

# Linking species distributions and thermal physiology to understand climate change impacts on ants

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## Abstract:

Linking species distribution changes with mechanisms for these changes is critical to better predict how species will respond to climate change. Historical studies provide information on past species distributions, allowing us to examine changes in distributions and potential drivers of temporal change. We leveraged a late 1950s study on ant species assemblages in a canyon in Boulder, CO to determine how the community has changed over a period of more than 60 years. We found high species turnover between time periods and biotic homogenization. Community composition differed significantly between the time periods, with aspect and tree cover influencing the set of species represented. Species that foraged in broader temperature ranges became more widespread between historical and contemporary time periods. Additionally, aspect may dictate the community that is present based on species thermal physiology. Here we show that large community shifts and biotic homogenization can occur in natural areas without strong habitat degradation, which may have implications for community interactions and ecosystem functioning. We also show the power of pairing historical and contemporary data and we encourage more mechanistic studies to predict species changes in light of climate change.

## Introduction:

Understanding how communities are affected by anthropogenic climate change is critical as global climate change is predicted to increase mean temperature, increase variance, and increase instances of weather extremes (Easterling et al 2000; Vasseur et al 2014; IPCC 2021; Jentsch and Beierkuhnlein 2008). With species declines that have been linked to changing temperature regimes in the Anthropocene (IUCN 2019; Halsch et al 2020) and the predicted future loss of biodiversity (Turvey and Crees 2019; Thomas et al 2004), a focus on how community compositions are altered is important.

Species respond to changing environments in a variety of ways, including shifting their ranges (e.g. along latitudinal or elevational gradients) (Parmesan et al 1999), acclimating to the environment (Somero 2010) or adapting (Hoffmann and Sgrò 2011) to new conditions, or potentially becoming extirpated from the area. Altered environments have caused changes in the geographical distributions of species. As a result of climate change, some species have experienced range shifts (Chen et al 2011; Diamond 2017; Hickling et al 2006), while others have incurred range losses (Kerr et al 2015; Davis and Shaw 2001; Devictor et al 2012). There has been increasing evidence that insect species are declining in abundance (Sánchez-Bayo and Wyckhuys 2019; Wagner 2020; Lister and Garcia 2018; Hallmann et al 2017; Wagner et al 2021). Many of the changes that we are seeing in insect communities are likely the

result of the changing climate. Increases in average daily minimum temperatures had the biggest negative effects on insects – even more so in dry years (Halsch et al 2020). Additionally, certain insect communities may be more susceptible under altered temperature regimes. For instance, climate change and drought have bigger impacts on communities at higher elevations (Halsch et al 2020). However, confounding factors potentially complicate our ability to understand the ecological effects of climate change (e.g. tree cover increases due to fire suppression practices).

Ants are a useful taxon for studying impacts of climate change on biodiversity as they can be found in almost all habitat types, are ectothermic, and vary in their environmental tolerances (Nowrouzi et al., 2016; Hölldobler and Wilson, 1990). Since ants have established colonies, their occurrence implies suitable environmental conditions in contrast to more vagile organisms whose occurrence could be incidental. This allows us to better understand the mechanisms of change. Temperature dictates the functioning of ant colonies. For example, temperature restricts activity periods for foraging and regulates the development of ant broods, with temperature extremes limiting the reproductive output of a colony (Kipyatkov & Lopatina, 2015). Therefore, the clear links between temperature and colony fitness suggest that changes in temperature can affect geographic distributions of ants. Understanding how climate affects ants could have implications for ecosystem functions such as nutrient cycling, seed dispersal, decomposition, and community regulation (Del Toro et al., 2012; Ness et al 2009; Folgarait, 1998; Wills and Landis 2017; Warren and Bradford 2012). Historical studies on distributions when paired with contemporary distribution and environmental data can provide a unique opportunity to examine how environmental change has affected communities over long time scales.

Browne and Gregg (1969) examined the distributions of ants in Gregory Canyon near Boulder, CO in 1957 and 1958. They sought to determine the differences among ant communities due to the differing habitats created by topography. Gregory Canyon varies in temperature and moisture between its aspects, specifically the north-facing slopes, south-facing slopes, and canyon bottom. This canyon spans an elevational range of approximately 1645m to 2485m. The records of collection sites in Gregory Canyon, dates, and methods were documented in Browne and Gregg (1969). They found that based on aspect, the ant communities did vary, likely in part to differences in species specific environmental tolerances. Four species, which made up 9% of the collections, were collected in low numbers, causing the determination of habitat preference to only be at most suggestive. Fifteen species, 33% of the collections, had no specific tie to an ecological complex within Gregory Canyon and were usually widely distributed throughout the various ecological complexes. These species are the ones that have wide tolerances to environmental conditions. Other species were found to be more limited in their range in the canyon and were associated with a specific ecological complex. Eleven species, which constituted 24% of the total collections in the canyon, were found primarily on south-facing slope sites indicating that they prefer to nest in more mesic areas. Fifteen species, 33% of the total collections, were restricted to the north-facing slope sites indicating that these species prefer to inhabit nesting areas with cooler temperatures and higher levels of moisture. Browne and Gregg determined that the environment acted as a filter for ant species locality within the canyon.

Historical studies on distributions when paired with contemporary data and species thermal physiology can provide a unique opportunity to examine how climate change has affected the distribution of species and predict distributions under future climate change scenarios. In this study, we examined changes in ant assemblages across 60 years; specifically changes in species richness and composition across sites and the potential drivers of change. We asked:

1. Have ant species distributions in Gregory Canyon, Boulder, CO changed since sampling done by Browne and Gregg in 1957/58?
2. Does ant species composition still vary between aspects?
3. Is temperature driving the changes in ant species site occurrence in Gregory Canyon?
4. Does change in tree cover in Gregory Canyon relate to changes in ant species site occurrence?
5. Does thermal tolerance vary between aspects?

To answer these questions, we revisited 33 sites where ant assemblages were sampled more than 60 years ago by Browne and Gregg to look at how recent climate change has impacted local ant communities and how they have changed over time. Linking distributional changes across spatial scales with mechanisms for these changes is critical to better predict how species will respond under future climate change scenarios.

## Material & Methods:

### *Study site:*

The study was conducted in Gregory Canyon, (who was it owned by/managed by at the time), which is currently maintained by the City of Boulder Open Space and Mountain Parks public lands, Boulder County, Colorado, USA (39.999143, -105.302537). The study consisted of 33 (1.9 to 10.0 ha,  $4.62 \pm 1.95$ ) ant collection sites (Figure 1). Gregory Canyon has variable topography and therefore habitat types are defined by slope aspect (13 south-facing slope, 14 north-facing slope sites, and 6 canyon bottom sites). South-facing slopes have a semi-arid savannah habitat with higher temperatures and lower moisture content. North-facing slopes are characterized by a forested habitat with lower temperatures and higher soil moisture dominated by Ponderosa pine (*Pinus ponderosa*) and Lodgepole pines (*Pinus contorta*) and Douglas fir (*Pseudotsuga menziesii*) at higher elevations. South-facing slopes are characterized by lower soil moisture content and higher temperatures. The landscape on south-facing slopes generally is mixed shrubland and perennial graminoids with Kentucky Bluegrass (*Poa pratensis*), Canadian Brome (*Bromopsis canadensis*), Yucca (*Yucca glauca*), and New Mexico prickly-pear cactus (*Opuntia phaeacantha*). Canyon bottoms are characterized by riparian vegetation including narrowleaf cottonwoods (*Populus angustifolia*), willows (*Salix* spp.), American plum (*Prunus americana*), and wild raspberry (*Rubus idaeus*).

### *Environmental Conditions:*

To measure percent soil moisture Browne and Gregg collected soil samples along two north-south transects. Each transect had a collection point for each aspect. Soil samples were collected 3 times in 1958 (May, June, July) at each transect and transect point. Three samples at a depth of 5 inches were taken from each point in both transects from underneath medium sized rocks. Samples were dried in an oven at 105°C and the weight (grams) was recorded. Soil was sampled on the same dates recorded in Browne and Gregg 1969 following the same methods.

All analyses were run in R, version 4.0.2 (R Core Team 2022). To quantify how environmental conditions differed between the sampling years, we analyzed soil moisture and air temperature. To calculate percent soil moisture, we used the gravimetric soil water content equation (Gravimetric soil water content (%) =  $[\text{mass of moist soil (g)} - \text{mass of oven-dried soil (g)}] / \text{mass of oven-dried soil (g)} \times 100$ ). To determine if percent soil moisture differed between aspects and timeframes, we ran a two-way

ANOVA with percent soil moisture as the response variable and timeframe and aspect as the predictor variables. We then used a post-hoc Tukey test to determine which aspects differed.

