Effects of Mound Nests of the Ant *Formica podzolica* on Soil Moisture, Nitrogen, and Vegetation

By
Madison Sankovitz
Ecology and Evolutionary Biology, University of Colorado at Boulder

Defense Date: March 24, 2017

Thesis Advisor:
Dr. Michael Breed, Ecology and Evolutionary Biology

Defense Committee:
Dr. Michael Breed, Department of Ecology and Evolutionary Biology
Dr. Pieter Johnson, Department of Ecology and Evolutionary Biology
Dr. Sarah Massey-Warren, Program for Writing and Rhetoric

Table of Contents

Abstract

Ants are widely regarded as 'ecosystem engineers' because they change the biological, chemical, and physical properties of the soil around their nests through nest construction and contributions to nutrient cycling. These processes were investigated in a species of alpine ant, *Formica podzolica*, to quantify its effects on soil structure and vegetation composition in an alpine ecosystem in Nederland, Colorado. Measurements of vegetation percent cover, biomass, species diversity and abundance, soil moisture and pH, and ^{15}N in plants were taken at various distances from nests. A stable isotope analysis was utilized to determine the amount of ^{15}N in vegetation surrounding nests, which provides information on the relatedness of ants and vegetation in a trophic web. Results showed that proximity to a nest had a positive influence on plant abundance and soil moisture. Additionally, high plant ^{15}N concentrations near nests, compared to control sites (away from nests), aligned with high ^{15}N in ant samples. *F. podzolica* has relatively large-scale impacts on the alpine ecosystem surrounding their nests. This research reveals how the daily foraging and excavation activities of an ant colony can lead to long-term changes in soil and vegetation structure, and suggests that *F. podzolica* ants are having similar ecosystem effects across North America.

Introduction

Ants as Ecosystem Engineers

Ants constantly modify their surrounding ecosystems. Just as organisms change and grow throughout their lives, whole ecosystems constantly morph; they shift in response to outside pressures and fluctuate as the needs of their inhabitants change. Although dwarfed by large-scale drivers of environmental change such as human-caused pollution and weather patterns, insects often very effectively modify the world around them (Jones et al. 1994). They are incredibly diverse in form and function, existing in almost every niche and habitat imaginable on Earth. Some insects (such as butterflies and house flies) are solitary, feeding and traveling on their own. Others are social and rely on collaboration within a colony to successfully collect food, take care of young, and build a protective nest. Social insects, such as ants, are often considered by scientists as ‘ecosystem engineers’: organisms that significantly create, change, or destroy a habitat (Jones et al. 1994). Ants do this by affecting the biological, chemical, and physical properties of the soil in and around their nests through nest construction and contributions to nutrient cycling (Frouz & Jilková 2008).

‘Ecosystem engineer’ may seem like a lofty term to describe a tiny ant, but these insects are mightier animals than their size suggests. At the colony level, ants are such massive forces of ecosystem change that scientists often depart from the study of the individual insect and instead regard the colony as an organism itself. The collective behavior and ecology of ant colonies have been studied to better understand complex systems such as cancer and the internet, which, like social insects, function successfully without centralized control (Gordon 2003). Over 9,500 species of ant are known (with thousands likely still undiscovered) and they make up about twenty percent of all terrestrial animal biomass on Earth (Shultz 2000). With these great numbers, their presence alone has significant consequences for ecosystems. But ants are also great workhorses; they live in constant motion, communicating and pursuing the wellbeing of the colony through foraging, caring for young, excavating a nest, and defending it from predators. Just as a human city imports large amounts of food and exports waste, so too does an ant colony. And just as humans constantly excavate land and build structures, so too do ants manipulate their environment to make habitable nests. The effects of the sum of their daily activities on their surrounding environments is an understudied topic, one that could offer clues into vegetation patterns, soil composition, and animals that cohabitate with ants (Beattie & Culver 1977, Culver & Beattie 1983, Sagers et al. 2000, Romero et al 2015).

Ants change the biological, chemical, and physical makeup of their surroundings through a variety of methods. Constant foraging brings an influx of food into the nest, usually in the form of pieces of vegetation, nectar, and small invertebrates. The import of nutrients and the resulting excretion of nitrogenous food waste (in the form of uric acid) changes the chemical, and therefore biological, composition of their nest area. For example, a study on *Azteca* ants (Sagers et al. 2000) found, through a stable isotope analysis, that although ants consume plants, they provide more carbon and nitrogen to their surrounding environment (by way of deposited debris) than they receive. Another way that ants add to the organic material in the surrounding area is by discarding corpses outside their nests (Beattie 1989, Sun et al. 2013). These daily activities often result in a shift of pH in the soil surrounding the nest towards neutral in acidic soils, and constant excavation of nests leads to more porous soil in nest areas (Frouz & Jilková 2008). Similarly, Nkem et al. (2000) found that nest building and foraging activities have both long- and short-term effects on soil through structural alterations, nutrient accumulation and release, and possible enhancements of soil organic matter.

Ants not only affect soil properties, but also influence vegetation community structure. Lesica and Kannowski (1998) showed that ant mounds offer a warm, aerated, nutrient-rich environment for plants. Consequentially, deserted nests create habitat for numerous species of plants that otherwise could not propagate in cold, water-logged soil. In addition to soil structural changes, ants influence plants by maintaining nutrient flow throughout their biomes. For example, wood ants (*Formica rufa*), increase nitrogen cycling in boreal forests of eastern Finland through foraging behaviors (Finér et al. 2012). These small arthropods have proven themselves to be quite powerful organisms of change, and scientists are continually discovering new information about how they operate within their complicated nest systems (Del Toro et al. 2015, Dorn 2014, Gonçalves et al. 2016).

Along with soil and vegetation alterations, ants can influence atmospheric conditions. In the modern age of climate change, it is more important than ever to understand how insects, which live among humans worldwide, play key roles in our changing Earth while under the influence of morphing atmospheric and geologic conditions. A recent study conducted in Arizona and Texas revealed that ants enhance the process of mineral dissolution in Ca-Mg silicate weathering approximately 50-300 times greater than controls, which assists in the gradual breakdown of atmospheric CO_2 (Dorn 2014). Dorn speculated that ant enhancement of Ca-Mg silicate dissolution might have been an influence on Cenozoic cooling since ants underwent a great expansion in biomass and diversity during that period. This evidence shows that ants affect ecosystems not only at the local scale, but at a much larger atmospheric scale than historically thought possible, which has strong implications for the future of our ecosystems in the face of climate change (Roura-Pascual et al. 2014, Jenkins et al. 2011).

Stable Isotope Analysis of Nitrogen

Stable isotope analysis identifies the isotopic signature, or distribution of certain stable isotopes, of a substance. This analysis allows scientists to trace nutrients, such as nitrogen, through a food web and link trophic levels (Feldhaar et al. 2010). ^{15}N is concentrated as nutrients pass up a food chain, causing animals at higher trophic levels to be more enriched with this isotope (on the order of ~3-5% per trophic transfer, DeNiro & Esptein 1981). Measurement of the relative abundance of nitrogen isotopes in various organisms, therefore, can provide an unbiased estimate of their position within a food web compared to other methods such as analyzing stomach contents, which can be highly variable (Atwell et al. 1998). Stable isotope analysis is an important process in understanding how ants interact with and change their ecosystems because it provides an analysis of their trophic level and role in nutrient cycling. The procedure uses technology to separate isotopes based on their mass, identifying the heavier isotopes such as ^{15}N resulting from ant excretions that exist in the soil and plants (UC Davis Stable Isotope Facility).

