Vachellia Collinsii: Assessing the Below-Ground Dynamics in an Ant-Plant Relationship

Abstract

The mutualistic interaction between the bullhorn acacia (*Vachellia collinsii*) and its resident ants (*Pseudomyrmex*) is well-documented for its above-ground dynamics, where the plant provides food and shelter to the ants in exchange for protection and vegetation clearance. This study explores the under-studied below-ground impacts of this mutualism, focusing on the hypothesis that the ants' above-ground clearing activities affect fine root mass beneath *V. collinsii* canopies. Conducted in June 2024 at Estacion Experimental Horizontes in the tropical dry forest of northwestern Costa Rica, this study compares fine root mass within *V. collinsii* clearings to that in adjacent control sites without *V. collinsii*. Fine root samples were collected from six cores per site in ten experimental sites. Statistical analyses revealed no significant difference in fine root mass between *V. collinsii* and control sites ($p = 0.246$). However, a significant positive relationship was found between clearing size and the difference in root mass ($R^2 = 0.64$, $p = 0.013$), suggesting that larger clearings are associated with greater differences in fine root mass. These results indicate that while *V. collinsii* clearings do impact below-ground dynamics, the effects are more pronounced with larger clearing sizes.

Intro

Perhaps one of the most widely studied mutualistic interactions occurs between a particular ant and plant species that are native to Central America (Davidson, 1993; Heil&McKey, 2003; Janzen, 1966; Rico-Gray & Oliveira, 2007). The plant species *Vachellia collinsii* or the "bullhorn acacia" is particularly fascinating, as it possesses many unique structural traits that foster interactions between itself and the ants, *Pseudomyrmex,* that cohabitate within it.

The mutualistic interaction between *V. collinsii* and the ants can be described through the benefits that are exchanged between the two species. *V. collinsii* provides the *Pseudomyrmex* with food in the form of extrafloral nectaries (Barton, 1986), and Beltian bodies (Belt, 1874), a detachable tip found on the

leaves of *V. collinsii*, containing lipids, proteins, and sugars (Rickson, 1975). Likewise, *V. collinsii* provides the *Pseudomyrmex* with shelter within the swollen thorns of the tree that resemble bullhorns, which the colonies inhabit (Fonseca, 1993). In exchange, the *Pseudomyrmex* aggressively defend the tree against herbivores and encroaching vegetation. In fact, the *Pseudomyrmex* often maintain a clearing on the soil surface surrounding the stem of the tree, keeping the tree free from all competing vegetation (Janzen, 1966). However, the size of the clearing varies among different ant species, with larger clearing sizes presumably indicative of a better plant defense and a closer symbiotic relationship between the ant and the plant (Amador-Vargas, 2019).

Previous studies have focused on ant-plant relationships from a variety of perspectives, such as the patterns of defense of *V. collinsii* across varies Pseudomyrmex species (Suarez, et al., 1998; Amador-Vargas, 2021), the evolutionary ecology of the relationship itself (Davidson, 1993; Heil&McKey, 2003), as well as the ant-plant relationship and recruitment of host species seedlings (Amador-Vargas, 2011). The common thread that weaves between these studies is their focus on the maintenance of the symbiotic relationship based on above-ground processes and characteristics. Yet, the relationship between *V. collinsii*, it's mutualism with Pseudomyrmex, and the system below the ground has yet to be explored.`

The symbiotic interaction not only shapes the above-ground processes, but also has the potential to have an impact on the below-ground dynamics. Above-ground canopy gaps, defined as an opening in the forest canopy (Runkle 1982), have been studied extensively, detailing the patterns that shape the spatial heterogeneity that these structures can cause (Denslow 1980; Denslow&Hartshorn 1994). Fine root mass, growth, and nitrogen content have been studied in other parts of Costa Rica, with information about a fabaceae species, but have not focused specifically on *V. collinsii* (Valverde-Barrantes et al., 2007). However, there are no published studies that explore the symbiotic relationship below-ground between *V. collinsii* and Pseudomyrmex.

