

The Effects of Ants on Ecosystem Dynamics: Investigating the Ecological Influence of Ant Nests in the Rocky Mountains

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Full-Time Independent Research
Summer 2016

Abstract

Formica obscuripes, or the Western Thatching Ant, is a native North American ant species commonly found throughout the central and western US. Like many ant species, *F. obscuripes* acts as an ecosystem engineer, as colonies can significantly alter the nutrient content, moisture content, temperature, and pH of the soil when constructing and maintaining their nests. And while the immediate effects of *F. obscuripes* on the local environment are well known, it is not quite well understood how these shifts in the physical and chemical properties of the substrate affect larger ecosystem processes. As many plant and fungal species are sensitive to factors such as soil moisture and nutrient availability, we hypothesized these biotic communities would alter in composition, abundance, and diversity as ants altered the immediate environment. We explored the effect of ant nests on soil properties, plant community composition, and belowground fungal communities. As part of that, we sampled and assessed plant community composition, pathogen loads, and fungal colonization of abundant plant species in a sagebrush meadow in Colorado's Almont Triangle. While we found that soil below ant nests was indeed drier and warmer than the surrounding soil, we did not find a significant difference in arbuscular mycorrhizae (AMF) or dark septate endophyte (DSE) colonization for our focal species, *Chrysothamnus viscidiflorus*. However, we did find that the presence of ant nests was related to an increase in the abundance of oomycete root pathogens for *C. viscidiflorus*. Furthermore, we found that plant diversity on and around ant nests increased as nests aged and became inactive, while the presence of woody species, especially, *Symphoricarpos rotundifolius*, increased with nest proximity and activity. Moreover, we found that not only was the presence of *S. rotundifolius* correlated with ant nest activity, its roots also saw elevated levels of AMF colonization. And though its abundance was unaffected by nest presence, *A. tridentata* saw increased rates of colonization by DSE when occurring on ant nests. Thus, while ants may create warmer, drier, and more nutrient dense environments, fungal and plant responses will differ between species, as mutualisms and unique mechanisms lead species and partners to experience differential success.

Introduction

Although small in size and stature, ants can transform the land around them during the construction of large and elaborate nesting mounds. An influx of organic materials into mounds by workers leads to the accumulation of an abundance of soil nutrients, the concentrations of which can well surpass the stores contained by both vegetated and bare soils (Jones & Wagner, 2006; Laakso & Setälä, 1998). Ammonium and nitrate concentrations up to 6 times higher than in nearby soils have been seen, and elevated levels of K, Na, Ca, Mg, and P have also been found in various studies (Snyder, Crist, & Friese, 2002; Jilkova, Sebek, & Frouz, 2012; McGinley, Dhillon, & Neumann, 1994). Moreover, the effects that ant mounds confer upon soils are extensive, as they can alter the physical, and not just chemical, properties of the substrate. For example, soil moisture levels are generally reduced in and around ant mounds (Jones & Wagner, 2006; Laakso & Setälä, 1997; Wardle et al, 2011). Likewise, ants have been observed to keep their mounds warmer than the adjacent ground, as they thermoregulate their nests through a number of processes, such as metabolic heating (Rosengren et al, 1987; Laakso & Setälä, 1998). Furthermore, this surplus of nutrients and warm temperatures can encourage the proliferation of microorganisms in the soil, which in turn speeds the rate of decomposition and CO₂ production in mounds (Jilkova, Pícek, & Frouz, 2015; Jones & Wagner, 2006). In certain mounds, the total microbial biomass has been found to be up to three times higher in mound material than in the surrounding soil (Laakso & Setälä, 1997).

And just as ant mounds have been seen to bolster the communities of microorganisms taking residence within, so too have belowground fungi been shown to benefit. Ant mounds have been found to play host to increased abundances of AMF, with intra-mound roots often exhibiting extensive AMF colonization (Snyder & Friese, 2001). In extreme cases, root-mats, which develop inside of colonies, can host AMF at densities orders of magnitude greater than found in the surrounding soil (Friese & Allen, 1993). And though the formation of root-mats within colonies is somewhat rare, typical root material collected from ant mounds often still exhibits

higher levels of colonization than roots from the surrounding soil (Snyder, Crist, & Frieese, 2002). Although AMF colonization has been relatively well studied around ant-mounds, colonization and abundance patterns of other fungal functional groups, like dark septate endophytes, fungal pathogens, and interactions between fungal groups have not been investigated. Although only colonization by AMF has been observed thus far, another group of root-colonizing fungi, DSE, often co-occurs with AMF. To date, no work has been conducted on abundance changes in DSE or interactions between AMF and DSE in ant mounds. However, because AMF and DSE differ in the nutrient resources they allocate to host plants, it is likely that mound mediated soil nutrient changes will lead to changes in fungal co-occurrence patterns.

A combination of altered soil chemistry, increased soil fungal density and fungal relative abundance could feedback to re-shape plant community composition on or near ant mounds. A number of hypotheses have been made for the selection of mycotrophic plant species over non-mycotrophic plant species near ant mounds, as an abundance of soil fungi might drive selection for mutualistic plant partners (Frieese & Allen, 1993; Snyder, Crist, & Frieese, 2002). Moreover, changes in soil-nutrient content could affect the competitive ability of plants in the area, as species more adept at utilizing nutrient abundances may displace less well-adapted species (Thedose & Bowman, 1997). Furthermore, a number of prior studies have argued that one of the most significant effects that ants confer upon the local plant community is a selection woody over herbaceous species (Wardle et al., 2011).

However, while many of these effects are fairly well documented, most are also sensitive to environmental variables, and the interactions between them are not well understood. If the temperature or moisture content of a mound were to drop, the rate of decomposition may also decrease, slowing the release of nutrients to the soil and the release of CO₂ to the environment during soil respiration (Jilkova, Picek, & Frouz, 2015; Jilkova & Frouz, 2014; Jones & Wagner, 2006). Likewise, in cool or water-limited environments, AMF may have a difficult time outcompeting the more stress-tolerant DSE within a mound (Newsham, 2010). Moreover, slight changes to the concentrations of soil nutrients, such as P or N, could alter both the fungal and plant communities in and around ant mounds (Johnson et al, 2003). We explored the direct and indirect effects of ant activity on soil properties, aboveground and belowground organisms, and overall ecosystem function. We measured the direct changes in soil moisture and soil temperature as well as indirect interactions between co-occurring plant symbionts (arbuscular mycorrhizal fungi, dark-septate endophytes, and root pathogens) and the resulting changes in the plant community around ant mounds in the Almont Triangle. Moreover, we utilized our study of plant community composition to investigate whether ants are selective when choosing their nesting sites, and what the consequences of that selection might be. We asked:

- 1. What is the effect of ant mounds on soil temperature and moisture content?**
- 2. How do ant mounds influence the abundance patterns of arbuscular mycorrhizal fungi, dark septate endophytes, and plant root pathogens in the roots of common host plants?**
- 3. How does the presence of ant mounds affect plant community composition?**

Methods and Materials

Study Site

Our study site was located in the Almont Triangle Wilderness Area in Almont, Colorado at 11 mi N, 5 mi E of Gunnison, Gunnison County, Co. US. Latitude: 38.66 N Longitude: -106.84 W. The site was an arid, alpine meadow which was bordered by stands of aspen on both the western and eastern sides. The area was principally dominated by woody shrubs, such as Sagebrush (*Artemisia tridentata*), Round-leaved Snowberry (*S. rotundifolius*), and Rabbitbrush (*Chrysothamnus viscidiflorus*), however a number of herbaceous species were abundant, such as *Wyethia amplexicaulis*, *Castilleja miniata*, and *Calochortus gunnisonii*. Temperatures range over the course of the year from lows of -9 to highs of 24 °C, with rainfall averaging 25.4 cm for the months of

June and July. Overall, the meadow was dense with ant nests, with perhaps the majority being within just a few meters of one another. In total, within the 375m x 75m (2.8 ha) study site there were well over 200 nests present.

Of those 200+ nests, we selected 30 in total to sample, 10 of which were active in 2016, 10 of which were last active in 2013, and 10 of which were last active in 2010. The hope with this sample group was that it would not only allow us to understand the effects of ants on ecosystem function, but also that it would allow us to understand the legacy of those effects (Figure 1).

Study Species

The ant species looked at in this study was *Formica obscuripes*, or the Western Thatching Ant, one of the most abundant ants in western North America (Conway, 1996). While these ants are diurnally active year round, it is only during the summer that they are able to collect honeydew from the aphids which they tend (Crutsinger & Sanders, 2005). In Gunnison County, Colorado, the location of our study site, they are often found nesting in areas that are dominated by communities of sagebrush and rabbitbrush (Conway, 1996).