The eusocial organization of ant colonies presents interesting implications for the use of stable isotope analysis in determining the trophic position of a single ant, for high levels of trophic variation are present both within and among colonies. Tillberg et al. (2006) address this variation in a study of colonies of *Lasius alienus* and *Aphaenogaster rudis* from Trelease Woods, Champaign, Illinois. They found that *L. alienus* workers were enriched in ^{15}N when compared to their brood, a distinction created either by differences in resources assimilated by the two groups, or processing error. A difference was also found in the ^{15}N composition of ants taken from the two species' colonies, leading to the conclusion that they take prey from different trophic levels. The study was replicated at the colony level, and a difference in ^{15}N was also found among colonies of the same species, suggesting that a single species' trophic position can span up to two trophic levels. Tillberg et al. (2006) also examined methods of preparing specimens for stable isotope analysis, concluding that storage in ethanol may affect results. This study highlights the importance of taking care when selecting and preparing groups of eusocial insects to analyze for trophic level, because samples must be handled cautiously to yield correct results and high variation may occur within and among colonies (Tillberg et al. 2006). It also addresses the legitimacy of using stable isotope analysis in determining the trophic position of ants by examining the amount of nitrogen within various specimens of a food web.

The Study Species: Formica podzolica

Given their many thousands of species, ants come in a huge variety of form and behavior and live in vastly different terrestrial habitats around the world, including dry deserts, tropical rainforests, and high alpine ecosystems. A major example of this is *Formica*, the most common genus of ant in the north temperate zone, commonly known as wood ants, mound ants, and field ants. *Formica* ants have likely had such widespread success due to their ability to build nests in a broad range of habitats, including urban environments, heavily wooded areas, grasslands, swamps, and ocean-side areas across every continent except Antarctica ("Genus: *Formica*", 2017).

Formica nesting habits and behavior have been studied extensively, and many observations have contributed to understanding the genus' success in such a wide variety of environments. These ants reside in nests with either one (monodomous) or multiple (polydomous) mounds. There can be one (monogynous) or many (polygynous) egg-laying females. If multiple queens are present, this provides a great deal of genetic variation in colonies that can range from hundreds to millions of individuals (Hölldobler & Wilson 1990, Pamilo & Rosengren 1984). Colonies have higher temperatures within the core of their mound nests during cool weather, allowing them to withstand the climates of northern regions of the globe and reside in nests for up to forty years. *Formica* workers forage at temperatures between 0ºC and 55ºC, but they prefer high temperatures and humidity when foraging (Hölldobler & Wilson 1990, Klotz 1984, Schumacher & Whitford 1974). Primarily diurnal, they tend to increase activity just before dawn (Klotz 1984, Rosengren & Fortelius 1986a, Schumacher & Whitford 1974). Foraging behaviors exemplify sustainable and robust practices in load carrying and navigation. When *Formica* workers forage individually, prey size has been found to be positively correlated with worker size (Hölldobler & Wilson 1990). Group transport of food and enemies has also been observed (Hölldobler & Wilson 1990). Polarized light has been identified as a mechanism of navigation (Dias & Breed 2006), including a notable moon compass response demonstrated by Jander (1957). Persistence in navigation while foraging is likely partially due to a tendency towards habituation, as found in experiments on maze learning in *Formica* (Schneirla 1941). During these experiments, workers' progress through a maze occurred through a simultaneous decrease in excited and erratic behavior and an increase in the tendency to continue running when obstacles were encountered. This tendency may be useful in defense, as *Formica* evacuate their nest in a rapid and well-organized way in the presence of formidable enemies or flooding (Hölldobler & Wilson 1990). Their ability to work efficiently as a group is a skill that aids them well in survival.

A prominent species found throughout the Rocky Mountains is *F. podzolica*. This is a mound-building, medium-sized, black ant that lives throughout the high latitude regions of North America. *F. podzolica* nests can be found within pine and aspen stands in Colorado, at altitudes near 10,000 feet. This species is a particularly good study organism because of its inability to sting and its conspicuous soil mounds, which can exceed two meters in diameter (Deslippe & Savolainen 1994). Estimates of colony size of *F. podzolica* range from 5,000 to 100,000 workers. They tend aphids for honeydew, scavenge, and prey on a wide variety of invertebrates from March through October (Deslippe & Savolainen 1994). Winged, sexual individuals mate outside their nests from July through September, after which males die and females start new colonies (Deslippe & Savolainen 1994). About one-third of *F. podzolica* colonies contain a single queen, while most have more than one queen, with low average nestmate relatedness (DeHeer & Herbers 2004). *Formica* ants have well-honed navigational skills, which help worker efficiency in meeting large foraging needs. Although workers often leave the nest to forage, they make use of a combination of polarized light and nest-specific information (possibly landmarks) throughout early stages of returning to their nest, similar to other *Formica* species. When polarized light in the sky is unavailable, or it contradicts landmark information, the ant appears to reorient solely by means of nest-specific landmarks, ignoring polarizing information altogether (Dias & Breed 2008). This navigational strategy allows them to forage efficiently in complex and varying environments.

Along with foraging for small arthropods and plant material, *F. podzolica* tend aphids to harvest their honeydew in a mutualistic relationship. In exchange for honeydew, the ants protect the aphids from arthropod predators (but lead to an increase in avian predators, Mooney 2006). A 2010 study conducted in Ithaca, New York found *F. podzolica* protecting milkweed aphids, *Aphis asclepiadis*, from deadly fungal infections caused by an aphid pathogen, *Pandora neoaphidis*. Through focused cleaning and quarantining behavior, the ants were shown to reduce disease spread in aphid colonies (Nielsen et al. 2009). The ants devote a lot of energy to aphid farming because the honeydew reward is large.

Although *F. podzolica* take advantage of many different food sources, the ants have a high sensitivity to nutrient availability. A 2012 study found that a macronutrient imbalance affected their aggression and interactions with other ants. Excess carbohydrates relative to protein escalated *F. podzolica* aggressiveness, predatory inclination, and foraging activity. Additionally, it caused reduced collection of aphid honeydew and plant nectar (Petry et al. 2012). Food is such an important factor in colony success that *F. podzolica* nest location has been shown to directly correlate with natural food availability. Deslippe and Savolainen (1994) found nest densities and reproductive yield to be greatest along forest edges (where natural food levels were high), and lowest in overgrazed meadows. The ants feed, on average, at or slightly above the trophic position of a primary predator (Mooney & Tillberg 2005). These findings add to a large collection of evidence that *F. podzolica* obtain food through complex systems of farming, foraging, and predacious activity.

With their widespread distribution, large mound nests, and complicated foraging activity, *F. podzolica* likely plays an important role in its broader ecosystem dynamics. This prediction is supported by a 1997 study conducted in Illinois (Beattie & Culver 1977), one of the main inspirations for this thesis. Beattie and Culver (1977) collected soil and vegetation data (largely including grass and juniper) from land immediately surrounding *F. obscuripes* mound nests at three different sites and calculated plant species richness, diversity, and abundance. They found that, although plants were scattered throughout the study site, most plant species were positively associated with the location of mounds nests, and species abundance reached a peak at 1.5 meters from nests in juniper-dominated areas. Additionally, altered soil conditions correlated with plant heterogeneity. *F. obscuripes* is similar in habitat and range to *F. podzolica*; they both are omnivores that harvest honeydew from aphids, build mound nests made of soil and plant material, and live in a wide variety of habitats across North America (Beattie & Culver 1977). In a second study, Culver and Beattie (1983) found many of the same effects of *F. canadensis* on soils and vegetation at the Rocky Mountain Biological Laboratory, Gothic, Colorado.

