). After collecting traps at the end of sampling, we replaced the trapping solution with 95% ethanol. Later, we identified all ants to species or to species complexes with keys and species descriptions in (MacGown 2003).

Ant Functional Groups

In addition to identifying ants to species, we also classified each species into functional groups according to Andersen (1997), a classification scheme used previously for determining how ecological differences across species may influence environmental effects on ant distributions (e.g., Izhaki et al. 2003). Anderson's classifications separate species into categories based on their associations with environmental attributes and their competitive abilities (Andersen 1997). There are eight functional groups present in our experimental landscapes: dominant Dolichoderinae, subordinate Camponotini, hot climate specialists, cold climate specialists, tropical climate specialists, opportunists, generalized Myrmicinae, and cryptic species. The dominant Dolichoderinae favor open areas and are aggressive and competitively dominant with other ant species. Subordinate Camponotini are larger species that usually forage at night and are subordinate to dominant ants including the Dolichoderinae. The climate specialists all typically avoid the dominant Dolichoderinae. Hot climate specialists prefer arid conditions, cold climate specialists prefer cooler conditions, and tropical climate specialists prefer warmer conditions. Opportunists are ants that establish rapidly after disturbance but are weak competitors. The generalized Myrmicinae are cosmopolitan ants that recruit and defend clumped resources rapidly. Finally, cryptic ant species forage mostly underground and in the leaf litter with few interactions with ants foraging above ground.

Statistical Analysis

We conducted all analyses in R version 4.2.2 (R Core Team 2023). For all analyses, we summarized data across pitfall traps calculating the incidence of each species across pitfall traps in each patch within each site for each year. To examine how ant taxonomic and functional group diversity differed across patch types over time, we used three forms of Hill numbers estimated with the hillR package (Li 2018). Hill numbers calculate diversity by summarizing the relative abundances of species in a community at different orders of q which scale diversity according to the importance of rare species (Hill 1973, Jost 2006). We calculated Hill numbers at q orders of 0, 1, and 2, which are similar to diversity indices commonly used in ecology: species richness (q = 0), Shannon's entropy (q = 1), and Simpson's Index (q = 2); however, Hill numbers place these three diversity metrics on the same scale (Jost 2006, Roswell et al. 2021). When q = 0, rare species are more important and their importance decreases at q = 1 and q = 2 (Jost 2006, Roswell et al. 2021). To test for potential effects of connectivity and patch shape (i.e., relative edge amount) on Hill numbers, we fit linear mixed effects models with patch type, years since site establishment, and their interaction as fixed effects; we also included experimental landscape as a random effect. We natural log transformed response variables when assumptions of normality and homoscedasticity were not met. We fit linear mixed effects models with the 'lmer' function from the lme4 package (Bates et al. 2015). For hill diversity at q = 2 calculated with functional group data, transforming variables did not address issues with normality and homoscedasticity of residuals, so we instead used a generalized mixed effects model with a poisson distribution (Bates et al. 2015). To further assess significant differences among treatments, we used the 'emmeans' function from the 'emmeans' package to estimate marginal means and contrasts with the Tukey method and Kenward-Roger degrees of freedom (Lenth 2020). When our models showed evidence of an interaction between patch type and years since site establishment, we used the 'emtrends' function from the 'emmeans' package to perform estimated marginal means of linear trends analysis to determine if trends through time were statistically different across patch types. We also subsetted our data by patch type and performed linear mixed effects models with years since site establishment as a fixed effect and experimental landscape as a random effect.

To determine how taxonomic and functional group composition differed with connectivity and patch shape over time we conducted multivariate community analyses with the vegan package (Oksanen 2015, Oksanen et al. 2018). We conducted permutational analysis of variance (PERMANOVA) with the ‘adonis2’ function with patch type, years since site establishment, and their interaction as main effects. We accounted for potential site-level effects by setting the ‘strata’ option to constrain permutations within experimental landscapes and all PERMANOVAs ran over 999 permutations. We conducted separate PERMANOVAs with distance matrices using Bray-Curtis dissimilarity and the Morisita-Horn index. We did additional PERMANOVAs with the Morisita-Horn index because Bray-Curtis dissimilarity is known to have potential issues with differences in the number of sampled individuals (Wolda 1981). We found qualitatively similar results for the Morisita-Horn index as when we used Bray-Curtis dissimilarity, so we present all results using the Morisita-Horn index in the supplemental materials (Tables S2 and S3). When our PERMANOVAs showed evidence of patch type effects, we performed pairwise comparisons using Bonferroni corrections with the ‘pairwise.perm.manova’ function from the RVAideMemoire package (Herve 2023). Because parameters within PERMANOVAs can be significant because of differences in means as well as differences in variance, we also performed multivariate analyses of homogeneity using the ‘betadisper' function from vegan to assess variance in community composition among patch types (i.e. beta diversity). We followed up these tests with Tukey multiple comparisons of means tests with the ‘TukeyHSD’ function to assess pairwise comparisons among levels of significant predictor variables. To assess the effects of significant interactions between patch type and time, we performed additional PERMANOVAs on our data subdivided by patch type. For these additional PERMANOVAs, we used time since site establishment (years) as the main effect and again accounted for potential site-level effects by setting the ‘strata’ option to constrain permutations within experimental landscapes. For all PERMANOVAs, we visualized results with non-metric multidimensional scaling (NMDS) with the 'metaMDS' function in the vegan package. As was used in the PERMANOVAs, we used both the Bray-Curtis dissimilarity and the Morisita-Horn index for our distance matrix in our NMDS plots. Each NMDS used three dimensions (i.e., $k = 3$), because with scree plots we determined that this was where the stress values began to decrease more slowly with additional dimensions.

Results

Over five years of annual sampling we collected and identified 144,826 individual ant specimens from 62 species and 8 functional groups. The most common species we detected in our experimental landscapes were *Solenopsis invicta*, *S. molesta* grp, *Dorymyrmex bureni*, *Pogonomyrmex badius*, and *Forelius pruinosus*.

Effects of connectivity and patch shape on ant taxonomic and functional group diversity

For Hill number estimates of taxonomic diversity at q orders of 0, 1, and 2, patch type, time since site establishment, and their interaction were all significant predictors (Fig. 2A-C, Table 1). Hill diversity at each level of q increased with time since site establishment (all $p < 0.05$; Table 1). For Hill diversity at $q = 0$, the only significant difference among patch type means occurred between the connected and rectangular patches (Table 2). However, when rare species were weighted less at $q=1$ and $q=2$, we found significant differences in diversity between connected and rectangular patch types, as well as between the rectangular and winged patch types (Table 2). With estimated marginal means of linear trend analyses we found that Hill diversity for all orders of q increased over time for taxonomic diversity in connected and winged patch types, but not rectangular patch types (Table 3). For each order of q, we also found differences between slopes when comparing connected vs. rectangular and connected vs. winged, indicating that taxonomic diversity in connected patches accumulated more quickly over time than both isolated patch types (Table 4). When Hill diversity estimates gave the greatest weight to rare species at $q = 0$, the rate of increase was 2.4 and 4.6 times faster for connected patches compared to isolated winged and rectangular patch types, respectively. When rare species were weighted less at $q = 1$, the rate of increase was 2.3 and 4.3 times faster for connected patches compared to isolated winged and rectangular patch types, respectively. When rare species were weighted the least at $q = 2$, the rate of increase was 2.0 and 3.7 times faster for connected patches compared to isolated winged and rectangular patch types, respectively. We found no differences in how fast taxonomic diversity accumulated in patches when comparing winged and rectangular patch types (Table 4). When we subsetted the data by each patch type to investigate the interaction between time and patch type, we found that Hill diversity at each level of q increased with time for each patch type (all $p < 0$).

For functional group diversity, we found no effects of patch type, time since site establishment, and their interaction when Hill diversity gave the most weight to rare functional groups at $q = 0$ (Fig. 3A, Table 1). When diversity estimates gave less weight to rare functional groups at $q=1$ and $q=2$, we detected effects of patch type, time since site establishment, and their interaction on functional group diversity (Fig. 3B and 3C, Table 1). At $q = 1$ and $q = 2$, Hill diversity increases through time after site establishment (Table 1). When $q = 1$, connected patches had significantly lower functional group diversity than winged patches, and for $q = 2$, we found that connected patches had marginally less functional group diversity than winged patches (Table 2). All other comparisons across patch types were not statistically different (Table 2, $p > 0.05$ for all). As with taxonomic diversity, Hill diversity at $q = 1$ and $q = 2$ increased with time since site establishment for all patch types (all $p < 0.05$; Table 1). Additionally, functional group Hill diversity increased over time in all patch types for all orders of q (Table 3). However, for $q = 1$ and $q = 2$, the linear increase in functional group Hill diversity was higher in both connected patches and winged patches than in rectangular patches but did not differ between connected and winged patches (Table 4). For $q = 1$, the rate of increase was 2.7 times faster for connected patches compared to isolated rectangular patches and 2.3 times faster for winged patches compared to rectangular patches. When comparing functional group diversity at $q = 2$, we found that the rate of increase was 2.4 times greater for connected than rectangular patches and 2.1 times greater for winged than rectangular patch types. After subsetting by patch type to further investigate the interaction between patch type and time, we again found that Hill diversity at each level of q increases with time for each patch type (all $p < 0.05$). We report the coefficients from these mixed effects models in Table S1 in the supplement.

Effects of connectivity and time on ant taxonomic and functional group composition

Taxonomic species composition summarized with Bray-Curtis dissimilarity, showed significant effects of both patch type and the interaction between patch type and time since site establishment, however no main effect of time since site establishment on its own (Table 5, Fig. 4). Because we detected that patch type and time since site establishment interacted in our PERMANOVA, we also performed separate PERMANOVAs on our data subsetted by patch type. These separate PERMANOVAs showed a time since site establishment effect only for rectangular patch types (F = 1.40, p = 0.02), and not for connected or winged patch types (connected: F =7.57, p = 0.95; winged: F = 2.47, p = 0.28). Post-hoc pairwise comparisons among patch types showed a difference between high edge isolated (winged) and low edge isolated (rectangular) patches (p = 0.009) and a marginal difference between connected and low edge isolated patches (p = 0.08). However, our multivariate analysis of homogeneity showed differences in dispersion among patch types with ant communities in connected patches exhibiting greater dispersion than those in winged patches, but not pairwise differences in dispersion between winged vs. rectangular or connected vs. rectangular patch types (Table 6, Fig. 4c).

For functional group composition, our PERMANOVA based on Bray-Curtis dissimilarity showed effects of patch type, time since site establishment, and their interaction (Table 5, Figure 4d and 4e). Although patch type was a significant predictor in the PERMANOVA, further post hoc pairwise tests comparing centroids found no significant differences between patch types (all p>0.05). Our multivariate analysis of homogeneity also showed no differences in dispersion across patch types (Table 6, Figure 4f), but did show significant differences across years (F = 5.18, p = 0008). Our PERMANOVAs subsetted by patch type only showed a significant effect of time since site establishment for rectangular patches (F = 1.93, p = 0.001), not for connected or winged patches (Connected: F = 9.82, p = 0.16; Winged: F = 4.38, p = 0.17).

Discussion

Although habitat corridors are often used to reconnect habitat patches that are isolated because of fragmentation (Hilty et al. 2006), the mechanisms by which corridors function are often not well understood. A general lack of understanding of biodiversity responses to landscape change combined with the potential for confounding effects of area, edges, and connectivity within fragmented systems obscures these mechanisms (Ewers and Didham 2006). With long-term monitoring of the ant community within a large-scale habitat corridor experiment, we found evidence for effects of both connectivity and edge amount on ant diversity and community composition. However, these effects depended on how we measured diversity (i.e., taxonomic vs. functional group diversity) and how we partitioned diversity (alpha- vs. beta-diversity). Taken together, these results provide evidence of how corridors can have important impacts on the assembly of ant communities through time.

Prior to site creation, each of our experimental landscapes was maintained as a mixed pine plantation. Upon the establishment of our experiment, management activities switched to those instead aimed at restoring the landscape patches to longleaf pine savanna ecosystems (e.g. introduced frequent prescribed fire and removal of resprouting hardwoods). The ant communities within our experimental landscapes, thus, have been assembling over time since the establishment of our patches. We found that both taxonomic and functional group diversity increased through time for all patch types, but connectivity and patch shape effects depended on whether we measured taxonomic or functional group diversity. While taxonomic diversity increased faster over time in connected than isolated patch types, change over time in functional group diversity depended on patch shape with high edge isolated (winged) patches accumulating functional groups faster than low edge isolated (rectangular) patches. This mismatch suggests that the mechanisms structuring ant taxonomic diversity and functional group diversity may differ in fragmented ecosystems. One possible explanation for this mismatch is that corridors promote species colonization and dispersal into connected patches leading to higher taxonomic diversity over time, but changes to patch shape may affect patch quality attributes that are better captured by our ant functional groups. Indeed, previous studies have generally found that disturbance typically impacts ant communities by increasing opportunist species and decreasing specialists (Leal et al. 2017, Andersen 2019). Although functional group diversity is a great first step for exploring the potential effects of landscape connectivity via corridors on ant community assembly, we may gain different insights from measuring different functional traits, such as dispersal ability (Helms and Kaspari 2013, Parr et al. 2017).

Although we found no difference in alpha diversity (number of species within patches) between isolated and connected patch types, connected patches did have higher taxonomic beta-diversity (turnover across patches) than isolated patches. That is, we found more variation in ant communities among patches with corridors than among isolated patches. This result suggests a function of connectivity provided by corridors that may be scale dependent with connectivity effects evident at larger scales than at the local patch scale. Connectivity may not have an effect at the scale of individual patches because at this scale diversity may be impacted more so by local environmental conditions. This result from our study contrasts results from a previous study that took place during the early years of this same experiment where Orrock et al. found the largest amounts of beta diversity in Hymenoptera in isolated low edge (rectangular) patches, not connected patches (Orrock et al. 2011). Our contrasting results here suggest that connectivity effects via corridors may take time to accrue.

Diversity is often summarized as taxonomic diversity, yet functional diversity may provide greater insights into the mechanisms driving differences across communities (Safi et al. 2011, Cardoso et al. 2014). For ants, functional groups based on species' biogeography, environmental requirements, and dominance of species are well-defined (Andersen 1997). We found differences in diversity among patch types that depended on whether we measured taxonomic or functional group identity. Patch shape, but not connectivity, was a driver of taxonomic diversity with isolated low edge (rectangular) patches containing more species than isolated high edge (winged) patches. However, connectivity, not patch shape, was a driver of mean functional group diversity with fewer functional groups in patches with corridors than in isolated high edge (winged) patches. In an earlier study within this same experiment, corridors initially facilitated the spread of the polygyne social form of *Solenopsis invicta* (red imported fire ants), coinciding with a decrease in native ant species in connected patches compared to isolated patches (Resasco et al. 2014). However, this effect on taxonomic diversity was transient, as species diversity in connected patches eventually caught up to that of isolated patches once polygyne fire ants also established in isolated patches (Resasco et al. 2023). Our finding that functional group diversity is still significantly lower within connected than isolated patches suggests that communities may take longer to recover functionally than taxonomically.

Taken together, our results suggest that corridors can function in landscapes via changes to patch connectivity and edge effects. The trajectory of ant diversity growth in our sites shows a greater rate of increase in patches with corridors. Combined with our finding of greater beta diversity among patches with corridors, this suggests that ant diversity in connected patches may eventually surpass that of isolated patch types. Furthermore, our results underscore the importance of long-term monitoring and large scale experiments for disentangling the underlying mechanisms key for ant community assembly in fragmented landscapes.

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Figures and Tables

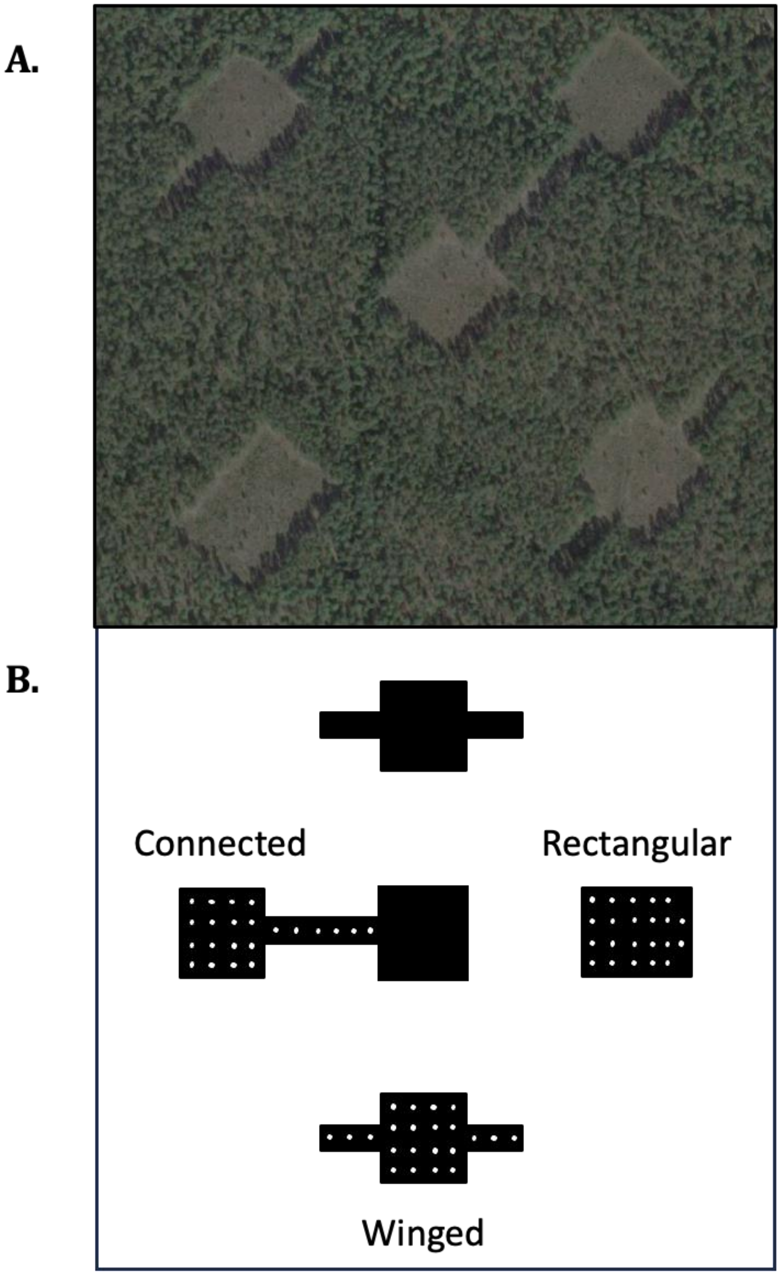

Figure 1. A. Aerial view of one experimental landscape (N=7; Photo Credit: Google Earth). **B.** Diagram of experimental landscapes. Comparison between connected and winged patches test for connectivity effects, while comparisons between winged and rectangular patches test for patch shape (i.e., edge:area ratio) effects.

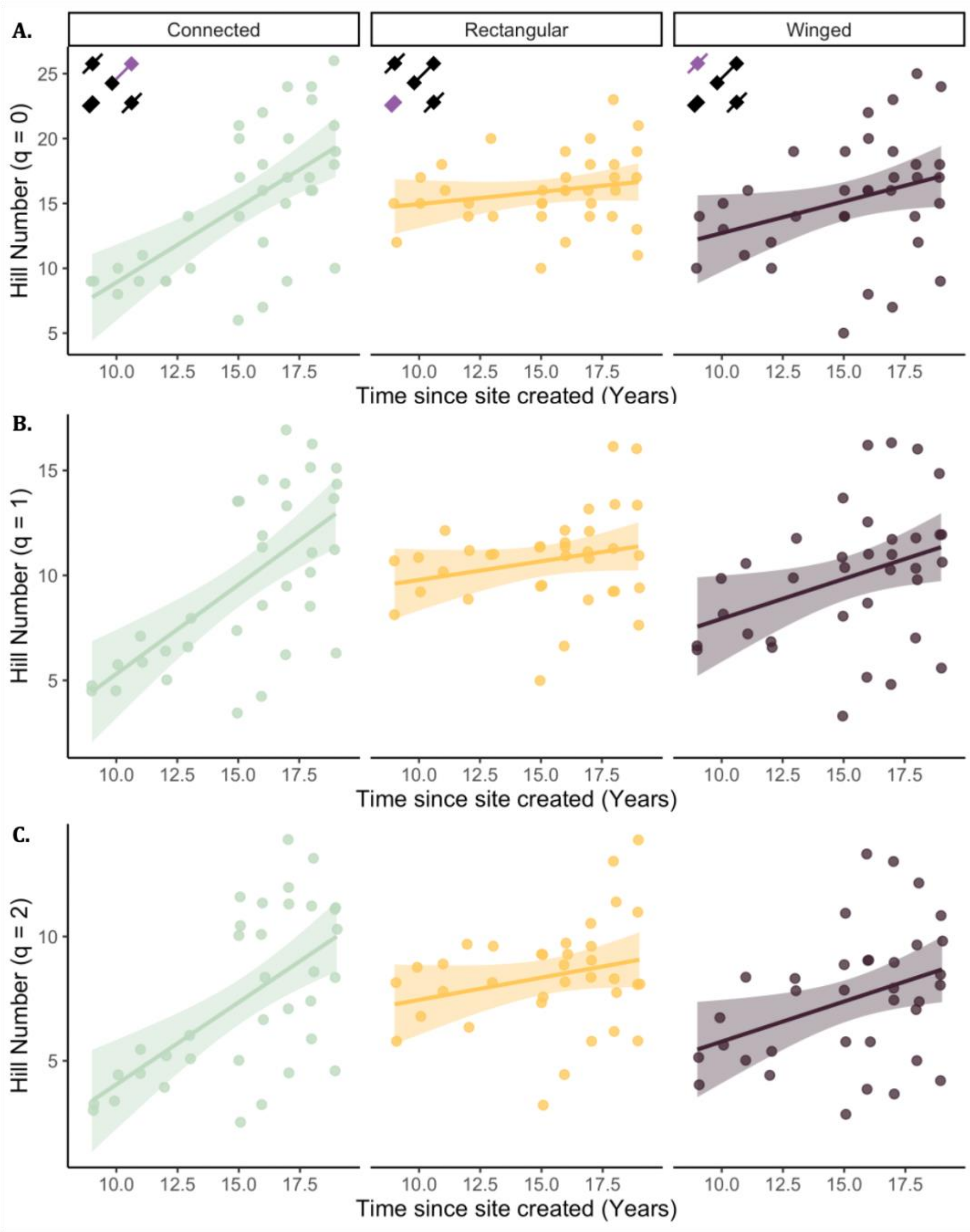

Figure 2. Hill numbers based on taxonomic diversity over time by patch type. We present results from q = 0 (A.), q =1 (B.), and q = 2 (C.). Each point represents diversity summarized across pitfall traps within each patch in each experimental landscape. Shaded areas represent 95% confidence intervals around the regression slope.