The nests made by *F. obscuripes* are generally very large, varying in height from 2 to 46 cm, and in depth from 0.3 to 1 meter, often being restricted by the depth of the water table (Weber, 1935). The nests themselves are composed of a thatching material, for the most part twigs and fecal pellets, but the composition of which varies depending on the composition of the immediate vegetation (Weber, 1935; Conway, 1996). The diet of *F. obscuripes* is multifaceted, as these omnivorous ants will hunt for live insects, and scavenge the remains of dead ones, as well as collect honeydew from aphids, and harvest plant matter when necessary. While their prey of preference likely varies from location to location, it has been noted that they commonly feed on the members of Orthoptera. Moreover, they have been observed tending to aphids found on sagebrush (Conway, 1996).

Finally, the effect of this species on the surrounding vegetation is non-trivial, as they actively crop the vegetation around their nests. While vegetation will often be allowed on the periphery of nests, the center of the nest is almost always kept clear of plants (Beattie & Culver, 1977). In areas with sagebrush, mounds are often found with dead or stunted sagebrush on them. This is accomplished by workers which chew the bark at base of the plant and then spray formic acid on the cambium to kill the plant (Conway, 1996). This behavior is thought to be the response of ants to prevent the overgrowth of nests, which would have deleterious consequences (Beattie & Culver, 1977; Berg-Binder & Suarez, 2012).

Soil Measurements

In mid-June we collected soil moisture (percent volumetric water content) measurements directly adjacent to ant mounds (on mound measurement) and at 1m distances from the ant mounds (away from mound measurement). At the same time, we also recorded soil temperature in the same locations using a 10cm analog immersion thermometer.

Fungal Community

In order to sample the soil fungal community, soil cores (2.5cm x 15cm) were taken from each plot. Since *C. viscidiflorus* was present at nearly every nest, it was chosen as a focal species in order to ensure a controlled comparison. Moreover, cores were taken from the edge of each nest, as well as at 0.5m and 1m distances from each nest. However, because other shrub species were abundant at our site, cores of *A. tridentata* and *S. rotundifolius* we also collected (at 0m and 1m distances for each nest at 10 nest-sites). All cores were kept refrigerated at around 5 °C until they were processed.

Soil samples were passed through 2mm mesh to extract roots from the soil core. Fine roots were then collected and put into cassettes for clearing and staining. After being repeatedly rinsed with deionized water, the

root cassettes were then placed in 10% KOH solution to bleach root pigments, and were stained using Trypan blue (Koske and Gemma 1989). Finally, the root cassettes were then flushed repeatedly with deionized water in order to remove any excess stain before slide preparation.

Stained roots were then placed onto slides, with as many roots as possible (typically around 15) from each cassette being used in order to ensure thorough, randomized sampling. The roots were then glued-down using PVLG and a coverslip. Finally, the slides were scored using the gridline-intercept method in order to quantify fungal abundance and diversity (McGonigle et al, 1990).

Plant Community Composition

Plant community composition was recorded for all 30 mounds, with one measurement being taken at the edge of each mound, and then a second, control measurement being taken one meter away (generally to the north unless restricted by a neighboring mound). Measurements were made using a 0.25m² quadrat. All species that fell within the area of that quadrat were identified and then an estimation of percent cover for each species was made. Moreover, the percent of bare ground, animal esker, woody cover, rock cover, and plant litter was recorded for each plot. Finally, percent shrub cover was recorded for each mound, with estimations being made for a circle (of 1m radius) centered on the nest. As three shrubs dominated the study-site, this percent shrub cover measurement was broken down further into percent cover of *Chrysothamnus viscidiflorus*, *Artemisia tridentata*, and *Symphoricarpos rotundifolius*.

Statistical Analysis

Plant community composition and fungal colonization data were analyzed using linear mixed-effect models. For our analyses of plant diversity, plant richness, total shrub cover, and percent cover by individual plant species, we constructed linear mixed-effect models with soil temperature, soil moisture, and plot (for blocking the data) as random-effects. For these models our fixed effects included distance from nest, nest activity, and age since last nest activity. For our analyses of cover, soil temperature, and soil moisture, linear mixed-effect models were still used, but fixed and random effects in the models varied as applicability of variables fluctuated. For our cover analyses, nest activity and nest age were used as fixed effects, and temperature and moisture were used as random effects. For temperature and moisture models, activity, distance, and age were used as fixed effects. For our temperature model the random effects were moisture and plot, and then for our moisture model the random effects were temperature and plot. For our analysis of AMF, DSE, and oomycetes, we compared colonization rates on, 0.5m from the ant mound, and 1m from the ant mound using linear mixed-effect models. We constructed models with plot as a random-effect and then distance, age, and activity as fixed effects. Since temperature and moisture readings were taken only on nests, and then at a 1 meter distance away, inclusion of these variables into our colonization models would have meant the exclusion of colonization data collected at 0.5m from the nests. However, since we only took cores of *S. rotundifolius* and *A. tridentata* at 0 and 1m distances we were able to incorporate moisture and temperature as random effects into those respective colonization models. When determining best-fit models, we varied both fixed and random effects in order to assess all feasible models, ultimately deciding on a final, best-fit model using AIC scores and performing likelihood-ratio tests.

To understand changes in plant community composition between active and non-active ant mounds and at different distances from the ant mounds, we visualized community composition using bray's dissimilarity index using non-linear multidimensional scaling ordination.

Results

Soil Properties

As we hypothesized, we found that ant activity increased soil temperature but decreased soil moisture. We found soil temperature decreased with increasing distance from nests, with nests on average being 4.3% (about 1 degree Celsius) warmer than the surrounding soil ($n=60$, $\text{ChiSq}=17.6$, $p > 0.001$). Moreover, this effect was even greater for active nests, which were found to be 7.2% warmer than the surrounding soil.

However, we found that soil moisture decreased just as distance to a nest decreased. Measurements taken on and off of nests revealed ant nests to be 14.8% drier than the surrounding ground ($n=58$, $\text{ChiSq}=7.07$, $p=0.001$). Again, this difference was exacerbated for active nests, with active nests being 29.4% drier than the surrounding area ($n=58$, $\text{ChiSq}=6.79$, $p=0.015$).

Fungal Colonization

While we expected to see differences in fungal colonization as a function of nest activity and proximity, we found that the response of AMF, DSE, and pathogen colonization differed between plant species. Surprisingly, we found no differences in AMF ($n=88$, $\text{ChiSq}=0.872$, $p=0.520$) or DSE ($n=88$, $\text{ChiSq}=1.56$, $p=0.222$) colonization rates of *Chrysothamnus viscidiflorus* regardless of nest proximity or activity (Figure 2). However, we found the presence of oomycotan pathogens on *C. viscidiflorus* to significantly increase as a function of increasing nest proximity (Figure 3). Colonization of roots by oomycetes was 2.74 times greater for intra-mound roots than for roots 1.0 meter away, and still 2.34 times greater for intra-mound roots just 0.5 meters away ($n=88$, $\text{ChiSq}=4.40$, $p=0.037$). Unlike with *C. viscidiflorus*, the level of colonization of *S. rotundifolius* by AMF was 48% greater for roots on ant nests relative to roots 1m away from ant nests ($n=20$, $\text{ChiSq}=7.46$, $p=0.017$) (Figure 4). However, we didn't observe any differences in colonization patterns of DSE ($n=20$, $\text{ChiSq}=2.52$, $p=0.130$) or pathogens ($n=20$, $\text{ChiSq}=1.35$, $p=0.297$) in *S. rotundifolius* roots by distance or activity levels. And though the level of colonization of *A. tridentata* by AMF ($n=18$, $\text{ChiSq}=1.36$, $p=0.258$) or pathogens ($n=18$, $\text{ChiSq}=0.624$, $p=0.441$) were not found to be significantly affected by ant nest proximity, colonization by DSE was 88% greater for on-nest roots ($n=18$, $\text{ChiSq}=6.38$, $p=0.047$) (Figure 5).

Plant Community Composition

When looking at the data relating to plant community composition, the extent of the effects of ants upon the local environment quickly became evident. Overall, plant diversity, as measured by Simpson's Diversity Index, was found to be lowest on active ant nests, and we found plant diversity was correlated with nest activity, and with an interaction between activity and distance (activity: $n=58$, $\text{ChiSq}=4.23$, $p=0.003$; distance: $n=58$, $\text{ChiSq}=5.60$, $p=0.026$). We found active nests to be the least diverse environments, with inactive and off-nest sites being up to 12.6% more diverse (Figure 6).

