Hemiparasitic plants increase alpine plant richness and evenness but reduce arbuscular mycorrhizal fungal colonization in dominant plant species

Michael McKibben 1, 2, Jeremiah A Henning Correspond. 2, 3, 4

1 Department of Biology, University of Tampa, Tampa, Florida, United States
2 Rocky Mountain Biological Laboratory, Gothic, Colorado, United States of America
3 Department of Ecology & Evolutionary Biology, University of Tennessee - Knoxville, Knoxville, Tennessee, United States
4 Department of Ecology, Evolution, and Behavior, University of Minnesota, Saint Paul, Minnesota, United States

Corresponding Author: Jeremiah A Henning
Email address: jhenning@umn.edu

Hemiparasitic plants increase plant biodiversity by reducing the abundance of dominant plant species, allowing for the establishment of subordinate species. Hemiparasites reduce host resources by directly removing nutrients from hosts, competing for light and space, and may indirectly reduce host resources by disrupting plant associations with symbiotic root fungi, like arbuscular mycorrhizal fungi and dark-septate endophytes. Here we explored how a generalist hemiparasite, Castilleja, influences plant richness, evenness, community composition, and mycorrhizal colonization patterns across a ~1000m elevational gradient in the North American Rocky Mountains. We hypothesized that the presence of Castilleja would be associated with increased plant richness and evenness, shaping plant community composition, and would reduce mycorrhizal colonization within dominant plant taxa. However, the magnitude of the effects would be contingent upon climate contexts i.e. elevation. Overall, we found that the presence of Castilleja was associated with an 11% increase in plant richness and a 5% increase in plant evenness, regardless of elevation. However, we found that the presence of Castilleja influenced plant composition at only two of the five sites and at the remaining three of five sites, plot pairing was the only predictor that influenced composition. Additionally, we found that the presence of Castilleja reduced mycorrhizal fungal colonization within dominant plant species by ~20%, regardless of elevation. Taken together, our results suggest that hemiparasites regulate plant diversity, evenness, and interactions with mycorrhizal fungi independent of abiotic and biotic contexts occurring at the site, although overall effect on community composition is likely driven by site-level factors.
Hemiparasitic plants increase alpine plant richness and evenness but reduce arbuscular mycorrhizal fungal colonization in dominant plant species.

Michael McKibben¹,², Jeremiah A. Henning²,³,⁴*

¹ Department of Biology, University of Tampa, 401 West Kennedy Blvd., Tampa, FL 33606
² Rocky Mountain Biological Laboratory, P.O. Box 519, Crested Butte, CO 81224
³ Ecology and Evolutionary Biology, University of Tennessee, 569 Dabney Hall, Knoxville, TN 37996.
⁴ Ecology, Evolution, and Behavior, University of Minnesota, 140 Gortner Laboratory, 1479 Gortner Avenue, Saint Paul, MN 55108

*jhenning@umn.edu
Michael.McKibben@spartans.ut.edu
fax: 1-612-625-5700

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Hemiparasitic plants increase plant biodiversity by reducing the abundance of dominant plant species, allowing for the establishment of subordinate species. Hemiparasites reduce host resources by directly removing nutrients from hosts, competing for light and space, and may indirectly reduce host resources by disrupting plant associations with symbiotic root fungi, like arbuscular mycorrhizal fungi and dark-septate endophytes. Here we explored how a generalist hemiparasite, *Castilleja*, influences plant richness, evenness, community composition, and mycorrhizal colonization patterns across a ~1000 m elevational gradient in the North American Rocky Mountains. We hypothesized that the presence of *Castilleja* would be associated with increased plant richness and evenness, shaping plant community composition, and would reduce mycorrhizal colonization within dominant plant taxa. However, the magnitude of the effects would be contingent upon climate contexts *i.e.* elevation. Overall, we found that the presence of *Castilleja* was associated with an 11% increase in plant richness and a 5% increase in plant evenness, regardless of elevation. However, we found that the presence of *Castilleja* influenced plant composition at only two of the five sites and at the remaining three of five sites, plot pairing was the only predictor that influenced composition. Additionally, we found that the presence of *Castilleja* reduced mycorrhizal fungal colonization within dominant plant species by ~20%, regardless of elevation. Taken together, our results suggest that hemiparasites regulate plant diversity, evenness, and interactions with mycorrhizal fungi independent of abiotic and biotic contexts occurring at the site, although overall effect on community composition is likely driven by site-level factors.
Introduction

Root hemiparasitic plants regulate plant community composition and ecosystem function by reducing the abundance of dominant plant species, increasing co-existence of subordinate species, resulting in greater plant evenness (Davies et al. 1997; Press & Phoenix 2005; Bardgett et al. 2006; Westbury et al. 2006; Reed 2012). However, see (Press & Phoenix 2005; Westbury & Dunnett 2007) for examples that counter this general pattern. Hemiparasites range from host specialists to complete generalists, but typically impact dominant plant species to a greater degree compared to lower abundance plant taxa (Davies et al. 1997; Westbury et al. 2006; Reed, 2012). However, generalist hemiparasites can have taxa specific effects independent of dominance patterns (Adler 2003; Demey et al. 2015). Hemiparasites directly reduce host plant biomass by parasitizing carbon and nutrients from the host through specialized structures called haustoria (Adler 2000; Salonen et al. 2001). Although, hemiparasites parasitize resources from their hosts, they are not wholly reliant on hosts for carbon and nutrient resources and fix carbon via photosynthesis and can obtain soil resources through absorptive roots (Ducharme & Ehleringer 1996; Press & Phoenix 2005). As a result, hemiparasites impact hosts by directly parasitizing host resources as well as competing for light and nutrients (Press & Phoenix 2005). Additionally, hemiparasite associations with root-associated fungal communities like arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) (Davies & Graves 1998, Li & Guan 2008) may provide an additional pathway for hemiparasites to acquire resources and compete with surrounding plant taxa.