In the tropical wet forest, areas below gaps in the canopy had less fine root length and biomass than the closed canopy sites. Whether the clearing of vegetation above-ground by ants results in below-ground gaps underneath the Vachellia plant is not known. Understanding these below-ground gaps could be important for predicting and assessing patterns of regeneration and structure in a secondary-succession forest, as *V. collinsii* show up as an early succesional species in these ecosystems (Janzen, 1974).

Fine roots were chosen for this study because they account for a high proportion of energy and nutrient fluxes within the terrestrial biome (Jackson et al., 1997).

Our study has two objectives. First, we test the hypothesis that above-ground clearing by ants reduces fine root mass underneath the *V. collinsii* crowns. We did this by comparing fine root stocks sampled from clearings to fine root stocks two meters away but outside the clearing zones, predicting lower fine root mass under *V. collinsii*. Second, we asked whether the reduction in fine root mass underneath *V. collinsii* crowns is proportional to clearing sizes, predicting a larger reduction in fine root mass with larger clearing sizes.

Methods

Study Site

This investigation was conducted in June 2024 in the tropical dry forest located in Estación Experimental Horizontes of Área de Conservación Guanacaste (10.71"N 85.55"W), in northwestern Costa Rica. We located ten individual *V. collinsii* plants, noting that the plant has a clonal growth form, where a clump of stems may have originated from the same individual. We restricted our sampling to plants that had distinguishable clearing. To sample nearby “non-Vachellia” root stocks, we paired each of the ten individuals with a corresponding control site lacking Vachellia that was located in a random direction 2m away from the edge of the clearing.

Fine Root Sampling

Within each *V. collinsii* clearing, we haphazardly selected three points to extract root cores from in order to collect representative samples of the root mass within the cleared zone. We followed a similar sampling protocol for the control zones, restricting the three haphazard root samples to a ~1 m^2 area. Soil samples were extracted by pounding a 10 cm long r steel ring with a diameter of 8 cm into the ground, as a total of six cores were collected at each site, three from the *V. collinsii* zone, and three from the control.

Fine roots (≤2 mm in diameter) were carefully washed and removed from each soil sample, and individually stored in coin envelopes for drying. We noted the presence of any potential root nodules in all samples. After drying in a toaster oven at 100℃ for one hour, viable and dead root mass was measured. A traditional drying oven was unable to be used for this investigation, as the oven located at the field station was broken due to heavy rainfall and a gecko nesting inside the fuse box.

Ancillary Variables

Additional metrics were recorded at each experimental site to better describe variation in vegetation within and among the ten sites: canopy cover using CanopyApp, diameter at breast height, ant species, clearing size, and total number of *V. collinsii* stems > 5cm diameter breast height. Clearing size was taken as two measurements, the diameter from North to South, and East to West. Clearing area in units of m^2 was calculated as the area of an ellipse.

Data Analysis

In order to examine the correlation between root mass and site type, graphs and statistical tests were calculated and visualized using the ggplot2 package in RStudio. The data was sorted into two separate composites, one containing the individual values of each site, and the other aggregating the three data points from each site into mean values in order to reduce replication and confusion within the R program. To test our first hypothesis, we used a paired T-test to determine the relationship between the root mass and site type, using the average root mass data as the response variables. A violin plot was

created for further visual aid. To test the second hypothesis, we ran a paired T-test between clearing size and the difference in root mass between the experimental and control site. A visual regression model was created, then R^2 was calculated in order to determine how well the model fits the data.

Results

In total, ten different experimental sites were examined, collecting 30 total samples from *V. collinsii* sites and 30 samples from control plots, 60 samples in total. Throughout the drafts of various visualizations, it was clear that there appeared to be an outlier, and ultimately, Site 10 was excluded because it was calculated to be 3.44 standard deviations away from the mean. Almost no *V. collinsii* sites contained nodules, and those that did only had dead nodules.