And while plant diversity was seen to decrease on active nests, total shrub cover was found to increase. Percent shrub cover, a metric combining cover by *Symphoricarpos rotundifolius*, *Artemisia tridentata*, and *Chrysothamnus viscidiflorus*, was found to increase quite dramatically for plots as the date of last nest activity became more recent ($n=58$, $\text{ChiSq}=6.62$, $p=0.017$) (Figure 7). Moving from 2010 nests to 2013 nests, total shrub cover increased by 55%, and when comparing 2010 nests with 2016 nests, shrub cover increased by 95%. One of the driving factors behind an increase in total shrub cover was a strong relationship between ant nest activity and the presence of *S. rotundifolius*. Percent cover of *S. rotundifolius* was 2.8 times greater on active nests versus inactive nests ($n=58$, $\text{ChiSq}=6.05$, $p=0.001$). Moreover, the percent cover of *S. rotundifolius* was twice as high on nests last active in 2013 versus nests last active in 2010, and 3.65 times higher on mounds active in 2016 versus mounds last active in 2010 ($n=60$, $\text{ChiSq}=20.5$, $p > 0.001$) (Figure 8). Additionally, percent cover of *S. rotundifolius* increased 23% when off-nest and on-nest areas were compared ($n=58$, $\text{ChiSq}=6.21$, $p=0.020$) (Figure 9).

Looking at our nMDS plot of community composition it seems that there is a potential weak grouping of similar plot types (Figure 10). The basis of this grouping seemed to be most related to plot age, as plots active in 2016 were grouped to the left in the nMDS, whereas the 2013 plots were grouped in the middle, and the 2010

plots grouped to the right. Active ant mounds seem to be associated with higher cover of *Chenopodium sp.*, *Lathyrus leucanthus*, *Festuca thurberi*, *Mertensia fusiformis*, and *Galium septentrionale*, whereas inactive nests seem to have higher cover of *Sedum lanceolatum*, ANDSEP, and *Alopecurus pratensis* (Figure 10).

Finally, we found ground cover of litter increased as nests aged. Litter cover decreased by 41% between nests last active in 2013 versus 2016, with that difference widening for nests last active in 2010 versus 2016, with a decrease of 66% ($n=58$, $\text{ChiSq}=7.55$, $p=0.012$)(Figure 11).

Discussion

Overall, we found warmer temperatures and lower soil moisture content on ant nests as compared to off nest sites, which is consistent with what has been observed by other researchers (Rosengren et al, 1987; Laakso & Setälä, 1998). Since soil temperature and moisture were found to vary between on-nest and off-nest sites, we decided to incorporate these factors into our models of fungal colonization and plant community composition. In addition to changes to soil properties, we also observed a decrease in total plant diversity on ant nests (Figure 6), which was likely linked to an increase in total shrub cover on ant nests (Figure 7). Principally driving this increase in total shrub cover was an increase in the abundance *S. rotundifolius* on nests (Figure 8). And although all three shrub species were abundant around ant nests, we found that the species differed greatly in their associations and interactions with fungal symbionts and root pathogens. We found that while *S. rotundifolius* hosted elevated levels of AMF on ant nests, *A. tridentata* instead hosted elevated levels of DSE. Surprisingly, we found no difference in the colonization of *C. viscidiflorus* by either AMF or DSE as a function of nest activity or proximity. However, we did observe a significant increase in root pathogens for *C. viscidiflorus* individuals growing on ant mounds. As AMF and DSE are generally both mutualistic fungi, it seems possible that the presence of ant nests may inadvertently select for certain plant communities, which could affect community structure on a long-term basis. Moreover, these shifts at the soil, fungal, and plant community levels may lead to feedback cycles, as shifts in one community affects others in a domino-like effect.

While a number of prior studies suggested that the rate of AMF colonization is frequently higher on ant nests, our results, which focused on the roots *C. viscidiflorus*, *S. rotundifolius*, and *A. tridentata*, suggested that the situation was more complicated than that (Snyder & Fries, 2001; Snyder et al., 2002). Usually an increased colonization rate is attributed to soils being enriched with available nutrients, such as phosphates, nitrates, and labile carbon. Moreover, it is also thought that since harvester ants are consistently incorporating new plant matter into their nests, they may be inadvertently carrying AMF or DSE inoculum into the nest with them (Fries & Allen, 1993). While we did not assess what the inflow of inoculum might be like, we have previously observed soil enrichment for nests at our study-site, with elevated levels of available nitrogen, phosphorus, and potassium being seen for active mounds (Wood et al., 2011). However, even with soil enrichment, we found that colonization of *C. viscidiflorus*, *S. rotundifolius*, and *A. tridentata* did not increase across the board. While we found no significant differences in the rate of colonization by DSE for *C. viscidiflorus* and *S. rotundifolius*, we did see an increase in the level of colonization of *S. rotundifolius* by AMF and *A. tridentata* by DSE. These differences could perhaps be due to *S. rotundifolius* and *A. tridentata* being more adept than *C. viscidiflorus* at forming mutualisms, or because *C. viscidiflorus* was limited by an environmental factor that *S. rotundifolius* and *A. tridentata* were not affected by. Moreover, these abundances could also be explained by an ant-plant-fungi feedback cycle. As ants enrich the soil, they may, for example, favor the establishment of *S. rotundifolius* or encourage AMF colonization, which in turn could lead to even greater AMF abundances or *S. rotundifolius* establishment. While perhaps not conclusive, our data does seem to suggest that there is some interplay between increased AMF abundance and increased *S. rotundifolius* abundance, like what might be seen as the result of a feedback. In order to understand to what extent feedbacks were responsible for the results we saw, further experimentation would ultimately be needed.

One of the more unexpected results seen in this study was an increase in the level of oomycete colonization for *C. viscidiflorus* on ant nests. Moreover, colonization of oomycetes was found to be dependent on proximity to nests, rather than as a function of nest age or activity. One possibility to explain the heightened colonization of oomycetes is that during nest maintenance and construction, ants carried in viable oospores from infected plants or soils. Transportation of oomycete propagules by ants and other arthropods has been documented in previous studies, and successful infection has been seen to occur after oospore movement (EI-Hamalawi & Menge, 1996; Alvarez et al., 2009). Another possibility is that oomycete colonization was elevated due to increased signaling between host plants, AMF, and DSE. Typically chemical signaling between AMF, DSE, and host plants concludes with the production of cutin monomers by the host plant, which allow for hyphopodia formation by the fungus. However, oomycetes are also dependent upon a production of cutin monomers to induce appressoria formation, and thus they often coopt the same signaling process (Gobbato, et al., 2013; Wang, et al., 2012). Perhaps then if elevated amounts of cutin monomers were being produced by host plants in response to altered fungal loads, oomycete colonization might be elevated as well, as appressoria formation is made easier. Thus, under this hypothesis, an increase in oomycete colonization may echo an underlying shift in the function or abundance of AMF or DSE that we were unable to detect. One such functional shift that might affect plant-fungal signaling would be a role reversal of AMF on ant mounds, with the once mutualistic fungi becoming parasitic as its host plant is no longer nutrient limited (McGinley et al., 1994). Prior studies have shown that when there are high levels of bioavailable N and P in the soil, often no effect is seen on total vascular plant biomass, as mutualistic fungi begin to slow nutrient exchange in order to retain a bargaining advantage (Wardle et al., 2011). While an effect of this type may be a significant effect of ant nests, its presence may not be immediately apparent in fungal colonization rates. Future studies are likely necessary in order to disentangle the ecological roles of fungal communities within our ant mounds, as well as how an increased pathogen load affects the fitness of plants around ant mounds.

The decreased plant diversity we observed was consistent with prior studies, which have shown that the presence of active ant nests is negatively correlated with diverse plant communities (Beattie & Culver, 1977). This decrease in diversity on and around ant nests seems to be tied to an increase in shrub presence and cover, which we observed to increase as time since last nest activity decreased. This prevalence of a few woody shrubs over a diverse herbaceous community on ants has been echoed in other studies, which have shown that herbaceous species respond positively, increasing in abundance and diversity, when ants are excluded from an area (Wardle et al., 2011). In our study, *S. rotundifolius* was the woody species whose presence was most correlated with the presence of active ant nests. However, the abundance of *S. rotundifolius* on nests was not only correlated to nest activity, being found more often on active nests, it was also correlated with the age of the nest, being found more frequently on nests which were more recently active. Considering these two correlations, it seems that an abundance of *S. rotundifolius* and other shrubs on ant nests could be brought about by a number of causes.