Hemiparasite interactions with AMF and DSE may regulate or exacerbate parasitic effects of hemiparasites on plant communities (Gworgwor & Weber 2003; Lendzemo et al. 2007; Bouwmeester et al. 2007; Li et al. 2012). AMF and DSE are ubiquitous across most ecosystems.
globally and provide nutrient resources to plant hosts in return for plant-derived carbon, leading
to the promotion of plant growth (Smith & Read 2008; Mandyam, & Jumpponen, 2014; Vergara
et al. 2017; Jumpponen et al. 2017). Hemiparasites may interact with AMF and DSE in a number
of ways. For instance, hemiparasites may benefit from a host plant’s association with AMF and
DSE by indirectly accessing excess resources through the plant host, directly associating with the
common mycorrhizal network, or by capitalizing on plant-mycorrhizal signaling pathways to
colonize host roots (Davies and Graves 1998; Bouwmeester et al. 2007; Stein et al. 2009; De
Vega et al. 2010). If hemiparasites are superior competitors for host carbon resources, the
reduction in available carbon resources may lead to feedbacks between plant hosts and fungal
symbionts (Stewart & Press 1990; Davies and Graves 1998; & Phoenix 2005). Conversely,
plants may allocate more carbon resources to mycorrhizal fungi to increase nutrient access,
offsetting hemiparasite effects. However, the effect of hemiparasites on AMF and DSE carbon
allocation has not been directly tested. Conversely, AMF can inhibit the germination of
hemiparasite seeds and the attachment of hemiparasites on host plants, reducing the effect of
hemiparasites on host plants (Gworgwor & Weber 2003; Lendzemo et al. 2007; Li et al. 2012).
Thus, the interaction among hemiparasites, AMF, DSE, and plant hosts will likely have
cascading effects on plant community diversity and composition, however the impact of
hemiparasites on AMF and DSE remains a significant knowledge gap.

Environmental contexts shape the composition and function of plant and fungal
communities (Callaway at al. 2002; Kivlin et al. 2011; Sundqvist et al. 2013; Jumpponen et al.
2017). Because hemiparasites influence plant community richness, evenness, and community
composition, it is likely that environmental contexts may regulate the effect of hemiparasites on
plant-fungal interactions (Marx et al. 1970; Press & Phoenix 2005; Pennings & Callaway 1996;
For example, in low productive ecosystems, hemiparasites typically have a stronger influence on plant diversity and community composition because light competition with surrounding plants is low (Matthies & Egli 1999; Pennings & Callaway 2002; Press & Phoenix 2005; Těšítel et al. 2011; 2018). However, light competition in more productive ecosystems may outweigh negative parasitic effects, reducing the effect of hemiparasites (Matthies & Egli 1999; Pennings & Callaway 2002; Press & Phoenix 2005; Těšítel et al. 2011; 2018). Additionally, it has been hypothesized that hemiparasite effects on plant evenness and the promotion of subordinate species should be greatest in areas with high competitive asynchronies among taxa (Pennings & Callaway 1996; Press & Phoenix 2005). Thus, site-level abiotic and biotic factors likely regulate the effects of hemiparasites on plant community composition and associations with fungi.

Elevational gradients have a long history of usage to understand how biotic and abiotic contexts shape species interactions, community composition, and ecosystem function (MacArthur 1972; Callaway et al. 2002; Sundqvist et al. 2013; Read et al. 2017). Moving from low to high elevation, abiotic properties like: temperature, precipitation, pH, nutrient availability and biotic factors like: plant taxa present, herbivore abundance, symbiont abundance shift while often maintaining a similar disturbance history, soil parent material, and regional species pool (reviewed in Körner 2007; Sundqvist et al. 2013). Together, biotic and abiotic factors shape the outcome of species interactions (Callaway et al. 2002) and productivity (Whittaker et al. 1974; Sundqvist et al. 2013) from low to high elevation. Across many elevational gradients, low elevation soil fungal communities are dominated by host carbon-reliant AMF communities, while high elevation ecosystems are often dominated by less host carbon-reliant DSE and ericoid mycorrhizal fungi are more abundant relative to AMF (Haselwandter & Read 1980; Schmidt et
Thus, the effect of hemiparasites on plant diversity, community composition, and soil fungal communities may shift across an elevational gradient, with effects on plant diversity and fungal colonization exacerbated at lower elevation communities (Choler et al. 2001; Bardgett et al. 2006; Reed 2012).

In the North American Rocky Mountains hemiparasites in the genus *Castilleja* (Orobranchaceae) commonly occur in a wide-variety of montane ecotones (Hersch & Roy, 2007). *Castilleja*, in this region, are perennial, generalist hemiparasites (Sweatt 1997; Adler 2003), that receive up to 40% of their carbon from plant hosts (Ducharme & Ehleringer 1996). *Castilleja* parasitize a wide variety of host plants, often increase plant community evenness, and increase nitrogen cycling in nutrient poor soil (Ducharme & Ehleringer 1996; Adler 2003; Reed 2012; Spasojevic & Suding 2012). Across an elevational gradient near Gothic, Colorado, USA, three species of *Castilleja*, *C. angustifolia* (2,480 – 2,740 m), *C. miniata* (3,392 m), and *C. sulphurea* (3200 – 3460 m) are distributed in different elevational clines. Here, we measured how the presence of *Castilleja* was related to: 1) plant richness and evenness of the surrounding plant community, 2) the presence of *Castilleja* would alter community composition, and 3) the presence of *Castilleja* would reduce dominant plant associations with AMF and DSE at 5 sites along a ~1000m pre-established elevational gradient (Read et al. 2017) near Gothic, Colorado, USA. We hypothesized that: 1) the presence of *Castilleja* would increase plant richness and evenness, 2) which would re-shape community composition and that 3) *Castilleja* would reduce the colonization of host carbon reliant AMF while increasing colonization of less carbon reliant DSE in the roots of the dominant plant species. However, 4) the effects of *Castilleja* on plant and fungal communities would be contingent on climate contexts, with stronger *Castilleja* effects at low elevation, relative to higher elevation sites (increasing abiotic stress).
Methods

Study Site
We utilized pre-existing sites along an elevational gradient that spanned from 2480m to 3460m (~1000m) near Gothic, CO, USA (Read et al. 2017, Table 1). The sites are located on USDA Forest Service land and are covered under Forest Service Special Use Permit #GUN1120. The gradient site receives about 439 to 668 mm yr$^{-1}$ of precipitation and has a mean annual temperature of -1.6 to 1.5 °C (Hijmans et al., 2005; Read et al. 2017; Table 1). Additionally, nutrient availability shifts across the gradient, with high elevation sites having more available phosphorus but lower available nitrogen relative to low elevation sites (Read et al. 2017). Plant diversity is highest at middle elevation sites, while plant community composition transitions from a sagebrush steppe at low elevation to montane meadows at high elevation (Read et al. 2017).