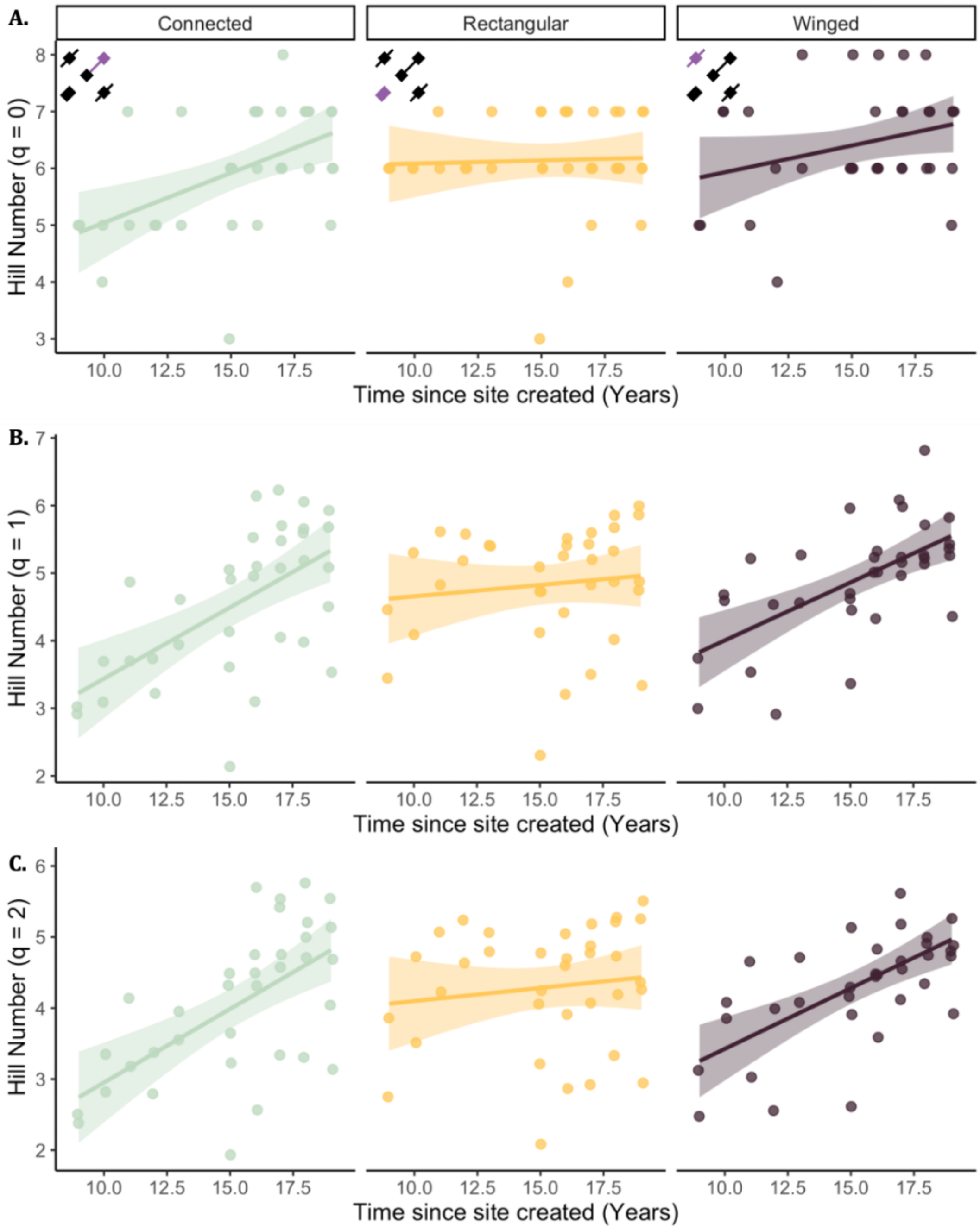

Figure 3. Hill numbers based on functional group diversity over time separated by patch type. We present results from q = 0 (A.), q =1 (B.), and q = 2 (C.). Each point represents diversity summarized across pitfall traps within each patch in each experimental landscape. Shaded areas represent 95% confidence intervals around the regression slope.

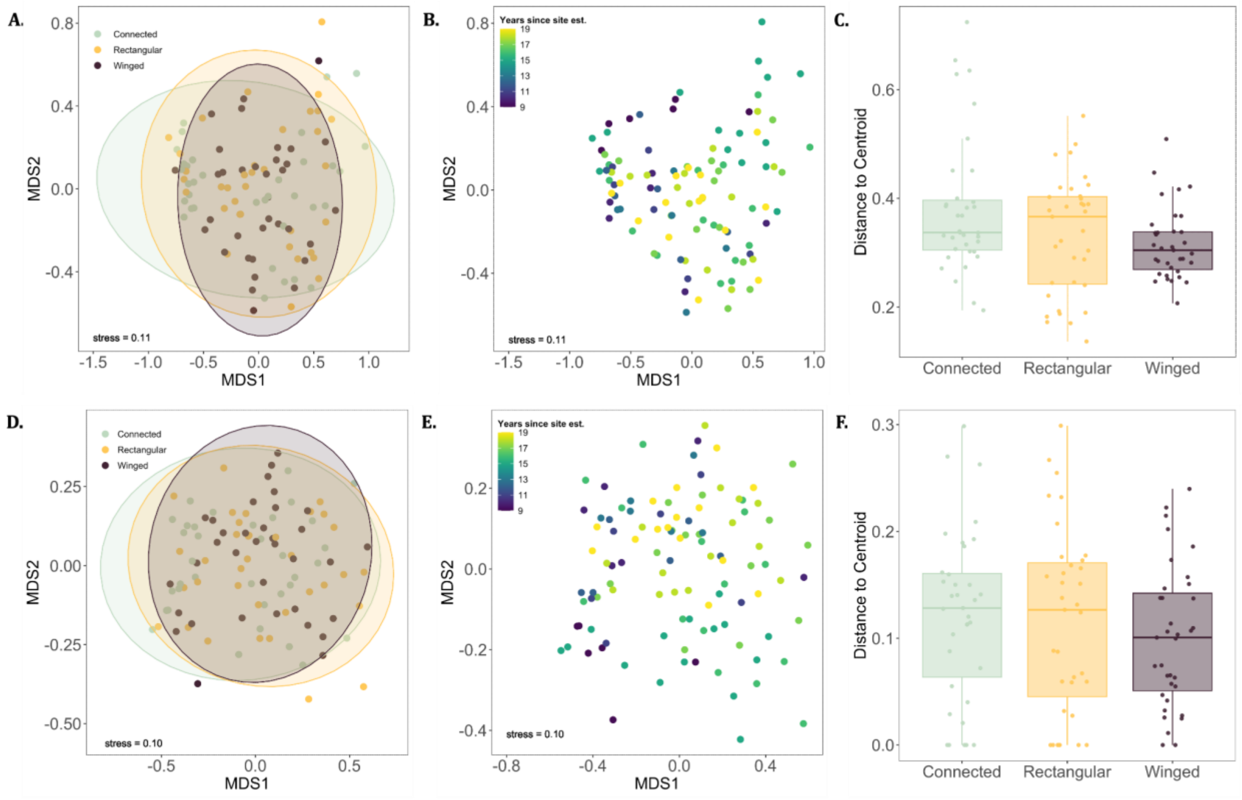

Figure 4. Nonmetric multidimensional scaling (NMDS) plots for taxonomic and functional group diversity conducted on Bray Curtis dissimilarity matrix showing patch type (A. taxonomic diversity, D. functional group diversity) and time (B. taxonomic diversity, E. functional group diversity) effects. The shaded ellipses in A. and D. show 95% confidence intervals. Boxplots (C. taxonomic diversity, F. functional group diversity) show the distance to centroids for each patch shape. The shaded regions of these boxplots show the median and upper and lower quartiles.

Table 1. Output from mixed effects models for taxonomic and functional group diversity calculations. For each model we specified block as a random effect. We log transformed taxonomic diversity Hill diversity to improve normality and homoscedasticity of model residuals. Model coefficients are presented in table S1 in the supplement.

	Hill Number	**Fixed Effects**	**Chi-square**	**df**	**p-value**
Taxonomic Diversity	**q = 0**	Patch Type	29.73	2	**<0.0001**
		Time since est.	38.76	1	**<0.0001**
		Patch Type: Time since est.	25.75	2	**<0.0001**
	q = 1	Patch Type	44.51	2	**<0.0001**
		Time since est.	56.40	1	**<0.0001**
		Patch Type: Time since est.	37.26	2	**<0.0001**
	q = 2	Patch Type	39.02	2	**<0.0001**
		Time since est.	54.20	1	**<0.0001**
		Patch Type: Time since est.	31.93	2	**<0.0001**
Functional Group Diversity	**q = 0**	Patch Type	0.59	2	0.75
		Time since est.	1.35	1	0.24
		Patch Type: Time since est.	0.78	2	0.68
	q = 1	Patch Type	18.76	2	**<0.0001**
		Time since est.	38.87	1	**<0.0001**
		Patch Type: Time since est.	16.90	2	**0.0002**
	q = 2	Patch Type	19.02	2	**<0.0001**
		Time since est.	45.42	1	**<0.0001**
		Patch Type: Time since est.	17.35	2	**0.0002**

Table 2. Estimated marginal means for patch type for taxonomic and functional group diversity. We only present contrasts from estimated marginal means for models in which patch type was a statistically significant predictor of the diversity metric.

	Hill Number	**Contrast**	**Estimate**	**SE**	**df**	***t***	***p***
Taxonomic Diversity	**q = 0**	Connected - Rectangular	-0.12	0.045	93	-2.65	**0.03**
		Connected - Winged	-0.04	0.045	93	-0.86	0.67
		Rectangular - Winged	0.08	0.045	93	1.79	0.18
	q = 1	Connected - Rectangular	-0.16	0.043	93	-3.80	**0.0008**
		Connected - Winged	-0.051	0.043	93	-1.18	0.47
		Rectangular - Winged	0.11	0.043	93	2.62	**0.03**
	q = 2	Connected - Rectangular	-0.18	0.046	93	-3.94	**0.0005**
		Connected - Winged	-0.032	0.046	93	-0.71	0.76
		Rectangular - Winged	0.15	0.046	93	3.24	**0.005**
Functional Group Diversity	**q = 1**	Connected - Rectangular	-0.29	0.14	93	-2.07	0.10
		Connected - Winged	-0.36	0.14	93	-2.61	**0.03**
		Rectangular - Winged	-0.08	0.14	93	-0.55	0.85
	q = 2	Connected - Rectangular	-0.25	0.13	93	-1.87	0.15
		Connected - Winged	-0.28	0.13	93	-2.15	**0.09**
		Rectangular - Winged	-0.037	0.13	93	-0.28	0.96

Table 3. Estimated marginal means of linear trends for patch type through time for analyses with taxonomic and functional group diversity. A p-value of less than 0.05 indicates a slope significantly different from zero. Confidence limits are 95% confidence intervals.

	Hill Number	**Patch Type**	**slope**	**SE**	**df**	**Lower CL**	**Upper CL**	***t***	***p***
Taxonomic Diversity	**q = 0**	Connected	0.092	0.015	97.7	0.062	0.12	6.06	**<0.0001**
		Rectangular	0.020	0.015	97.7	-0.0099	0.050	1.33	0.19
		Winged	0.039	0.015	97.7	0.0086	0.069	2.55	**0.01**
	q = 1	Connected	0.11	0.015	99	0.079	0.14	7.36	**<0.0001**
		Rectangular	0.025	0.015	99	-0.0042	0.054	1.70	0.09
		Winged	0.047	0.015	99	0.018	0.076	3.20	**0.002**
	q = 2	Connected	0.11	0.016	99	0.081	0.14	7.23	**<.0001**
		Rectangular	0.030	0.016	99	-0.00062	0.061	1.94	**0.05**
		Winged	0.055	0.016	99	0.024	0.086	3.53	**0.0006**
Functional Group Diversity	**q = 1**	Connected	0.28	0.047	95.8	0.19	0.37	6.04	**<0.0001**
		Rectangular	0.10	0.047	95.8	0.01	0.20	2.24	**0.03**
		Winged	0.24	0.047	95.8	0.15	0.34	5.21	**<0.0001**
	q = 2	Connected	0.29	0.045	97.4	0.20	0.38	6.55	**<0.0001**
		Rectangular	0.12	0.045	97.4	0.03	0.21	2.73	**0.008**
		Winged	0.26	0.045	97.4	0.17	0.34	5.75	**<0.0001**

Table 4. Contrasts between patch types for linear trends for taxonomic and functional group diversity over time. A p-value of less than 0.05 represents a significant difference between slopes.

	Hill Number	**Contrast**	**Estimate**	**SE**	**df**	***t***	***p***
Taxonomic Diversity	**q = 0**	Connected - Rectangular	0.071	0.015	93	4.89	**<0.0001**
		Connected - Winged	0.053	0.015	93	3.63	**0.001**
		Rectangular - Winged	-0.018	0.015	93	-1.26	0.42
	q = 1	Connected - Rectangular	0.083	0.014	93	5.89	**<0.0001**
		Connected - Winged	0.061	0.014	93	4.33	**0.0001**
		Rectangular - Winged	-0.022	0.014	93	-1.57	0.27
	q = 2	Connected - Rectangular	0.082	0.015	93	5.51	**<0.0001**
		Connected - Winged	0.057	0.015	93	3.85	**0.0006**
		Rectangular - Winged	-0.025	0.015	93	-1.65	0.23
Functional Group Diversity	**q = 1**	Connected - Rectangular	0.18	0.045	93	3.91	**0.0005**
		Connected - Winged	0.04	0.045	93	0.86	0.67
		Rectangular - Winged	-0.14	0.045	93	-3.05	**0.008**
	q = 2	Connected - Rectangular	0.17	0.043	93	3.95	**0.0004**
		Connected - Winged	0.036	0.043	93	0.83	0.68
		Rectangular - Winged	-0.13	0.043	93	-3.12	**0.007**

Table 5. Results of permutational analyses of variance (PERMANOVA) with patch type, time since site establishment (years), and their interaction as main effects. For the distance matrix, we used a Bray-Curtis and a Morisita-Horn dissimilarity matrices. Separate PERMANOVAs were conducted for species-level and functional groups-level identities.

	Predictor	**df**	**Sum of Squares**	**R^2**	**F**	**p-value**
Taxonomic **Bray Curtis**	**Patch Type**	2	0.29	0.02	1.18	**0.03**
	Time since est.	1	0.73	0.05	6.02	0.27
	Patch Type: Time since est.	2	0.74	0.05	3.05	**0.001**
Functional Groups **Bray Curtis**	**Patch Type**	2	0.10	0.02	1.43	**0.06**
	Time since est.	1	0.37	0.09	10.03	**0.02**
	Patch Type: Time since est.	2	0.19	0.04	2.62	**0.007**

Table 6. Results of pairwise comparisons of dispersion between patch types after multivariate analyses of homogeneity with Tukey multiple comparisons of means tests. Confidence limits are 95% confidence intervals.

	Predictor	**Difference**	**Lower CI**	**Upper CI**	**p-adj**
Taxonomic **Bray Curtis**	**Rectangular - Connected**	-0.050	-0.11	0.0096	0.12
	Winged - Connected	-0.066	-0.12	-0.0071	**0.02**
	Winged - Rectangular	-0.017	-0.076	-0.042	0.78
Functional Groups **Bray Curtis**	**Rectangular - Connected**	-0.0016	-0.040	0.037	0.99
	Winged - Connected	-0.012	-0.050	0.027	0.75
	Winged - Rectangular	-0.010	-0.049	0.029	0.81

Supplementary Materials for Chapter 2

Figure S1. Pitfall trap installed in field under white pizza stacker. Photo Credit: M. Burt.

Table S1. Output from general linear mixed effects models for analyses with Hill diversity. For each model with Hill diversity calculated from taxonomic species identity we log transformed the response variable to address issues with normality and heteroskedasticity of model residuals.

Response Variable	**Parameter**	**Estimate**	**SE**	**t**
Taxonomic Diversity **q = 0**	Intercept	1.24	0.24	5.08
	Patch Type Rectangular	1.21	0.23	5.31
	Patch Type Winged	0.85	0.23	3.72
	Time Since Site Created (years)	0.091	0.02	6.23
	Patch Type Rectangular:Time Since Site Created (years)	-0.071	0.02	-4.89
	Patch Type Winged:Time Since Site Created (years	-0.053	0.02	-3.63
Taxonomic Diversity **q = 1**	Intercept	0.53	0.25	2.16
	Patch Type Rectangular	1.44	0.22	6.52
	Patch Type Winged	0.99	0.22	4.47
	Time Since Site Created (years)	0.11	0.01	7.51
	Patch Type Rectangular:Time Since Site Created (years)	-0.083	0.01	-5.89
	Patch Type Winged:Time Since Site Created (years	-0.061	0.01	-4.33
Taxonomic	Intercept	0.20	0.26	0.75

Response Variable	Parameter	Estimate	SE	t
Diversity **q = 2**	Patch Type Rectangular	1.43	0.23	6.17
	Patch Type Winged	0.91	0.23	3.92
	Time Since Site Created (years)	0.11	0.02	7.36
	Patch Type Rectangular:Time Since Site Created (years)	-0.082	0.01	-5.51
	Patch Type Winged:Time Since Site Created (years	-0.057	0.01	-3.85
Functional Group Diversity **q = 0**	Intercept	1.32	0.37	3.56
	Patch Type Rectangular	0.47	0.51	0.92
	Patch Type Winged	0.31	0.51	0.62
	Time Since Site Created (years)	0.030	0.02	1.30
	Patch Type Rectangular:Time Since Site Created (years)	-0.029	0.03	-0.88
	Patch Type Winged:Time Since Site Created (years	-0.015	0.03	-0.48
Functional Group Diversity **q = 1**	Intercept	0.25	0.74	0.34
	Patch Type Rectangular	2.99	0.71	4.24
	Patch Type Winged	0.955	0.71	1.35
	Time Since Site Created (years)	0.28	0.05	6.23
	Patch Type Rectangular:Time Since Site Created (years)	-0.18	0.05	-3.91
	Patch Type Winged:Time Since	-0.039	0.05	-0.86

Response Variable	Parameter	Estimate	SE	t
	Site Created (years			
Functional Group Diversity **q = 2**	Intercept	-0.41	0.72	-0.57
	Patch Type Rectangular	2.85	0.67	4.24
	Patch Type Winged	0.83	0.67	1.24
	Time Since Site Created (years)	0.29	0.04	6.74
	Patch Type Rectangular:Time Since Site Created (years)	-0.17	0.04	-3.95
	Patch Type Winged:Time Since Site Created (years	-0.036	0.04	-0.83

Community Composition analyses using the Morisita-Horn Index

Our PERMANOVAs using the Morisita-Horn index showed similar patterns, with a significant effect of the interaction between patch type and time since site establishment on taxonomic composition, but no main effects of patch type or time since site establishment (Table S2). Because we detected that patch type and times since site establishment interacted in our PERMANOVA using the Morisita-Horn index, we again performed separate PERMANOVAs on our data subsetted by patch type. We found no effect of time since site establishment for any patch type (Connected: F = 8.38, p = 0.95; Winged: F = 2.53, p = 0.51; Rectangular: F = 0.56, p = 0.34). Our PERMANOVAs using the Morisita-Horn index showed significant effects of the interaction between patch shape and time since site establishment, but only marginal effects of both patch shape and time since site establishment on functional group composition (Table S2). Our multivariate analysis of homogeneity showed no difference in dispersion (i.e., beta-diversity) among patch shapes or time since site establishment (Table S3). As we found with Bray-Curtis dissimilarity our PERMANOVAs subsetted by patch type found no significant effect of time since site establishment for connected (F = 8.38, p = 0.97) or winged patch types (F = 2.53, p = 0.46), but did detect an effect of time for rectangular patch types (F = 0.91, p = 0.002).

Table S2. Results of permutational analyses of variance (PERMANOVA) with patch type, time since site establishment (years), and their interaction as main effects that used the Morisita-Horn index.

	Predictor	**df**	**Sum of Squares**	**R^2**	**F**	**p-value**
Taxonomic **Morisita-Horn**	**Patch Type**	2	0.09	0.01	0.54	0.37
	Time since est.	1	0.67	0.07	8.32	0.59
	Patch Type:Time since est.	2	0.41	0.05	2.57	**0.004**
Functional Groups **Morisita-Horn**	**Patch Type**	2	0.06	0.03	1.83	**0.05**
	Time since est.	1	0.23	0.12	14.60	0.08
	Patch Type:Time since est.	2	0.09	0.05	2.98	**0.03**

Table S3. Results of pairwise comparisons of dispersion (i.e., beta-diversity) between patch types after multivariate analyses of homogeneity with Tukey multiple comparisons of means tests. Confidence limits are 95% confidence intervals.