Due to the limited number of temperature records in Browne and Gregg (1969) and uncertainty surrounding the exact locations and timing of the temperature measurements included in the publication, we used a local weather station (NOAA 2019) to characterize climatic changes in the study area. The weather station (GHCND:USC00050848) is located approximately 1700m from the closest sampling site and 4000m from the farthest sampling site. Maximum daily temperature was acquired from the weather station for the general sampling season (May 1st to October 31st). To determine how climatic variables differed between all sampling years (1957, 1958, 2021, 2022) and throughout the sampling season, we used a quadratic regression with maximum daily air temperature recorded from the local weather station as the response variable and year, Julian date, and Julian date squared as predictor variables. We used the emmeans package (Lenth 2022) to obtain estimated marginal means.

To see how aspect temperatures differed from one another we used a quadratic regression with surface temperatures and air temperatures collected in the field as the response variable and year, Julian date, and Julian date squared as predictor variables. We used the emmeans package (Lenth 2022) to obtain estimated marginal means.

*Question 1: Have ant species distributions in Gregory Canyon, Boulder, CO changed since sampling done by Browne and Gregg in 1957/58?*

Historical field collections: Sites were sampled from late May to late October in 1957 and 1958. Ants were hand collected by opening soil or thatch nests, turning rocks or logs, and picking up foragers, which is an effective method of sampling ant diversity (Agosti et al., 2000). The length of time spent collecting in each site by Browne and Gregg is unknown, but collection times ranged from 8:30 AM to 2:30 PM. Ants were identified to species (Creighton 1950) then stored in 85% ethyl alcohol.

Georeferencing & Occurrence Records: Browne and Gregg (1969) included a map of Gregory Canyon and the surrounding area, with drawn polygons of the sampling locations. To determine the locations of the sites and ant species collections based on a modern coordinate system, we used the Georeferencing Tool in ArcGIS (Figure 1; ESRI 2011). We projected the historical aerial images to NAD 83, UTM 13N. Other layers used for georeferencing were a City of Boulder Open Space and Mountain Parks streams and ditches layer (City of Boulder 2021), a Bureau of Land Management Public Land Survey System First division layer (BLM 2018), and NAIP (National Agriculture Imagery Program) imagery from 2015 (United States Forest Service, National Agriculture Imagery Program 2015). 8 control points (locations that can be accurately identified on the historical map and in contemporary imagery and coordinates) were used and a 2nd order polynomial transformation. To verify and adjust images the control points used included landmarks, road intersections, PLSS lines, and stream confluences. Polygons of the sampling sites and points of species collections were then created.

Contemporary field collections: Each site was sampled on approximately the same date (+/- 1.22 days) that it was sampled in the original study. Fieldwork was conducted from late May to late October of 2021 in Gregory Canyon for roughly 6 hours during daylight hours (8:00 AM to 5:00 PM). We collected ants using the same methods as Browne and Gregg (1969). Ants were taken back to the lab for curation and species-level identification using the dichotomous keys (Mackay & Mackay 2002; [www.antwiki.org](http://www.antwiki.org), 2015; Gregg 1963). Browne and Gregg's species determination were updated to contemporary names.

Species identifications were compared to those of Gregg's using his collections housed at the Field Museum as well as the synoptic collection at the CU Boulder Museum of Natural History to ensure that species-level identifications matched.

*Question 2: Does ant species composition still vary between aspects (north-facing slopes, south-facing slopes, canyon bottom)?*

To test whether community composition differed between timeframes and across aspects, we used a richness-based approach using the site occurrence of species. We next analyzed the effects of timeframe, aspect, and percent tree cover on ant species composition by site using a permutational multivariate analysis of variance (PERMANOVA) on distance matrices (Anderson 2001). This analysis was only done with the historical data and the 2021 samples. We first transformed ant species composition into a distance matrix using Jaccard's dissimilarity based on incidence (presence/absences). We determined significance using a PERMANOVA using the adonis function (Oksanen et al 2019) with 999 permutations and used an NMDS (non-metric multidimensional scaling; Oksanen et al 2019) to visualize the differences. To determine which species are driving the compositional differences between time periods we used pairwise comparisons using the *simpser* function from the *vegan* package (Oksanen et al 2019) for each aspect to find the contribution of each species to the overall dissimilarity.

To determine the extent to which changes in species composition between historical and contemporary time periods could be attributed to simply short-term fluctuations in ant communities, we randomly selected and resampled a subset of 8 sites (2 canyon bottom, 3 north-facing slope, 3 south-facing slope) in the summer of 2022. To determine the level of interannual variation in ant community composition between years we used a permutational multivariate analysis of variance (PERMANOVA) on distance matrices (Anderson 2001). This analysis was only done with the subset of 2021 and 2022 sites. We first transformed ant species composition into a distance matrix using Jaccard's dissimilarity based on incidence (presence/absences). We determined significance using a PERMANOVA using the adonis function (Oksanen et al 2019) with 999 permutations.

*Question 3: Is temperature driving the changes in ant species site occurrence in Gregory Canyon?*

Measures of environmental variables (air temperature and surface temperature - °C) were recorded for each individual ant collection. Air temperatures were recorded approximately 6-8 inches above the collection location of the ant using a Kestrel Instruments 3500 weather meter. Surface temperatures were recorded at the location where the ant was collected using a Fluke 62 MAX+ Handheld Infrared Laser Thermometer. Specifically, if an ant was found under a rock, the surface temperature would be immediately measured where the ant is and then the ant would be collected. Ant collections were categorized as surface foragers (individuals who were on the surface and exposed to ambient conditions) or non-surface foragers (individuals who were in a thermally buffered environment when they were collected; e.g. under a rock, inside a log, etc.).

To determine if temperature was driving the differences in species composition between timeframes, we compared the ranges of temperatures ants were collected in, to their change in site occurrence. We used temperature data and site occurrence data from both 2021 and 2022 collections for temperature analyses. Species used in these analyses were ones that had 10 or more observations and were present in both timeframes. To standardize the change in site occurrence and account for variability between more abundant species and more rare species, z-scores ( $z\text{-score} = [\text{observational}$

value - mean value]/standard deviation of values) were used. Z-scoring centers the mean for each value of change in site occurrence and converts the measurements into standard deviations away from the mean.

We regressed species change in site occurrence between contemporary and historical time periods against the 95% range of species foraging surface and air temperatures and against the 95% range of air temperatures for ants in both thermally buffered and exposed environments. Additionally, we tested the relationship between site occurrence and the 95% range of air and surface temperatures for individuals in thermally buffered environments and air temperatures for all collected individuals.

*Question 4: Does change in tree cover in Gregory Canyon relate to changes in ant species site occurrence?*

*Tree Cover Quantification:*

The historical images used were acquired during flights commissioned by the U.S. Forest Service and Soil Conservation Service in 1938 and 1940. The contemporary images used were acquired by NAIP (National Agriculture Imagery Program) in the fall of 2015 (United States Forest Service, National Agriculture Imagery Program 2015). Images were classified by Rodman et al 2019. (see Acknowledgments). To quantify percent tree cover at each site we calculated the sum of 1 m pixels on the classified image layers for 1938 and 2015 (1 = forest presence, 0 = forest absence) using the zonal statistics tool in ArcGIS, then divided the sum by the total number of 1 m pixels within each site (ArcGIS).

To determine if tree cover was driving the changes observed in community composition, we used occurrence data from all collection years and aerial imagery of Gregory Canyon from 1940 and 2015. Percent tree cover was quantified for each site and each timeframe by dividing the number of 1m pixels in which tree cover was present in a site by the total number of 1m pixels within that site. To determine how percent tree cover differed between timeframes and across aspects, we ran a two-way ANOVA with percent tree cover as the response variable and timeframe and aspect as the predictor variables. We then used a post-hoc Tukey test to determine which aspects differed. Change in percent tree cover (2015 percent tree cover - 1940 percent tree cover) for each site was compared to species richness.

We used a linear regression with species richness as the response variable and percent tree cover and timeframe as predictor variables. We used linear regressions to determine if there was a significant relationship between the change in percent tree cover by site and the change in species richness by site. To determine if tree cover influenced community composition, we used the `vegdist` function from the `vegan` package (Oksanen et al 2019) to get Bray-Curtis dissimilarity values, then the dissimilarity value of each site to the change in percent tree cover.