Plant Community Sampling
At each elevation, we identified all *Castilleja* present within a 20m × 20m area. Next, we haphazardly-selected 10 *Castilleja* individuals of similar size and identified an adjacent “*Castilleja* free” area that was located within 2 m distance from our focal *Castilleja*, but contained no *Castilleja* within a 1.5 m diameter around the centroid. Thus, we sampled 100 total plots (10 plots ×2 *Castilleja* treatments × 5 elevations). From our sampling design, we were unable to determine if plots were truly absent of *Castilleja* influence, as shallow-rooted *Castilleja* can infect across a semi-broad spatial area (Ducharme & Ehleringer 1996). Logistical
157 constraints of the pre-existing elevational gradient and the observational focus of our study
158 prevented us from removing Castilleja individuals, which would have provided a more direct
159 measurement of Castilleja effects on plant community composition and ecosystem function.
160 To measure plant community composition, we identified all plant species present within a
161 0.5 × 0.5 m quadrat in each plot. Plant community composition was measured in the Castilleja
162 present plots, but placing the focal Castilleja in the center of our quadrat and Castilleja-free
163 surveys were conducted by placing the quadrat in the center of the Castilleja-free area to
164 maximize the distance from all surrounding Castilleja plants. Next, we visually estimated the
165 cover of each species present in the quadrat. We estimated percent coverage to the nearest 1%
166 for species < 20% and the nearest 5% for species > 20% coverage. We also estimated the amount
167 of bare ground and rock within each plot.
168
169 Root sampling
170 From our cover data, we identified the most abundant plant species that occurred in all 20
171 plots at each elevation to sample for fungal colonization. At 1 of the 5 sites (2,480m), our
172 sampled plant species (Balsamorhiza sagittata) was also the dominant taxa, however at 4 of the 5
173 sites, we sampled either the 2nd or 3rd most abundant taxa, as the dominant taxa did not occur in
174 all 20 plots. We collected a single 2.5 × 15 cm core from our focal individual to measure the
175 colonization of AMF and DSE. A single focal plant (Table 1) was haphazardly selected near the
176 center of each plot (10 plots × 2 Castilleja treatments × 5 elevations = 100 total soil cores). We
177 transported the soil cores back to the Rocky Mountain Biological Laboratory on ice and stored
178 them at 4° C in lab until being processed within 24 hours. Next, we extracted live roots from the
179 core using a 0.5 mm mesh sieve and placed roots in Fisher brand Histosette II tissue cassettes
Tissue cassettes were then placed in deionized water to remove any remaining soil. Next, we cleared pigment from roots in a 10% potassium hydroxide (KOH) solution, acidified root samples in a 2% hydrochloric acid (HCl) solution, and then stained roots in a 0.01% trypan blue solution (Koske & Gemma 1989). We then mounted roots on microscope slides using PVLG (polyvinyl lactic acid glycerol) glue (INVAM 2017) and slides were oven dried at 50° C for 48 hours. We removed two root samples (Castilleja present plant 5 and 8) from our 3,200m elevation site because of low root biomass. Next, we quantified the presence of AMF hyphae, DSE hyphae, as well as AMF vesicles, arbuscules, coils, and spores, DSE microsclerotia, and any potential pathogens, of at least 50 root intersections per slide, using the magnified grid-line intercept method (McGonigle et al. 1990). Finally, we calculated percent colonization of AMF, DSE, and any structures and potential pathogens by dividing the number of positive observations by the total number of root intersections observed.

**Statistical Analysis**

All analyses were conducted in R (R Development Core Team, 2008) and RStudio (RStudio Support Team, 2015), with packages cited within. To test for differences in plant richness, evenness, and community composition independent of the contribution of Castilleja we removed the Castilleja cover from all analyses. However, Castilleja cover is located in Table 1. We calculated species richness using the `specnumber` function in the “vegan” package (Oksanen et al. 2017) and species evenness as the probability of interspecific encounter (PIE, Simpson’s Evenness) (Hurlbert 1971) as: PIE = N/(N-1)×(1-∑ p_i^2), where N = total number of individuals, and p_i = is the relative abundance of species i. within each plot using the `calcPIE` function in the
“mobr” package (McGlinn et al. 2018). We chose to calculate PIE as our evenness measurement because of its independence from sample size when comparing across elevation sites (Chase & Knight 2013). We tested for differences in plant richness and evenness between plots associated with the presence of Castilleja across sites by constructing linear mixed-effect models with elevational site, Castilleja presence (absent or present), and their interaction as fixed factors using the nlme package (Pinheiro et al. 2014). Within each mixed model, we allowed the intercept to vary by plot pairings (random effect). Next, we constructed mixed effect models with and without fixed factors (elevation and Castilleja presence) and compared AIC scores to determine if adding each fixed factor improved model fit. Next, we calculated the deviance and compared the inclusion of each factor with a likelihood ratio test using the Anova function (“car” package, Fox & Weisberg, 2011).