Several hypotheses have been put forward to explain ant activity leading to higher woody biomass. The first hypothesis is that ants select for areas with woody species when establishing nest sites (Heikkinen, 1999). This could come about for a number of reasons, such as the root systems of woody species providing the foundation for nest creation, or because those species provide a good site for tending aphids (Weber, 1935). Moreover, support for this theory often arises because the shrubs, which are found on ant nests, take years to establish themselves, and though nests can remain active for up to 15 years, many see much quicker turnover times. A secondary hypothesis is that the presence of ant nests actively encourages the establishment of woody rather than herbaceous species. This could be due to an increase in soil nutrients, which large shrubs need in greater volumes to maintain their greater biomass, or because the ground around ant nests is drier, therefore favoring the establishment of species which are more frugal with their water use. Moreover, as nest proximity

was correlated with an increase in both *S. rotundifolius* cover and AMF colonization of *S. rotundifolius*, perhaps a heightened level of AMF colonization is conferring a fitness benefit upon *S. rotundifolius*. That is, an inadvertent bolstering to the belowground community of AMF by ants may contribute to a selection for *S. rotundifolius* and cause a positive feedback. A third hypothesis is that ants alter the environment in favor of woody species, encouraging the proliferation, rather than the establishment, of shrubs. As ants actively crop vegetation which encroaches on their nests (as it can be a threat to nest homeostasis and the structure itself) it may be that the more easily cropped vegetation, such as herbaceous species, are removed in greater volumes than their tougher, woody counterparts (Beattie & Culver, 1977). The inadvertent selection for woody species, along with a bolstering of soil nutrients and fungal inoculum, and the creation of a more arid environment, might end up encouraging the growth of existing shrubs. Moreover, even if the proliferation of shrubs was not encouraged, the removal of herbaceous species might give the illusion of either of the two previous hypotheses.

Regardless of what factors are driving these decreases in diversity and increases in shrub cover for young, active nests, it does appear that ant nests seem to confer a sort of legacy upon their environment. For community composition, groupings in the nMDS seem to shift over time. Younger, active nests show a selection for species like *Chenopodium* sp. *Lathyrus leucanthus*, and *Festuca thurberi*, whereas inactive or older nests exhibit higher coverage of *Sedum lanceolatum*, ANDSEP, and *Alopecurus pratensis*. This suggests that the legacy of nests, at least with regard to community composition, lasts for at least six years (the age difference between our oldest and youngest nests), and potentially longer. This same sort of trend can be seen with percent plant litter cover, which decreased gradually moving from 2010, to 2013, to 2016. Again, at a minimum there exists a six year legacy of ant nests, however perhaps in a very different way. As the rate of change for litter cover is likely much quicker than the rate of change for community composition, the two are likely not experiencing a legacy effect from the same factor. Whereas community composition may be controlled by a nutrient legacy, litter cover may be controlled by the lack of workers maintaining the area. This suggests that the legacy of ant nests may not be controlled by just one factor, but by a number of factors, each with their own legacy and time frame.

Overall the effects of ants upon their environment seem to be extensive and tiered, as impacts are visible when looking at soil, fungi, and plants responses. And while each of these effects could be caused independently, it ultimately seems more likely that there is a feedback between the three, with a web of interactions controlling the visible results. Further study would be necessary to elucidate these interactions and truly understand the flow of effects. With a better understanding of the possible feedback cycles at play, it would also be interesting to investigate whether the effects of ants upon ecosystem function are just unintended consequences of activity, or whether they might end-up conferring some benefit to the ants themselves.

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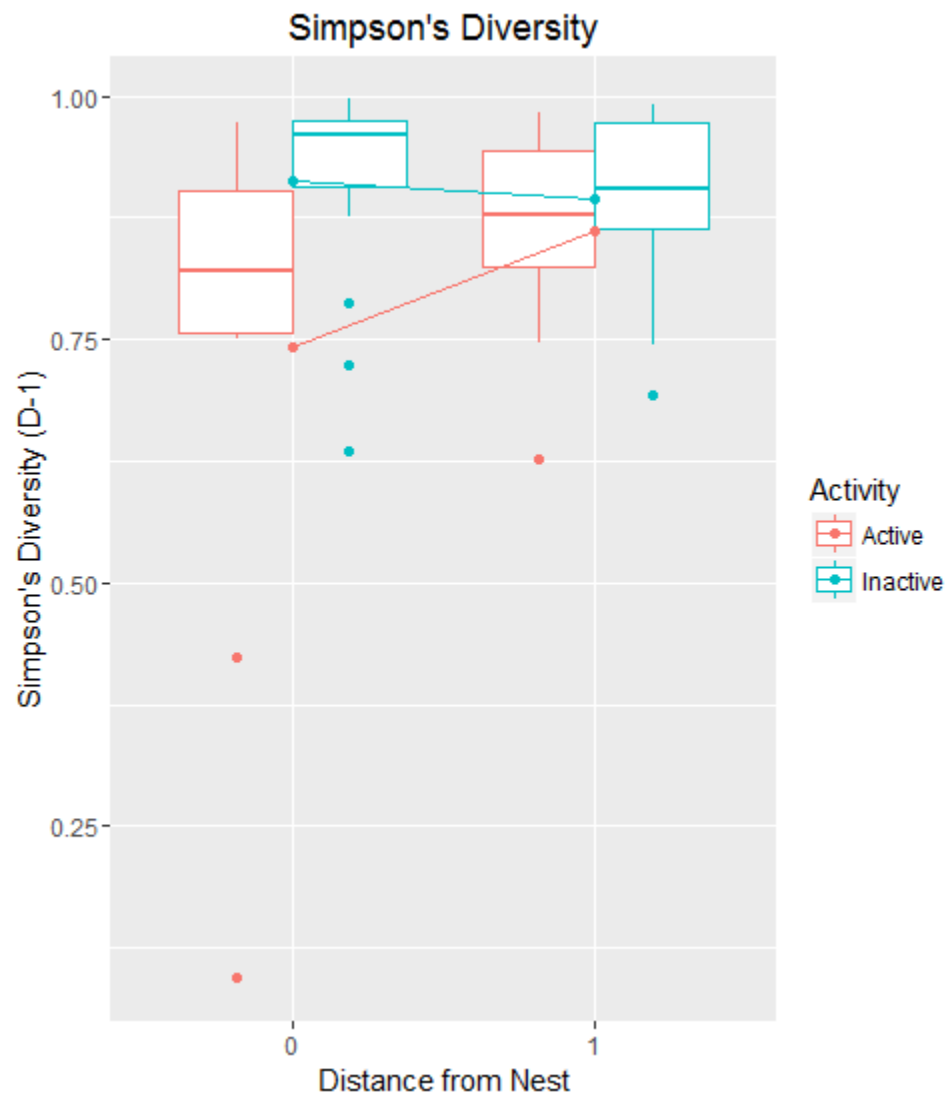


Figure 6: Simpson's diversity index for ant nests of varying activities and distances. The greater the (D-1) index is, the more diverse the community is. Inactive nests are more diverse than either plots on or off active nests.

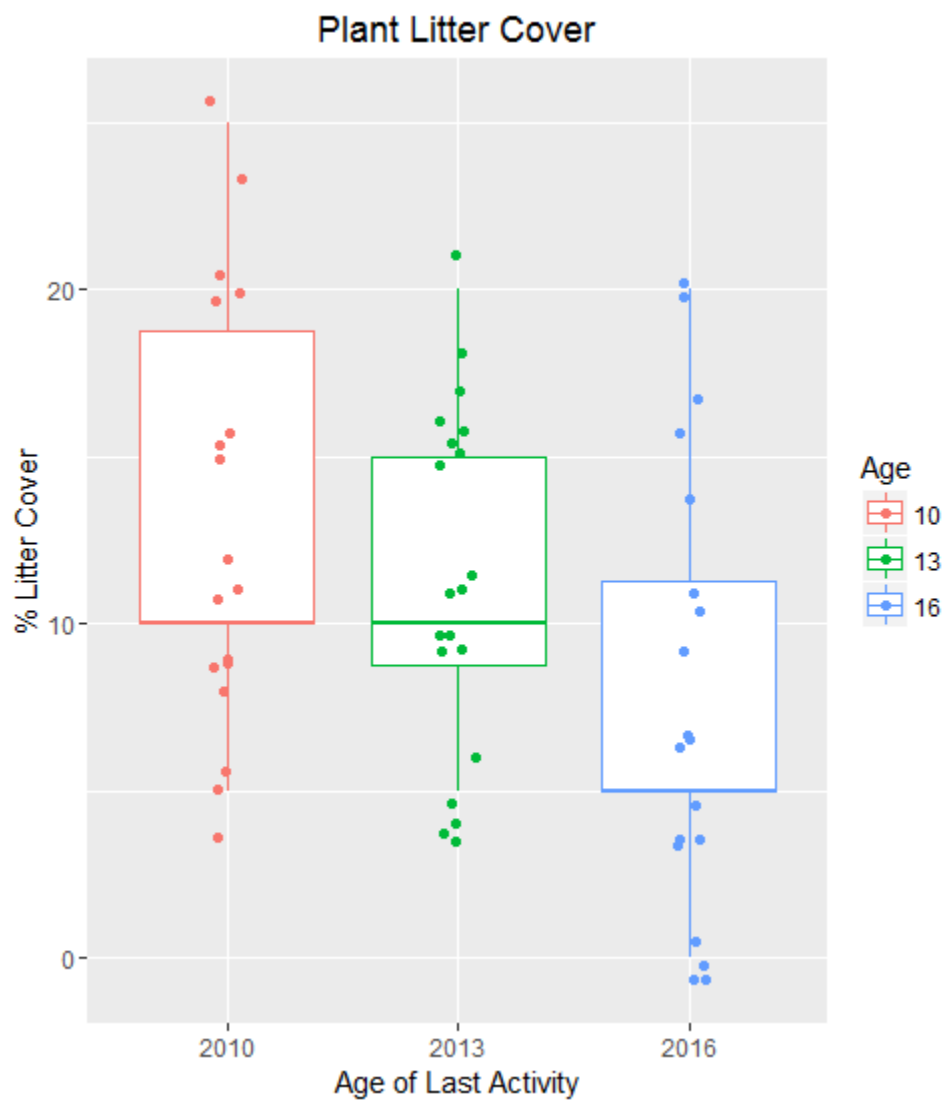


Figure 11: Litter cover measured during our community composition analysis. Nests more recently active show a lower % of litter cover than nest last active in 2013 or 2010.