*Question 5: Does thermal tolerance vary between aspects?*

Thermal tolerance testing to determine critical thermal minima and maxima was conducted in 2022. A subset of species that ranged from widely abundant to less abundant were selected. Ants were collected from multiple aspects (if they colonized multiple aspects) and over the course of 4 sampling rounds (early June, mid/late July, late August, mid/late October). Every aspect was represented for each sampling round.

At least 10 ants were collected in 50ml centrifuge tubes from an individual colony and taken back to the lab for thermal tolerance testing. All ants were tested within 6 hours of collection. We used a dynamic ramping protocol that is commonly used to measure thermal tolerance (Bujan et al. 2020A; Roeder et al. 2021). We placed 10 random ants from a colony into individual 1.5 ml microcentrifuge tubes that had been modified with cotton to remove a thermal refuge in the top. 5 of these tubes had the critical thermal maxima tested, and 5 tubes had the critical thermal minima tested. Tubes were placed in Thermo-Scientific dry baths (one for cold tolerance testing and one for heat tolerance testing). The ants were checked every 10 minutes to see if they had reached their critical thermal limit by rotating the vials and looking for a righting response (Diamond et al. 2012; Kaspari et al. 2015). The heat dry bath was increased 2°C every 10 minutes from a baseline of 36°C. The cold dry bath was decreased 2°C every 10 minutes from a baseline of 20°C. The process was repeated until all ants had lost muscle control. To determine if ant species critical thermal limits varied between aspects, we used a two-way ANOVA with CTmin/CTmax as the response variable and sampling round and aspect as the predictor variables. We then used a post-hoc Tukey test to determine which aspects differed (if any).

## **Results:**

### *Environmental Conditions:*

Soil moisture differed significantly between aspect ( $F = 27.06$ ,  $df = 2$ ,  $P < 0.001$ ) and timeframes ( $F = 87.22$ ,  $df = 1$ ,  $P < 0.001$ ) with percent moisture being on average 9.96% lower in the contemporary time period compared to the historical. North-facing slope sites had the highest percent soil moisture (28.92% - historical; 14.21% - contemporary) and south-facing slopes had the lowest (15.52% - historical; 8.44% - contemporary). Maximum daily temperatures throughout the sampling season differed significantly between years ( $t = 2.013$ ,  $P = 0.045$ ) with temperatures being higher on average for 2022. Contemporary surface temperatures differed between all aspects ( $P < 0.01$ ), with south-facing slopes having higher surface temperatures on average and north-facing slopes having the lowest average temperatures. Contemporary air temperatures differed between aspects ( $P < 0.001$ ) except between Canyon Bottoms and North-facing slope sites. South-facing slope sites had higher air temperatures overall.

*Question 1: Have ant species distributions in Gregory Canyon, Boulder, CO changed since sampling done by Browne and Gregg in 1957/58?*

Overall, we documented 57 species of ants, an increase from the 43 species documented in the historical study (Table 1). 31 species were found in both time periods. 10 ant species decreased in their site occurrence, 20 increased, and 1 exhibited no change (Figure 2). Additionally, after accounting for differences in sampling effort between the two time periods using rarefaction via the iNEXT package in R, species richness still exhibits an overall increase (Figure 3).

*Question 2: Does ant species composition still vary between aspects (north-facing slopes, south-facing slopes, canyon bottom)?*

The PERMANOVA indicated that overall communities differed significantly between aspects (Figure 4;  $F = 22.42$ ,  $df = 2$ ,  $P < 0.001$ ), among timeframes ( $F = 42.42$ ,  $df = 1$ ,  $P < 0.001$ ), and with percent tree cover ( $F = 108.73$ ,  $df = 1$ ,  $P < 0.001$ ). There was a significant interaction between timeframe and aspect ( $F = 3.51$ ,  $df = 2$ ,  $P = 0.009$ ), timeframe and percent tree cover ( $F = 26.09$ ,  $df = 1$ ,  $P < 0.001$ ), as

well as aspect and percent tree cover ( $F = 3.94$ ,  $df = 2$ ,  $P = 0.008$ ). The subset of sites sampled in 2022 did not differ significantly from 2021 sites (Figure 5). The dissimilarity in community composition between the historical and the 2021 contemporary time periods was 0.62. The bray-curtis dissimilarity value for the 2021 and 2022 subset of sites was 0.32 compared to 0.68 for the same sites for the historical and 2021 communities.

*Question 3: Is temperature driving the changes in ant species site occurrence in Gregory Canyon?*

There was a significant positive relationship between change in species site occurrence and both the range of surface temperatures ( $t = 2.75$ ,  $P = 0.023$ ; Figure 6A) and the range of air temperatures ( $t = 3.14$ ,  $P = 0.01$ ; Figure 6B) foragers were collected at. There was not a significant correlation between change in site occurrence and the 95% range of surface and air temperatures experienced by ants in thermally buffered environments. The number of sites a species occurred in was not related to the 95% range of surface temperatures for buffered ants ( $t = 1.92$ ,  $P = 0.09$ ; Figure 6C); however, it was positively related to the range of air temperatures for buffered ants ( $t = 3.52$ ,  $P = 0.007$ ; Figure 6D). When looking at air temperature for both ants that were in buffered and exposed environments, there was a significant positive relationship for both change in site occurrence ( $t = 2.28$ ,  $P = 0.033$ ; Figure 6E) and the number of sites a species occurred in ( $t = 3.501$ ,  $P = 0.002$ ; Figure 6F).

*Question 4: Does change in tree cover in Gregory Canyon relate to changes in ant species site occurrence?*

Contemporary tree cover was 20% greater than it was in 1940 ( $F = 19.50$ ,  $df = 1$ ,  $P < 0.001$ ). Additionally, percent tree cover differed significantly between aspects ( $F = 10.87$ ,  $df = 2$ ,  $P < 0.001$ ) with roughly 20% more tree cover on north-facing slopes than south facing slopes. However, there was not a significant interaction between aspect and timeframe, nor was there a relationship between change in species richness and change in percent tree cover. There was a significant positive relationship between species richness and percent tree cover ( $t = 3.68$ ,  $P < 0.001$ ; Figure 7), but no relationship with timeframe. However, there was a significant interaction between percent tree cover and timeframe ( $t = 2.07$ ,  $P = 0.043$ ). There was no relationship between site species composition and change in percent tree cover (Figure 8).

*Question 5: Does thermal tolerance vary between aspects?*

Minimum critical thermal limits for the ant community differed significantly between aspects ( $F = 32.05$ ,  $df = 2$ ,  $P < 0.001$ ). The canyon bottom minimum thermal tolerances did not differ from the south slopes. Maximum critical thermal limits for the ant community differed significantly between aspects ( $F = 6.37$ ,  $df = 2$ ,  $P < 0.002$ ). The canyon bottom maximum thermal tolerances did not differ from the south slopes. There were some between species differences in critical thermal minima and critical thermal maxima (Figure 9).

**Discussion:**

Browne and Gregg sought to determine differences among ant communities in Gregory Canyon due to its differing habitats. First, I decided to quantify how these habitats differed in terms of their environmental conditions. I found that soil moisture was lower among south slopes and air/surface temperatures were higher. North slopes tended to have higher soil moisture and lower air/surface temperatures. The canyon bottom sites did not differ from the north slopes, but did differ from the



south slope sites. This confirms, that between timeframes, the south slopes still represent a more arid environment, while north slopes are cooler.

#### Species richness and distributions:

Ant species richness increased from 43 species documented in the historical study to 57 species for the contemporary collections (Table 2). The observed increase in ant species richness may be a consequence of differences in sampling effort between studies, which we accounted for using rarefaction. Another study conducted in proximity to Gregory Canyon documenting ant community differences between ecotones (plains-foothills) identified a total of area 25 ant species (Conklin 1972). A larger scale study documenting elevational shifts in ant species in the Front Range documented 105 ant species in their 2010-2012 collections (Szewczyk and McCain 2019). Other studies looking at changes in ant communities over time have found modest increases in ant abundance (Kaspari et al. 2019). Additionally, they found only a marginal increase in local diversity 20 years after the initial sampling (Kaspari et al. 2019). Increases in local ant abundance could be a consequence of marginal increases in local temperatures as it can increase ant worker activity (Prather et al 2018). While many ground ant communities are regulated by temperature, the changes in the community we see may be attributed to competitive interactions between more abundant dominant species that primarily occupy shrubland/forested areas (Retana & Cerda 2000). We only documented one invasive species, *Tetramorium immigrans*, which was mainly found in areas that had higher levels of human traffic (along roadways, parking areas, or major trail junctions).