To test whether the presence of Castilleja was associated with changes in plant community composition, we performed a PERMANOVA using the adonis function in the vegan package (Oksanen et al. 2017) with Castilleja presence, elevation, and plot pairing as predictors. To separate the effects of Castilleja presence on abundance shifts among dominant taxa versus changes in taxa gains and losses, we performed PERMANOVAs using abundance-weighted Bray-Curtis distances as well as presence-absence data using Jaccard’s distance. During our initial PERMANOVA fitting across all sites (Table S2), we observed significant interaction terms within both elevation × Castilleja presence ($F_{(1,99)} = 2.532, p = 0.01$) and elevation × plot pairing ($F_{(1,99)} = 13.48, p = 0.01$), which suggested that effect of Castilleja presence on plant community composition differed by site. To explore when and where Castilleja was associated with plant community composition, we conducted separate PERMANOVAs for each elevation.
Within our site level PERMOANOVAs, we included *Castilleja* presence, plot pairing, and their interaction as predictor variables.

To visualize the results of our PERMANOVAs, we performed non-metric multidimensional (nMDS) scaling based on Bray-Curtis distances using the *metaMDS* function in the *vegan* package (Oksanen et al. 2017) for each elevational site.

Finally, we compared fungal colonization patterns within the dominant plant species with mixed effect models as described above, with *Castilleja* presence/absence, elevational site and the interaction between *Castilleja* presence and elevation as predictor variables. For fungal mixed models, we constructed AMF and DSE models separately. Structures of AMF and DSE (vesicles, arbuscules, coils, spores, microsclerotia) were extremely rare within our samples, therefore we present only AMF and DSE hyphal colonization data.

**Results**

*Castilleja association with plant richness and evenness*

Overall, we found that the presence of *Castilleja* and elevation were retained within our best models to predict plant richness and evenness (Table S1). Taken as a whole, we found that the presence of *Castilleja* was associated with an increase in plant richness of 11% ($\chi^2 = 52.360$, $p < 0.0001$, Table 2), and an increase in plant evenness of 5% ($\chi^2 = 6.805$, $p = 0.009$, Table 2).

As expected, elevation significantly impacted plant richness and evenness, with the highest plant species richness and evenness values observed at the middle elevation sites (3200 m: richness $\mu = 11.8$, evenness $\mu = 0.835$; 3392 m: richness $\mu = 11.2$, evenness $\mu = 8.46$) (Table 2)(Fig 1).

Surprisingly, we observed no significant interaction terms between *Castilleja* presence and plant richness or evenness (Table 2).
Castilleja association with plant community composition changes

Overall, we found that elevation was the best predictor of plant community composition change, accounting for 30% of the data variation in community composition (Table S2). Using abundance-weighted measures, Castilleja presence was associated with a significant ($p = 0.01$), but weak effect ($R^2 = 0.021$) on overall plant community composition (Table S2). Site-level heterogeneity (plot pairings) explained an additional 9% of data variation across plant community composition.

At the site level, we found that the presence of Castilleja was associated with plant composition shifts in abundance-weighted (Bray-Curtis distance) at two (2,740m and 3,200m) of the five elevation sites (Table 3; Fig 2). At 2,740m, we observed higher abundance of Adenolinum lewissii, Phacelia sericea, Antennaria rosea and reduced abundance of shrub species Artemisia tridentate and Symphoricarpos rotundifolia, and forb Delphinium nuttallianum when Castilleja was present (Fig 2). At 3,200m, we observed reduced cover of invasive species Bromopsis inermis, when Castilleja was present (Fig 2). At three of the five sites (2,480m, 3,392m, and 3,460m), we observed a significant relationship of plot pairing, suggesting that spatial heterogeneity in plant community composition may cloud our ability to observe the effect of Castilleja on community composition. Unlike our abundance-weighted results, we found no effect of Castilleja and only weak effects of plot pairings on plant community composition when sites were compared using Jaccard’s index (Table S3).

Castilleja association with mycorrhizal and dark-septate endophyte colonization
Overall, we found that *Castilleja* presence was associated with reduced mycorrhizal colonization at each site by 20% (Table 2, Fig 3) and was the only factor retained within our best fit model (Table S1). At our four lowest elevation sites *Castilleja presence* was correlated with a reduction in AMF colonization in *Balsamorhiza sagittata* (2480 m), *Chrysothamnus viscidiflorus* (2740 m), *Viola adunca* (3200 m), *Ligusticum porteri* (3392 m), and at our highest (3460 m) elevation site, *Castilleja* presence was correlated to a reduction in ericoid mycorrhizae colonization within *Arctostaphylos uva-ursi*. We found that climate contexts had no impact on *Castilleja* effects, as elevation was not retained within our best-fit model for mycorrhizal colonization (Table S1). Surprisingly, we found that *Castilleja* presence was not related to DSE colonization and only elevation was retained within the best fit model predicting DSE colonization. Overall, we found that DSE colonization was highest ($\mu = 51.9\%$) at our high elevation site (3,460m), and colonization rates were similar at the four other elevations, ranging from 27-33% (Table 2, Fig 3).

**Discussion**

*Castilleja increases plant richness and evenness*

Overall, we found the presence of *Castilleja* was associated with an 11% increase in plant richness and a 5% increase in plant evenness. Our results are in line with several studies that have found increased plant richness and evenness with the presence hemiparasites (Davies et al. 1997; Press & Phoenix 2005; Bardgett et al. 2006; Westbury et al. 2006; Reed 2012), although this result can be contingent on hemiparasite identity and the identity of the surrounding plant community (Press & Phoenix 2005; Westbury & Dunnett 2007). Interestingly, we found the effect of *Castilleja* associated with plant richness and evenness did not differ by site, suggesting
in this system *Castilleja* effects may be consistent despite the shifting biotic and abiotic contexts along the elevational gradient. Background plant richness and evenness shifted across the elevational gradient, displaying the characteristic unimodal pattern with highest richness and evenness at middle elevations. Additionally, the species of *Castilleja* present differed by elevation, with *C. angustifolia* present at 2,480 m and 2,740 m, *C. miniata* at 3,392 m, and *C. sulphurea* at 3,200 m, 3,392 m, and 3,460 m.