I was eager to examine the ecological effects of another abundant ant species and the findings of Beattie and Culver (1977) and Culver and Beattie (1983) inspired my own investigation of the effects of the mound nests of *F. podzolica* on surrounding soil, nitrogen, and vegetation. Using similar methodology, I set out to investigate whether vegetation effects can be generalized across species and habitats. Upon review of the literature, some studies exist on the effects of ant populations on the environment, but the very common species *F. podzolica* has yet to be studied in relation to vegetation structure in alpine regions of North America (Beattie & Culver 1977, Del Toro et al. 2015, Finér et al. 2012). Additionally, there is a lack of comprehensive studies that combine soil, vegetation, and nitrogen measurements. Beattie and Culver's (1977) findings have interesting implications for insect-ecosystem interactions in the face of climate change; how will ant-influenced vegetation patterns morph as shifting climates compel changes in ant colonies? This is a far-reaching research question, and this project, which serves as a first step to answering it, provides insight into the impact these ants have on their surrounding ecosystem by revealing the extent to which they change soil properties and plant community structure.

Experimental Questions and Hypotheses

I focus on how a close-to-home ant species might manipulate our environment. My overall hypothesis was that colonies of *F. podzolica*, through their construction, foraging, and waste management, modify the ecosystem around each colony in ways that affect vegetation community structure. In areas surrounding *F. podzolica* nests I analyzed:

1. *Vegetation abundance and diversity*
2. *Soil moisture and pH*
3. *Vegetation biomass and percent cover*
4. *^{15}Nitrogen content*

I predicted that I would find correlations between these variables and distance from nests. Specifically, I predicted that (1) vegetation abundance and diversity would be greatest close to nests, (2) soil moisture would increase and pH will decrease with distance from nests, (3) vegetation biomass and percent cover would be greatest close to nests, and (4) ^{15}Nitrogen content would be greatest close to nests.

Methods

Study Sites and Location

My study site, University of Colorado's Mountain Research Station, is located at an elevation of 9,500 feet in Nederland, Colorado and is an ideal location to study alpine ecology. With a fully equipped alpine laboratory, it sits below Niwot Ridge and is surrounded by aspen and pine stands that contain many *F. podzolica* mound nests. Preliminary observations of 24 nests revealed complex vegetation structure in areas surrounding nests, with little, but some, plant growth on the nests themselves (Figure 1). In addition, soil appeared to be sandier in nest areas than elsewhere.

Figure 1. A representative *F. podzolica* mound nest (left) and an up-close view of vegetation living on a nest (right).

My research was conducted in the meadows and lodgepole pine-dominated forests around the Mountain Research Station. A total of 24 nests were used for data collection: nine in Elk Meadows (alpine meadows dominated by grasses and forbs), nine in areas off the Sourdough Trail (a hiking trail through a closed-canopy pine forest with limited undergrowth), and five near C1 Meteorological Station (a meadow-like clearing in the forest with many grasses, Figure 2). Elevation is approximately constant among all sites. Measurements and ant, plant, and soil samples were collected during the summer of 2016. Ant and plant samples were processed at University of Colorado Boulder and sent to the UC Davis Stable Isotope Facility for stable isotope analysis during the autumn of 2016.

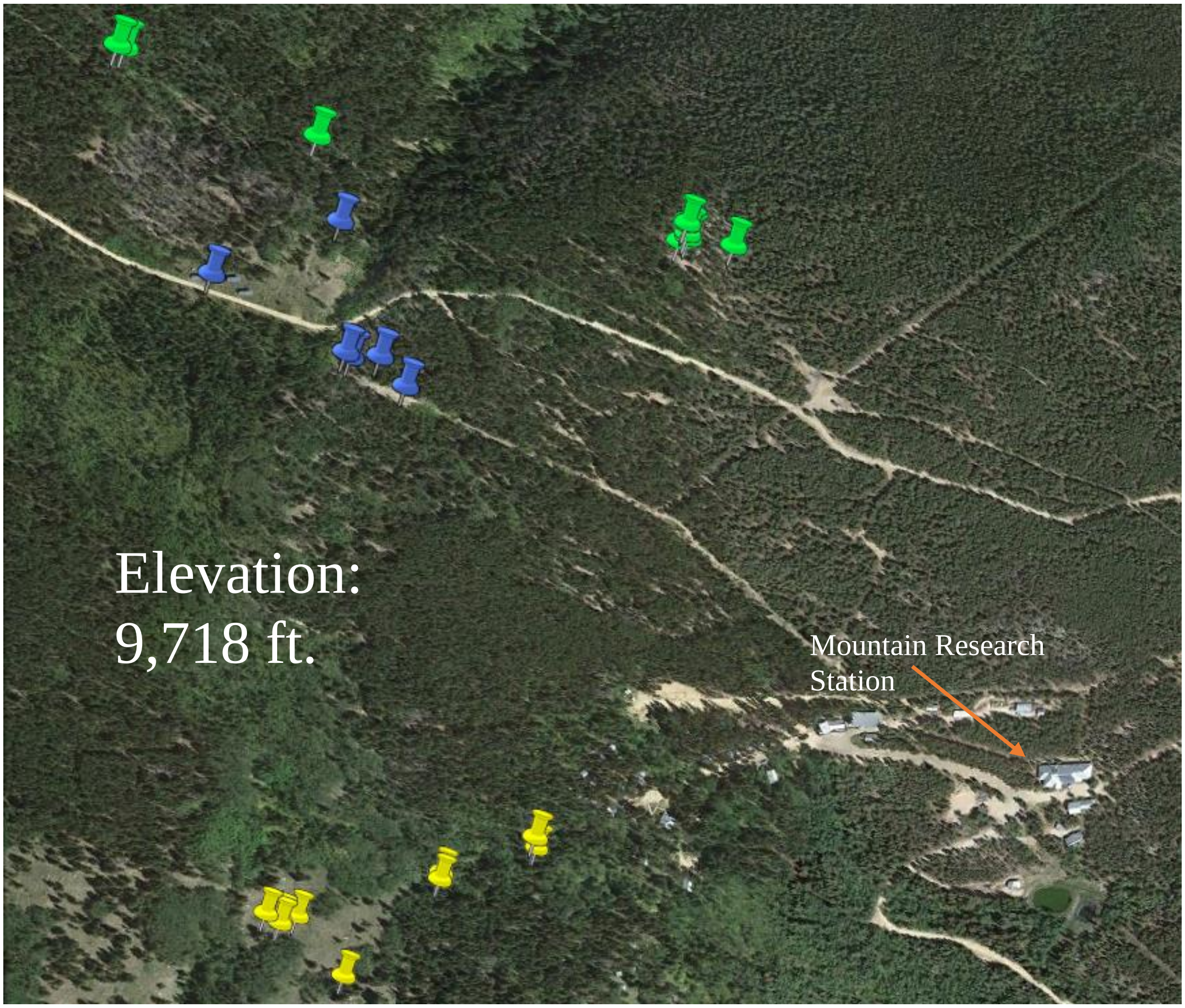

Figure 2. Map of nest locations (Google Earth). Yellow pins: Elk Meadows, blue pins: C1, green pins: Sourdough Trail.

1. *Vegetation Abundance and Diversity*

At each nest, vegetation abundance and diversity was determined within 21 quadrats ($0.5m^2$) surrounding the nest: five quadrats along a 2.5-meter transect on the uphill, downhill, and two adjacent slopes to the nest, and one control quadrat at a location chosen by a random number generator (within 2.5 and 10 meters away from the nest in any direction). Each transect started at the edge of the nest and runs linearly in a predetermined direction (Figure 3). The number of stems per species was noted. Degree of slope was noted for each nest.