	Predictor	**Difference**	**Lower CI**	**Upper CI**	**p-adj**
Taxonomic **Morisita-Horn**	**Rectangular - Connected**	-0.075	-0.16	0.013	0.11
	Winged - Connected	-0.11	-0.19	-0.019	**0.01**
	Winged - Rectangular	-0.031	-0.12	0.056	0.67
Functional Groups **Morisita-Horn**	**Rectangular - Connected**	-0.0064	-0.051	0.038	0.94
	Winged - Connected	-0.022	-0.066	0.023	0.48
	Winged - Rectangular	-0.015	-0.060	0.029	0.70

Chapter 3 – Ants disperse seeds farther in habitat patches with corridors

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Abstract

Habitat fragmentation impacts ecosystems worldwide through habitat loss, reduced connectivity, and edge effects. Yet, these landscape factors are often confounded, leaving much to be investigated about their relative effects, especially on species interactions. In a landscape experiment, we investigated the consequences of connectivity and edge effects for seed dispersal by ants. We found that ants dispersed seeds farther in habitat patches connected by corridors, but only in patch centers. We did not see an effect on the total number of seeds moved or the rate ants detected seeds. Furthermore, we did not see any differences in ant community composition across patch types, suggesting that shifts in ant behavior or other factors increased ant seed dispersal in patches connected by corridors. Long distance seed dispersal by ants that requires an accumulation of short distance dispersal events over generations may be an underappreciated mechanism through which corridors increase plant diversity.

Keywords: ants, seed dispersal, myrmecochory, connectivity, corridors, edge effects, fragmentation

Introduction

Globally, habitat loss and fragmentation are major threats to biodiversity (Haddad et al. 2015, Morreale et al. 2021). In addition to the impacts of habitat loss, habitat fragmentation decreases connectivity among patches and increases potentially negative edge effects (Ries et al. 2004, 2017). These changes to habitat amount, habitat quality, and landscape structure can affect the colonization and extinction dynamics of species, leading to changes in community composition (Damschen et al. 2019). Compositional changes, as well as changes to the behavior of organisms, can transform interactions between species, including predation (Orrock et al. 2003, Orrock and Damschen 2005, Martinson and Fagan 2014, Hawn et al. 2018), pollination (Tewksbury et al. 2002, Townsend and Levey 2005, Hadley and Betts 2012, Dicks et al. 2021), and seed dispersal (Brudvig et al. 2015, Ripple et al. 2015, Nield et al. 2020). Although there is a large literature on fragmentation effects on seed dispersal by vertebrates (Tewksbury et al. 2002, Levey et al. 2005, Ripple et al. 2015), comparatively less is known about the effects of fragmentation on seed dispersal by ants (Mitchell et al. 2002, Crist 2009, Christianini and Oliveira 2013, Bieber et al. 2014). Here, we aim to increase the mechanistic understanding of how ant seed dispersal mutualisms respond to fragmentation with an experiment that separates the effects of fragmentation into connectivity and edge amount.

Ants could play an essential role in plant population and community responses to habitat fragmentation. With about 11,000 plant species dispersed by ants, myrmecochory is an important mechanism of seed dispersal structuring plant communities (Giladi 2006, Lengyel et al. 2010, Penn and Crist 2018). When ants disperse seeds, the collected seeds may be protected from seed predators, moved to nutrient-rich trash middens in or near the ant’s nest that are favorable germination sites, and escape from competition with the parent plant (Handel 1978, Culver and Beattie 1980, Giladi 2006, Lengyel et al. 2010, Penn and Crist 2018). In return, the ants receive a food resource in the form of a lipid-rich appendage on the seed coat called an elaiosome (Beattie 2010). However, there is not always evidence that consuming elaiosomes is essential to the ants (Mitchell et al. 2002, Warren et al. 2019, Schultz et al. 2022). The elaiosome may also attract seed predators, but there is evidence this adaptation has evolved in plants for selecting dispersers over predators or for manipulating the behavior of the dispersers (Levey and Byrne 1993, Giladi 2006, Warren II and Giladi 2014). Because ants typically disperse seeds shorter distances, ant-mediated seed dispersal is especially vulnerable to disruption due to fragmentation.

Unlike prior studies that have focused on the effects of fragment size (Mitchell et al. 2002, Bruna et al. 2005) and dynamics at boundaries across habitat edges (Ness 2004, Ness and Morin 2008, Warren II et al. 2015), ours focuses on the individual and interactive effects of edge and connectivity. Mitchell et al. (2002) found that the composition of seed dispersing ant communities differed between large and small forest fragments. Bruna et al. (2005) found no evidence that fragment size affected ant-plant interactions (i.e., plants that ants use for shelter in exchange for protection for the plant) or ant species richness, but found lower densities of ant-plants in smaller fragments.

With fragmentation, the amount of edge relative to interior habitat proportionately increases. This results in potential changes to abiotic and biotic gradients at habitat edges that can have consequences for seed dispersing ants and their plant mutualists (Crist 2009). For example, in eastern deciduous forests, habitat edges typically have fewer ant-dispersed plants and mixed effects of edges on the major seed dispersing ant, *Aphaenogaster spp* (Ness 2004, Ness and Morin 2008, Warren II et al. 2015). Shorter seed dispersal has been found at distances closer to an edge (Ness 2004) and ants direct seed dispersal away from the edge towards the habitat interior (Ness 2004, Warren II et al. 2015) suggesting that altered ant behavior plays a role in edge effects on ant-dispersed plant distributions.

In a landscape-scale experiment that manipulated habitat connectivity and edge amount across habitat patches, we investigated the consequences of fragmentation on seed dispersal by ants. Specifically, we asked: 1) How is seed dispersal by ants impacted by (a) connectivity and edge amount? and (b) local-scale proximity to a habitat edge? and 2) To what extent might differences in ant-mediated dispersal across patches be driven by (a) changes in ant community composition?, and (b) changes in the behavior of individual ant species?

Materials and Methods

Experimental Landscapes

We conducted this study at the Savannah River Site (SRS) - a Department of Energy (DOE) National Environmental Research Park in New Ellenton, SC, USA. Our experimental landscapes are managed by the US Department of Agriculture Forest Service - Savannah River and were established by clearing patches of mature trees within pine plantations in the winter of 1999 and 2000. The habitat patches within our experimental landscapes have been managed as restored longleaf pine savannas. Thus, the habitat patches are relatively open woodlands embedded in more shaded pine plantations. Our experimental design creates patches and corridors of suitable habitat surrounded by a matrix of unsuitable habitat. Previous work on the effects of fragmentation on seed dispersal by ants has largely focused on dynamics within forested fragments (Giladi 2006, Warren II and Giladi 2014). Given that ants disperse seeds in a wide range of ecosystems (Lengyel et al. 2010), more studies expanding outside forests are needed to determine whether these results apply more generally. Longleaf pine savannas of the southeastern US are one of the most imperiled ecosystems in North America with less than 3% of its original range remaining today (Noss 1988, 2013, Frost 2006).

To reduce woody shrub encroachment and maintain an open canopy, prescribed burns are implemented every 2-3 years and herbicide and brush-cutting are applied periodically. Each experimental landscape block consists of a one-hectare square center patch surrounded by four peripheral patches that are equal in area (~1.4 ha) but differ in the amount of habitat connectivity or edge (Fig. 1). One peripheral patch is connected to the center patch by a 150mx25m corridor (hereafter "connected"). The isolated patches are also 150m from the center patch and vary in their edge amount. The winged patch type (hereafter "isolated high edge") is a 100*100m square with two 75m*25m projections on either side of the patch. The rectangular patch type (hereafter "isolated low edge") consists of a 100*100m square with an area equal in size to the corridor added to the part of the patch farthest from the center patch. Each block contains one of each patch type and a duplicate of one of the isolated patch types. To test effects of connectivity, we compare the high edge and connected patch types. To test effects of edge amount, we compare the high edge and low edge patch types. We investigated edge effects within the patch as a function of proximity to the matrix. We conducted seed dispersal assays in four experimental blocks because these had pre-existing populations of *P. cistoides* in each patch type.

Focal plant species

Seed dispersal assays (described below) were conducted with the forb, *Piriqueta cistoides* (L.) Griseb. (Common Name: pitted stripeseed; Family: Turneraceae). It can be an annual or perennial and is endemic to the southeastern United States (USDA NRCS 2020). Populations are distributed across the coastal plain of South Carolina, Georgia, and Florida in sandy soils located along woodland borders. They flower and disperse seeds from May through September (Radford et al. 1964). On average seed capsules contain 12-20 seeds that are 1.8-2.2mm long (Ornduff and Perry 1964, Radford et al. 1964). Each seed has an elaiosome along the entire length of its seed (Radford et al. 1964) and the seeds are dispersed by ants (Cumberland and Kirkman 2013).

Ant community of longleaf pine savannas

The open habitats of the SRS are home to a diverse ant community dominated by *Solenopsis invicta, Dorymyrmex bureni, Crematogaster lineolata,* and *Pheidole* species (Resasco et al. 2014, Resasco and Fletcher 2021), which have each been documented as seed dispersers in longleaf pine savanna (Stuble et al. 2010). The most common species in our study site, *S. invicta*, is an invasive non-native species (Tschinkel 2006). *Solenopsis invicta* has been shown to both disperse and predate seeds in their invaded range, potentially disrupting seed dispersal mutualisms with native ant seed dispersers (Zettler et al. 2001, Ness 2004, Stuble et al. 2010). Although *S. invicta* outcompetes native ants foraging for myrmecochorus seeds, in longleaf pine savanna they move seeds distances commensurate with native ant seed dispersers (Stuble et al. 2010). Additionally, a large proportion of seeds moved to *S. invicta* trash middens were not damaged (Cumberland and Kirkman 2013).

Seed dispersal assays

We conducted seed dispersal assays in July-August of 2018 during peak seed production in *P. cistoides* (personal observation, MAB). In each patch type within each block, we conducted observations of seed removal at four seed depots, two located at the center of each patch (~37.5m from the nearest edge) and two located at the edge of each patch (~12.5m from the nearest edge) (4 blocks x 3 patch types x 2 edge distances x 2 replicates = 48 depots total; Fig. 1). Each seed depot was made with an open 10cm petri dish in which we melted four ~1cm openings around the edge to facilitate movement of ants carrying seeds into and out of the depot. The largest ants, major workers of *Pogonomyrmex badius,* are 7-9mm long (Deyrup 2016). This method has been used in previous studies and facilitates observing the seeds on sandy soil during the observation (e.g., Stuble et al. 2010). We also conducted a pilot experiment in 2016 where we observed that even the smaller ants visiting seed caches were able to climb up and over the sides of the petri dishes while holding a seed in their mandibles (Methods and Results in Appendix S1). In each depot, we placed ten *P. cistoides* seeds that we collected from local populations at SRS during the same growing season. We chose to use consistently use 10 seeds in depots to limit density dependent variance across observations. Additionally, while seed capsules typically contain between 12-20 seeds (Ornduff and Perry 1964), the seed gradually fall away from the capsule when in opens which would decrease the number of seeds on the ground at any given time. We observed each depot individually until all seeds were removed or up to 60 min, whichever occurred first. During each observation, we recorded the amount of time it took for ants to discover seed depots (i.e., when ants first entered the depot and interacted with seeds), the time when ants removed each seed, the distance ants dispersed seeds, and the ant species that dispersed the seeds. We collected voucher specimens of ants visiting depots for later confirmation of identity with a microscope. Because temperature change associated with time of day impacts ant activity, we randomized the order in which we conducted observations within a block, and only conducted observations when ants were active (~800h–1200h). Observations for individual blocks were done over two days.

Statistical Analyses

To test how patch type and edge proximity affected ant seed dispersal (Q1), we used linear mixed models (LMM) and generalized linear mixed models (GLMM) for four response variables: time to discovery, the count of seeds dispersed from a depot, ant species richness visiting seed depots, and seed dispersal distance. We averaged seed dispersal distance across seeds within a seed depot and treated each depot as a replicate. If assumptions of normality and homoscedasticity were met, we used LMMs. When assumptions were not met, we either transformed the response variable or we used GLMMs with an appropriate error distribution (see Table S4 for more details). For each model, we checked relevant assumptions to that test including testing for overdispersion in the GLMMs. For each mixed effects model, we specified patch type, edge proximity, and the interaction between patch type and edge proximity as fixed effects and patch nested within block as a random effect. We used Wald chi-square tests to assess fixed effects using type III tests when the interaction between patch type and edge proximity was at least marginally supported ($p < 0.10$) and type II tests when it was not. If the interaction between patch type and edge proximity was a significant predictor, then we split the analysis into two separate models analyzing the data from seed depots located along the edge and within the center of patches separately. When fixed effects were significant predictors of the response variable, we used a Tukey post-hoc test to assess differences between levels.

To investigate the extent that differences in ant-mediated dispersal were driven by changes in ant community composition and ant behavior (Q2), we conducted additional analyses. First, to determine if the relative frequency that different ant species were observed moving seeds from seed depots was different across patch types and edge proximity, we performed Fisher's exact tests on contingency tables. Second, to test whether differences across patch types were driven by shifts in behavior of individual ant species, we used mixed effects models similar in structure to those described above for analyses across species for the seed depot data in which we dropped individual species one at a time from the models. If the behavior of an individual species was driving the overall pattern, we predict that dropping that species would qualitatively change the pattern relative to the pooled dataset.

All analyses were conducted in R version 4.1.1 (R Development Core Team 2011). We used the lme4 package for mixed effects models, the car package for Wald chi-squared tests, and the emmeans package for Tukey multiple comparison post hoc tests (Bates et al. 2015, Fox and Weisberg 2019, Lenth 2020).

Results

Approximately 84% of our seed dispersal depots were visited by ants. We observed 11 ant species dispersing seeds (Table S3, Fig. 2). The most common species were *S. invicta* (visited 36% of seed depots) and *C. lineolata* (visited 20% of seed depots). We found no effects of patch type, edge proximity, or their interaction on the amount of time it took for ants to discover seed depots, the number of seeds removed from seed depots, or the number of ant species that removed seeds from seed depots (all p>0.05, Table S4).

On average, ants dispersed seeds ~96 cm in connected patches versus ~48 cm in isolated high edge patch types, and this apparent difference was caused by even higher differences in dispersal in the centers of connected patches. In analyses pooled across species, we found a marginally significant interaction between patch type and edge proximity such that seed dispersal distance was positively affected by connectivity, but only in patch centers (Fig. 3, Table S4). When considered separately, ants dispersed seeds about four times as far in the center of connected patches than in the center of isolated patches (connected centers: Mean: ~125 cm, Median: 67.7 cm; isolated centers: Mean: ~32 cm, Median: 31.0; $X^2 = 11.14$, $p = 0.004$, Fig. 3, Table S4), but there was no difference in seed dispersal among patch types for seed dispersal depots at patch edges ($X^2 = 0.56$, $p = 0.76$; Fig. 3, Table S4).

When we conducted analyses with individual species removed, the overall pattern of longer seed dispersal distances in the center of connected patches generally holds (see Appendix S2 for full details). This is despite mixed results when different species were lost. We also found no association between the relative abundance of species visiting seed depots and patch type (Fisher's Exact Test: p = 0.24; Fig. 2A) or edge proximity (Fisher's Exact Test: p = 0.95; Fig. 2B).

Discussion

Our results show that connecting fragmented habitat patches via corridors may have important effects on ant-mediated seed dispersal. We found ants dispersed seeds farther in habitat patches with corridors, an effect that depended on within-patch proximity to an edge. Variation in seed dispersal distance across habitat patches was not explained by fragmentation-mediated changes to ant community composition, but instead seemingly resulted from changes in behavior across multiple individual species in the ant community. The differences we observed in dispersal distances suggests fragmentation likely has important consequences for plant colonization dynamics.

We found that ants dispersed seeds about twice as far in connected patches, a result caused by dispersal in patch centers that was four times farther than in isolated patches. This difference of ~48 cm at the patch scale and ~93 cm in patch centers, represents quite a large difference relative to total ant seed dispersal. Previous studies find that ants usually disperse seeds ~75-200 cm on average (Culver and Beattie 1980, Stuble et al. 2010, Gómez and Espadaler 2013). Thus, in unconnected patches dispersal is lower than average suggesting that isolation reduces dispersal distance. These seemingly short distance dispersal events by ants accrue over generations yielding more rapid colonization of distant patches over time. Previous work has shown that the dispersal of seeds by ants results in less clumped distributions of offspring (Pudlo et al. 1980, Mitchell et al. 2002, Zelikova et al. 2011) potentially because of less intraspecific competition (Culver and Beattie 1980). Therefore, our finding that greater dispersal distances in connected patches suggests that greater connectivity between habitat patches could lead to plant communities in which intraspecific competition would be less limiting for ant-dispersed plants. Future work should follow seedlings germinating from myrmecochorous seeds to determine if this connectivity effect produces cascading consequences for populations of myrmecochorous plants.

Our results help to resolve two unexplained long-term dynamics of plant communities also observed in this experiment. Previous work shows plant species richness continues to increase through time through the positive effects of connectivity on colonization (Damschen et al. 2006, 2008, 2019). Additionally, plants with no obvious long-distance dispersal mechanism, such as *P. cistoides*, colonize new areas at a similar rate as bird and wind dispersed species (Damschen et al. 2008, 2019). The results of our ant seed dispersal experiment suggest seed dispersal by ants as one potential mechanism that would result in a higher chance of colonization. Although *S. invicta*, the most prevalent seed dispersing ant in our experiment, may damage seeds of other myrmecochorus plant species (Zettler et al. 2001), Cumberland and Kirkman (2013) found little damage to *P. cistoides* seeds in their aboveground trash middens. This suggests that *S. invicta* may not limit the accumulation of species in connected patches.

How does greater connectivity via corridors lead to greater dispersal distances? We hypothesized that patch isolation or edge effects would affect seed dispersal through effects on ant community composition or ant behavior. In our experiment, however, we did not detect evidence of an effect of patch connectivity on ant community compositional changes. Conversely, we found that patch type and edge proximity did not affect the composition of ant species visiting seed dispersal depots, suggesting that differences in seed dispersal distance are not mediated by ant community compositional differences. Further, the robustness of our seed dispersal patterns to dropping individual ant species suggests that the changes in seed dispersal difference are not mediated by the seed dispersal abilities of any one species alone. Instead, changes to seed dispersal behavior seem to be occurring across the community as a whole. While not measured in this study, it is possible that changes to worker or colony density in the center of connected patches could cause workers to travel farther from their nest to forage for resources. A higher nest density may result in workers having to travel further to forage if resources are limited and a lower nest densities may mean that colonies were more likely to be further away from our seed depots.

Our finding that seeds were dispersed for similar distances along the edges of both connected and isolated patches suggests that corridors are not enough to overcome the negative effect of being close to an edge. Our experimental habitat patches are fragments of restored longleaf pine savanna surrounded by a timber plantation matrix. Thus, plant and ant communities and their mutualisms are affected by shading caused by the densely spaced trees at patch edges. Past observational studies have also found strong effects of edges on the direction of seed dispersal and the distribution of surviving adult plants after dispersal (Ness 2004, Ness and Morin 2008), with areas in close proximity to an edge typically having fewer ant-dispersed plants than interior sites (Ness and Morin 2008, Warren II et al. 2015).

Taken together, our results demonstrate that increasing connectivity via corridors can positively impact seed dispersal by ants. It has long been known that corridors increase seed dispersal by birds at the patch scale (Tewksbury et al. 2002, Levey et al. 2005), and this work now demonstrates that this connectivity even impacts short distance seed dispersal events by ants. Given that ant-dispersed plants are globally widespread, the implications of this result suggest seed dispersing ants and myrmecochorous plants will benefit from restoring connectivity.

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Figures

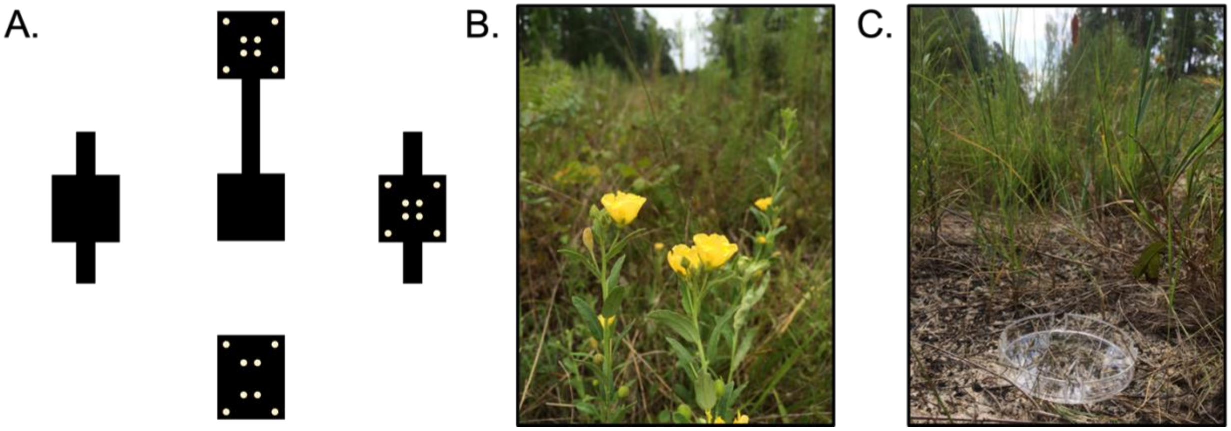


Figure 1. Experimental landscapes (A), focal species (B), and seed depot setup (C). In (A), circles indicate the potential locations of seed dispersal depot observations (4 locations per patch type). B. Photo of *Piriqueta cistoide*s. C. Seed dispersal depot (Photo credit: Melissa Burt).