**Castilleja has inconsistent effects on plant community composition**

The presence of *Castilleja* was associated with a significant, but weak, effect on overall plant community composition across the elevational gradient. However, site-level analyses revealed that the effect of *Castilleja* on abundance-weighted plant community composition differed by site. For example, at two of our middle elevation sites (2,740 m and 3,200 m), the presence of *Castilleja* was the strongest predictor of plant community composition. The presence of *Castilleja* was correlated with a reduction in aboveground cover of shrub species *Artemisia tridentata* and *Symphoricarpos rotundifolia* at 2,740 m and invasive grass *Bromopsis inermis* at 3,200 m. The reduction of shrub and grass taxa was matched with an increase in low abundant forb taxa: *Adenolinum lewisii*, *Phacelia sericea*, and *Antennaria rosea*. However, it is important to note the nature of our study did not allow us determine which hosts *Castilleja* were parasitizing, how much carbon was being parasitized from hosts, or the long-term dynamics of *Castilleja* in an ecosystem comprised mostly of long-lived perennial plant taxa. In a previous study by Ducharme and Ehlerginer (1996), authors found *Castilleja* received ~40% of its carbon from critical host *Artemisia tridentata*. However, *Castilleja* had no effect on the photosynthetic rates or water potentials of *Artemisia* over a two-year period. Thus, it is unclear how detrimental
Castilleja is for host production and survival (Ducharme & Ehlerginer 1996; but see Reid et al. 1994 and Bowie & Ward 2004).

The association of Castilleja presence on plant community composition was only apparent when we compared plant community composition using abundance-weighted Bray-Curtis distance metrics. When we compared plant community composition using presence/absence data we found no association of Castilleja presence. This suggests that the association of Castilleja is primarily driven by abundance changes among dominant plant taxa and less by taxa gains and losses. Taken together, our results align with previous studies demonstrating that few hemiparasites reduce host resources enough to cause mortality, but can reduce host growth rate allowing for the establishment of subordinate taxa (Reid et al. 1994; Watson 2009).

Although our study is not a direct test of the effect of Castilleja on plant community composition, our results align with previous direct, manipulative hemiparasite removal and addition experiments that report a strong negative effect of hemiparasites on dominant taxa abundance with an overall increase in plant diversity, especially with forb taxa (Pennings & Callaway 1996; Davies et al. 1997; Press 1998; Press & Pheonix 2005; Bardgett et al. 2006; Těšitel et al. 2015; 2018). For instance, sowing of hemiparasite Rhinanthus into a Calamagrostis invaded grassland reduced Calamagrostis cover from ~45% to ~2% within two growing seasons (Těšitel et al. 2018). Overall, plant diversity increased to compensate for the reduction in Calamagrostis biomass and cover, although authors found that Rhianthus had inconsistent effects on overall plant community composition. Taken together, this suggests that hemiparasite effects on overall plant community composition are likely driven by the identity of the surrounding plant species, competitive ability of these taxa, and abundance levels of those taxa.
(Press & Phoenix 2005; Watson 2009; Těšitel et al. 2018). To confirm previous findings, a direct test manipulating both plant community composition and the presence of hemiparasites needs to be performed.

Site-level spatial heterogeneity was a strong driver of plant community composition

At three of the five sites (2,480m, 3,392m, 3,460m), plot pairing was the strongest predictor of plant community composition explaining ~13%, 18%, and 13% respectively, of the data variation in community composition. At each of these three sites, the presence of Castilleja had no effect on plant community composition, suggesting that site-level spatial heterogeneity obscures our ability to detect changes in community composition mediated by hemiparasites.

This was surprising because we observed a similar increase in plant richness and plant evenness between plot pairings at all five sites. The overall richness and evenness effects, but limited compositional effects likely reflects the generalist hemiparasite life history of Castilleja (Ducharme & Ehleringer 1996; Adler 2003; Spasojevic & Suding 2012). Additionally, the effect of plot pairing was stronger using abundance-mediated measures community composition (Bray-Curtis distance), compared to presence-absence (Jaccard’s distance). With the exception of our low elevation site, we observed no effect of plot pairing on plant community composition using Jaccard’s distance metrics. This suggests that at each site, differences in community composition were driven by abundance differences in taxa plot to plot, and less by taxa turnover among plots.

Hemiparasitic plants shape abundant plant interactions with mycorrhizal fungi

Overall, we found that the presence of Castilleja was associated with a reduction in AMF colonization by 18% and ericoid mycorrhizal fungi (ERM) by 17%. However, we found no
effect of *Castilleja* presence on DSE colonization within focal taxa roots. We found that *Castilleja* reduced mycorrhizal colonization similarly across elevation sites even though the overall rate of colonization differed by elevation, suggesting a ubiquitous pattern independent of biotic and abiotic contexts, counter to our expectations. However, our results provide an indirect test of the effect of *Castilleja* on mycorrhizal colonization because we did not confirm if sampled plants were actively parasitized by *Castilleja*. Additionally, we were unable to determine if every single root we quantified colonization was from the target individual. However, even with the indirect measure and the uncertainty of host identity, we still observed a consistent reduction in mycorrhizal colonization when *Castilleja* was present.

Our results align with previous studies that observed a reduction in mycorrhizal fungal colonization with hemiparasite colonization (Gehring & Whitham 1992; Davies & Graves 1998; reviewed in Press & Phoenix 2005). One potential explanation for the reduction in mycorrhizal colonization is that hemiparasites outcompete mycorrhizal fungi for host carbon resources (Gehring & Whitham 1992; Davies & Graves 1998; reviewed in Press & Phoenix 2005). Because AMF have a higher reliance on host-derived carbon resources relative to DSE and ERM, competition with hemiparasites for host carbon may explain why we observed reduced AMF colonization but no difference in DSE colonization (Caldwell et al. 2000; Jumpponen, 2001; Usuki & Narisawa 2007; Knapp et al. 2018). Although at our high elevation site, we observed reduced ERM colonization in *Arctostaphylos uva-ursi* even though ERM produce a suite of carbon-degrading enzymes making them less reliant on plant-derived carbon (Read et al. 2004; Smith & Read 2008; Averill et al. 2014). Future studies should directly test whether hemiparasites reduce carbon allocation to mycorrhizal fungi and whether the differences in the
reliance on host carbon resources will determine the response of fungal colonization to
hemiparasite presence.