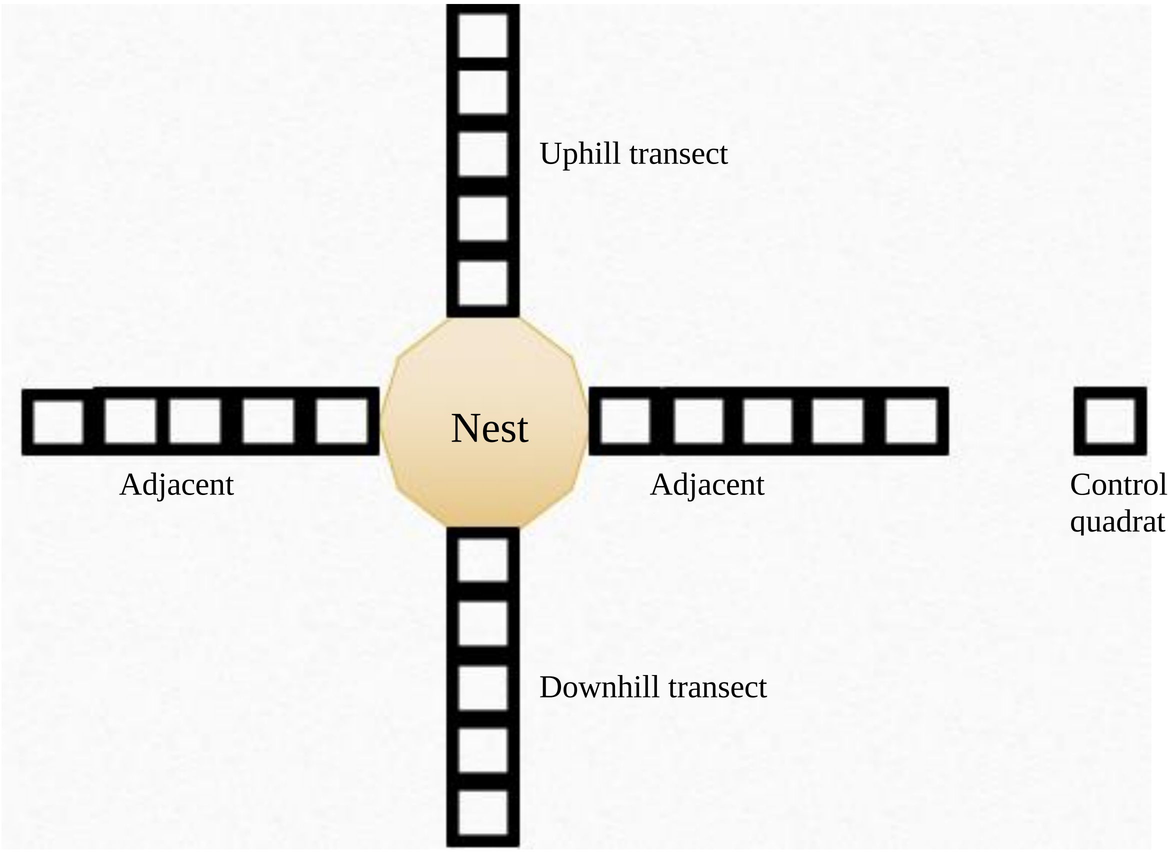

Figure 3. Transect layout around each nest. Each transect contains five 0.5m^2 quadrats. A control quadrat is placed randomly beyond the transects.

2. *Soil Moisture and pH*

Soil samples were collected from each quadrat at all nests. Samples were taken from a depth of 10 cm using a trowel. Wet weight was immediately taken and then samples were dried in an oven at 105°C for 24 hours. Dry weight was then taken and the difference between the two weights was determined as the moisture content. After making a 2:1 slurry of deionized water and soil, pH of each sample was determined using a pH meter.

3. *Vegetation Biomass and Percent Cover*

All vegetation was clipped above ground within 0.25m^2 quadrats, chosen randomly from within a 1m^2 quadrat at locations of 0.5 meters and 2.5 from nests on the uphill and downhill slopes. Plants were immediately dried in an oven at 60°C for 24 hours and the dry weight was taken to determine biomass.

Percent cover was calculated by laying a 10x10 grid over each quadrat and counting the number of squares containing vegetation.

4. *Stable Isotope Analysis for ^{15}N Content*

Vegetation samples taken from control, uphill and downhill transects at 0.5 and 2.5 meters from nests were pulverized using a ball mill, creating a homogenized mixture of the plant material. Twenty ants were collected from each nest during the summer and frozen for preservation. The ground vegetation samples and ants were packed into tin capsules (one vegetation tin per quadrat and two ant tins per nest) and sent to the UC Davis Stable Isotope Facility for a ^{15}N stable isotope analysis. By using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer, amount of ^{15}N was determined for each sample (UC Davis Stable Isotope Facility).

Statistical Analysis

Linear mixed-effects models (for distance effects, with site and nest as random effects) and t-tests (for nest vs. control and downhill vs. uphill comparisons) were used to analyze the soil, vegetation, and ant data. Shannon's Diversity Index was used to calculate the diversity of plant species within each quadrat. A chi-square test was applied to each species of plant to examine dependence of abundance on distance from nest.

Results

1. *Vegetation Abundance and Diversity*

The results of this study illustrate how the soil and plant communities surrounding nests are composed. The mean number of stems per quadrat decreased from approximately 24 (SE=2) stems per quadrat at 0.5 meters from the nest to 18 (SE=2) stems per quadrat at 2.5 meters from the nest (linear mixed-effects model, -2.292 stems per quadrat, p=0.0004, df=455). Mean number of stems per control quadrat was 23.25 (Figure 4, SE=3.23).

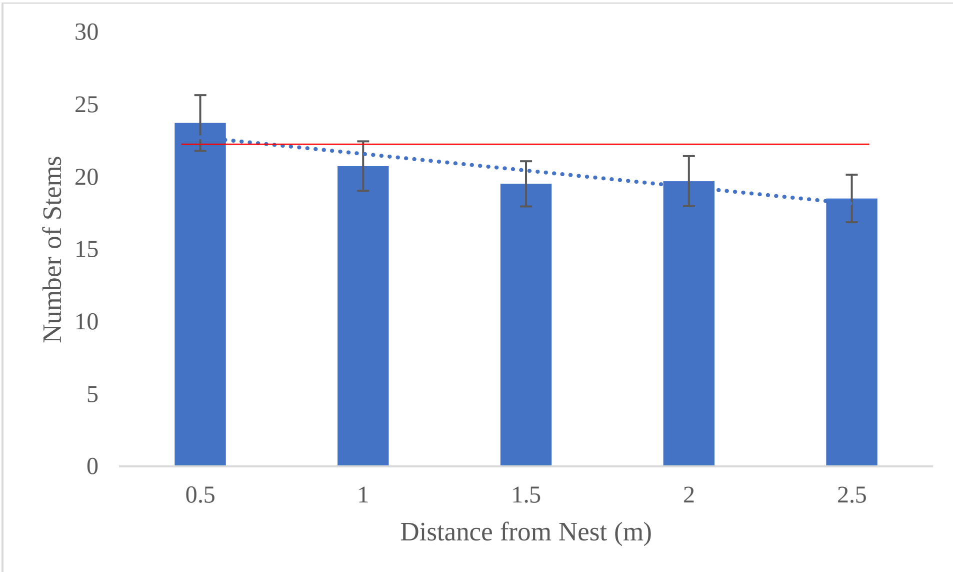

Figure 4. Number of stems per quadrat (linear mixed-effects model, p=0.0004, df=455). Mean number of stems per control quadrat is marked by the red line.

Table 1 shows the plant species found within each quadrat. Overall, there was a high abundance of plants that grow well in dry soil conditions in quadrats 0.5 meters from nests, such as *Juncus arcticus* (a rush) and *Pentaphylloides floribunda* (a species in the rose family, Stevens et al. 2012, The University of Texas at Austin 2015). Additionally, quadrats 2.5 meters from nests contained a higher proportion of large fruited plants such as *Fragaria virginiana* (a strawberry) and plants that require moister soil like *Achillea lanulosa* (The University of Texas at Austin 2012). Five species showed a significant pattern of stems per quadrat in response to distance, while nearly half showed a moderately strong pattern.