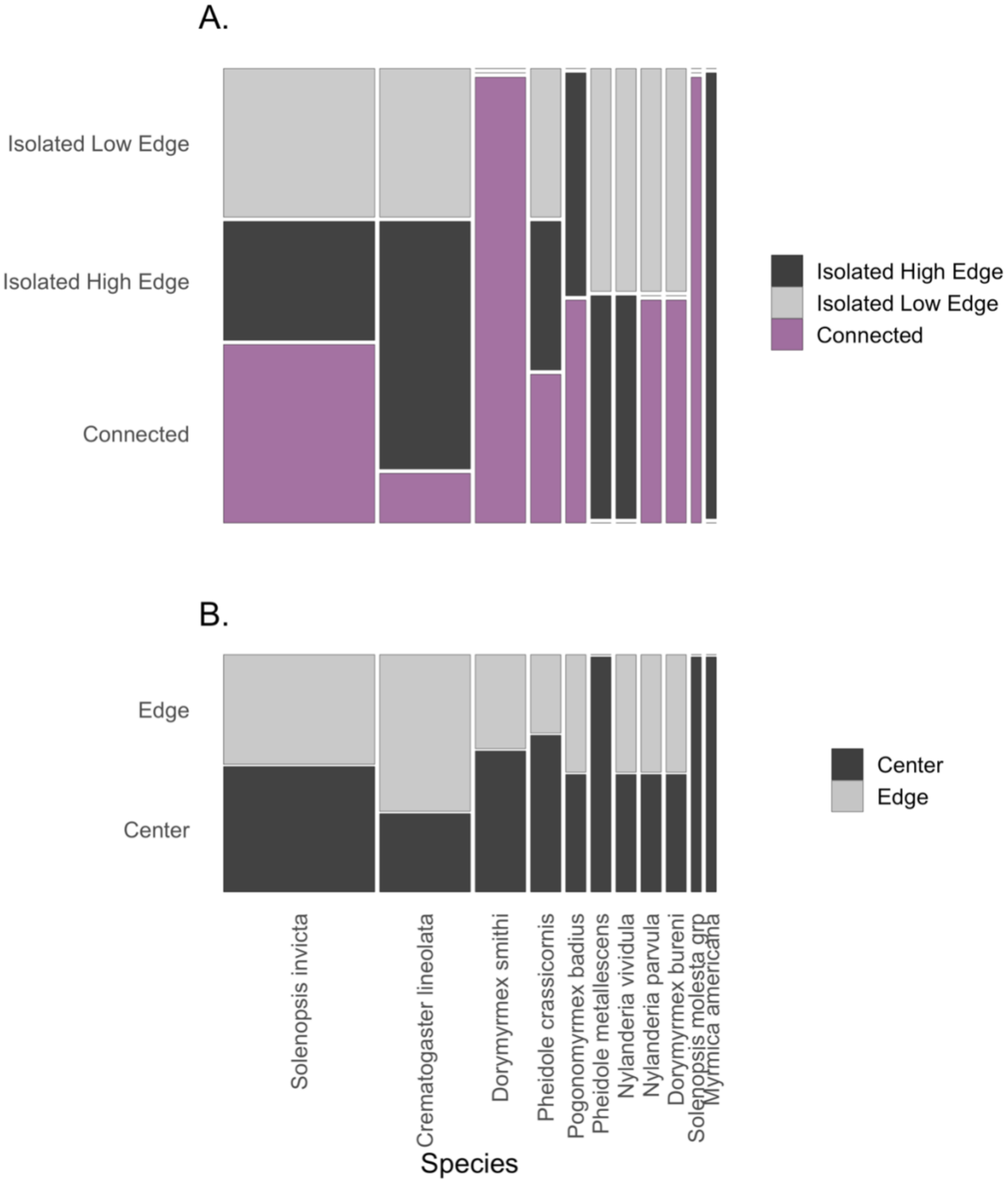


Figure 2. Mosaic plots showing frequency of seed depots visited by each seed dispersing ant species across patch types and edge proximities. Horizontal widths of bars indicate the relative abundance of each ant species, and the vertical height of bars indicate the relative abundance across patch type (A) and edge proximities (B). Single lines

indicate patch types and edge proximities where the species was not observed visiting seed dispersal depots.

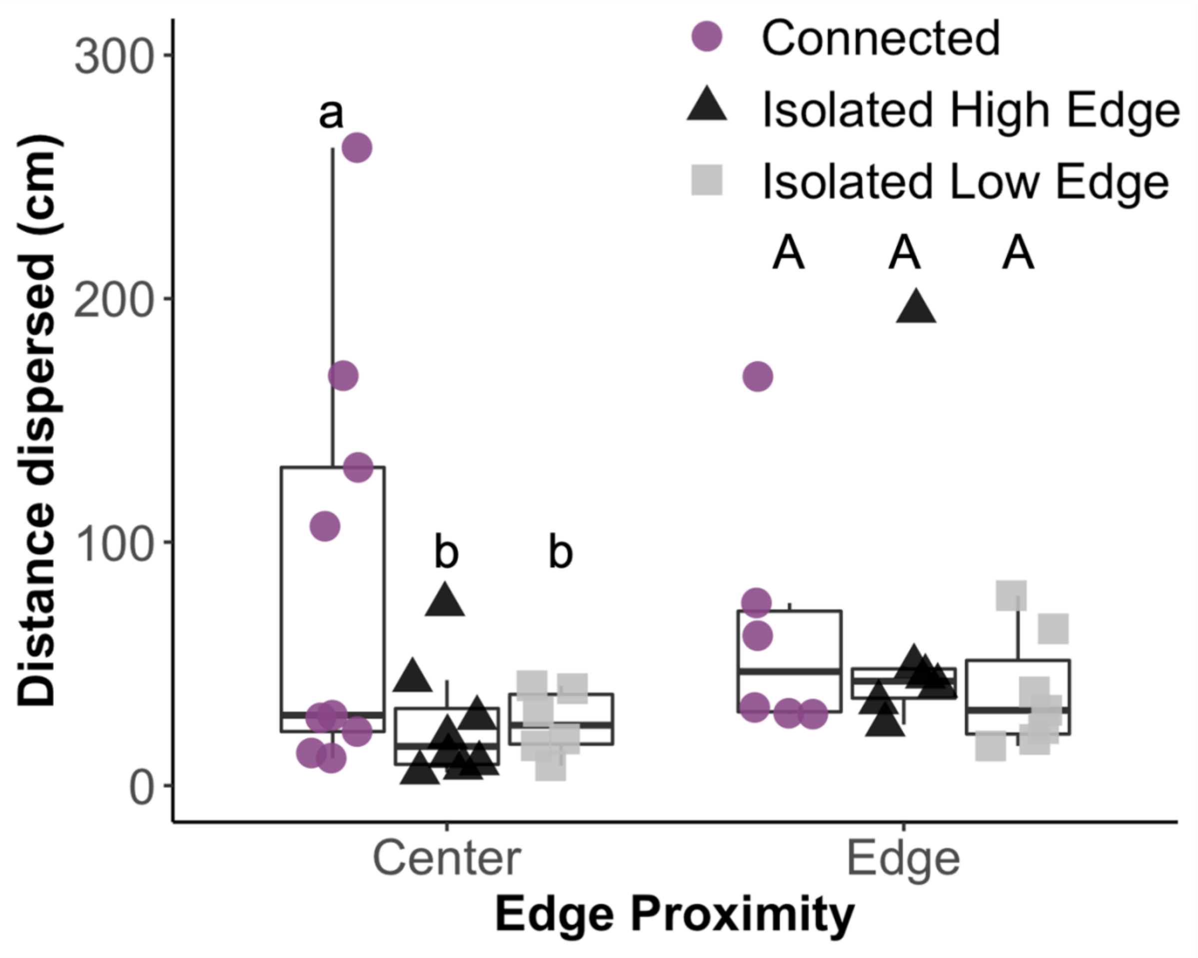


Figure 3. Distance seeds dispersed from dispersal depots. Patch type is designated by color and point shape: purple circles for connected, black triangles for isolated high edge, and gray squares for isolated low edge. Significant differences among patch types within edge proximity categories indicated by different letters.

Supplementary Materials for Chapter 3

Appendix S1

2016 Pilot Study

Methods

In August 2016, we conducted pilot observations of seed dispersal by ants in the same experimental landscapes where we conducted the 2018 seed dispersal observations. We measured the rate ants removed seeds with seed depots made with uncovered plastic petri dishes (10 cm in diameter, 1.5 cm depth), the same depots used in the 2018 experiment. Each petri dish had four ~1cm wide openings melted into the raised sides of the petri dish to facilitate ant movement in and out of the depot. In each block, we established two transects of four seed dispersal depots in each patch type (N = 8 depots/patch type/block) each containing 20 *P. cistoides* seeds. Within each patch type, the two transects were arranged such that two of the depots were located at the edge of the patch near the pine plantation matrix and two depots were located at the center of the patch. For each patch type within an experimental landscape, we conducted observations simultaneously with one observer in each patch type conducting the observations of transects at the same time. For each seed depot along a transect, we recorded the number of seeds removed every 16 minutes for 48 minutes. We also collected a specimen of the ants visiting seed depots for later identification under a microscope and recorded notes about their behavior within the seed depot. We only collected specimens if it was clear that it was not the first ant of a species discovering the seeds.

Statistical analyses:

Seed Removal To test for an effect of patch type, proximity to an edge, and their interaction on seed removal, we first summarized seed removal as the count of seeds removed and the percent seeds removed at each observation time point (i.e., at 16 minutes, at 32 minutes, and at 48 minutes). For seed count and percent seeds removed at each time point we used separate generalized linear mixed effects models with a negative binomial error distribution. In these models, patch type and edge proximity were fixed effects and patch nested within block was a random effect. We initially included the interaction between patch type and edge proximity for each of these models but removed that term when it was not significant.

Richness of Ants Dispersing Seeds To test for an effect of patch type and proximity to an edge on the number of ant species that visited seed depots, we used a generalized linear mixed effects model with a poisson error distribution. In these models, patch type and edge proximity were fixed effects and patch nested within block was a random effect. For each of these models, we initially included the interaction between patch type and edge proximity but removed that term when it was not significant.

Results

We observed a total of 14 different ant species dispersing seeds from seed depots in 2016 (Table S1). The most common species that removed seeds from depots were *Solenopsis invicta* (57% of seed depots) and *Crematogaster lineolata* (21% of seed depots).

We found no effects of patch type nor edge proximity on the number of ant species visiting seed dispersal depots or the number of seeds removed from seed dispersal depots for each time point during our observations (Fig. S1 and S2; Table S2).

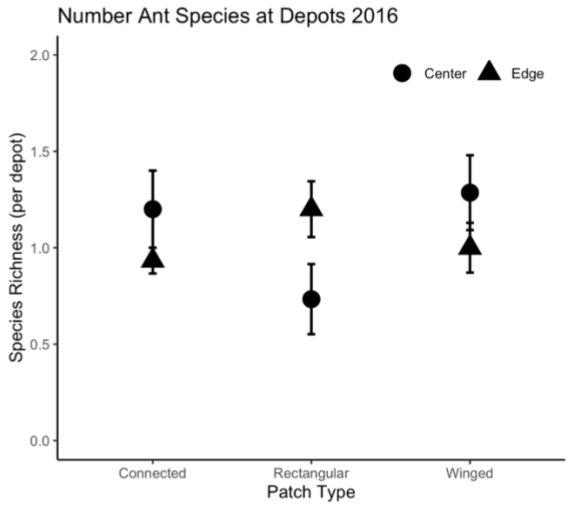


Figure S1. Ant species richness per depot in 2016. Points represent the mean number of ant species across seed depots per patch. Error bars show standard error around the mean.

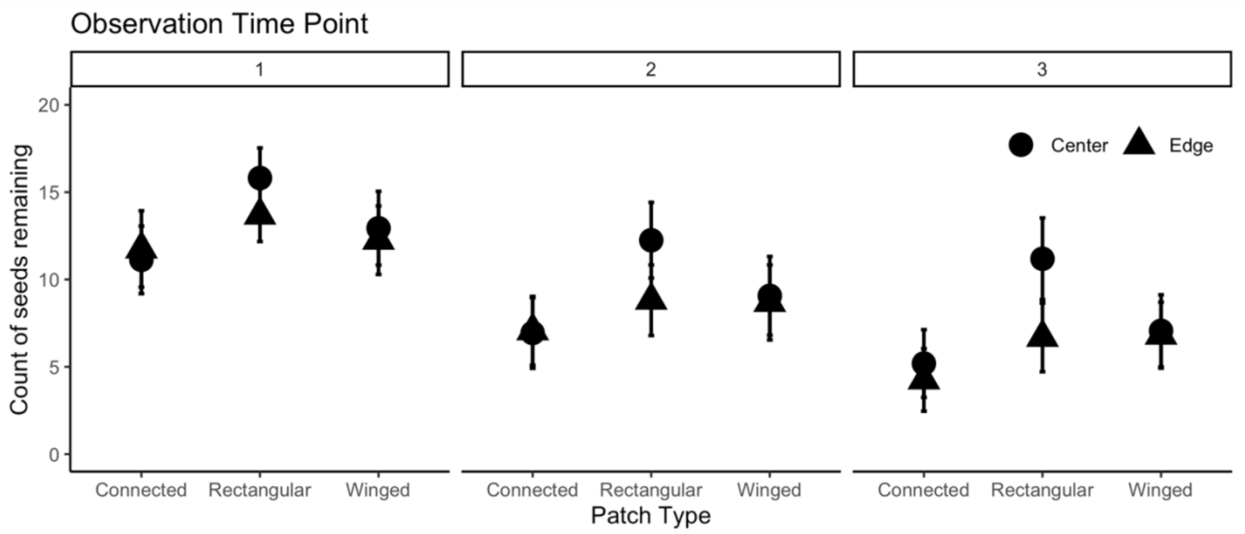


Figure S2. Count of seeds removed from seed depots at each time point. Points represent the mean number of ant species across seed depots. Error bars represent standard error around the mean.

Table S1. Composition of ant species visiting seed depots in 2016.

Species	**Count of Depots 2016**	**Proportion Depots 2016**
Solenopsis invicta	44	0.57
Crematogaster lineolata	16	0.21
Dorymyrmex smithi	7	0.09
Pheidole metallescens	5	0.06
Nylanderia parvula	4	0.05
Crematogaster ashmeadi	3	0.04
Forelius pruinosus	3	0.04
Pheidole crassicornis	3	0.04
Aphaenogaster floridana	2	0.03
Dorymyrmex bureni	2	0.03
Nylanderia vividula	2	0.03
Pogonomyrmex badius	2	0.03
Dorymyrmex grandulus	1	0.01
Trachymyrmex septentrionalis	1	0.01

Table S2. Results of generalized linear mixed effects models for 2016

Response Variable	**Fixed effect**	X^2	**df**	***p***
Ant Species Richness	Patch Type	0.15	2	0.93
	Edge Proximity	0.64	1	0.42
Count of Seeds Removed after 16 minutes	Patch Type	1.31	2	0.52
	Edge Proximity	0.06	1	0.80
Count of Seeds Removed after 32 minutes	Patch Type	1.46	2	0.48
	Edge Proximity	0.15	1	0.70
Count of Seeds Removed after 48 minutes	Patch Type	2.09	2	0.35
	Edge Proximity	0.36	1	0.55

Appendix S2

2018 Experiment Supplemental Results and Figures

Table S3. Composition of ant species visiting seed depots. Count of depots is the total number of seed depots in which a species visited and removed any number of seeds from the depot.

Species	Count of Depots				Proportion Depots Visited
	Total	Connected Patch	Isolated, High Edge Patch	Isolated, Low Edge Patch	
Solenopsis invicta	20	6	5	9	0.36
Crematogaster lineolata	11	1	5	5	0.20
Dorymyrmex smithi	5	5	0	0	0.09
Pheidole metallescens	2	0	1	1	0.04
Nylanderia parvula	2	1	0	1	0.04
Pheidole crassicornis	3	1	1	1	0.05
Dorymyrmex bureni	2	1	0	1	0.04
Nylanderia vividula	2	0	1	1	0.04
Pogonomyrmex badius	2	1	1	0	0.04
Solenopsis molesta grp	1	1	0	0	0.02
Myrmica americana	1	0	1	0	0.02

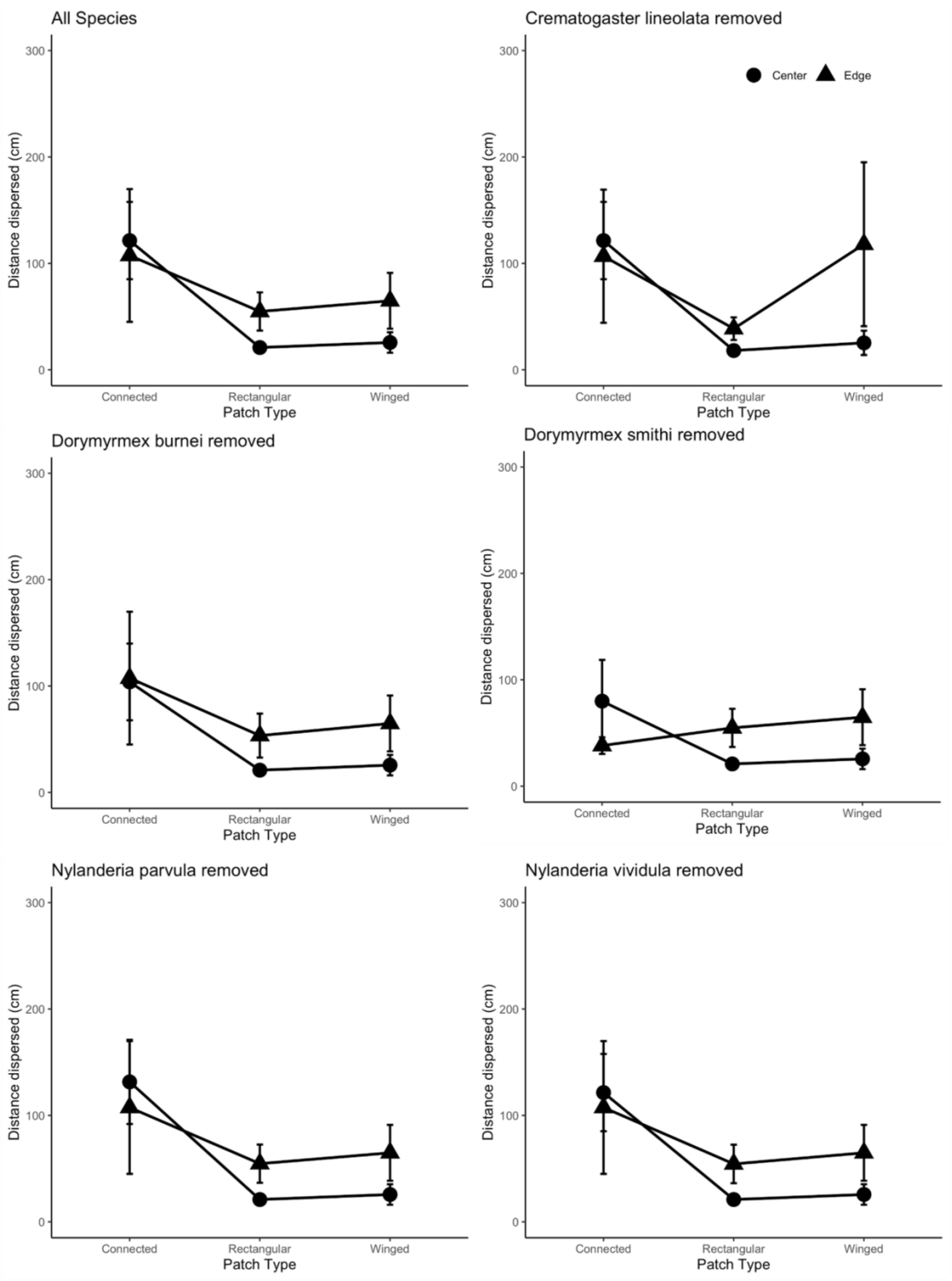
Table S4. Statistical analyses for 2018 myrmecochory assays.

Response Variable	**Distribution**	**Fixed effect**	X^2	**df**	**p-value**
Time to Discovery (sec); ln transformed	**Gaussian**	**Patch Type**	0.65	2	0.72
		Edge Proximity	0.62	1	0.43
		Patch Type: Edge Proximity	0.24	2	0.88
Count of Seeds Removed	**Negative binomial**	**Patch Type**	1.09	2	0.58
		Edge Proximity	0.04	1	0.83
		Patch Type: Edge Proximity	0.90	2	0.64
Species Richness	**Negative binomial**	**Patch Type**	0.82	2	0.66
		Edge Proximity	0.08	1	0.77
		Patch Type: Edge Proximity	0.55	2	0.76
Seed Dispersal Distance (All species; ln transformed)	**Gaussian**	**Patch Type**	9.46	2	**0.01**
		Edge Proximity	0.43	1	0.51
		Patch Type:Edge Proximity	5.48	2	**0.06**
Seed Dispersal Distance Center Depots Only (All species; ln transformed)	**Gaussian**	**Patch Type**	11.14	2	**0.004**
Seed Dispersal Distance Edge Depots Only (All species; ln transformed)	**Gaussian**	**Patch Type**	0.56	2	0.76

Sensitivity Analyses:

Results

When we conducted analyses with individual species removed, results were mixed. When removing infrequent visitors, such as *Myrmica americana*, *Nylanderia parvula*, *Nylanderia vividula*, *Pheidole crassicornis*, and *P. metallescens,* did not qualitatively change the patterns we found (Figure S3, Table S5). However, removing frequent visitors qualitatively changed seed dispersal patterns. We found that individually excluding *C. lineolata*, *D. bureni*, *P. badius*, and *S. invicta* caused the interaction term (Patch Type:Edge Proximity) to explain less variation in seed dispersal distance. In the model without *C. lineolata,* the significant patch type effect is driven by a difference between the connected and low edge isolated patch types, a comparison that confounds the effects of edge amount and isolation. In the model without *D. bureni* and *P. badius*, we found that ants dispersed seeds farther along patch edges than in patch centers, regardless of patch types. Conversely, in the model without *S. invicta*, we found that seed dispersal distance varied across patch types, but not across edge distances. This difference is driven primarily by a significant difference in seed dispersal distance between the connected and low edge isolated patch types ($p = 0.03$). Finally, when we removed *D. smithi* from analyses, we found a qualitatively similar pattern to the one we observed in the full dataset; however, the differences between patch types are less pronounced.


