

The effects of hemiparasitism by *Castilleja* spp on community structure in alpine ecosystems

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Introduction

Plant community ecologists have examined the effects of competition and herbivory on plant community structure and ecosystem processes for at least decades while generally ignoring whether parasitic or hemiparasitic plants might also have effects. Parasitic plants are comprised of two main groups, holoparasites (plants that are completely parasitic and contain virtually no chlorophyll) and hemiparasites (plants that are both parasitic and photosynthetic), and each have the potential to play major roles in shaping community structure and driving ecosystem functions (Press and Phoenix 2005). Globally there are over 3000 parasitic flowering plant species, and most plant assemblages contain one or more of these species (Marvier 1996; Bardgett et al. 2006; Quested 2008). Parasitic plants are also represented in every major ecosystem (Adler 2002). Given their ubiquity and diversity, it is surprising that so few studies have examined whether they have had impacts on communities and ecosystems.

Though few studies have examined community-level impacts, several experiments on the impacts of parasitic plants on host performance have been performed (see Bardgett et al. 2006). Hemiparasitic plants obtain some resources, including water, fixed carbon compounds, nitrogen, etc., from their host plants, but they also compete with their hosts for light, water and soil nutrients (Pennings and Callaway 2002; Press and Phoenix 2005; Marvier 1996). Hemiparasitic plants have the potential to determine the productivity and structure of the plant communities in which they are present because hemiparasitic plants can decrease the growth of host species, which might indirectly affect other plants by altering the competitive balance between hosts and non-hosts species in a community (Press and Phoenix 2005). Put another way, when they have detrimental effects on their hosts, this allows non-host plants to gain a competitive advantage and become more abundant in the community (Bardgett et al. 2006). Hemiparasitic plants, therefore, have the potential to alter community biomass and the relative abundance of particular species by reducing the competitive advantage and abundance of their hosts, even if the hemiparasitic plants represent only a small percentage of the cover or biomass.

Nutrient availability in the system, density of parasitic plants, and parasitic plant host preference can all drive changes in community composition, which is why more studies on the community effects of hemiparasitic plants are needed to better understand their potential role in plant communities (Spasojevic and Suding 2010; Quested 2008). Most parasitic plants are thought to be generalists, meaning that they parasitize many different plant species, and

Castilleja spp. are even thought to parasitize over 100 different hosts (Press 1998). It is common, however, for parasitic plants to behave more as specialists because they show various levels of host preference (Press and Phoenix 2005).

Although *Castilleja* species are common in alpine systems in Colorado and the Rocky Mountains, their community and ecosystem effects have not been widely studied. One exception is the work of Spasojevic and Suding (2010), who conducted an experiment in the Front Range of the Rocky Mountains focusing on the effects of *Castilleja occidentalis*. They confirmed that litter effects of long-lived hemiparasitic plants in nutrient poor systems may increase the availability of nitrogen in the system. This positive nutrient effect would compensate for the negative effects of parasitism on the host by significantly increasing productivity and slightly increasing diversity. This is one study that shows the importance of hemiparasitism in shaping community structure and driving ecosystem processes in Rocky Mountain ecosystems.

Importantly, Spasojevic and Suding (2010) and most other studies of the effects of parasitism and hemiparasitism in plant communities more generally, have focused on single sites while it is clear that the effects of interspecific interactions in plant communities can vary with elevation (Callaway et al. 2002). Distributions and abundances of plant species may vary depending on their interactions with other plant species and the elevation (Callaway et al. 2002). In general, competition is more important for interactions within plant communities at lower elevations, while interactions among plants at high elevations are positive (Callaway et al. 2002). According to Pennings and Callaway (2002), parasitic and hemiparasitic plants could have varying types of effects at different elevations depending on their differences in virulence, host-preference, and community interactions.