We predicted that species richness will increase in Gregory Canyon, with more generalist or abundant species will increase in their site occurrence (or distribution) throughout the canyon (Finderup et al. 2019). We see that most species increased in their distribution throughout Gregory Canyon since their site occurrence increased since the time of the historical study. This could also be tied to increases in ant abundance/activity from increased temperatures (Prather et al. 2018). We see a large increase in site occurrence for more dominant competitive generalist species such as *Tapinoma sessile* (Menke et al. 2010), *Lasius pallitarsis*, *Liometopum luctuosum*, and *Camponotus laevigatus*, which has been documented in other studies as well (Roeder et al. 2021). Reductions in more specialized species, such as the seed dispersers *Aphaenogaster occidentalis* and *Pheidole pilifera* could have implications for ecosystem functioning as reductions in seed dispersing species means that plants will limit their distribution and abundance (Ness et al. 2009; Warren et al. 2010). Shifting competitive interactions could influence community regulation and ecosystem services. There is evidence that changes we see in community composition are driven by competitive interactions from increasingly abundant dominant species (Retana and Cerda 2000). Additionally, under altered temperature regimes more cold-adapted species are outcompeted by the increasing warm-adapted species (Urban et al. 2012). However, this is not necessarily the pattern we see in this study as many cold-adapted species increased in their site occurrence as well (e.x. *Stenamma diecki*), which could be potentially tied to increasing tree cover providing additional suitable microhabitats for species to colonize.

The contemporary ant community is more similar in its composition across sites and aspects than the historical community. In the NMDS plot we see that the historical communities have more spread (more distance between points) than the contemporary communities indicating that historical communities had more differences in species composition across sites, while the contemporary community tends to be more similar or homogenized across sites. A lot of what might be driving this

differentiation between the communities is the number of unique species that occurred in each time period. We see that the points for the contemporary data tend to be very tight together showing that the species representation is more similar across sites, suggesting that only specific species might be occurring in certain environments. This may be due to these species having environmental tolerances that more closely match this habitat. It is possible that temperatures are potentially limiting the species that can colonize in these specific locations. Additionally, the expansion of dominant species across Gregory Canyon, may explain some of the differences we see in the ant communities between timeframes.

Habitat openness is a key driver of variation in ant communities (Andersen 2019). Increases in tree cover in Gregory Canyon may lead to increases in species richness (Gaytán et al 2021; Martins et al 2022). While there was not a significant relationship between the change in species richness and the change in tree cover, there was a relationship between contemporary tree cover and species richness, meaning that habitat openness and temperature is likely acting as an environmental filter for ant species in Gregory Canyon.

#### Temperature:

At the microsite level, species that foraged in a wider range of surface and air temperatures exhibited larger changes in their site occurrence. However, the surface temperatures species were present at in thermally buffered environments did not relate to the number of sites species occurred in, indicating that species are able to behaviorally thermoregulate by retreating to nests and reducing the temperature variation they experience (Jones and Oldroyd 2006). Since the range of temperature variation underground is largely dampened, buffered nest temperatures may not be the best predictor of species persistence. Species present in a wider range of air temperatures exhibited increased in their site occurrence and occurred in a greater number of sites throughout Gregory Canyon.

Temperatures are increasing in the Boulder area; however, tree cover in Gregory Canyon may complicate the interpretation of temperature influence on the ant community. Increasing tree cover throughout the canyon may be buffering the impact of increased temperatures (De Frenne et al. 2019; Kašpar et al. 2021). Another consideration is that the thermal space (the range of air and surface temperatures recorded for species collections) we documented species in, may not be the same as it was in the historical study. Indeed, some ant species have exhibited plastic (Bujan et al. 2020B) or adaptive responses (Diamond et al. 2017), to temperature, indicating that the species documented in this study potentially are utilizing different thermal space than they were historically.

Species thermal physiology may be determining the increases and decreases in species site occurrence. According to thermal performance theory, insect physiological performance increases with increasing temperature until it reaches a maximum; then performance quickly declines, eventually resulting in death (Angilletta 2009; Kingsolver and Huey 2008). Following this theory, it is possible that we are seeing increases in ant species richness and abundance, but with continued temperature increases locally, an abrupt decline in species persistence may result. Alternatively, warming temperatures could impact the windows of time for favorable foraging temperatures (Jayatilaka et al. 2011; Roeder et al. 2022). Changes in community composition over time have been linked to increasing temperatures (Lewthwaite et al. 2017). Species distributions can be limited by their physiological tolerances, as species decreasing in their ranges tend to have lower thermal tolerances (Roeder et al. 2021). Additionally, thermal tolerance can influence daily foraging patterns, with species with lower

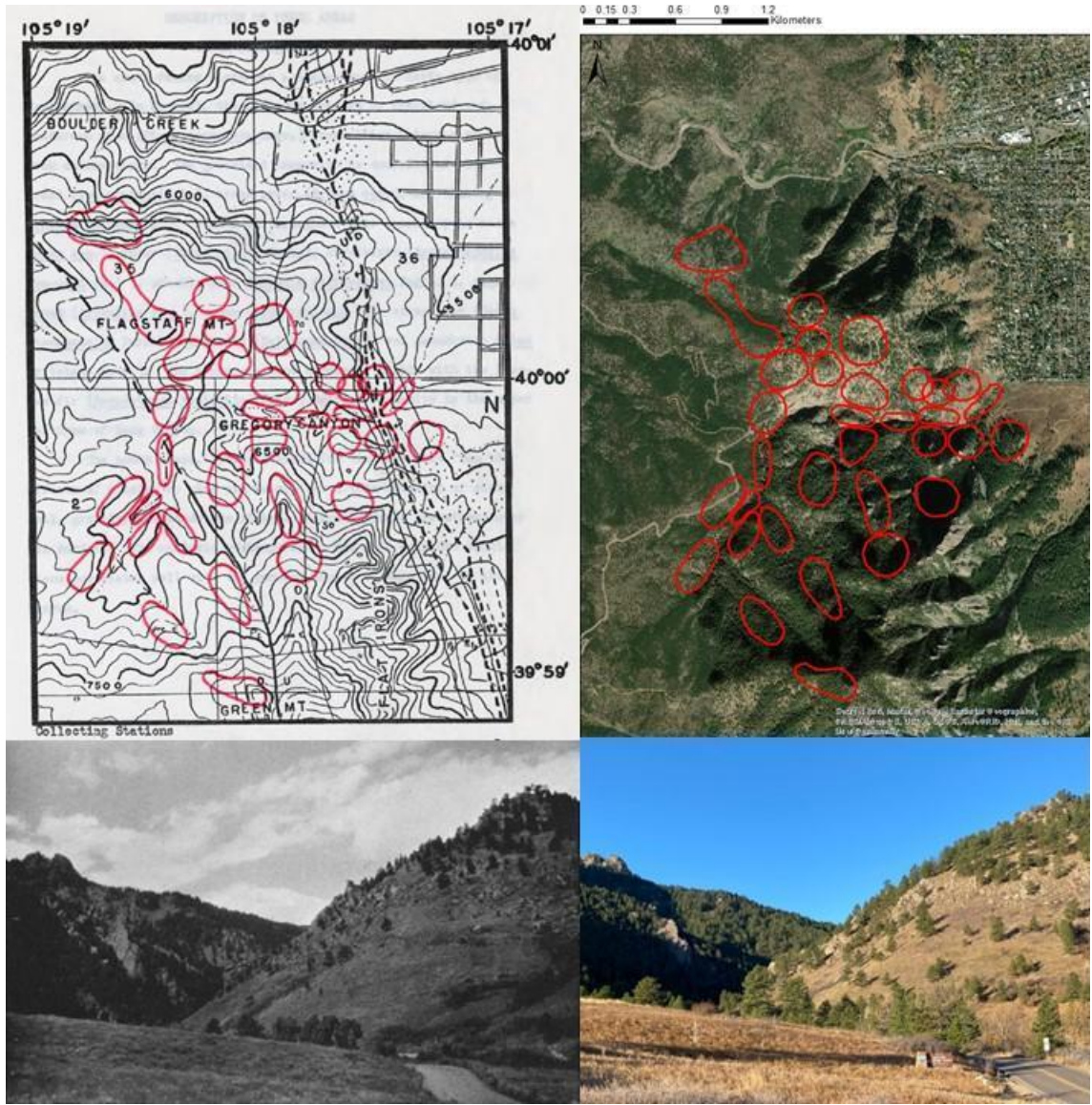
thermal tolerances showing preference to more thermally buffered areas (Wittman et al. 2010). The range of temperatures that a species can forage in increases the likelihood of a species occupying a site (Braschler et al. 2020), which is evident in our results. Increases in temperature increases ant activity and running speed (Gillooly et al. 2001; Hurlbert et al. 2008; Prather et al. 2018), which increases the likelihood of ants entering new areas of the canyon. The biggest implication for increased temperatures is shifts in competitive interactions. Species that are heat-intolerant typically are negatively affected by interactions with more dominant heat-tolerant species (Diamond et al. 2017; Retana and Cerda 2000), indicating that increased temperatures will increase competition with heat-intolerant species (Warren and Chick 2013) potentially leading to declines in the range of heat-intolerant ant species.