All Species
Crematogaster lineolata removed
● Center ▲ Edge
Dorymyrmex burnei removed
Dorymyrmex smithi removed
Nylanderia parvula removed
Nylanderia vividula removed
Distance dispersed (cm)
0
100
200
300
Connected
Rectangular
Winged
Patch Type

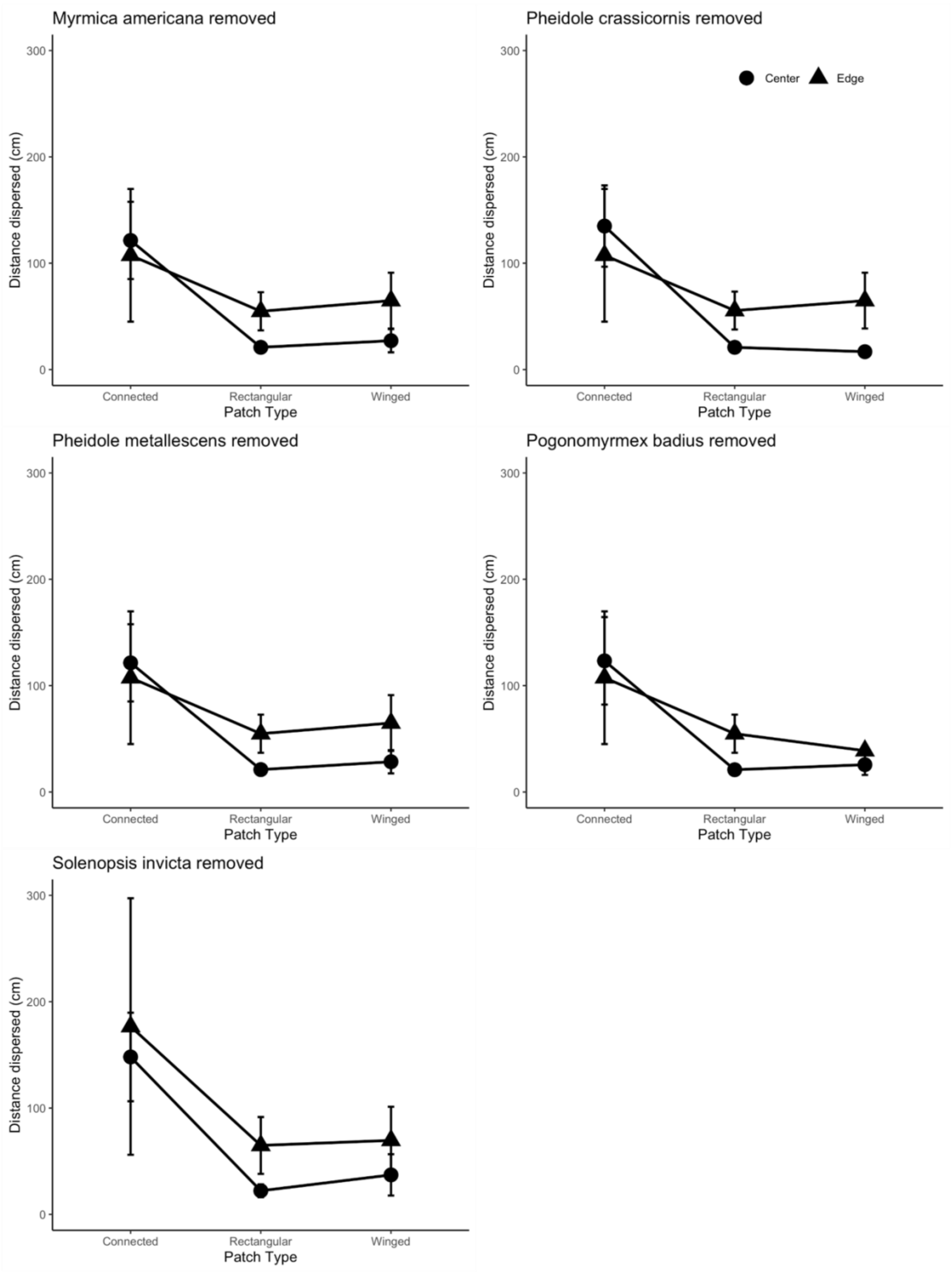


Figure S3. Distance seeds moved by ants. Top left panel: Mean distance moved of all distance observations. The other panels show the results of separate analyses conducted by removing individual species from dispersal distance analyses.

Table S5. Sensitivity of seed dispersal distance to ant species identity.

Response Variable	**Fixed effect**	X^2	**df**	**p-value**
All species; ln transformed	**Patch Type**	9.46	2	**0.01**
	Edge Proximity	0.43	1	0.51
	Patch Type:Edge Proximity	5.48	2	**0.06**
All species; ln transformed **Center plots only**	**Patch Type**	11.14	2	**0.004**
All species; ln transformed **Edge plots only**	**Patch Type**	0.56	2	0.76
Crematogaster lineolata **removed; ln transformed**	**Patch Type**	12.22	2	**0.002**
	Edge Proximity	0.66	1	0.42
	Patch Type: Edge Proximity	3.60	2	0.17
Dorymyrmex bureni **removed; ln transformed**	**Patch Type**	3.02	2	0.22
	Edge Proximity	6.44	1	**0.01**
	Patch Type: Edge Proximity	3.53	2	0.17
Dorymyrmex smithi **removed; ln transformed**	**Patch Type**	2.82	2	0.24
	Edge Proximity	5.44	1	**0.02**
	Patch Type: Edge Proximity	4.38	2	0.11
Dorymyrmex smithi **removed; ln transformed** **Center Only**	**Patch Type**	5.80	2	**0.06**

Dorymyrmex smithi **removed; ln transformed Edge Only**	**Patch Type**	0.54	2	0.76
Myrmica americana **removed; ln transformed**	**Patch Type**	9.23	2	**0.01**
	Edge Proximity	0.43	1	0.51
	Patch Type:Edge Proximity	5.30	2	**0.07**
Myrmica americana **removed; ln transformed Center Only**	**Patch Type**	10.63	2	**0.005**
Myrmica americana **removed; ln transformed Edge Only**	**Patch Type**	0.56	2	0.76
Nylanderi parvula **removed; ln transformed**	**Patch Type**	8.68	2	**0.01**
	Edge Proximity	0.39	1	0.53
	Patch Type:Edge Proximity	5.11	2	**0.08**
Nylanderi parvula **removed; ln transformed Center Only**	**Patch Type**	9.63	2	**0.008**
Nylanderi parvula **removed; ln transformed Edge Only**	**Patch Type**	0.57	2	0.75
Nylanderia vividula **removed; ln transformed**	**Patch Type**	9.42	2	**0.01**
	Edge Proximity	0.43	1	0.51
	Patch Type:Edge Proximity	5.29	2	**0.07**
Nylanderia vividula **removed; ln transformed Center Only**	**Patch Type**	11.14	2	**0.004**

Nylanderia vividula **removed; ln transformed** **Edge Only**	**Patch Type**	0.59	2	0.74
Pheidole crassicornis **removed; ln transformed**	**Patch Type**	25.9	2	**2.348e-06**
	Edge Proximity	1.06	1	0.30
	Patch Type:Edge Proximity	8.53	2	**0.01**
Pheidole crassicornis **removed; ln transformed** **Center depots only**	**Patch Type**	28.1	2	**8.09e-07**
Pheidole crassicornis **removed; ln transformed** **Edge depots only**	**Patch Type**	0.52	2	0.77
Pheidole metallescens **removed; ln transformed**	**Patch Type**	8.75	2	**0.01**
	Edge Proximity	0.40	1	0.53
	Patch Type:Edge Proximity	4.90	2	**0.09**
Pheidole metallescens **removed; ln transformed** **Center Only**	**Patch Type**	10.87	2	**0.004**
Pheidole metallescens **removed; ln transformed** **Edge Only**	**Patch Type**	0.56	2	0.76
Pogonomyrmex badius **removed; ln transformed**	**Patch Type**	4.17	2	0.12
	Edge Proximity	4.26	1	**0.04**
	Patch Type: Edge Proximity	4.04	2	0.13
Solenopsis invicta	**Patch Type**	9.96	2	**0.007**

removed; ln transformed	**Edge Proximity**	2.08	1	0.15
	Patch Type: Edge Proximity	1.10	2	0.58

Chapter 4 – Positive effects of multivariate climate change on seed dispersal by ants: a mesocosm approach

Authors: Melissa A. Burt, Annika S. Nelson, Charles Kwit, and Susan R. Whitehead

Abstract

Ongoing anthropogenic climate change is drastically restructuring ecosystems. Yet, understanding these impacts is challenging because climate shifts are predicted to be highly multidimensional. Mutualisms may be especially impacted by climate change owing to their highly context dependent nature, yet most climate change experiments have focused solely on temperature. Here, we used a greenhouse-based mesocosm experiment in which we manipulated temperature and precipitation to determine the effects of multidimensional climate change on ant seed dispersal mutualisms. We specifically asked how warming and precipitation affected the number of seeds dispersed, the re-dispersal of seeds out of ant nests after initial collection, and the distance that seeds were dispersed. Initially, we found an effect of warming on seed removal by ants that depended on the precipitation treatment. While ants removed more seeds from ambient than warmed treatments in the historical precipitation treatment, they removed more seeds from warmed than ambient mesocosms in the higher magnitude, less frequent precipitation treatment. Initial differences in seed dispersal between treatments equalized over 24 hours, indicating that multidimensional climate change may alter mutualisms by shifting the timing rather than the magnitude or variance of interactions. Overall, these findings

imply that the responses of species interactions to climate change may be contingent upon concurrent changes in multiple climate factors.

Introduction

Globally, ecosystems are already seeing the effects of human-caused climate change, yet predicting the consequences for organisms and interactions among species is challenging because shifts to climate are expected to be highly multidimensional (IPCC 2018, 2022, Malhi et al. 2020). Alongside shifts in mean conditions, climates are expected to become more variable, with extreme weather events occurring more frequently (Easterling et al. 2017, IPCC 2018, 2022). Our ability to predict the cascading impacts of these complex changes to climate remains hindered by a lack of controlled experiments that incorporate the multifactorial nature of climate change and its effects on organisms and communities (Beier et al. 2012, Kreyling and Beier 2013, Korell et al. 2020).

In addition to the challenges associated with simultaneous changes to multiple climate factors, ecological communities themselves are multidimensional. Individual species may engage in complex interactions within communities where the abiotic environment is not the only factor driving their ability to adapt to changing conditions (Ockendon et al. 2014, Bairey et al. 2016, Chomicki et al. 2019, Hale et al. 2020, Åkesson et al. 2021). Mutualisms, interactions in which both partners benefit because of their interaction (Bronstein 2015), are potentially especially at risk because of the differences among individual species' responses to climate change (Tylianakis et al. 2008, McConkey et al. 2012, Travis et al. 2013, Northfield and Ives 2013, Jamieson et al. 2017). However, predicting the outcomes of mutualisms under climate change is difficult because the basic mechanisms that drive mutualisms are not always well understood (Vidal et al. 2021). The coevolution of mutualists under common environmental conditions may mean that species engaged in mutualisms will respond similarly to changing environmental conditions (Northfield and Ives 2013). However, diverging responses of partners may result in the breakdown of these interactions (e.g., Hoegh-Guldberg et al. 2007, Aslan et al. 2013, Miller-Struttmann et al. 2015). An improved mechanistic understanding of mutualisms will be essential for predicting community and ecosystem-level responses to climate change (Urban et al. 2016, Åkesson et al. 2021).

The dispersal of seeds by ants, also known as myrmecochory, is a critical mutualism that shapes plant populations and community dynamics (Lengyel et al. 2009, Zelikova et al. 2011, Prior et al. 2020). Seeds of plants that are ant-dispersed typically have a fleshy lipid-rich appendage on the outside of their seeds called an elaiosome. Ant seed dispersal behavior is driven by both nutritional rewards and chemical cues produced within the seeds and elaiosomes with fatty acids, especially oleic acid, driving their behavior (Kusmenoglu et al. 1989, Nelson et al. 2019, Miller et al. 2020, Nelson and Whitehead 2021). After collecting seeds from myrmecochorous plants, ants consume the elaiosome and either store seeds within their nest or re-disperse them to aboveground trash middens (Beattie 1985). Given that ants are ectotherms, changes to thermal conditions as a result of climate change may alter their seed dispersal behavior (Zelikova et al. 2008, Pelini et al. 2011, Stuble et al. 2014). However, impacts to seed dispersal may depend on the range of a species' thermal tolerances (Stuble et al. 2013, Roeder et al. 2021). Additionally, ultimate outcomes of seed dispersal by ants under anthropogenic climate change may depend on tradeoffs between thermal tolerance of species and desiccation resistance (Bujan et al. 2016, Parr and Bishop 2022).

In eastern deciduous forests of North America, approximately 30% of plant species in the understory are dispersed by ants (Beattie and Culver 1981, Warren II et al. 2021). Although ants are typically moving seeds an average of just 0.5 meters, their dispersal services are a key factor shaping the distribution of plants within populations and communities (Ness et al. 2009, Canner et al. 2012). Changes to climate within eastern deciduous forests are predicted to bring changes to two major climate axes, temperature and precipitation, which are expected to shift significantly in coming decades in magnitude and variability. Mean annual temperatures in eastern North America are expected to rise by ~3°C by 2100, with simultaneous increases of up to 10% in mean annual precipitation (Easterling et al. 2017, Vose et al. 2017, IPCC 2018, 2022). Although previous studies have examined the potential effects of climate change on ant activity and seed dispersal, most have only focused on temperature (Parr and Bishop 2022). These studies focused on warming temperatures have found variable effects with some finding positive effects (Deutsch et al. 2008, Zelikova et al. 2008, Boukal et al. 2019), no effects (Stuble et al. 2014), or even negative effects of warming (Pelini et al. 2011). Given that both the ant and plant partners participating in myrmecochory are both affected by temperature and moisture, experiments factoring in more realistic climate change scenarios will provide insights into the mechanisms that structure ant-mediated seed dispersal now and in the future. The small spatial scale of ant seed dispersal interactions makes it an ideal model mutualism for experiments that are investigating the effects of climate change because both partners fit within the confinement of small scale mesocosms, balancing the need for experimental control of conditions and realism (Prior et al. 2014a, Meadley-Dunphy et al. 2019, Prior et al. 2020).

Here we present results from a mesocosm experiment in which we crossed temperature treatments with altered precipitation treatments to investigate the effects of multidimensional climate change on the seed dispersal mutualism between a wildflower native to eastern deciduous forests, *Sanguinaria canadensis* (bloodroot), and their seed dispersing ants, *Aphaenogaster rudis* (winnow ants). For our first objective, we assessed the impact of warming and altered precipitation patterns on seed removal and re-dispersal by ants. Second, we investigated whether changes to overall ant activity underlie changes to their seed dispersal services. Finally, we explored whether changes to seed dispersal activity by ants had consequences for the distance seeds were dispersed. We predicted that warming would increase rates of ant-mediated seed dispersal by increasing ant foraging behavior. Ant foragers should be more active in warmed treatments relative to ambient treatments because warmed temperatures fell under reported thermal maxima of *A. rudis* (Stuble et al. 2013, Warren II and Chick 2013). We also predicted that because ants are intolerant to dry conditions, that foragers would be less active and disperse fewer seeds in decreased frequency precipitation treatments relative to treatments with a higher frequency of precipitation events.

Materials and Methods

Study System

Our experiment focused on the seed dispersal mutualism between two common and co-occurring native species in eastern deciduous forests, *Sanguinaria canadensis* (bloodroot; Papaveraceae) and ants in the *Aphaenogaster rudis-picea* complex (winnow ants, hereafter *A. rudis;* Formicidae). *Aphaenogaster rudis* is considered a keystone seed disperser in eastern deciduous forests, with ~30% of understory plants dispersed by ants and ~74% of all ant seed removal events attributed to *A. rudis* (Beattie and Culver 1981, Zelikova et al. 2008, Ness et al. 2009, Warren II et al. 2021). *Sanguinaria canadensis* is one of the earliest emerging herbaceous perennials, producing conspicuous white flowers between March to April. The fruits are elongated capsules that split open when ripe to reveal 20-40 seeds, each with a fleshy, lipid-rich elaiosome. All parts of the plant, but especially the rhizomes and seeds, are rich in isoquinoline alkaloids that confer the bright orange-red color that gives bloodroot its name (Bennett et al. 1990, Salmore and Hunter 2001, Campbell et al. 2007). These compounds provide defense against insect herbivores and pathogens (Marshall et al. 1979, Skidmore and Heithaus 1988, Gunther and Lanza 1989). As with other myrmecochorous plant species, the elaiosomes of *S. canadensis* are also rich in oleic acid and a diversity of other fatty acids that attract ants (Kusmenoglu et al. 1989). Removal of elaiosomes on *S. canadensis* seeds dispersed by ants has been found to increase their germination rates (Lobstein and Rockwood 1993), and dispersal by ants has important consequences for *S*. *canadensis* population densities and spatial distributions (Pudlo et al. 1980).

Mesocosm Construction and Experimental Design

Our mesocosm experiment took place in the greenhouses managed by the Department of Biological Sciences at Virginia Tech (Blacksburg, VA, USA). In late spring 2023, we constructed mesocosms in plastic children's pools (diameter = 0.82 m) similar to those used in previous studies (Prior et al. 2014b, 2020, Meadley-Dunphy et al. 2019). To allow for drainage, we drilled 0.25-in holes in the bottom of each mesocosm and added a layer of mosquito netting polyester fabric to the inside of each pool to prevent ants from escaping from out of the bottom of the mesocosm. Each pool was filled with 6 cm of a mixture of 60% topsoil, 30% compost, and 10% sand purchased and sourced locally (American Mulch & More, Christiansburg, VA) with a 5 cm layer of 100% sand on the bottom to aid in drainage. Each mesocosm contained three *S. canadensis* individuals that we planted along a line through the center of each mesocosm and a colony of *A. rudis* housed in a wooden nest box placed along the edge of the mesocosm equidistant from each plant (see "Focal species collection" below for more details). To limit direct sun exposure, 50% Shade cloth was suspended above the mesocosms.

In the greenhouse, mesocosms were arranged in blocks of six (7 total blocks) with mesocosms within each block randomly assigned to temperature and precipitation treatments. We arranged the mesocosms in blocks to account for any variation in temperature in the greenhouse. We assigned each mesocosm to one of six treatment combinations in a randomized, crossed design with seven replicates per treatment combination (N = 42 mesocosms total; Fig. 1). Treatments included two levels of warming: 1) ambient, and 2) warmed to ~3°C above ambient conditions; these were crossed with three levels of precipitation: 1) historical regime: precipitation events based on an average year from historical records (1950-1990), 2) increased mean: frequency of events as in (1), but with 10% increase in total precipitation for each precipitation event, 3) increased mean and decreased frequency: same total amount of precipitation as in (2), but with 50% fewer precipitation events. To decrease precipitation frequency while keeping the same total precipitation as the increased mean treatment, we combined sets of two precipitation events that occurred adjacent to one another in time (e.g., two events in one week of 12mm and 14mm rain would combine to a single event of 26mm), similar to the approach in Knapp et al. (2008). This means that over the course of the experiment both the increased magnitude treatments and the decreased magnitude and decreased frequency treatments received the same total amount of precipitation over time. We began precipitation and warming treatments on 01 May 2023.

We chose these specific treatments to match predictions within the overlapping ranges of *S. canadensis* and *A. rudis* in eastern temperate forests. Here, climate models predict an increase of about 3°C above historical mean annual temperatures by 2050 as well as an increase to the magnitude of annual precipitation up to ~10% above historical means, but a decrease in the frequency of individual rain events (i.e., more rain annually that will fall as less frequent, higher magnitude precipitation events) (Wang et al. 2016, Easterling et al. 2017, IPCC 2018). Our historical precipitation regime was based on the historical climate data for our region available from the National Oceanic and Atmospheric Administration's (NOAA) National Centers for Environmental Information. We calculated annual precipitation, as well as the mean and variation in event size and frequency within seasons (Spring: March-May, Summer: June - August, Fall: September - November, and Winter: December-February) over a period from 1950-1990. Then, for each season, we randomly chose a year that was within one standard deviation of the mean for both magnitude and frequency of precipitation events and based our historical precipitation treatment on the records for those years (Spring: 1959, Summer: 1984, Fall: 1981, and Winter: 1976; precipitation schedule Table S1). For precipitation treatments, we added water at the predetermined quantity and frequency with a watering can that had a low flow rose.

To achieve ~3°C of warming above ambient mesocosms, we warmed mesocosms with ceramic bulbs, controlling temperature with dimmer switches. We hung two ceramic warming lamps ~ 54 cm above the center of each warmed mesocosm, with ambient mesocosms receiving the same experimental setup (i.e. lamp with no bulb) to account for any potential shading caused by the lamp.

Focal Species Collection

We collected *S. canadensis* individuals after they had flowered and were beginning to develop fruit from three different local naturally occurring populations in and near Blacksburg, VA: Graham Farm (Elliston, VA), Bishop Road (Blacksburg, VA), and Floyd County (Floyd, VA). All populations were located on private land and were collected with landowner permission.

We collected whole ant colonies from Mill Creek Springs Natural Area Preserve with permits from the Virginia Department of Conservation and Recreation and The Nature Conservancy. To collect colonies, we used wooden nest boxes made with two 20cm x 9cm x 1.8cm pieces of untreated pine lumber (Lubertazzi 2012). One of the two pieces of wood contained a U-shape engraved to 1.3cm deep and 1cm wide connected to the outside edge with a 0.3 cm deep and 1 cm wide engraving. The two pieces of wood were screwed together with the u-shaped engraving on the inside. Nest boxes were placed at least 10 meters apart in a grid at Mill Creek Springs Natural Area Preserve during the Fall of 2022 and then were collected on 5/2/2023, 5/4/2023, and 5/6/2023. Prior to adding the ant colonies to our mesocosms, we counted the number of workers and assessed whether colonies contained a queen, eggs, larva, and pupa. Within each block of mesocosms, we added colonies that were similar in initial size to control for potential variation due to initial colony size. Additionally, we culled each colony to 300 workers so that each colony started at the same size. For one block of mesocosms, we culled colonies to 270 workers because three of the colonies had fewer than 300 workers initially. We transferred one colony to each mesocosm within the nest box, leaving the nest box in the mesocosm for the entirety of the experiment. Each ant colony in each mesocosm was given plain water, 20% sugar water, ~5 g ant diet (recipe modified from Bhatkar and Whitcomb 1970, Dussutour and Simpson 2008, Lash et al. 2020; instructions for recipe in supplement), and mealworms 1-2 times per week.