Hemiparasites also directly associate with mycorrhizal fungi and DSE (Bouwmeester et al. 2007; Li & Guan 2008; Stein et al. 2009; Li et al. 2012). Associations with mycorrhizal fungi and DSE may provide an additional mechanism for hemiparasites to access soil and plant-derived resources (Li & Guan 2008), so it is unclear whether a reduction fungal colonization in dominant plant species would be detrimental to hemiparasites. In a previous field census, we found both AMF and DSE actively colonizing the roots of *C. angustifolia*, *C. miniate*, and *C. sulphurea* across this elevational gradient (Henning unpublished data). However, we did not measure fungal colonization rates of *Castilleja* in this study. Our results highlight an interesting pattern, however further study is required to determine if interactions among hemiparasites, mycorrhizal fungi, and plant hosts could cascade to influence plant community composition and ecosystem function.

**Hemiparasite effects on plant diversity, plant community composition and mycorrhizal colonization are independent of climate contexts**

We sought to explore how climate contexts shape the impact of hemiparasites on plant richness, evenness, community composition, and the relationship of dominant plant species with root symbionts. Although we found that the presence of *Castilleja* or elevation were always retained in the best fit models for plant richness, evenness, mycorrhizal colonization, and DSE, we did not observe any significant interactions between the presence of *Castilleja* and elevation for any response variable. This suggests that hemiparasites increase plant richness, increase plant evenness and reduce mycorrhizal fungal colonization within dominant species independent of
biotic and abiotic contexts. Our results are counter to several studies, which have found hemiparasite effects are contingent on-site properties like: plant composition; nutrient availability, soil moisture, mycorrhizal fungi present, plant productivity (Callaway & Pennings 1998; Matthies & Egli 1999; Stein et al. 2009; Tĕšitel et al. 2015; 2018). Thus, across a wide array of ecosystems, hemiparasites may be critical for the maintenance of plant diversity and the regulation of competitively dominant plant taxa.

Conclusions

Overall, we found that the presence of *Castilleja* was consistently associated with increased richness and evenness within the plant community, while reducing colonization by mycorrhizal fungi across a wide-spread elevational gradient. This suggests that the effect of *Castilleja* on plant and fungal communities is consistent across climate contexts, the species of *Castilleja* present, and differences in plant community composition, and underlying differences in overall diversity, even if the overall effect of *Castilleja* on plant community composition was inconsistent. Although our study was observational by design, our study provides testable hypothesis to explore within future mechanistic experiments exploring the interaction between hemiparasites, mycorrhizal fungi, and host plants, across climatic gradients.

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Figure Legends

Figure 1: Plant richness and plant evenness (probability of interspecific encounter, PIE) within a 0.5 m × 0.5 m quadrat across five elevational sites with *Castilleja* present (blue) or *Castilleja* absent (orange). Each boxplot consists of ten plots at each elevation site. The midline represents the median value, with lower and upper hinges corresponding to the 25th and 75th percentiles. The upper and lower whiskers extend from the hinges to highest to the lowest values but no further than 1.5× the inter-quartile range (IQR).

Figure 2: Non-metric multidimensional scaling of plant community composition based on Bray-Curtis distances with either *Castilleja* present (blue) or absent (orange) across 5 elevational sites.

Plant species identities: ACHMIL = *Achillea millefolium*, ADELEW = *Adenolinum lewisii*, AGOGLA = *Agoseris glauca*, AGO_SP = *Agoseris sp.*, ALLSEP = *Allium sp.*, ANDSEP = *Androsace septentrionalis*, ANTROS = *Antennaria rosea*, ARCUVA = *Arctostaphylos uva-ursi*, ARNMOL = *Arnica mollis*, ARTTRI = *Artemisia tridentate*, BALSAG = *Balsamorhiza sagittata*, BOESTR = *Boechera stricta*, BROINE = *Bromopsis inermis*, CAPBUR = *Capsella bursa-pastoris*, CARALB = *Carex albonigra*, CAR_SP = *Carex sp.*, CHRVIS = *Chrysothamnus viscidiflorus*, CLATON = *Claytonia lanceolata*, DELNUT = *Delphinium nuttallianum*, DRAAUR = *Draba aurea*, ELYELY = *Elymus elymoides*, ERECON = *Eremogone congesta*, ERISPE = *Erigeron speciosus*, ERI_SP = *Erigeron sp.*, ERYGRA = *Erythronium grandiflorum*, FESTHU = *Festuca thurberi*, FRAVIR = *Fragaria virginiana*, GALSEP = *Galium septentrionale*, HELQUI = *Helianthella quinquenervis*, IPOAGG = *Ipomopsis aggregate*, JUNDRU = *Juncus drummondii*, LIGPOR = *Ligusticum porteri*, MAHAQU = *Mahonia repens*,...
Figure 3: Colonization rates of A) mycorrhizal fungi as either arbuscular mycorrhizal fungi (AMF – at 2480m, 2740m, 3200m, 3392m, 3460m) or ericoid mycorrhizal fungi (ERM – 3460m) and B) dark-septate endophytes (DSE) across all five elevational sites with Castilleja present (blue) or Castilleja absent (orange). Each boxplot consists of ten plots at each elevation site. The midline represents the median value, with lower and upper hinges corresponding to the 25th and 75th percentiles. The upper and lower whiskers extend from the hinges to highest to the lowest values but no further than 1.5× the inter-quartile range (IQR).