Thermal tolerance testing indicated that some species in Gregory Canyon have variable thermal physiology based upon the environmental conditions that they experience. We found that conditions between the south-facing slopes and canyon bottoms did not differ significantly, meaning that ant species might be exhibiting similar temperature conditions. However, they are likely experiencing differing moisture conditions that acts as a filter on the community since canyon bottoms generally represent a riparian habitat. This is the same pattern that we see in species thermal tolerance, as species who have a closer association to the environmental conditions have physiology and thermal limits that match the temperature fluctuations associated with that environment. Overall, temperature and habitat structure changes within Gregory Canyon have acted as an environmental filter for ant species, many of them limited by their physiological tolerances resulting in the different ant community that we see today compared to the community sampled by Browne and Gregg.

### Conclusions:

Historical data gives us insight on past community structure and functioning. Browne and Gregg stated in their 1969 publication that, “there are no physical barriers in Gregory Canyon to prevent the spread of colonizing individuals.” Increasing presence of generalist competitors indicate that the colonization of individuals is not being limited in this area. Temperature and habitat structure changes within Gregory Canyon have acted as an environmental filter for ant species, many of which are limited by their physiological tolerances, resulting in the different ant community that we see today compared to the community sampled by Browne and Gregg. Increases in the richness and occurrence of thermally generalist species may destabilize ecosystem functioning (Wang et al. 2021), resulting in the decline of other species (McKinney and Lockwood 1999). How species interact may determine the structure and outcome of ant communities in the face of climate change. Future studies should focus on how species are occupying thermal space and how it relates to competition, foraging behavior, and resource acquisition to better understand the mechanisms underlying changing communities.

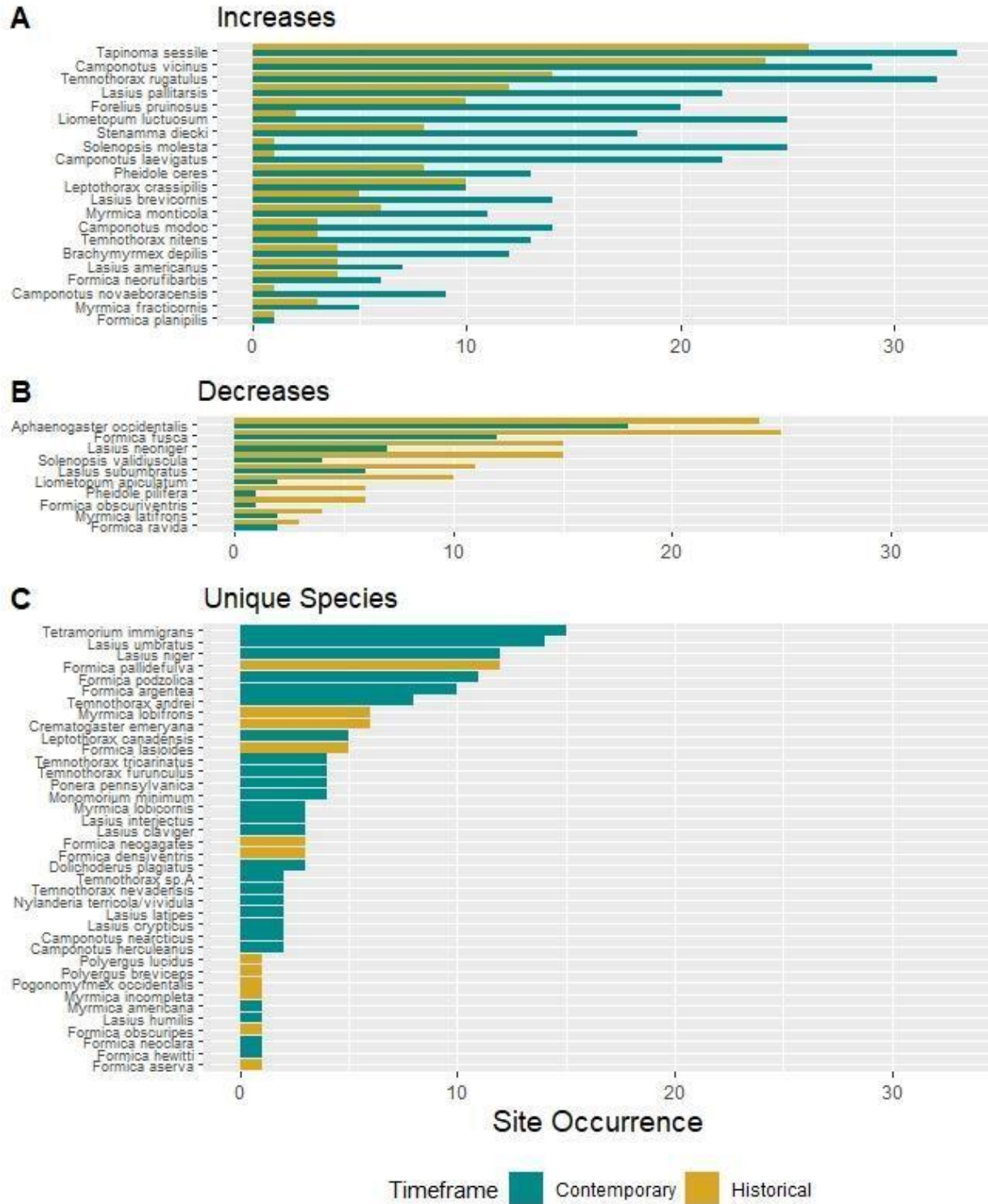
**Figure 1:** Top-left: the map of sampling sites from Browne and Gregg (1969). Top-right: georeferenced sampling sites overlaid on 2015 satellite imagery. Bottom-left: photograph of the entrance to Gregory Canyon included in the 1969 publication. Bottom-right: a 2020 photograph of the entrance of Gregory Canyon from roughly the same location (Photo credit: Julian Resasco).



**Table 1:** Species richness observed (and jackknife estimation) for each aspect and timeframe.

	Historical	Contemporary
All Sites	43 (52.69)	57 (63.79)
North-facing Slope Sites	27 (32.57)	52 (65.93)
South-facing Slope Sites	32 (44.92)	39 (45.46)
Canyon Bottom Sites	28 (37.17)	34 (42.33)