In this study, I employed a manipulative experiment and conducted surveys over an extensive elevational gradient to examine the relationship between the density of *Castilleja* spp. and a suite of community-level properties. In particular, I asked:

1. Is there a relationship between the abundance (both percent cover and biomass) of *Castilleja* and species diversity and community composition? If there are clear relationships, are they positive, negative, or neutral? I predicted that if *Castilleja* species have an effect on plant communities, then there would be a visible relationship between *Castilleja* abundance and species diversity and community composition. If there was a

high abundance of *Castilleja* species, then the richness and evenness of the rest of the plant community would be higher.

2. Are these relationships between *Castilleja* abundance and richness and evenness represented across an elevational gradient? I predicted that the relationships will remain relatively the same because all elevational gradient sites are similar in plant communities, but I predict that all plots, regardless of presence of *Castilleja*, will decrease in richness and evenness with increasing elevation, as abiotic stress is higher, and this will cause certain competitive species, like *Artemisia tridentata*, to become more abundant, while other species cannot thrive under such abiotic stress.

Overall, this research leads to a better understanding of the roles of parasitic plants in shaping community structure and ecological processes in alpine systems.

Methods

Natural History

Castilleja species are facultative root hemiparasites. Their roots grow until they touch roots of other plants, and then they penetrate the roots of the host plant with haustoria and obtain a portion of its nutrients (Bardgett et al. 2006; Press and Phoenix 2005; Cameron et al. 2009; Quested 2008). Hemiparasitic plants like *Castilleja* species can “forage” by selectively growing in a specific direction (towards or away from host plants) or by selectively penetrating a host’s roots upon contact (Pennings and Callaway 2002). It is difficult to determine the host for most *Castilleja* individuals, but it is understood that *Castilleja* species can host on a wide range of plant species in montane and alpine communities (Spasojevic and Suding 2010), including deeply rooted sagebrush, fast growing grasses, lupines, and other plants (Darrow 2006). Adler conducted a study using *Castilleja indivisa* in Texas and found that lupines benefit the *Castilleja* host more than grass hosts, contributing to the idea that though *Castilleja* can parasitize a wide range of species, some hosts have stronger positive effects on the hemiparasite’s productivity than others (Adler 2003).

*Is there a relationship between the abundance (both percent cover and biomass) of *Castilleja* and species diversity and community composition? If there are clear relationships, are they positive, negative, or neutral?*

I conducted this research in at the Rocky Mountain Biological Laboratory in Gothic, Colorado. My study site was located at approximately 3000 meters in elevation in a dry montane meadow near Judd Falls. Common plant species at the site included *Populus tremuloides*, *Artemisia* spp., *Potentilla hippiana*, *Mahonia repens*, *Lomatium dissectum*, etc.

To determine if *Castilleja* influences the structure of plant communities and ecosystem processes 18 1-m² plots were established in six blocks with a 0.5 × 0.5 m buffer zone surrounding each of the plots. Each plot within a block was approximately one to two meters away from adjacent plots, but the exact distance was not measured, as it depended on the topography and vegetation of the area. Within each block, I implemented three treatments: a control plot in which nothing was manipulated; a *Castilleja* removal plot, in which *Castilleja* was removed by clipping to ground level each week during the growing season (primarily July 2011); and a random biomass removal plot, in which a comparable amount of biomass (compared to the *Castilleja* removal plot within the block) was removed. I included a random biomass removal plot in each block to account for the removal of *Castilleja* biomass and the impacts that removing biomass in general could have on plant community structure. I air dried the biomass removed from the *Castilleja* removal plots and random biomass removal plots in paper bags and then dried for them for 72 hours at 70°C in a static air oven. I then weighed the biomass to the nearest 0.01 gram.

For each of the 18 1-m² plots I measured richness and evenness on three occasions (June 28th, July 14th and July 28th) during the 2011 growing season. I measured richness in all plots by identifying and recording each plant species present in the plots, and I measured evenness (Evenness = $(1/\sum p_i^2)(1/S)$; where p_i is the proportion of each species and S is the total number of species) by estimating the relative abundance of each species based on percent cover in five percent intervals. Note that in all cases the response variable does not include *Castilleja*; instead, the focus is on the rest of the plant community.