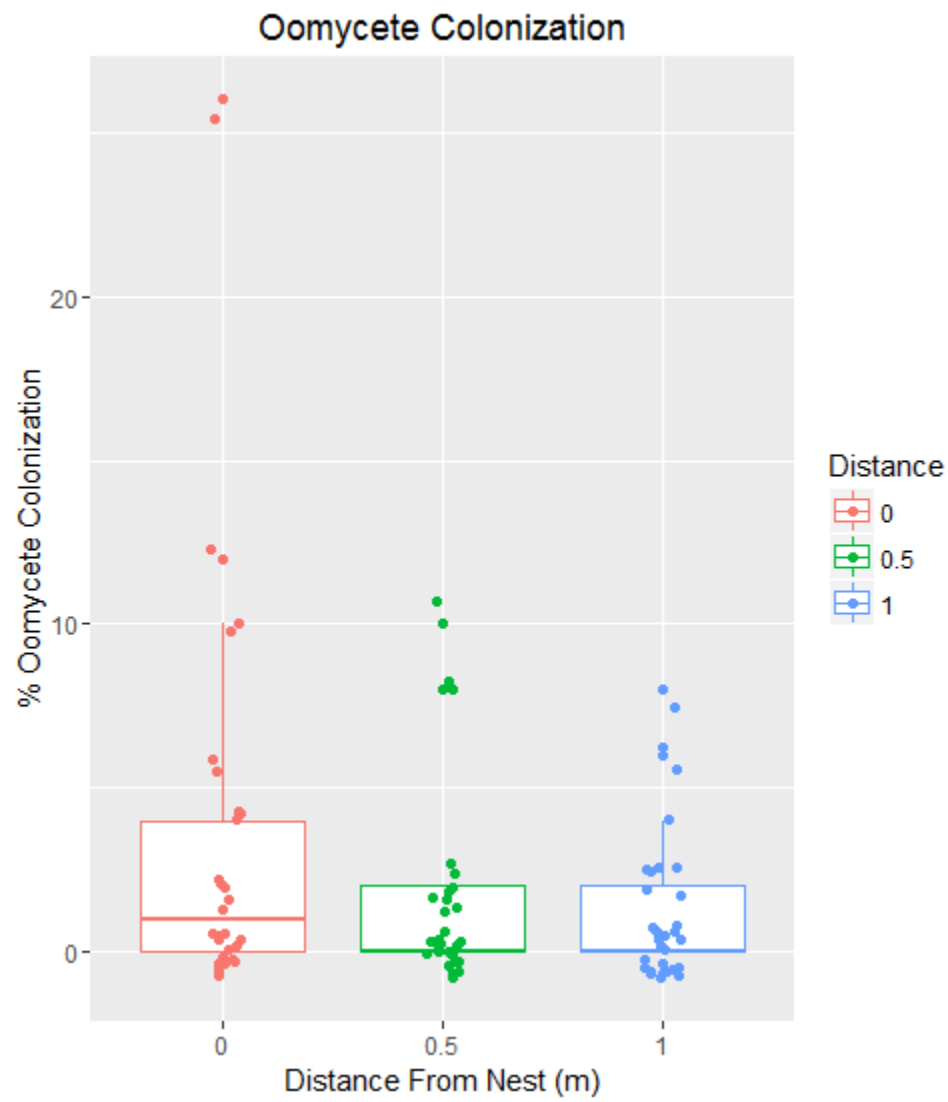


Figure 3: Percent oomycete colonization at varying distances from ant nests. Oomycete colonization increases with increasing proximity to nests, peaking when directly on the nests.

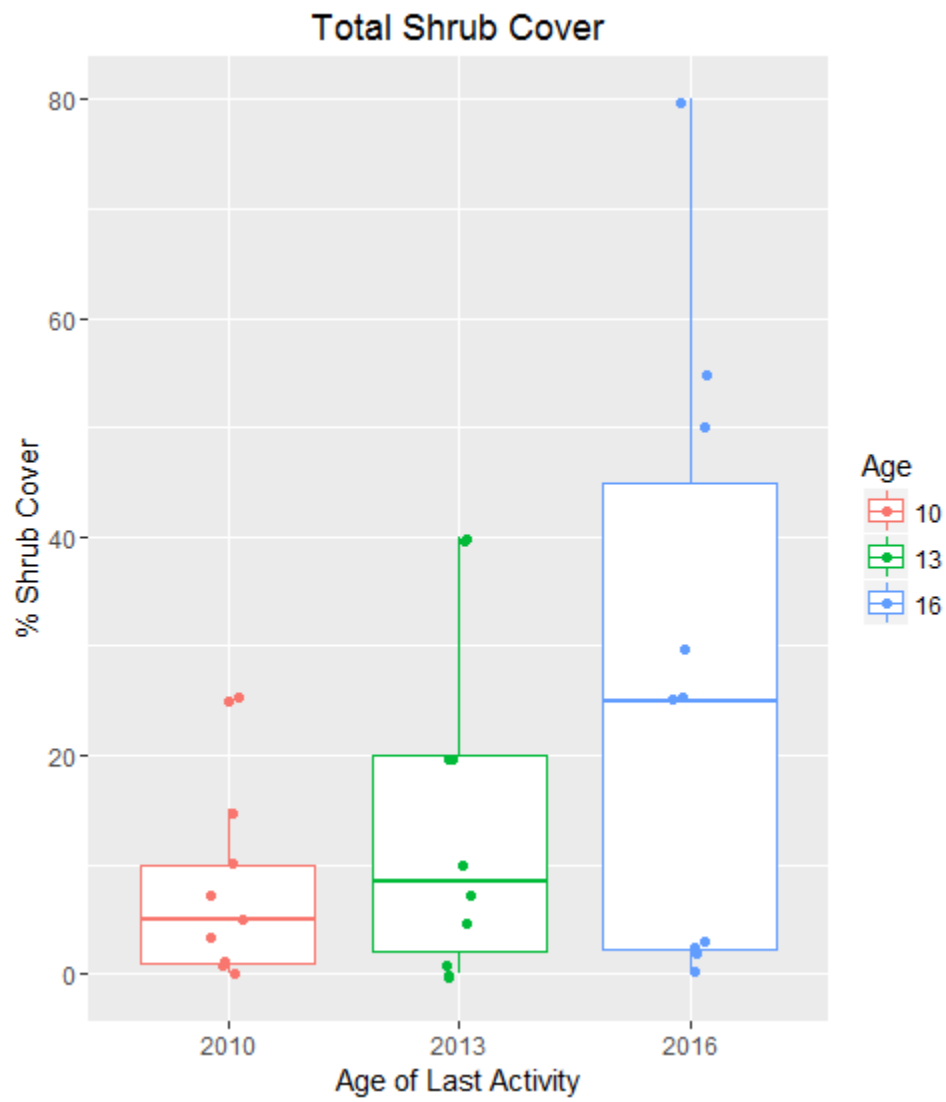


Figure 7: Total shrub cover, a statistic combining the cover of three woody species, is shown to increase dramatically as the year of last nest activity becomes more recent.