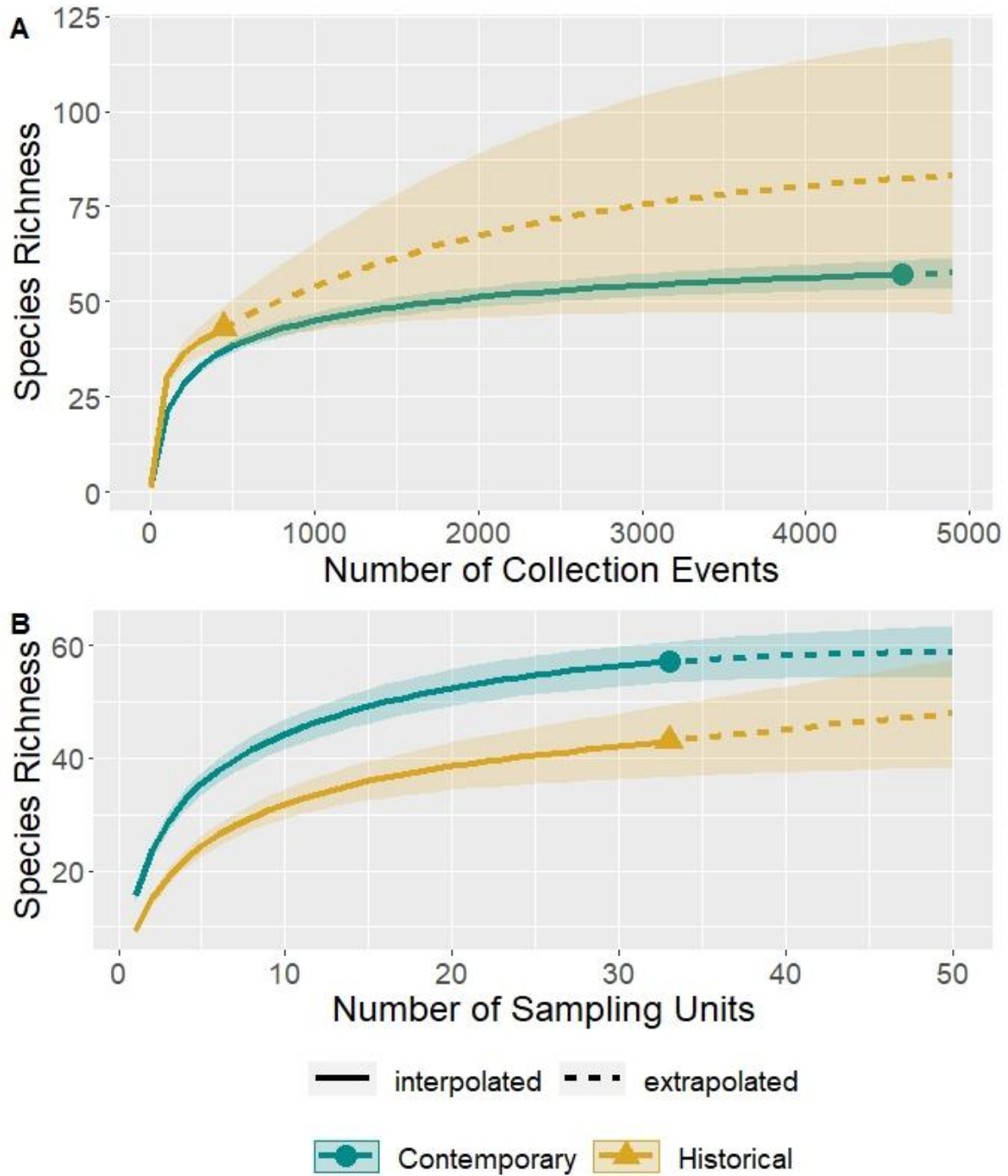
**Figure 2:** A) Observed increases in species site occurrence. B) Observed decreases in species site occurrence. C) Unique species site occurrence (species that were only found in one time period).



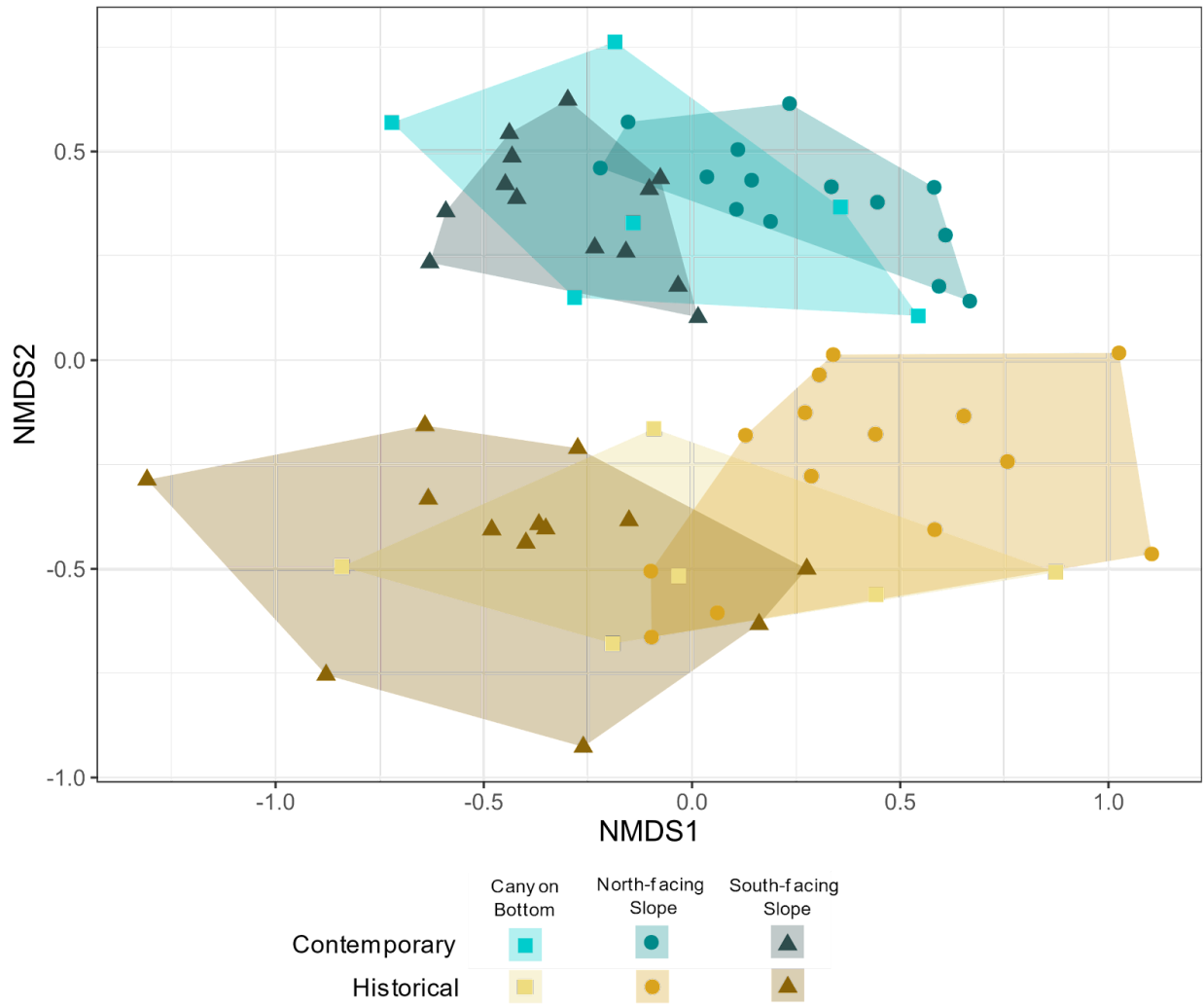
**Table 2:** Studies conducted in the Gregory Canyon area looking at the ant community.

Species	1936	1956	1957-58	1965-67	2021	Species	1936	1956	1957-58	1965-67	2021
Aphaenogaster occidentalis		X	X	X	X	Lasius latipes				X	X
Aphaenogaster rudis	X					Lasius neoniger		X	X	X	X
Brachymyrmex depilis		X	X		X	Lasius niger		X			X
Camponotus herculeanus		X			X	Lasius pallitarsis		X	X	X	X
Camponotus leavigatus			X		X	Lasius subumbratus		X	X		X
Camponotus modoc		X	X		X	Lasius umbratus				X	X
Camponotus nearcticus					X	Leptothorax canadensis		X			X
Camponotus novaeboracensis		X	X		X	Leptothorax crassipilis		X	X		X
Camponotus vicinus	X	X	X	X	X	Liometopum apiculatum	X		X		X
Crematogaster coarctata	X					Liometopum luctuosum			X	X	X
Crematogaster emeryana		X	X			Monomorium minimum					X
Crematogaster lineolata	X			X		Myrmica americana		X			
Dolichoderus plagiatus					X	Myrmica brevinodis		X			
Forelius pruinosus	X		X	X	X	Myrmica discontinua		X			
Formica altipetens		X				Myrmica fracticornis		X	X		X
Formica argentea	X			X	X	Myrmica incompleta	X		X		
Formica aserva		X	X			Myrmica latifrons			X	X	X
Formica criniventris	X					Myrmica lobicornis		X			X
Formica densiventris		X	X			Myrmica lobifrons		X	X		
Formica fusca		X	X	X	X	Myrmica monticola		X	X		X
Formica hewitti					X	Myrmica scabrinodis	X				
Formica incerta	X					Nylanderia terricola/vividula					X
Formica integra		X				Pheidole ceres	X		X		X
Formica lasioides		X	X	X		Pheidole pilifera	X		X	X	X
Formica limata				X		Pogonomyrmex occidentalis	X		X		
Formica neoclara					X	Polyergus breviceps	X		X		
Formica neogagates			X			Polyergus lucidus			X		
Formica neorufibarbis		X	X		X	Ponera pennsylvanica		X			X
Formica obscuripes			X	X		Solenopsis molesta	X		X		X
Formica obscuriventris		X	X	X	X	Solenopsis truncorum		X			
Formica pallidefulva		X	X	X		Solenopsis validiuscula		X	X	X	X
Formica planipilis			X		X	Stenamma diecki		X	X		X
Formica podzolica					X	Stigmatomma pallipes		X			
Formica ravida			X		X	Tapinoma sessile	X	X	X	X	X
Formica rubicunda		X				Temnothorax andrei					X
Formica subpolita	X					Temnothorax furunculus					X
Lasius americanus	X		X	X	X	Temnothorax nevadensis					X
Lasius brevicornis		X	X	X	X	Temnothorax nitens			X		X
Lasius claviger		X		X	X	Temnothorax rugatulus	X	X	X	X	X
Lasius crypticus					X	Temnothorax sp.A					X
Lasius humilis					X	Temnothorax tricarinatus					X
Lasius interjectus					X	Tetramorium immigrans					X

**Figure 3:** A) Extrapolated and interpolated species richness based off of the number of collections for each time period. B) Extrapolated and interpolated species richness for contemporary and historical collected based off of the raw incidence data.