To understand the relationship between the root mass within a *V. collinsii* clearing and without, we modeled the mean root mass values from both *V. collinsii* plots and the control plots, or "Non." The mean root mass in the *V. collinsii* group and control were found to be 95.39 g/m^2 and 117.9 g/m^2, respectively. The p-value for the paired t-test between the two site's root mass means was found to be 0.246. The individual values and distributions are represented by Figure 1, with blue dots denoting the mean value for each individual site.

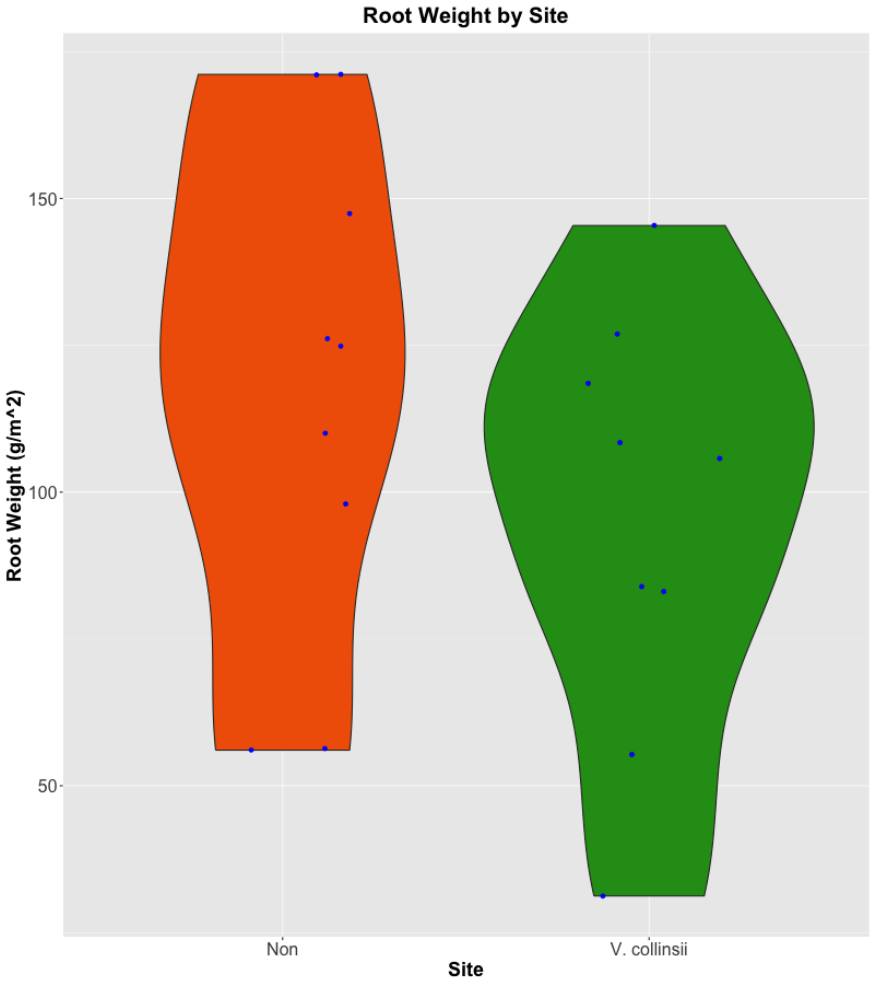

Figure 1. Mean Root Mass (g/m^2) for sites 1-9 of both V. collinsii (orange) and Non (green).