Vegetation diversity increased with distance from nest, on average. Shannon's H values increased from 1.2506 (SE=0.0424) at 0.5m to 1.7488 (SE=0.8903, df=455) at 2.5m from nests.

	0.5 m	*1 m*	*1.5 m*	*2 m*	*2.5 m*	*Control*	*Chi-square / p-value*	*Ecological Info.*
Muhlenbergia montana	163	239	149	165	161	64	17.047 / 0.0044***	Grass, dry habitat
Lupinus argenteus	41	77	93	81	71	14	12.366 / 0.0301**	Lupine
Fragaria virginiana	66	58	87	75	93	20	5.607 / 0.3464	Strawberry, moist habitat
Juncus arcticus	1610	1095	1036	1040	902	234	122.392 / <0.0001***	Rush, dry habitat
Sedum lanceolatum	0	4	2	1	1	4	2.263 / 0.8117	Spearleaf stonecrop, rocky mountainous habitat
Taraxacum officinale	20	23	33	21	18	11	4.334 / 0.5024	Dandelion, moist habitat
Rumex acetosella	60	90	79	81	113	27	9.437 / 0.0929*	Sheep's sorrel, sandy habitat
Thermopsis divaricarpa	102	165	147	154	142	43	9.576 / 0.0882*	Flowering plant
Pseudocymopterus montanus	12	19	24	18	15	18	10.619 / 0.0595*	Alpine false spring parsley, alpine environment
Juniperus communis	4	5	4	4	4	2	0.375 / 0.996	Common juniper, cool temperate habitat
Abies lasiocarpa	0	0	1	2	13	0	8.092 / 0.1512	Subalpine fir, alpine habitat
Aquilegia coerulea	0	0	1	0	0	0	N/A	Colorado blue columbine,
Achillea lanulosa	86	97	97	118	141	36	9.792 / 0.0813*	Mountain yarrow, moist habitat
Populus tremuloides	4	3	5	15	8	9	11.128 / 0.0489**	Quaking aspen
Antennaria rosea	0	2	9	9	5	0	5.045 / 0.4104	Rosy pussytoes
Campanula rotundifolia	8	24	37	13	16	5	12.375 / 0.03**	Bluebell, cold habitat
Pentaphylloides floribunda	74	74	58	69	49	15	3.959 / 0.5553	Hardy deciduous flowering shrub, dry habitat
Stachys byzantina	10	6	6	16	7	5	4.569 / 0.4707	Lamb's-ear
Artemisia ludoviciana	0	2	3	3	5	6	4.453 / 0.4862	White sagebrush
Castilleja sulphurea	17	9	6	7	14	5	4.868 / 0.4322	Sulphur Indian paintbrush
Shannon's H	**1.2506**	**1.6436**	**1.6836**	**1.6783**	**1.7488**	**2.0934**		

Table 1. Total number of stems, Shannon's H, chi-square/p-value, and ecological information for each plant species within 0.5m, 1m, 1.5m, 2m, and 2.5m, and control quadrats. Significant p-value for the chi-square tests are marked: *=notable, **=significant, ***=highly significant.

2. Soil Moisture and pH

Soil moisture increased from approximately 6% (SE=0.01) at 0.5 meters to approximately 9% (SE=0.01) at 2.5 meters away from the nest (Figure 5, linear mixed-effects model, p=0.0001, df=455, t=4.0937). While mean soil moisture was greater in the most distant quadrat from the nest within each transect, the 2.5 meters surrounding nests contained a total average of 4% more moisture than control quadrats (t-test, p=.0032, df=455, t=-3.2182). No significant pattern of soil pH was found (linear mixed-effects model, t=-0.4125, p=0.6801).

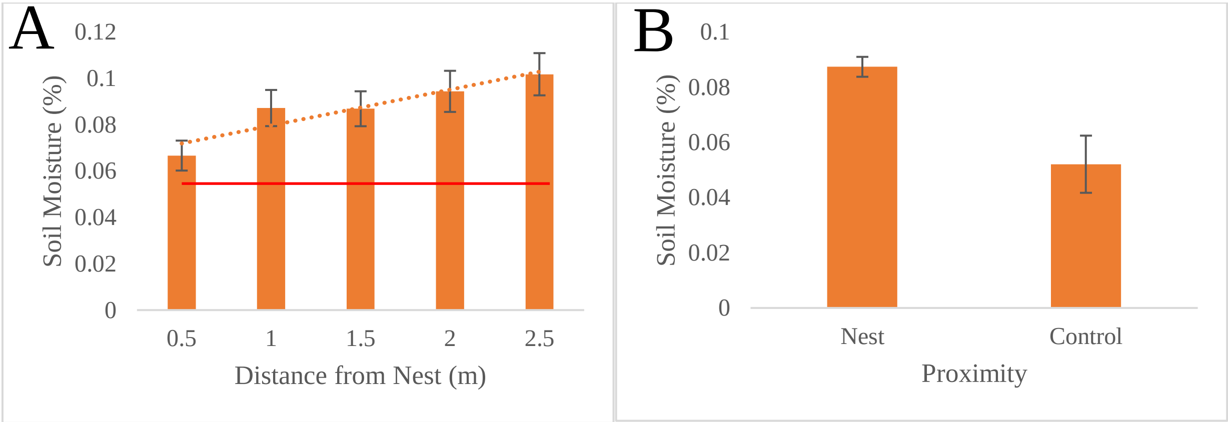

Figure 5. (A) Soil moisture per quadrat (linear mixed-effects model, p=0.0001, df=455, t=4.0937) and (B) soil moisture in the 2.5m around the nest vs. the control areas beyond 2.5m (t-test, p=0.0032, df=455, t=-3.2182). Control mean soil moisture (0.0521, SE=0.01) is marked by the red line.

3. Vegetation Biomass and Percent Cover

Slope was an important factor in the way moisture and nutrients were distributed around ant nests, with greater biomass and plant cover observed downhill from nests than uphill (Figure 6). There was a mean of approximately 3 grams more biomass in the downhill transects than the uphill transects (paired t-test, p<0.0001, SE= 0.41, df=47, t=7.9845). Additionally, plant cover was about 10% higher in the downhill transects than the uphill transects (paired t-test, p<0.0001, df=119, t=4.9702).

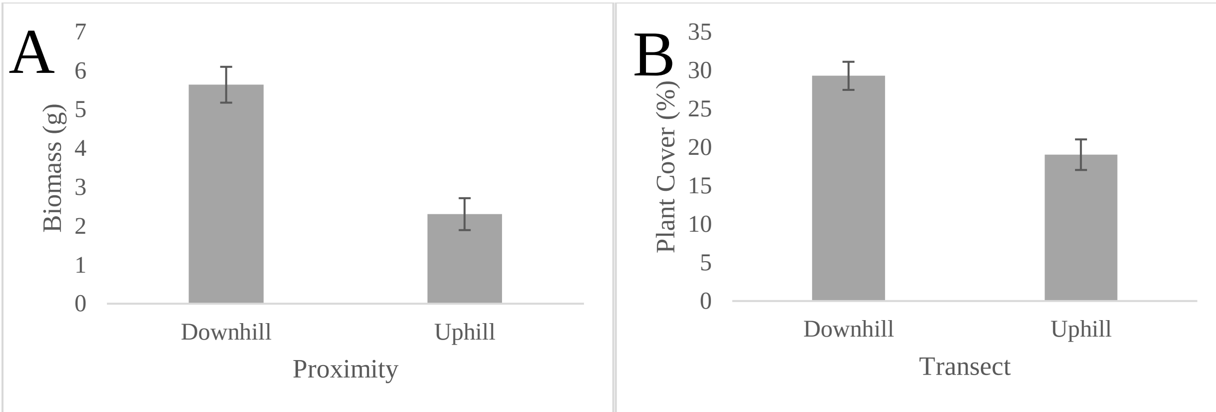

Figure 6. (A) Biomass (g) uphill vs. downhill from nests (paired t-test, $p<0.0001$, df=47, t=7.9845) and (B) plant cover (%) uphill vs. downhill from nests (paired t-test, $p<0.0001$, df=119, t=4.9702).

