

The Role of *Castilleja spp.* In Plant and Mycorrhizae Communities Within Various Climates

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Abstract

Hemiparasites such as those in the genus *Castilleja* have important effects on the plant communities they are found in. For example, hemiparasitic plants may reduce the abundance of dominant plant species, opening niche space for subordinates. This effect may be driven by direct parasitism of *Castilleja* on dominant hosts species or maybe mediated through indirect interactions as *Castilleja* compete with root-associated fungal communities, like arbuscular mycorrhizal fungi and dark septate endophytes, for host carbon. Here we explored how *Castilleja* affects plant diversity, richness, and community composition, and how *Castilleja* influenced the relationship between dominant plants in the community and their root-associated fungal communities. Overall, we found that the presence of *Castilleja* increased plant diversity 9%, increased species richness 11%, without influencing plant community composition. Additionally, we found that *Castilleja* affected dominant plant interactions with mycorrhizal fungi, leading to an 18% reduction in arbuscular mycorrhizal fungal colonization within dominant root tissues.

Introduction

Hemiparasitic plants may be key regulators of plant community composition and ecosystem function through their interactions with surrounding plant species (Reed, 2012). For instance, *Castilleja* is a genus of hemiparasitic plants in the family Orobanchaceae that inhabit large portions of North and South America (Hersch & Roy, 2007). Across the western range of the Rocky Mountains three species of *Castilleja*, *Castilleja miniata*, *Castilleja rhexifolia*, and *Castilleja sulphurea* occur in several biomes and commonly hybridize (Hersch-Green et. al 2008). Hemiparasites, like *Castilleja*, can increase plant diversity in a community by reducing the relative abundance of dominant species, thus allowing subordinate species to increase in abundance (Westbury et. al, 2006; Reed, 2012). However, the effects of hemiparasites on plant community composition and ecosystem function may differ across climate contexts. For example, plant community composition in stressful environments is typically driven by beneficial, facilitative interactions between plant species, whereas in less stressful environments, antagonistic, competitive interactions drive the composition of plant communities (Callaway, 1998). To minimize multiple biotic and abiotic factors, while varying temperature and water availability ecologists have used altitudinal gradients to study species interactions for several decades (Körner 2007, Sundqvist et al. 2013). By measuring at multiple sites along an altitudinal gradient, we could explore the effect of *Castilleja* on plant diversity and productivity under different climate contexts (Callaway, 1998). For instance, facultative interactions at high elevation may reduce *Castilleja*'s ability to regulate dominant species abundance and increase plant diversity. Where as, reduction of dominant plant status at low elevations may lead to a greater gain in plant diversity, richness, and community composition when *Castilleja* is present.

Root inhabiting fungi, like Arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE), are critical associates of alpine plants; providing nutrients, water, and protecting plants against pathogens (Kiers et. al, 2011; Mandyam, & Jumpponen, 2014). At larger spatial-scales root-associated fungi positively-influence plant richness, diversity, and shape community composition (Van der Heijden et. al, 1998, Read, et al. 2017). However, it is not known how *Castilleja* influence the relationship between dominant host plants and their root-associated fungal communities. *Castilleja* may compete with AMF and DSE for host carbon resources leading to a negative interaction between hosts and AMF and DSE. Additionally, *Castilleja* may benefit from AMF and DSE associations directly by gaining nutrient resources from AMF and DSE (Cripps & Eddington 2005), or indirectly by parasitizing AMF and DSE-provided nutrient resources from the host plants (Salonen et. al, 2001). Thus, the ability of *Castilleja* to disrupt dominant plants relationship with root-associated fungi, like AMF and DSE, may provide a possible mechanism in how *Castilleja* can influence plant diversity, community richness, and composition. To understand the interactions between climate, plant hosts, fungal symbionts, and *Castilleja*, two related questions were explored:

- 1) How does climate mediate *Castilleja* effects on plant biodiversity and community composition?
- 2) How does *Castilleja* influence host interactions with root-inhabiting fungi?

To answer these questions, we measured plant diversity and composition in adjacent areas with and without *Castilleja* along an elevation gradient near Gothic, CO. Additionally in each plot we collected root samples from the dominant plant species at each site, which was determined using the plant community data. From these samples we measured root associated fungal communities.

Methods

Location of Study

We measured plant diversity with and without *Castilleja* at five sites that varied in elevation from 2480m to 3460m. By selecting sites that vary in ~1000m in elevation, we could maximize climatic variation among our sites (Table 1, Fig 1). Previous work demonstrated that there was ~3 C° in mean temperature difference between low and high elevation and large differences in water availability between low and high elevation (Read et al. 2017).