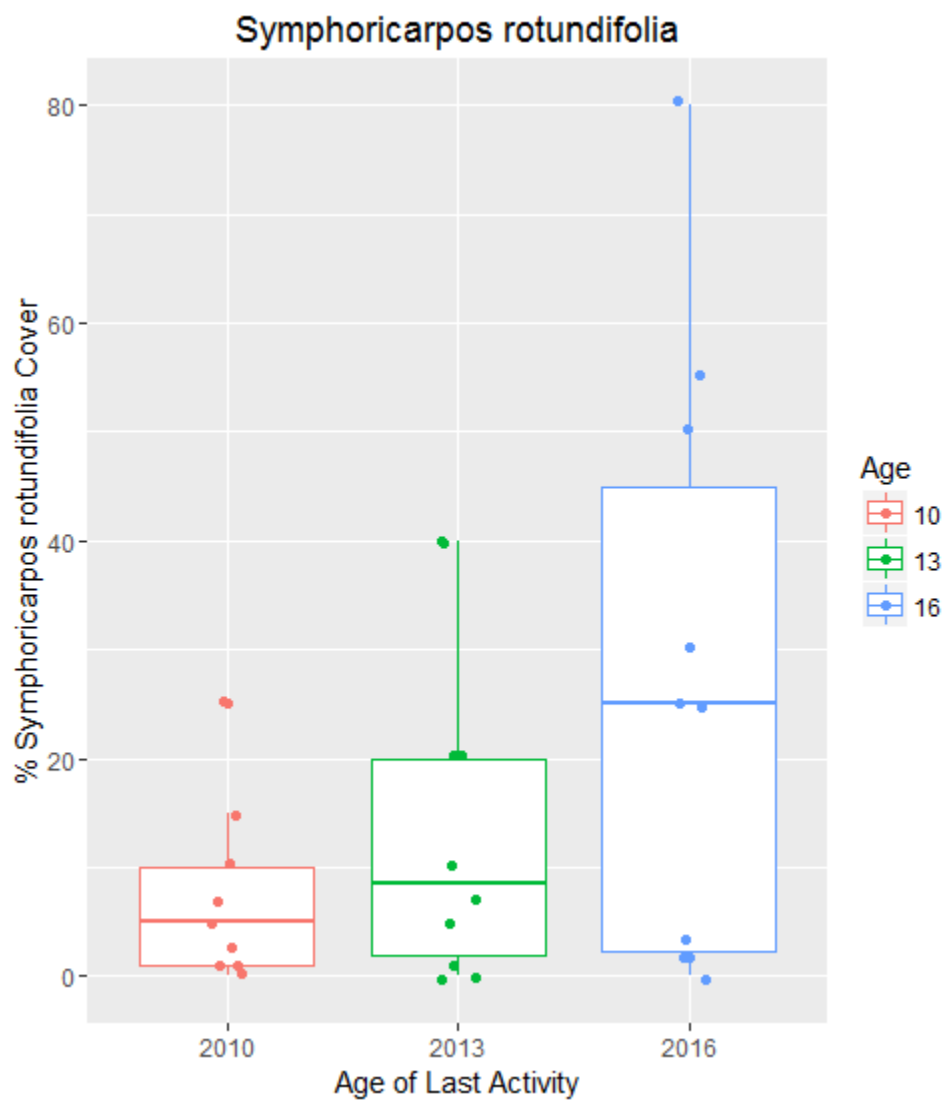


Figure 8: Differences in percent cover by *S. rotundifolius* between active and inactive nests. Cover of *S. rotundifolia* was much greater the area on and around (in a circle with a 1m radius) for recently active nests than for inactive nests.

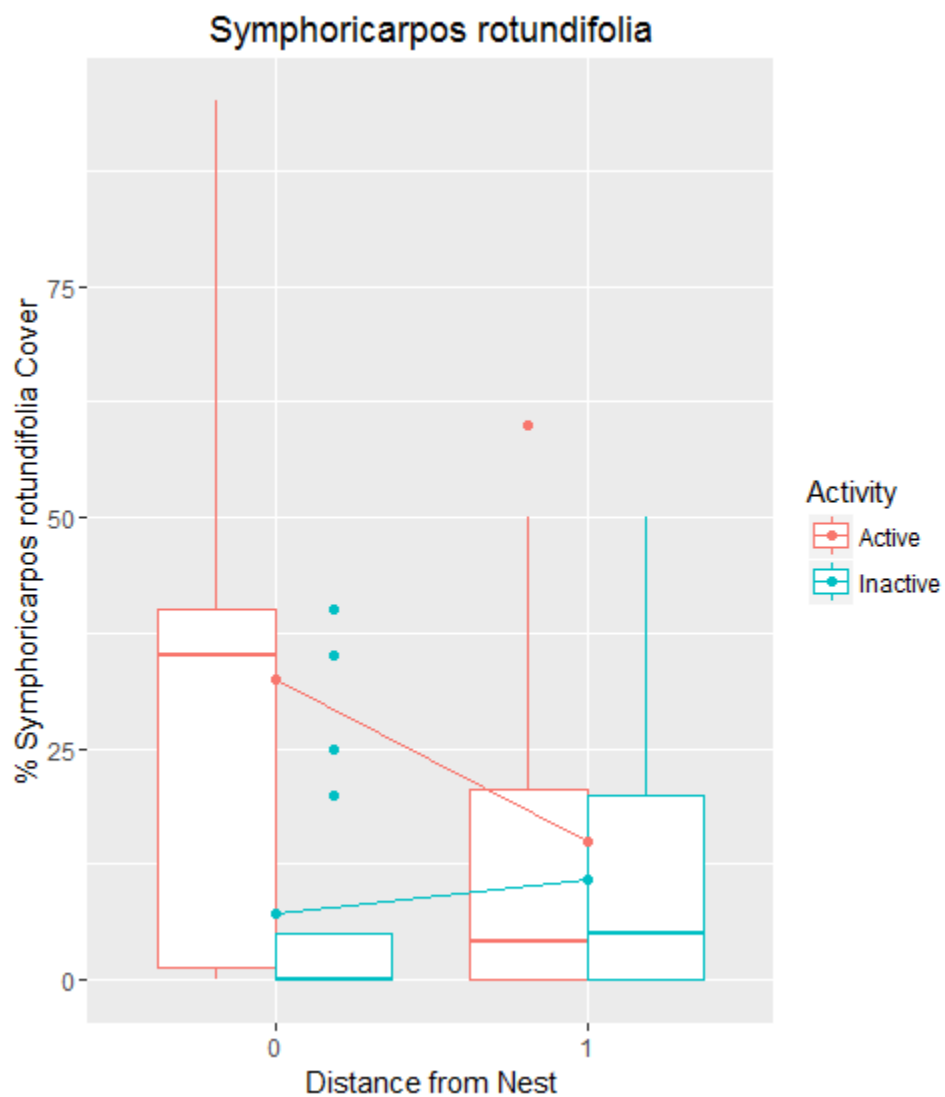


Figure 9: *S. rotundifolius* cover as assessed during the plant community composition analysis. Not only is this species found more often on active nests, it is also found to be more abundant on more recently active nests, as seen in Figure 5.

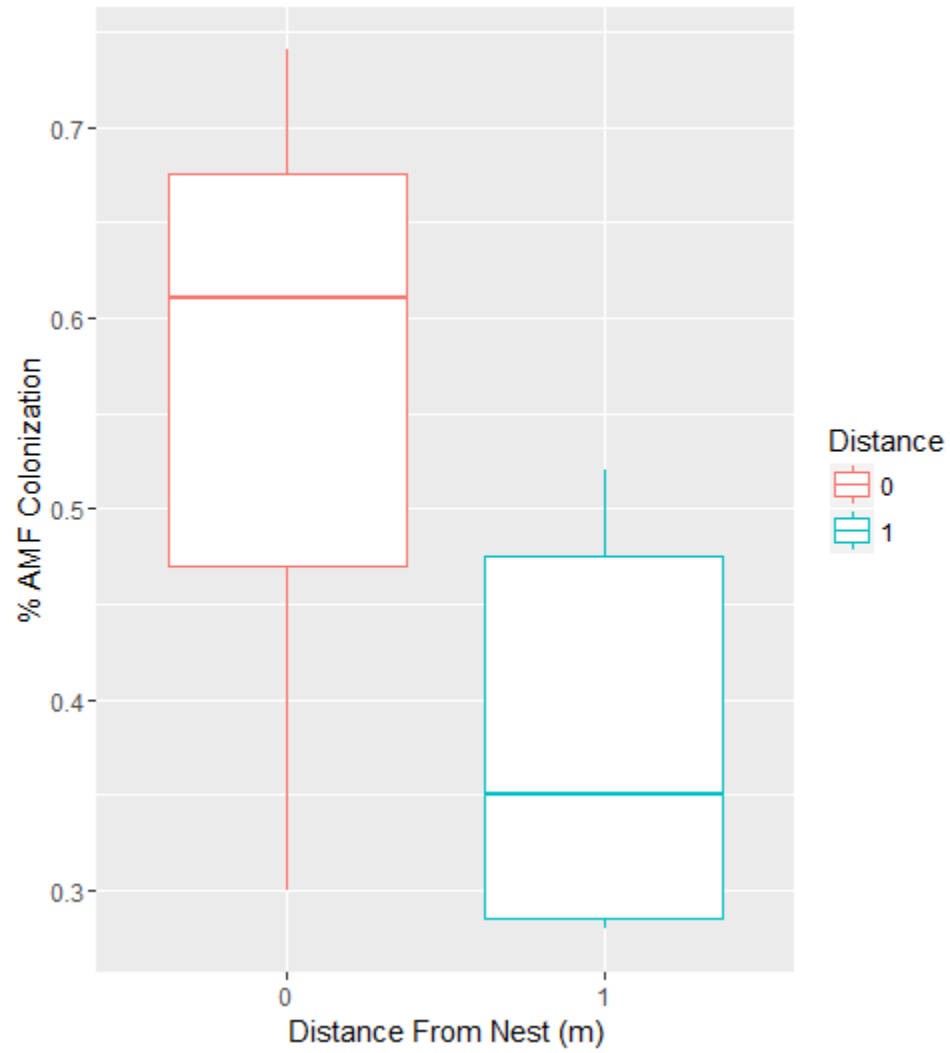
AMF Colonization of *Symphoricarpos Rotundifolius*