4. ^{15}Nitrogen Content

The stable isotope analysis revealed a slight decrease in $\partial^{15}N$ with distance from nest. Plants 0.5 meters from nests had a mean of -1.17 (SE=0.26) and those 2.5 meters away had a mean of -1.78 (SE=0.17) $\partial^{15}N$, a value similar to that of the control quadrats, which, with a mean of 4.9 meters from nests, had a mean of -1.58 $\partial^{15}N$ (linear mixed-effects model, p-value=0.0176, df=214, t=-2.3927, SE=0.33, Figure 7). $\partial^{15}N$ is the ratio of stable isotopes (^{15}N:^{14}N), or the proportion of ^{15}N in the sample relative to a standard reference material. The negative $\partial^{15}N$ values of these results indicate that there is less ^{15}N in the plants than an environmental control. Ant samples had a $\partial^{15}N$ mean of 3.01 (SE=0.07), much higher than the plant samples.

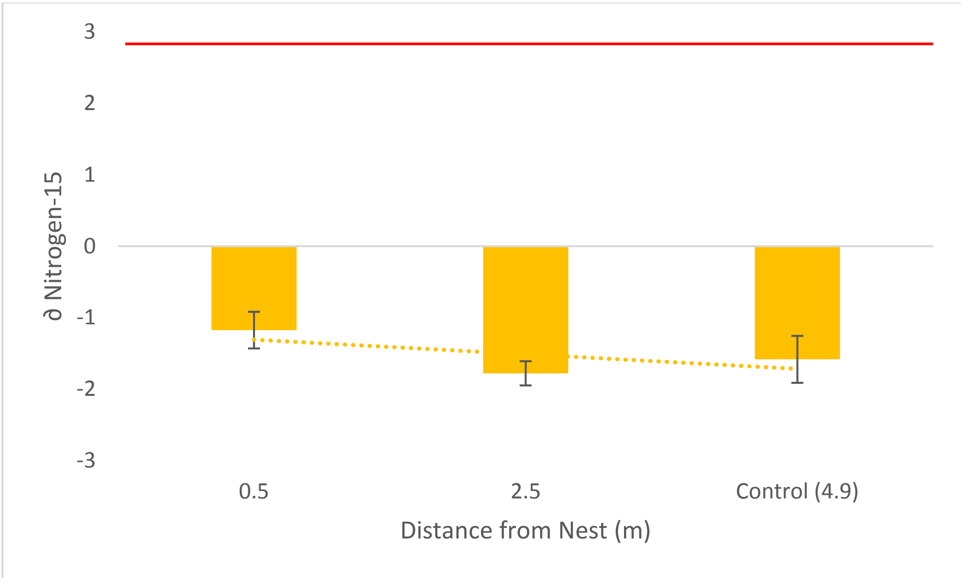

Figure 7. ∂^{15}N 0.5 and 2.5 m from nests, as well as control values averaged at 4.9 m from nests (linear mixed-effects model, p-value=0.0176, df=214, t=-2.3927). The red line indicates mean ∂^{15}N of ant samples.

Effect of site was controlled for in the linear mixed-effect model, but the Sourdough Trail area had significantly lower ∂^{15}N values than the other two sites (Figure 8). In addition, the Sourdough Trail showed significantly different measurements for biomass, plant cover, and number of stems per quadrat (including both nest and control quadrats).

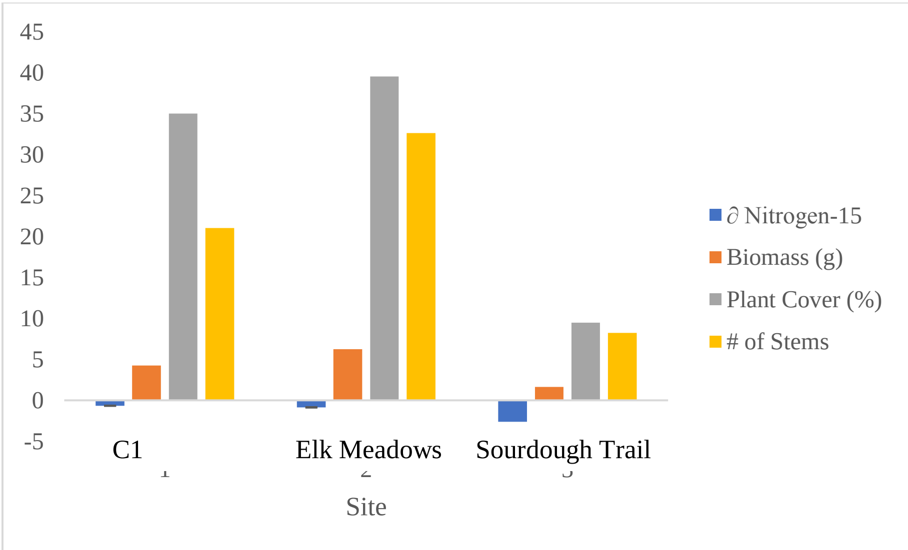

Figure 8. ∂^{15}N, biomass (g), plant cover (%), and number of stems per site.

Discussion

The activities of an ant colony can have significant community and ecosystem impacts over time. Just as a human engineer changes land to build various structures, ants also shape their world on a relatively comparable scale. Soil, vegetation, and nitrogen patterns around the mound nests of this study tell a detailed story of how *F. podzolica* ants are affecting their alpine biome. These results suggest that they are ecosystem engineers, impacting vegetation patterns beyond their nests.

1. Vegetation Abundance and Diversity

Plant abundance and soil moisture per quadrat have an inverse relationship; while areas directly surrounding nests had greater plant abundance, they were also drier. This helps to explain the species data (Table 1), with a pattern of dry-soil-dwelling plants near the nest (such as *J. arcticus* and *P. floribunda*) and plants requiring moister conditions more distant from the nest (for example, *A. lanulosa*). Additionally, plant species diversity increases slightly with distance. Species diversity is also correlated with soil moisture, leading to the conclusion that many different species thrive in moister conditions, but a small number of species can proliferate in dry soil conditions.

Some of my species abundance data reflects similar results in Culver and Beattie's 1983 study. Just as *M. montana* (a grass) was significantly more abundant close to nests than in control areas in this study, Beattie and Culver found *Bromus polyanthus* (another grass) to be greater in nest than non-nest areas. Similarly, *L. argenteus* (lupine) was found to have the same pattern of decreasing abundance with distance as *Collomia linearis* (trumpet) in the 1983 study. Not many plant species exist in both studies because Gothic, Colorado (the site of Culver & Beattie 1983) is in a different geographic zone than Nederland, Colorado. *F. canadensis* (the study organism of Culver & Beattie 1983) and *F. podzolica* are not closely related ant species, which could explain some variation in their vegetation preferences. Additionally, whereas *F. canadensis* forages largely for seeds, *F. podzolica* does not rely on seeds as much, based on my observations.