Procedure

Question 1: How does climate mediate *Castilleja* effects on plant biodiversity and community composition?

To address how climate mediates *Castilleja* effects on plant diversity and community composition we measured plant composition in adjacent areas with and without *Castilleja*. Within each plot we identified each plant species and visually

estimated cover within a 0.5 × 0.5 m quadrat in 10 paired plots. We analyzed composition data with using paired t-tests in R (R Development Core Team, 2008). Additionally, we calculated Cohen's D effect sizes on diversity and richness responses to *Castilleja* and conducted a linear regression to explore diversity responses across the altitudinal gradient. Finally, we conducted principal component analysis ordination to explore plant species responses and changes in community composition in response to *Castilleja* at each site individually.

Question 2: How does *Castilleja* influence host interactions with mycorrhizal fungi?

To identify how root fungal communities are influenced by *Castilleja* spp. we collected a single 2.5 × 15cm core from each plot that contained the roots of the dominant plant species found across that site. (10 cores with *Castilleja* present, 10 cores without *Castilleja* present). We stored soil cores at 4° C in lab until processed. We extracted plant roots from the core using a 0.5 mm mesh sieve. Live roots were placed within tissue cassettes. Roots were cleared in a 10% potassium hydroxide (KOH) solution, acidified in a 2% hydrochloric acid (HCl) solution, and then stained in a 0.01% trypan blue solution. We mounted the roots on microscope slides using PVLG (polyvinyl lactic acid glycerol) glue, and assessed the root material for the amount of AMF, DSE, and pathogenic hyphae colonizing the root tissues. To assess the roots, we viewed 50 root intersections per sample, noting AMF, DSE, fungal structures, and pathogen presence/absence using the grid-line intercept method (McGonigle et al. 1990, Fig 2). We analyzed the colonization data by performing a paired t-test between the root samples from *Castilleja* containing plots and their *Castilleja* absent pair. Similarly to the plant community data we performed a Cohen's D effect size analysis across the altitudinal gradient to assess if there was any variation in the affects across the altitudinal gradient.

Results

***Castilleja* increases plant diversity and richness**

Overall we found an 11% increase in plant richness ($t=3.710$, $P=0.0005$, Table 2, Fig 3) and a 9% increase in Shannon's Diversity ($t=2.877$, $P=0.006$, Table 2, Fig 3) when *Castilleja* was present within plant communities. Surprisingly when we compared effect sizes across sites to explore differences in *Castilleja* effects in different climate contexts, we found no differences in diversity or richness (Simpson's, $ANOVA_4, F=0.5402$, $P=0.707$)(Shannon's, $ANOVA_4, F=1.4537$, $P=0.232$) (Richness, $ANOVA_4, F=1.3925$, $P=0.252$). Thus, *Castilleja* increases diversity and richness in plant communities regardless of climate context.

To explore changes in community composition in response *Castilleja* we performed a principal component analysis ordination to compare plant composition with and without *Castilleja*. Overall, we found that no individual species were driving the diversity increases and changes in diversity were driven by the presence or absence of rare species at each site (4-8). For instance, at our high elevation site, we observed

that low abundant species like *Carex alba*, *Potentilla gracilis* and *Ligusticum porteri* were observed when *Castilleja* was present, but not when *Castilleja* was absent (Fig 4). This pattern continued into the lower sites. For example, at our lowest elevation site, we found in *Castilleja* plots that *Elymus* sp., *Taraxicum*, and *Capsella* were much more common relative to *Poa* sp., *Chrysanthamnus*, and *Draba* at our *Castilleja*-absent plots (Fig 7). Similar patterns can be observed within our other elevation sites (Fig 4-8).

***Castilleja* reduces mycorrhizal colonization in dominant plant hosts**

At all sites *Castilleja* reduced AMF colonization by 18% ($t=-9.63$, $p<0.0001$, Table 3, Fig 9). However, we found significant difference DSE colonization ($t=-1.53$, $p=0.133$, Table 3, Fig 9) or within any AMF structures (Vesicle, $t=-.502$, $p=0.618$; Arbuscle, $t=0.256$, $p=0.799$, Table 3, Fig 10) or pathogens ($t=1.273$, $p=0.209$, Table 3) with or without *Castilleja*. At our highest elevation site, our dominant plant species (*Arctostaphylos uva-ursi*) is strongly associated with ericoid mycorrhizal fungi, and we found that *Castilleja* also significantly reduced ericoid abundance within their roots ($t=-2.64$, $p=0.027$). The decrease in AMF/EMF caused by *Castilleja* was shown to not differ across the altitudinal gradient in a Cohen's D effect size analysis (ANOVA, $F=0.796$, $p=0.534$).