I also measured soil moisture for all plots periodically using a soil moisture probe to determine if all plots were similar in this abiotic factor. I took two measurements in each plot at each sampling event, and the probe measured volumetric water content as a percent. The moisture of the experimental plots was the same across all treatments (Fig. 2)

To determine the relationship between abundance of *Castilleja* species and a suite of community-level variables, species composition and diversity, I performed several statistical

analyses with the data. I analyzed the effects of the presence of *Castilleja* on these diversity measures using Analysis of Variance and Analysis of Covariance statistical tests. I also did a one-way analysis on evenness and richness by treatment and date, and compared values to the control using Dunnett's method. The significance threshold for all statistical tests was $\alpha = 0.05$. All above analyses were performed using the statistical program JMP.

Are these relationships between Castilleja abundance and richness and evenness represented across an elevational gradient?

For the elevational gradient portion of this study, I surveyed plant communities along an elevational gradient at 2450 m, 2700 m, 2750 m, 3000 m, and 3260 m (See Table 1.). To determine if there is a relationship between *Castilleja* abundance and species richness and evenness along an elevational gradient, I conducted an observational study related to the experimental study explained above. Specifically, I chose sites that were dry montane meadows, and thus similar in plant composition, and where *Castilleja* was present. We randomly placed twelve 1×1 m quadrats at each site, with two conditions: six of the twelve plots at each site contained *Castilleja*, and six did not. I measured richness and evenness within each 1m^2 plot. This correlational study was conducted to draw parallels between the results of the experimental study and the results of a natural, observational study.

To determine if there is a relationship between *Castilleja* abundance and species richness and evenness along an elevational gradient, I used Analysis of Variance and Analysis of Covariance statistical tests. The model included *Castilleja* abundance, elevation, and their interactive effects as independent variables. Separate analyses were performed for richness and evenness of plot as dependent variables. I also compared all elevation evenness and richness using Tukey-Kramer HSD. The significance threshold for all statistical tests was $\alpha = 0.05$. All data met the assumptions of normality.

Results

There is a positive relationship between the abundance (both percent cover and biomass) of Castilleja and community composition or evenness.

Before plant removals, richness and evenness did not differ among treatments (Fig. 1a and 1d). Similarly, volumetric soil moisture content was consistent across all treatments (Fig. 2).

Richness in the experimental plots did not vary among treatments or through time (Fig. 1d, 1e, 1f), but evenness in the *Castilleja* removal plots was significantly different than in the control plots after plants were removed (Fig. 1b and 1c). Using Dunnett's method for comparing treatments to a control, I found that evenness was lower in *Castilleja* removal plots by 34% (Fig. 1b) and 25% (Fig. 1c) when compared to the control plots.

There are no relationships between Castilleja abundance and richness and evenness across an elevational gradient.

At the community level, I found no significant difference in richness within the *Castilleja* present plots ($F=0.7041$, $P=0.5966$), or richness within the *Castilleja* absent plots ($F=0.2137$, $P=0.9283$) across the elevational gradient (Fig. 3). Neither elevation nor the presence or absence of *Castilleja* had an effect on the richness of the plant communities. I found that elevation does have an effect on overall evenness ($F=3.46$, $P=0.014$) (Table 2), but it does not have an effect on evenness within the *Castilleja* present plots ($F=1.9667$, $P=0.1306$) or within the *Castilleja* absent plots ($F=1.9753$, $P=0.1292$) over the elevational gradient (Fig. 4). In other words, elevation matters for evenness, but *Castilleja* presence does not. There is no trend in the percent cover or estimated biomass of *Castilleja* and richness or evenness.