2. *Soil Moisture and pH*

The soil moisture results show a snapshot of the water retention in the soil at a small scale (within the 2.5 meters surrounding nests) and on a larger scale (up to 10 meters away from nests). At a small scale, it is apparent that nest excavation creates dry, aerated soil, a finding that is supported by previous studies that have found soil directly under mound nests to be porous and relatively dry (Laundré 1990, Scherba 1959). Moister soil at 2.5 meters away from nests could be a result of what happens just outside the nest's boundary: unwanted organic items such as dead ants and other small arthropods are discarded, adding to the soil microbiome and nurturing a habitat for plants, bacteria, and small animals that thrive in relatively moist conditions. Another explanation is that *F. podzolica* concentrates organic material, such as food items, in the far-reaching corridors of their nests (approximately 2.5 meters from the center of the nest) and keep the more central tunnels clear. A surplus of organic material compared to control quadrats is likely to result in higher soil moisture measures. Yet another explanation is that porous soil could maintain moisture longer when infiltrated compared to other soils. But examining the data at a broader scale, it appears that the nest processes of *F. podzolica* contribute to the retention of soil moisture in the 2.5 meters surrounding nests compared to control areas within 2.5 and 10 meters away from nests (the question of cause and effect will be discussed later). This pattern could be explained by a similar study which measured the soil moisture below ant nests at different depths, and found soil moistures between 50-110 cm below nests to be higher than controls (Laundré 1990). Laundré attributed this finding to the high amount of organic material that ants store in the tunnels of their nests.

3. Vegetation Biomass and Percent Cover

The biomass and plant cover results suggest that slope of the study site might be an important factor in how vegetation is distributed around *F. podzolica* nests. With both biomass and plant cover being significantly greater in the downhill transects than the uphill transects of the nest sites, it is apparent that vegetation thrives below the nests. A plausible explanation is that water and nutrients naturally flow and settle through the force of gravity. Ants might also tend to travel downhill when discarding organic material from their nests. It cannot be said from my investigation whether ants purposefully choose to build nests on slopes, but my results suggest that these nests significantly influence the surrounding vegetation regardless of the ants' choice to live on sloping land.

4. ^{15}Nitrogen Content

∂^{15}N values indicate a positive influence by ants on nitrogen composition of nearby vegetation, as it is significantly higher in areas next to nests (at 0.5 meters) than farther away (at least 2.5 meters away). This finding supports my hypothesis that *F. podzolica* increases the nutrients around its nests through foraging inputs and waste disposal. The possibility that foraged food items add to the macronutrients in areas surrounding nests is illustrated by the significant positive correlation between proximity to nest and ∂^{15}N.

Nests near the Sourdough Trail yielded significantly different measurements of $\partial^{15}N$, biomass, plant cover, and plant abundance then other sites. This is likely because the trail is situated in a rockier part of the forest, an exposed ridge subject to higher rates of erosion. Elk Meadows and the C1 meteorological station are in areas with greater amounts of topsoil and on slopes with lower erosion rates. Linear mixed-effects models controlled for the effects of site in my analyses.

The subject of 'cause and effect' must be examined in this study; do *F. podzolica* ants have a positive effect on their surrounding vegetation, or do the ants purposefully choose nest sites that provide the conditions described in the results? It is difficult to say with certainty, but this study and previous studies suggest the former. The significant relationships found between soil moisture, biomass, and plant cover data within the 2.5 meters surrounding nests and control quadrats (between 2.5 and 10 meters away from nests) leads to the conclusion that nests are an influential factor; it is very unlikely that the landscapes of C1 meteorological station, Elk Meadows, and the Sourdough Trail would contain pockets of nest-like soil and plant conditions, each having been discovered by an ant colony. This conclusion is supported by other research showing that ant colonies change and manipulate their broader environments, such as Frouz and Jilková's 2008 finding that daily activities of ants result in a shift of pH in the soil surrounding the nest towards neutral, and the work of Finér et al. (2013) that revealed an increase in nitrogen cycling in boreal forests of eastern Finland as a result of wood ant (*Formica rufa*) activity.

The relationships between plant abundance, biomass, plant cover, soil moisture, and $\partial^{15}N$ support the conclusion that *F. podzolica* play a role of ecosystem engineers. As Jones and Lawton (1994) defined, ecosystem engineers are organisms that significantly create, change, or destroy a habitat. *F. podzolica* mound nests and interactions with the surrounding environment provide evidence for these processes taking place. The increase in vegetation in areas surrounding nests can likely be attributed to a variety of factors identified in the literature, including the addition of organic matter to soil just outside nests as a result of discarded carcasses and foraging behavior (Beattie 1989, Sun et al. 2013) and the creation of a warm, aerated environment below the soil surface (Lesica & Kannowski 1998). Results of the stable isotope analysis support the work of Sagers et al. (2000), who concluded that ants provide large amounts of nutrients (in the form of nitrogen) to their environment. Additionally, my results suggest stable isotope analysis can be an effective method of assessing the trophic level of ants and of nitrogen movement in their habitats, as laid out by Tillberg et al. (2006) and DeNiro and Esptein (1981). This study verifies the techniques they employed with their research.

The findings of this study reveal the impact of *F. podzolica* on pine forest ecosystems of the Colorado Rocky Mountains, but leave room for future studies. Recommendations for studies to follow include gathering measurements of vegetation below the surface level of soil and investigating soil quality within nests at various depths underground. Soil moisture measurements can vary with organic matter within the soil below the surface level, a difficult variable to control. Additionally, soil properties vary greatly within the nest than in the surrounding areas. Taking samples from within the cavities of the nest itself would be an invasive procedure, but the findings would add more pertinent data to this study.

To fully assess the range of ecosystem impacts of *F. podzolica*, this study should be carried out in other climates and habitats, such as urban areas and meadows. A more in-depth stable isotope analysis should be completed utilizing soil samples in addition to ant and vegetation samples. This would provide a clearer understanding of the path of ^{15}N through the ecosystem surrounding the nest. Lastly, similar methodology should be applied to not only other species of ants, but other nest-building insect species (i.e. termites). This study has potential to be applied at a much broader scale and aid in the greater understanding of the role of ecosystem engineers in a variety of environments.

Small organisms can have large influences on their ecosystems. This idea is supported by the information gained from studying *F. podzolica* ants living in the alpine forests and meadows in Nederland, Colorado. Although their nest effects are small compared to the area of the entire forest, *F. podzolica*'s impacts of altered nitrogen and soil moisture on vegetation structure is large compared to the ants' size. The extent of their ecosystem impacts is comparable to the size of a city for humans. Just as we are constantly innovating, building, and altering the environments in which we live, *F. podzolica* ants are true ecosystem engineers as they constantly change their habitats. A 2015 meta-analysis of 122 studies showed that the general influence of ecosystem engineers on diversity is positive and parallels an increase in species richness globally (Romero et al. 2015). My study builds on other ant-ecosystem interaction studies like Beattie and Culver's research (1977, 1983), adding to the evidence that ants can cause widespread change in their environments by pulling together a wealth of evidence from vegetation and soil measurements. It shows that they can influence multiple levels of the ecosystem, and have the potential to have far-reaching effects. This is exciting and pertinent knowledge as we face climate change and realize that all organisms on Earth are extremely interdependent. The ways in which ants shape and control our environments is likely greater than we understand today, and we must maintain a diligent watch on the ways by which our Earth is shaped by seemingly-small, yet mighty ant colonies.

Acknowledgements

This study would not have been possible without the support of many people and organizations. First and foremost, thank you to Dr. Michael Breed, my REU faculty mentor and advisor throughout the school year. He helped me get started on this project, and gave me valuable advice and guidance the whole way through. Thank you to the National Science Foundation for their financial support through the Research Experiences for Undergraduates program and University of Colorado's Mountain Research Station for housing, food, and access to study sites during the program. My gratitude goes to University of Colorado Boulder's Undergraduate Research Opportunity Program, which provided me with the funding for my stable isotope analysis. Thank you to the Rocky Mountain Biological Laboratory for their support through the REU Travel Grant, which gave me the opportunity to present a poster of my initial results at the International Union for the Study of Social Insects 2016 Colloquium. I am also appreciative of the Department of Arts and Sciences Honors Program. Lastly, thank you to all those who have helped me with various pieces of my research: Dr. Pieter Johnson for teaching the EBIO Honors Seminar, Dr. William Bowman for leading the REU program, Dr. Sarah Massey-Warren for writing suggestions, Helen McCreery for statistics help, Amber Churchill for teaching me how to use the ball mill and pack tins for stable isotope analysis, Jay Arehart for help in preparing plant samples, and David Zonana and Francie Saunders for writing feedback.