Discussion

Hemiparasitic plants increase plant diversity

Overall, we found *Castilleja* increased diversity and richness within plant communities when they were present. The increase in diversity and richness is also supported by past research on *Castilleja* in controlled experiments (Reed, 2012). However, we found inconsistent results at individual sites, which suggests that the strength of this relationship is highly variable. Despite the variability, the effect size is consistent across the altitudinal gradient. Consistent effect sizes would support the notion that *Castilleja* consistently is a major contributor to plant community dynamics and diversity as a biotic factor. The differences within each site could be explained by microclimates or thousands of other variables, however such variables do not appear to alter the effects *Castilleja* has on larger scales. Over all, our PCA results demonstrate that *Castilleja* effects was not driven by any single species, rather that *Castilleja* broadly benefited low abundance or rare species in the community. A potential explanation for this relationship is how *Castilleja* is able to decrease the biomass of any potential dominant, allowing for other novel or subordinate species to inhabit previously occupied niches.

Hemiparasitic plants shape dominant plant interactions with mycorrhizal fungi

We found that *Castilleja* reduced AMF colonization by 18.3% in dominant species across the gradient ($t=-9.63$, $p=8.49e-13$, Table 3, Fig 3). It is possible that *Castilleja* competes with AMF for host carbon resources. AMF are completely reliant on plant hosts for carbon resources, thus reduced carbon from the host results in

lower colonization. Whereas, DSE contain carbon-degrading enzymes, which allow them obtain carbon resources from the soil, leaving DSE less reliant on their plant hosts for carbon. As such, it may not be surprising that DSE are affected less by *Castilleja* as AMF. Since AMF potentially have less carbon resources, we anticipated observing a reduction in storage organs (vesicles), however we observed no differences in vesicles with or without *Castilleja* ($t=-.502$, $p= 0.618$, Table 3, Fig 4). With less AMF colonizing a host plant, the host is less able to acquire phosphorus and water resources. With a reduction in resources for the host, we conclude that reduction of AMF in dominant plant roots may be a feasible mechanism in how dominant species are reduced by *Castilleja* presence. However, future studies need to tease apart the relative contribution to dominant suppression by direct parasitism of dominants by *Castilleja* and the indirect effects mediated by competition and reduction of root-associated fungal communities.

We sought to tease apart the importance and role of *Castilleja* within the plant communities they reside. Our results match past experiments which found *Castilleja* increased biodiversity and species evenness within plant communities (Reed, 2012). In conjunction with the plant community findings, *Castilleja* negatively impacts host plant mycorrhizal communities. Because global change is shaping the climatic contexts, the composition of species, and the dominance patterns within plant communities, future research should aim at how *Castilleja*-community diversity/productivity interactions are regulated by global change. Research on other hemiparasites like *Castilleja* should also be considered as shifts in hemiparasitic plant abundance may shape the diversity and functioning of future ecosystems.

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Figures And Tables

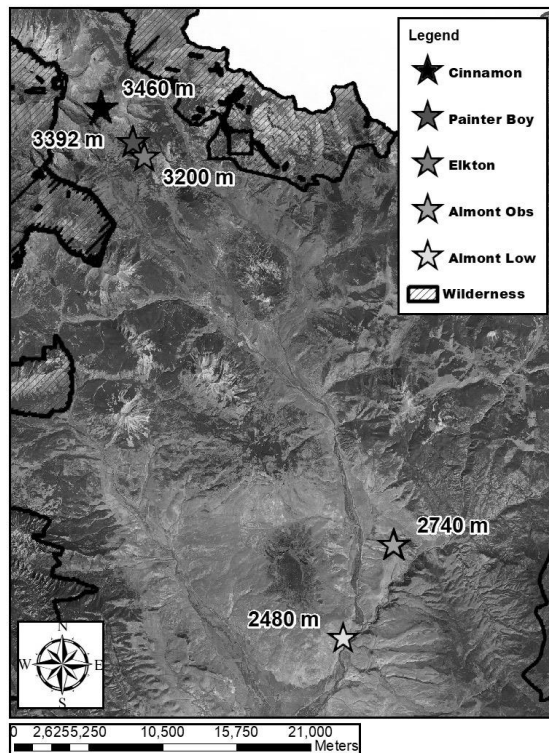


Figure 1: A map of the site of this study located within Gunnison County. Note elevations labeled by site.



Figure 2: Sample root slide showing DSE (red square) and AMF (green square) colonization. Note that DSE tend to have regular width forms and cell walls (red circles), while the AMF do not.