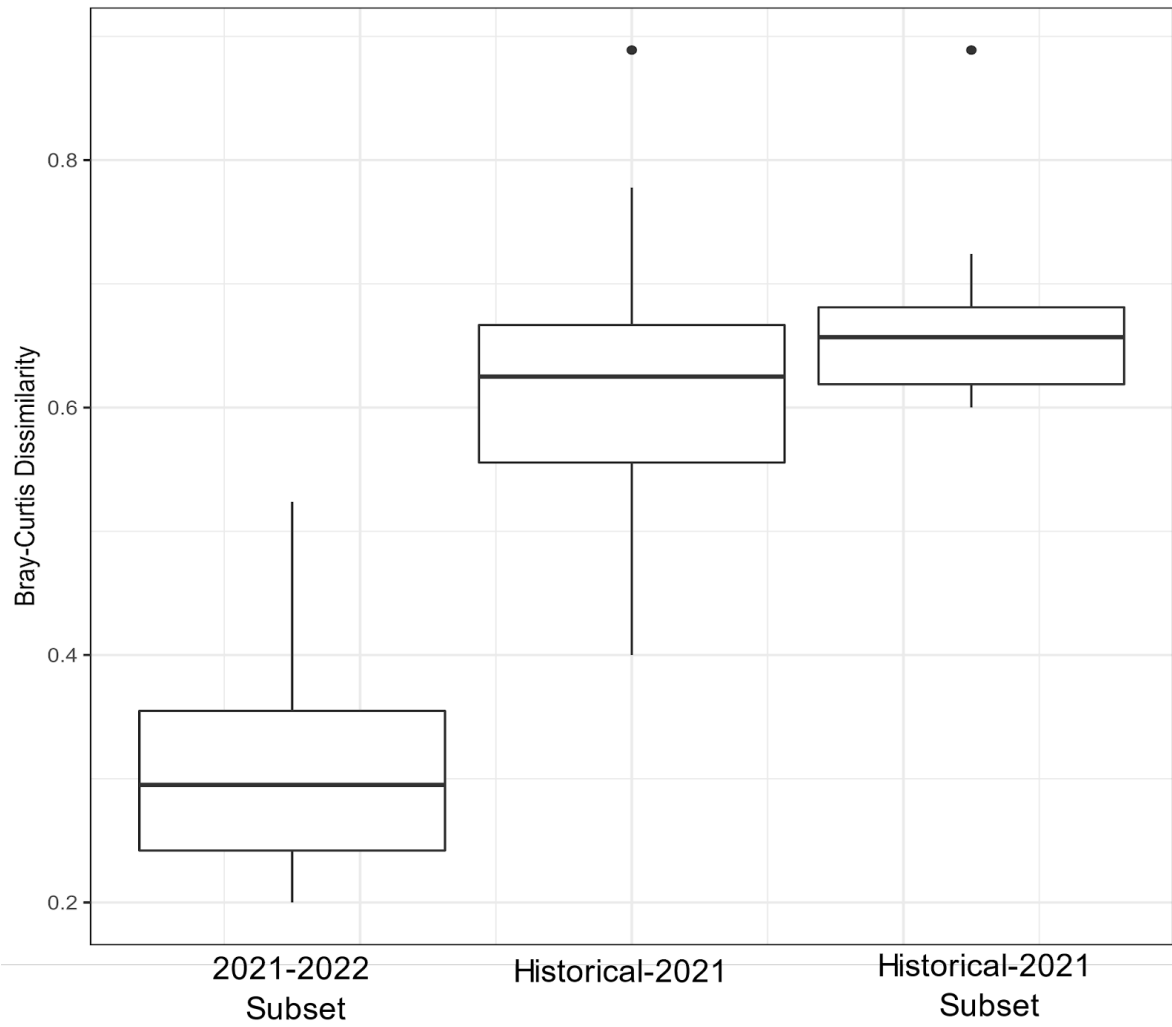


**Figure 4:** Dissimilarity of ant species composition between the historical and contemporary communities across sites. Each polygon represents the ant community for the historical samples and the contemporary. Within each polygon, the dots represent the individual sites. Polygons that do not overlap indicate that each community is fairly distinct in its representation of species.