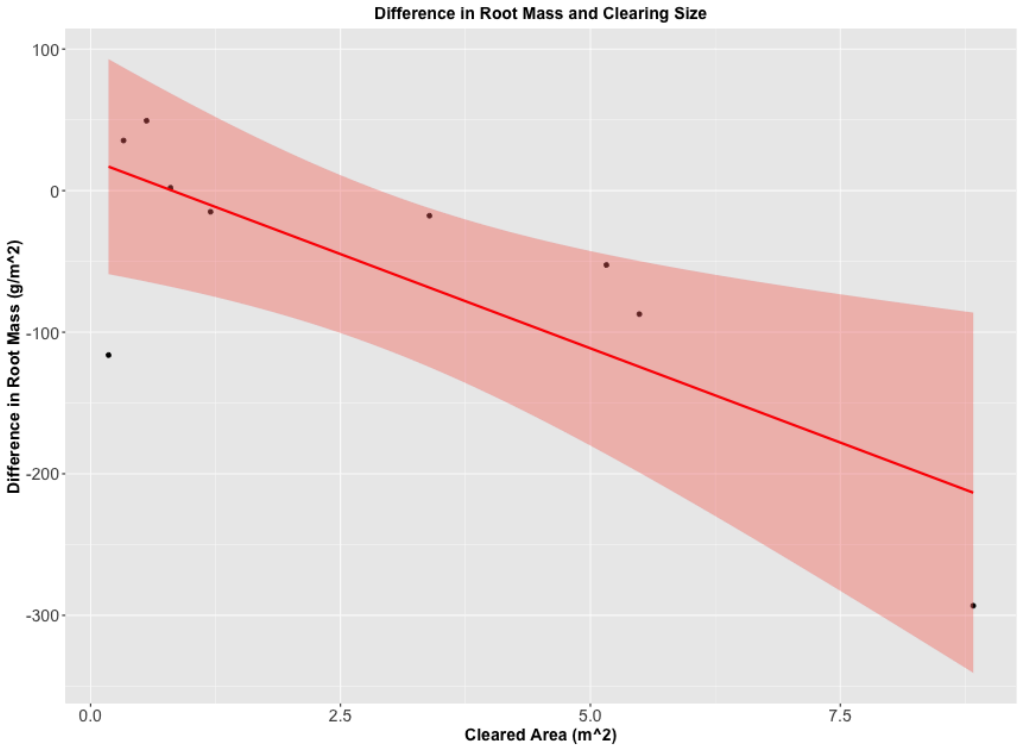

Figure 2: Regression plot of the difference in Root Mass (g/m^2) between experimental and control sites and Clearing Size of experimental plots

This plot illustrates the relationship between the variation in root mass and the size of the cleared area. This visualization indicates that as the clearing size increases, the difference in root mass also increases. The red shading denotes the confidence interval, while the red line represents the trendline. The coefficient of determination (R^2) for this analysis was 0.64, and the p-value is 0.013, providing statistically significant evidence that the difference in root mass increases with clearing size.

Discussion

This investigation operated under two assumptions. We assume roots under *V. collinsii* come from *V. collinsii*, but we did not verify this through deeper analysis. Second, we assume ants help reduce non vachellia roots below ground.

These metrics are a stepping stone to explaining the parameters of success of Vachellia collinsii in the tropical dry forest. From the results, it can be concluded that there exists a reduction of root mass in *V. collinsii* clearings, as opposed to the control plots that did not contain *V. collinsii*. However, there's

variation among sites, and in general the non *V. collinsii* sites had higher root mass, but due to the large variability this was not considered to be significant.

It is suggested that 64% of the variance in root mass differences can be attributed to the size of the clearing. Root mass difference was found to be proportional with clearing size, indicating that the larger the system and mutualism, the more below-ground exclusion. A strong relationship between size of clearing and reduction in root mass tells us how more competitive ants above ground translate to more competition below ground within the gap.

In the context of secondary succession and the ways that *V. collinsii* makes itself successful in this environment, we can confidently say that *V. collinsii* plays a role in shaping its environment through the mechanism of establishing a below-ground gap, as evidenced by the experimentation.

For future study, it would be interesting to run the same experiment again across a farther and more varied landscape, collecting more data points and possibly addressing the variance within the sites and data. Likewise, taking nutrients into account, and examining how *V. collinsii* might be establishing a below-ground gap as a means to acquire nutrients, and whether that is influencing the dynamics surrounding the clearing.

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