Discussion

The purpose of this study was to investigate if there is a relationship between *Castilleja* spp. abundance and species diversity and composition. Richness was not affected by the presence of *Castilleja* in the experimental plots or associated with the presence of *Castilleja* across the elevational gradient, but evenness was affected in some cases. Evenness in plots where *Castilleja* was removed was significantly reduced, and this decrease in evenness compared to the control plots persisted throughout the 2011 growing season (Fig. 1b. and 1c.). In other words, the presence of *Castilleja* matters for evenness. This result supports the hypothesis that there is a positive relationship between *Castilleja* abundance and the community composition, because *Castilleja* presence increases the evenness of the rest of the plant community in the manipulated plots. The elevational gradient correlational study did not support this conclusion, however, because there was no significant difference in richness or evenness in plots that contained *Castilleja* at all elevations.

Seeing only shifts in evenness was somewhat expected because the removal of *Castilleja* simply decreased the evenness or percent cover of plant species, rather than making new species appear or others become extinct in removal plots over one growing season. Studies that have seen larger effects of hemiparasitism on community structure were longer experiments that extended over several growing seasons (See Bardgett et al. 2006 and Marvier 1998), so it may be beneficial to extend this study over more growing seasons to see if any richness or evenness measurements change over more time. Effects also may be limited depending on how selective *Castilleja* is for its host and how virulent it is in montane meadows and alpine ecosystems in general (Pennings and Callaway 2002). If *Castilleja* is largely a generalist, then it would parasitize multiple plant species, and have little effect on plant structure. If *Castilleja* is primarily a specialist, then it would have more effect on the abundance of a few species, causing a shift in the evenness and possibly richness of the community over time. I found that, in general, plots that had *Castilleja* present increased species evenness, while leaving species richness unchanged, so it is logical to consider *Castilleja* more of a specialist hemiparasite than a generalist in alpine ecosystems. Considering *Castilleja* a specialist when referring to its host preference, accounts for the significant increase in evenness and relatively no change in richness.

Parasitic and hemiparasitic plants are a largely ubiquitous group of plants that have been largely excluded from community-level studies. Hemiparasites have the potential to largely influence community structure and composition, so it is important that plant community ecologists consider their roles in various ecosystems in order to obtain a better understanding of their potential impacts. Through several studies, we now understand that hemiparasites have varying effects on their plant communities depending on factors, such as their abundance, their host preference, and soil fertility of the system. According to this study, *Castilleja* spp generally increase the evenness of the rest of the plant community, which is similar to the finding that *Rhinanthus minor*, also a hemiparasite, in grassland ecosystems increases species diversity (Bardgett et al. 2006). *R. minor* is a facultative root hemiparasite, that like *Castilleja*, affects the community composition (Bardgett et al. 2006). This study, however, was conducted in low-to-moderately productive grassland, so it is interesting to see how similar effects of hemiparasites can extend across different ecosystems. In a study by Spasojevic and Suding (2010), *Castilleja occidentalis* was associated with slight changes in plant diversity in the alpine tundra, similarly to that of *Castilleja* spp. in montane meadows.

After finding that the hemiparasite *Castilleja* does significantly increase evenness in alpine ecosystems, this enforces the idea that hemiparasites can have large effects on community structure. Hemiparasites have largely been excluded from community-level studies, yet several studies show their importance in shaping and changing communities (Bardgett et al. 2006; Press and Phoenix 2005; Spasojevic and Suding 2010). Our findings suggest that hemiparasitic plants act as major drivers in shaping plant communities in alpine ecosystems, and given these findings and the fact that almost all terrestrial ecosystems support hemiparasites and parasites, they are likely to have unknown effects on plant communities and ecosystems. It is also possible that with a changing world and changing ecosystems, hemiparasitic and parasitic plants could have increasingly stronger effects on community composition and structure. Because of the possible influences on plant communities, hemiparasitic plants should not be excluded from community theory and should be more widely studied.