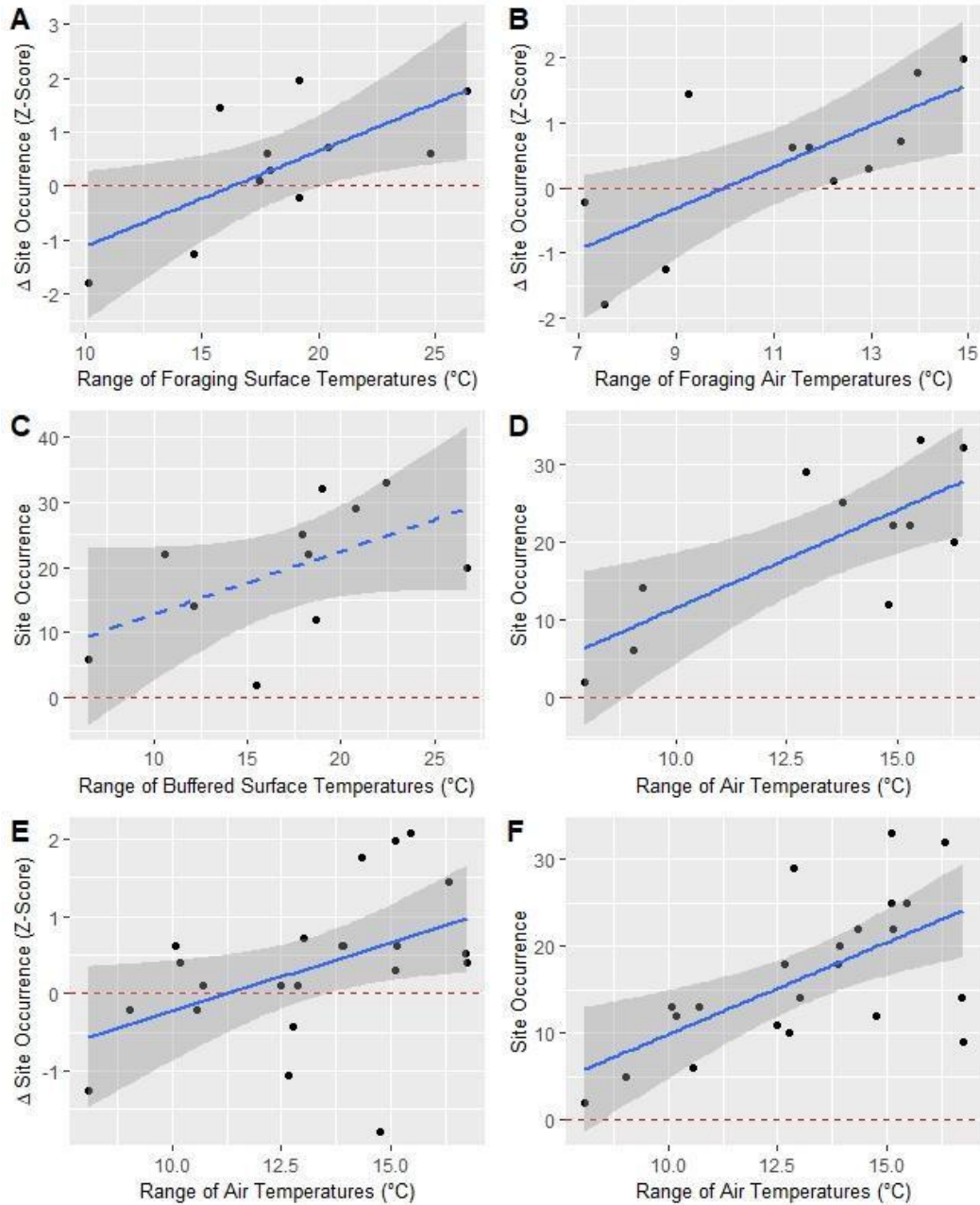




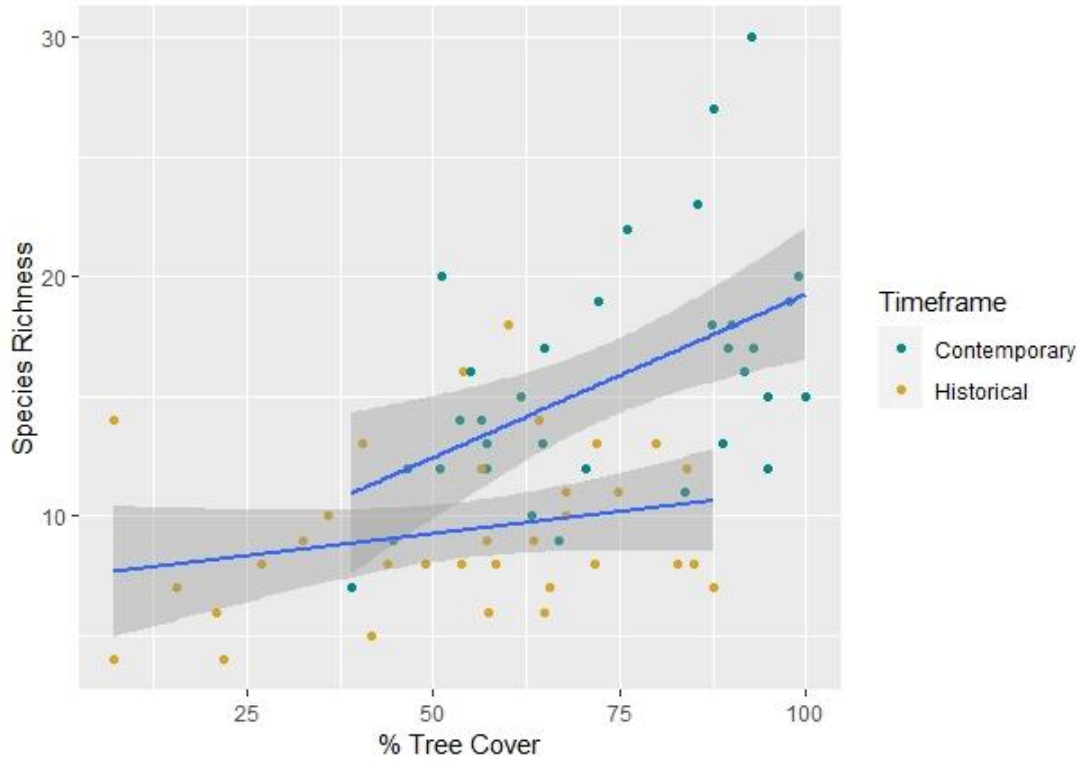
**Figure 5:** Bray-Curtis dissimilarity of the 2021-2022 subset of 8 sites, all 33 sites sampled historically and in 2021, and using the sites that were resampled.



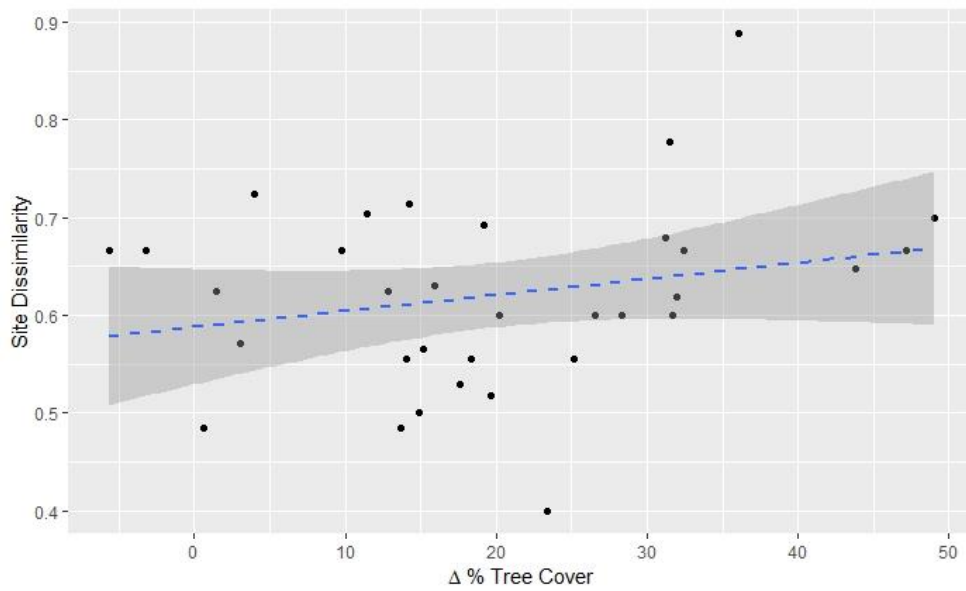
**Figure 6:** A) Change in site occurrence by the 95% range of surface temperatures for foraging ants. B) Change in site occurrence by the 95% range of air temperatures for foraging ants. C) The number of sites a species occurred in contemporarily by the 95% range of surface temperatures for ants found in thermally buffered environments. D) The number of sites a species occurred in contemporarily compared to the 95% range of air temperatures for ants collected from thermally buffered environments. E) Change in site occurrence compared to the 95% range of air temperatures ant species were collected at (exposed and buffered) F) The number of sites a species occurred in compared to the 95% range of air temperatures ant species were collected at (exposed and buffered).



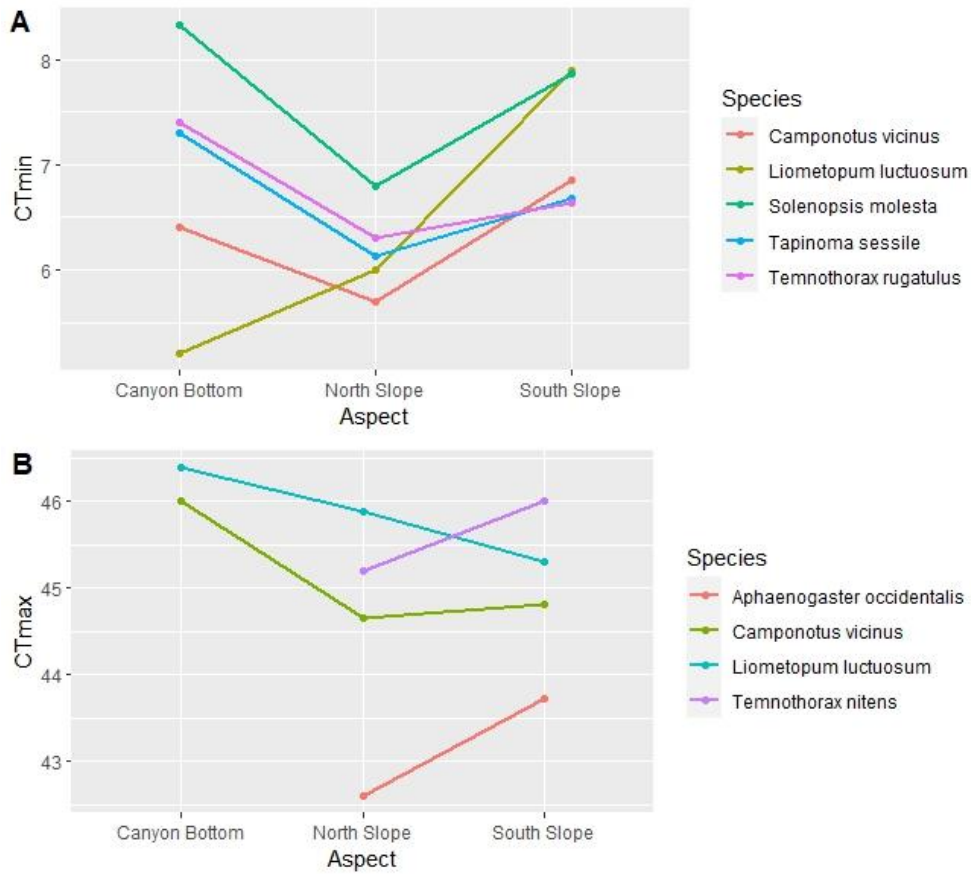
**Figure 7:** Percent tree cover and observed species richness for both the contemporary and the historical timeframe.



**Figure 8:** Bray-Curtis dissimilarity between historical and contemporary sites and site percent change in tree cover.



**Figure 9:** A) Critical thermal minima for ant species collected on all three aspects (canyon bottom, north-facing slope, south-facing slope). B) Critical thermal maxima for ant species collected on multiple aspects.



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