Figure 4: Differences in AMF colonization of *S. rotundifolius*. Colonization can be seen to be much greater on nests as compared with off-nest locations. (Percent out of 1.0).

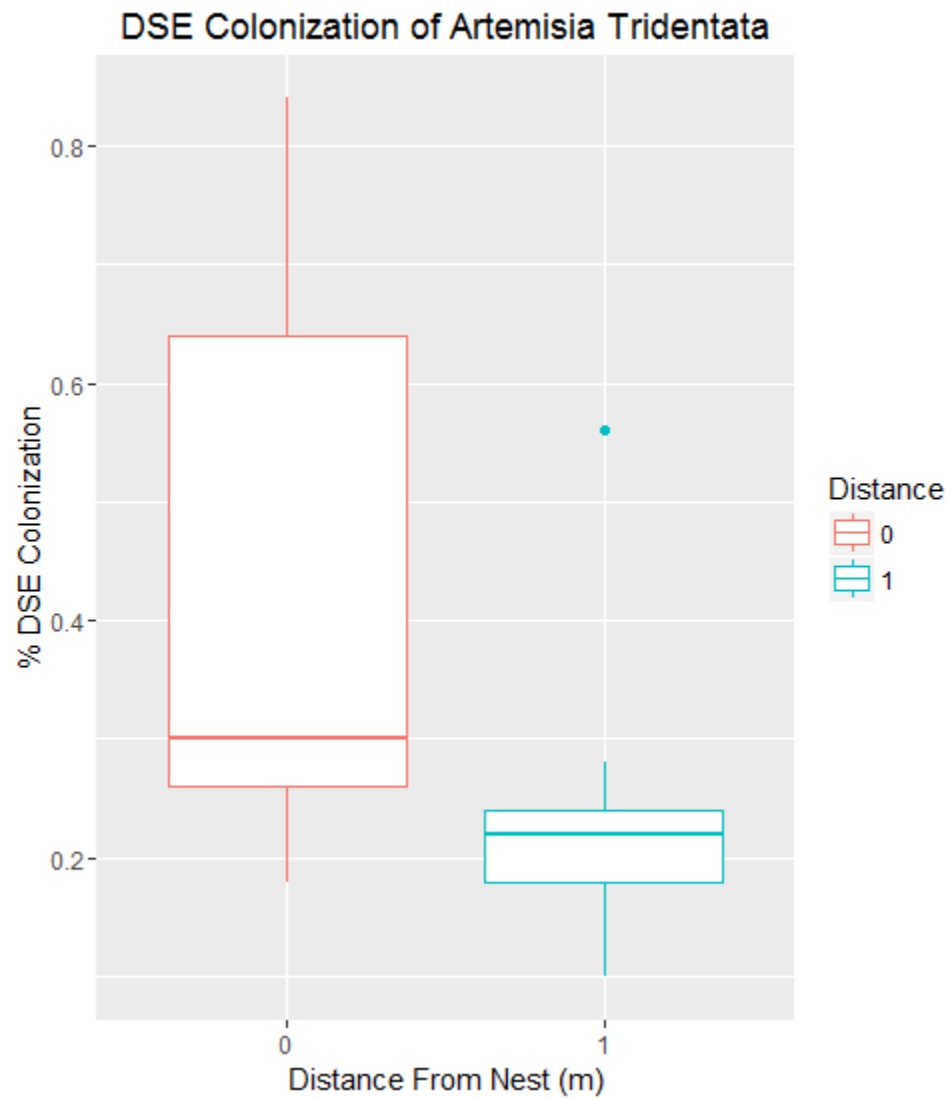


Figure 5: DSE Colonization of *A. tridentata*. A clear difference can be seen between the level of colonization on-nest and off-nest. (Percent out of 1.0).

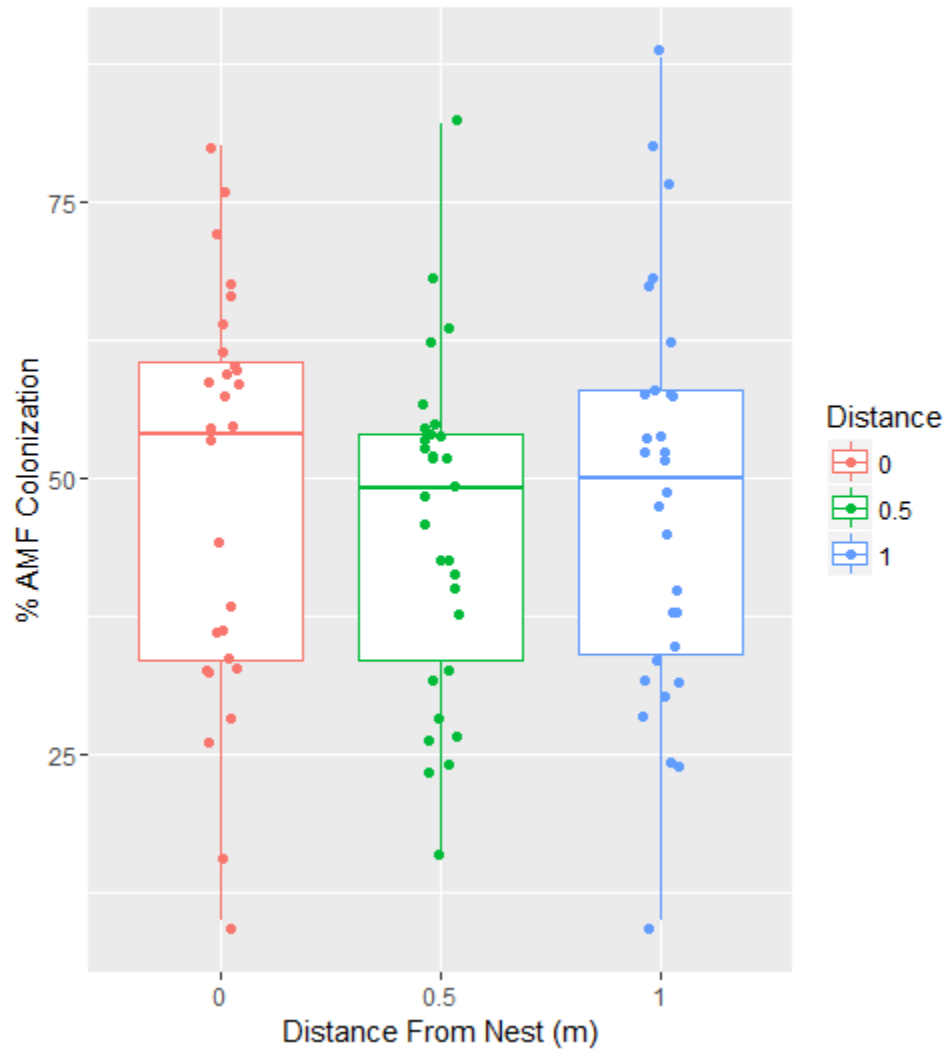
AMF Colonization of *Chrysothamnus viscidiflorus*

Figure 2: Colonization of *C. viscidiflorus* by AMF. Little to no difference is seen across distances, and the graph of DSE appears virtually the same.