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Location	Elevation (m)	Dominant Plant Species
Almont	2470	<i>Artemisia tridentata</i> , <i>Achillea millefolium</i> , <i>Wyethia amplexicaulis</i>
Cement Creek	2700	<i>Artemisia tridentata</i> , <i>Antennaria rosea</i> , <i>Ipomopsis aggregata</i>
Nordic Center	2750	<i>Artemisia tridentata</i> , <i>Eriogonum umbellatum</i> , <i>Lupinus argenteus</i>
Judd Falls	3000	<i>Paxistima myrsinites</i> , <i>Heterotheca villosa</i> , <i>Lomatium dissectum</i>
Schofield Pass	3260	<i>Chamerion angustifolium</i> , <i>Aquilegia coerulea</i> , <i>Lupinus argenteus</i>

Table 1. Locations, elevations, and dominant plant species of the elevational gradient sites.

Source	Sum of Squares	F Ratio	P-value
Castilleja	0.00110697	0.06936057	0.79335239
Elevation	0.22072545	3.45754929	0.01431903
Castilleja*Elevation	0.03099767	0.48556231	0.74622545

Table 2. Analysis of the effect of *Castilleja* and elevation on evenness in plots.

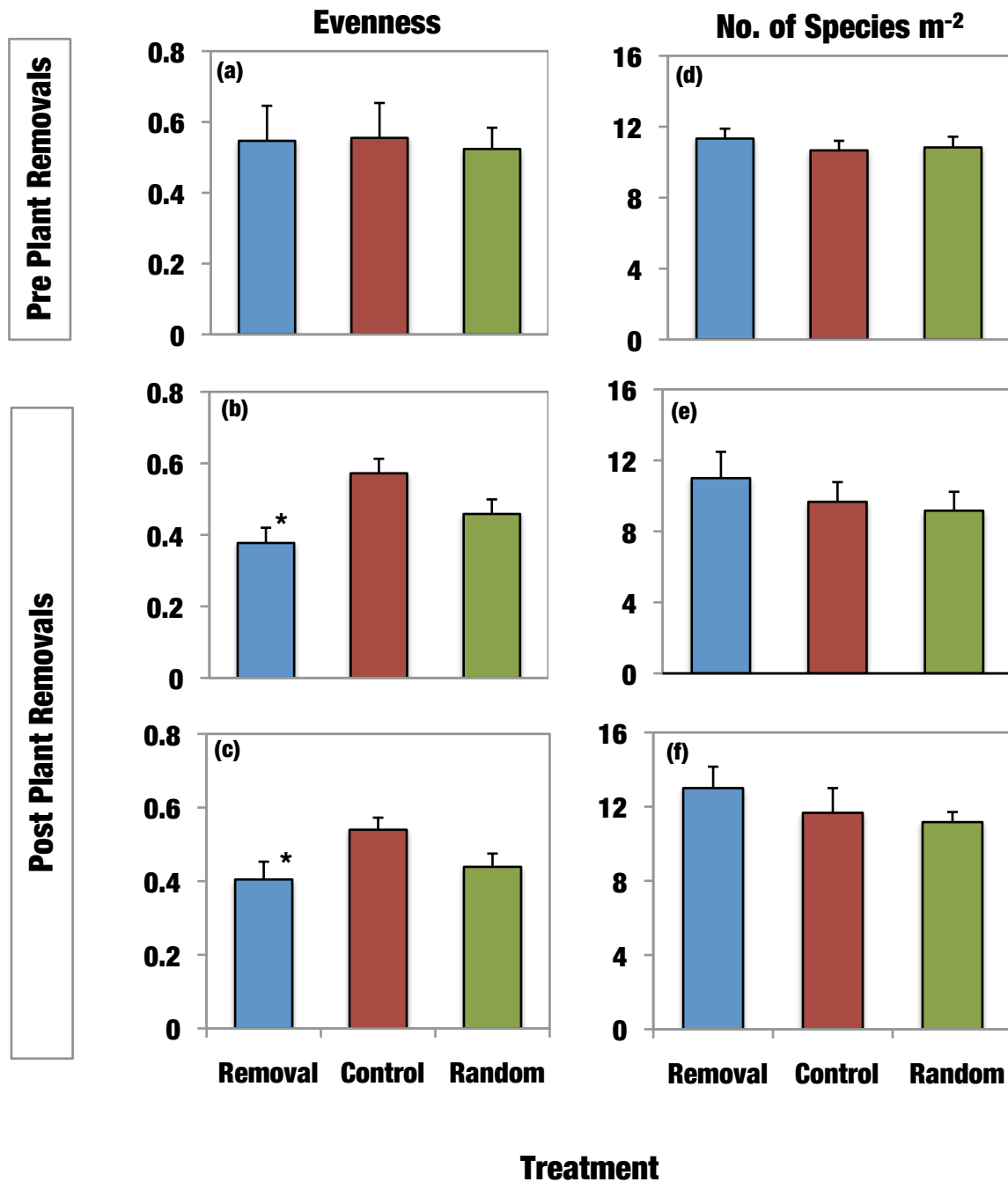


Figure 1. Evenness of species (a), (b) and (c) and species richness measured as the number of species m⁻² (d), (e) and (f) for *Castilleja* removal plots, control plots, and random biomass removal plots. Each bar represents the mean (+ SE) of six plots. Bars marked with * are significantly different at p=0.05 from the control.

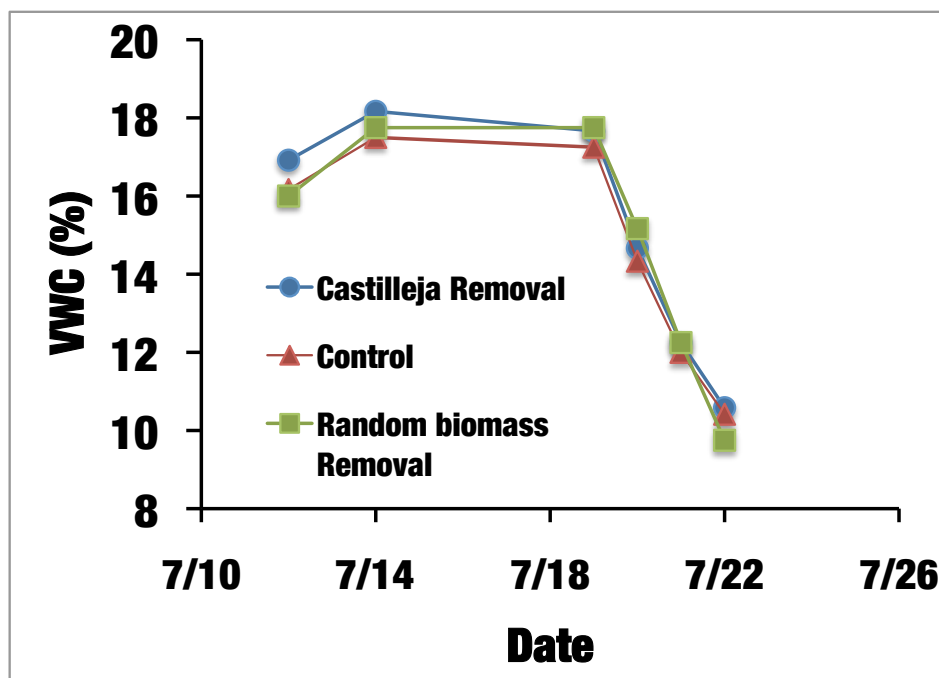


Figure 2. Average Volumetric Water Content at 12cm depth for the three treatment plots over time.