Seeds for seed dispersal assays

From late May through early June 2023, we collected seeds from the *S. canadensis* individuals that were planted in the mesocosms for use in the bioassays described below. Since *S. canadensis* seed pods open upon full development, spilling their seeds on the ground, we placed mesh bags on each seed pod for seed collection prior to seed dispersal assays. We collected seed pods only after they had dehisced within the mesh bags. After collection, seeds were stored in a refrigerator at ~4°C prior to assays to prevent desiccation. Transplanted plants from the Graham Farm and Bishop Road populations produced an average of 15.0 +/- 7.2 and 7.5 +/- 6.3 seeds per pod, respectively. The plants from Floyd County produced no seeds. We aimed to conduct seed dispersal assays at the location of each transplanted *S. canadensis* with seeds from the plant at that location, so we supplemented with seeds collected from Mill Creek Springs Natural Area Preserve and Wildwood Park (Radford, VA) when necessary. For the Graham Farm and Bishop Road populations, we used 10 and 8 seeds for seed dispersal assays, respectively (17% of plants required supplemental seeds for the Graham Farm population and 48% of plants required supplemental seeds for the Bishop Road population). All assays near Floyd County plants were performed with supplemental seeds with 10 seeds per seed dispersal assay. We report the total seeds produced per seed pod prior to subsetting for seed dispersal assays in the supplemental materials.

We collected supplemental *Sanguinaria canadensis* seeds from Mill Creek Springs Natural Area Preserve with permits from the Virginia Department of Conservation and Recreation and The Nature Conservancy. Seeds from Wildwood Park were collected with permission from Radford Parks and Recreation.

Seed dispersal assays

We ran three separate experimental rounds of seed dispersal assays within each mesocosm, one for each of the three *S. canadensis* parent plants collected from different populations. We conducted assays when all seed pods had opened for plants in each population across all mesocosms. We found variation in the timing of seed pod development across populations such that the Graham Farm seed pods developed more quickly than the Bishop Road population. Thus, the Graham Farm's population seed dispersal assays started 06/01/2023, the Floyd County population's seed dispersal assays (with seeds from Mill Creek Springs Natural Area Preserve, see above for explanation) started 06/06/2023, and Bishop Road's population seed dispersal assays started 06/09/2023. Prior to use in the seed dispersal assays, we placed a dot of fluorescent paint on each seed with a randomly assigned separate color assigned to each plant's seeds within each mesocosm (i.e., paint color was randomly assigned to a plant's seeds for each mesocosm such that each plant's seeds within a mesocosm had a different color). This aided in seed recovery after re-dispersal and allowed us to separate seed identity by plants after re-dispersal.

For each assay, we placed seeds in a seed depot near the base of the corresponding parent plant. We made seed depots out of 85mm petri dishes with four 1-cm melted openings placed at equal distances around the dish to aid ants in entering and exiting. We observed seed removal from the seed depots by ants every hour for 6 hours after placing seeds into each mesocosm and then again at 24 hours after seed placement. At 24 hours, remaining seeds in the depots were dumped into the mesocosm at the location of the depot (i.e., the base of the plant). At each observation we counted the number of seeds remaining in the seed depot, the number of ants out of the nest box in the mesocosm, and the temperature of the soil surface near the seed depot with an infrared thermometer. At 24 hours we also measured volumetric water content (%) with a Fieldscout TDR 150 soil moisture meter.

At 48, 72, and 96 hours after seed depots were deployed to mesocosms, we measured re-dispersal of seeds by ants out of their nests into the mesocosms marking each seed with a plastic toothpick where the color of the toothpick corresponded to the color of paint marked on the seeds. We measured the distance between the re-dispersed seeds and both the ant nest box and the location of the seed depot.

<u>Mesocosm abiotic variable measurements</u>

We measured temperature in each mesocosm in two ways. First, we used Mouser Electronics DS1921G-F5 iButton thermochron temperature data loggers to measure temperature every 15 minutes. Data loggers were shielded in a ~10cm length x ~1.9cm diameter PVC pipe, and one data logger was placed at the center of each mesocosm

(Maclean et al. 2021). Second, prior to each precipitation event we measured ground surface temperature (°C) at each planted *S. canadensis* with an Etekcity infrared thermometer. Prior to each precipitation event, we also measured soil volumetric water content (%) with a Fieldscout TDR 150 soil moisture meter fitted with 3in (7.62cm) probes. When precipitation events occurred with longer intervals between events, we supplemented our usual soil moisture and surface temperature measurements with additional measurements in between precipitation events.

Statistical analyses

We performed the following analyses in R version 4.3.1 (R Core Team 2023). For hypothesis testing for the fixed effects for all models, we used the "Anova'' function from the "car" package to perform Wald Chi Square tests using type III tests when the interaction term was significantly different and type II tests when it was not (Hector et al. 2010, Fox and Weisberg 2019). For all models we used the 'emmeans' function from the 'emmeans' package to perform post-hoc tests to assess differences among factor levels for models with significant predictor variables (Lenth 2020).

To investigate the effectiveness of our experimental treatments during seed dispersal assays, we used general linear mixed effects models to determine the effects of experimental treatments on air temperature, ground temperature, and soil moisture measured during the seed dispersal assays. In these models we included temperature treatment, precipitation treatment, the interaction between temperature treatment, and assay round as fixed effects in the model and mesocosm nested within block as random effects. To understand how experimental treatments affected these variables between May through June of 2023, we performed separate general linear mixed effects models with temperature treatment, precipitation treatment, the interaction between temperature treatment and precipitation treatment, and time as fixed effects. We specified mesocosm within block as random effects. Here we used time as a fixed effect instead of within the structure of the random effect because models with time as a random effect had a singular fit.

To investigate the effects of our experimental treatments on ant mediated seed dispersal, we assessed treatment effects on the proportion of the initially placed seeds removed from the seed depots at 6 hours and 24 hours and the proportion of initially placed seeds re-dispersed from the ant colony's nest into the mesocosms by 96 hours after seed depot placement at the start of each assay. We used separate generalized linear mixed effects models (GLMMs) for each of these response variables. All models included temperature treatment, precipitation treatment, the interaction between temperature treatment and precipitation treatment, and experimental round as fixed effects. Additionally, we included mesocosm nested within block specified as random effects. We include assay round within the fixed effects terms in these models and not as a random effect because we only had three different assay rounds in our experiment. For each response variable, the proportion of seeds is summarized at the level of the seed depot with three separate seed depots for each mesocosm (3 seed dispersal depots x 42 mesocosms = 126 seed dispersal depots). For each of these models we used a quasi-binomial family because models with a binomial distribution had overdispersed residuals. To specify these models, we used the 'glmmPQL' function from the 'MASS' package (Venables and Ripley 2002). When the interaction between temperature treatment and precipitation treatment was significant, we performed additional generalized mixed effects models on our data first subsetted by temperature treatment and then subsetted by precipitation treatment. These models were specified with the same random effect structure.

To assess the effects of treatments on the rate of seed removal from between 1 and 24 hours after seed depot placement in mesocosms, we also fit mixed effects Cox proportional hazards models. As with the GLMMs, we specified temperature treatment, precipitation treatment, the interaction between temperature treatment and precipitation treatment, and round of assay as fixed effects and mesocosm nested within block as random effects. We used the 'coxme' function from the 'coxme' package to specify these models (Therneau 2024). If the interaction between temperature treatment and precipitation treatment were significant, we performed additional mixed effects Cox proportional hazards models on our data subsetted by precipitation treatment and temperature treatment as with our GLMMs above.

To investigate the effects of experimental treatments on dispersal distance from seed depot, dispersal distance from the ant colony nest box, and the number of ants out of the nest box during seed dispersal observations we used general linear mixed effects models fit with the 'lmer' function from the 'lme4' package (Bates et al. 2015). We summarized the distance seeds were dispersed by ants from the nest box and the seed depot in two ways. First, we averaged dispersal distance at the level of the seed depot across all seeds in each mesocosm including non-dispersed seeds as dispersed 0 cm. Second, we averaged the distance for seeds that had been dispersed excluding non-dispersed seeds. We summarized ant activity by averaging the number of ants active outside the nest box across observations between 1 to 24 hours post-deployment of seeds into the mesocosms for each plant population. As with the models above, for each response variable we started with a model that had temperature treatment, precipitation treatment, the interaction between temperature treatment and precipitation treatment, and round of assay as fixed effects and mesocosm nested within block as random effects. If a singular fit occurred, we simplified the random effect term in the model to block and removed the mesocosm term (Bolker et al. 2009). Additionally, if model residuals did not meet assumptions, we log transformed variables to improve normality and homoscedasticity.

In each of our models we specified assay round as a fixed effect because we found that this term accounted for a significant proportion of the variation within the model. However, because populations were not randomly assigned to assays across rounds of seed dispersal assays, this factor confounds population identity of the plants with time. We are thus unable to distinguish if plant population identity effects are due to actual differences among plant populations or differences in abiotic factors over time or some other unmeasured variable changing over time. Thus, we do not perform post hoc tests to interpret differences across rounds.

Results

Effects of temperature and precipitation treatments on abiotic variables within mesocosms.

We found differences in surface temperature across temperature treatments demonstrating that our warming treatments worked as well as temporal variation across the periods when each assay was conducted (Table S1, Fig. 2). Temperatures were 3.1 +/- 0.2°C higher in warmed treatments than ambient treatments (t =-14.03, p = <0.0001).

Surface temperatures were lower during round 1 of seed dispersal assays than round 2 (t = 7.75, p = <0.0001) and round 3 of assays (t = 8.25, p = <0.0001). Additionally, surface temperature was lower during round 3 of assays than round 2 of assays (t = 6.64, p = <0.0001). For soil volumetric water content measured during seed dispersal assays, we also found a significant effect of temperature treatment, precipitation treatment, and assay round (Table S1, Fig. 2). Soil volumetric water content was ~21 percent lower in warmed than ambient temperature treatments (t = 4.76, p = <0.0001). Additionally, soil volumetric water content was ~17% lower in increased magnitude, decreased frequency precipitation treatments than historical precipitation treatments (t = 2.48, p = 0.05) during seed dispersal assays. Soil volumetric water content was not significantly different across other pairs of precipitation treatments (p > 0.05). We also found similar patterns among treatments in abiotic variables when we expanded our analyses to the abiotic data collected between May to June. We present these results in the supplemental materials.

Warming and precipitation effects on initial seed removal

At 6 hours after deployment of seed depots into mesocosms we found the proportion of seeds dispersed by ants depended on the interaction between temperature and precipitation treatments and the round of assay (Table 1, Fig. 3A). When we performed GLMMs across precipitation treatments separately for each level of temperature treatment, precipitation treatments only differed within warmed temperature treatments, such that the proportion of seeds removed from seed depots after 6 hours was about 2.08 times greater for increased magnitude, decreased frequency precipitation treatments than historical precipitation treatments (t = -2.89, p = 0.3). When we performed separate GLMMs across temperature treatments separately for each level of precipitation treatment, we found that the proportion of seeds dispersed 6 hours after the start of seed dispersal assays differed for historical precipitation treatments and increased magnitude decreased frequency treatments, but not for increased precipitation magnitude treatments. For historical precipitation treatments, ants dispersed about 2.3 times the proportion of seeds in ambient treatments than warmed treatments, however for increased magnitude decreased frequency precipitation treatments, ants dispersed ~2.3 times the proportion of seeds in the warmed temperature treatments than in the ambient temperature treatments. We found similar patterns in our mixed effects Cox proportional hazards models and present those results in the supplement.

Treatment effects on ant activity across mesocosms during seed dispersal assays

During initial dispersal of seeds between 1- and 24-hours post-deployment of seed dispersal depots, we found effects of temperature treatment, but not precipitation treatment on ant activity measured as the average number of ants out in the mesocosms during observations (Table 1, Fig. 4). We found that the average number of ants across observations was greater in ambient than warmed treatments ($t = 2.38$, $p = 0.02$).

No effects of warming or precipitation treatments on re-dispersal of seeds out of ant nests

We found that temperature treatment, precipitation treatment, and their interactions had no effects on the proportion of seeds re-dispersed after initial removal by ants (Table 1, Fig. 5C).

Marginal effects of precipitation treatment on seed dispersal distance

For each way we measured dispersal distance, we found that precipitation treatment only marginally affected distance dispersed from the ant colony nest box when we included seeds not dispersed (Table 2, Fig. 5A-B). We also found evidence of a marginal effect of precipitation treatment such that seeds were dispersed further away from the ant colony nest boxes in historical precipitation treatments than increased magnitude treatments (t = 2.16, p = 0.08).

Discussion

Warming temperatures and altered precipitation associated with human-caused climate change are expected to have wide reaching impacts on species interactions, especially mutualisms (Kiers et al. 2010, Magnoli et al. 2023). With our mesocosm experiment that crossed temperature and precipitation treatments, we investigated the potential effects of multidimensional climate change on ant seed dispersal mutualisms between the myrmecochorous plant, *S. canadensis*, and its seed dispersing ant, *A. rudis*. We found partial support for our predictions that warming would increase ant seed dispersal, however this effect of warming depended on the precipitation regime (Fig. 3). *A. rudis* dispersed greater than double the proportion of seeds under ambient than warmed conditions when they received precipitation at a historical magnitude and frequency. When *A. rudis* received projected future precipitation regimes (greater magnitude and decreased frequency), however, this pattern flipped and instead they dispersed greater than double the proportion of seeds in warmed than ambient conditions. Although this interaction effect diminished by 24 hours after starting the seed dispersal assays, we did still find a significant effect of precipitation treatment such that *A. rudis* dispersed more seeds in increased magnitude decreased frequency precipitation treatments than increased magnitude alone treatments. Although we found higher seed dispersal under anticipated future climate scenarios, overall ant activity in mesocosms was higher in ambient than warmed mesocosms during the first 24 hours of seed dispersal assays (Fig. 4). Finally, we found no treatment effects on the re-dispersal of seeds from ant nests or the distance those seeds were moved by ants within the mesocosms (Fig. 5).

One mechanism by which plants are often hypothesized to benefit from engaging in seed dispersal mutualisms with ants is via predator avoidance (Beattie 1985, Giladi 2006). This hypothesis states that quick removal of the seeds of myrmecochorous plants by ants away from the parent plant could limit the impact of seed predators for plants engaged in this mutualism (Giladi 2006) and has been invoked in many studies measuring the outcomes of ant-mediated seed dispersal (Bennett et al. 1990, Manzaneda et al. 2005, Boulay et al. 2007, Ness and Morin 2008, Kwit et al. 2012, Tanaka et al. 2015). Our finding that the rate of seed dispersal was higher in our treatments simulating future climate regimes suggests the possibility that removal rates and seed predator avoidance may be higher under future climate change scenarios. Field-based observations of *A. rudis* dispersal of *S. canadensis* seeds that we have conducted suggest that the majority of seed predation takes place at night by slugs, snails, and small mammals (unpublished data, A. Nelson). Future studies are needed that capture the impacts of climate change on seed predation in addition to seed dispersers to get a better understanding of whether we would see the same pattern with seed predators in the system.

Although we found positive effects of warming and decreased precipitation frequency on initial seed removal rates, we found no effect of our climate change treatments for re-dispersal of seeds and the distance that seeds were dispersed within the mesocosm. Increased initial removal of seeds by ants may be related to increased ant activity rates in warmed treatments. Although we found that more ant workers were active in ambient treatments than warmed treatments (Fig. 4), activity of individual ants, which we did not measure in this study, could increase with warming temperatures leading to greater rates of seed dispersal. Under dryer conditions present with warmed temperatures and decreased precipitation frequency, ant activity may be higher as ants forage for limited water resources. This dynamic has also been shown in ant-plant defense mutualisms (Pringle et al. 2013). We may not have detected treatment effects on re-dispersal of seeds by ants because our re-dispersal observations occurred with lower frequency over longer time scales (every 24 hours) where the ants potentially had time to wait for preferred temperature conditions across ambient and warming treatments. Although dispersal distances generally fell within distances previously reported, one reason we may not have found effects of treatments on dispersal distance may be that the size of our mesocosms limited the movement of ants to be within the mesocosm. We may have been more likely to observe differences in seed dispersal distances across treatments if the mesocosms were larger encompassing the upper limits of how far *A. rudis* can disperse seeds.

Our finding that warming-mediated effects flipped direction depending on precipitation treatment suggests that warming effects on ant-mediated seed dispersal mutualisms may depend on how precipitation regimes are also altered under anthropogenic climate change. For example, eastern deciduous forests are predicted to experience precipitation events of greater magnitude, but decreased frequency, by 2100 (Easterling et al. 2017, Vose et al. 2017, IPCC 2018, 2022), which may result in greater rates of ant-mediated seed dispersal. However, ant seed dispersal mutualisms may falter in locations expected to be warm and dry, such as in Brazilian Caatinga ecosystems where increasing aridity has been found to be correlated with decreased rates of ant-mediated seed dispersal and decreased seed dispersal distances (Oliveira et al. 2019). Ant species often exhibit tradeoffs between thermal tolerance and desiccation tolerance experiencing lower critical thermal maxima when water is a limiting resource (Johnson and Stahlschmidt 2020). Moreover, our experiment shows the utility for experiments that take a multifactor approach to climate change. If we had not manipulated precipitation in addition to temperature, as has been done for most experiments focused on the effects of climate change on ants (Parr and Bishop 2022), we may conclude negative warming-related effects on the number of seeds dispersed, when we found a positive effect of warming for increased magnitude, decreased frequency treatments.

Our study here focused primarily on the ant side of this mutualism (seed removal rate by ants, distance ants moved the seeds, ant activity), but mechanisms from the plant side of the mutualism could also drive responses of myrmecochory to anthropogenic climate change (e.g., shifting plant phenology, changes to attractive or defensive plant secondary metabolites, etc.). Another possible factor determining the outcome of ant-mediated seed dispersal could be associated changes to plant traits. In some cases, warming has been shown to increase the production of defensive secondary metabolites in plant tissues, with cascading consequences for insect herbivores (Bidart-Bouzat and Imeh-Nathaniel 2008, Kuczyk et al. 2021). If similar shifts occur in seed secondary metabolites, this could reduce the preferences of seed-dispersing ants and ultimately plant reproductive success. To better predict the impacts of climate change on seed dispersal mutualisms, additional studies are needed that incorporate mechanisms from the plant perspective into controlled experiments.

Conclusions

Our mesocosm experiment underscores the importance of using experiments in testing the potential mechanisms that will drive how species and species interactions may respond to ongoing changes to climate. This experiment allowed us to assess the effects of a combination of climate factors predicted to change with human-caused climate change, while also preserving a degree of reality of how ants may encounter myrmecochorous seeds in a natural system. Assessing ant seed dispersal mutualisms over a combination of climate factors allowed us to determine when warming and precipitation effects might be context dependent. Overall, the positive effects of predicted climate change scenarios on the rate of seeds initially dispersed by ants and the lack of detectable effects on re-dispersal of seeds and dispersal distance suggest that this mutualism may be buffered against risks that could come from the progression towards climates that are warmer and have less predictable precipitation regimes.

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Tables and Figures

Table 1. Output from generalized mixed effects models for initial dispersal and re-dispersal of seeds. Models included a random effect for mesocosm nested within experimental block. Models for proportion of seeds dispersed used a quasi-binomial error distribution. For probability of seeds dispersed we used the same model predictor structure, but instead used a Cox proportional hazards mixed effects model.

Response	**Predictor**	X^2	**df**	***p***
Proportion seeds removed at 6 hours	Temperature Treatment	0.003	1	0.96
	Precipitation Treatment	0.66	2	0.72
	Temperature Treatment: Precipitation Treatment	10.40	2	**0.006**
	Plant Population ID	102.18	2	**< 2.2e-16**
Proportion seeds removed at 6 hours - Warmed Only	Precipitation Treatment	9.34	2	**0.009**
	Plant Population ID	51.56	2	**6.37e-12**
Proportion seeds removed at 6 hours - Ambient Only	Precipitation Treatment	4.65	2	0.10
	Plant Population ID	44.35	2	**2.34e-10**
Proportion seeds removed at 24 hours	Temperature Treatment	0.23	1	0.63
	Precipitation Treatment	5.71	2	**0.06**
	Temperature Treatment: Precipitation Treatment	1.42	2	0.49
	Plant Population ID	155.58	2	**< 2.0e-16**
Proportion re-dispersed	Temperature Treatment	0.64	1	0.43

seeds between 48-96 hours	Precipitation Treatment	3.55	2	0.17
	Temperature Treatment: Precipitation Treatment	0.64	2	0.72
	Plant Population ID	71.59	2	**2.99e-16**
Probability seed removed between 0 and 24 hours	Temperature Treatment	0.16	1	0.69
	Precipitation Treatment	1.16	2	0.56
	Temperature Treatment: Precipitation Treatment	11.44	2	**0.003**
	Plant Population ID	1020.02	2	**< 2.2e-16**
Average ant activity per observation between 1 and 24 hours	Temperature Treatment	5.65	1	**0.02**
	Precipitation Treatment	0.61	2	0.74
	Temperature Treatment: Precipitation Treatment	1.42	2	0.49
	Plant Population ID	102.60	2	**< 2.0e-16**

Table 2. Output from general linear mixed effects models investigating the effects of treatments on seed dispersal distance Models included a random effect for mesocosm nested within experimental block.