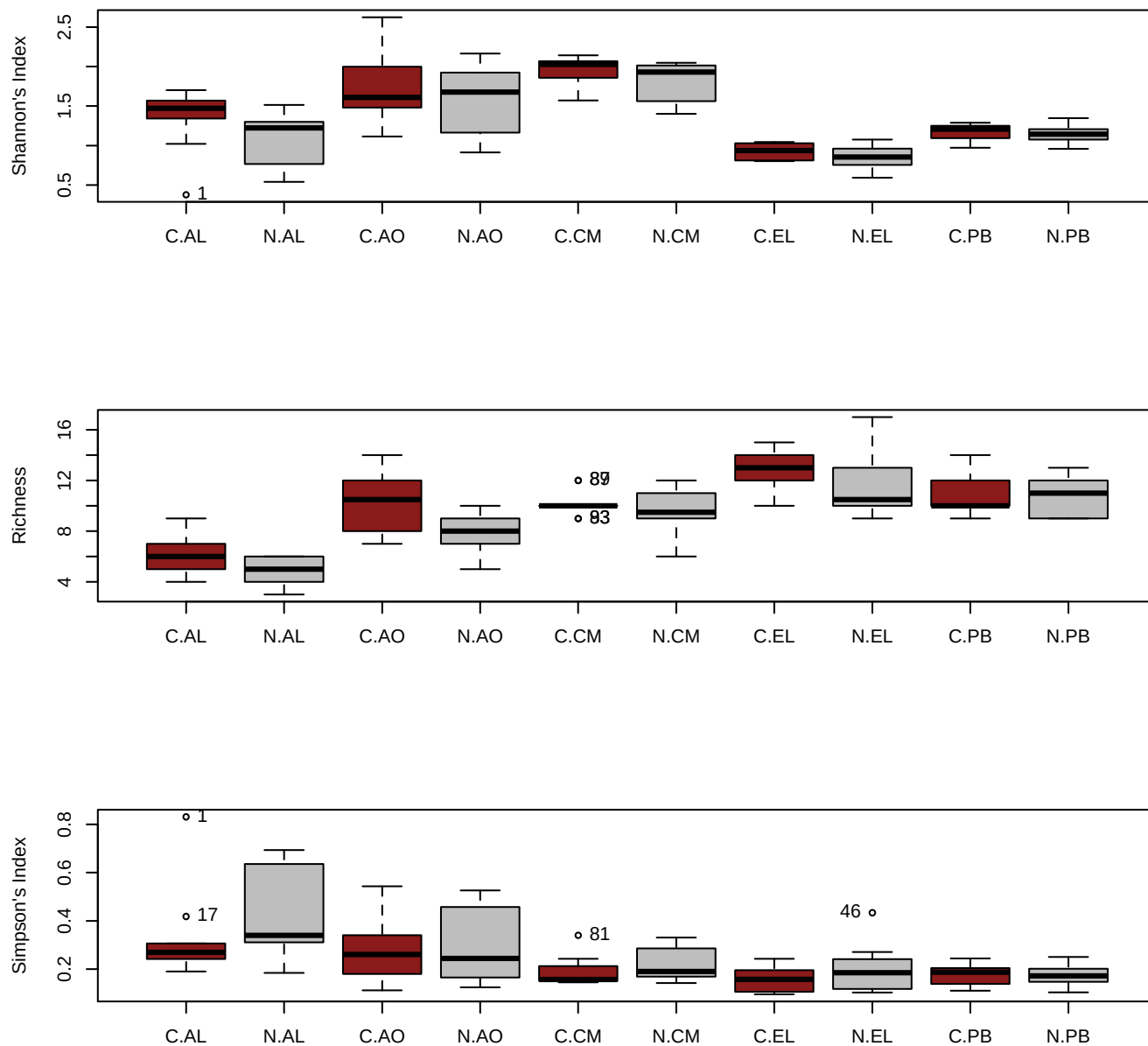


Figure 3: Box and whisker plots for Shannon's Index, Simpson's Index, and Richness across all sites. Note that *Castilleja* present sites are in red, while absent sites are in grey.