Table 1: Site characteristics, dominant plant species, functional group of the dominant species, and root associated fungi within dominant plant roots. Elev = Elevation, MAT = mean annual temperature, MAP = mean annual precipitation. AMF = arbuscular mycorrhizal fungi, DSE = dark-septate endophytes, ERM = ericoid mycorrhizal fungi.

Table 2: Analysis of deviance table from “best-fit” mixed-model results exploring how site (elevation), the presence of Castilleja (Castilleja present), and the interaction of elevation and Castilleja presence on plant richness, plant evenness (probability of interspecific encounter, PIE), mycorrhizal fungal colonization, and dark septate endophyte (DSE) colonization. Full
Table S1: Model selection to determine “best” fit mixed model to predict plant richness, plant evenness, mycorrhizal colonization, and dark septate endophyte colonization. Full mixed models were constructed with Castilleja presence and elevation as fixed factors and intercepts were allowed to vary by plot pairings (random effect). Fixed effects were sequentially removed from the full model until we only fit the random effect. We report the number of model parameters (df) and AIC score (AIC).

Table S2: PERMANOVA results to partition the effect of site (Elevation), Castilleja presence (Castilleja present) and spatial structure (plot pairing) on plant community composition using Bray-Curtis distances. Df = degrees of freedom, SS = Sequential sums of Squares, MeanSqs = Mean squares, $F = F$ statistic, $R^2 = \text{partial } R^2$, $P = p$ value.

Table S3: Site level (elevation) PERMANOVA results to partition the effect of Castilleja presence (Castilleja present) and spatial structure (plot pairing) on plant community composition
using Jaccard’s distances. Df = degrees of freedom, SS = Sequential sums of Squares, MeanSqs = Mean squares, $F = F$ statistic, $R^2 = \text{partial } R^2$, $P = \text{p value.}$
Figure 1

Plant richness (A) and plant evenness (B, probability of interspecific encounter) within a 0.5 m × 0.5 m quadrat across five elevational sites with *Castilleja* present (blue) or *Castilleja* absent (orange)

Each boxplot consists of ten plots at each elevation site. The midline represents the median value, with lower and upper hinges corresponding to the 25th and 75th percentiles. The upper and lower whiskers extend from the hinges to highest to the lowest values but no further than 1.5× the inter-quartile range (IQR).
Manuscript to be reviewed

**A**

Plant richness

**B**

Plant evenness (PIE)
Figure 2

Non-metric multidimensional scaling of plant community composition based on Bray-Curtis distances with either *Castilleja* present (blue) or absent (orange) across 5 elevational sites.

(A) 2480 m, (B) 2740 m, (C) 3200 m, (D) 3392 m, (E) 3460 m. Plant species identities:

ACHMIL = *Achillea millefolium*, ADELEW = *Adenolinum lewisii*, AGOGLA = *Agoseris glauca*,
AGO_SP = *Agoseris sp.*, ALLSEP = *Allium sp.*, ANDSEP = *Androsace septentrionalis*, ANTROS = *Antennaria rosea*, ARCUVA = *Arctostaphylos uva-ursi*, ARNMOL = *Arnica mollis*, ARTTRI = *Artemisia tridentata*, BALSAG = *Balsamorhiza sagittata*, BOESTR = *Boechera stricta*, BROINE = *Bromopsis inermis*, CAPBUR = *Capsella bursa-pastoris*, CARALB = *Carex albonigra*, CAR_SP = *Carex sp.*, CHRVIS = *Chrysothamnus viscidiflorus*, CLATON = *Claytonia lanceolata*, DELNUT = *Delphinium nuttallianum*, DRAAUR = *Draba aurea*, ELYELY = *Elymus elymoides*, ERECON = *Eremogone congesta*, ERISPE = *Erigeron speciosus*, ERI_SP = *Erigeron sp.*, ERYGRA = *Erythronium grandiflorum*, FESTHU = *Festuca thurberi*, FRAVIR = *Fragaria virginiana*, GALSEP = *Galium septentrionale*, HELQUI = *Helianthella quinquenervis*, IPOAGG = *Ipomopsis aggregate*, JUNDRU = *Juncus drummondii*, LIGPOR = *Ligusticum porteri*, MAHAQU = *Mahonia repens*, MUHMON = *Muhlenbergia montana*, PHASER = *Phacelia sericea*, POA_SP = *Poa sp.*, PHLOX = *Phlox hoodii*, POTGRA = *Potentilla gracilis*, PSEMON = *Pseudocymopterus montanus*, PULPAT = *Pulsatilla patens*, ROSWOO = *Rosa woodsii*, SEDLAN = *Sedum lanceolatum*, SENCRA = *Senecio crassulus*, SYMROT = *Symphoricarpos rotundifolia*, TAROFF = *Taraxiacum officinale*, THAFEN = *Thalictrum fendleri*, VIOADU = *Viola adunca.*
Manuscript to be reviewed

A

2480 m

B

2740 m

C

3200 m

D

3392 m

E

3460 m

Castilleja present

Castilleja absent
Figure 3

Colonization rates of (A) mycorrhizal fungi and (B) dark-septate endophytes (DSE) across all five elevational sites with *Castilleja* present (blue) or *Castilleja* absent (orange).

Mycorrhizal fungal colonization consists of arbuscular mycorrhizal fungi (AMF – at 2480m, 2740m, 3200m, 3392m, 3460m) or ericoid mycorrhizal fungi (ERM – 3460m). Each boxplot consists of ten plots at each elevation site. The midline represents the median value, with lower and upper hinges corresponding to the 25th and 75th percentiles. The upper and lower whiskers extend from the hinges to highest to the lowest values but no further than 1.5× the inter-quartile range (IQR).
Table 1 (on next page)

Site characteristics, dominant plant species, functional group of the dominant species, and root associated fungi within dominant plant roots.