Response	Predictor	X^2	df	*p*
Distance between re-dispersed seeds and seed depot - including zeros	Temperature Treatment	0.30	1	0.58
	Precipitation Treatment	4.12	2	0.13
	Temperature Treatment: Precipitation Treatment	3.13	2	0.21
	Plant Population ID	170.16	2	**<2e-16**
Distance between re-dispersed seeds and colony nest box - including zeros	Temperature Treatment	0.059	1	0.81
	Precipitation Treatment	4.86	2	**0.09**
	Temperature Treatment: Precipitation Treatment	2.58	2	0.28
	Plant Population ID	138.18	2	**< 2e-16**
Distance between re-dispersed seeds and seed depot - not including zeros	Temperature Treatment	0.56	1	0.46
	Precipitation Treatment	0.18	2	0.91
	Temperature Treatment: Precipitation Treatment	1.54	2	0.46
	Plant Population ID	9.08	2	**0.01**
Distance between re-dispersed seeds and colony nest box - not	Temperature Treatment	0.41	1	0.52
	Precipitation Treatment	0.93	2	0.63

including zeros	Temperature Treatment: Precipitation Treatment	0.61	2	0.74
	Plant Population ID	7.03	2	**0.03**

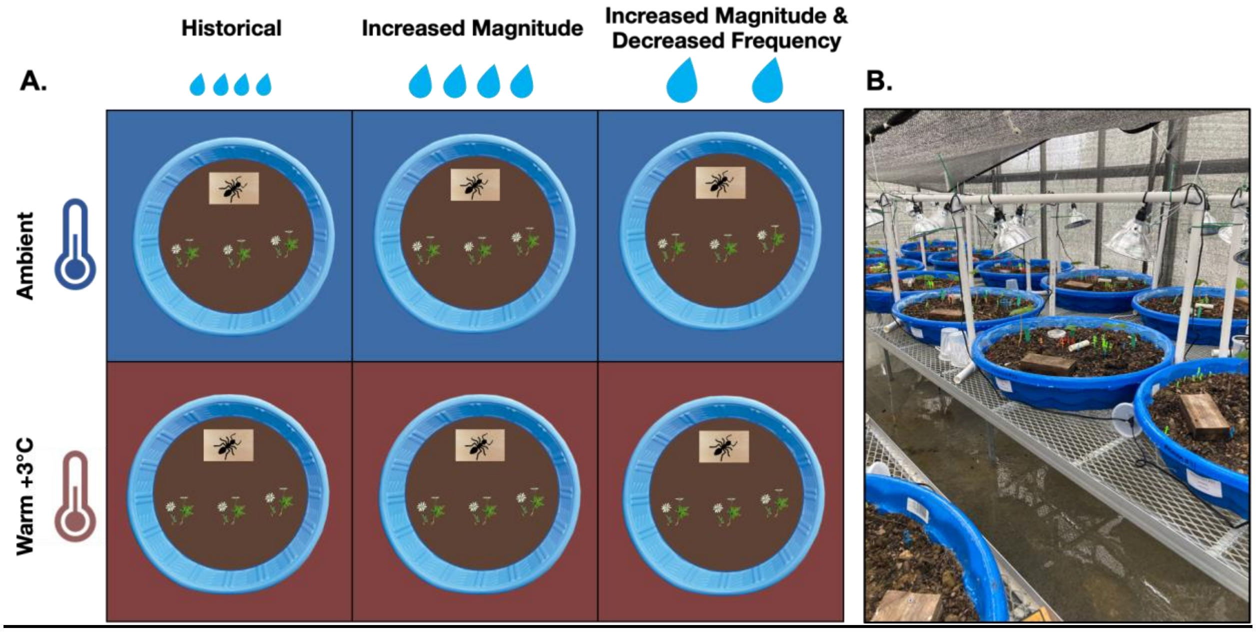

Figure 1. A. Design of mesocosm experiment. We constructed ant seed dispersal mesocosms and exposed them to temperature treatments crossed with altered precipitation magnitude and frequency. Temperature treatments included ambient and warmed to 3°C above ambient. Our three precipitation treatments included a historical, increased magnitude, and an increased magnitude plus decreased frequency treatments. Mesocosms were constructed in 0.82m diameter plastic children's pools and each had one *A. rudis* colony and three *S. canadensis* individuals from each of three populations. Each treatment was replicated 7 times for a total of 42 mesocosms. **B.** Picture of a subset of the mesocosms in the greenhouse.

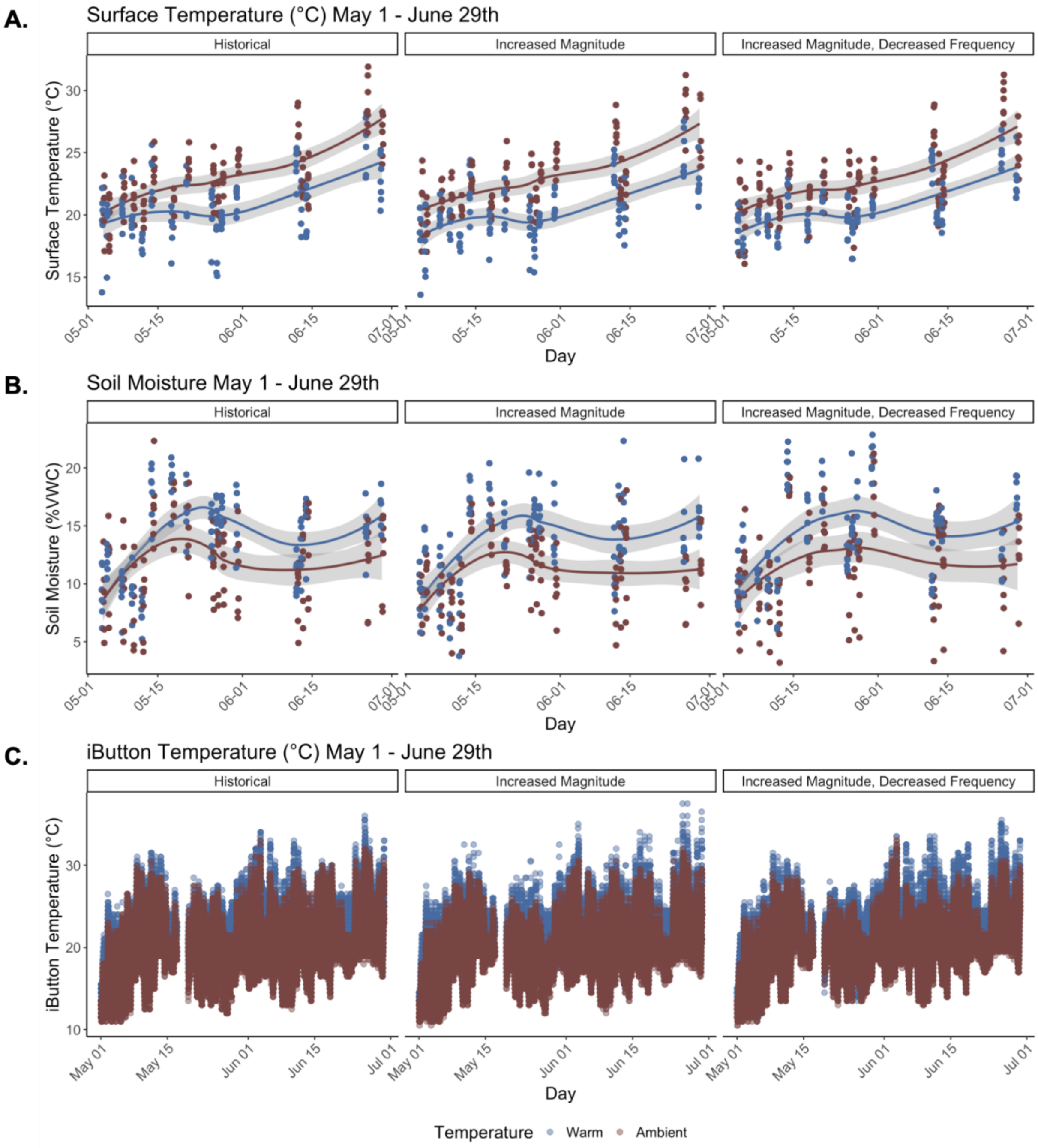

Figure 2. A. Surface temperature (°C) and **B.** soil volumetric water content (%) measure, and **C.** iButton temperature (°C) measured prior to precipitation events between May and June 2023.

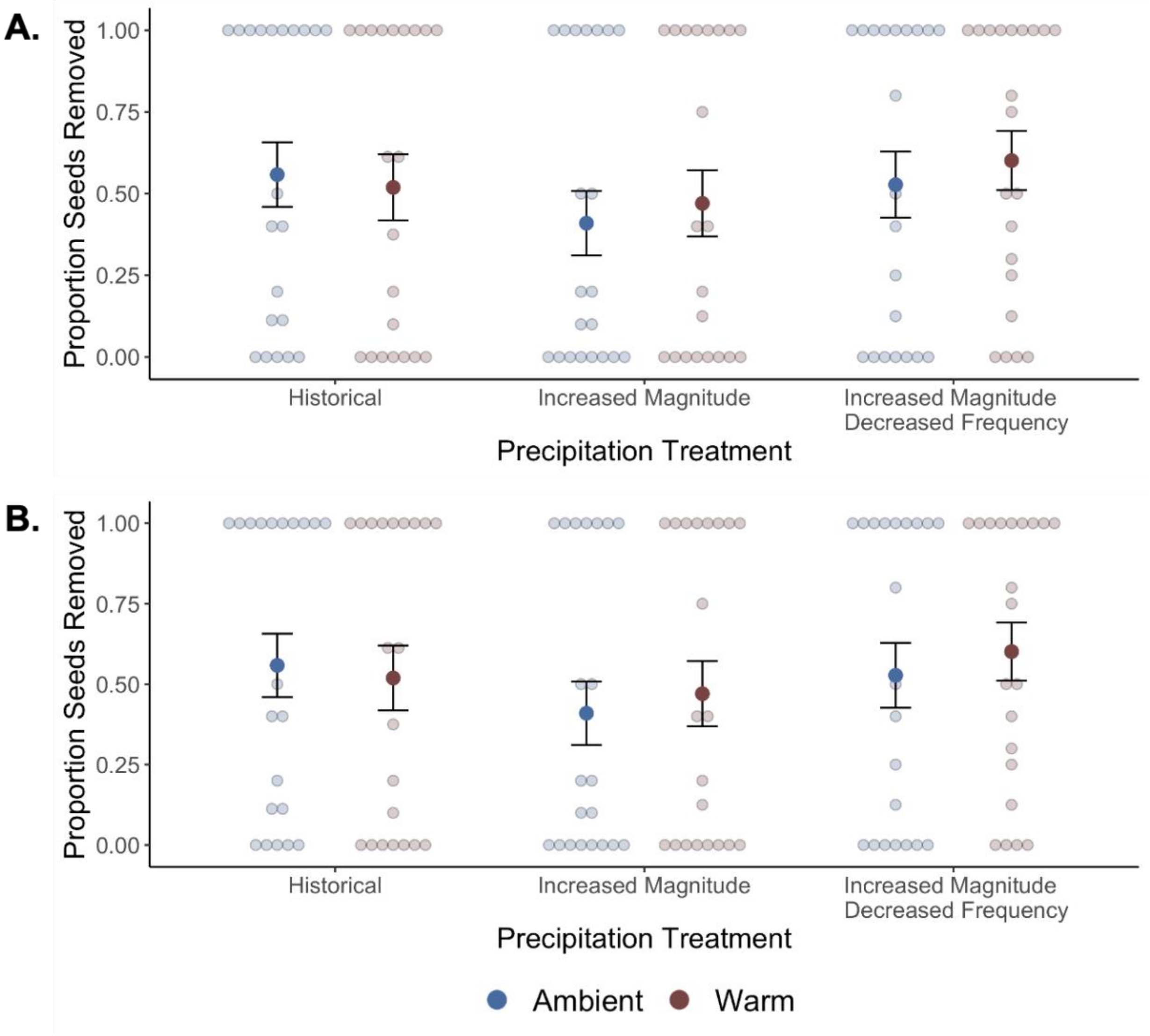

Figure 3. Dot plots showing the mean proportion of seeds dispersed at 6 hours **(A.)** and 24 hours **(B.)** for each treatment combination. Each point corresponds to the proportion of seeds removed from an individual seed depot. Error bars represent standard error.

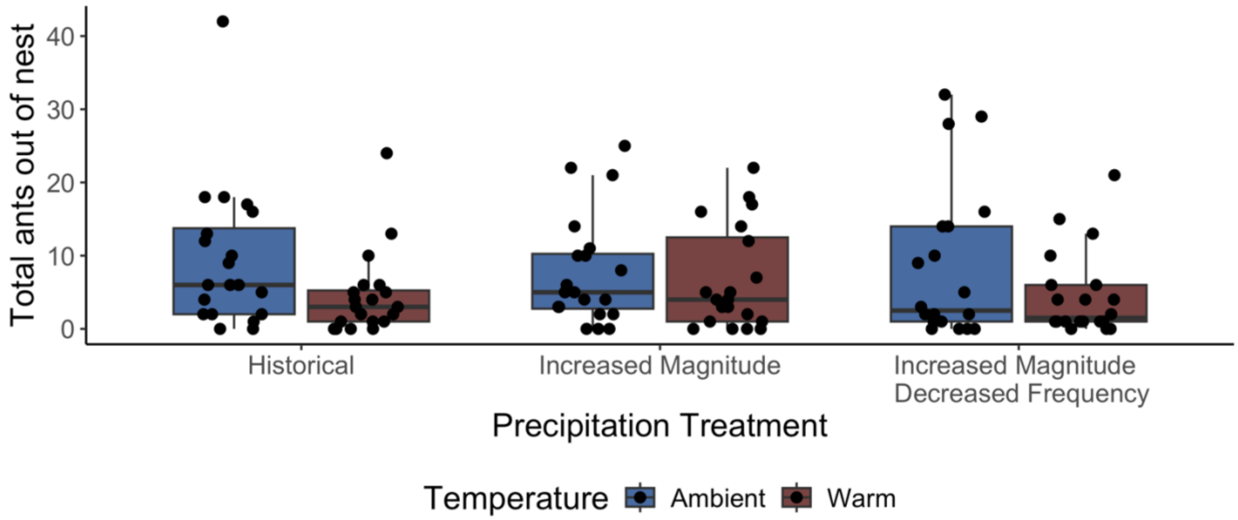

Figure 4. Boxplots showing the number of ant workers active in mesocosm outside the nest over observations made from 0 to 24 hours during seed dispersal assays. The shaded areas of the boxplots show the median and upper and lower quartiles.

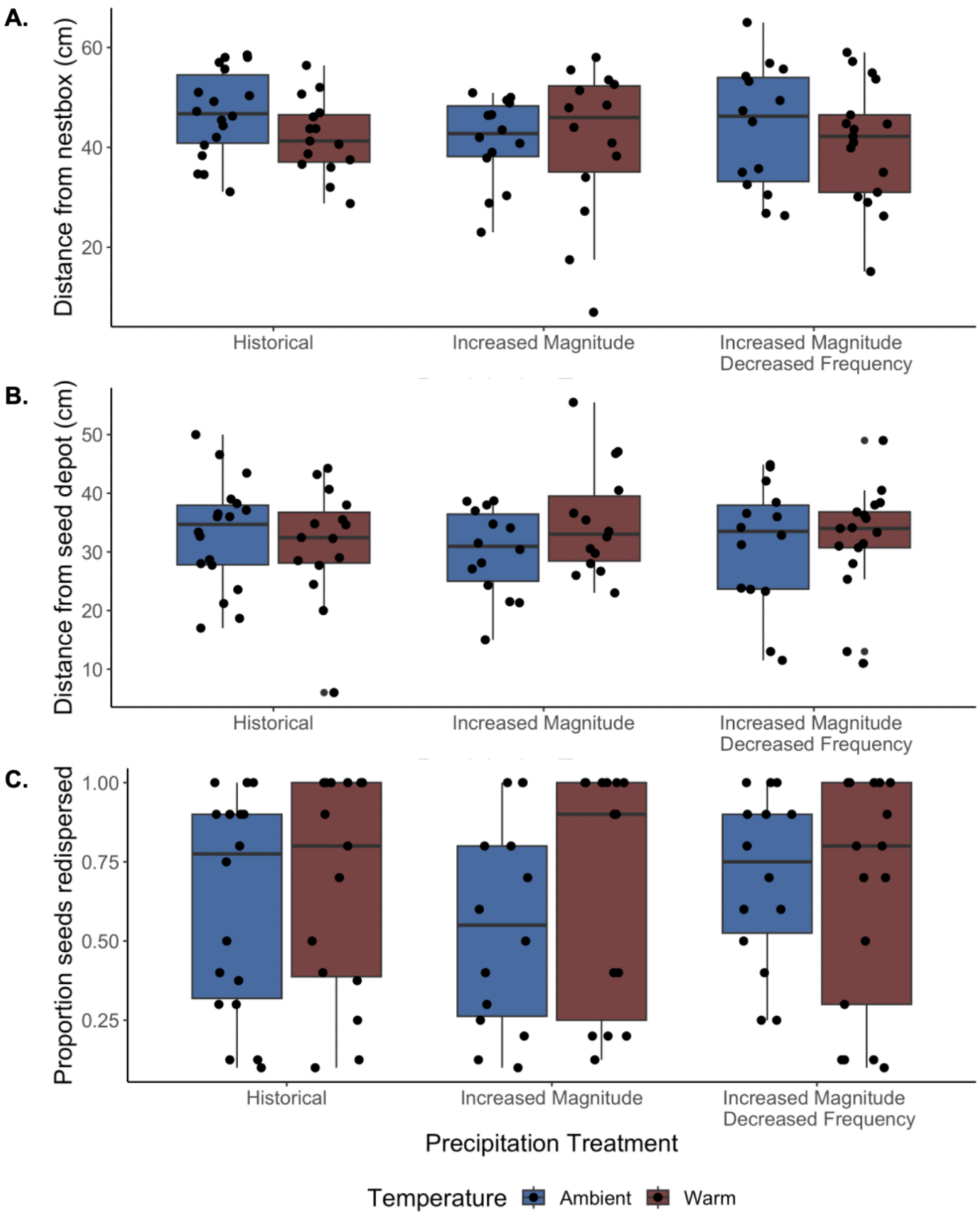

Figure 5. A. Distance seeds dispersed from ant colony nest boxes averaged across seeds within seed depot **B.** Distance seeds dispersed from seed depot averaged across seeds

within seed depot **C.** Proportion of seeds redispersed after initial removal by ants from seed dispersal depots. The shaded areas of the boxplots show the median and upper and lower quartiles.

Supplementary Materials for Chapter 4

Table S1. Precipitation treatment schedule May-June 2023.

Historical Date	Date Treatment Applied	Historical Treatment (cm)	Historical Volume in Mesocosm (mL)	Increased Magnitude Treatment (cm)	Increased Magnitude Volume in Mesocosm (mL)	Decreased Frequency Treatment (cm)	Deceased Frequency Volume in Mesocosm (mL)
5/1/59	5/1/23	0	0	0	0	0	0
5/2/59	5/2/23	0	0	0	0	0	0
5/3/59	5/3/23	0	0	0	0	0	0
5/4/59	5/4/23	0.46	2369	0.51	2606	0.56	2889
5/5/59	5/5/23	0	0	0	0	0	0
5/6/59	5/6/23	0	0	0	0	0	0
5/7/59	5/7/23	0	0	0	0	0	0
5/8/59	5/8/23	0	0	0	0	0	0
5/9/59	5/9/23	0	0	0	0	0	0
5/10/59	5/10/23	0.05	258	0.06	283	0.00	0
5/11/59	5/11/23	0	0	0	0	0	0
5/12/59	5/12/23	0	0	0	0	0	0
5/13/59	5/13/23	1.6	8241	1.76	9065	2.99	15410
5/14/59	5/14/23	1.12	5768	1.23	6345	0.00	0
5/15/59	5/15/23	0	0	0	0	0	0
5/16/59	5/16/23	0	0	0	0	0	0
5/17/59	5/17/23	0	0	0	0	0	0
5/18/59	5/18/23	0.15	773	0.17	850	0.89	4589
5/19/59	5/19/23	0	0	0	0	0	0
5/20/59	5/20/23	0	0	0	0	0	0
5/21/59	5/21/23	0.66	3399	0.73	3739	0.00	0
5/22/59	5/22/23	0	0	0	0	0	0
5/23/59	5/23/23	0	0	0	0	0	0
5/24/59	5/24/23	0	0	0	0	0	0
5/25/59	5/25/23	0	0	0	0	0	0
5/26/59	5/26/23	0.28	1442	0.31	1586	0.36	1870
5/27/59	5/27/23	0.05	258	0.06	283	0.00	0
5/28/59	5/28/23	0.03	155	0.03	170	1.34	6912
5/29/59	5/29/23	0	0	0	0	0	0
5/30/59	5/30/23	0	0	0	0	0	0
5/31/59	5/31/23	1.19	6129	1.31	6742	0.00	0
6/1/84	6/1/23	0	0	0	0	0	0
6/2/84	6/2/23	0	0	0	0	0	0

Historical Date	Date Treatment Applied	Historical Treatment (cm)	Historical Volume in Mesocosm (mL)	Increased Magnitude Treatment (cm)	Increased Magnitude Volume in Mesocosm (mL)	Decreased Frequency Treatment (cm)	Deceased Frequency Volume in Mesocosm (mL)
6/3/84	6/3/23	0	0	0	0	0	0
6/4/84	6/4/23	0	0	0	0	0	0
6/5/84	6/5/23	0	0	0	0	0	0
6/6/84	6/6/23	0	0	0	0	0	0
6/7/84	6/7/23	0	0	0	0	0	0
6/8/84	6/8/23	0	0	0	0	0	0
6/9/84	6/9/23	0	0	0	0	0	0
6/10/84	6/10/23	0	0	0	0	0	0
6/11/84	6/11/23	0	0	0	0	0	0
6/12/84	6/12/23	0.51	2627	0.56	2889	0.89	4589
6/13/84	6/13/23	0.3	1545	0.33	1700	0.00	0
6/14/84	6/14/23	1.42	7314	1.56	8045	1.89	9745
6/15/84	6/15/23	0	0	0	0	0	0
6/16/84	6/16/23	0	0	0	0	0	0
6/17/84	6/17/23	0	0	0	0	0	0
6/18/84	6/18/23	0.3	1545	0.33	1700	0.00	0
6/19/84	6/19/23	0	0	0	0	0	0
6/20/84	6/20/23	0	0	0	0	0	0
6/21/84	6/21/23	0	0	0	0	0	0
6/22/84	6/22/23	0	0	0	0	0	0
6/23/84	6/23/23	0	0	0	0	0	0
6/24/84	6/24/23	0	0	0	0	0	0
6/25/84	6/25/23	0	0	0	0	0	0
6/26/84	6/26/23	0.76	3914	0.84	4306	1.25	6459
6/27/84	6/27/23	0	0	0	0	0	0
6/28/84	6/28/23	0	0	0	0	0	0
6/29/84	6/29/23	0.38	1957	0.42	2153	0.00	0
6/30/84	6/30/23	0	0	0	0	0	0

Modified Bhatkar-Whitcomb Diet Recipe

Supplies

Purified water

LB Agar Miller (Fisher)

Vanderzant vitamin mixture for insects (Sigma)

Large raw eggs

Honey

Directions for preparing diet

1. Boil 500-mL distilled water with 10 g LB agar. Mixture should boil for at least 1 minute. Set aside to cool. This will take about 20 minutes.
2. With the immersion blender, mix 500-mL water, 2-g Vanderzant vitamin mixture for insects, 2 eggs, and 170g honey.
3. Once agar solution is cool, mix it with the mixture from step 2. Use the immersion blender to mix thoroughly.
4. We poured the final mixture into deep petri dishes so that it will set up. Store in the refrigerator at ~4°C until needed.