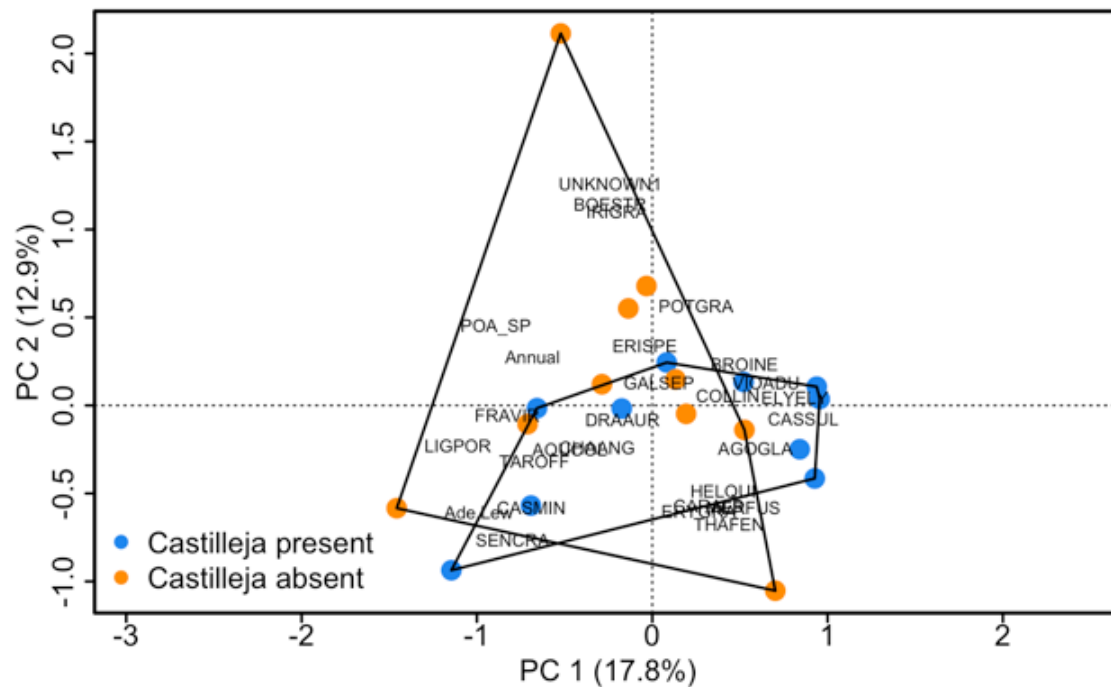


Figure 4: Sample principal component analysis for the Painter Boy Mine site.

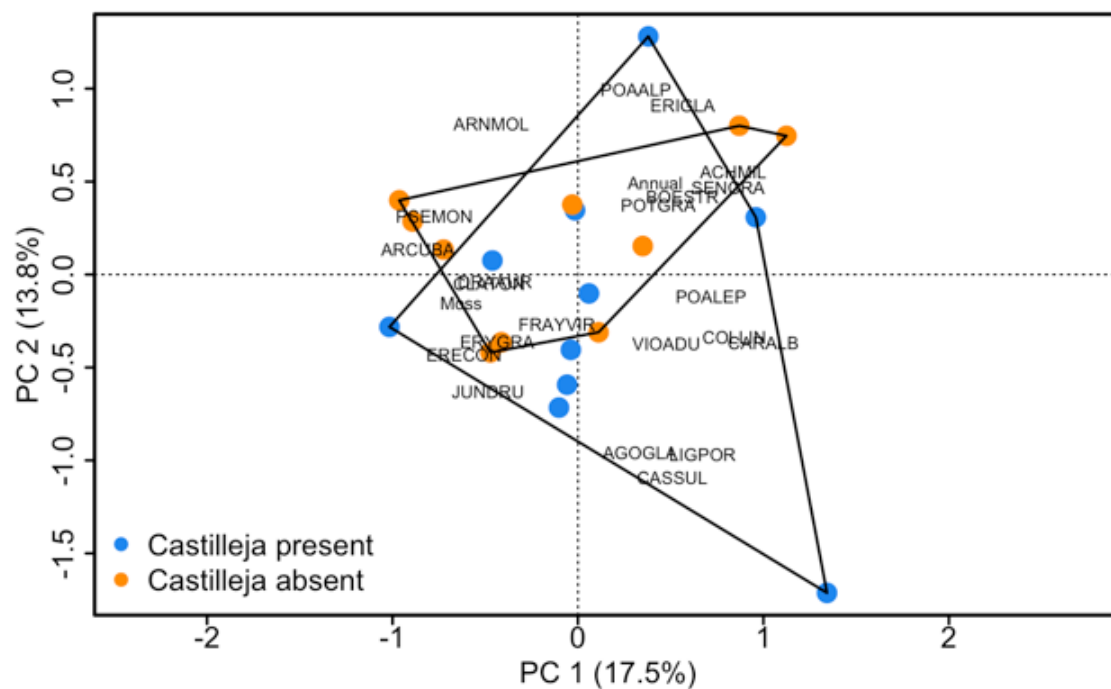


Figure 5: Sample principal component analysis for the Cinnamon site.