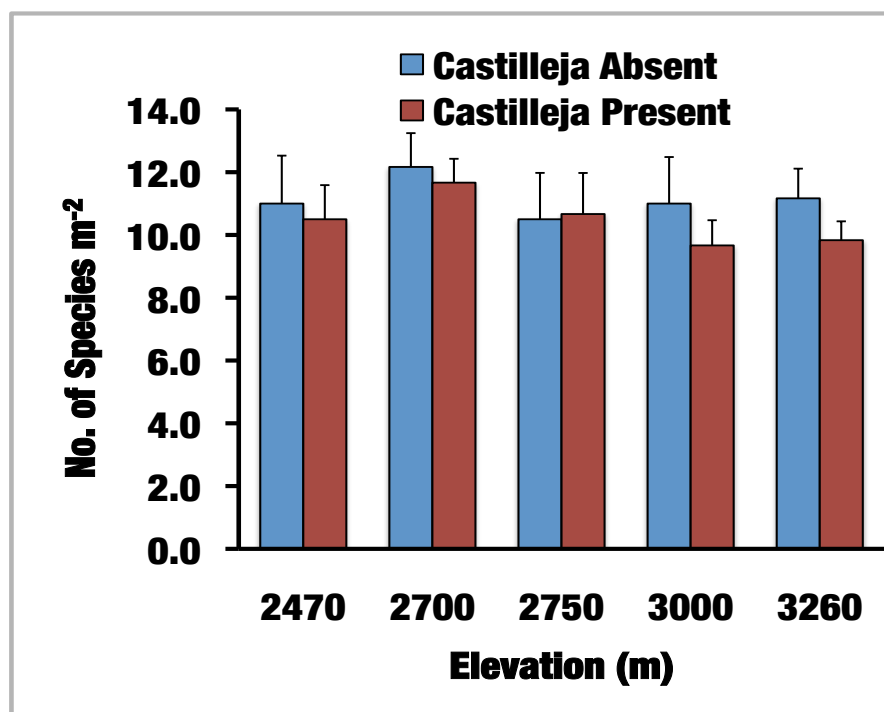


Figure 3. Richness of Castilleja present and Castilleja absent plots

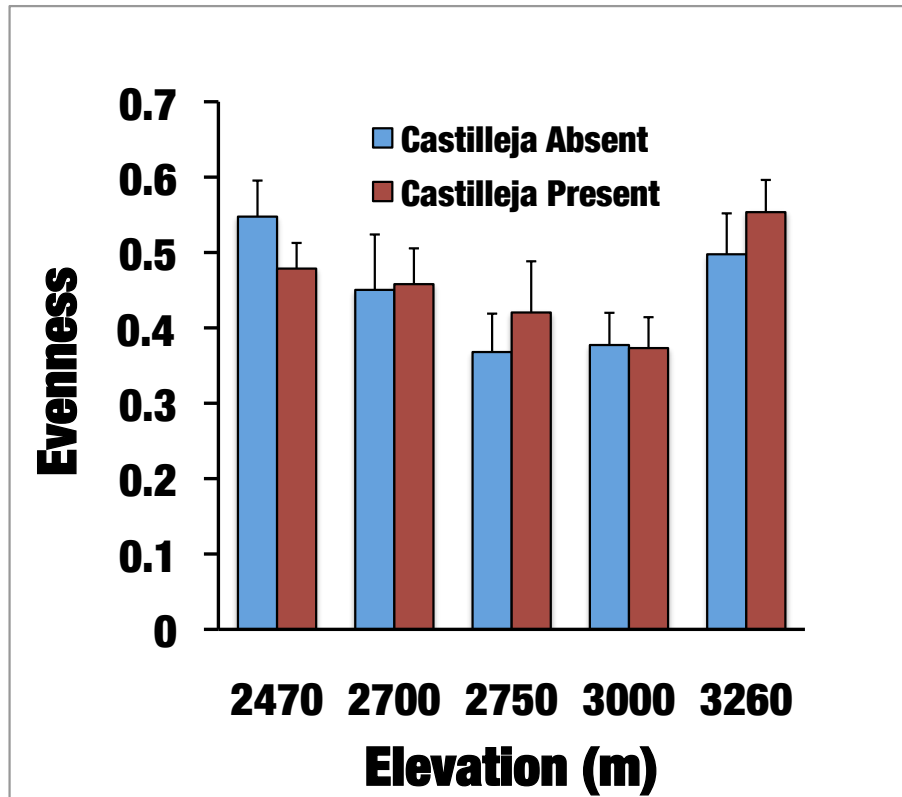


Figure 4. Evenness of Castilleja present and Castilleja absent plots.