Table S2. Site origin of transplanted *Sanguinaria canadensis* and the original number of seeds produced. To avoid issues with variation across seed density per mesocosm, we only used 10 seeds per pod for assays for both Graham Farm and Floyd County individuals and 8 seeds for Bishop Road individuals.

Block ID	**Mesocosm ID**	**Seed Depot Location**	**Origin of Plant**	**Number of Seeds in Pod**	**Seeds from Supplemental Pod** ***1 = Seeds from supplemental pods**
1	1	1	Floyd County	21	1
1	1	2	Bishop Road	10	0
1	1	3	Graham Farm	18	0
1	2	1	Bishop Road	0	1
1	2	2	Graham Farm	11	0
1	2	3	Floyd County	13	1
1	3	1	Graham Farm	21	0
1	3	2	Floyd County	22	1
1	3	3	Bishop Road	9	0
1	4	1	Graham Farm	18	0
1	4	2	Bishop Road	0	1
1	4	3	Floyd County	35	1
1	5	1	Graham Farm	16	0
1	5	2	Bishop Road	0	1
1	5	3	Floyd County	17	1
1	6	1	Floyd County	22	1
1	6	2	Graham Farm	16	0
1	6	3	Bishop Road	8	0
2	7	1	Bishop Road	9	0
2	7	2	Floyd County	35	1
2	7	3	Graham Farm	16	0
2	8	1	Floyd County	35	1
2	8	2	Bishop Road	0	1
2	8	3	Graham Farm	15	0
2	9	1	Bishop Road	11	0
2	9	2	Floyd County	17	1
2	9	3	Graham Farm	5	1
2	10	1	Floyd County	22	1
2	10	2	Graham Farm	15	0

Block ID	Mesocosm ID	Seed Depot Location	Origin of Plant	Number of Seeds in Pod	Seeds from Supplemental Pod *1 = Seeds from supplemental pods
2	10	3	Bishop Road	19	0
2	11	1	Bishop Road	9	0
2	11	2	Graham Farm	24	0
2	11	3	Floyd County	23	1
2	12	1	Graham Farm	23	0
2	12	2	Floyd County	15	1
2	12	3	Bishop Road	10	0
3	13	1	Graham Farm	13	0
3	13	2	Bishop Road	12	0
3	13	3	Floyd County	31	1
3	14	1	Bishop Road	0	1
3	14	2	Floyd County	24	1
3	14	3	Graham Farm	14	0
3	15	1	Graham Farm	24	0
3	15	2	Floyd County	29	1
3	15	3	Bishop Road	0	1
3	16	1	Bishop Road	0	1
3	16	2	Graham Farm	11	0
3	16	3	Floyd County	11	1
3	17	1	Bishop Road	5	1
3	17	2	Floyd County	15	1
3	17	3	Graham Farm	27	0
3	18	1	Graham Farm	13	0
3	18	2	Bishop Road	9	0
3	18	3	Floyd County	38	1
4	19	1	Graham Farm	12	0
4	19	2	Bishop Road	15	0
4	19	3	Floyd County	42	1
4	20	1	Bishop Road	0	1
4	20	2	Graham Farm	10	0
4	20	3	Floyd County	27	1
4	21	1	Graham Farm	25	0
4	21	2	Floyd County	24	1
4	21	3	Bishop Road	4	1
4	22	1	Floyd County	27	1
4	22	2	Graham Farm	13	0

Block ID	Mesocosm ID	Seed Depot Location	Origin of Plant	Number of Seeds in Pod	Seeds from Supplemental Pod *1 = Seeds from supplemental pods
4	22	3	Bishop Road	8	0
4	23	1	Graham Farm	20	0
4	23	2	Bishop Road	0	1
4	23	3	Floyd County	57	1
4	24	1	Bishop Road	7	1
4	24	2	Graham Farm	8	1
4	24	3	Floyd County	14	1
5	25	1	Graham Farm	12	0
5	25	2	Bishop Road	6	1
5	25	3	Floyd County	31	1
5	26	1	Bishop Road	9	0
5	26	2	Graham Farm	21	0
5	26	3	Floyd County	22	1
5	27	1	Graham Farm	0	1
5	27	2	Floyd County	12	1
5	27	3	Bishop Road	0	1
5	28	1	Bishop Road	21	0
5	28	2	Floyd County	15	1
5	28	3	Graham Farm	4	1
5	29	1	Bishop Road	NA	1
5	29	2	Graham Farm	7	1
5	29	3	Floyd County	16	1
5	30	1	Graham Farm	18	0
5	30	2	Floyd County	11	1
5	30	3	Bishop Road	NA	1
6	31	1	Bishop Road	17	0
6	31	2	Floyd County	19	1
6	31	3	Graham Farm	21	0
6	32	1	Graham Farm	11	0
6	32	2	Floyd County	20	1
6	32	3	Bishop Road	8	0
6	33	1	Bishop Road	18	0
6	33	2	Graham Farm	16	0
6	33	3	Floyd County	27	1
6	34	1	Graham Farm	31	0
6	34	2	Floyd County	25	1

Block ID	Mesocosm ID	Seed Depot Location	Origin of Plant	Number of Seeds in Pod	Seeds from Supplemental Pod *1 = Seeds from supplemental pods
6	34	3	Bishop Road	4	1
6	35	1	Graham Farm	16	0
6	35	2	Bishop Road	16	0
6	35	3	Floyd County	38	1
6	36	1	Graham Farm	21	0
6	36	2	Bishop Road	14	0
6	36	3	Floyd County	20	1
7	37	1	Floyd County	10	1
7	37	2	Graham Farm	16	0
7	37	3	Bishop Road	0	1
7	38	1	Graham Farm	11	0
7	38	2	Bishop Road	9	0
7	38	3	Floyd County	27	1
7	39	1	Bishop Road	13	0
7	39	2	Floyd County	28	1
7	39	3	Graham Farm	13	0
7	40	1	Bishop Road	NA	1
7	40	2	Floyd County	19	1
7	40	3	Graham Farm	0	1
7	41	1	Floyd County	15	1
7	41	2	Graham Farm	22	0
7	41	3	Bishop Road	11	0
7	42	1	Bishop Road	0	1
7	42	2	Graham Farm	0	1
7	42	3	Floyd County	41	1

Effects of experimental treatments on abiotic environment in mesocosms between May and June 2023

For surface temperature between May and June 2023, we found a significant effect of temperature treatment such that warmed treatments were ~2.3 °C higher than ambient treatments (t = -11.79, p = <0.0001). We also found differences in soil moisture among temperature treatments such that soil moisture was ~17 percent lower in warmed than ambient treatments (t = 4.55 , p = 0.0001), but we found no significant differences in soil volumetric water content among precipitation treatments (Table S1). Air temperature, measured by iButtons temperature loggers every 15 minutes, was ~2.4 °C higher in warmed than ambient temperature treatments (t = -15.23, p = <0.0001).

Table S3. Effects of experimental treatments on abiotic variables measured during seed dispersal assays. We used general linear mixed effects models with mesocosm nested within experimental block.

Response	Predictor	X^2	df	*p*
Ground surface temperature (°C) - during seed dispersal assays	Temperature Treatment	196.93	1	**< 2.2e-16**
	Precipitation Treatment	0.62	2	0.73
	Temperature Treatment: Precipitation Treatment	0.87	2	0.65
	Plant Population ID	78.75	2	**< 2.2e-16**
	Date Time	65.12	1	**7.0e-16**
Soil volumetric water content (%) - during seed dispersal assays	Temperature Treatment	22.85	1	**2.0e-06**
	Precipitation Treatment	6.21	2	**0.05**
	Temperature Treatment: Precipitation Treatment	0.37	2	0.83
	Plant Population ID	7.10	2	**0.03**
	Date Time	4.98	1	**0.03**
Ground surface temperature (°C) - May-June 2023	Temperature Treatment	139.04	1	**<2e-16**
	Precipitation Treatment	2.18	2	0.34
	Temperature Treatment: Precipitation Treatment	1.13	2	0.57
	Date Time	495.30	1	**<2e-16**
Soil volumetric water	Temperature Treatment	20.73	1	**5.284e-06**

content (%) - May-June 2023	Precipitation Treatment	0.75	2	0.69
	Temperature Treatment: Precipitation Treatment	0.25	2	0.88
	Date Time	56.5	1	**5.607e-14**
iButton temperature May-June 2023	Temperature Treatment	232.06	1	**<2e-16**
	Precipitation Treatment	2.38	2	0.30
	Temperature Treatment: Precipitation Treatment	1.63	2	0.44
	Date Time	47580.75	1	**<2e-16**

Treatment effects on probability seeds removed by ants - results of mixed effects Cox proportional hazards models

In addition to our GLMMs performed that assessed differences among treatments at 6 and 24 hours after the start of seed dispersal assays, we also performed mixed effects Cox proportional hazard models as complementary analyses to investigate the effects of treatments on the proportion of seeds removed by ants from 1 through 24 hours after seed dispersal depots were deployed to mesocosms during our assays. With these models, we also found a significant interaction between the temperature and precipitation treatments as we did in the GLMMs assessing the effects of our treatments at 6 hours after seed dispersal assays began (Table 1, Figure 4). To further investigate the significant interaction term between temperature and precipitation treatments, we conducted separate mixed effects Cox proportional hazards models subsetting the data by temperature treatment and precipitation treatment. We found a significant effect of precipitation treatment for warmed temperature treatments, but not for ambient treatments (Table S4, Fig. S1). For warmed treatments we found that the probability of dispersal was higher for increased magnitude, decreased frequency treatments than both historical (z =-4.17 , p = 0.0001) and increased magnitude treatments (z = 2.43, p = 0.04). There was not a difference between historical and increased magnitude treatments (z = -1.75, p>0.05). When we subsetted data by precipitation treatments, for both historical precipitation and increased magnitude decreased frequency treatments, but not increased magnitude precipitation treatments, we found a significant effect of temperature treatment (Table S3). For historical precipitation treatments, we found a greater probability of seed dispersal in ambient than warmed temperature treatments (z = 3.31, p = 0.0009). Alternatively, for increased magnitude decreased frequency treatments we found a higher probability of seed dispersal in warmed than ambient temperature treatments (z = -2.07 , p = 0.04).

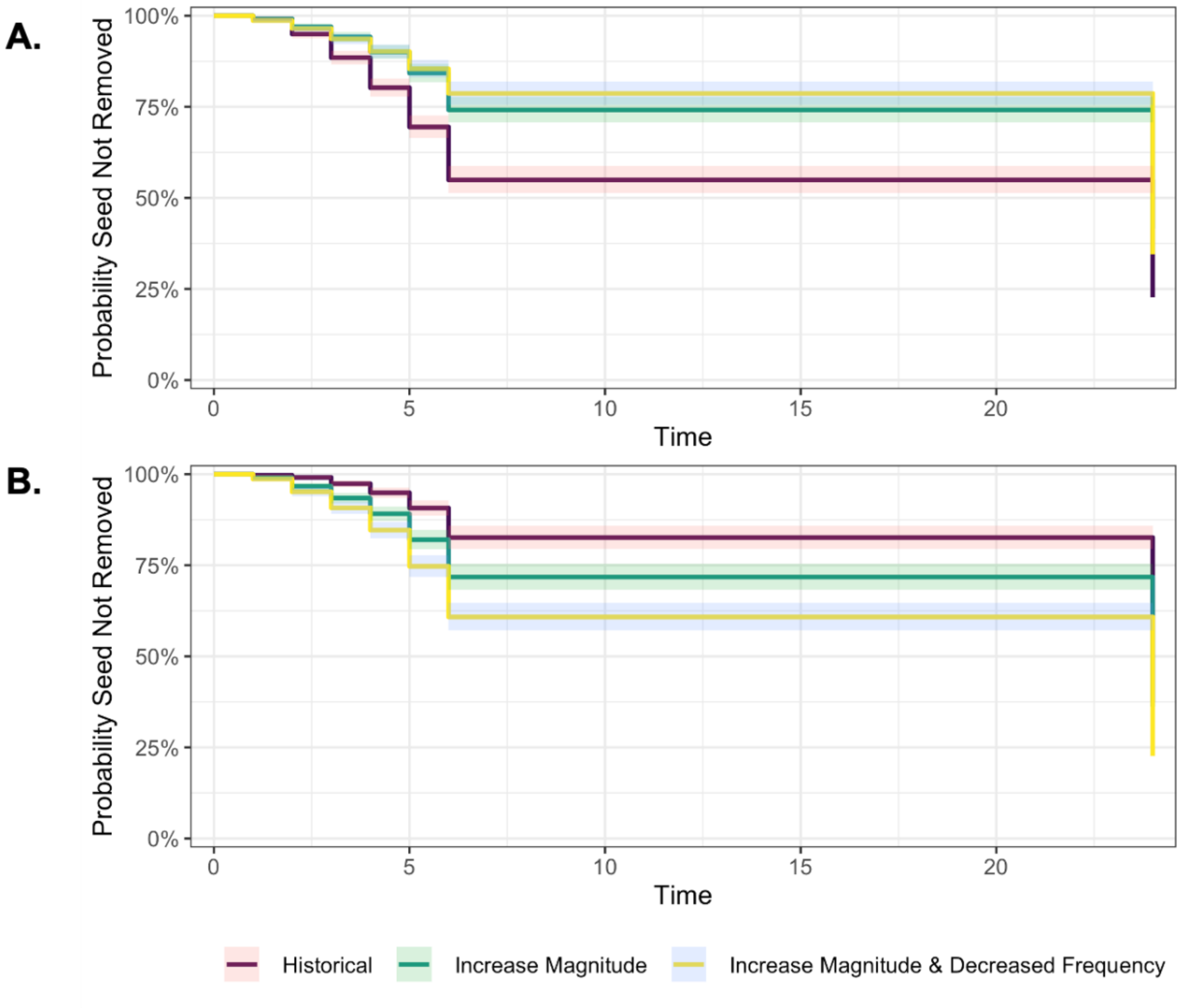

Figure S1. Survival curves showing probability that seeds remained over time for ambient (A.) and warmed (B.) treatments. The shaded area around each curve represents 95% confidence intervals.

Table S3. Output from mixed effects Cox proportional hazards models. We specified temperature treatment, precipitation treatment, the interaction between temperature treatment and precipitation treatment, and plant population identity as fixed effects in the model. Mesocosm nested within block were specified as random effects. We used this model as a complementary analysis to our generalized mixed effects models that investigated the effects of the same predictors on the proportion of seeds removed at 6 and 24 hours after the start of seed dispersal assays. We additionally performed separate analyses with subsets of our data to assess the interaction between temperature treatment and precipitation treatment.

Response	**Predictor**	**X^2**	**df**	***p***
Probability seed not removed between 0 and 24 hours	Temperature Treatment	0.16	1	0.69
	Precipitation Treatment	1.16	2	0.56
	Temperature Treatment: Precipitation Treatment	11.44	2	**0.003**
	Plant Population ID	1020.02	2	**< 0.0001**
Probability seed not removed between 0 and 24 hours - Warm only	Precipitation Treatment	17.70	2	**0.0001**
	Plant Population ID	647.77	2	**< 0.0001**
Probability seed not removed between 0 and 24 hours - Ambient only	Precipitation Treatment	4.26	2	0.12
	Plant Population ID	394.10	2	**<0.0001**
Probability seed not removed between 0 and 24 hours - Historical Precip Treatment Only	Temperature Treatment	10.97	1	**0.0009**
	Plant Population ID	313.61	2	**< 0.0001**
Probability seed not removed between 0 and 24 hours - Increased Magnitude Precip Treatment Only	Temperature Treatment	0.81	1	0.37
	Plant Population ID	343.93	2	**<0.0001**
Probability seed not removed between 0 and 24 hours - Increased Magnitude and Decreased Frequency Precip Treatment Only	Temperature Treatment	4.28	1	**0.04**
	Plant Population ID	341.74	2	**<0.0001**

Chapter 5 - Conclusions

Summary and Synthesis

In my dissertation, I used field and greenhouse-based experiments to investigate the effects of two of the largest threats to biodiversity, habitat fragmentation and human-caused climate change on ant seed dispersal mutualisms. Chapter 2 of my dissertation investigated how restoring connectivity with habitat corridors impacts ant diversity and community change over time in restored longleaf pine savannas. In this study, we found that habitat patches with corridors accumulated taxonomic diversity at a faster pace than isolated patches. Additionally, we found evidence that corridors may increase beta-diversity across patches. Taken together, these results suggest that connectivity could play an important role in the assembly of ant communities. Chapter 3 of my dissertation took place in the same experimental landscapes as that of Chapter 2. With this chapter, we investigated the effects of corridors on ant seed dispersal mutualisms finding that ants dispersed seeds further in patches with corridors than isolated patch types. Within the paper published on that work we found that corridor effects on the diversity of seed dispersing ant species did not appear to be mediating that effect (Burt et al. 2022), but results from our long-term monitoring of the ant community in Chapter 2 suggest that these results may have been mediated through changes to ant alpha and beta diversity including non-seed dispersing ants as well. For my final data chapter, Chapter 4, I used an experiment that crossed warming with altered precipitation to investigate the effects of different climate change scenarios on ant seed dispersal mutualisms. In the greenhouse we constructed mesocosms with *Sanguinaria canadensis,* a myrmecochorous plant species and colonies of their seed dispersal mutualists, *Aphaenogaster rudis.* Our mesocosm design allowed us to closely follow the major parts of the process of ant-mediated seed dispersal: initial collection of seeds by ants and then re-dispersal of seeds after they removed seed. Determining the final dispersal location of seeds is often a metric that has not been measured in field-based studies owing to the difficulty in finding seeds after ants re-disperse them from their nests. However, our mesocosms provided a set area to focus on for finding the re-dispersed seeds. Overall, we found that the effect of warming on the number of seeds ants dispersed depended on precipitation treatment. In historical precipitation treatments, ants collected more seeds from depots in ambient temperature treatments than in warmed temperature treatments. However, for precipitation regimes expected with ongoing climate change with ants moving more seeds in warmed treatments than ambient treatments. Surprisingly, we did not find differences among our treatments in the proportion of seeds re-dispersed into the mesocosms by ants or the distance seeds were dispersed from the ant's nest box or the *S. canadensis* plants.

Taken together, the results of the experiments that make up my dissertation suggest various mechanisms by which global change factors might affect ant seed dispersal services. In fragmented landscapes, our results suggest evidence that reconnecting isolated fragments may be important for maintaining quality seed dispersal (greater seed dispersal distances) in longleaf pine savanna ecosystems. Importantly, ant taxonomic diversity is increasing faster with connectivity which may have consequences for these seed dispersal distance patterns. With environmental change predicted as a consequence of human-caused climate change, our results provide evidence that the combination of climate factors simultaneously changing as a result of climate change is important to consider when designing experiments to predict ant seed dispersal mutualisms' responses. When we increased warming alone, we found evidence that ants moved more seeds in ambient conditions than warmed conditions. However, when we measured seed dispersal under precipitation regimes predicted with current ongoing climate change, we found that this pattern flips to ants dispersing more seeds in warmed than ambient conditions. Overall, these results may suggest that ant-mediated seed dispersal could be limited by habitat fragmentation, but that climate change may have positive or perhaps at worst neutral effects on ant seed dispersal mutualisms. Future studies investigating the demographic outcomes for seeds in the context of global change would benefit our understanding of the ultimate impacts of environmental change-mediated effects on seed dispersal (Wang and Smith 2002).

In addition to independently affecting ecological systems, the major factors of global change may also have interactive effects on communities and species interactions that are difficult to predict. For instance, species that can move in response to increasing temperatures from climate change may be unable to disperse across patches in fragmented landscapes. Conservation and restoration strategies under a changing climate could require consideration of habitat connectivity (Doerr et al. 2011). Additionally, climate change and habitat fragmentation have both been shown to promote the invasion of non-native species (Didham et al. 2007, Warren et al. 2017). Although these interactive effects across factors of global environmental change are predicted to have substantial effects, most studies that investigate the effects of global change on species interactions, including the ones that make up my dissertation, consider these global change factors independently (McConkey et al. 2012, Jamieson et al. 2017, Parr and Bishop 2022). Additional studies that incorporate the possible interactions between factors will lead to a better understanding of the mechanisms structuring species interactions responses to human-mediated environmental change.

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