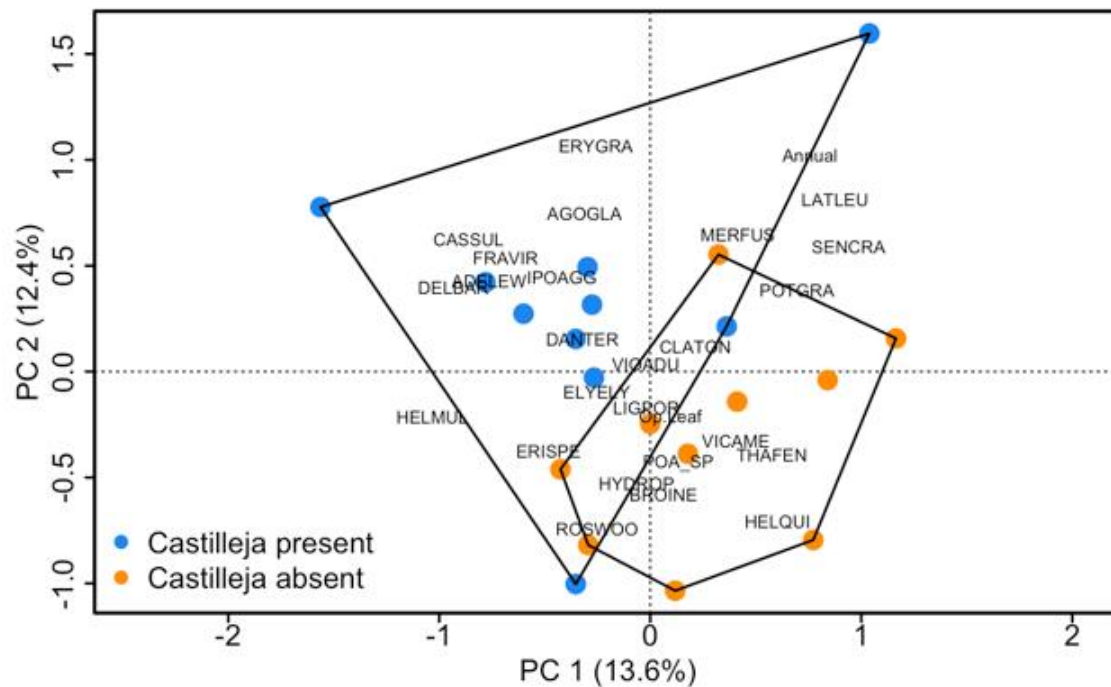


Figure 6: Sample principal component analysis for the Elkton site.

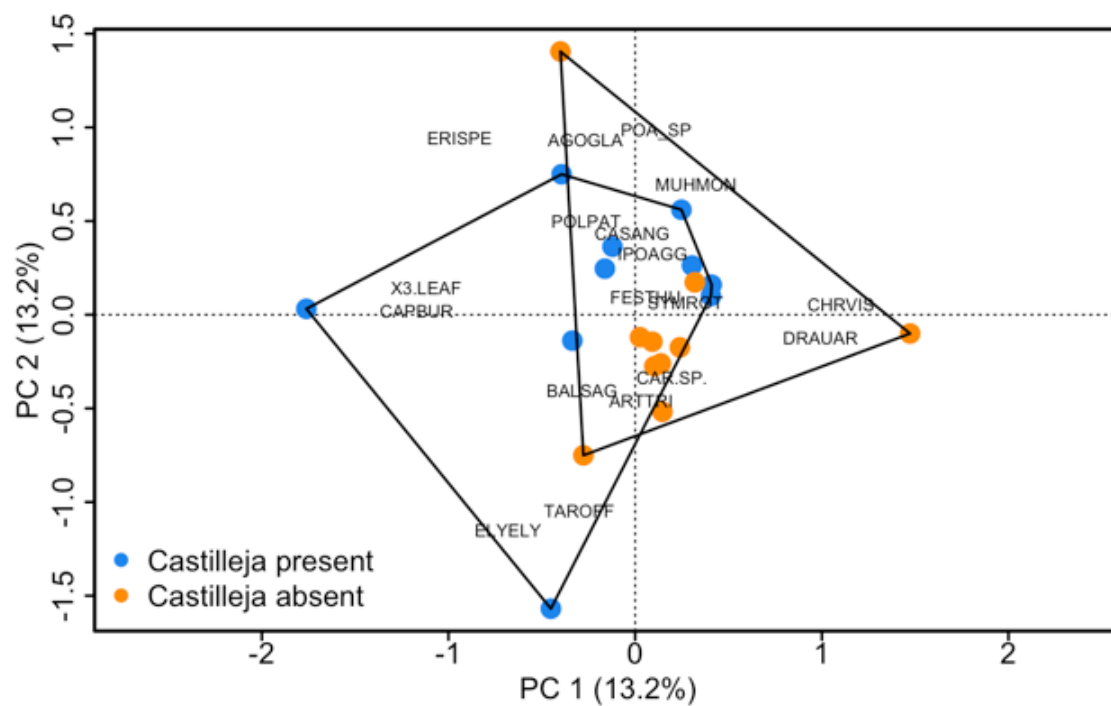


Figure 7: Sample principal component analysis for the Almont Low site.