Elev = Elevation, MAT = mean annual temperature, MAP = mean annual precipitation. AMF = arbuscular mycorrhizal fungi, DSE = dark-septate endophytes, ERM = ericoid mycorrhizal fungi.
Table 1:

| Elev (m) | 2480  | 2740  | 3200  | 3392  | 3460  |
|----------|-------|-------|-------|-------|-------|
| Latitude | 38.65391 | 38.71533 | 38.96133 | 38.97005 | 38.99158 |
| Longitude| -106.86198 | -106.82264 | -107.03147 | -107.03987 | -107.06656 |
| MAT (C)  | 1.36  | 1.52  | -0.80  | -0.70  | -1.62  |
| MAP (mm) | 443.4 | 439.2 | 599.0  | 592.0  | 667.8  |
| *Castilleja sp* present | C. angustifolia | C. angustifolia | C. sulphurea | C. miniata | C. sulphurea |
| *Castilleja* cover (%) | 3.6 | 2.5 | 11 | C. m. – 1.2 | C. s – 4.5 |
| Targeted species | Balsamorhiza sagittata | Chrysothamnus viscidiflorus | Viola adunca | Ligusticum porteri | Arctostaphylos uva-ursi |
| Functional group | Forb | Forb | Forb | Forb | Forb |
| Fungi present | AMF, DSE | AMF, DSE | AMF, DSE | AMF, DSE | ERM, DSE |
Table 2 (on next page)

Analysis of deviance table from “best-fit” mixed-model results exploring how site, the presence of *Castilleja*, and the interaction influence plant richness, plant evenness, and fungal colonization.

Cast present = presence of *Castilleja*, Sum Sq = sum of squares, Df = Degrees of freedom, $F = F$ statistic, $P = P$ value.
Table 2:

| Response                        | Retained factors          | $\chi^2$ | Df | $P$   |
|---------------------------------|---------------------------|----------|----|-------|
| Plant richness                  | *Castilleja* present      | 52.360   | 1  | <0.0001|
|                                 | Elevation                 | 38.905   | 1  | <0.0001|
|                                 | *Castilleja* × Elevation  | 0.4467   | 1  | 0.504  |
| Plant evenness (PIE)            | *Castilleja* present      | 6.8050   | 1  | 0.009  |
|                                 | Elevation                 | 36.627   | 1  | <0.0001|
|                                 | *Castilleja* × Elevation  | 1.5041   | 1  | 0.220  |
| Mycorrhizal colonization (%)    | *Castilleja* present      | 163.17   | 1  | <0.0001|
| DSE colonization (%)            | Elevation                 | 11.497   | 1  | 0.0007 |
Table 3 (on next page)

Site-level (elevation) abundance-weighted (Bray-Curtis) PERMANOVA results to partition the effect of *Castilleja* presence and spatial structure (plot pairing) on plant community composition.

Df = degrees of freedom, SS = Sequential sums of Squares, MeanSqs = Mean squares, $F = F$ statistic, $R^2 = $ partial $R^2$, $P = \text{p value}$. 

| Castilleja Presence and Spatial Structure (Plot Pairing) | Df | SS  | MeanSqs | F  | R² | P |
|--------------------------------------------------------|----|-----|---------|----|----|---|
|                                                        |    |     |         |    |    |   |
## Table 3:

| Elevation | Factor                | Df | SS    | MeanSqs | F      | R²     | P     |
|-----------|-----------------------|----|-------|---------|--------|--------|-------|
| 2480m     | Castilleja present    | 1  | 0.211 | 0.21099 | 1.087  | 0.0529 | 0.37  |
|           | Plot pairing          | 1  | 0.500 | 0.50037 | 2.577  | 0.1255 | 0.05  |
|           | Cas. present × Pairing| 1  | 0.170 | 0.16982 | 0.875  | 0.0426 | 0.54  |
|           | Residuals             | 16 | 3.106 | 0.19414 | 0.7790 |        |       |
|           | Total                 | 19 | 3.987 |         | 1.0000 |        |       |
| 2740m     | Castilleja present    | 1  | 0.291 | 0.29112 | 2.173  | 0.1004 | 0.03  |
|           | Plot pairing          | 1  | 0.243 | 0.24259 | 1.811  | 0.0837 | 0.14  |
|           | Cas. present × Pairing| 1  | 0.222 | 0.22188 | 1.656  | 0.0765 | 0.09  |
|           | Residuals             | 16 | 2.143 | 0.13397 | 0.7394 |        |       |
|           | Total                 | 19 | 2.899 |         | 1.0000 |        |       |
| 3200m     | Castilleja present    | 1  | 0.481 | 0.48098 | 2.637  | 0.1257 | 0.01  |
|           | Plot pairing          | 1  | 0.278 | 0.27842 | 1.527  | 0.0727 | 0.11  |
|           | Cas. present × Pairing| 1  | 0.150 | 0.14983 | 0.822  | 0.0391 | 0.55  |
|           | Residuals             | 16 | 2.918 | 0.18237 | 0.7624 |        |       |
|           | Total                 | 19 | 3.827 |         | 1.0000 |        |       |
| 3392m     | Castilleja present    | 1  | 0.101 | 0.10117 | 0.756  | 0.0355 | 0.69  |
|           | Plot pairing          | 1  | 0.523 | 0.52344 | 3.911  | 0.1837 | 0.01  |
|           | Cas. present × Pairing| 1  | 0.084 | 0.08361 | 0.625  | 0.0293 | 0.76  |
|           | Residuals             | 16 | 2.141 | 0.13382 | 0.7514 |        |       |
|           | Total                 | 19 | 2.849 |         | 1.0000 |        |       |
| 3460m     | Castilleja present    | 1  | 0.229 | 0.22856 | 1.076  | 0.0536 | 0.37  |
|           | Plot pairing          | 1  | 0.546 | 0.54636 | 2.573  | 0.1280 | 0.04  |
|           | Cas. present × Pairing| 1  | 0.094 | 0.09421 | 0.444  | 0.0221 | 0.90  |
|           | Residuals             | 16 | 3.398 | 0.21239 | 0.7963 |        |       |
|           | Total                 | 19 | 4.267 |         | 1.0000 |        |       |