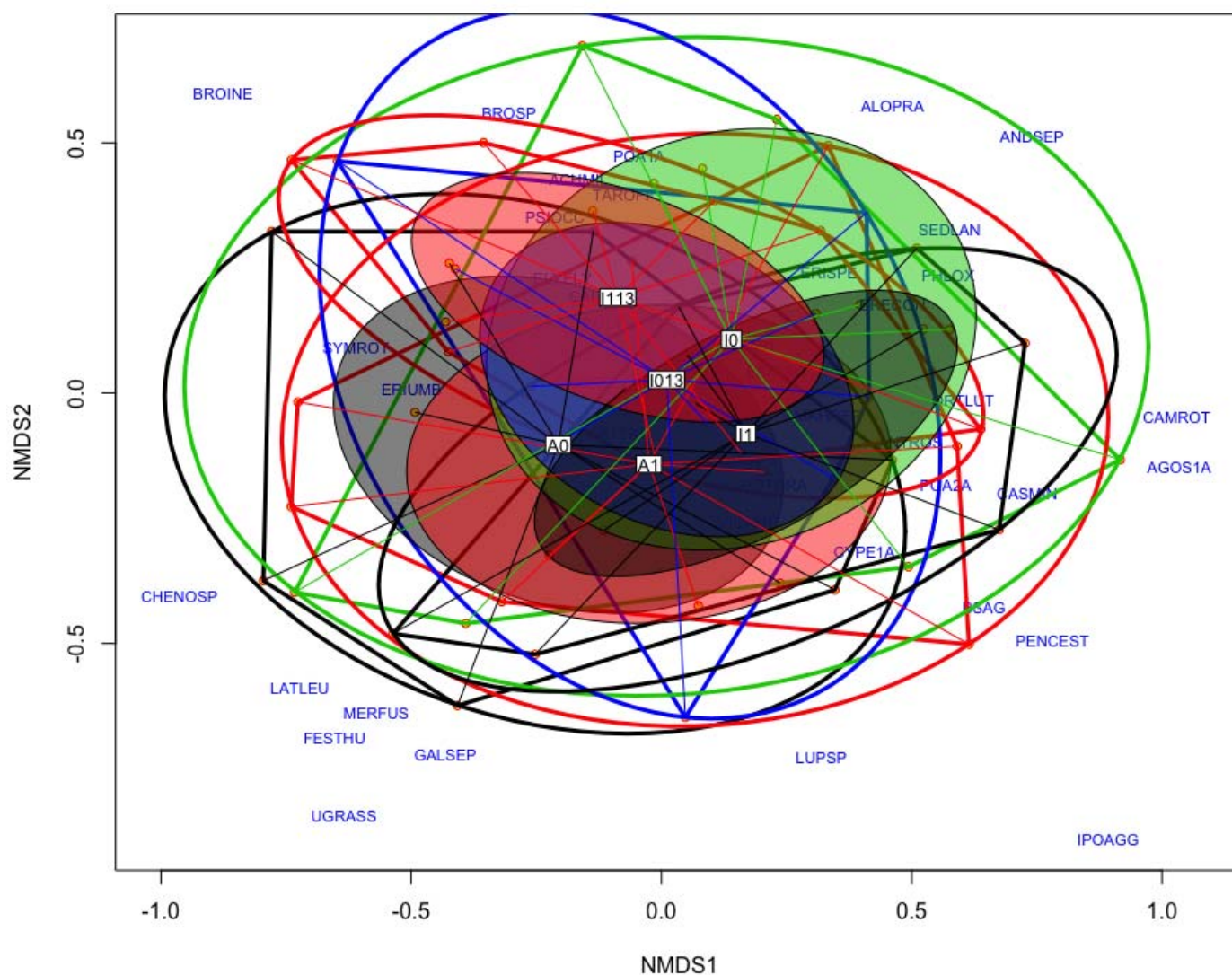


Figure 10: NMDS Plot showing grouping of plots by ages. A refers to active in 2016, I13 refers to last active in 2013, and I refers to last active in 2010. 0 and 1 following the letter codes designate distance from nest. In blue are the code names of the 42 species that were included in our community composition assessment. We see a slight grouping for A0/A1, I013/I113, and I0/I1.



Figure 1: Map of the research site in the Almont Triangle. The features in the map are the 30 ant nests that were the focus of the study. The nests are broken down into three categories by age of last activity.

<i>S. rotundifolia</i>	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	p < 0.05
1	•	•				•	•	•	522.30	
2		•	•			•	•	•	524.08	
3	•					•	•	•	520.95	
4		•				•	•	•	525.72	
5			•			•	•	•	522.73	
6				•		•	•	•	522.57	
7					•	•	•	•	518.81	*
8						•	•	•	524.38	
9							•	•	522.38	
10						•		•	522.38	*
7A					•	•	•	•	518.81	
7B					•		•	•	516.81	
7C					•	•		•	516.81	

Table 1: *S. rotundifolius* cover model selection

<i>S. rotund Cover</i>	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	p < 0.05
1	•					•	•		254.11	
2			•			•	•		256.46	*
3						•	•		263.27	
4							•		261.27	
5						•			261.27	
2A			•			•	•		254.11	*
2B			•				•		252.11	
2C			•			•			258.51	

Table 2: *S. rotundifolius* cover (from the shrub cover metric) model selection

Shrub Cover	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	P < 0.05
1	•	•				•	•	•	538.13	
2		•	•			•	•	•	536.79	*
3	•					•	•	•	536.40	*
4		•				•	•	•	541.27	
5			•			•	•	•	535.02	*
6				•		•	•	•	537.90	
7					•	•	•	•	539.35	
8						•	•	•	539.45	
9							•	•	537.45	*
10						•		•	538.14	
5A			•			•	•	•	535.02	
5B			•				•	•	533.02	
5C			•			•		•	534.19	

Table 3: Shrub cover model selection

Litter	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	P < 0.05
1	•	•				•	•	•	381.97	*
2		•	•			•	•	•	380.88	*
3	•					•	•	•	380.61	*
4		•				•	•	•	386.14	
5			•			•	•	•	379.52	*
6				•		•	•	•	382.81	
7					•	•	•	•	383.82	
8						•	•	•	384.78	
9							•	•	382.78	
10						•		•	382.78	
5A			•			•	•	•	379.52	
5B			•				•	•	377.52	
5C			•			•		•	377.52	

Table 4: Litter cover model selection

Moisture	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	P < 0.05
1	•	•					•	•	226.60	
2		•	•				•	•	226.61	*
3	•						•	•	229.93	
4		•					•	•	224.71	*
5			•				•	•	229.97	
6				•			•	•	226.46	
7					•		•	•	221.93	*
8							•	•	228.06	

Table 5: Soil moisture model selection

Temperature	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	P < 0.05
1	•	•				•		•	214.83	
2		•	•			•		•	215.01	*
3	•					•		•	226.96	
4		•				•		•	213.23	*
5			•			•		•	227.15	
6				•		•		•	214.86	
7					•	•		•	213.55	*
8						•		•	225.37	

Table 6: Soil temperature model selection

Simpson's	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	P < 0.05
1	•	•				•	•	•	-57.345	
2		•	•			•	•	•	-57.685	*
3	•					•	•	•	-58.684	
4		•				•	•	•	-55.697	
5			•			•	•	•	-59.474	*
6				•		•	•	•	-56.938	
7					•	•	•	•	-60.092	*
8						•	•	•	-56.866	
9							•	•	-46.621	
10						•		•	-58.862	*
7A					•	•	•	•	-60.092	
7B					•		•	•	-51.630	
7C					•	•		•	-59.847	

Table 7: Simpson's Diversity Index model selection

Oomycetes	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	P < 0.05
1	•	•						•	489.37	*
2		•	•					•	489.89	*
3	•							•	491.77	
4		•						•	488.08	*
5			•					•	492.30	
6				•				•	490.62	*
7					•			•	491.28	
8								•	490.48	

Table 8: *C. viscidiflorus* infection by oomycetes model selection

AMF	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	P < 0.05
1	•	•				•	•	•	-13.821	
2		•	•			•	•	•	-13.821	
3	•					•	•	•	-11.808	
4		•				•	•	•	-13.977	*
5			•			•	•	•	-11.808	*
6				•		•	•	•	-11.856	
7					•	•	•	•	-11.856	
8						•	•	•	-12.075	
9							•	•	-14.075	*
10						•		•	-8.8250	
4A		•				•	•	•	-13.977	
4B		•					•	•	-15.977	
4C		•				•		•	-14.582	

Table 9: Colonization of *S. rotundifolius* by AMF model selection

DSE	Activity	Distance	Age	Distance*Age	Distance*Activity	Moisture	Temperature	Plot	AIC	P < 0.05
1	•	•					•	•	-0.2633	
2		•	•				•	•	-0.2633	
3	•						•	•	2.8391	
4		•					•	•	0.1213	*
5			•				•	•	2.8391	
6				•			•	•	1.7096	
7					•		•	•	1.7096	*
8							•	•	3.3974	
9								•	1.9131	
4A		•				•	•	•	2.1213	
4B		•					•	•	0.1213	
4C		•				•		•	0.1213	

Table 10: Colonization of *A. tridentata* by DSE model selection