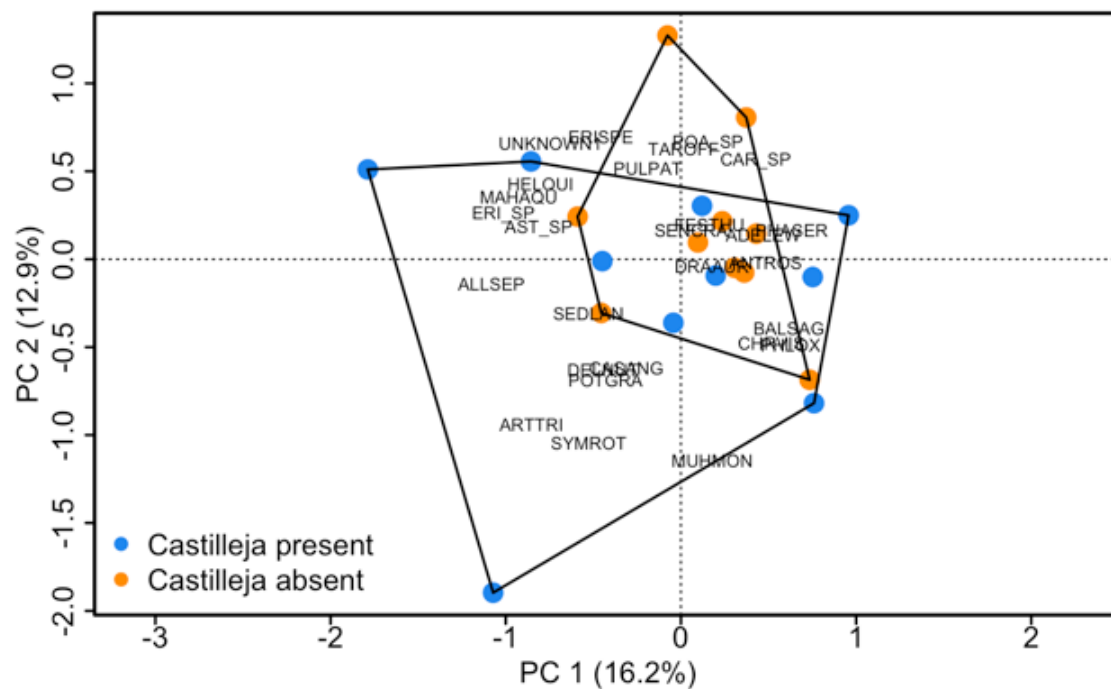


Figure 8: Sample principal component analysis for the Almont Obs site.