References

Achillea millefolium var. occidentalis (Western Yarrow). (2012). *The University of Texas at Austin*. Retrieved 19 January 2017, from http://www.wildflower.org/plants/result.php?id_plant=ACMIO.

Atwell, L., Hobson, K. A., & Welch, H. E. (1998). Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *55*(5), 1114-1121.

Beattie, A. J., & Culver, D. C. (1977). Effects of the mound nests of the ant, *Formica obscuripes*, on the surrounding vegetation. *The American Midland Naturalist*, *97*(2), 390–399.

Beattie, Andrew. "Myrmecotrophy: plants fed by ants." *Trends in Ecology & Evolution* 4.6 (1989): 172-176.

Carbon (^{13}C) and Nitrogen (^{15}N) Analysis of Solids by EA-IRMS. Retrieved July 19, 2016, from http://stableisotopefacility.ucdavis.edu/13cand15n.html.

Culver, D., & Beattie, A. (1983). Effects of ant mounds on soil chemistry and vegetation patterns in a Colorado montane meadow. *Ecology, 64*(3), 485-492.

Dasiphora fruticosa ssp. floribunda. (2015). *The University of Texas at Austin*. Retrieved 19 January 2017, from http://www.wildflower.org/plants/result.php?id_plant=DAFRF.

DeHeer, C. J., & Herbers, J. M. (2004). Population genetics of the socially polymorphic ant Formica podzolica. *Insectes sociaux*, *51*(4), 309-316.

Del Toro, I., Ribbons, R. R., & Ellison, A. M. (2015). Ant-mediated ecosystem functions on a warmer planet: effects on soil movement, decomposition and nutrient cycling. *Journal of Animal Ecology*, *84*(5), 1233–1241. http://doi.org/10.1111/1365-2656.12367

Deslippe, R. J., & Savolainen, R. (1994). Role of food supply in structuring a population of Formica ants. *Journal of Animal Ecology*, 756-764.

Dias, C. M., & Breed, M. D. (2008). Untangling the interplay among navigational strategies used by the ant *Formica podzolica*. *Annals of the Entomological Society of America*, *101*(6), 1145-1149.

Dorn, R. I. (2014). Ants as a powerful biotic agent of olivine and plagioclase dissolution. *Geology*, *42*(9), 771-774.

Feldhaar, H. F., Gebauer, G. G., & Blüthgen, N. B. (2010). Stable isotopes: past and future in exposing secrets of ant nutrition (Hymenoptera: Formicidae). *Myrmecological News*, *13*, 3–13.

Finér, L., Jurgensen, M. F., Domisch, T., Kilpeläinen, J., Neuvonen, S., Punttila, P., … Niemelä, P. (2012). The role of wood ants (*Formica rufa* group) in carbon and nutrient dynamics of a boreal Norway spruce forest ecosystem. *Ecosystems*, *16*(2), 196–208.

Frouz, J., & Jilková, V. (2008). The effect of ants on soil properties and processes (Hymenoptera: Formicidae). *Myrmecological News*, *11*, 191–199.

Genus: Formica. (2017). *AntWeb*. Retrieved 14 January 2017, from http://www.antweb.org/description.do?name=Formica&rank=genus&project=allantwebants.

Gonçalves, A. Z., Oliveira, R. S., Oliveira, P. S., & Romero, G. Q. (2016). Species-specific effects of ant inhabitants on bromeliad nutrition. *Plos One*, *11*(3).

Gordon, D. (2003). *The emergent genius of ant colonies.* Presentation, Long Beach, CA.

Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Harvard University Press.

Jenkins, C. N., Sanders, N. J., Andersen, A. N., Arnan, X., Brühl, C. A., Cerda, X., ... & Gove, A. D. (2011). Global diversity in light of climate change: the case of ants. *Diversity and Distributions*, *17*(4), 652-662.

Jones, C. G., Lawton, J. H., & Shachak, M. (1994). Organisms as ecosystem engineers. *Nordic Society Oikos*, *69*(3), 373–386.

Laundré, J. W. (1990). Soil moisture patterns below mounds of harvester ants. *Journal of Range Management*, 10-12.

Lesica, P., & Kannowski, P. B. (1998). Ants create hummocks and alter structure and vegetation of a Montana fen. *The American midland naturalist*, *139*(1), 58-68.
Mooney, K. A. (2006). The disruption of an ant–aphid mutualism increases the effects of birds on pine herbivores. *Ecology*, *87*(7), 1805-1815.
Mooney, K. A., & Tillberg, C. V. (2005). Temporal and spatial variation to ant omnivory in pine forests. *Ecology*, *86*(5), 1225-1235.
Nielsen, C., Agrawal, A. A., & Hajek, A. E. (2009). Ants defend aphids against lethal disease. *Biology Letters*.
Nkem, J. N., de Bruyn, L. L., Grant, C. D., & Hulugalle, N. R. (2000). The impact of ant bioturbation and foraging activities on surrounding soil properties. *Pedobiologia*, *44*(5), 609-621.
Pamilo, P., & Rosengren, R. (1984). Evolution of nesting strategies of ants: genetic evidence from different population types of Formica ants. *Biological Journal of the Linnean Society*, *21*(3), 331-348.
Petry, W. K., Perry, K. I., & Mooney, K. A. (2012). Influence of macronutrient imbalance on native ant foraging and interspecific interactions in the field. *Ecological Entomology*, *37*(3), 175-183.
Romero, G. Q., Gonçalves-Souza, T., Vieira, C. and Koricheva, J. (2015), Ecosystem engineering effects on species diversity across ecosystems: a meta-analysis. Biol Rev, 90: 877–890.
Roura-Pascual, N., Suarez, A. V., Gómez, C., Pons, P., Touyama, Y., Wild, A. L., & Peterson, A. T. (2004). Geographical potential of Argentine ants (*Linepithema humile Mayr*) in the face of global climate change. *Proceedings of the Royal Society of London B: Biological Sciences*, *271*(1557), 2527-2535.
Sagers, C. L., Ginger, S. M., & Evans, R. D. (2000). Carbon and nitrogen isotopes trace nutrient exchange in an ant-plant mutualism. *Oecologia*, *123*(4), 582–586.
Scherba, G. (1959). Moisture regulation in mound nests of the ant, *Formica ulkei Emery*. *American Midland Naturalist*, 499-508.
Schultz, T. R. (2000). In search of ant ancestors. *Proceedings of the National Academy of Sciences*, *97*(26), 14028-14029.
Stevens, M., Hoag, C., Tilley, D., and L. St. John. 2012. Plant Guide for mountain rush (*Juncus arcticus* ssp. *littoralis)*. USDA-Natural Resources Conservation Service, Aberdeen Plant Materials Center. Aberdeen, Idaho 83210.
Sun, Qian, and Xuguo Zhou. Corpse management in social insects. *International journal of biological sciences* 9.3 (2013): 313.
Tillberg, C. V, Mccarthy, D. P., Dolezal, A. G., & Suarez, A. V. (2006). Measuring the trophic ecology of ants using stable isotopes. *Insectes Sociaux*, *53*, 65–69.
Wagner, D., Jones, J. B., & Gordon, D. M. (2004). Development of harvester ant colonies alters soil chemistry. *Soil Biology & Biochemistry*, *36*, 797–804.
Wardle, D. A., Hyodo, F., Bardgett, R. D., Yeates, G. W., & Nilsson, M.-C. (2011). Long-term aboveground belowground consequences of red wood ant exclusion in boreal forest. *Ecology*, *92*(3), 645–656. http://doi.org/10.1890/10-1223.1