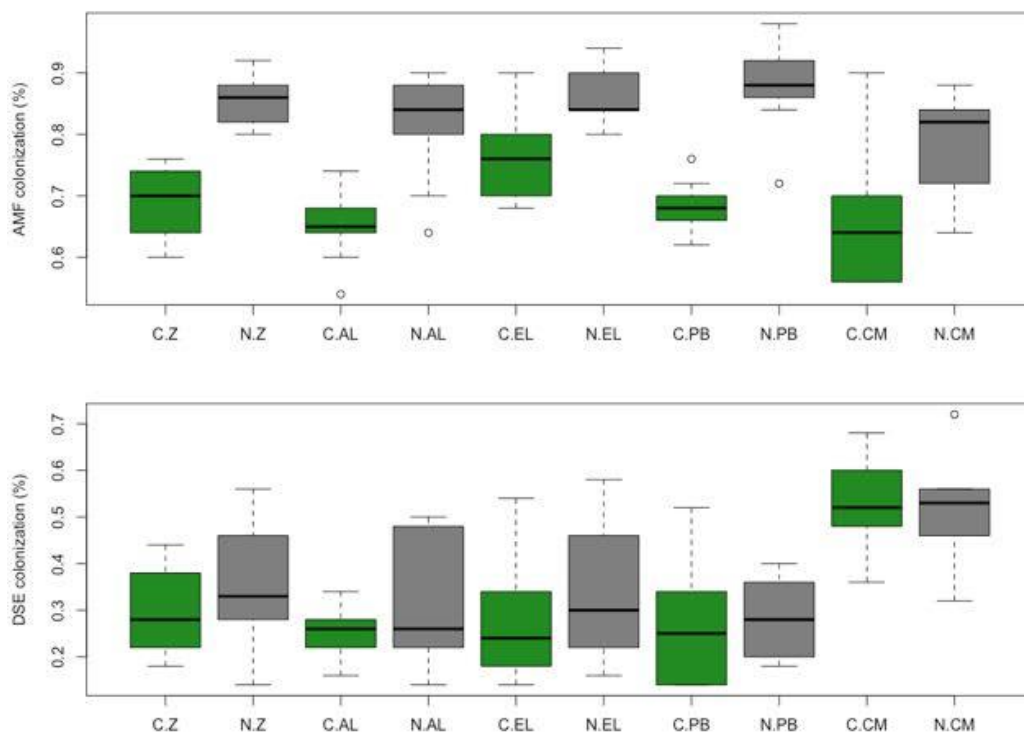


Figure 9: Box and whisker plots for AMF and DSE colonization rate as a decimal. Note that *Castilleja* present plots are denoted in green and absent plots are denoted in grey.

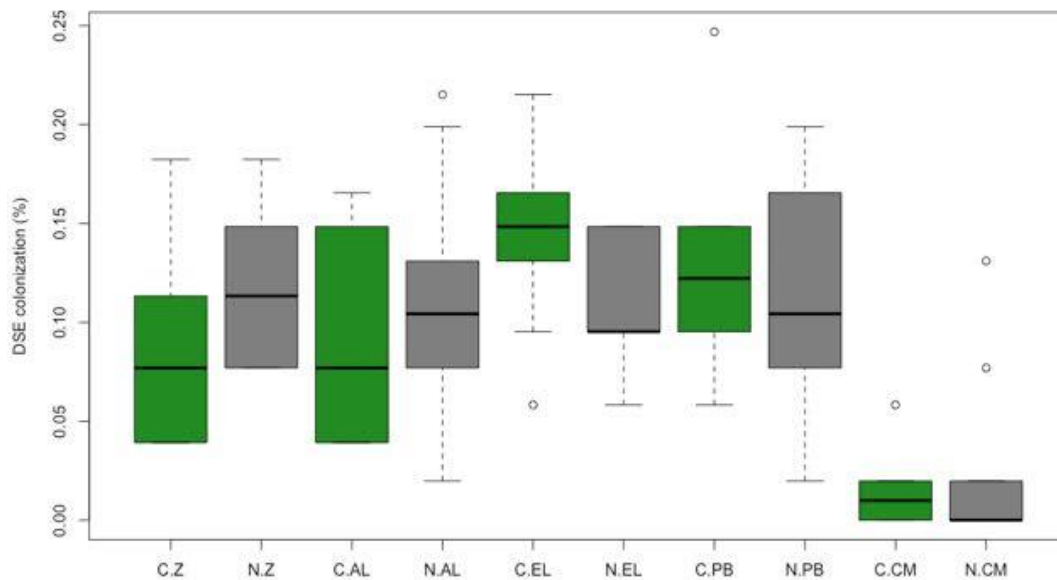


Figure 10: Box and whisker plots for vesicle abundance rate as a decimal. Note that *Castilleja* present plots are denoted in green and absent plots are denoted in grey.

Table 1: Elevation and GPS information for the six sites targeted in this study

Site	Elevation (m)	Latitude	Longitude
Almont low	2480	38.65390833	-106.861977
Almont Obs	2740	38.71533333	-106.8226389
Elkton	3200	38.96133333	-107.0314722
Paint Boy	3392	38.97005	-107.0398667
Cinnamon Obs	3460	38.99158333	-107.0665556

Table 2: Table of Paired t-test results for diversity matrices of *Castilleja* versus non-*Castilleja* plots for the total data across all sites. Note the significant p-values for Richness and Shannon's Diversity.

Diversity Measure	T Statistic	P value
Shannon's Diversity	2.877	0.006
Richness	3.710	<0.0001
Simpson's Diversity	-1.989	0.052

Table 3: Table of Paired t-test results for AMF/DSE colonization and structures from *Castilleja* versus non-*Castilleja* plots for the total data across all sites. Note the significant p-value for AMF colonization.

Structure	T Statistic	P value
AMF	-9.63	<0.0001
DSE	-1.53	0.133
Vesicle	-0.502	0.618
Arbuscle	0.256	0.799
Pathogen	1.26	0.209